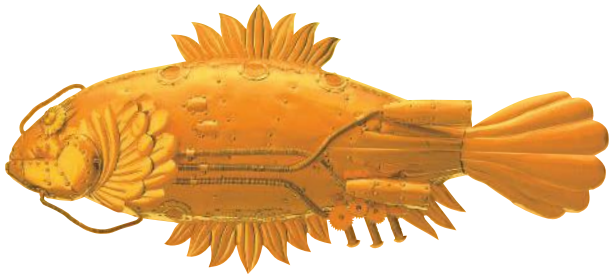
Shodex™



HPLC Columns

MANUAL

ORpak CRX-853

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™ ORpak™ CRX-853

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

1. Introduction

Shodex ORpak CRX-853 is designed to be used for the separation of chiral compounds by HPLC. The column is suitable for the analysis of compounds, which make a complex with copper, such as amino acids and acid hydroxyl groups. The ORpak CRX-853 column is packed with polymer-based gels bonded with L-amino acid derivatives as the ligand.

2. Specifications

Column type	Ligand	Particle size (micron)	Pore size (Angstrom)	Size (mm) IDxLength
ORpak CRX-853	L-amino acid derivative	6	100	8.0x50
ORpak CRX-G	Guard column	-	-	4.6x10

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

In-column Solvent: 0.25mM $CuSO_4$-aq.

Column material: SUS-316.

Packing material: Polyhydroxymethacrylate gel.

Max. pressure: 1.5 MPa.

Max. flow rate: 2.0 mL/min.

Usable temperature: 4 to 60 deg-C.

3. Eluent

0.05 to 10 mM $CuSO_4$ or Cu $(CH_3COO)^2$ which contain copper ion is used as eluent of CRX-853. Amino acids are eluted earlier when the concentration of CuS04 is higher.

Almost compounds are separated well under the concentration of $CuSO_4$ between 0.25 mM and 1 mM.

Addition of organic solvents, such as methanol and acetonitrile, to the eluent is recommended to decrease the hydrophobic absorption. Though in that case hydrophobic compounds are eluted earlier, the resolution is not good.

4. Preparation of eluent

1) Remove extraneous matter by passing the eluent through 0.45um filter.

Use of the disposable filter unit Shodex DT is recommended.

2) Thoroughly degas the eluent, by subjecting it and ultrasonic vibration and simultaneous heating or pressure reduction with an aspirator. Use of solvent degassing devices of Shodex DBGAS KT series will facilitate the degassing work.

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 that is to be used as the eluent.

When a solvent containing salt is remaining in the liquid chromatograph be careful not to allow the salt to remain in the liquid chromatograph and block the flow path.

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

Caution! It is recommended to start from a slow flow rate. And, after confirming that the pressure does not exceed the maximum flow rate, the flow rate can be raised at the flow rate to be used.

Note: In case of the separation of optical isomers it is recommended to use slower flow rate for better separation.

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 chromatograph.

Note: In case of the separation of optical isomers it is recommended to use lower temperature for better separation.

6. Pre-treatment of sample

1) The sample should be dissolved in the eluent to be used.

2) Remove extraneous matter by passing the eluent through a 0.45um filter.

Use of the disposable filter unit Shodex DT is recommended.

7. Guard column

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

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

8. Safekeeping

1) When the column is heated after completing analysis, keep pumping the eluent at a flow rate of 0.5ml/min. 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 on the following day.

3) In the case of 3 or more days of suspension of chromatography in which a saline solution was used as eluent replace the eluent with 50mM phosphate buffer (pH 5), setting the flow rate at 0.5ml/min.

4) In the case of its suspension over a long period of time take the same action as in 3) above and dismount the column from the chromatograph. Then, blank off both ends of the column.

5) Package it as delivered from the manufacturer.

6) Store it in a room that has little temperature fluctuation.

9. Trouble shooting

Table. 1 below gives troubles likely to occur during use of the column and the corrective actions to take. It is not guaranteed that the corrective actions as given in the table always solve the trouble. Therefore, after taking the actions, check the column resolution.

Please note that removal of the end fittings will allow the air or other extraneous substances to enter the column probably to deteriorate the column performance.

Trouble	Cause	Corrective action
Column pressure increase.	Plugged endfitting.	Reverse the column on chromatograph and pass the eluent through it for one hour.
	Inclusion of extraneous substances in packing.	Irreparable.
Rapid deterioration of resolution.	Void produced in the upstream end of column.	Irreparable.
	Liquid flow disturbance caused	Reverse the column on chromatograph and

	by extraneous matter clogging endfitting.	pass the eluent through it for one hour.
No elution of sample.	Sample adsorbed.	Change the separation conditions.
	Malfunctioning detector.	Check the detector.