

ULTRON AF-HILIC-DA

Instruction Manual

1. Introduction

Thank you for purchasing an ULTRON AF-HILIC-DA column for High-performance liquid chromatography. The ULTRON AF-HILIC-DA is a Hydrophilic Interaction Chromatography (HILIC) column based on 5 μm silica gel bonded with diamine group via a spacer. The ULTRON AF-HILIC-DA column is able to retain and separate highly polar compounds that are not retained in reversed phase chromatography, with a mobile phase containing high concentration of organic solvent. In general, amino type columns have poor retention ability for highly polar compounds compared to the other HILIC columns, but the ULTRON AF-HILIC-DA shows greatly improved selectivity.

The ULTRON AF-HILIC-DA columns, which are manufactured under highly controlled conditions, must pass a series of strict tests before being accepted for shipment. To ensure optimal performance and durability of the column, please read these instructions carefully before using this column.

2. Specification

Descriptions	Details
Particle size	5 μm
Fittings	Waters compatible
pH range	Recommended range: 2.0~7.0
Organic solvent concentration range	Recommended range: 80~95% (Allowable range: 70%~95%)
Analytical temperature range	Recommended range: 25~40°C (Maximum: 50°C)
Analytical pressure range	Maximum: 20 MPa

- ※ The degradation of column performance is likely to occur when used at higher temperatures and lower concentrations of organic solvents.
- ※ Avoid using a column repeatedly near the pressure limit or abrupt change in pressure to prevent shortening of the column life.

3. Shipping Solvent

The ULTRON AF-HILIC-DA columns are shipped containing 100% hexane. When using the column, please replace the shipment solvent with ethanol before installation in your LC system, and then use your desired mobile phase.

Note: In case of insufficient replacement with ethanol may lead to contamination of hexane in your LC system, potentially causing various system related issues.

When using a mobile phase containing buffer salts, care must be taken to avoid salt precipitation.

4. Mobile phase and sample

- The most suitable mobile phase in HILIC mode is acetonitrile/water or buffer.

[The relative solvent strength for HILIC mode]

Acetone < Acetonitrile < 2-propanol < Ethanol < Methanol < Water

- The lower polarity of mobile phase and the higher concentration of organic solvents increase the retention.

It is recommended to contain at least 3% water in a mobile phase to enhance the separation reproducibility by forming a stable hydrated layer on the surface of packing material.

- Suitable buffer for HILIC mode is ammonium acetate or ammonium formate.

A buffer concentration in the range 10 – 20 mM is recommended.

(Depending on separation or solubility, adjustment in the range of 5 – 200 mM can be made.)

- In case of gradient elution, each mobile phase composition should be adjusted to maintain a constant salt concentration during analysis.

- Avoid phosphate salt and other low solubility buffers to organic solvents.
- Ensure to filter samples and mobile phases using a membrane filter with a mesh size of 0.45 μm or smaller before using. Failure to filter mobile phases, etc. can lead to blockages of column filters and increases in analytical pressures.
- Ensure to thoroughly degas mobile phases prior to use. Insufficient degassing of mobile phases can lead to the formation of bubbles inside analytical instruments and columns resulting in problems with analyses.
- It is recommended to dissolve the sample in a solvent that is of the same composition as the initial mobile phase. Dissolving the samples in a stronger solvent than the initial mobile phase will result in distorted peak symmetry and degraded resolution.
- The pH of the sample solution must be set in acceptable pH range for packing material.

5. Precautions for usage

- Before installation the column, replace the liquid in the system with the mobile phase to be used.
- Tubing must have flat ends and must bottom out in the column endfitting. Tubing must be connected to the column correctly to avoid creating a void between the column frit and tubing, which can cause a leak and result in poor column performance.
- Install the column according to the direction of the arrow.
- Do not remove the column from LC system before the pressure drops zero.

6. Column cleaning and storage

- When shipped, the column is closed with end stop SUS plugs tightly. After replacing the shipment solvent with the mobile phase, the provided PEEK plugs can be used.
- When using buffer solutions as a mobile phase, replace the buffer solution of the mobile phase with purified water, and then clean the column using a solution with an organic solvent concentration the same as the mobile phase.
- When the column is stored for a long period, firstly, clean the column following the above method and then replace with ethanol. Finally, replace ethanol with hexane. After the process, please close the column with end stop SUS plugs tightly in order to maintain air-tightness. After tightening the SUS plugs by hand until they no longer turn, use a wrench to lightly tighten it again.
- Performance of the column should be carried out in accordance with the enclosed "Performance Report".

ULTRON series packed columns are shipped under highly controlled conditions. However, if you should find any defect, please contact your dealer or Shinwa.

Note that Shinwa does not warrant the product against column life or deterioration caused by the failure to follow the above instructions.



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