SHIMADZU
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ERAD_0001-9043A

# High Performance Packed Column for HPLC

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<br>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 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 series have stable quality products for customers by QC tests. 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 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. 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.

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.

## ■ 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 within 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.

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

Aqueous or non-aqueous solvents can be used as mobile phase, but repetitive replacement among solvents with large difference in polarities might degrade the column performance. Acetonitrile, methanol, and tetrahydrofuran (THF) can be used. For THF use, please take care of the solvent resistance of PEEK tubing, etc.
Depending on the pH of the eluent, the pH may effective the column life. Usually use between 20°C and 40°C. For at high pH use, it is recommended to use low concentration organic buffer solution such as 1 to 10 mM at low temperatures (e.g., <30°C).
Shim-pack Scepter HD C18 has a highly hydrophobic packing material, which may make equilibration or replacement with mobile phase containing low concentration of organic solvent difficult. The organic solvent should be contained 15% or more of methanol and 10% or more of low polar organic solvent. Replacement of methanol/aqueous solution with acetonitrile/aqueous solution may occur the abnormality to retention time or peak shape if the acetonitrile composition ratio is 20% or less. In such cases, replace with mobile phase after replacing with 60% acetonitrile aqueous solution.

## ■ Preparative Column Handling Precautions

When using the preparative columns above ambient, irregularities in peak shapes, such as peak broadening, or peak splits might happen, because temperature in the column is not kept uniformly. It is recommended that you preheat the mobile phase to avoid those phenomena.

| Products name | Scope of use pH | Temperature Limit (Maximum) | |
|---|---|---|---|
| Shim-pack Scepter C18<br>Shim-pack Scepter HD-C18 | 1.0-12.0 | 70 ℃<br>(pH 1-7) | 50 ℃<br>(pH 7-12) |
| Shim-pack Scepter C8 | 1.0-12.0 | 70 ℃<br>(pH 1-7) | 50 ℃<br>(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 ℃<br>(pH 1-7) | 50 ℃<br>(pH 7-10) |

## ■ Flow rate of column

The recommended flow rate of columns are the following.

| 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.

## ■ 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 0.2 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.

## ■ 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 1.9μm 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 manner.

- If the mobile phase does not contain buffer solution or 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. A 50 mM degree of buffer solution or salts can be directly replace with 60% acetonitrile aqueous solution.
- Rinse with only water after the use of mobile phase with pH-limited around 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 adsorb to column, they are generally difficult to remove 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 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, replace 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.