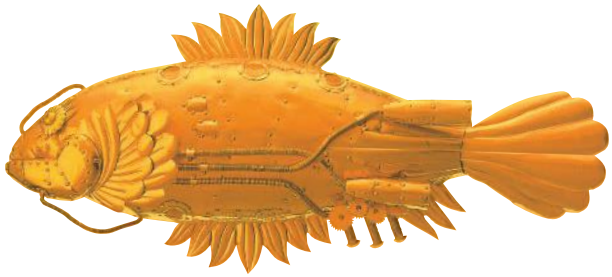
Shodex™



HPLC Columns

MANUAL

RSpak series

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™ series

(DS-613, 2013/DE-613, 2013/DM-614/DC-613, 2013)

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

1. Introduction

Rspak DS-613, DE-613, DM-614 and DC-613 are high-performance HPLC columns for adsorption/partition chromatography packed with totally porous spherical resin.

The columns are highly effective in analyzing samples such as pharmaceuticals, surfactants, food additives, saccharides, sugar alcohols, watersoluble vitamins, amino acids, nucleosides and organic acids.

2. Specifications

Nomenclature	Column size (I.D. x length)	Theoretical plates	pH range	Temp. range	Max. pressure	Max. flow rate
DS-613	6mmx150mm	6,000min.	2-12	RT-80°C	100kg/cm²	3.0ml/min
DE-613	6mmx150mm	7,000min.	2-12	RT-70°C	70kg/cm²	3.0ml/min
DM-614	6mmx150mm	4,000min.	2-10	RT-60°C	20kg/cm²	2.0ml/min
DC-613	6mmx150mm	5,000min.	2-14	40-85°C	50kg/cm²	3.0ml/min
DS-2013	20mmx300mm	7,000min.	2-12	RT-80°C	35kg/cm²	6.0ml/min
DE-2013	20mmx300mm	10,000min.	2-12	RT-70°C	50kg/cm²	20.0ml/min
DC-2013	20mmx300mm	7,000min.	2-14	40-85°C	50kg/cm²	20.0ml/min
DS-613P	4.6mmx50mm	Precolumn	2-10	RT-80°C		
DE-613P	4.6mmx50mm	Precolumn	2-12	RT-70°C		
DM-614P	4.6mmx50mm	Precolumn	2-10	RT-60°C		
DC-613P	4.6mmx50mm	Precolumn	2-14	40-85°C		
DS-2013P	8mmx50mm	Precolumn	2-12	RT-80°C		
DE-2013P	8mmx50mm	Precolumn	2-12	RT-70°C		
DC-2013P	8mmx50mm	Precolumn	2-14	40-85°C		

Packing material:
DS-613, DS-2013: Hydrophobic polystyrene gel.
DE-613, DE-2013: Polymethacrylate gel.
DM-614: Hydrophilic polyhydroxymethacrylate gel.

	DC-613, DC-2013:	Sulfonated polystyrene gel of counter ion Na^+ type.
Solvent packed:	DS-613, DS-2013:	Acetonitrile/THF/water = 40/30/30.
	DE-613, DE-2013:	Water.
	DM-614:	0.005M H_3PO_4.
	DC-613, DC-2013:	Acetonitrile/water = 70/30.
Endfitting:	Internally-threaded type, No. 10-32 UNF.	
Column material:	SUS 316	

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 desgned level for a long period of time.

In case of gradient, steep gradient sometime causes high pressure.

2) Do not impact or bend the column.

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

4) Install precolumn 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.

5) Do not use eluents or samples which react with the packing materials. For example, the gel of DC-613 reacts with strong basic substances or heavy metal ions.

3. How to use RSpak columns

1) The following factors affect the separation by RSpak columns:

i. Composition of the eluent.

ii. pH of the eluent.

iii. Concentration of salt.

iv. Column temperature

2) Chromatographic data, obtained by use of column packed with silicabase gels, are helpful in determining the eluent condition for the RSpak columns. Generally speaking, it is necessary that the eluent condition of RSpak columns should be a slightly faster condition in elution than that of the silica-base column.

