Shodex®

充てんカラム取扱説明書

Standard Operation Procedure

SUGAR シリーズ

SUGAR Series

SH1011 ,SH1821 ,SC1011 ,SC1821 ,SP0810 ,SC1211 ,SZ5532

［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。

Thank you for purchasing a Shodex product. Prior to use,be sure to read this instruction manual so that the maximum service life and performance of your column can be achieved.

SHOWA
DENKO

1. Introduction

Packed with rigid styrene/DVB-based strong cation-exchange resin designed exclusively for separation of sugars, the columns of the Shodex SUGAR series demonstrate high performance in separation and analysis of sugars. They are best suited to the application in food, biochemicals and natural products.

Although size-exclusion chromatography is the basic mode of separation for those columns, partition chromatography and interaction, i. e., ligand exchange, between counterions and the sample are combined with the basic mode to enable subtle separation like that of monosaccharides.

Also, the columns of the SH type enable simultaneous separation of organic acids and sugars because of their marked effectiveness in excluding ions.

2. Types and specifications

The columns come in seven types, each different in counterions, pore size, pore structure and sugar selectivity.

Table 1 Types and specifications

Type[1]	Counterion	Separation mode	Size (mm)	Exclusion limit	Plate number per column	Solvent[2]
SH1011	H^+	SEC+IE[3]	8×300	1,000	≧17,000	H_2O
SH1821	H^+	SEC+IE	8×300	10,000	≧17,000	H_2O
SC1011	Ca^{2+}	SEC+LE[4]	8×300	1,000	≧13,000	H_2O
SC1821	Ca^{2+}	SEC+LE	8×300	10,000	≧13,000	H_2O
SP0810	Pb^{2+}	SEC+LE	8×300	1,000	≧11,000	H_2O
SC1211	Ca^{2+}	P[5]+LE	6×250		≧5,500	25%CH_3CN /H_2O
SZ5532	Zn^{2+}	P+LE	6×150		≧5,500	70%CH_3CN /H_2O

Notes: 1) Material: stainless steel, SUS-316
End fittings: Internally-threaded type, No.10-32 UNF
2) Solvent filled by manufacturer
3) Stands for ion exclusion
4) Stands for ligand exchange
5) Stands for partition

The following six guard columns are available for the columns listed in Table 1 above.

Table 2 Guard columns

Type	Counterion	Size(mm)	Solvent[1)]	Applicable column
SH-G	H^+	6×50	H2O	SH group
SC-LG	Ca^{2+}	6×50	H2O	SC and SP group
SP-G	Pb^{2+}	6×50	H2O	SP group
SC-G	Ca^{2+}	4.6×10	25%CH_3CN/H_2O	SC1211
SZ-G	Zn^{2+}	4.6×10	70%CH_3CN/H_2O	SZ5532
KS-G[2)]	Na^+	6×50	H2O	SC and SP group

Notes: 1) Solvent filled by manufacturer
2) Primarily, guard column for columns of the SUGAR KS-800 series.

3. Use conditions and usable range

1) Standard conditions of use

Table 3 Standard conditions of use

Type	Mobile phase	Flow rate (mL/min)	Column temperature (℃)
SH1011	0.01N H_2SO_4	0.5～1.0	50～60
SH1821	0.01N H_2SO_4	0.5～1.0	50～60
SC1011	H_2O	0.5～1.0	70～80
SC1821	H_2O	0.5～1.0	70～80
SP0810	H_2O	0.5～1.0	70～80
SC1211	25%CH_3CN/H_2O	0.5～1.0	50～60
SZ5532	70%CH_3CN/H_2O	0.5～1.0	50～60

2) Usable range

Table 4 Usable range

Type	Mobile phase	Flow rate (mL/min)	Column temperature (℃)	Column pressure (MPa)
SH1011	H_2O～0.05N H_2SO_4	1.5max.	95max.	5.0max.
SH1821	H_2O～0.05N H_2SO_4	1.5max.	95max.	5.0max.
SC1011	H_2O～20%CH_3CN/H_2O	1.5max.	95max.	4.0max.

