

High Performance Packed Column for HPLC

Shim-pack

Scepter Diol-HILIC Series

INSTRUCTION MANUAL

■ Introduction

To maintain and maximize peak performance of Shim-pack Scepter Diol-HILIC series columns, and to ensure the long life and stability of columns, please read the following instructions before use.

■ Specifications

The product specifications of this product are as follows.

Products name	Chemical bonding group
Shim-pack Scepter Diol-HILIC	Dihydroxypropyl Groups

■ Operating Precautions

Check if anything is missing or damaged. If there are any signs of damage, notify your local Shimadzu representative at once.

Each of the Shim-pack Scepter Diol-HILIC column is delivered with a Column Performance Report. The information supplied in the report include the column serial number, and chromatographic test conditions. Please keep the report for future reference.

■ Column performance

The Shim-pack Scepter Diol-HILIC column have stable quality products for customers by QC tests. Shim-pack Scepter Diol-HILIC columns are shipped with the solvent used for the final QC test of the column, as detailed in the Column Performance Report delivered with the column.

When switching between solvents with significantly, please take care of different polarities, the miscibility and precipitation of salts.

■ Column Installation

The flow direction of the column is shown on the column (→). When installing the column, ensure that the flow direction matches the mobile phase flow direction.

Use PEEK tubing (UHPLC: SUS tubing) with an inner diameter of 0.25 - 0.3 mm (UHPLC: 0.1 - 0.2 mm) and an outer diameter of 1.6 mm. The 1.9 μm particle packing column has a higher pressure than the 5 μm or 3 μm particle packing column. Please take care of the maximum pressure of analysis systems and connect tubing. Generally, UHPLC systems that have a maximum pressure above the 60 MPa level is appropriate.

Use the shortest possible tubing connection from the injector to the column to minimize peak broadening.

The column should be connected with male nuts. Ensure that the fittings are connected properly to avoid creating dead volume between the tubing and the column interface. Male nuts can be ordered by referring to the part number below.

Item name	P/N	Remarks	Pressure
Male nut, PEEK	228-18565-84	5 pcs	20 MPa
Male nut 1.6 MN	228-16001	1 pc	130 MPa
Ferrule 1.6 F	228-16000-10	1 pc	130 MPa
UHPLC Fitting 2 S	228-56867-41	1 pc	130 MPa
Nexlock fittings	228-62544-90	1 pc	130 MPa

NOTE Stains or air in the flow line may deteriorate the column. Before connecting the column, be sure to flow the mobile phase to flush the flow line.

If peaks are tailing more on the early eluting compounds than later eluting compounds, there is a possibility that there is a dead volume. In such case, check that all column connections are properly connected.

Also, make sure to use appropriate internal diameter and length size of tubing at the injector and detector, especially when using semi-micro size columns, to avoid system dead volumes.

■ Metal-free column connection

Be sure to connect by hand. Tightening more than required by wrench may cause damaged. Install and remove the tubing or sealing plug in a status where the end fitting is held down, not the stainless steel pipe. Leakage may occur if the end fitting loosens.

In the case of the part for connect of general-purpose ferrule integrated model, it is rare that the fritted part will damaged if you use it with too tight a status. Column connect recommends Nexlock use without ferrule.

In the case of component breakage inside the end fitting, it is not covered by the warranty (exchange).

■ Column Handling Precautions

Do not drop or bump the columns, to avoid a deterioration of the column performance. To maximize column life, use the columns within the pressure shown in the following table.

Particle size	Column Dimension	Maximum pressure limit
1.9 μm	2.0–3.0 mm	100 MPa
3 μm, 5 μm	2.1–4.6 mm	45 MPa*

*Use the columns at a pressure of 30MPa or less for regular use.

Avoiding using a column repeatedly near the pressure limit or sudden change in pressure, which may cause shortening of in the column life.

Since the pressure varies depending on the column length, column temperature, type of organic solvent, etc., adjustment the Flow rate as appropriate.

Column should be disconnected from the system after the pressure drop to “0”.

Please note that operating the sample injection valve slowly or using an auto-sampler with slow valve switching speed will also generate a rapid pressure increase at the column inlet, which will cause premature column deterioration.

Recommendations of pH and temperature for column are the following.

Range of pH	Operating Temperature Range	
	(Recommended)	(Maximum)
2.0-10.0	20–40°C	50°C

※ Column life varies greatly depending conditions of use such as temperature and mobile phase compositions, as well as pH.

Generally, the high concentration of the buffer solution and the additive, the high column temperature, and the low organic solvent concentration can shorten the column life lower.

