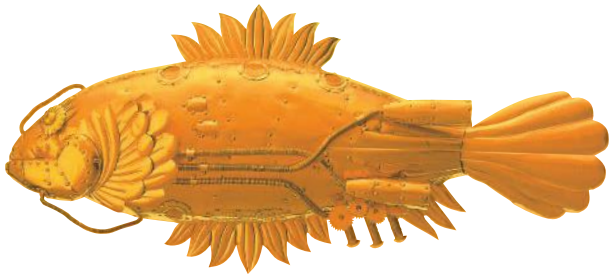
Shodex™



HPLC Columns

MANUAL

IC Y-521

Shodex HPLC Columns
Europe, Middle East, Africa, Russia

For technical support please use contact details shown below:

SHOWA DENKO EUROPE GmbH
Shodex Business
Konrad-Zuse-Platz 3
81829 Munich, Germany

E-mail: support@shodex.de
Phone: +49 (0)89 93 99 62 37
www.shodex.de

SHOWA DENKO EUROPE

Columns manufactured by Showa Denko K.K Japan
Made in Japan

Operation Manual

Shodex™ IC Y-521

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

1. Introduction

Shodex IC Y-521 is designed for exclusive use in ion chromatography for cationic separation. Packed with a polystyrene-based strong cation exchange resin prepared specially for ion chromatography, the column is suited for use in separation of alkaline metals, alkaline earth metals and amines.

2. Specification

1) Size: ID, 4.6 mm; length, 150 mm.

2) Material: SUS 316

3) End fittings: Swagelok-type, No. 10-32 UNF

4) Packing: Polystyrene-based cation exchange resin with low exchange capacity

5) Plate number: 3,000/15 cm

Note: The plate number was calculated on the following conditions.

i) Test specimen: 20-ppm Na^{+}.

ii) Injection volume: 50 µl.

iii) Mobile phase: Aqueous 4-mM nitric acid.

iv) Flow rate: 1.5 ml/min.

v) Column temperature: 40°C.

3. Mobile phase

1) For separation of alkaline metals and amines

Aqueous nitric acid in a range of 2 to 15 mM is used as the mobile phase. No organic solvents other than methanol can be added to it and the concentration of such methanol must be 1096 maximum.

CAUTION! Water for use in separation of alkaline metals must be ion exchange water with electrical conductance of 1 µS/cm maximum that has passed through a 0.22-µm-mesh filter.

2) For separation of alkaline earth metals

A solution of ethylene diamine and tartaric acid is the mobile phase.

NOTE: Recipe for mobile phase of 2-mM ethylene diamine/4-mM tartaric acid

i) Dissolve 6.01 g of ethylene diamine and 30.02 g of tartaric acid in ion exchange water that has passed through a 0.45-µm-mesh filter to obtain one liter of the solution.

ii) Dilute the solution 50 times and pass it through a 0.22-µm-mesh filter to prepare the mobile phase.

3) Degassing

Upon heating the mobile phase to 60 to 70 C, degas it by means of an ultrasonic bath and an aspirator.

4. Preinjection treatment of test specimen

1) Be sure to pass the test specimen through a 0.45-µm-mesh filter.

NOTE: Disposable filter, Shodex DT ED-13 or ED-03, is recommended for this purpose.

2) Be sure to remove protein, if contained in the specimen.

3) Remove any metal ions other than those of alkaline metals from the specimen that is to undergo alkaline metal separation.

Injection of test specimens containing alkaline earth metals or heavy metal ions will temporarily deteriorate the column performance for separation of alkaline metals.

5. Procedure to follow before column installation

1) Purge the chromatograph thoroughly with the mobile phase.

2) Remove pressure gages and dampers, if any, from the chromatograph, to wash them with the mobile phase, and restore.

Pressure gages and dampers or a now-through type are recommended for your use.

CAUTION! When a Bourdon tube gage is used, be sure to install line filter column IC Y-521L between the gage and the injector.