SC1821	H_2O～20%CH_3CN/H_2O	1.5max.	95max.	4.0max.
SP0810	H_2O～20%CH_3CN/H_2O	1.0max.	95max.	3.0max.
SC1211	H_2O～40%CH_3CN/H_2O	1.0max.	95max.	4.0max.
SZ5532	H_2O～95%CH_3CN/H_2O	2.0max.	80max.	4.0max.

Notes: Special care must be exercised in using SP0810 Follow the attached instructions in handling the column.

4. Mobile phase

The pure water used as the mobile phase must be deionized water that has been passed through a 0.45 μ m membrane filter.

Before use, it must also be degassed to prevent generating bubbles in the column and at its outlet, because all columns of this series are heated being used. Use of degassing device is recommended.

SH type

Although deionized water can be used alone as the mobile phase, adsorption of alkalis on the packing will change the chromatogram. Addition of sulfuric or phosphoric acid to the mobile phase is, therefore, recommended to prevent that change.

SC type and SP0810

Basically, deionized water is used as the mobile phase. A polar organic solvent, if added to the mobile phase, will improve separation of sugar alcohols and reduce hydrophobic adsorption. The concentration of the polar organic solvent must, however, be 20% maximum.

SC type

Addition of a salt to the mobile phase will improve separation of ionic substances. The salt must be either $Ca(NO_3)_2$ or $CaSO_4$, and its pH, in the 3-7 range. Also, in the case of analysis of samples containing large quantities of heavy metals, addition of about 10 – 50 ppm of Ca-EDTA will prevent deterioration of column performance.

The mobile phase used with SC1211 and SZ5532 is basically a mixture of water and organic solvent, generally Acetonitrile, and it is used in partition chromatography.

See Table 4 for the usable range.

5. Flow rate

Normally, use of columns at the flow rate given in Table 3 is recommended. See Table 4 for the usable range.

Generally speaking, sugars disperse in liquid more slowly than do other substances. The lower the flow rate, therefore, the higher the resolution. In separation of oligomers, more favorable results are obtained are obtained at lower flow rates.

6. Column heating

As stated in 5. above, sugars are slow in dispersing. A higher degree of resolution can, therefore, be obtained by heating the column to as high a temperature as the mobile phase permits. In normal operation, however, the standard conditions of use in Table 3 should be observed.

If columns of the SC type or SP0810 is not heated to 50℃ when in use, the anomers in sugar will separate from each other. Full attention must, therefore, be paid to column temperature.

The columns of the SH type are not suited for high-temperature separation of sucrose and raffinose because their packing acts as a decomposing catalyst on those sugars. In case, however, use of the SH type for separation of such sugars is unavoidable, the column temperature should be lowered to about 40℃ to prevent their decomposition.

When using a guard column of SP0810 or those of the SC type, they also have to be heated; otherwise, separation of anomers and/or preparation of broadening will occur. If they cannot be heated, use of SUGAR KS-G is recommended.

7. Combination of one type of column with another

When separation cannot be satisfactorily performed, use series of an additional column of the same or a different type will improve separation. Such combination, however, is possible only among SP0810

and columns of the SC and SUGAR KS type, and they must always be arranged in the following order.

SUGAR KS column + SC column + SP0810

When a guard column is used for any combination of columns, it must be one designed for use with the first column in combination.

See the appropriate data sheet and/or other technical information for selection of columns for combination.

8. Pretreatment of sample

The sample is usually dissolved in the mobile phase. Pass the dissolved sample through a 0.45 μm membrane filter and inject it into the column. Use of disposable filter unit is recommended.

Any proteins contained in the sample must be removed before filtering.

Samples containing heavy metal ions, surfactants (emulsifiers) or oily substances that adsorb on the packing should be passed through a mass of H-type cation-exchange resin before filtering.

