User Guide

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Agilent AdvanceBio Amide HILIC 1.8 µm Columns

HILIC columns for analysis of N-glycans and other polar analytes

Introduction

Agilent AdvanceBio Amide HILIC columns are designed for HILIC separations of N-glycans and other polar analytes. The totally porous, 1.8 µm silica particles and unique bonding chemistry provide for exceptional long-term stability and reproducibility, while providing optimal separation of complex N-glycan mixtures.

Getting started

A column performance report, including a column-specific QC test chromatogram and a batch-specific glycan separation, is enclosed with every Agilent column.

Agilent's 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 AdvanceBio Amide HILIC packings are respirable. Opening columns is strongly discouraged due to the safety risk and likelihood of reducing column performance. If you must, open columns only in a well-ventilated area.

Other tips

- Flow direction is marked on the column.
- Small injection volumes are best for HILIC separations. Injection volumes of more than 1 µL will compromise peak shape and resolution unless sample solvent formulation is adjusted to match the starting conditions of the gradient.

Using your column

Installation

Attach columns to the instrument using a short 3/8-inch wrench. This will help prevent overtightening of the end fittings, which can damage the column.

Tip: Use an Agilent InfinityLab Quick Connect LC fitting (part number 5067-5966) to quickly connect the column to your LC instrument.

Column conditioning

Agilent AdvanceBio Amide HILIC columns are designed for separating N-linked glycans cleaved from glycoproteins and glycopeptides. For the HILIC mechanism to work effectively, you must fully equilibrate the column before use.

Caution: Do not overpressure the fluorescence detector (FLD) flow cell. Agilent recommends disconnecting the FLD completely during column conditioning so that all mobile phase is diverted to waste.

Agilent recommends that you condition the column in this sequence:

- Mobile phase A: 50 mM Ammonium formate, pH 4.4
- Mobile phase B: Acetonitrile
- Flow rate: 0.5 mL/min
- Column temperature: 60 °C

1. Condition at 80% A for 15 minutes.
2. Equilibrate at initial gradient conditions for 10 minutes.

Optional conditioning for maximum MS signal

- Mobile phase A: 5 mM Ammonium formate, pH 4.4
- Mobile phase B: Acetonitrile
- Flow rate: 0.9 mL/min
- Column temperature: 80 °C

1. Condition at 90% A for 70 minutes.
2. Equilibrate at initial gradient conditions for 10 minutes.
3. Divert all LC flow to waste.

Note: If optional conditioning is desired, perform optional conditioning prior to standard conditioning and equilibration.

Instructions for use

Operating parameters: pH, temperature, and pressure

- The maximum operating pressure is 1,200 bar.
- Optimal column life is achieved by operating only up to 80% of the maximum pressure. The typical operating temperature is 60 °C. Temperatures up to 80 °C can be used but will shorten column lifetime.
- The operating pH range is 2 to 7.

Note: Using the column above pH 7 and 60 °C reduces column lifetime. AdvanceBio Amide HILIC columns are silica-based with a HILIC amide phase. All silica has some solubility in pH > 6 aqueous mobile phases, and solubility is increased at elevated temperatures.

Shipping solvents and compatibility

- AdvanceBio Amide HILIC columns are shipped with acetonitrile and are ready to use for HILIC separations. HILIC columns require more equilibration than reversed-phase columns. They are compatible with buffers and acetonitrile, which are most commonly used for glycan analysis.
- A typical mobile phase for glycan analysis is acetonitrile and ammonium formate buffer, pH 4.4. You can use this phase with fluorescence or MS detection.

Recommended starting conditions

- Mobile phase A: 50 mM Ammonium formate, adjusted to pH 4.4 with formic acid
- Mobile phase B: Acetonitrile
- Column temperature: 60 °C
- Flow rate: 0.5 mL/min

Note: Examples shown using 1 µL aqueous injections. For larger volume injections, consult your sample preparation user manual for guidance.

Table 1. Recommended starting gradient for highly complex or sialylated glycan samples.

