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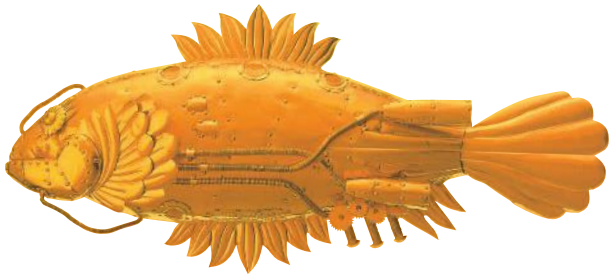

HPLC Columns

MANUAL

RSpak NN-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™ RSpak™ NN-814

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

1. Introduction

RSpak NN-814 is a high-performance HPLC column of which separation mode is a mixed mode of mainly partition and adsorption mode and partly cation exchange mode. Since the packing material has a little amount of cation exchange group, the column is suited to separate basic substances because the elution time of the basic substances are delayed by the cation exchange group. The column is suited for the separation of watersoluble substances such as nucleotides, nucleosides, nucleobases, watersoluble vitamins and amino acids.

2. Specifications

Nomenclature	Column size (ID x length)	Theoretical plates
Shodex RSpak NN-814	8mm x 250mm	8,000 min.
Shodex RSpak NN-814P	6mm x 50mm	Precolumn

Solvent packed: 0.1M NaH_2PO_4, pH 3.0 adjusted by phospholic acid.

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

Column material: SUS 316.

Packing material: Polyhydroxy methacrylate gel with a small amount of cation exchange group.

Max. temperature: 60 °C.

Max. pressure: 20kg/cm².

Max. flow rate: 1.5 ml/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 water to be used as the eluent should be newly distilled and deionized.

3) The temperature of the column should be between 10°C and 60°C.

4) Do not impact or bend the column.

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

6) Install precolumn, NN-810P 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.

3. Eluent

Following eluents can be used:

① Buffer.

② Buffer + salt.

③ Buffer + polar organic solvent.

④ Buffer + salt + polar organic solvent.

Separation of the sample can be controlled by changing eluent.

1) Water/polar organic solvent

The elution time of substances which have long alkyl chain or benzene ring is rather long. The elution time of such substances can be shorten by raising the concentration of polar organic solvent.

2) pH

In case of basic substances, the lower the eluent pH value, the faster the elution time.

3) Salt concentration

In case of basic substances, the higher the salt concentration of the eluent, the faster the elution time.

4) Column temperature

The elution time of ionic substances changes according to the temperature.

Caution! ① The eluent should be filtrated before being used. Especially, in case of the eluent in which salt is dissolved, it is important to filtrate the eluent.

② The maximum concentration of organic solvents, methanol, ethanol and acetonitrile is 50%.

③ The maximum concentration of salt is 0.5M.

④ The pH range of eluent should be 2.0 to 12.0. In case of eluent containing chlorine ions, the pH should be 4.0 or higher.

⑤ Strong acids, strong bases and perchloric acid should not be used.

⑥ When the eluent is replaced from the eluent containing no organic solvent to the eluent containing organic solvent, or reverse way, flow rate should be 0.5ml/min. or slower.

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.

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 solvent 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 polar organic solvent concentration of sample solution should be as same as those of the eluent.

4) Fats should be eliminated from the sample because they will be adsorbed by the packing materials.

6. Safekeeping

1) After completing analysis, keep pumping the eluent at a flow rate of 0.3 to 0.5ml/min. until the column is cooled down to room temperature.

2) If the column is to be used in two or three days, the column can 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 using the flow rate of 0.5ml/min. or slower.

4) If the column will not be used for a long period, replace the eluent according to the procedure in 3) and disconnect the column 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: Water containing 0.13% of uracil.

2) Injection volume: 10µl.

3) Eluent: 0.1M NaH_2PO_4 pH 3.0 adjusted with phospholic acid.

4) Flow rate: 1.0 ml/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 15cm 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