



MANUAL

RSpak KC-811



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[™] KC-811

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

1. Introduction

Designed for chromatography by a combination of ion exclusion and partition *I* adsorption, the Shodex RSpak KC-811 column is packed with ion-exchange resin gels of sulfonated rigid styrenedivinylbenzene co-polymer and demonstrates an excelling performance in separating low-molecular weight organic acids and water-soluble organics such as alcohols, aldehydes and nitriles.

2. Specifications

1) Nomenclature and number of theoretical plates

<u>Nomenclature</u>	Number of theoretical plate / 30cm ¹⁾
RSpak KC-811	>17,000
RSpak KC-G	Guard column

NOTE 1): Specimen, 5 μL of 3% acetic acid; mobile phase, 0.1% phosphoric acid; flow rate, 1mL/min; column temperature, 40°C.

2) Size: ID, 8mm; length, 300mm (exclusive of KC-G, which is 6mm in ID and 50mm in length.)

- 3) End fitting: Internally-threaded type, No.10-32 UNF.
- 4) Material: SUS-316
- 5) Packing: Strong cation-exchange resin gels.
- 6) In-column mobile phase: 0.1% phosphoric acid aqueous solution.
- 7) Maximum working pressure per column: 5MPa
- 8) Maximum working flow rate when column is heated to 40 85°C: 1.5mL/min.
- 9) Maximum working temperature: 85°C.

3. Mobile phase

1) An aqueous solution containing 0.1% phosphoric or perchloric acid is normally used as the mobile phase.

Caution! Do not use hydrochloric acid aqueous solution as the mobile phase since it corrodes the column.

2) Remove extraneous substances and insolubles from the mobile phase by passing it through a 0.45 μ m filter into a bottle and thoroughly degas it by, for instance, heating to about 60°C in a hot water bath and shaking in an ultrasonic washing vessel, with the ambient air pressure being simultaneously reduce by an aspirator. Use of solvent degassing device will facilitate the degassing work.

^{Note}: Molecules of highly hydrophobic specimens are too slow to flow out of the column due to their adsorption onto the packing. In such a case, add ethanol in an amount up to 20% to the mobile phase to reduce the adsorptivity.

4. Mounting and start-up

1) Before mounting the column on the liquid chromatograph, replace the solvent in the chromatograph with the mobile phase.

Caution! In replacing a water-insoluble organic solvent in the chromatograph, replace it first with a water-soluble organic solvent, such as acetone or ethanol, and then replace the water-soluble solvent with the mobile phase.

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

3) Connect the column to the chromatograph in such a way that the arrow mark on the column will point to the flow direction.

Caution! Do not let the air into the column during the connection.

4) Heat the column to 40- 50°C and start the pump.

Caution!

- i) Keep the column pressure below 5MPa per column; otherwise, the column will be ruined.
- ii) The Maximum working flow rate depends on the working temperature, as follows:

Working temperature (°C)	Maximum working flow rate
40 – 85	1.5 mL/min
30 – 39	1.0 mL/min
29 or below	0.5 mL/min

iii) Do not abruptly change the column pressure or flow rate.

iv) Do not heat the column higher than 85°C.

5. Pre-treatment of specimen

1) When the specimen is solid, dissolve it, using some of the degassed mobile phase.

^{Note}: Do not use any other solvent; otherwise, the solute sometimes settles in the mobile phase to plug the column.

2) When the specimen is liquid and contains an organic solvent such as alcohol, dilute it with the mobile phase so as to reduce concentration of the organic solvent to 20% maximum; otherwise, the column performance will deteriorate.

3) Pass the specimen through a 0.45 μ m filter to remove extraneous substances and insolubles. Use of the disposable filter unit is recommended.

4) When the specimen contains proteins, add 2 or 3 drops of distilled water in which sulfosalicylic acid is dissolved at the rate of 20g per deciliter, to 5mL of the specimen, stir and pass it through a 0.45μm filter to remove the proteins.

6. Dismounting and storage

1) Set the flow rate at 0.2mL/min, turn off the heater and let the column stand as is until it cools down to room temperature.

2) Stop the pump and dismount the column.

3) Cap both ends of the column, put it back in the same case in which it was delivered from the manufacturer, and store the case in a place where the temperature does not fluctuate more than ±5°C.

4

Caution!

- Do not remove the end fittings of the column; otherwise, its performance will deteriorate or the column may become unserviceable.
- ii) Do not let the in-column solvent freeze during storage; otherwise, the column will swell and become unserviceable.
- iii) Storage at abnormally high temperatures will also cause the swelling to render the column unserviceable.

7. Guard column

It is recommended that Guard column RSpak KC-G he installed immediately upstream of the column to protect the packing from contamination by dirt or readily adsorbable substances in the specimen, such as metal ions and amino compounds.

8. Performance test

The column performance can be checked by calculating the number of theoretical plates under the following conditions:

1) Specimen	3% Acetic acid
2) Injection amount	5 μL
3) Mobile phase	0.1% Phosphoric acid aqueous solution
4) Flow rate	1mL/min
5) Column temperature	40°C
6) Detector	High-sensitivity refractive index detector such as Shodex RI
7) Calculation formula	$NTP = 5.54 \text{ x} (Rt/W)^2$
	where NTP: Number of theoretical plate
	Rt: Retention time
	W: Peak half width



9. 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.