No.1141-3

Shodex®

充塡カラム取扱説明書

Standard Operation Procedure

ＯHpak ＳＢ－８００ ＨＱシリーズ
ＳＢ－２０００シリーズ

OHpak SB-800 HQ Series
SB-2000 Series

［必ずお読み下さい］

この度は Shodex 製品をお買い上げいただき誠にありがとうございます。
カラムライフや性能を永く保持してご使用いただくために、この取扱説明書を読んでからご使用ください。

Please read this manual carefully before using the column for keeping shelf life of the column.

SHOWA
DENKO

Contents

1. Introduction

The packed columns of Shodex OHpak SB-800 HQ series are designed for use with high resolution, aqueous gel filtration chromatography. And Shodex OHpak SB-2000 series are designed for use with preparative columns. The packed columns are best suited for analysis of particularly, water soluble polymers, proteins and enzymes.

2. Specifications

Column type	Exclusion limit (Pullulan)	NTP*	Column type	Exclusion limit (PEG) (Pullulan)	NTP*
SB-802 HQ	4×10^3	>12000	SB-2002	4×10^3	>9000
SB-802.5 HQ	1×10^4	>16000	SB-2002.5	1×10^4	>12000
SB-803 HQ	1×10^5	>16000	SB-2003	1×10^5	>12000
SB-804 HQ	1×10^6	>16000	SB-2004	1×10^6	>12000
SB-805 HQ	4×10^6	>12000	SB-2005	4×10^6	>12000
SB-806 HQ	(2×10^7)	>12000	SB-2006	(2×10^7)	>12000
SB-806M HQ	(2×10^7)	>12000	SB-2006M	(2×10^7)	>12000
SB-G	Guard column for SB-800 HQ series		SB-LG	Guard column for SB-2000 series	

*Number of theoretical plates(NTP),calculated as shown in Section 7 below for conditions as given in the Inspection Data sheet supplied with each column.

Column type	SB-800 HQ series	SB-G	SB-2000 series	SB-LG
Shipping solvent	Ion exchanged water			
Max. flow rate	1.2 mL/min.		5 mL/min.	
Usable temperature	4 – 70 ℃		15 – 60 ℃	
Usable pH range	3 – 10			
Usable salt concentration	≦ 0.5 M			
Column size (I.D.× length)	8mm×300mm	6mm×50mm	20mm×300mm	8mm×50mm
Column material	SUS 316			
End fitting	Internally-threaded type, No.10 32 UNF			
Packing material	Polyhydroxymethacryrate gel			
Max. pressure	SB-802,802.5,803 HQ :5.0 MPa SB-804,805,806,806M HQ :3.0 MPa		2.0 MPa	

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 pulse less 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 5.0MPa (SB-802, 802.5, 803 HQ),
3.0MPa (SB-804, 805, 806, 806M HQ),
2.0MPa (SB-2000 series)

③ The temperature of the column should generally be between 4℃ and 70℃(SB-800 HQ series), 15℃ and 60℃(SB-2000 series).

④ Do not impact or bend the column.

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

⑥ Install guard column immediately upstream of the main column to protect it from contamination by the sample. Guard column is intended to maintain the column performance as designed for a long period of time and not to improve its resolving power.

3. Eluent

Some nonionic sample can be analyzed with the use of ion exchanged water as the eluent. It is a general practice to use, as the eluent, either an aqueous salt solution or buffer solution with or without a polar organic solvent added to it.
The followings are how to select the eluent to be used.

1) Ionic and nonionic hydrophilic samples.
Salt solutions or buffer solutions are generally used as the eluent.

Typical aqueous salt solutions are as follows:

<Aqueous salt solutions>

Sodium chloride aqueous solution
Sodium nitrate aqueous solution
Sodium sulfate aqueous solution
Potassium sulfate aqueous solution
Ammonium sulfate aqueous solution

<Buffer solutions>

Phosphoric acid buffer solution
tris-hydrochroric acid buffer solution
acetic acid buffer solution
citric acid buffer solution

Caution!

① In the range of 0.05M to 0.3M,the salt concentration is recommended. Maximum usable salt concentration is 0.5M. When the eluent contains salt, flow rate should be slower than 0.5mL/min. When the eluent contains salt more over 0.2M, flow rate should be slower than 0.3mL/min.
② pH of eluent should be 3.0 to 10.0.
③ pH of eluent should be higher than 6.0 when the eluent contains chloride ions.
④ Boric acid buffer solution is not recommended because boric acid makes complex with di-ol groups of packing (SB-804, 805, 806, 806M HQ,SB-2004,2005,2006,2006M).

2) Nonionic and ionic hydrophobic samples

Addition of polar organic solvents in the eluent is recommended to decrease the hydrophobic adsorption. Addition of salts in the eluent is also recommended when the sample is ionic.

The recommended concentration of polar organic solvents is below.

Column type	Methanol	Acetonitrile	DMF
SB-802 HQ	0%	0%	0%
SB-802.5*,803 HQ	0 - 100%	0 - 75%	100%
SB-804,805,806,806M HQ	0 - 75%	0 - 75%	100%
SB-2002,2002.5,2003, 2004, 2005,2006,2006M	Recommended polar organic solvents :less than 50%		

* SB-802.5 HQ is recommended to use 100% DMSO.

Caution!

Flow rate should be slower than 0.5mL/min. when in-column solvent is changed to the containing polar organic solvent.

In case of using DMSO(SB-802.5 HQ), flow rate should be slower than 0.3mL/min. And column temperature should be from 50℃ to 70℃.

