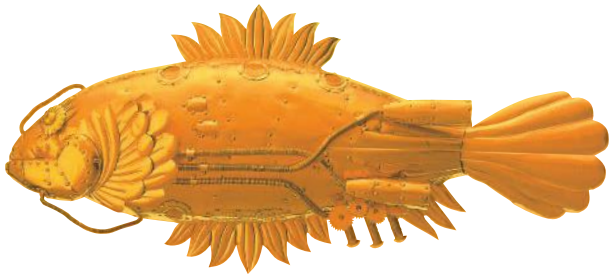
Shodex™



HPLC Columns

MANUAL

HILICpak VG-50

SHOWA DENKO EUROPE

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

Shodex HPLC Columns
Europe, Middle East, Africa, Russia

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Operation Manual
Shodex™ HILICpak™ VG-50

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

1. Introduction

The Shodex HILICpak VG series is packed with a polymeric gel containing chemically bonded amino groups and hydrophilic groups. Under the chromatographic conditions generally employed for the silica-base amino columns, the VG column provides equal resolution and far greater reproducibility.

2. Handling Instructions <Important>

Caution! Please consult the MSDS for the reagents and solvents used with the columns for health concerns caused by acute exposure due to leakage from the column or adjoining tubing.

Attention! Use the column within the regular range of flow rate, pressure and temperature. The column performance may deteriorate when it is handled beyond the permissible range even for a short time. See the Section 4. Usable Conditions for the permissible ranges.

3. Specifications

Product code	Product name	Size (mm)		Particle size (μm)	Plate number (per column)
		ID	Length		
F7630200	VG-50 4D	4.6	150	5	>5,500
F7630100	VG-50 4E	4.6	250		>7,500
F6711100	VG-50G 4A	4.6	10		(Guard column)

Packing material:	Polyvinyl alcohol.
Functional group:	Amino, hydrophilic group.
Column material:	Stainless steel (SUS 316).
Screw type:	Unified Thread Standard No. 10-32 UNF.
Shipping solvent:	Acetonitrile / H_2O = 80 / 20.

4. Usable conditions

Product name	Flow rate (mL/min)		Max pressure (MPa)	pH range	Temperature range (°C)
	Standard	Max			
VG-50 4D	0.5-1.0	1.5	10	2-13	4-60
VG-50 4E	0.5-1.0	1.5	15		
VG-50G 4A	0.5-1.0	1.5	-		

Attention! High-temperature operation may result in the generation of an air bubble, necessitating degassing. Low-temperature operation may require reduced flow rates, because of increased eluent viscosity.

5. System clean up

Clean up the LC system including the injector and the sample loop by switching the valve, and flow the eluent before column installation.

Attention! The previous eluent used for analyses in the system may damage the column, if it is not compatible with the column.

Attention! When replacing nonpolar solvent to water, replace first with methanol and then replace with water. When replacing buffer solution to acetonitrile/water, replace first with 100% water and then replace with eluent. Substances adsorbed in the pump and tubing may not be compatible with the column.

6. Column Installation

Install and use the VG column with the flow through the column matching the flow direction arrow on the column tag. Set the flow rate at 0.3mL/min, and connect the column. Flow at the low rate until the column temperature increases to the setting temperature and then increase the flow rate to the analytical condition.

Attention! The column should always be installed in the manner above, for safe and effective operation.

7. Eluents

	HILIC mode
Operational solvents	Aqueous solutions of acetonitrile (or methanol[*1]) at any ratio, is used as eluent.[*2] Any salts[*3] soluble in the solution above are available. [*1] With aqueous methanol solutions, the flow rate should be lower than normal, due to relatively high viscosity. [*2] Other organic solvents are not guaranteed as the eluent. [*3] Maximum concentration of the buffer depends on the ratio of acetonitrile (or methanol). Please note any precipitation of salt when using buffer!
Eluent modes	Isocratic, gradient, or stepwise elution.

Attention! Filter the eluent with a membrane filter (0.45μm) to prevent chromatogram noise and column performance deterioration by small particles or undissolved materials.

Attention! When the shipping solvent (Acetonitrile/ H2O = 80 / 20) is replaced with a buffer solution, please flow 100% water to the column before flowing the buffer solution to avoid the precipitation of the buffer. When replacing buffer solution to acetonitrile solution, also flow 100% water to the column before flowing acetonitrile solution.

Attention! Column equilibration for using acetonitrile/buffer eluent.

Anions in the buffer interact with the amino functional groups of VG packing gel. The reproducibility of the analysis will not be attained until the ionic equilibrium is attained between the anions and the amino groups. In such case, the equilibration time may be shortened by the operation below. This equilibrium operation is not needed when acetonitrile/water or buffer eluents are used.

	HILIC mode	
Eluent	Acetonitrile / buffer	Acetonitrile / water
Solution	The same buffer without acetonitrile	Unnecessary
Quantity	10 to 20 times the column volume	
Flow rate	Lower than half of the normal flow rate	

Attention! When the eluent conditions change from buffer solution to acetonitrile/water, please use the standard cleaning method. See Section 9.Column Cleaning.

