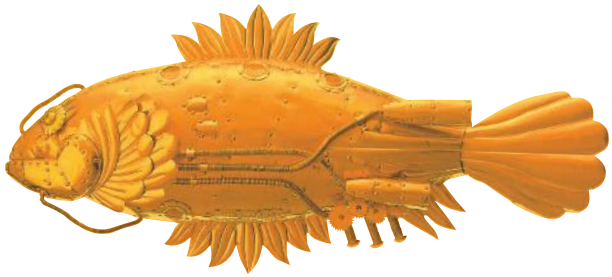
Shodex™



HPLC Columns

MANUAL

IC T-521

SHOWA DENKO EUROPE

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

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

Operation Manual

Shodex™ IC T-521

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

1. Introduction

The Shodex IC T-521 is designed for exclusive use in ion chromatography for transition metal separation. It can also be applied for separation of alkaline metals, alkaline earth metals and amines by changing the eluent conditions. For best separation of these ions, however, it is recommended to use Shodex IC Y-521

2. Specifications

1) Column size:	ID, 5mm; length, 150mm.
2) Fluid contact materials:	Pyrex glass, Tefron (filter).
3) Endfittings:	Shodex type (1/16 inch, Swagelok type, No. 10-32 UNF).
4) Packing:	Polystyrene-based cation exchange resin with low ion exchange capacity.
5) In-column fluid at the time of shipment:	3 mM HNO_3.
6) Plate number:	3,000/15cm.

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

i) Test specimen: 10 ppm Na^{+}.

ii) Injection volume: 50 µl.

iii) Mobile phase: Aqueous 3-mM nitric acid.

iv) Flow rate: 1.0 ml/min.

v) Column temperature: 40°C.

3. Eluent and reagent

Procedures for preparation of eluents used in separation of transition metals, alkaline metals and alkaline earth metals and the reagent for detection of transition metals are described

below. The column can be used reversibly by replacing the eluent in accordance with the ions to be analyzed. Equilibration may take a long time when switching to the eluent for alkaline metals from the eluent for other ions. In that case, injecting 100µ of 1 N HNO_3 several times will shorten the equilibration time.

3.1 For separation of transition metal ion (Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+}, etc.)

Normally the eluent is 6 mM oxalic acid + 3 mM citric acid + 0.1 mM sodium azide + 16mM KOH. For detection, 4-(2-pyridyl azo)resorcinol (PAR) is mixed post-column for generation of a visual color which is detected at 530 nm by a UV-VIS detector.

1) Reagent (3 M NH_4OH + 1 M CH_3COOH + 0.2 mM PAR)
    a. Put 150g of 28% ammonia water in a 500ml measuring flask and dilute with ion exchange water to approximately 400 ml.
    b. Put 30g of reagent grade acetic acid in a 100ml beaker and add ion exchange water to approximately 80 ml.
    c. Add the diluted acetic acid to the above mentioned 500 ml flask.
    d. Add 0.021g of PAR to the 500ml flask and dissolve ultrasonically. Add ion exchange water to the flask to precisely 500ml. Pass the solvent through a 0.45 µm mesh filter to prepare the reagent.
2) Eluent
    a. Put 0.756g of reagent grade oxalic azide, 0.63 of reagent grade citric azide and 0,0065g of reagent grade sodium azide in a one liter measuring flask and add ion exchange water to approximately 900ml.
    b. Add 16ml of 1 N KOH to the above mentioned one liter measuring flask, add ion exchange water to precisely one liter and dissolve the whole. Pass the solvent through a 0.45µm mesh filter to prepare the eluent.

Notes: 1. The 0.1 mM sodium azide is added as a preservative. When the column is to be stored for more than two weeks, be sure to replace the above eluent with 3 mM HNO_3 ; otherwise, minute particles may be generated and invoke a column pressure rise in subsequent use.

