

# Shimadzu Packed Column for HPLC

# Shim-pack VP-ODS / C8 / Phenyl

# **Instruction Manual**

#### ■ Introduction

Shim-pack VP-ODS / -C8 / -Phenyl are high performance reversed-phase columns for HPLC. The packing material is high purity spherical silica particles and the surfaces of the silica particles are chemically bonded with functional groups and thoroughly endcapped.

### Specifications

Item	Contents		
Silica particles	Spherical, porous, high purity silica particles		
Particle size	4.6 μm		
Pore size	12 nm		
Surface Modification	VP-ODS : Octadecylsilyl groups VP-C8 : Octyl groups VP-Phenyl : Phenylpropyl groups (Mono-functional)		
Other modification	Endcapping		
Carbon contents	VP-ODS : about 20 % VP-C8 : about 12.5 % VP-Phenyl : about 12.3 %		
Type	Stainless steel packed column		
Storage solvent	Please see the Column performance report.		
pH range	$2 - 7.5^{*1}$		
Maximum Pressure	About 20 MPa		
Operating temperature	$80^{\circ}C^{*1}$ When mixtures of water or acidic aqueous solution (pH 3 or greater) and acetonitrile are used.		

\*1. Refer to "■ Column Handling Precautions".

#### Certificate of Compliance

These columns come with a quality assurance certificate that refers to the physical properties, chromatographic and column performance. These items are shown in " Description Items of the Certificate".

#### ■ Description Items of the Certificate

## Physical Properties

Item	Contents		
Particle Size	The particle size (µm) indicated is that of the halfway point of the particle size distribution.		
Pore Size	The average pore size (nm) is determined by the nitrogen adsorption method.		
Pore Volume	The pore volume (mL/g) is determined by the nitrogen adsorption method.		
Specific Surface	The specific surface area (m <sup>2</sup> /g) is determined		
Area	by the nitrogen adsorption method.		
Trace Metal	The total and individual trace metal content		
Contents	(ppm) of the silica is determined for six different metals.		
Carbon Loading	The carbon loading (%) of the octadecyl and methyl groups in the packing determined by		
	CHN measurement.		
C18 Surface	The even distribution of octadecyl groups per		
Coverage	unit of packing surface area is certified.		
	$(\mu mol/m^2)$		

#### Chromatographic Performance

<u> </u>	Chromatographic refformance		
Item	Contents		
Hydrophobic	The relative retention $(\alpha)$ of amylbenzene and		
Interaction	butylbenzene is calculated to determine how		
	hydrophobic the stationary is.		
Basic	The tailing factor (symmetry factor, Tf) and		
Compound	relative retention ( $\alpha$ ) of N-acetylprocainamide		
	against phenol are examined to determine the		
	elution characteristics of basic compounds.		
Acidic	The tailing factor (symmetry factor, $Tf$ ) and		
Compound	relative retention ( $\alpha$ ) of salicylic acid against		
	phenol are examined to determine the elution		
	characteristics of acidic compounds.		
Chelating	The interaction of chelating compounds and the		
Compound	bonded phase is measured by examining the		
	theoretical plate number $(N)$ and relative		
	retention ( $\alpha$ ) for 8-quinolinol against toluene.		

#### Column Performance

Column reno		
Item	Contents	
Retention Time	The retention time of naphthalene ( $t_R$ ) is used to determine whether the column meets hydrophobilevel requirements.	
Plate Number	The number of theoretical plates ( $N$ ) is calculated for naphthalene to ensure that the column is packed properly. The following formula is used to calculate the number. $N = 5.54 \times (t_R / W_{1/2})^2$ $t_R$ : retention time $W_{1/2}$ : peak width at 1/2 height	
Tailing Factor	The tailing factor (symmetry factor, Tf) of naphthalene is used to determine that the column is uniformly packed. The following formula is used to calculate the factor. $Tf = W_{0.05} / 2f$ $W_{0.05}$ : peak width at 5 % height $f$ : width from peak upslope to peak apex at 5 % height	
Pressure	The column head pressure (MPa) is measured to ensure that the column is packed properly.	