Organic acids can be removed by passing the sample through a mass of OH-type anion-exchange resin. In case of columns of the SH type, however, samples containing organic acids require no pretreatment. Alkaline samples must be neutralized before injection; otherwise, column performance will deteriorate.

9. Restoration of column performance

Since columns of this Shodex series are packed with a variety of counterion exchange resin, ions contained in the sample adsorb on the resins and ion exchange occurs in the column, causing a change in the counterions and deformation of the chromatogram.

The following table shows the method of correcting and preventing such deterioration in column performance.

Table 5 Restoration of column performance

Type	Restoration liquid	Column temperature (°C)	Procedure
SH group	0.005N H_2SO_4	50	Send in 50mL at 0.5mL/min
SC1011 SC1821	0.1M $Ca(NO_3)_2$	50	Send in 50mL at 0.5mL/min
SP0810[1)]	0.2M $Pb(NO_3)_2$	70～95	Inject 50 μL 3-4 times.
SC1211[2)]	0.1M $Ca(NO_3)_2$	50	Send in 30mL at 0.5mL/min
SZ5532[2)]	0.1M $Zn(NO_3)_2$	50	Send in 30mL at 0.5mL/min

NOTE: 1) While it is desirable to restore the performance of SP0810 in the same manner as in the case of columns of the SC group, utmost care must be exercised to treat the waste restoration liquid because lead salt is highly toxic.
2) In the case of this column, follow the restoration procedure after completely replacing the mobile phase with deionized water. After going through the procedure, purge the column with deionized water and gradually replace it with the solvent to be used as the mobile phase.

10. Dismounting and storing of column

Upon completion of the analysis, lower the flow rate to 0.3 – 0.5 mL/min. range, turn off the constant temperature bath and keep pumping until the column cools down to room temperature.

Upon cooling of the column to that temperature, stop the pump, dismount the column and blank off both ends. Place the column in the box used for its delivery from the manufacturer and store it in a place where there is no direct sunlight and little temperature fluctuation.

11. Checking of column performance

The following table lists the conditions under which column performance is checked.

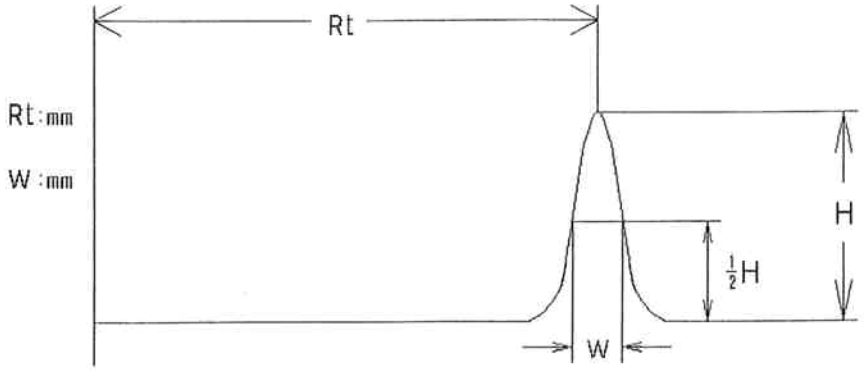
Table 6 Checking conditions

Type	Mobile phase	Flow rate (mL/min)	Column temperature (℃)	Sample	Injection volume (μL)
SH type	0.01N H_2SO_4	1.0	50	1% Glycerol	5
SC1011,SC1821	H_2O	1.0	80	1% Glycerol	5
SP0810	H_2O	1.0	80	1% Glycerol	5
SC1211	25%CH_3CN/H_2O	1.0	50	1% Glycerol	10
SZ5532	70%CH_3CN/H_2O	1.0	50	1% Glucose	5

The following formula is for use in calculating the plate number.

$$NTP=5.54\times(Rt/W)^2$$

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



Injection

12.