2. The appropriate reagent flow rate is 0.9 – 1.0 ml/min.

3.2 For separation of alkaline metal ions and amines.

Alkaline metals and amines can be separated by using 2 to 10 mM nitric acid as the eluent. Do not add an organic solvent to the eluent. Use a conductivity detector for analysis.

3.3 For separation of alkaline earth metal ions

Normally the eluent is 10mM oxalic acide + 10mM EDTA 2Na + 21mM KOH. Do not use eluent containing tartaric acid and ethylenediamine, which are normally used for alkaline earth metals; otherwise the characteristics of the column may change. Use a conductivity detector for analysis.

3.4 Procedure for preparation of eluent

1) Put 1.26g of reagent grade oxalic acide and 3.72g of ethylendiamine tetraacetic acid disodium salt in a one liter measuring flask and add ion exchange water to approximately 900ml.
2) Add 21ml of 1 N KOH and then ion exchange water to precisely one liter.
3) Dissolve completely. Pass the solvent through a 0.45µm mesh filter to prepare the eluent.

4. Preinjection treatment of test specimen

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

Note: Disposable filter, named Shodex DT ED-13 or ED-03 is recommended for this perpose.

2) Install the precolumn IC T-521P before the column to prevent contamination of the specimen in it.
3) Be sure to remove any proteins contained in the sample.

5. Precautions in use of column

1) It is recommended that the column be heated at a fixed temperature for better reproducibility of the data. The column may be heated to maximum 70°C, but a temperature of 40 to 50°C is recommended for normal operation.
2) The maximum operation pressure of the column is 50 kg/cm^2. Since this is due to the material of the fittings, use a pressure less than 50 kg/cm^2 when ever connecting two or more columns. The maximum flow rate of the eluent is 1.5 ml/min. In case the pressure

exceeds 50 kg/cm² even if the flow rate does not reach 1.5 ml/min, reduce the flow rate and use a column at a pressure less than the maximum operation pressure.

3) Observe the following when installing, dismounting and storing the column
   a. Purge the chromatograph thoroughly with the eluent before installation.
   b. When the column is to be heated, start to flow the eluent after the column temperature has reached the target.
   c. Dismount the column after the column temperature has reached room temperature.
   d. In case the column is to be stored longer than two weeks, do not leave it filled with an eluent for transition metals. Be sure to replace the eluent with 3 mM HNO_3, cap both ends, then store in a place where there is little fluctuation in the ambient temperature.
4) Install between the pump and the injector a line filter Shodex IC Y-521L for alkaline metal analysis and IC I-610L for alkaline earth metal and transition metal analysis.

6. Deterioration of column performance and corrective actions.

The cause given in the table below temporarily deteriorates the column performance, expending elution of the constitution substances of the test specimen to unsatisfactory separation. A special caution, therefore, must be exercised in separating alkaline metals.

Cause	Corrective Action	Reactivation
1) Contaminants in eluent, e.g., those from chromatograph.	1) Use a precolumn[a)].	1) Injection 100µl of aqueouse 1N nitric acid 4 to 6 times.
	2) Use a line filter column[b)].	2) Pass aqueous 0.1M sodium tartarate and 0.1M nitric acid in that order[c)].
2) Metal ions in test specimen.	1) Use a precolumn.	1) Same as a) and b) above.
3) Proteins and nitrogen compounds in test specimen.	1) Remove proteins.	1) Pass aqueous 0.1N sodium hydroxide and 0.1N nitric acid in that order[d)].
	2) Use a precolumn[a)].	

Notes:

a) Shodex IC T-521P is recommended.

b) Shodex IC Y-521L is recommended (for alkaline metal analysis only).
Since it is filled with ion exchange water by the manufacturer, be sure before use to pass ca. 30 ml of the eluent through it.
The line filter column can be reactivated by passing through it 50 ml each oh aqueous 0.5N sodium hydroxide, 0.1M tartaric acid and 0.5N nitric acid in that order.

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

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