#### Lineup

Size	VP-ODS	VP-C8	VP-Phenyl
4.6 mm <i>i.d.</i> × 150 mm	228-34937-91	228-59927-91	228-59928-91
4.6 mm i.d. ×250 mm	228-34937-92	228-59927-92	228-59928-92
6.0 mm <i>i.d.</i> $\times$ 150 mm	228-34937-93	-	-
2.0 mm i.d. ×150 mm	228-34937-94	228-59927-93	228-59928-93
2.0 mm i.d. ×250 mm	228-34937-95	228-59927-94	228-59928-94
4.6 mm <i>i.d.</i> × 50 mm	228-36849-91	-	-
20 mm <i>i.d.</i> × 50 mm	228-36849-92	-	-
20 mm i.d. × 100 mm	228-36849-93	-	-
10 mm <i>i.d.</i> × 250 mm	228-36849-94	-	-

#### Column Installation

- The flow direction of the column is shown as (→) on the column. When installing the column, ensure that this flow direction matches the mobile phase flow direction.
- The column is connected with PEEK male nuts. Ensure that the fittings are connected properly to avoid creating dead volume between the tubing and the column interface. The product name and parts number of the spare PEEK male nuts are as follows;

Item name	P/N	Comments	
Male nut, PEEK	228-18565-94	5 /pkg	

#### NOTE:

The presence of air in the flow line may damage the column. Before connecting the column, be sure the flow line is completely filled with mobile phase.

#### ■ Mobile Phase Solvent

- Generally, in reversed-phase chromatography, the mobile phase consists of a mixture of methanol or acetonitrile and water. Filter the mobile phase and sample solutions through a less than 0.45 μm membrane filter, or an equivalent, before use.
- 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 acetic acid. However, the pH must be carefully monitored to ensure that it is within an acceptable range for stationary phase stability.
- The solute retention can also be controlled by the addition of an ion-pair reagent, such as a tetrabutyl-ammonium salt or 1- pentanesulfonate salt. Select conditions such that the solute retention remains constant, even if the ion-pair concentration fluctuates.

#### ■ Column Handling Precautions

- Do not overtighten the column (male) nuts during installation. This may damage the fittings.
- Observe the pressure and temperature limits given in "■ Specifications". The steep pressure change over the column may cause deterioration.
- Adjust the pH of mobile phase within the range described in "■ Specifications". Optimum lifetime is obtained at pH
   2.5 pH 7.0 and at 40 °C or less when a buffer is used.
- To remove the column from the system, be sure to confirm the temperature of the column becomes the room temperature and the pressure of the column becomes zero.
- ●Do not shock the column by banging it or dropping it.

#### NOTE:

Filter the sample solutions through a less than  $0.45~\mu m$  membrane filter, or an equivalent, before use. Suspended particles will lead to column clogging, which will increase the system pressure.

# Flushing the Column

- To remove neutral, low polarity substances from the column, flush with methanol or acetonitrile at a flow rate of 1.0 mL/min (0.2 mL/min for 2.0 mm i.d. column) for 30 minutes. (If organic salts are present, first flush the column with water at these flow rates for 10 minutes so that the salt does not precipitate.)
- For flushing out an ion-pair reagent or other water-soluble material, 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 the guard column (replace periodically) or ultrafiltration prior to injection in order to remove macromolecules from the sample.

#### ■ Column Storage

Do not allow the column packing material to dry out. When removing the column from the system, cap both ends of the column so that the solvent cannot evaporate. For long-term storage, first flush the column (see Flushing the Column, above), replace the mobile phase described in the Column performance report, then cap both ends of the column before storage. Remember to flush with water first if salt buffers were used as the mobile phase.

# ■ Technical Support

It is the customer's responsibility to develop and validate analytical conditions for a particular application. However, Shimadzu offers technical support by e-mail and phone for customers who need help.

Write specific questions to <u>analytic@group.shimadzu.co.jp</u> or call your local representative.

X The contents of this instruction sheet are subject to change without notice.