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SHIMADZU
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High Performance Packed Column for HPLC

CoreFocus

Shim-pack Scepter™ Diol-HILIC-120
Shim-pack Scepter Claris Diol-HILIC-120

INSTRUCTION MANUAL

■ Introduction

Shim-pack Scepter Diol-HILIC-120 and Shim-pack Scepter Claris Diol-HILIC-120 are "Hydrophilic Interaction Chromatography (HILIC)" columns based on an organosilane hybrid particle bonded with a dihydroxy propyl ligand. To maintain and maximize peak performance of Shim-pack Scepter Diol-HILIC-120 / Shim-pack Scepter Claris Diol-HILIC-120, and to ensure the long life and stability of columns, please read the following instructions before use.

■ Operating Precautions

- Inspect the column for anything is missing or damaged. If there are any signs of damage, notify your local Shimadzu representative at once.
- Each Shim-pack Scepter Series / Shim-pack Scepter Claris Series Diol-HILIC column is individually tested and includes a Column Performance Report.
- The report includes the column Lot number, column serial number, and chromatographic test conditions. Please keep the report for future reference.

■ Column Performance

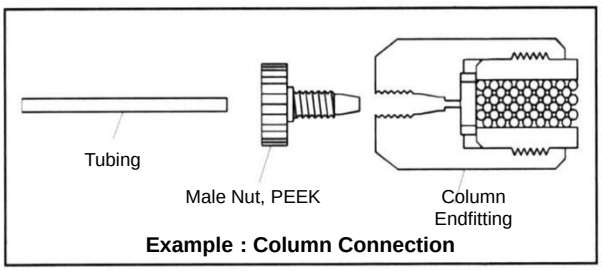
- The Shim-pack Scepter Series / Shim-pack Scepter Claris Series Diol-HILIC columns undergo rigorous QC testing to ensure quality and stability. The shipping solvent is acetonitrile: water = 90/10 (v/v). Refer to the table below for the physical properties of the Shim-pack Scepter Diol-HILIC-120 / Shim-pack Scepter Claris Diol-HILIC-120.

Column	Bonded Phase	Pore Size (nm)	Surface Area (m^2/g)	End-capping
Scepter Diol-HILIC-120 / Scepter Claris Diol-HILIC-120	Dihydroxy propyl	12 (120 Å)	360	No

■ Column Installation

- The flow direction of the column is indicated by an arrow on the column label (→). When installing the column, ensure that the flow direction arrow matches the mobile phase flow direction.
- Use PEEK or 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 (1/16".)
- This column is intended for HILIC analysis with recommended mobile phases of acetonitrile/water or buffer. Never use PEEK tubing with Tetrahydrofuran (THF), chloroform, hexafluoroisopropanol (HFIP), concentrated sulfuric acid, concentrated nitric acid, dichloroacetic acid, acetone, dichloromethane or dimethyl sulfoxide (DMSO) as the mobile phase.
- The 1.9 µm particle column produces higher backpressure than the 5 µm or 3 µm particle packing. Please be aware of the maximum pressure of the HPLC systems and connect the appropriate tubing. Generally, UHPLC systems that have a maximum pressure above 60 Ma/600 bar/8700 psi are appropriate for 1.9 µm columns and require SUS tubing and fittings.
- Use the shortest possible tubing connection from the injector to the column to minimize peak broadening.
- The column should be connected with PEEK or SUS fittings. Ensure that the fittings are connected properly to avoid creating dead volume between the tubing and the bottom of the column port. Refer to the table below for PEEK and SUS fitting options.

Item Name	P/N	Remarks	Pressure Limit
Male Nut, PEEK	228-18565-84	5 pcs	20 MPa
Male Nut Fitting Kit	228-45717-01	2 pcs	35 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



Example : Column Connection

NOTE
Particulates or air in the system flow line may deteriorate the column. Before connecting the column, be sure to filter all solvents and flush the flow line up to the column with mobile phase.

- If peaks are tailing more on the early eluting compounds than later eluting compounds, it's that there is dead volume. Check the inlet and outlet connections to ensure the tubing is flush with the bottom of the column port. The length of the tubing from the ferrule tip to the bottom of the column port is about 2.4 mm as a guide.

- Make sure to use appropriate internal diameter and tubing length from the injector and to the detector, especially when using semi-micro size columns, to reduce system dead volume and peak broadening.

■ Column Hardware and Precautions When Using Metal Free Columns

Shim-pack Scepter Diol-HILIC -120 and Shim-pack Scepter Claris Diol-HILIC -120 have 3 types of column hardware. Refer to the table below for each type.

Columns	Column Hardware
Shim-pack Scepter Diol-HILIC-120	Stainless steel column
Shim-pack Scepter Diol-HILIC-120 [Metal free]	PEEK-lined stainless steel column
Shim-pack Scepter Claris Diol-HILIC-120	Inert coated stainless steel column

When using the Shim-pack Scepter Diol-HILIC-120 [Metal free], note the following and handle with care.

