SHIMADZU

High Performance Packed Column for HPLC

CoreFocus

[Reversed phase column] Shim-pack Scepter™ Series
[Reversed phase column] Shim-pack Scepter Claris Series

INSTRUCTION MANUAL

■ Introduction

Shim-pack Scepter Series / Shim-pack Scepter Claris Series are based on an organosilane hybrid particle. To maintain and maximize peak performance of Shim-pack Scepter Series / Shim-pack Scepter Claris Series, and to ensure the long life and stability of columns, please read the following instructions before use.

■ Operating Precautions

- Inspect the column for anything 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 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 undergo rigorous QC testing to ensure quality and stability. The shipping solvent is 100% acetonitrile. Refer to the table below for the physical properties of the Shim-pack Scepter Series / Shim-pack Scepter Claris Series columns.

Column	Bonded Phase	Pore Size (nm)	Surface Area (m^2/g)	Carbon Content (%)	End-capping
Scepter C18-120 / Scepter Claris C18-120	Octadecyl groups	12	360	20	Yes
Scepter C18-300 / Scepter Claris C18-300		30	N.D.	N.D.	Yes
Scepter HD-C18-80 / Scepter Claris HD-C18-80		8	430	25	Yes
Scepter C8-120 / Scepter Claris C8-120	Octyl groups	12	360	17	Yes
Scepter Phenyl-120 / Scepter Claris Phenyl-120	Phenyl butyl groups		360	17	Yes
Scepter PFPP-120 / Scepter Claris PFPP-120	Pentafluoro phenyl propyl groups		360	15	No
Scepter C4-300 / Scepter Claris C4-300	Butyl groups	30	N.D.	N.D.	Yes

■ 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”.) 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 system and connect the appropriate tubing. Generally, UHPLC systems that have a maximum pressure above 60 MPa/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 installed 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
UHPLC Fitting 2 S	228-56867-41	1 pc	130 MPa

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 possible that there is dead volume. Make sure the connection tubing is fully inserted into the column joint. As a guideline, tighten the connection when the tubing extends about 5 mm from the tip of the ferrule, and when removed, the tubing should extend 2-3 mm from the tip of the ferrule.
- 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 Series and Shim-pack Scepter Claris Series have 3 types of column hardware. Refer to the table below for each

Column	Column Hardware
Shim-pack Scepter Series	Stainless steel column
Shim-pack Scepter Series [Metal free]	PEEK-lined stainless steel column
Shim-pack Scepter Claris Series	Inert coated stainless steel column

When using the Shim-pack Scepter Series [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-k-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 column within the pressure limit 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/1000 bar/14,500 psi
3 µm 5 µm	2.1 - 4.6 mm	45 MPa/450 bar/6,500 psi*2
5 µm	10 mm	10 MPa/100 bar/1,450 psi
	20 - 30 mm	30 MPa/300 bar/4,300 psi
	50 mm	20 MPa/200 bar/2,900 psi

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

*2 Use the column 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 / 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, or baseline noise and drift 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. Around the limit of pH range, conditions such as temperature and eluent composition may shorten the life of the column.

Column	pH Range	Recommended Operating Temperature Range	
		Routine Use	Maximum
Scepter C18-120 Scepter Claris C18-120	1-12	20 - 40℃	90℃ (pH 1-7) 50℃* (pH 7-12)
Scepter C18-300 Scepter Claris C18-300			
Scepter HD-C18-80 Scepter Claris HD-C18-80			
Scepter C8-120 Scepter Claris C8-120			
Scepter Phenyl-120 Scepter Claris Phenyl-120	1-10		50 ℃*
Scepter PFPP-120 Scepter Claris PFPP-120	1-8		
Scepter C4-300 Scepter Claris C4-300	1-10		90℃ (pH 1-7) 50℃* (pH 7-12)

*At pH 7 or higher, it is possible to use the column at temperatures above the recommended upper limit of 50℃, but the column lifetime will be shortened.

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

- Both aqueous and organic solvents can be used as mobile phase, but frequent switching between solvents of largely different polarities may degrade the column performance. Acetonitrile, methanol, and tetrahydrofuran (THF) are some common organic mobile phase solvents. When using THF, only SUS tubing should be used.

NOTE
When switching from the current solvent to one with significantly different polarity , first flush the column at 50% of the operating flow rate with at least 10 times the column volume of a mutually miscible solvent with intermediate polarity such as 2-propanol or dioxane (e.g. 25 mL for a 150 mm x 4.6 mm I.D. column.) Then switch the solvent to the one you want to use. Gradually ramp up the flow rate of the new solvent from 50% to 100% of the operating flow rate for the analysis.

- Conditions such as pH and temperature extremes affect the column lifetime. Typical operating temperature is between 20 °C and 40 °C. When using a column at a high pH for a long time, using low concentration organic buffer solution from 1 to 10 mM at low temperatures is recommended (e.g., < 30 ℃).
- The Shim-pack Scepter HD-C18 is a highly hydrophobic phase and is not compatible with 100% aqueous or < 10% of certain organic mobile phase conditions. The mobile phase should contain 15% or more methanol, or 10% or more of an organic solvent with lower polarity such as acetonitrile.
- Direct replacement of methanol/aqueous mobile phase with acetonitrile/aqueous mobile phase may cause abnormal retention time or peak shape when the acetonitrile composition is 20% or less. In such cases, first flush the column with 60% acetonitrile/ 40% aqueous mobile phase before replacing with acetonitrile/aqueous mobile phase of 20% acetonitrile or less.

■ Column Flow Rate

The recommended flow rates are as follows.

Particle Size	Column I.D.	Recommended Flow Rate
1.9 µm	2.0/2.1 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
5 µm	10 mm	5.0 mL/min
	20 mm	20.0 mL/min
	30 mm	42.0 mL/min

■ Preparative Column Handling Precautions

- When heating preparative columns above ambient temperature, irregular peak shapes such as peak broadening or splitting may occur because the temperature throughout the column is not uniform. Preheating the mobile phase is recommended to avoid these phenomena.
- The flow rate will be higher than that for analytical columns, so larger ID tubing (0.5 mm to 1.0 mm ID) should be used depending on the column ID.
- An injection valve with a bypass which reduces pressure shock to the column is recommended to prolong the column lifetime.

■ Sample Considerations

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 time. 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 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 0.45µm 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.
- 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.

- 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 column, purge the pump and close the drain valve tightly and check the LC system for leaks, or contact your local Shimadzu representative for additional assistance.

■ 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 does not contain buffer solutions and salts, increase the concentration of the organic solvent in the mobile phase up to 100% and hold at 100% for at least 5 column volumes to flush any sample material remaining in the column. The addition of THF may be effective, especially when highly lipid-soluble components are adsorbed.
- 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. Then, rinse the column in the same manner as above.
- Note that rinsing with 100% water after the use of mobile phase near the upper pH limit may cause column deterioration. Rinse with the last used water /organic solvent mixture (free of salts and ion-pairing reagents) or 60% acetonitrile aqueous solution.
- When macromolecular compounds such as proteins and polysaccharides are adsorbed on the 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 100% acetonitrile.
- 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 Series / Shim-pack Scepter Claris Series 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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