3) Protein samples

Urea or 6M guanidine aqueous solution, which is commonly used as protein modifier, can be used as the eluent. The eluent containing surfactant, such as SDS or Brij-35, is

recommended for the samples, such as membrane proteins, of which when their solubility in water is poor.

Caution!
Frequent replacement of the in-column eluent from normal solvent to the above solvent will make the column life short. In such case, it is recommended to use one column specifically for the purpose.

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 0.45 μm 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 pressure reduction with as aspirator. Use of solvent degassing devices of DEGASSER will facilitate the degassing work. Usually, the flow rate of 1.0 mL/min. should be used.
4) After replacing the solvent in the chromatograph, set the flow rate.(refer to 2) Regarding the flow rate to be used when as eluent containing salts or polar organic solvents is used, refer to 3.
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. 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 0.45 μm filter. Use of the disposable filter unit is recommended.

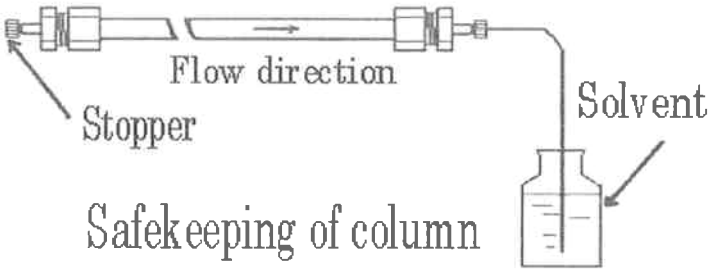
6. Safekeeping
1) After completing analysis, keep pumping the eluent at a flow rate of 0.5mL/min. until the column is cooled down to room temperature.
2) When the column is used for next day, the column is connected to the chromatograph as it is.
3) When the column is not to be used for more than a week, store the column by the following procedure:
① Replace the eluent by 0.02% sodium azide solvent which is degassed completely.
② Detach the detector inlet line from the column and connect a Teflon tube of 1/16 inch in outside diameter, 0.8mm in inside diameter and 500mm in length to the

column outlet.

③ Start pumping the solvent at a flow rate of 0.5mL/min.(SB-800 HQ series), 1.5mL/min.(SB-2000 series) and stop the pump as soon as it begins to flow out from the free end of the tube.

④ Put the solvent into a bottle and soak the free end of the tube in the bottle to prevent air from entering the column.

⑤ Dismount the column from the chromatograph, blank the column's inlet end store it in a room that has little temperature fluctuation.



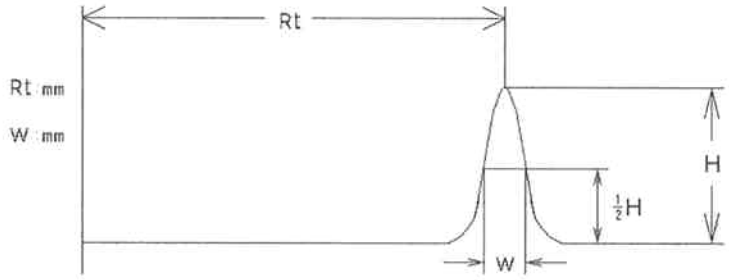
Safekeeping of column

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: ethylene glycol aqueous solution
2) Eluent: Ion exchanged water
3) Flow rate: 1.0mL/min.(SB-800 HQ series),3.0mL/min.(SB-2000 series)
4) Detector: Shodex RI
5) Column temperature: Ambient
6) Calculation formula: $N=5.54\times(Rt/W)^2$

where N: Number of theoretical plate
Rt: Retention time
W: Peak half width



Injection

8. Warranty

8-1.Showa Denko K. K. warrants that the Shodex Column, at the time of delivery to the user, will conform tothe specification of the attached Certificate of Analysis, if the Shodex Column is used in accordance withthe 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.

8-2.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.

8-3.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 remodelled by anyone other than person or firm designated by Showa Denko K.K.
3) If the Shodex Column is disposed of
4) If the Shodex Column is resold by the user without giving prior written notice to Showa Denko K.K.
5) 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, crime, riot, act of terrorism, war or radioactive contamination

8-4.In no event is Showa Denko K.K. liable for (i) the results of analyses or preparations using the ShodexCoiumn 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.

8-5.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.

総発売元

昭光通商株式会社

ショウデックス部

〒105-8432 東京都港区芝公園1丁目7番13号

■本　　社	TEL:03-3459-5104	FAX:03-3459-5081
■大阪支店	TEL:06-6314-0775	FAX:06-6314-0772
■福岡支店	TEL:092-711-9575	FAX:092-781-5277
■技術相談専用電話	TEL:03-3459-5110	FAX:03-3459-1221
(Shodex 110番)	E-mail:shodex.lab@shoko.co.jp	

製造元

SHOWA DENKO

昭和電工株式会社

化学品部門 特殊化学品事業部

ショウデックス（分離・分析機器）グループ

〒210-0867 神奈川県川崎市川崎区扇町 5-1

SHOWA DENKO K.K.

Chemicals Division

Shodex(Separation & HPLC) Group

5-1, Ogimachi, Kawasaki-ku, Kawasaki Kanagawa 210-0867 Japan

SHOKO AMERICA INC.

2853 Janitell Road
Colorado Springs,CO 80906 USA
Tel : +1-719-576-1834
Fax: +1-719-576-1837
E-mail: shodex@shoko-america.com

SHOWA DENKO EUROPE GmbH

Chemicals & Electronics Dept
Martin-Kollar-Str.1.D-81829 Munich
Tel : +49-(0)89-939-962-34
Fax : +49-(0)89-939-962-50
E-mail : shodex@sde.de

http://www.shodex.com