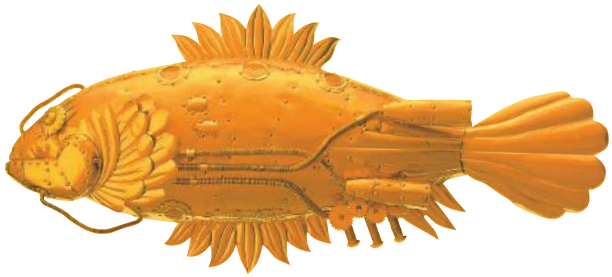
Shodex™



HPLC Columns

MANUAL

HIC PH-814

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™ HIC PH-814

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

1. Introduction

Filled with the packing prepared by bonding phenyl groups to hydrophilic and totally porous gels. Shodex HIC PH-814 column is designed for use in high-speed hydrophobic chromatography.

Being polymeric, the column packing is more advantageous than silicic packings in that it can be washed with a solution of a higher pH.

The column is especially suited for separation of proteins and enzymes.

2. Specifications

1) Size: ID, 8mm; length, 75 mm.

2) End fittings: Internally-threaded type, No. 10-32 UNF.

3) Packing: Hydrophilic polymethacrylate gels to which phenyl groups are bonded.

4) Plate number: 2000 minimum*)

5) In-column eluent: Ion-exchange water

NOTE:

a) See section 7 below for calculation of the plate number.

b) Each lot of the packing is tested for capability of separating proteins.

3. Mobile phase

Hydrophobic chromatography is a process of separating a specimen by hydrophobic interaction between the specimen and the packing in the mobile phase.

Generally speaking, a specimen is retained by a packing in the mobile phase that is high in salt concentration and is eluted by decreasing the salt concentration. The salt used in the mobile phase is normally ammonium sulfate or sodium sulfate.

Urea, guanidine hydrochloride or a surfactant is used as modifier.

Caution!

a) Control the pH of the mobile phase to a range of 2.0~12.0.

b) Keep the salt concentration in the mobile phase at 3M maximum.

c) Keep an addition of a polar organic solvent at 50% maximum.

4. Filtration and degassing of mobile phase

1) Pass the mobile phase through a 0.45 μm membrane filter to remove extraneous and insoluble substances.

2) The mobile phase has to be degassed. For example, upon heating it in a hot water bath of ca.50°C, place it in an ultrasonic bath and degas it with an aspirator.

Use of solvent degassing device of Shodex DEGAS KT series will facilitate the degassing work.

5. Column mounting

1) Before mounting the column on a liquid chromatograph, throughly replace the solvent in the chromatograph with the mobile phase to be used subsequently.

2) Set the flow rate of the pump at 1 ml/min.

Caution!

a) Do not increase the flow rate to more than 1.5 ml/min.

b) The maximum column pressure per column is 25 kg/cm². Any higher pressure will deteriorate column performance.

3) Connect the column to the chromatograph in such a way that the flow mark on the column will point to the flow direction, and then start the pump.

Caution!

Do not let the air get into the column while connecting the column to the chromatograph.

4) Heat the column as required.

Caution!

The column temperature must be kept in a range of 10 to 50 °C.

6. Pretreatment of specimen

1) Dissolve the specimen, if possible, in the mobile phase to be used. In gradient elution, dissolve it in the initial mobile phase. If it does not dissolve, gradually decrease the salt

concentration until it does satisfactorily. Care must be exercised, however, so as not to decrease the salt concentration unnecessarily.

2) Pass the specimen through a 0.45 µm membrane filter to remove insoluble substances.

NOTE: Use of the disposable filter unit Shodex DT ED-03, 13 or 25 is recommended.

Caution! The specimen must be adjusted to bring its salt concentration and pH as close to those of the mobile phase as possible. Also, the polar organic solvent content of the sample solution must be compatible with the mobile phase.

Do not inject the specimen in large quantities without observing said caution.

7. Dismounting and storage

1) When the column is heated, reduce the flow rate to 0.5 ml/min and stop heating. Keep flowing the mobile phase into the column until it cools down to room temperature.

Caution! Do not dismount the column before it cools down to room temperature; otherwise, the air will enter the column to deteriorate its performance.

2) Stop the pump and leave the column on the chromatograph, if it is to be reused 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 mobile phase, replace the mobile phase with an ion-exchange water, setting the flow rate at 0.5ml/min maximum.

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 and store it in a place where temperature does not markedly fluctuate.

Caution! Do not allow the column temperature to go below 0°C; otherwise the column will freeze to deteriorate its performance.

8. Calculation of plate number

The plate number is calculated under the following conditions.

1) Specimen: Aqueous 0.5% acetone solution

2) Injection volume: 20 µQ

3) Mobile phase: Ion-exchange water

4) Flow rate: 1 ml/min

5) Detector: UV detector (280 nm)

6) Calculation formula: $N = 5.54 (t_R/W) 2$

Where N = Plate number

t_R = Retention time (min)

W = Half value width (min)

9. Troubleshooting

Table 1 below gives troubles likely to occur during use of the column and the corrective actions to take. After taking the corrective actions as given in the table, check the column resolution. The column performance can sometimes be restored. Please note that removal of the end fittings will allow the air or other extraneous substances to deteriorate the column performance.

Table 1. Troubleshooting

Trouble	Cause	Corrective Action
1. Column pressure increase.	1-1. Plugged end fitting.	1-1-1. Reverse the column on the chromatograph and pass the mobile phase through it at the rate of 0.5 ml/min for one hour. 1-1-2. Inject several times 1 or 2 ml of aqueous 0.1N sodium hydroxide with an injector equipped with a 2 ml loop. 1-1-3. Inject several times 1 or 2 ml of aqueous 30% acetic acid with an injector equipped with a 2 ml loop. 1-1-4. Replace the end fitting
	1-2. Inclusion of extraneous substances in packing.	1-2-1. Take the same action as given in 1-1-2 or 1-1-3 above.
2. Rapid deterioration of resolution.	2-1. Void produced in the upstream end of column.	2-1-1. Irreparable.
	2-2. Liquid flow disturbance caused by extraneous matter cologging end fitting.	2-2-1. Remove and wash end fitting in ultrasonic bath.
	2-3. Accumulation of adsorbed substances.	2-3-1. Reverse the column on chromatograph and take the same action in 1-1-2 or 1-1-3 with flow rate set at 0.5ml/min.
3. No elution of specimen.	3-1. Specimen adsorbed.	3-1-1. Change the separation conditions 3-1-2. Take the same action as given in 2-3-1 above.
	3-2. Malfunctioning detector.	3-2-1. Check the detector.