Instructions for Shodex SUGAR SP0810

(MISUSE will trigger deterioration of column performance.)

Since the column is fifed with somewhat softer packing than in the case of other columns of Shodex SUGAR series, special care must be exercised in handing it, particularly, when starting or stopping the pump.

Before using it, acquaint yourself with the following instructions as well as those in the operation manual for columns of the Shodex SUGAR series.

I . Mounting and dismounting of column

(1) Mounting of column, and start-up of pump

1) Set the flow rate at 0.2-0.3mL/min.
2) Remove the blank caps from both ends of the column and, before connecting the column to the tubing, heat it to degas the solvent. Make sure that solvent flows out from both ends.
3) Make sure the flow rate is proper and start the pump to degas the tubing.
4) Keep pumping and so mount the column that the mobile phase flows in the direction indicated by the arrow on the column. Upon mounting, make sure that no solvent leaks from either end.
5) Keep heating the column until it reaches 70℃ minimum.
6) After that temperature is attained, gradually increase the flow rate to the given level.

(2) Post analysis procedure when chromatography is to be recommenced the following day.

1) Lower the flow rate to 0.2-0.3mL/min and keep pumping.
2) Turn off the constant temperature bath.
3) Stop the pump when the column has cooled down to the temperature deemed appropriate.
4) Leave the column mounted on the chromatograph.

(3) Recommencement of analysis

1) Make sure that the flow rate is in the 0.2-0.3mL/min range.

2) Turn on the constant temperature bath to heat the column.
3) Keep heating until the column reaches the prescribed temperature.
4) Start the pump as soon as that temperature is reached.
5) Gradually raise the flow rate to the prescribed level.

(4) Completion of analysis, upon which the column will be dismounted.
1) Lower the flow rate to 0.2-0.3mL/min.
2) Turn off the constant temperature bath.
3) Keep pumping until the column cools down to room temperature.
4) Stop the pump when the column cooled to that temperature, and dismount the column.
5) Blank off both ends of the column and store it in a place where is little temperature fluctuation.

Ⅱ. Care to be taken in column handling

(1) The organic solvent addable to the mobile phase is either ethanol or Acetonitrile and its concentration must not exceed 20%.

(2) Under no circumstance should the column be subjected to a sudden change in pumping pressure or flow rate. Use a pulsation-free pump or install pulse dampers in the pumping system so that the mobile phase will flow without pulsation.

(3) Keep the column temperature in the 70-95℃; range; otherwise, anomers will separate to cause a broadening of the peaks.

(4) The maximum working pressure is 3.0MPa per column.
Use of a limiter-equipped pressure gauge with a full scale of 10MPa is recommended.

(5) Although the maximum working flow rate is normally 1.0mL/min., it is 0.5mL/min. when the column is used at 40℃ maximum.
Follow the instructions given in Section Ⅰ above for starting and stopping of the pump.

(6) It is recommended that guard columns, i.e., guard columns be used because they are effective not only in keeping the column from being contaminated by foreign matter in the sample but also in protecting it to some extent from pulsation or pressure change.
For guard column selection, refer to the operation manual for columns of the Shodex SUGAR series.

CAUTION

Do not use the column under any pressure or at any flow rate in excess of the specification; otherwise, the column performance will deteriorate beyond restoration.

13. 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 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, 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 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.

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.

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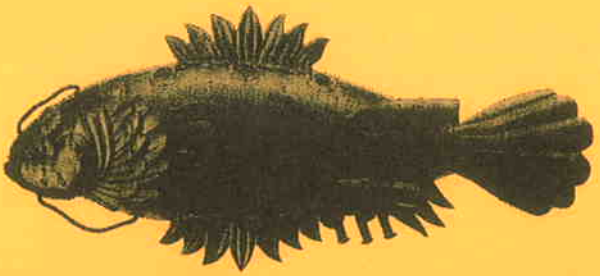

総発売元

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

昭和電工株式会社

SHOWA DENKO

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