SHIMADZU
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High Performance Packed Column for HPLC

CoreFocus

Shim-pack Scepter™ Series

INSTRUCTION MANUAL

■ Introduction

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

■ Specifications

The specifications of this product are as follows.

Products name	Chemical bonding group
Shim-pack Scepter C18 Shim-pack Scepter HD-C18	Octadecyl Groups (C18)
Shim-pack Scepter C8	Octyl Groups (C8)
Shim-pack Scepter Phenyl	Phenyl Groups
Shim-pack Scepter PFPP	Pentafluorophenyl Groups
Shim-pack Scepter C4-300	Butyl Groups (C4)

■ 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 series columns is delivered with a Column Performance Report. The information in the report includes the column serial number, and chromatographic test conditions. Please keep the report for future reference.

■ Column performance

All the Shim-pack Scepter series columns have passed QC tests and products with stable quality are brought to customers. Shim-pack Scepter series 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 solvents, please check the miscibility of the solvents and be careful not to precipitate 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 check the maximum pressure of analysis systems and connect tubing. Generally, UHPLC stems that have a maximum pressure above the 60 MPa level is appropriate.

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

The column should be connected with male nuts. Ensure to connect the fittings properly to avoid 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 more peaks tail on the early eluting compounds than later eluting compounds, there is possibly a dead volume. In such case, check if all columns are properly connected.

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

■ Metal-free column connection

Be sure to connect by hand. Do not over-tighten the fitting to column by wrench. Install and remove the tubing or sealing plug in holding the end fitting, not the stainless steel column body. Leakage may occur if the end fitting loosens.

When using a general-purpose connection part integrated with ferrule, over-tight may damage the frit. Nexlock which has no ferrule is recommended to be used.

■ 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.

■ 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 under the pressure shown in the following table.

Particle size	Column Dimensions	Maximum pressure limit
1.9 µm	2.0-3.0 mm	100 MPa
3 µm, 5 µm,	2.1-4.6 mm	45 MPa*
5 µm	10 mm	10 MPa
	20 – 30 mm	30 MPa
	50 mm	20 MPa

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

Using a column repeatedly near the pressure limit and a sudden change in pressure may shorten the column life.
Column should be disconnected from the system after the pressure drops to “0”.
Please note that slow operation of the sample injection valve and using an auto-sampler with slow valve switching speed will also increase pressure rapidly at the column inlet, which will cause premature column deterioration.

Both aqueous and non-aqueous solvents can be used as mobile phase, but repetitive replacement between solvents of largely different polarities might degrade the column performance. Acetonitrile, methanol, and tetrahydrofuran (THF) can be used. For THF use, please check the solvent resistance of PEEK tubing, etc.
Some conditions, such as pH of the eluent, may affect the column life. Usually use the columns between 20 ° C and 40 ° C. When you use it 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 ℃).
As Shim-pack Scepter HD C18 has a highly hydrophobic packing materials, equilibration and replacement with mobile phase of low concentrated organic solvent may be difficult. The organic solvent should contain 15% or more methanol, or 10% or more of the organic solvent with lower polarity. Replacement of methanol/aqueous solution with acetonitrile/aqueous solution may cause abnormal retention time or peak shape when the acetonitrile composition ratio is 20% or less. In such cases, replace mobile phase after replacing with 60% acetonitrile aqueous solution.

Products name	Scope of use pH	Temperature Limit (Maximum)	
Shim-pack Scepter C18 Shim-pack Scepter HD-C18	1.0-12.0	70 ℃ (pH 1-7)	50 ℃ (pH 7-12)
Shim-pack Scepter C8	1.0-12.0	70 ℃ (pH 1-7)	50 ℃ (pH 7-12)
Shim-pack Scepter Phenyl	1.0-10.0	50 ℃	
Shim-pack Scepter PFPP	1.0-8.0	50 ℃	
Shim-pack Scepter C4-300	1.0-10.0	90 ℃ (pH 1-7)	50 ℃ (pH 7-10)

■ Preparative Column Handling Precautions

When heating the preparative columns above ambient temperature, irregular peak shapes, such as peak broadening, or peak splits might happen, because temperature in the column is not kept uniformly. Preheating the mobile phase is recommended to avoid these phenomena.

■ Flow rate of column

Particle size	Column Tubing ID	Recommended flow rate Range
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

The flow rate of preparative columns will be larger than that of analytical columns. Use a 0.8 mm or 1.0 mm I.D. tubing accordingly. We recommend you to use an injection valve with a bypass to prevent the column from deterioration.

■ Precaution using UHPLC column

Sample diffusion in the passage in a system, extra-column diffusion, may deteriorate column performance.. In particular, when you use a column of the I.D. 2 mm, optimize LC system as shown below.

1) Shorten the tubing between injector and column, and between column and detector, as much as possible. The tubing I.D. should be small (0.15 mm or less). Be careful not to form voids in the connect.
2) Use low volume types of flow cell in the detector, such as semi-micro type or micro type. Use minimize sample loop.

Optimize the response of the detector and the data sampling speed of the data processor to be higher than 10 data points per peak according to the peak width. For UHPLC with 1.9 µm 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.

■ Clogging of column

The most common cause of the increase of column back pressure and split peaks is blockage and stains of the inlet filter by sample particulates.

- Filtrate the mobile phase using a 0.2 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 (0.2 µm) 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.

■ Washing the column

Generally, rinse the column in the following manner.

- If the mobile phase does not contain buffer solution and salts, increase the concentration of the organic solvent in the mobile phase and rinse the material remaining in the column. You can use up to 100% of the organic solvent. The addition of THF may be effective, especially when highly lipid-soluble components are adsorbed.
- If mobile phase contains buffer solution or salts, replace them with water /organic solvent mixtures (mobile phase in the same proportions as those of the non-containing products). Then, rinse them in the same manner as above. Approximately 50 mM of buffer solution or salts can be directly replaced with 60% acetonitrile aqueous solution.
- Rinse with only water after the use of mobile phase near pH-limit may cause column deterioration. Rinse with the above water /organic solvent mixtures or 60% acetonitrile aqueous solution.
- When macromolecular compounds such as proteins and polysaccharides are adsorbed to column, they are generally difficult to be removed by rinse. It is recommended to pretreatment or use the guard column in advance if the sample contains a large amount of these compounds or impurities.

■ Storage of Columns

After using reversed-phase columns with the eluent containing buffer or ion-pair reagent, wash the column thoroughly with a salt-free eluent before storing. When storing the column for a long period of time, replace mobile phase with 100% organic solvent such as methanol.
Completely seal the column with the plugs provided, and store it in a temperature stable place.

■ Technical Support

Shim-pack Scepter series 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.