

Shodex™



HPLC Columns

MANUAL

OHpak SB-2000 series

SHOWA
DENKO
EUROPE

Columns manufactured by Showa Denko K.K Japan
Made in Japan

Shodex HPLC Columns
Europe, Middle East, Africa, Russia

For technical support please use
contact details shown below:

SHOWA DENKO EUROPE GmbH
Shodex Business
Konrad-Zuse-Platz 3
81829 Munich, Germany

E-mail: support@shodex.de
Phone: +49 (0)89 93 99 62 37
www.shodex.de

Operation Manual

Shodex™ OHpak™ SB-2000 series

(Please read this manual carefully before using the column to keep its good performance and life.)

1. Introduction

The packed columns of Shodex OHpak SB-2000 series are designed to be used for semi-preparative GPC of water-soluble polymers.

2. Specifications

| Column type | Exclusion limit (Pullulan) | Theoretical plate number |
|-------------|----------------------------|--------------------------|
| SB-2002 | 4,000 | 8000< |
| SB-2002.5 | 10,000 | 10000< |
| SB-2003 | 100,000 | 10000< |
| SB-2004 | 400,000 | 10000< |
| SB-2005 | 4,000,000 | 10000< |
| SB-2006 | (20,000,000)* | 10000< |
| SB-2006M | (20,000,000)* | 10000< |

*: Figures in parentheses are estimated values.

Column material: SUS-316.

Column size: 20 x 300mm.

Endfitting: Internally-threaded type, No. 10-32 UNF.

Packing material: Porous hydrophilic polrhydroxymethacrylate gel.

In-column solvent: 0.02% Sodium azide aqueous solution.

| Column type | Usable pressure (kgf/cm ²) | Usable flow rate (ml/min) | Recommended flow rate (ml/min) |
|-------------|--|---------------------------|--------------------------------|
| SB-2002 | Max. 25 | Max. 5 | 3 |
| SB-2002.5 | Max. 25 | Max. 6 | 3 |
| SB-2003 | Max. 25 | Max. 6 | 3 |
| SB-2004 | Max. 25 | Max. 6 | 3 |
| SB-2005 | Max. 25 | Max. 6 | 3 |
| SB-2006 | Max. 25 | Max. 5 | 3 |
| SB-2006M | Max. 25 | Max. 5 | 3 |

| Column type | Usable temperature °C | Usable pH | Usable salt concentration | Usable polar organic solvent |
|-------------|-----------------------|-----------|---------------------------|------------------------------|
| SB-2002 | 15-60 | 3-10 | Max. 0.5M | 0% |
| SB-2002.5 | 15-60 | 3-10 | Max. 0.5M | Max. 60% |
| SB-2003 | 15-60 | 3-10 | Max. 0.5M | Max. 50% |
| SB-2004 | 15-60 | 3-10 | Max. 0.5M | Max. 50% |
| SB-2005 | 15-60 | 3-10 | Max. 0.5M | Max. 50% |
| SB-2006 | 15-60 | 3-10 | Max. 0.5M | Max. 50% |
| SB-2006M | 15-60 | 3-10 | Max. 0.5M | Max. 50% |

3. Eluent

The separation performance of SB-2000 series will be largely affected by the eluent conditions.

0.1M NaNO₃ aqueous solution, a buffer, an organic solvent or a mixture of buffer and organic solvent can be used as an eluent. Among them, 0.1M NaNO₃ aqueous solution is most commonly used as an eluent.

The followings are buffers and organic solvents usually used.

1) Buffer

(1) Phosphate buffer

- Caution!**
- ① The buffer pH range should be between 3 and 10.
 - ② When a salt is added, the salt concentration should be lower than 0.5 mole/L.
 - ③ When an eluent whose salt concentration is more than 0.2 mole/L is used, flow rate should be lower than 3 ml/min.
 - ④ A salt containing chloric ion should not be added because it corrodes metallic parts. Even when it is essential to add it, the time used with it should be shorter than 8 hours and pH range should be within the range higher than 4.
 - ⑤ Do not use an eluent which contains boric acid or borate, because borate makes a complex with diol group of packing material.

2) Organic solvent

- (1) Acetonitrile
- (2) Methyl alcohol

- Caution!**
- ① When an organic solvent is added to an eluent, the eluent viscosity will become higher. Consequently, the column pressure will also become higher. Therefore, be careful that maximum pressure will not exceed 25kgf/cm².
 - ② When an eluent whose organic solvent concentration is more than 30% with SB-2003, SB-2004, SB-2005, SB-2006 and SB-2006M, flow rate should be lower than 3 ml/min.

