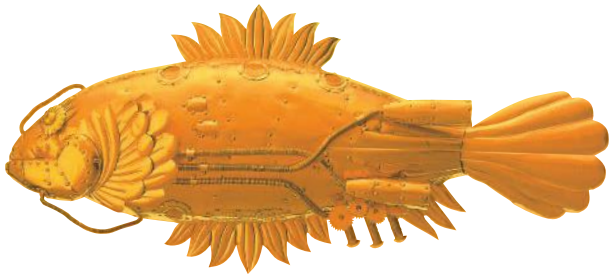
Shodex™



HPLC Columns

MANUAL

AXpak WA-624

Shodex HPLC Columns
Europe, Middle East, Africa, Russia

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

Operation Manual
Shodex™ AXpak™ WA-624

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

1. Introduction

Packed with an ion-exchange resin made by ion-exchange group to hydrophilic and totally porous gels. Shodex AXpak WA-624 column is designated for use in high-speed anion-exchange chromatography.

The column is well suited for separation of peptides and components of nucleic acids.

2. Specifications

Nomenclature	Column size (ID x length)	Theoretical plates
Shodex AXpak WA-624	6mm x 150 mm	1500 min.
Shodex AXpak WA-624P	4.6mm x 10mm	Precolumn

Solvent packed: 0.1M NaH_2P0_4 / CH_3CN = 80 / 20

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

Column material: SUS 316

Packing material: Weak anion exchanger

Ion exchange group: Dimethylaminolethyl

Ion exchange capacity: 1.2meq/g

Max. temperature: 60°C

Max. pressure: 25kg / cm²

Max. flow rate: 1.2ml/min

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) The temperature of the column should be between 4°C and 60°C.

3) Do not impact or bend the column.

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

5) Install precolumn, WA-624P immediately upstream of the main column to protect it from contamination by the sample.

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

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

3. Eluent

1) The pH of the eluent should be between 3.0 and 12.0

2) Maximum salt concentration of the eluent is 1.0M.

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

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) 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 KT series will facilitate the degassing work.

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

4) Connect the column to the chromatograph as that the arrow mark on the column will face downstream.

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

6) In gradient elution, equilibrate the column beforehand according to the following procedure:

① Pass 10ml of buffer A (low ionic strength) through the column.

② Pass 40ml of buffer B (high ionic strength) for replacement with right counter ions.

③ Heat the column as required.

5. Pre-treatment of sample

1) Dissolve the sample in the same solvent that is to be used as the 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 0.45µm filter.

Use of the disposable filter unit Shodex DT is recommended.

3) The pH, salt concentration and organic solvent concentration of sample solution should be as same as those of the eluent.

4) Remove sebaceous substances completely from the sample; otherwise, they will be adsorbed by the packing materials to deteriorate the column performance.

6. Safekeeping

1) After completing analysis, keep pumping the eluent at a flow rate of 0.5ml/min. until the column is cooled down to room temperature.

Caution! Do not dismount the column before it is cooled down to room temperature; otherwise, the air will enter into the column to deteriorate the column.

2) If the column is to be used in two or three days, the column ca be connected to the chromatograph as it is.

3) If the column is connected to the chromatograph for more than three days, the eluent in the chromatograph and column should be replaced with purified water setting the flow rate at 0.5ml/min. maximum.

4) If the column will not be used for a long period, replace the eluent according to the procedure in 3) and disconnect it from the chromatograph and cap both ends of the column to

prevent the eluent from leaking out. And, package it as delivered from the manufacturer and store it in a room that has little temperature fluctuation.

7. 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) Sample: Uridine 5-monophosphate
2) Injection volume: 20ul
3) Eluent: 0.1M NaH_2P0_4 pH3.0 / CH_3CN = 80/20
4) Flow rate: 1.0ml/min
5) Detector: UV-260nm
6) Chart speed: 1.5 to 2.0 cm/min
7) Detector sensitivity: The sensitivity must be so adjusted as to obtain a peak of 10 to 15 cm in height
8) Calculation formula: $N = 5.54 \times (t_R/W)^2$
   Where N: Theoretical plate number
   t_R: Retention time
   W: Peak half width