Operation Manual
Shodex OHpak SB-2000 series

(Please read this operation manual carefully to achieve the best and consistent column performance for a long time.)

Important Handling Instructions

Caution!

· Please consult the Safety Data Sheet (SDS) of reagents and solvents used with the column and understand their proper handling methods to prevent potential health hazards or death from occurring.

· Please wear appropriate personal protective equipment such as lab goggles and gloves when handling organic solvents and acid and alkaline reagents. Avoid any direct physical contact to prevent chemical injuries.

Before Using the Column

(1) Please visually inspect the column package and the column surface for any damage.

(2) Please check the product name and serial number (Serial no. or S/N) written on the column package and adhesive label on the column body.

(3) Please download the Certificate of Analysis (CoA) for the purchased product. The CoA can be downloaded from Shodex website (https://www.shodex.com/download/). You will be asked to enter the serial number.

1. Introduction

Thank you for purchasing the Shodex product. Shodex OHpak SB-2000 series is size exclusion chromatography columns used with aqueous solvents. The column series is suitable for preparative isolation of various water-soluble polymers, proteins, and oligomers. Please select columns with target molecular weight ranges (see below) that meet the molecular weight range of your samples.

2. Column Components

Please refer to the Shodex website: https://www.shodex.com/en/da/07.html

3. Column Specifications

Product Code	Product Name	Column Size (mm)		Particle Size (μm)	Theoretical Plate Number (Per Column)	Exclusion Limit[*1]
		I.D	Length			
F6516011	**OHpak SB-2002**	20.0	300	15	≥ 9,000	1,000
F6516012	**OHpak SB-2002.5**	20.0	300	10	≥ 12,000	10,000
F6516013	**OHpak SB-2003**	20.0	300	10	≥ 12,000	100,000
F6516014	**OHpak SB-2004**	20.0	300	18	≥ 12,000	1,000,000
F6516015	**OHpak SB-2005**	20.0	300	20	≥ 12,000	(4,000,000)[*2]
F6516016	**OHpak SB-2006**	20.0	300	20	≥ 12,000	(20,000,000)[*2]
F6516017	**OHpak SB-2006M**	20.0	300	20	≥ 12,000	(20,000,000)[*2]
F6709555	**OHpak SB-G 8B**	8.0	50	18	(Guard Column)	-

SB-2006M is a mixed-gel column, provides a wider molecular-weight analysis range.

[*1] Reference value only / Measured with pullulan
[*2] Estimated value

Base Material : Spherical porous particles of polyhydroxymethacrylate
Column Housing : SUS-316
Screw Type : Internally-threaded type No.10-32 UNF
Shipping Solvent : 0.02 % Sodium Azide aq. Solution

4. Usable Conditions

4.1 System Settings

Product Name	Flow Rate (mL/min)		Maximum Pressure (MPa)(Per Column)	pH Range	Temperature Range (°C)
	Recommended	Maximum			
OHpak SB-2002	3.0	5.0	2.0	3 - 10	15 - 60
OHpak SB-2002.5					
OHpak SB-2003					
OHpak SB-2004					
OHpak SB-2005					
OHpak SB-2006					
OHpak SB-2006M					
OHpak SB-G 8B					

4.2 List of Applicable Solvents

Product Name	Maximum Usable Solvent Concentration (%)		
	Methanol	Acetonitrile	N,N-Dimethylformamide (DMF)
OHpak SB-2002	0	0	0
OHpak SB-2002.5 - SB-2006M, OHpak SB-G 8B	50	50	50

(1) Buffers and aqueous solutions of different salts can be used separately or together. Usable buffers include phosphate, acetate, citrate, and Tris. Usable aqueous salt solutions include sodium chloride, sodium sulfate, potassium sulfate, and ammonium sulfate. Their recommended concentration ranges are 0.05 to 0.3 M. Please keep total concentration of salts under 0.5 M.

(2) Protein denaturants such as urea and guanidine hydrochloride can also be used. However, their required concentration are usually high, which may damage the column during the solvent replacement. Thus, it is recommended to dedicate the column for these specific uses.

(3) Surfactants such as SDS and Brij-35 can also be added to the eluent. However, since surfactants tend to remain on the column, solvent replacement after their use takes a longer time than replacement from general solvents. The replacement time can be shorten by using 30 to 50 % (v/v) methanol (Note that organic solvents cannot be used with SB-2002).

Attention!

- Use the column within above stated flow rate, pressure, and temperature ranges. Using the column outside the given range may damage the column and lower its performance.
- When using a mixture of buffer (or aqueous solution of salt) and organic solvent, make sure there is no precipitation of salt.
- When adding chloride ions, make sure the eluent pH is 6 or higher.
- When using highly corrosive salts such as sodium chloride, wash out the salts at the end of analysis. The metal parts of the devices and/or the columns may rust.
- Do not use borate buffer as it forms a complex with diol group of the packing material.
- Column pressure is influenced by eluent composition, flow rate, and column temperature. When changing the eluent compositions, adjust the flow rate and column temperature so that the column pressure remains below the usable maximum pressure..

