Operation Manual
Shodex SUGAR KS-800 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 SUGAR KS-800 series is high performance chromatography columns which is specifically designed for separation and analysis of sugars. A strong acid cation exchange type packing gel, prepared from a rigid resin of styrene divinylbenzene copolymer provides a mixed separation mode. Smaller sugars, such as mono and disaccharides, are separated by a combination of ligand exchange and size exclusion modes. On the other hand, larger sugars, such as oligo saccharides and polysaccharides, are separated by size exclusion mode, and thus it is suitable for molecular weight analysis. Columns with different exclusion limits are available (see below). Please select the columns by comparing the molecular weight ranges of your interests and the exclusion limits.

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*
		I.D.	Length			
F6378010	**SUGAR KS-801**	8.0	300	6	≥ 17,000	1,000
F6378020	**SUGAR KS-802**	8.0	300	6	≥ 17,000	10,000
F6378025	**SUGAR KS-803**	8.0	300	6	≥ 17,000	50,000
F6378035	**SUGAR KS-804**	8.0	300	7	≥ 17,000	400,000
F6700020	**SUGAR KS-G 6B**	6.0	50	10	(Guard Column)	-

*Reference value only / Measured with pullulan

Base Material : Spherical porous particles of styrene divinylbenzene copolymer modified with sulfo group (counter ion: Na^+)

Column Housing : SUS-316

Screw Type : Internally-threaded type No.10-32 UNF

Shipping Solvent : Water

4. Usable Conditions

Product Name	Flow Rate (mL/min)		Maximum Pressure (MPa/column)	Temperature (°C)	
	Recommended	Maximum		Recommended*	Maximum
SUGAR KS-801	0.5 - 1.0	1.5	5.0	50 - 85	85
SUGAR KS-802					
SUGAR KS-803					
SUGAR KS-804					
SUGAR KS-G 6B		-	-		

* Increased temperature slows down the formation of sugar anomers, and thus prevents peak splitting.

Usable solvents are listed below.

(1) The standard eluent is water.

(2) Aqueous solutions of sodium chloride or sodium nitrate salts can be used instead of water. Keep the pH of solution between 3 and 7. For the analysis of samples containing large amount of heavy metals, addition of 10 to 50 µg/mL EDTA-4Na will prevent the lowering of column performance.

(2) Up to 20 % (v/v) acetonitrile and ethanol are usable for KS-801 to KS-803 and up to 50 % (v/v) are usable for KS-804.

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.
- Do not use salts other than sodium salts. Use of other salts may replace the sodium ion attached to the sulfo functional group with other cation and may deteriorate the column.
- When using a mixture of aqueous salt solution and organic solvent, make sure there is no precipitation of salt.
- 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 solvent of guaranteed quality, which can be used without problems in HPLC is recommended. If organic solvent 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. For gradient elution, samples are recommended to be dissolved or diluted using the eluent used at the beginning of the gradient method.

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

(3) Suggested injection volume for monosaccharides to oligosaccharides is less than 20 μL and for polysaccharides is between 50 and 100 μL.

(4) 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 %

(5) When analyzing an acidic or a basic sample, make sure to neutralize the sample prior to the injection.

(6) When a sample contains cations other than sodium ions (the pretreated sample after neutralization), remove them by using a cation exchange resin.

(7) When a sample contains protein or lipid, make sure to remove them prior to the injection. Proteins may be removed by ultrafiltration or by adding acid or acetonitrile. If using acid to remove the protein, neutralize the sample before injection. If using acetonitrile, make sure that the final acetonitrile concentration is less than 20 % (v/v).

(8) When a sample contains a large amount of organic acids, use an OH-form anion exchange resin to remove them.

(9) When a sample contains hydrophobic substances or surfactants, use a reversed-phase solid phase extraction to remove them.

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.

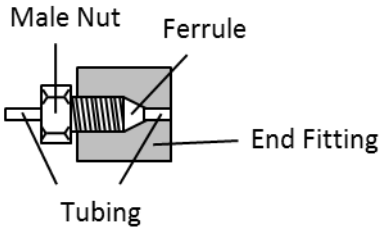
Attention! · If the eluent left in the HPLC system is not compatible with the column to be used, it may damage the column.

· 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 at 0.2 to 0.3 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 temperature.

(4) At the end of analysis, reduce the flow rate to 0.2 to 0.3 mL/min and turn the column oven off. Make sure that the column oven temperature is at room temperature before turning off the pump.

(5) 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.

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 0.2 to 0.3 mL/min flow rate. A rapid increase in pressure can damage the column.

· 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. This may create an empty space in the column and may deteriorate the column.

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 column temperature at 50 °C and start the system at 0.2 to 0.3 mL/min flow rate. Recommended solvent volume to introduce at each step is 3 to 5 times of the column volume.

Attention! · Frequent eluent replacement may damage the column, and thus not recommended.

7.4 Column Cleaning (Regeneration)

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 (regenerating) 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 (regenerate) the guard column.

If the problem remains even after removing the guard column, clean (regenerate) both guard and analytical columns. Make sure to clean (regenerate) the guard and the analytical columns separately. In case multiple number of analytical columns are used together, wash (regenerate) them separately. During the column cleaning (regeneration), 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 (regeneration), please replace the column with a new one.

<Cleaning method>

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.

<Regeneration Method>

Presence of cations other than sodium ion in the sample may replace the sodium ion attached to the sulfo functional group. This can change the separation patterns and peak shapes. Following regeneration procedure reattaches the sodium ions and may resume the column performance.

Method 1: Set the column temperature at 50 °C and the flow rate at 0.5 mL/min. Introduce 50 mL of 0.2-M aqueous sodium hydroxide solution. Wash the column thoroughly with water.

Method 2: Set the column temperature at 50 °C and the flow rate at 0.5 mL/min. Intorudce water and inject 40 µL of 1-M aqueous sodium hydroxide solution.

Attention!

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

· Strong alkaline solvents such as aqueous sodium hydroxide solution can damage the detector cell. Do not connect the detector while regenerating the column and collect the elute directly from the column outlet to a waste container.

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).