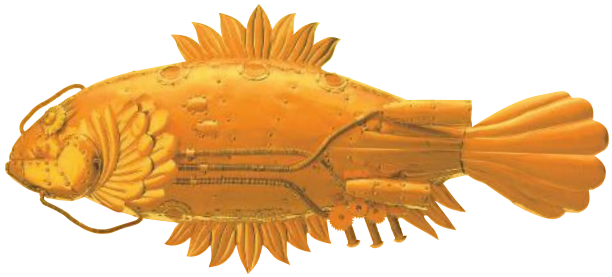
Shodex™



HPLC Columns

MANUAL

IEC 420N

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-N series

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

1. Introduction

The packed columns of Shodex IEC NP series are designed to be used for the separation of bio-polymers such as proteins and nucleic acids by ion-exchange mode.

The packing material is an ion-exchange resin made by bonding ion exchange group to hydrophilic and non-porous gels.

2. Specifications

Nomenclature	Type	Ion-exchange group	Ion-exchange capacity	Theoretical plates
IEC DEAE-420N	Weakly basic	Diethylaminoethyl	0.4 meq/g	500 min.
IEC SP-420N	Strongly acid	Sulfopropyl	0.3 meq/g	100 min.

Note: 1) Refer to Section 8 below for calculation of theoretical plate number.

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

Column size: 4.6mm I.D. x 35mm length.

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

In-column solvent: Ion-exchange water.

Column material: SUS316.

Packing material: Non-porous hydrophilic .

Max. temperature: 60 °C.

Max. pressure: 200 kg/cm².

Max. flow rate: 2.0 ml/min. (See the “Caution” below).

Caution! 1) Do not abruptly change the column pressure or the flow rate while the liquid chromatograph is in operation. Use a damper-equipped or pulseless pump to maintain the performance of the column at the designed level for a long period of time.

2) Check the column pressure from time to time and never allow the pressure to go above 200kg/cm².

3) Although the maximum flow rate is 2.0 ml/min, slower flow rate should be when highly viscous eluent is used or measurement temperature is low. Usually, use of the flow rate range of 1.0 to 1.5 ml/min. is recommended.

4) Tile temperature of the column should generally be between 0°C and 60°C. Avoid a temperature above 60° C.

5) Do not impact or bend the column.

6) Do not remove the endfittings of the column under any circumstances; otherwise, its performance will deteriorate.

3. Eluent

1) 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 gives some example of such buffers.

pH	DEAE-420N	SP-420N
6.0	20mM Piperazine HCl	20mM Na malonate
7.0	20mM Bis-Tris propane HCl	20mM Na phosphate
7.5	20mM Tris HCl	
8.0		20mM HEPES(*)
9.0	20mM Ethanolamine HCl	
10.0	20mM 1,3-Diaminopropane HCl	

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

Generally, the eluent pH is higher than the isoelectric point of the sample in the case of DEAE-420N and lower in the case of SP-420N.

Caution! ① Each column must be used in a pH range of 2.0 to 12.0.

② Total concentration of salt in the eluent should be lower than 1. 5M.

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

④ Use highly purified water and reagents for the preparation of eluent. Do not use eluent containing extraneous substances of small particle size or bacteria.
⑤ Prepare the eluent just before it is used. Do not use the eluent stored for a long time after the preparation.

4. 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.

If the liquid chromatograph is equipped with a device in which complete replacement of the solvent is not possible, e.g., a Bourdon pressure gauge, disassemble the device and wash it with the solvent that is to be used as the eluent.

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

3) Thoroughly degas the solvent that is to be used as the eluent, by subjecting it and ultrasonic vibration and simultaneous heating or pressure reduction with an aspirator.

Use of solvent degassing devices of Shodex DEGAS 11 series will facilitate the degassing work.

4) After replacing the solvent in the chromatograph, set the flow rate at 1.0ml/min.

5) 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.

6) Upon completion of the connection, start the pump, watching for any sudden change in the column pressure or the flow rate.

5. Pre-treatment of sample

1) Dissolve the sample in the same solvent that is to be used as the eluent. In gradient elution, dissolve it in the initial eluent.

To make the blank peaks as small as possible when a detector such as a differential refractometer is used, it is recommended that the sample be dissolved in the eluent obtained from the reservoir.

2) Remove extraneous matter or gels from the dissolved sample by passing it through a 0.45um filter.

Use of the disposable filter unit Shodex DT is recommended.

6. 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 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 eluent, replace the eluent 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.

5) Package it as delivered from the manufacturer.

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

7. Regenaration

In the event of adsorption of impurities to the packing material, they may be removed by one of the following procedures:

1) Inject 500ul of 0.1N NaOH for several times.

2) Inject 500ul of 50% formic acid water solution for several times.

3) Inject 500ul of 20% acetonitrile water solution or 20% dimethyl sulfoxide for several times.

4) Connect the column upside down.

8. Calibration

The column is calibrated by ensuring that the specified plate number is maintained.

Following are the conditions for calculation of the plate number:

1) IEC DEAE-420N

Eluent: 50mM phosphate buffer + 0.17M NaCl + 1% MeOH (pH 6. 0).

Sample: Oligodeoxyadenylic acid ($pd(A)_7$), 0.01 unit.

Flow rate: 1.0 ml/min.

Temperature: 30°C.

Detection: UV-260nm x0.04.

Chart speed: 1.0 cm/min.

2) IEC SP-420N

Eluent: 20mM Acetic acid buffer + 0.5M Na_2SO_4 (pH 5. 0).

Sample: 0.2% Glycyl-L-tyrosine.

Flow rate: 1.0 ml/min.

Temperature: Room temperature.

Detection: UV-280nm, x0.16.

Chart speed: 2.0cm/min.

3) Calculation formula

$N = 5.54 \times (t_R/W)^2$

where N: Theoretical plate number

t_R: Retention time

W: Peak half width