· Shear degradation occurs more likely in larger molecular weight compounds. The result of shear degradation may appear as lower molecular weight measurement than the actual value and/or low reproducibility. If shear degradation is suspected, use a lower flow rate.

5. Eluent Preparation

(1) Degas the eluent fully to prevent the formation of air bubbles.

(2) Presence of small debris or insoluble substances may result in deterioration of columns and/or they may appear as noise on chromatograms. Filter the eluent with a 0.45-μm disposable filter to prevent the problems from occurring.

Attention!

· Whenever water is required, use ultra-pure water freshly generated by a water purification system or water from a newly opened HPLC grade distilled water bottle. Use of HPLC grade organic solvents of guaranteed quality, which can be used without problems in HPLC is recommended. If organic solvents with different grades are used together, make sure that their qualities are all suitable for the analysis prior to the use. Solvents left in opened bottles for a long time should not be used. The content may have been changed, absorbed moisture, or has been contaminated.

· Always use freshly prepared solvents. Solvents stored for a long time may have changed their compositions and may influence elution patterns and/or damage the column.

Note

· Use of an on-line degasser is recommended.

6. Sample Preparation

(1) If possible, use the eluent for analysis to dissolve or dilute samples. If this is difficult, use a solvent which has a composition that is as close as possible to the eluent composition and which fully dissolves or dilutes the sample.

(2) Filter diluted sample solutions using disposable 0.45-μm filters to prevent the column from clogging or deteriorating.

(3) To prepare samples with molecular weights larger than 1,000,000, first allow the sample to stand in the eluent of analysis for 1 day until it becomes fully swollen. Next, slowly agitate the sample solution to completely dissolve the sample. Be careful as aggressive agitation can cut the polymer chains of the analyte.

(4) Suggested injection volume is 250 to 1,000 μL.

(5) Viscosity of high molecular weight compound is largely influenced by its molecular weight and concentration. Samples with high viscosity cause peak broadening and elution delay, and this makes it difficult to obtain their accurate molecular weight distributions. In general, the larger the molecular weight of the compound, the higher its viscosity becomes. To suppress the influence from high viscosity, it is recommended to lower the sample concentration. Please use the below table as a reference when preparing samples for molecular weight distribution analyses.

Molecular Weight Range	Optimal Concentration (w/v)
≤ 5,000	≤ 1.0 %
5,000 - 25,000	≤ 0.5 %
25,000 - 200,000	≤ 0.25 %
200,000 - 2,000,000	≤ 0.1 %
≥ 2,000,000	≤ 0.05 %

Attention!

· When a sample is dissolved in a solvent other than the eluent and if the sample matrix contains components which do not dissolve in the eluent fully, precipitates may form and clog the column.

Note

· Use of a guard column is recommended to protect the analytical column.

7. Column Usage Procedure

7.1 HPLC System Preparation

Wash the entire HPLC system prior to column installation, including all flow-lines and sample loop by switching the valves, and then replace the washing solution with the eluent to be used. If desired new eluent has low miscibility/solubility to the eluent of previous analysis, first use the eluent that is miscible/soluble to both eluents, and then replace it with the desired eluent.

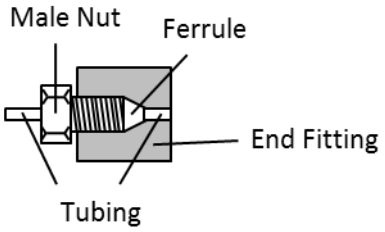
Attention!

- If the eluent left in the HPLC system is not compatible with the column to be used, it may damage the column.
- A drastic change in the eluent compositions may remove substances adsorbed on the HPLC system and they may enter and deteriorate the column.

7.2 Column Installation

(1) Connect the column to HPLC system by following the “flow direction arrow” (➞) indicated on the column adhesive label. If a guard column is used, position the guard column in front (before the inlet) of the analytical column.

(2) Make sure to insert the tubing all the way to the end fitting and secure it with the male nut. It is important that there is no extra space between the tubing and the column side of the end fitting. Presence of an extra space will let the sample to spread out and may result in wide peaks.



(3) Set the initial flow rate to less than 1.0 mL/min and start the system. If the column is to be heated during the analysis, keep the low flow rate until the column temperature reaches to the set temperature, and then gradually increase the flow rate to the desired.

(4) Multiple number of columns can be connected in series. When connecting multiple columns with different exclusion limits, set the column with higher exclusion limit at the upper stream position. When using SB-2006M, use multiple number of SB-2006M columns in series. This is because mixed-gel columns are prepared by using several different gels with different exclusion limits, designed to provide linear calibration curves over the set molecular weight ranges. If different columns were used together, this changes the mixing ratio of the gels and consequently it may deform the linear calibration curve.

