SHIMADZU

228-30531A

Shimadzu High Performance Packed Column for HPLC

**Shim-pack** **FC-ODS**

# Instruction Sheet

## 1 Introduction

The Shim-pack FC-ODS is a high performance reversed-phase column for HPLC. The packing material is composed of 3um to tally porous spherical silica particles. These silica particles are chemically bonded with octadecylsilyl (ODS) groups and thor oughly end-capped.

Features of the Shim-pack FC-ODS column include:

- High throughput analysis is obtained.
- High theoretical plates are obtained.
- Excellent peak shapes and superior chromatography are assured.
- Reproducibility between columns or column batches is assured.

## 2 Specifications

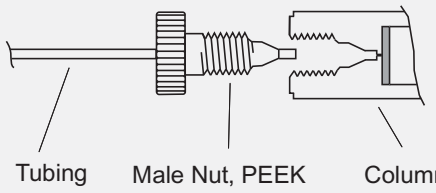
| Packing | |
|---|---|
| Silica particles | Spherical, porous, high purity silica gel |
| Particle size | 3um |
| Trace metal contents | Less than 30ppm |
| ODS functional group | Multi-functional |
| Other | End-capped |

| Column | | | | | |
|---|---|---|---|---|---|
| Dimensions | 30 x 4.6 mm | 75 x 4.6 mm | 150 x 4.6 mm | 75 x 2 mm | 150 x 2 mm |
| Product No. | 228-40511-91(ALL) | 228-40511-92(ALL) | 228-40511-93(ALL) | 228-40511-94(ALL) | 228-40511-95(ALL) |
| Max. operating pressure | 20 MPa | | | | |
| pH range | 1.5 - 9 | | | | |
| Storage solvent | acetonitrile /water /acetic acid = 60/40/0.1 | | | | |

## 3 Column Installation

- The flow direction of the column is shown on the column □ ( FLOW ). When installing the column, ensure that this flow direction matches the mobile phase flow direction.
- The column is connected with PEEK malenuts as is shown in the figure. Ensure that the fittings are connected tightly to avoid leaking liquid from the connection. If the leakage occurs when mobile phase is flowed, check the following points:
  - The connection of the malenuts becomes loose.
  - The tubing is not inserted fully to the column interface.
  - The edge of the tubing is not flat.
- The product name and parts number of the PEEK male nuts used for connection are as follows;

Column Connection




| Item Name | Part No. | Comment |
|---|---|---|
| Male Nut, PEEK | 228-18565-84 | 5/pkg |

## 4 Mobile Phase

- Generally, in reversed-phase chromatography, the mobile phase consists of a mixture of methanol or acetonitrile and water.
- When analyzing ionic substances, the separation characteristics of the compounds are kept uniform by the addition of salts, such as potassium dihydrogen phosphate, or pH modifiers, such as phosphate buffer or trifluoroacetic acid. However, the pH must be carefully monitored to ensure that it is within an acceptable range for stationary phase stability.
- The retention of ionic substances can also be controlled by the addition of an ion-pair reagent, such as a tetrabutylammonium or pentane sulfonate. Set conditions such that the solute retention remains constant, even if the ion-pair concentration fluctuates.

## 5 Chromatographic conditions

- The Shim-pack FC-ODS is a high-performance column having packing materials smaller than those of conventional ODS columns. Pay attention to the followings when determining the chromatographic conditions.
- Basically, the same flow rate used for the conventional columns can be set. However, it may be reduced to 0.5-0.6mL/min for 4.6mm ID, or 0.1-0.15mL/min for 2mm ID, in case of the column 150mm long.
- Set the flow rate not to exceed the maximum pressure (20MPa).
- Set the column temperature in the range of 15 - 65°C considering peak shape and column lifetime.
- When preparing sample solutions, dilute the sample with an appropriate solvent to make the composition and pH close to those of the mobile phase. It leads the peak shape sharp.
- Inject a sample of 100uL or less. Too large volume injection may distort the peak shape of polar compounds.

# 6 Flushing the Column

- To remove neutral, low polarity substances from the column, flush with methanol or acetonitrile at a flow rate of 0.5-1.0 mL/min (4.6mm i.d. column) or 0.1-0.2mL/min (2mm i.d. column) for 10-20 minutes. If salts insoluble in such organic solvents are present, first flush the column with water at these flow rates so that the salt does not precipitate.
- For flushing out an ion-pair reagent or other ionic substances, use a 0.1% acetic acid solution of methanol or acetonitrile.
- It is difficult to remove hydrophilic polymers, such as proteins and nucleic acids, from the column. For such applications, use ultrafiltration prior to injection in order to remove macromolecules from the sample.

# 7 Column Handling Precautions

- Do not overtighten the column malenuts during installation. This may damage the fittings.
- Do not shock the column by banging it or dropping it.
- Mobile phase and sample solutions must be filtered with a membrane filter, or an equivalent, before use. Suspended particles will lead to column clogging, which will increase the column pressure.

# 8 Column Storage

- Do not allow the column packing material to dry out. When removing the column from the chromatograph, plug both ends of the column so that the solvent cannot evaporate.
- For long-term storage, replace the mobile phase with a 60% acetonitorile aqueous solution, then plug both ends of the column before storage.

# Certificate of Analysis

- This column comes with a quality assurance certificate that refers to the physical properties of packing materials and column performance. These items are described below.

## Packing Materials

**D50 [µm]**
The halfway point of the particle size distribution of the silica base material.

**D90/D10**
The ratio of the 90% and 10% point of the particle size distribution indicating the equality coefficient of the distribution.

**Pore size [nm]**
The average pore size of the silica gel.

**Specific surface area [$m^2$/g]**
The specific surface area of the silica gel.

**Pore volume [mL/g]**
The pore volume of the silica gel.

**Trace metal contents [ppm]**
The individual trace metal contents of the silica gel are measured to ensure that the contents are within the criteria.

**tR(1) [t0, min]**
The retention time of the t0 marker (a non-retained compound).

**k'(7)**
The capacity factor of butylbenzoate is measured to ensure that the hydrophobicity of the packing materials meets the specification.

**$\alpha$(7/4)**
The separation factor of butylbenzoate to amitriptyline is measured to ensure that the packing materials were well end-capped.

**$\alpha$(7/5)**
The separation factor of butylbenzoate to propylbenzoate is measured to ensure that the packing materials have the fixed hydrophobic recognition ability.

**$\alpha$(7/6)**
The separation factor of butylbenzoate to progesterone is measured to ensure that the hydrophilicity of the packing materials meets the specification.

**N(2)**
The number of theoretical plates is calculated for 4-hydoxybenzoic Acid to evaluate the peak shape of acidic compounds.

**N(3)**
The number of theoretical plates is calculated for 8-quinolinol to evaluate the peak shape of chelating compounds.

**N(4)**
The number of theoretical plates is calculated for amitriptyline to evaluate the peak shape of basic compounds.

**N(6)**
The number of theoretical plates is calculated for progesterone to evaluate hydrophilic interaction.

**Pressure [MPa]**
The pressure indicates that the particle size distribution is within the criterion.

## Column Performance

**Retention time [min]**
The retention time of uracil as a t0 marker is measured to evaluate the packing density.

**Capacity factor**
The capacity factor of the second eluting compound is measured to determine whether the column meets hydrophobic level requirements.

**Plate number**
The number of theoretical plates is calculated for the second peak by the JP method to ensure that the column is packed properly.

**USP Tailing factor**
The tailing factor of the second peak calculated by the USP method is used to determine that the column is uniformly packed.

**Column pressure [MPa]**
The column head pressure is measured to ensure that the column is packed properly.



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