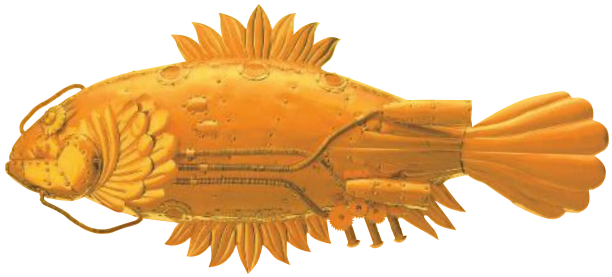
Shodex™



HPLC Columns

MANUAL

IEC series
(QA, DEAE, SP, CM)

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™ IEC series

(QA-825, DEAE-825, SP-825, CM-825)

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

1. Introduction

Packed with an ion-exchange resin made by bonding ion-exchange group to hydrophilic and totally porous gels, Shodex IEC series columns are designed for use in high-speed ion-exchange chromatography.

Being polymeric, the column packing is more advantageous than silicic packings in that it can be used in a wider pH range.

Those columns are well suited for separation of proteins, peptides, and the components of nucleic acids among others.

2. Specifications

Grade	Type	Ion-exchange group	Ion-exchange capacity (meq/g)	Plate/column[a)]
IEC QA-825	Strongly base	Quaternary ammonium	0.45	2000 min
IEC DEAE-825	Weakly base	Diethylamino ethyl	0.60	2000 min
IEC SP-825	Strongly acid	Sulfopropyl	0.40	2000 min
IEC CM-825	Weakly acid	Carboxymethyl-	0.40	2000 min

NOTE:

a) See Section 8 below for calculation of the plate number.

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

1) Size: 8mmID x 75mmL

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

3) In-Column Liquid: 50mM Na_2SO_4

3. Eluent

The eluent buffer must be high in buffering capability for a given eluent pH and the buffering ions must have the same electric charge as the ion-exchanger.

The following Table 1 gives some examples of such buffers.

Table 1. Recommendable buffers.

pH	QA-825, DEAE-825	SP-825, CM-825
6	20mM piperazine HCl	20mM Na malonate
7	20mM Bis-Tris propanol HCl	20mM Na phosphate
7.5	20mM Tris HCl	20mM Na phosphate
8	20mM Tris HCl	20mM HEPES*
9	20mM Ethanolamine HCl	
10	20mM 1,3-Diaminopropane HCl	

* 4-(2-Hydroxyethyl)-1- piperazine ethanesulfonic acid

Generally, the eluent pH is higher than the isoelectric point of the specimen in the case of QA-825 and DEAE-825 column and lower the case of SP-825 and CM-825.

CAUTION!

1) Each column must be used in a pH range of 2 to 12.

2) Total concentration of the salts in the eluent is usually in a range of 20mM to 1M.

3) Water-soluble organic solvents, such as ethylene glycol and 2-Propanol, must not be added in the quantity of more than 20%.

4) Proteins are generally separated by gradient elution in which the ionic strength or pH of the eluent is changed for their separation.

Normally, however, only the ionic strength is changed or increased for the separation while the pH is kept at a given level because it is difficult to change the pH. Almost all proteins are eluted from the column by increasing the salt concentration in a 0.02 to 0.05M buffer from 0 to 0.5M.

4. Filtration and degassing of eluent

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

2) The eluent has to be thoroughly 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. Degassing is especially important when low-pressure gradient elution is performed because bubbles are more likely to from in the valves of the gradient system.

Use of solvent degassing device will facilitate the degassing work.

5. Column mounting

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

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

CAUTION!

1) Do not increase the flow rate to more than 1.5mL/min.

2) The maximum column pressure per column is 4 MPa for the QA-825 column or 2 MPa for the DEAE-825, SP-825, CM-825.

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

4) In gradient elution, equilibrate the column beforehand according to the following procedure.

a) Pass 10mL of buffer A (low ionic strength) through the column.

b) Pass 40mL of buffer B (high ionic strength) for replacement with right counter ions.

c) Heat the column as required.

NOTE:

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 eluent to be used.

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

NOTE: Use of the disposable filter unit is recommended.

CAUTION!

a) The specimen must be adjusted to bring its salt concentration and pH as close to those of the eluent as possible. Also, the polar organic solvent content in the sample solution must compatible with the eluent.

b) Remove sebaceous substances completely from the specimen; otherwise, they will be adsorbed by the packing deteriorate the column performance.

7. Dismounting and storage

1) When the column is heated, reduce the flow to 0.5mL/min and stop heating. Keep flowing the eluent 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 dose 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 µL.

3) Eluent: 50mM sodium sulfate.

4) Flow rate: 1mL/min.

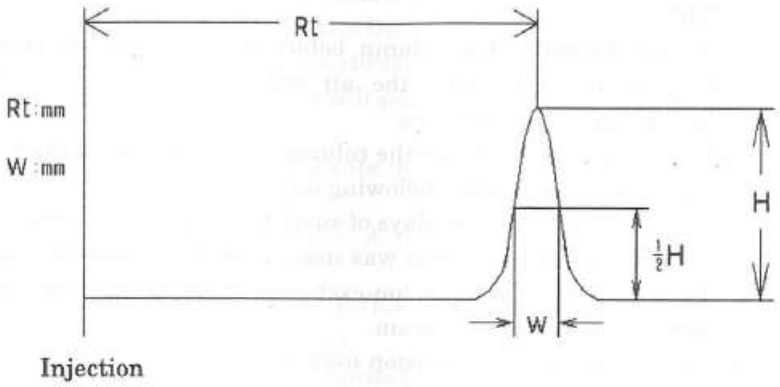
5) Detector: UV detector (280nm).

6) Calculation formula:

■ **Calculation of NTP**

$NTP=5.54\times(Rt/W)^2$

where NTP: Number of theoretical plate Rt: Retention time W: Peak half width



9. Troubleshooting

Table 2 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.

Table 2. Troubleshooting

Trouble	Cause/Corrective Action
1. Column pressure increase	1-1. Plugged end fitting. 1-1-1. Reverse the column on chromatograph and pass the eluent through it at the rate of 0.5mL/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 2mL loop. 1-1-3. Inject several times 1 or 2mL of aqueous 30% acetic acid with an injector equipped with a 2mL loop. 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 closing end fitting. 2-3. Accumulation of adsorbed substances. 2-4. 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 2-4 above. 3-2. Malfunctioning detector. 3-2-1. Check the detector.

10. 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.