Caution!

- Verify that there is no solvent leak. The solvent leak may cause electronic leakage, rust, and/or chemical injury.

Attention!

- Make sure not to let air bubbles enter the column while installing the column. The air bubbles may damage the column.
- When restarting the system after column installation or after holding the eluent flow, start the system at less than 1.0 mL/min flow rate. A rapid increase in pressure can damage the column.
- If the column was heated during the analysis, lower the flow rate to less than 1.5 mL/min at the end of analysis. Then, turn off the column oven to let the column temperature returns to room temperature before stopping the pump. This is to prevent creating an empty space in the column, which deteriorates the column. Since if the pump was stopped while the eluent inside the column is still hot, the eluent volume decreases and creates an empty space when the eluent temperature decreases.

Note

- It is recommended to set the pump limiter to avoid exceeding the maximum pressure.

7.3 Solvent Exchange

To replace the solvent, set the system at less than 1.0 mL/min. Recommended solvent volume to introduce at each step is 3 to 5 times of the column volume.

(1) Check miscibility/solubility of the desired new solvent and the solvent currently filled in the column.

(2) When replacing the current solvent with a miscible solvent, first introduce 1:1 mixture of the current solvent and the new solvent, and then replace it with 100 % new solvent.

e.g. When replacing highly concentrated buffer solution (or aqueous salt solution) to methanol, first run 1:1 mixture of buffer (or aqueous salt solution) and methanol, then replace it with methanol.

(3) When replacing the current solvent with a solvent with low miscibility/solubility to the current solvent, first use a solvent that is miscible/soluble to both solvents, and then replace it with the new solvent.

e.g. When replacing highly concentrated buffer solution or salt solution to water/acetonitrile, first run water and then replace it with the eluent.

Attention! · Frequent solvent replacement deteriorates the column, and thus not recommended.

7.4 Column Cleaning

Problems in peak shapes and elution time changes or elevated column pressure are often caused by the deposition of insoluble or adsorbing components from the sample/flow-line inside the column. These problems may be resolved by cleaning the column.

If a guard column is used with an analytical column(s), first remove the guard column and check the performance of the analytical column alone. If the problem is solved, most likely the cause was from the guard column. In this case, clean the guard column.

If the problem remains even after removing the guard column, clean both guard and analytical columns. Make sure to clean the guard and the analytical columns separately. In case multiple number of analytical columns are used together, wash them separately. During the column cleaning, disconnect the detector and collect the washing solution directly from the column outlet into a waste container (i.e., do not let the solution go through the detector).

If the column performance does not improve (recover) after performing the column cleaning, please replace the column with a new one.

<Cleaning method>

(1) Insoluble components that block the column inlet may be removed by reversing the flow direction, i.e., introducing the eluent from the column outlet, with flow rate at less than half of the recommended flow rate.

(2) Follow below cleaning steps for adsorbing components. For an efficient cleaning, reverse the flow direction. Set the flow rate at less than 1.5 mL/min. Recommended solvent volume to introduce is 5 to 10 times of the column volume.

Method 1: Adsorption of hydrophobic compounds (when using aqueous eluent)
Introduce the eluent with a high polar organic solvent (acetonitrile or methanol).

Method 2: Adsorption of ionic compounds
Introduce the eluent with a higher salt concentration.

Attention! · Keep the organic solvent and salt concentrations within the concentrations stated in 4.2 List of Applicable Solvents.

· Keeping the washing solution in the column for a long time will lead to column deterioration. Please replace the washing solution with the eluent immediately after cleaning.

8. Column Storage

Remove the column from HPLC system after replacing the in-column solvent with the initial shipping solvent. Securely tighten the end caps and store the column at a location with stable temperature (a cool and dark space is recommended). Refer to section 7.3 Solvent Exchange for how to replace the eluent.

Attention! · Never allow inside the column to dry. It can damage the column.

9. Column Inspection

Please refer to the inspection method described in the CoA. At Shodex, “half width method” is adopted for the calculation of plate count and “asymmetry factor (Fas)” is adopted for the calculation of peak symmetry. Please refer to the Shodex website for the detail: https://www.shodex.com/en/da/07.html

Attention!

· Plate count and Fas values change significantly depend on samples and/or analysis conditions being used. To check the initial column condition, please make sure to use the same sample and the analysis condition mentioned in the CoA.

10. Additional Warnings

(1) Do not remove end fittings.

(2) Do not make a strong impact on the column. Do not drop or hit the column on a hard surface.

(3) Please follow a proper waste disposal method specified by your local regulations.

Please refer to the Shodex website (https://www.shodex.com/) for product details and their applications. For additional assistance, contact the distributor from whom you purchased the column or contact your regional Shodex support office (https://www.shodex.com/en/support_office/list).