

For HPLC High Performance Packed Column

CoreFocus

Shim-pack™ Mix-HILIC

Instruction manual

■ Introduction

Shim-pack Mix-HILIC is a hydrophilic interaction chromatography (Hydrophilic Interaction Chromatography : HILIC) column packed with anion-exchange polymer gels for the separation of highly polar compounds.

■ Column hardware specification

Item	Shim-pack Mix-HILIC
Product number (P/N)	227-32751-01
Column dimensions	2.1 mm Inner diameter × 150 mm Length
Column body material	Stainless steel
Particle base material	Polymethacrylate
Particle size	5 μm
Functional group	Polyamine
Liquid sealed in the column for shipping or storage	Acetonitrile : [40 mmol/L Ammonium carbonate + 6 mmol/L Formic acid (approximately pH 7.0)] = 40 / 60 (v/v)

*Usage conditions are listed in "■ Column Handling Precautions".

■ Column Performance

Each column is shipped after undergoing a quality inspection. The test results are documented in the attached inspection report that accompanies each product. This report includes the column's serial number, performance metrics, and the conditions under which the inspection was conducted. Please retain it for future reference.

After purchase and before using the column for analysis, please replace the column's internal liquid according to the conditions specified in section 2) of the 'How to store columns' guide. If the product remains unused, it is recommended to replace the internal solution every month as per the conditions outlined in sections 1-2) of the 'How to store columns' guide.

While the product meets the specifications outlined in the report at the time of shipment, these specifications do not assure the retention times or peak shapes for all compounds to which this column will be applied.

NOTE: The elution time and peak shape of a compound can vary based on the conditions of use. Before you develop and validate your analytical method, ensure that your column meets the requisite specifications.

■ Operating Precautions

Please keep the following in mind when preparing or replacing the mobile phase and wash solution:

- Organic solvents, such as acetonitrile, are highly flammable and can be dangerous upon skin contact. Ensure to wear protective equipment like gloves and safety glasses, and work in a well-ventilated area.

■ Column Installation

Please keep the following points in mind when installing the column into the flow path:

- If dirt or air enters the column from the channel, it can cause deterioration. Before connecting a column, make sure to flush the mobile phase or wash solution into the flow path.
- Check the direction of the mobile phase flow (→) indicated on the column label and connect the pipe to the column.
- Typically, a SUS pipe with an inner diameter of 0.1mm and an outer diameter of 1.6mm is used for the connection pipe on the column inlet side. It's recommended to avoid using longer pipes than necessary to minimize peak broadening.
- PEEK tubing and male nuts have limited pressure resistance and are only suitable for use on the outlet side of the column.
- When connecting the column, ensure that no extra void is created at the end of the piping and secure it with a male nut or fitting. The male nut or fitting can be obtained by referencing the product name and product number (P/N) shown in the following table.

Item name	Product number (P/N)	Remarks	Pressure limit
Malenut fitting kit	228-45717-01	2 pc	35 MPa
UHPLC fitting 2 S	228-56867-41	1 pc	130 MPa
Male nut, PEEK	228-18565-84	5 pc	20 MPa

- If tailing is observed on sharply eluting peaks, dead volume could be a possible cause. Ensure that the connecting pipe is inserted all the way into the column joint, and that the tip of the pipe makes contact with the column joint without any gaps before tightening.
- Use a detector flow cell with small capacity, such as a semi-micro type or UHPLC type.

■ Column Handling Precautions

For long-term stable performance, operate within the pressure, pH, and temperature ranges shown in the following table. Avoid continuous use near the operational limit and sudden pressure changes as these can negatively impact performance.

Item	Value
Upper limit of pressure	35 MPa
pH range used	2.0 - 13.0
Upper limit of temperature	50 °C
Recommended flow rate	0.3 mL/min

- Be careful not to drop or bump the column. A severe shock can lead to column degradation.
- Column pressure rises when a salt-free neutral/acidic eluent or a non-buffer neutral/acidic eluent is used with a high water ratio (water ratio of 50% or more). Furthermore, the displacement from the salt-containing mobile phase typically takes longer to equilibrate, which causes the pressure to increase gradually.
- To prevent column damage due to pressure increase, when using a mobile phase with nearly 100% water content, it is recommended to use an aqueous solution or buffer solution with a salt concentration of 5 mmol/L or higher. Additionally, be sure to set the system's maximum pressure limiter appropriately.
- Please ensure that the flow rate is adjusted to stay within a range that does not exceed the upper limit of pressure.
- When changing mobile phases, particularly where salts may precipitate when mixed, it's recommended to use a mixed solution such as aqueous solution/acetonitrile = 1/1 as an intermediate solution.
- After using the column, replace the liquid within it according to the conditions outlined in the 'How to Store Columns' section.
- Halt the pump and ensure that the pressure gauge reads 0 MPa before detaching the column from the flow path.
- Avoid letting the inside of the column dry out. After detaching it from the flow path, ensure to securely seal it with the included plugs.

■ Mobile phase solvent

Generally, in HILIC separation mode, the retention increases as the organic solvent ratio increases. Further, with this column, anionic compounds are strongly retained in cationic functional groups modified on the stationary phase, even when the organic solvent ratio is decreased.

The mobile phase most suitable for this column is a mixture of acetonitrile and an aqueous solution containing salt (a buffer is recommended)

Utilize 0 to 95% acetonitrile and 5 to 100% aqueous solution. Employ a mobile phase that contains at least 5% aqueous solvent to form a stable hydration layer on the stationary phase surface, which enhances separation reproducibility.

Please take note of the following points when using this product.