※ In the case of high pH mobile phase, it is recommended to use a low concentrated buffer such as 5 to 10 mM and analyze at a low temperature (e.g., <30°C).

■ Flow rate of column

The recommended flow rate of columns are the following.

Particle size	Column I.D.	Recommended flow rate
1.9 μm	2.0 mm	0.2 ~ 0.8 mL/min
	3.0 mm	0.4 ~ 1.6 mL/min
3 μm, 5 μm	2.1 mm	~0.2 mL/min
	3.0 mm	~0.4 mL/min
	4.6 mm	~1.0 mL/min

■ Mobile Phase Selection

Acetonitrile/water or buffer solution (about 90/10 to 60/40 (v/v)) is the most suitable mobile phase, and water-soluble organic solvent shown below can be used.

In general, HILIC separation, contrary to reverse-phase, retention is increased by lower polarity of the mobile phase and increase of the organic solvent concentration. Use mobile phase containing at least 3% aqueous solution to form a stable layer of water on the surface of the packing material and enhance the separation reproducibility. [usable solvent and solvent strength (low-to-high)]

tetrahydrofuran (THF) < acetonitrile < 2-propanol < ethanol < methanol < water

※ For THF use, please take care of the solvent resistance of PEEK tubing, etc.

Ammonium acetate buffer solution or ammonium formate buffer solution are suitable for use as mobile phase. The final salt concentration in mobile phase should be about 10 to 20 mM, and the salt should be adjustment in the range of 5 to 200 mM depending on the separation and solubility. Using gradient elution, adjust the mobile phase compositions so that the concentration of salts in mobile phase remains constant. Be sure to check that there is no salt precipitation prior to use and replacement of the mobile phase. Avoid using salts that are not soluble in organic solvent, such as phosphoric acid.

■ Sample

Samples should be dissolved in an eluent or solvent weaker than the mobile phase, which helps avoid sample precipitation at column inlet/head and inconsistent retention values.

In order to prevent the precipitation of salts contained in sample or solvent, check the miscibility of these with mobile phase before injection.

■ Clogging of column

The most common cause of the increase of column back pressure or split peaks is blockage of the inlet filter by sample particulates, or large quantities of lipophilic compounds adsorbing to the head of the column.

- Filtrate the mobile phase using a 0.45 μm membrane filter before using the column.
- Installing “Ghost Trap DS” between the pump and injector can efficiently remove particulates or contaminants in the mobile phase.
- Filtrate the sample using a syringe filter before injecting to the column.
- Installing “Guard Column” or “Guard Column for UHPLC” can prevent column clogging problems.

Baseline drift and noise can be caused by defective pumping due to air bubbles in eluent or decrease of light intensity when using a UV detector. Note that bubbles can form in the detector flow cell if the eluent is not degassed properly before introduction into the column.

■ Precaution using UHPLC column

The extra column volume has a major effective on sample diffusion. In particular, if you use the column of the I.D. 2 mm, optimize LC system as shown below.

- 1) The tubing between injector and column, and between column and detector, should be as short as possible. The tubing I.D. should be small (0.15 mm or less). No voids are formed in the connect.
- 2) Use low volume types such as semi-micro or micro in flow cell of detector. Use minimize sample loop.

The data sampling rates of the response and data processing instrument of the detector should be optimal according to the peak so that they are higher than the 10 data points per 1 peak. For UHPLC with 1.9 μ m column, the response should be less than 0.1 sec and the data sampling speed should be 10 points or more per second in order to acquire appropriate sharp peak with short retention.

■ Washing the column

Generally, rinse the column in the following.

- Flow a mixtures of organic solvent /water with high elution power than mobile phase, such as acetonitrile /water (50/50), to rinse the substance remaining in the column. A water concentration of about 50% is appropriate. If further is required, please use acetonitrile /water (5/95).
- When macromolecular compounds such as proteins and polysaccharides adsorb to column, they are generally difficult to remove by rinse. It is recommended to perform pretreatment (cleanup) by solid phase extraction in advance for sample containing these compounds or impurities.

■ Column storage

If the column is stored for a long period, replace the packing solvent with the acetonitrile /water (90/10). If you are using a mobile phase containing buffer solution or salt, take care of the replacement procedure to prevent salt precipitation.

■ Technical Support

Shim-pack Scepter Diol-HILIC columns are manufactured, inspected, packaged and shipped under strict standards of quality control. Should you find any defect in performance, please contact your local Shimadzu representative, who will ensure your complete satisfaction.

We regret that we cannot guarantee the lifetime of columns, also that we cannot accept any claim when performance has deteriorated due to noncompliance with the operation procedures elucidated above, or as a result of normal aging.