8. Sample preparation

		HILIC mode	
Sample preparation	Solid sample	Eluent: Acetonitrile/ water	Dissolve sample in water or the eluent, and add acetonitrile to obtain 50% or higher acetonitrile aqueous solution.
		Eluent: Acetonitrile/ buffer	Dissolve sample in the buffer or the eluent; and add acetonitrile to obtain 50% or higher acetonitrile aqueous solution.
	Aqueous sample	Add acetonitrile to obtain 50% or higher acetonitrile aqueous solution.	
Injection volume		20 µl or less for analytical columns.	

Optimum separation efficiency is generally obtained with sample matrix similar to the eluent composition. Use acetonitrile in the sample matrix whenever possible.

Attention! Filter the sample with a membrane filter (0.45µm) to prevent blockage.

Attention! In case of gradient condition, dilute the sample with the initial eluent.

9. Column cleaning

Elution characteristics of a column may change considerably after long, repeated usage, due to the accumulation of pollution components on the packing material, for example metal ions from the LC system or the sample. The cleaning procedures outlined below may be used.

Clean the guard column and analytical column separately by flowing the cleaning solution in the opposite direction of the arrow on the column tag. The applied flow rate should be lower than 0.5 mL/min.

Cleaning method 1 (Standard cleaning)

Cleaning solution		VG-50 4D	VG-50 4E	VG-50G 4A
1	H_2O	6min	10min	1min
2	0.1M NaOH $_{aq.}$	75min	120min	5min
3	H_2O	12min	20min	2min
4	Eluent	40min	60min	3min

Cleaning method 2 (Cleaning metal ions)

Cleaning solution		VG-50 4D	VG-50 4E	VG-50G 4A
1	H_2O	6min	10min	1min
2	0.1M $NaOH_3$ $_{aq.}$	75min	120min	5min

3	H_2O	6min	10min	1min
4	0.1M NaOH $_{aq.}$	75min	120min	5min
5	H_2O	12min	20min	2min
6	Eluent	40min	60min	3min

Attention! After cleaning the column by alkaline, in some substances, there are cases that the theoretical plate number becomes higher than the initial plate number. In such cases, the plate number will get back to the initial level gradually by running the eluent or water.

Attention! Complete the washing procedure steps continuously. Do not store the column with either acid or alkaline solution as it will advance the deterioration of the column.

Attention! Flow the alkaline waste into a separate bottle without flowing to the detector. The strong alkaline solution may cause damage to the cell of the detector.

10. Column Inspection

Column inspection method is described in Certificate of Analysis (CoA).

Attention! Assessment of the column's functional integrity prior to initial and later use by standardized comparison of the certificate of analysis is recommended. Please see CoA for the detailed analysis conditions and sample preparation.

11. Attention

1) Do not remove the end fittings of the column to prevent performance deterioration and for safety reasons.

2) Do not make a strong impact on the column: such as hitting or dropping on the floor.

3) Replace the solvent in the LC system with the eluent to be used before connecting the column.

4) Connect the column so that the flow direction corresponds to the arrow mark on the tag.

5) When the column is not used for two weeks or more, replace the in-column solvent with the shipping solvent, remove it from the LC system, close each end with a stopper, and store it at controlled room temperature.

6) Contact Shodex website (http://www.shodex.com/) or Shodex partners regarding product and analysis applications.

12. Warranty

1) Showa Denko K. K. warrants that the Shodex Column, at the time of delivery to the user, will conform to the specification of the attached Certificate of Analysis, if the Shodex Column is used in accordance with the operating manual. The foregoing warranty is exclusive and is in lieu of all other warranties with respect to the Shodex Column, whether written, oral, implied, statutory or otherwise. No warranties by Showa Denko K. K. are implied or otherwise created, including, but not limited to, the warranty of merchantability and fitness for particular purposes.

2) Any claim of inconformity to the specification must be notified to Showa Denko K.K. within ten (10) days after delivery to the user. User's exclusive remedy and Showa Denko K.K.'s exclusive liability for such claim are limited to the replacement of the Shodex Column in question. In no event is Showa Denko K.K. liable for any indirect, incidental or consequential damage arising out of in connection with the Shodex Instrument, whether or not such damage is allegedly based on breach of warranty, negligence or otherwise.

3) No warranty is made in any of the following cases:

(1) If the Shodex Column is not used in accordance with the operating manual.

(2) If the Shodex Column is remodeled by anyone other than person or firm designated by Showa Denko K.K.

(3) If the Shodex Column is resold by the user without giving prior written notice to Showa Denko K.K.

(4) If the performance of the Shodex Column is not conform to the specification of the attached Certificate of Analysis due to any of the reasons below:

a) Computer virus

b) Impurities contained in the sample, reagent, gas air or cooling water provided by the user

c) Breakdown or malfunction of equipment, apparatus or component used in combination with the Shodex Column

d) Force majeure such as fire, earthquake, flood, other natural disaster, rime, riot, act of terrorism, war or radioactive contamination

4) In no event is Showa Denko K.K. liable for (i) the results of analyses or preparations using the Shodex Column or any portion of the same, including, but not limited to, the reliability, accuracy, efficacy and safety of said results, and (ii) the occupational hazard in the use of the Shodex Column, whether or not such use is made in accordance with the attached Conditions for use.

5) The Shodex instrument is for laboratory use only. It must not be used for clinical diagnosis. Showa Denko K.K. is not liable for any use of the Shodex Instrument except laboratory use.