3) In case the eluent is a mixture of water and a polar organic solvent, the sample can be best separated in most cases only by adjusting their mixing ratio. If the separation is still unsatisfactory, the pH of the eluent should be adjusted or ionization of the sample suppressed by adding acid or base to the eluent. In case the sample is acidic, its ionization must be suppressed by adding phosphoric acid or the like, and ammonium hydroxide or a similar substance in case it is basic.

4) It should be noted that in reverse-phase chromatography, if the sample has a long alkyl chain, the longer the chain, the slower the elution. Aromatic compounds are also slow in eluting, being firmly adsorbed to the packing.

5) The eluent must not contain salt in a quantity larger than 0.5M.

6) DS-613, DS-2013

The column is used principally in reverse-phase chromatography, in which the eluent is a mixed solution of water and a polar organic solvent or a mixed solution of the same components plus salt, acid or base. The column can also be used in normal-phase chromatography, in which case the eluent is mainly composed of n-hexane.

The following solvents are used as eluent.

Water:	In mixture with another solvent except chloroform and n-hexane.
THF:	In mixture with another solvent except chloroform.
Chloroform:	In mixture with another solvent except water and THF.
Methanol:	Singly or in mixture with another solvent.
Ethanol:	Singly or in mixture with another solvent.
Acetonitrile:	Singly or in mixture with another solvent.
n-Hexane:	Singly or in mixture with another solvent.

Caution! 1. Under no circumstances must water exceed 90% in the eluent, and THF, chloroform and toluene 50%, since they expand the packed gel.

2. The eluent must be in pH range of 2.0 to 12.0. If it contains any chlorine ions, its pH must be 4.0 or more.

The eluent condition of c18 -silica gel columns are helpful for the determination of the eluent condition.

7) DE-613, DE-2013

Water, methanol, ethanol, acetonitrile or their mixture is used as the eluent.

Caution! 1. Nonpolar organic solvents such as benzene, chloroform, n-hexane and TH F must not be used as the eluent.

2. The eluent must be in a pH range of 2.0 to 12.0. If it contains any chlorine ions, its pH must be 4.0 or more.

The eluent condition of C_8 and C_4-silica gel columns are helpful for the determination of the eluent condition.

8) DM-614

A mixture of water and a polar organic solvent such as acetonitrile, methanol and ethanol, or such mixture with salt added to it is most commonly used as the eluent.

An aqueous solution alone may also be used.

Caution! 1. Nonpolar organic solvents such as n-hexane, benzene and chloroform cannot be used as the eluent.

2. The pH value of the eluent must be in the range of 2.0 to 10.0. In case the eluent contains chlorine ions, the chlorine ions, the pH value must not be below 4.0.
3. Do not use any strong acids, strong bases or perchlorates as the eluent.

9) DC-613, DC-2013

The eluent consists of water and a polar organic solvent such as acetonitrile and ethanol can be used. Sodium salt is the only substance that can be added to the eluent.

A mixed solvent of 50/50 to 90/10 acetonitrile/water or ethanol/water is used as the eluent for the separation of saccharides. The higher the water content, the faster the elution of saccharides.

In the event metal ions are present in the eluent, they will in most cases exchange with the sodium ions of packing, thereby deteriorate the performance of the packing.

To prevent such ion exchange, EDTA 4Na is added in the amount of 50 to 100 ppm to the eluent to mask the metal ions.

Caution!

1. Do not use more than 95% of polar organic solvent in preparation of the eluent; otherwise the packing will shrink and cause the column performance to deteriorate.
2. Use deionized and distilled water in preparation of the eluent.
3. Do not inject any basic sample.
4. Do not inject any sample containing heavy metal ions; otherwise, Na^+ ions of the ion exchange resin will be exchanged with the heavy metal ions, thereby sometimes causing the column's separation performance to change.

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.

Caution!

1. 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.
1. When the chromatograph contains an aqueous salt solution, do not purge it with an organic eluent such as methanol.

Mixing an organic eluent with the aqueous salt solution will separate out the salt, thereby something clogging the tubing. In such case, purge it with water and then replace the water with the organic eluent.

2. In replacing the solvent which is insoluble in the eluent to be used, first replace the solvent with an intermediate solvent and then replace it with the eluent.

2) Remove extraneous and insoluble substances from the eluent by passing it through a 0.45µm filter.

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 KT series will facilitate the degassing work.