3) In case alkaline metals are to be separated with a chromatograph equipped with a stainless steel piping, be sure to passivate the inside surface of the pipes.

CAUTION! Although aqueous nitric acid can be used with such a chromatograph, the inside surface of the pipes is likely to partially dissolve out until it has been completely passivated.

Since the substances so dissolved in the aqueous nitric acid deteriorate the performance of the column, if passed through it, take the following procedure to purge the chromatograph before mounting the column on it.

i) Keep passing aqueous 20-mM nitric acid through the chromatograph at a low rate or 0.5 to 1.0 ml/min or a few hours.

ii) Stop the pump and leave the chromatograph as is overnight.

iii) Keep passing aqueous 20-mM nitric acid through it for two hours.

iv) Replace the aqueous nitric acid with the mobile phase for use in separation and keep passing the latter through the chromatograph for one hour.

6. Preheating of column

Preheat the column for better reproducibility, but not higher than 70 °C.

The temperature of 40 to 50°C is recommended for normal operation.

7. Start-up

Be sure to set the now rate at the specified level before starting the pump.

CAUTION! 1) Do not allow the flow rate to exceed 3.0 ml/min.

2) Do not build the pumping pressure/column above 50 kg/cm².

3) Do not abruptly change the pumping pressure or the flow rate during the separation.

8. Column dismounting and storage

1) In case the column was kept heated during ·the operation, reduce the flow rate to 1.0 ml/min maximum; turn off the column heater and keep the mobile phase flowing until the column cools down to room temperature.

2) Stop the pump and dismount the column.

3) Cap both ends and store the column in a place where no direct sunlight reaches and ambient temperature fluctuates little.

CAUTION!

i) Do not store the column with aqueous nitric acid or 10 mM or more contained in it.

ii) In case the column is to be stored longer than one month, pass aqueous 0.1-M sodium chloride through it at a flow rate of 0.5 ml/min to change the ion exchanger to an Na^+ type and then pass aqueous 0.1% sodium azide before capping both ends.

ii) Do not loosen the column ends under any circumstances or the column performance will deteriorate.

9. Deterioration of column performance and corrective actions

The causes given in the table below temporarily deteriorate the column performance, expediting elution of the constituent substances or the test specimen to unsatisfactory separation. A special precaution, therefore, must be exercised in separating alkaline metals.

Cause	Corrective Action	Reactivation
1) Contaminants in mobile phase, e.g., those from chromatograph.	1) Use a precolumn[i)]. 2) Use a line filter column[ii)].	1) Inject 100µl of aqueous 1-N nitric acid 4 to 6 times. 2) Pass aqueous 0.1-M sodium tartrate and 0.1-M nitric acid in that order[iii)].
2) Metal ions in test specimen.	1) Use a precolumn[i)].	1) Same as 1) and 2) above.
3) Proteins and nitrogen compounds in test specimen.	1) Remove proteins. 2) Use a precolumn[i)].	1) Pass aqueous 0.5-N sodium hydroxide and 0.1-N nitric acid in that order[iv)].
4) Use for separation of alkaline metals of the column that was used for alkaline earch metals separation	1) Use a column designed exclusively for the specific purpose.	1) Inject 100 l of aqueous 1-N nitric acid 4 to 6 times.

Notes: i) Shodex IC Y-521P is recommended.

ii) 10 Shodex IC Y-521L is recommended.

Since It Is filled with ion exchange water by the manufacturer, be sure before use to pass ca. 30 ml or the mobile phase through it.

The line filter column can be reactivated by passing through it 50 ml each or aqueous 0.5-N sodium hydroxide, 0.1-M tartaric acid and 0.5-N nitric acid in that order.

iii) Flow rate, 0.5 ml/min; volume, 30 ml each.

iv) Flow rate, 0.5 ml/min; volume, 30 ml each. There are some cases where the column cannot be reactivated because or adsorption of the test specimen on the packing.