- Connect the tubing and fittings by hand. Tightening more than recommended by wrench may cause damage to the column. Install and remove the tubing or sealing plug by holding the end fitting, not the column body. Leakage may occur if the end fitting loosens.
- When using 1-piece fittings, the column frit can be damaged if the fitting is overtightened. Tubing with a torque limiting fitting such as the MarvelXACT or "PEEK Lining Pipe" (e.g. PN:228-74344-46) is recommended for column inlet and outlet connections.
- If a fitting breaks inside the column port, it is not covered by the warranty (no exchange will be provided).

For more information, please use the URL/QR code of "Take care when connecting Metal Free Column to the piping".
https://www.shimadzu.com/an/products/liquid-chromatography/hplc-consumables/shim-pack-scepter-lc-columns/index.html#anchor_manuals_0

■ Column Handling Precautions

- To avoid damage to the column and prevent deterioration in performance, do not drop or bump the column. To maximize column life, use the columns within the pressure shown in the following table.
- Pressure limits are the same for all column hardware types.

Particle size	Column I.D.*1	Maximum Pressure Limit
1.9 µm	2.0 - 3.0 mm	100 MPa/1,000 bar/14,500 psi
3 µm 5 µm	2.1 - 4.6 mm	45 MPa/450 bar/6,500 psi*2

*1 Please contact your local Shimadzu representative about the product with other size

*2 Use the columns at a pressure of 30 MPa/300 bar/6,500 psi or less for maximum lifetime. Avoid using a column consistently near the pressure limit or subjecting it to sudden changes in pressure, which can reduce the column lifetime. Since the pressure varies depending on the column length, column temperature, and mobile phase composition., adjust the flow rate to stay within the recommended pressure limit.

- The column should be disconnected from the system only after the pressure shows "0 MPa/0 bar/0 psi."
- Avoid excessive pressure fluctuation. This increases pressure rapidly at the column inlet, which may cause premature column deterioration. When using a preparative column, a bypass from the injector is recommended.
- If poor retention time reproducibility, baseline drift, or bleed noise are observed at the start of the analysis, the column equilibration may not be enough. In this case, flush it with at least 5 column volumes of the mobile phase initial conditions until the pressure and baseline signal are stable. If the performance is not improved, check the system for leaks, flow rate stability, etc. and contact your local Shimadzu representative for additional assistance.
- Temperature and pH recommendations are in the following table*.

pH Range	Recommended Operating Temperature Range	
	Routine Use	Maximum
2.0-10.0	20 - 40 ℃	50 ℃

* Column lifetime for the Diol-HILIC phase varies greatly depending on usage conditions such as temperature, mobile phase composition, and pH. In general, high concentrations of buffers or additives, high column temperature, and low organic solvent concentration can shorten the column life. When using high pH mobile phase (e.g. > pH 7), we recommended using low buffer concentrations (5 to 10 mM) and operating temperature < 30 ℃.

NOTE
The elution order, retention time, peak shape, etc. may change significantly when ion-pairing reagents are used in the mobile phase. These reagents are often difficult to completely remove, so we recommend that columns with a history of using ion-pair reagents be used exclusively for analyses with ion-pair reagents.

■ Mobile Phase Selection

- When using the Diol-HILIC column, acetonitrile/water or buffer solution (from 90/10 to 60/40 (v/v)) is the most common mobile phase, however, other water-soluble organic solvents shown below can be also used.
- In HILIC separations, contrary to reversed-phase, retention is increased by lower polarity of the mobile phase and high organic solvent concentration. Water is the "strong" solvent for eluting compounds in HILIC mode.
- Column conditioning before use and equilibration between injections is very important. Use mobile phase containing at least 3% aqueous to form a stable water layer on the surface of the packing material to ensure separation reproducibility. For initial conditioning, flush the column with at least 50 column volumes of mobile phase. Equilibrate with at least 10 column columns of mobile phase between injections.
- Ammonium acetate or ammonium formate buffer solutions are suitable for use as HILIC mobile phase. The initial salt concentration in the mobile phase is typically 10 to 20 mM, and can ranger from 5 to 200 mM depending on the solubility and separation performance.
- Usable solvents and solvent strength (low-to-high)
  [Tetrahydrofuran (THF)* < Acetonitrile < 2-propanol < Ethanol < Methanol < Water]

*When using THF, SUS tubing and fittings must be used.

- When using gradient elution, it is recommended to adjust the mobile phase composition so that the concentration of salts in the mobile phase remains constant.
- For any buffer (salt) additive, confirm that there is no precipitation in the mobile phase prior to use. Avoid using salts that are not soluble in organic solvent, such as phosphate compounds (including phosphoric acid.)

■ Column Flow Rate

The recommended flow rates are as follows. Note that these recommendations are for acetonitrile/water mobile phase. Adjust the flow rate appropriately because the pressure changes depending on the column length, temperature, types of organic solvent etc.