- Please ensure in advance that salt precipitation will not occur due to the interaction between the aqueous and organic solvents. For mobile phases with a high salt concentration and organic solvent ratio, we recommend preparing a mixed solution (premix).
- When the mobile phase is mixed online in a gradient mixer, a temperature drop may occur due to endothermic reactions and cause precipitation. It is advisable to place the gradient mixer in the column oven to maintain temperature. Optimal mixing performance is recommended.
- Whenever possible, utilize a fresh mobile phase prepared immediately before use. If storing the mobile phase, ensure the bottle is sealed tightly. If it is stored for an extended period under conditions with poor airtightness, such as when it is only covered with aluminum foil, the composition of the mobile phase may change.
- Column pressure will fluctuate due to changes in organic solvent concentration during gradient elution, so it is crucial to ensure that the upper limit is not exceeded.

[Notes on Organic Solvents]

Utilize acetonitrile as the organic solvent for the mobile phase.

Refrain from using other organic solvents as they might compromise the stability of the column.

[Precautions for Salt-Containing Aqueous Solutions]

- Compounds such as ammonium bicarbonate, ammonium acetate, and ammonium formate are preferable. Avoid salts with low solubility in organic solvents, such as phosphate. For pH adjustment, employ ammonia water, formic acid, or any substance that does not precipitate salt.
- Adjust the salt concentration of the entire mobile phase to be within the range of 5-100 mmol/L, based on the requirements for separation, solubility, and detection. Standard conditions recommend using 40 mmol/L.
- Before analysis, filter any remaining insoluble matter in the mobile phase through a membrane filter.

■ Sample

It is recommended to dissolve the sample in a solvent that has the same composition as the mobile phase (the initial solvent in gradient elution). Using a solvent with a higher elution power than the mobile phase can cause peak broadening and reduce resolution and reproducibility.

To prevent column clogging, filter the sample through a membrane filter (0.2-0.45 µm) prior to injection. Additionally, verify its miscibility with the mobile phase before injection to ensure that no precipitation occurs during mixing.

High molecular compounds, such as proteins and polysaccharides, tend to adhere to the columns and are typically difficult to remove, even with column cleaning. For samples that contain these substances or significant levels of impurities, it is recommended to perform a pre-treatment (cleanup) using techniques like solid-phase extraction before injection.

■ Column Cleaning

Residual components in the column may diminish its performance, hence they should be removed using the following method:

- Run the final gradient solution or a higher percentage of aqueous solvent (50-100% etc.) through at least 20 column volumes.
- Employ an aqueous solvent containing salts to elute anionic compounds that are strongly retained through ion exchange.
- If you suspect that the column inlet is clogged or dirty, connect the column in reverse and run the solution at low pressure (flow rate of 0.2 mL/min or less). (Please use forward connection under normal circumstances.)
- If the column is used under conditions with a high proportion of aqueous solvent and there is a possibility of adsorption of hydrophobic substances, increase the proportion of organic solvent when cleaning.

■ How to store columns

When the column is not in use for analysis, we recommend replacing the liquid within the column as described in this section.

If a new column is to remain unused for an extended period, we recommend periodic (monthly) fluid replacement, as described in section 1-2), to maintain performance.

When initiating the use of a new column or a column that has been in storage for an extended period, proceed with step 2) outlined in this section.

1-1) Storage after use for analysis

If you intend to pause system operation for a period, decrease the acetonitrile ratio of the solution in the flow path or column slightly (e.g., to 80%) to prevent salt precipitation.

If the column will remain unused for a week or longer, disconnect it from the flow path, seal it securely with the provided plugs, and store it in a cool, clean, dark environment with minimal temperature variations and low humidity. Replace the liquid chromatography flow path with a salt-free solution.

1-2) When storing columns in their initial factory state

To replace the current solution with a new storage solution, follow these steps:

1. A solution of acetonitrile and 40 mmol/L ammonium hydrogen carbonate aqueous solution* in a 40/60 (v/v) ratio is passed through the column for 20 minutes at a flow rate of 0.2 mL/min and a temperature of 40°C.
2. A solution of acetonitrile and 40 mmol/L ammonium bicarbonate + 6 mmol/L formic acid (approximately pH 7.0) in a 40/60 (v/v) ratio is passed through the column for 60 minutes at a flow rate of 0.2 mL/min and a temperature of 40°C.

2) When starting to use a new column/column after long-term storage

1. A solution of acetonitrile and 40 mmol/L ammonium bicarbonate aqueous solution* in a 40/60 (v/v) ratio is passed through the column for 60 minutes at a flow rate of 0.2 mL/min and a temperature of 40°C.
2. Switch to the mobile phase you intend to use and let it run for at least one hour. Equilibration using a mobile phase can also be stabilized by increasing the proportion of aqueous solvent in the mobile phase in advance (such as 50 to 100%) and passing at least 10 times the column volume.

*Preparation method of 40 mmol/L ammonium bicarbonate aqueous solution:

Dissolve 3.16 g of ammonium bicarbonate and 7.2 mL of 25% ammonia solution in 1 L of water, then mix thoroughly. (pH approx. 9.8)

■ Disposal Precautions

Separate the column from general industrial or household waste and dispose of it in accordance with local government regulations.

■ Technical Support

Shim-pack Mix-HILIC columns are manufactured, inspected, packaged, and shipped under stringent quality control standards. If you encounter any performance defects, please contact your local Shimadzu sales office for assistance. However, please note that we cannot guarantee the lifetime of the columns, nor can we accept any claims when performance has degraded due to non-compliance with the operation procedures outlined above, or as a result of normal aging.