CARE AND USE MANUAL

Ultisil HILIC Amphion II HPLC Column Care and Use Manual

Welch

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Dear User:

Thank you for choosing our liquid chromatography column products! This manual is designed to help you use our products under optimal conditions and maximize column lifespan. Please read the usage instructions carefully before operation.

Ultisil HILIC Amphion II is a zwitterionic HILIC column that employs covalent bonding technology to attach zwitterionic functional groups (containing both cations and anions). This eliminates the need for ion-pairing reagents while demonstrating exceptional hydrophilicity, enabling effective retention of highly polar ionic compounds and basic drug molecules that exhibit weak or no retention in reversed-phase chromatography.

Preparation Before Use

Column Identification

To ensure the quality of every column, each column sold by Welch Materials is assigned a unique identification code. This code allows us to trace quality-related issues. Upon receiving your column, please follow these steps:

1. Verify that the packaging is intact and matches the ordered column model.
2. Ensure the package contains a COA report with the inspector's signature.
3. Check the column surface for any damage and confirm that the protective plugs on both ends are intact.
4. Verify that the column body bears a Welch Materials identification label. Confirm that the model and serial number on the box match those on the column label.

Note:

The packing material inside the column is composed of fine particles. Do not attempt to open the column arbitrarily, as the material may become airborne and pose a health risk. If opening is necessary, use appropriate protective measures.

Installation

To achieve optimal column efficiency, use narrow-diameter tubing for connections. The connections to the column inlet and outlet must be tightly sealed, with the tubing ends polished and free of burrs or bevels. Use professional tools to cut and trim tubing.

1. Remove the column from its packaging. Check the direction of the eluent flow indicated by the arrow on the column body. Unscrew the protective plugs at both ends of the column. Position the column so that the eluent flow aligns with the direction indicated, as shown in Figure 1.

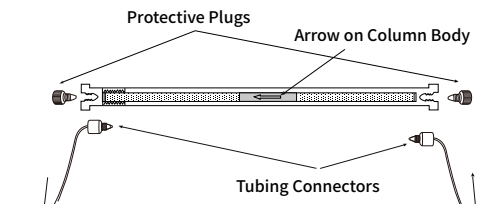


Figure 1. Column Installation

2. Carefully screw stainless steel or other compatible material fittings into both ends of the column. Ensure tight, zero-dead-volume connections to prevent leakage during operation.

Product Specifications

Column	Ultisil HILIC Amphion II
USP Code	L114
Pore Size	120 Å
Specific Surface Area	320 m^2/g
Carbon Loading	6 %
pH Range	2.0-8.0
Max. Temperature	60 °C (mobile phase pH ≤6.5)
	40 °C (mobile phase pH >6.5)
Max. Pressure Tolerance	40 MPa (≥5 μm)
	60 MPa (<5 μm)

Note:
When using silica-based columns at pH >7, use buffers with concentrations not exceeding 20 mM and temperatures not exceeding 40 °C to maximize column lifespan. Extreme pH or temperature conditions can significantly reduce column durability.

Column Activation

(Recommended for new columns or those unused for over a month)
Ultisil HILIC Amphion II columns are shipped in a solution of 80% acetonitrile in water. Activation should follow these steps:

Step 1: Activation

Activation Procedure		
Specification	ID⩽3 mm	ID＞3 mm
Flow Rate	0.1 mL/min	0.3 mL/min
Mobile Phase	100% Acetonitrile	
Time	4 hours	
Temperature	30 °C	

Step 2: Transition

If subsequent experiments involve buffer salts in the mobile phase, perform a transition procedure after Step 1.

Transition Procedure		
Specification	ID⩽3 mm	ID＞3 mm
Flow Rate	0.2 mL/min	1 mL/min
Mobile Phase	60% Acetonitrile	
Time	1 hour	
Temperature	30 °C	

Step 2 is skipped if no buffer salts are involved.

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Routine Column Washing (Backflush is Recommended):

Method Mobile Phase	Without acids, bases, salts	Containing acids, bases, salts
Washing Mobile Phase	95% Acetonitrile	60% → 95% Acetonitrile
Flow Rate	Method Flow Rate	
Time	30 mins each (Column Length ⩽100mm)	
	40 mins each (Column Length ＞100mm)	
Storage	Store the column in 95% acetonitrile in water, with protective plugs tightly sealed. Place in a cool, dry place.	

Column Regeneration:

If the column exhibits increased pressure, abnormal peak shapes, reduced efficiency, or decreased resolution, wash as follows:

Flow Rate	Method Flow Rate
Mobile Phase	60% Acetonitrile in water
Time	100 mins each (Column Length ⩽100mm)
	120 mins each (Column Length ＞100mm)

Glossary of Terms:

pH Range: The pH range tolerable for the column's mobile phase and sample solutions.

Specific Surface Area: The surface area per gram of silica.

Pore Size: The diameter of pores within silica particles, selected based on analyte molecular weight.

Carbon Loading: The percentage of carbon content in bonded functional groups relative to the total silica mass.

USP Code: Universal code for bonded phases in the USP chromatography column database.

Transition Mobile Phase: A solvent used to gradually adjust the type and ratio of mobile phase, typically free of buffers, acids, and bases.

Transition Procedure: A procedure to ensure miscibility between activation mobile phase and method mobile phases using transition mobile phase.

Maximum Pressure Tolerance: The upper pressure limit the column can withstand. Long-term use is recommended at pressures below 30 MPa. Monitor the instrument's pressure for safe operation.

Storage Solvent: Solvent used during storage or transport to maintain the activity of packing material. Handle with appropriate protective measures.