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

■ Sample

Samples should be dissolved in a solvent weaker than the initial conditions of the mobile phase, which helps avoid sample precipitation at column inlet/head, poor peak shape, and inconsistent retention times. In order to prevent the precipitation of salts contained in sample or solvent, check the miscibility of these with the initial conditions of the mobile phase before injection.

■ Column Clogging

The most common cause of the increased column backpressure or split peaks is blockage of the inlet frit by sample particulates, or large quantities of lipophilic compounds adsorbing to the head of the column.

- Filter the mobile phase using a 0.45 µm membrane filter before flushing the flow line and connecting the column.
- The "Ghost Trap DS" installed between the pump and injector can efficiently remove soluble mobile phase contaminants that pass through a filter. "Ghost Trap DS" can be ordered by referring to the part numbers below.

Item	Description	Dimensions	Internal Volume	Pressure
Ghost Trap DS* 228-59921-92	Two cartridges and one holder	7.6 mm ID × 30 mm	~700 µL	35 MPa 350 bar 5,075 psi
Ghost Trap DS* 228-59921-94	Two cartridges and one holder	4.0 mm ID × 20 mm	~150 µL	
Ghost Trap DS-HP 228-59931-91	Packed type	2.1 mm ID x 30 mm	~60 µL	100 MPa 1,000 bar 14,500 psi

NOTE
Avoid using Ghost Trap DS when using a mass spectrometer as the detector since it may induce bleed. Use of ion-pairing reagents is not recommended because they may be retained by the packing material which can affect retention times and peak shapes.

- Filter samples with a 0.2 – 0.45 µm membrane before injecting.
- Baseline noise and drift can be caused by air bubbles in the mobile phase or a decrease of light intensity when using a UV detector. Note that bubbles can form in the flow line and detector flow cell if the eluent is not degassed properly before introduction into the column. The detector also needs some restriction in the outlet tubing to prevent bubble formation in the flow cell. Always use the flow cell outlet tubing provided in the accessory kit. If the baseline noise and drift continue even after flushing the columns, please consider to check the system and the analytical condition
- Installing a guard column* for standard or UHPLC columns can prevent also prevent column clogging and prolong lifetime.

* The guard column is a cartridge type column and is sold separately from the analytical column. Note that we do not offer guard columns for Scepter Metal free or Shim-pack Scepter Claris columns. When using a cartridge guard column, a cartridge holder is required that is sold separately from the cartridge guard column. Choose from 2 types of column holders, one for standard analytical applications (3 µm, 5 µm) and one for high-speed analysis (1.9 µm.) Contact your Shimadzu representative for more information to select a guard column appropriate for the analytical column dimensions.

■ Precautions When Using Small ID and Small Particle Size UHPLC Columns

The extra-column volume has a significant effect on sample diffusion, especially with 2.0/2.1 mm ID columns. When using this size column, optimize the LC system as described below.

1) The tubing from injector - column and column – detector should be as short as possible to minimize dead volume. Recommended tubing ID is 0.15 mm or less. Take care when installing the tubing to the inlet and outlet ports that no voids are formed in the connection.
2) Use a semi-micro or micro flow cell in the UV or PDA detector. Use a fixed volume sample loop to minimize system volume from the injector.
3) The data sampling rate and detector response should be optimized according to the peak width in order to acquire sufficient data for quantitation of tall (sharp) and narrow peaks.

■ Cleaning and Storing the Column

Perform the following steps to clean and store the column. Use at least 5 column volumes of each solvent for the washing and replacement flushing before storage.

- If the mobile phase contains buffer solution, salts, or ion-pairing reagents, replace it with the same proportion of salt and reagent-free water /organic solvent.
- Flush the column with at lease 5 times the column volume of salt-free mobile phase when cleaning the column. For cleaning residual sample and matrix components from the column, a mixtures of organic solvent /water with higher elution power than the mobile phase (for example, acetonitrile : water = 50/50 (v/v)). A water concentration of about 50% is appropriate. If further cleaning is required, use up to acetonitrile : water = 5/95 (v/v).

- When macromolecular compounds such as proteins and polysaccharides are adsorbed on column, they can be difficult to remove, even when rinsing with 100% organic solvent or with the addition of some THF. It is strongly recommended to use SPE and/or a guard column if the sample contains a large amount of these compounds or impurities.
- For long-term storage, after completing the previous cleaning steps (especially to remove salts and ion-pairing reagents,) replace the solvent with acetonitrile : water = 90/10 (v/v) to match the shipping solvent.
- Seal the column with the plugs provided and store it in a temperature stable place.

■ Disposal Precautions

When disposing of the column, do so in accordance with your local processing standards determined by law, which may be separate from general industrial waste and household garbage requirements.

■ Technical Support

Shim-pack Scepter Diol-HILIC-120 and Shim-pack Scepter Claris Diol-HILIC-120 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 for assistance and to possibly arrange a replacement.

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