

## High Performance Packed Column for HPLC

*CoreFocus*

# Shim-pack™ Bio Diol

## INSTRUCTION MANUAL

### ■ Introduction

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

### ■ Specifications

The product specifications of this product are as follows.

Products name	Particle size	Pore size	Chemical bonding group
Shim-pack Bio Diol-60	3, 5 µm	6 nm	Dihydroxypropyl Groups
Shim-pack Bio Diol-120		12 nm	
Shim-pack Bio Diol-200		20 nm	
Shim-pack Bio Diol-250		25 nm	
Shim-pack Bio Diol-300		30 nm	

### ■ 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 Bio Diol column 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 Bio Diol column have stable quality products for customers by QC tests. Shim-pack Bio Diol columns are shipped in the 0.05% sodium azide aqueous solution. Wash with water sufficiently before replacing with the mobile phase.

### ■ 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 the shortest possible tubing connection from the injector to the column to minimize peak broadening.

Ensure that the fittings are connected properly to avoid creating dead volume between the tubing and the column interface.

Keep the piping connecting the guard column and the analysis column as short as possible.

**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.

### ■ 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.

Repeated sample injection may increase column pressure. Filter the sample 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.

#### Maximum pressure limit of shim-pack Bio Diol-60, 120, 200, 300

Particle size	Maximum pressure limit
2 µm	45 MPa* Usually at 30 MPa or less.
3 µm, 5 µm	20 MPa**

#### Maximum pressure limit of shim-pack Bio Diol-250

Column diameter	Maximum pressure limit
4.6 mm	14 MPa*
8.0 mm	12 MPa*

\*Since the pressure varies depending on the column length, column temperature, solvent, etc., adjust the Flow rate as appropriate.

\*\*Pressure tolerance of 20 mm inner diameter is 10 MPa.

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

Since the pressure varies depending on the column length, column temperature, type of organic solvent, etc., adjustment the Flow rate as appropriate.

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.

Use water-based eluent of the total salt concentration 0.7 M or less basically. Tris hydrochloride, citrate, etc. can be used as buffer solution, and can be used in combination with solution such as sodium chloride, sodium sulfate, ammonium sulfate, etc.

The aqueous solution of urea or guanidine hydrochloride can be used as protein denaturing agents. In addition, Tween80 or SDS in an amount of 0.1% or less can be used as surfactants. When these eluants are used, take precaution of the equilibration of column for long period than general.

The methanol and acetonitrile can be used, but take precaution of increase of pressure and precipitation of salts.

Recommendations of pH and temperature for column are the following.

Products name	Range of use pH	Temperature Limit (Maximum)
Shim-pack Bio Diol except 250	5.0-7.5	50 °C
Shim-pack Bio Diol-250		40 °C

The column lifetime varies greatly depending on the pH, temperature and eluent compositions. Generally, the higher concentration of the column temperature, the buffer solution, or the additive, can shorten the column life.

For storage, except for daily use, replace the column solvent with 0.05% sodium azide solution after washed with water. Later, the column should be sealed tightly at the both ends, and store the solution in a location with low temperature change. At next use, after washing column with the water thoroughly, replace it with the eluent.

#### Guard column

Particle Size	2 µm		3 µm				
	Bio Diol-200	Bio Diol-300	Bio Diol-60	Bio Diol-120	Bio Diol-200	Bio Diol-250	Bio Diol-300
4.0 mm i.d. x 10 mm L.	227-31202-02	227-31202-01	-	-	227-31202-04	-	227-31202-03

Particle Size	5 µm			
	Bio Diol-60	Bio Diol-120	Bio Diol-200	Bio Diol-300
8.0 mm i.d. x 30 mm L.	227-31007-04	227-31008-04	227-31009-06	227-31010-06
20 mm i.d. x 50 mm L.	227-31116-01	227-31117-01	227-31118-01	227-31119-01

\*The 4.0 mm i.d. x 10 mm L. guard column is a cartridge type and contains 5pc. Purchase and use a guard cartridge holder (227-31172-03, sold separately).

## ■ 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, particles created by aging pump seals, or large quantities of lipophilic compounds adsorbing to the head of the column.

- Filtrate the mobile phase using a 0.45 µm membrane filter before using the column.
- Filtrate the sample using a syringe filter before injecting to the column.
- Installing Guard Column (listed belows) 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

If hydrophobic proteins or hydrophobic substances are adsorbed or retained, use an eluent with a high salt concentration (about 0.5M) for rinse. At this time, take care of usable pH.

Except for the case of continuous use every day, after use, wash the column thoroughly with water, replace with 0.05% sodium azide aqueous solution, close both ends tightly, and store it at a stable temperature and moisture. When using the column next time, please replace store solvent with the mobile phase after passing enough water.

## ■ Technical Support

Shim-pack Bio Diol 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.