4. Preparation of eluent

- 1) Remove extraneous or insoluble matters by passing the eluent through a 0.45 μm filter.
- 2) Thoroughly degas the eluent, by ultrasonic vibration and simultaneous heating or pressure reduction with an aspirator.

Use of solvent degassing device, Shodex DEGAS KT series is recommended.

5. Installation and start-up

- 1) Prior to connection of the column to the liquid chromatograph, replace the solvent in the chromatograph with the solvent to be used as the eluent.

When a solvent containing salt is remaining in the chromatograph, wash all flow line with purified water before replacement with the eluent.

When a solvent containing organic solvent such as chloroform is remaining in the chromatograph, wash all flow line with acetone before replacing with the eluent.

- 2) After replacing the solvent in the chromatograph, set the flow rate at half of the flow rate to be used.

Caution! It is recommended to start from a slow flow rate to check column pressure does not exceed the maximum pressure.

- 3) Connect the column to the chromatograph as that the arrow mark on the column will face downstream. Do not let air get into the column while connecting the column to the chromatogram.

Then, start pumping.

Caution! After confirming that the column pressure does not exceed the maximum pressure, the flow rate can be raised at the flow rate to be used.

6. Pre-treatment of sample

- 1) The sample should be dissolved in the eluent to be used.
- 2) Remove extraneous or insoluble matters by passing the sample through a 0.45 μm filter.
Use of the disposable filter unit, Shodex DT series is recommended.

7. Guard column

Install a guard column just upstream of the main column to protect it from contamination by the sample.

The guard column is intended to maintain the main column performance as designed for a long period of time and not to improve resolving power.

8. Safekeeping

- 1) When the column is heated, after completing analysis, keep pumping the eluent at a low flow rate until the column is cooled down to room temperature.
- 2) Stop the pump and leave the column in the chromatograph, if it is to be used on the following day.
- 3) In case of 3 or more days of suspension of chromatograph in which a solvent containing salt was used as the eluent, replace the eluent with purified water, setting the flow rate at 0.5ml/min.
- 4) In case of its suspension over one week, take the same action of as in 3) above use 0.02% sodium azide aqueous solution instead of purified water and dismount the column from chromatograph.

Then, blank off both ends of the column.

9. Troubleshooting

Table 1 below gives troubles likely to occur during use of the column and the corrective actions to be taken. It is not guaranteed that the corrective actions given in the table always solve the trouble.

Therefore, after taking the actions, check the column resolution.

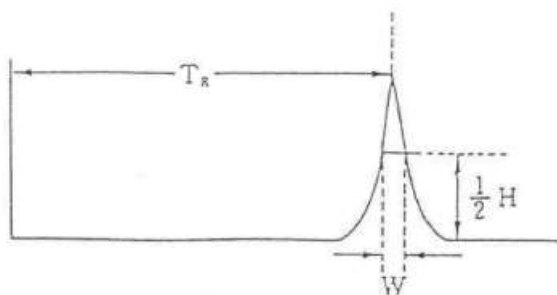
Please note that removal of the endfittings will allow the air or other insoluble matters to enter the column, probably to deteriorate the column performance.

| Trouble | Cause | Corrective action |
|------------------------------------|--|--|
| Column pressure increase. | Plugged endfitting. | Reverse the column on the chromatograph and pass the eluent through it for one hour. |
| | Inclusion of insoluble matters in the column. | Irreparable. |
| Rapid deterioration of resolution. | Void induced in the upstream end of the column or channel induced in the column. | Irreparable. |
| | Liquid flow disturbance caused by insoluble matters clogging endfitting. | Reverse the column on the chromatograph and pass the eluent through it for one hour. |
| | Adsorption of some impurity of sample or eluent on packing materials. | Pass acidic or alkaline buffer into the column at 0.5 ml/min for one hour. |
| No sample elution. | Sample adsorption. | Change the separation conditions. |
| | Malfunctioning detector. | Check the detector. |

10. Measurement of theoretical plate number (N)

The measurement conditions for N determination are described in attached inspection sheet.

Calculation of N



$$N = 5.54(T_R/W)^2$$

$$N = NTP$$

T_R = Retention time (min)
 W = Half width (min)

11. Warranty

1) Showa Denko shall replace any Shodex column,

1-1) If found damaged at the time of delivery.

1-2) If theoretical plate number (N) obtained by the purchase as per the operation manual is significantly smaller than the one given in the inspection sheet attached to the column.

Claims must be filed with Showa Denko within 10 days following delivery.

2) The following shall not be subject to warranty.

2-1) Service life.

2-2) Deterioration of column performance resulting from improper handling.