Time	%B	Flow (mL/min)
0	76	0.5
45	46	0.5
46	40	0.5
48	40	0.5
49	76	0.5
60	76	0.5

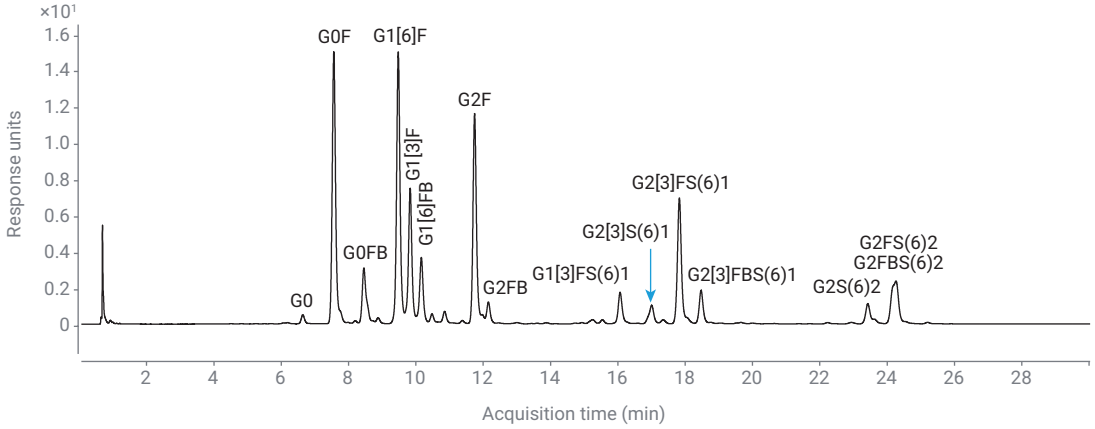

Figure 1. Sample chromatogram of Agilent AdvanceBio InstantPC Human IgG N-glycan library (part number GKPC-005) using the 60-minute gradient.

Table 2. Recommended starting conditions for simple neutral glycan samples.

Time	%B	Flow (mL/min)
0	76	0.5
17	62	0.5
18	40	0.5
20	40	0.5
21	76	0.5
30	76	0.5

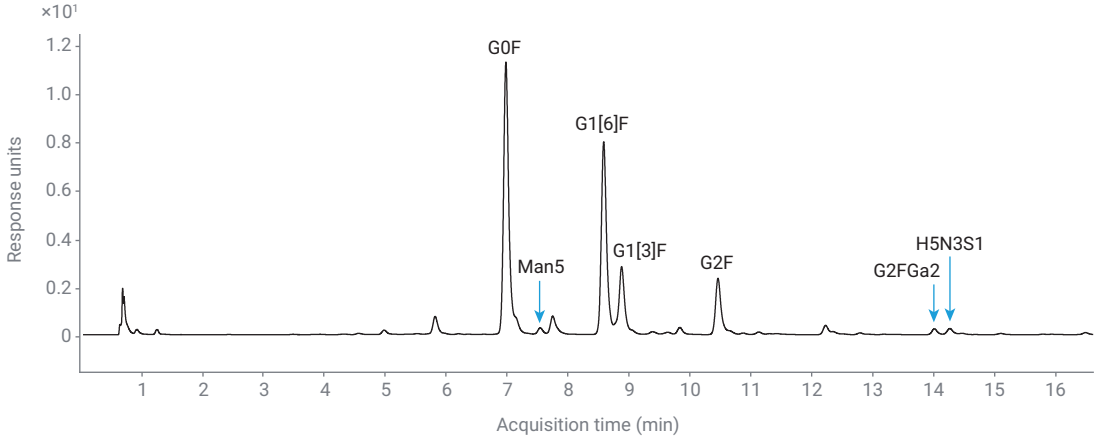

Figure 2. Sample chromatogram of AdvanceBio InstantPC-labeled N-glycans released from Agilent-NISTmAb using the 30-minute gradient.

Column care and cleaning

Extend the life of your column through proper cleaning:

- Separation methods should conclude with a flushing step using 60% mobile phase A (see "Recommended starting conditions"), followed by re-equilibration.
- If the solvent flow appears restricted (due to unusually high column backpressure), check to see that the flow is unobstructed up to the column inlet.
- If the restriction is before the column, replace the piece of tubing or filter that is plugged.
- If the column is plugged, replace the column.
- Do not backflush.
- Remove particulates before sample analysis to avoid blockage of the column inlet frit. If this is not possible, use an inline filter to protect the column and increase its lifetime. Be sure to filter samples before injecting them onto any column.

Recommended storage

Columns can be safely stored for short periods in the mobile phases. We recommend pure acetonitrile as the long-term storage solvent. Since higher-concentration buffers can precipitate when mixed with acetonitrile, it may be necessary to first flush the column with an acetonitrile/buffer mix that contains less than 10 mM total buffer prior to flushing with acetonitrile. Before storing the column, tightly cap the end fittings with end plugs to prevent the packing from drying out.

Ordering details

Description	Part Number
AdvanceBio Amide HILIC, 2.1 × 150 mm, 1.8 µm	859750-913
AdvanceBio Amide HILIC, 2.1 × 100 mm, 1.8 µm	858750-913
AdvanceBio Ammonium Formate Mobile Phase Concentrate (Makes 1 L of 50 mM Ammonium Formate, pH 4.4)	G3912-00000
AdvanceBio InstantPC Human IgG N-glycan Library	GKPC-005
Agilent-NISTmAb, 25 µL (10 mg/mL, 0.25 mg Total)	5191-5744
Agilent-NISTmAb, 4 × 25 µL (10 mg/mL, 1 mg Total, 0.25 mg per Aliquot)	5191-5745

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