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

5) Replace the in-column solvent with the eluent at 0.5ml/min. (DS-613, DE-613 and DC-613), 0.3ml/min. (DM-614), 1.0ml/min. (DS-2013, DE-2013 and DC-613) maximum.

6) Preheat the column for better separation but not exceed the upper temperature shown in the temperature range of the specification.

In case of DC-613 and DC-2013, it is essentially necessary to preheat the column to 40°C minimum.

7) Set the flow rate at 1.0ml/min. (DS-613, DE-613, DM-614 and DC-613) or 3.0ml/min. (DS-2013, DE-2013 and DC-2013) and 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 solvent that is to be used as the eluent. In case of gradient, dissolve the sample 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 resevoir.

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) Be sure to deprotenize a sample, possibly containing proteins, before injection.

6. Safekeeping

1) After completing analysis, keep pumping the eluent at a flow rate of 0.5ml/min. (DS-613, DE-613 and DC-613), 0.3ml/min. (DM-614) or 1.0ml/min. (DS-2013, DE-2013 and DC-2013) until the column is cooled down to room temperature.

2) 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.

3) When the column will not be used for a long time, keep it using the completely degassed solvents. The solvents to be used as the in-column solvent are shown below:

DS-613, DS-2013:	Methanol/water= 50/50
DE-613, DE-2013:	Purified water
DM-614:	Purified water
DC-613, DC-2013:	Acetonitrile/water = 70/30

7. Regeneration

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

1) DS-613, DS-2013

Pass 50ml (DS-613) or 1,000ml (DS-2013) of dioxane, acetonitrile or methanol at a flow rate of 0.5ml/min. (DS-613) or 3.0ml/min. (DS-2013).

2) DE-613, DE-2013

Pass 50ml (DE-613) or 1,000ml (DE-2013) of dioxane, acetonitrile or methanol at a flow rate of 0.5ml/min. (DE-613) or 3.0ml/min (DE-2013).

3) DM-614

Pass 50ml of methanol, acetonitrile or water at a flow rate of 0.3ml/min.

4) DC-613, DC-2013

Pass 50ml (DC-613) or 1,000ml (DC-2013) of methanol, ethanol , acetonitrile or 0.05N NaOH at a flow rate of 0.5ml/min. (DC-613) or 3.0ml/min. (DC-2013).

Or, inject 20 to 50µl (DC-613) or 200 to 500µl (DC-2013) 0.5N NaOH from the injector.

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) Sample: 0.5% Benzene (DS-613 and DS-2013).
1.0% Ethylene glycol (DE-613 and DE-2013).
0.2% Oxalic acid (DM-614).
2.0% Sucrose (DC-613 and DC-2013).

2) Injection volume: 5µl (DS-613, DE-613 and DM-614).
10µl (DC-613).
100µl (DS-2013 and DE-2013).
200µl (DC-2013).

3) Eluent: Acetonitriole/THF/water = 40/30/30 (DS-613 and DS-2013).
Water (DE-613 and DE-2013).
0.005M H_3PO_4 (DM-614).
Acetonitrile/water= 70/30 (DC-613, DC-2013).

4) Flow rate: 0.8ml/min. (DS-613, DE-613, DM-614 and DC-613).
4.0ml/min. (DS-2013).
9.0ml/min. (DE-2013 and DC-2013).

5) Detector: UV-254nm (DS-613 and DS-2013).
Shodex Rl (DE-613,DM-614,DC-613,DE-2013 and DC-2013).

6) Column temperature: Room temperature (DS-613, DE-613, DM-614, DS-2013 and DE-2013)
50°C (DC-613 and DC-2013).

7) Chart speed: 1.5 to 2.0 cm/min.

8) Detector sensitivity: The sensitivity must be so adjusted as to obtain a peak of 10 to 15cm in height.

9) Calculation formula: $N = 5.54 \times (t_R/W)^2$
where N: Theoretical plate number
tR: Retention time
W: Peak half width

9. Warranty

1. Showa Denko shall replace any Shodex column,

1) If found damaged at the time of delivery.

2) If the plate number obtained by the purchaser as per the operation manual is significantly smaller than the one given in the inspection sheet attached to the column.

Claims must be filed with Showa Denko within 10 days following delivery.

2. The following shall not be subject to warranty.

1) Service life.

2) Deterioration of column performance resulting from improper handling.