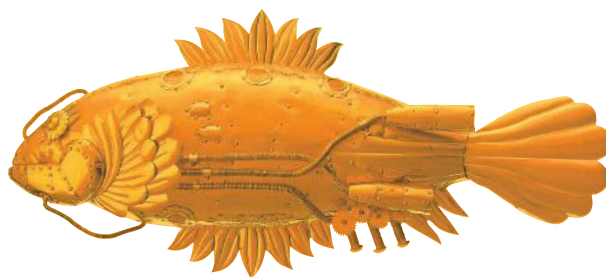


# Shodex™



## HPLC Columns

MANUAL

IC I-524A

**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™ IC I-524A

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

#### 1. Introduction

Shodex IC I -524A column was developed for use in ion chromatography for anionic separation. Packed with an ion exchange resin made by bonding a quaternary ammonium group to hydrophilic gels, the column is best suited for ion chromatography without use of a suppressor.

#### 2. Specifications

- 1) Size: ID, 4.6mm; length, 100mm.
- 2) Material: 316 stainless steel.
- 3) End fittings: Internally-threaded type, No. 10-32 UNF.
- 4) Packing: Ion exchange resin made by bonding a quaternary ammonium group to hydrophilic polymethacrylate type gels.
- 5) Number of theoretical plate: 2000/1 column.

<sup>Note</sup>: See Section 9 below for calculation of the plate number.

#### 3. Mobile phase

In general, the mobile phase is an aqueous solution of phthalic acid, salicylic acid, para-hydroxybenzoic acid, tartaric acid or citric acid.

Change from one concentration; pH or kind of the mobile phase to another will change the length of time and the order in which the specimen will be eluted.

When the mobile phase has the pH of 7 or higher, carbon dioxide gas will be adsorbed by the mobile phase to change the pH. It is, therefore, necessary to prevent the gas from going into the mobile phase by installing at the outlet of the mobile phase container a gas trap containing Ascarite or a similar adsorbent.

Normally, use of a stable mobile phase of a lower pH value is recommended.

- 1) Preparation of aqueous 2.5mM Phthalic acid (pH 4.0)

i) First, prepare aqueous 10mM Phthalic acid and aqueous 0.1M Tris (hydroxymethyl) aminomethane as follows.

a) Aqueous 10mM Phthalic acid.

Put 1.66g of pure Phthalic acid into a one-liter beaker and add 900mL of ion exchange water to dissolve the Phthalic acid while stirring and heating. After cooling the aqueous solution, empty the beaker into a one-Liter measuring flask and add ion exchange water to make it one liter.

b) Aqueous 0.1M Tris (hydroxymethyl) aminomethane.

Measure out 12.12g of pure Tris (hydroxymethyl) aminomethane on a precision balance; put it in a one-liter measuring flask and add water to make one liter of the aqueous solution.

ii) Prepare aqueous 2.5mM Phthalic acid with pH 4.0 as follows.

Put 250mL of the aqueous 10mM Phthalic acid into a one liter measuring flask; add 23mL of the aqueous 0.1M Tris(hydroxymethyl)aminomethane with a burette and increase the total quantity to one liter by adding water.

2) Filtration of mobile phase

Pass the mobile phase through a 0.45  $\mu$ m membrane filter immediately before feeding it into the column to remove particulate substances.

#### 4. Mounting of column

1) Before mounting the column on a liquid chromatograph, thoroughly replace the solvent in the chromatograph with the filtered mobile phase.

2) Set the-flow rate at 1.5mL/min.

##### **Caution!**

i) The maximum working flow rate is 2.0mL/min.

ii) The maximum working pressure/column is 2.5MPa.

3) Mount the column on the chromatograph in such a way that the flow mark on the column will point to the flow direction, and then start the pump.

**Caution!** Do not let the air into the column when mounting it on the chromatograph.

- 4) Heat the column, normally, to 40°C.

### 5. Pretreatment of specimen

- 1) Pass the specimen through a 0.45 µm membrane filter to remove particulate substances.
- 2) Remove proteins from the specimen, if it is expected to contain them.

### 6. Guard column

Use of the Shodex guard column IC IA-G immediately before the column is recommended to protect the packing from contamination by the specimen.

### 7. Dismounting and storage

- 1) Reduce the flow rate to 0.5mL/min and stop heating the column. Keep flowing the mobile phase into the column until it cools down to room temperature.

**Caution!** Do not dismount the column before it cools down to room temperature; otherwise, the air will enter the column.

- 2) Stop the pump and dismount the column from the chromatograph.
- 3) Blank off both ends of the column and store it in a place where temperature does not markedly fluctuate.

### 8. Column washing

The elution behavior markedly changes from time to time after repeated use of the column over a long period of time. This change is considered due to accumulation of substances adsorbed to the surfaces of the gels packed in the column, which will deteriorate the column performance.

If such change takes place, wash the column packing by flowing 30mL each of the following liquids through the column at the flow rate of 0.2mL/min.

- 1) In the case of accumulation of metals, aqueous 0.01M Tartaric acid (pH 3-4, adjust with 0.1N Sodium hydroxide).
- 2) In case of proteins, aqueous 20% Acetic acid and water.
- 3) In case of organics, a solution of 20% Acetonitrile/water and water, respectively.

## 9. Column performance test

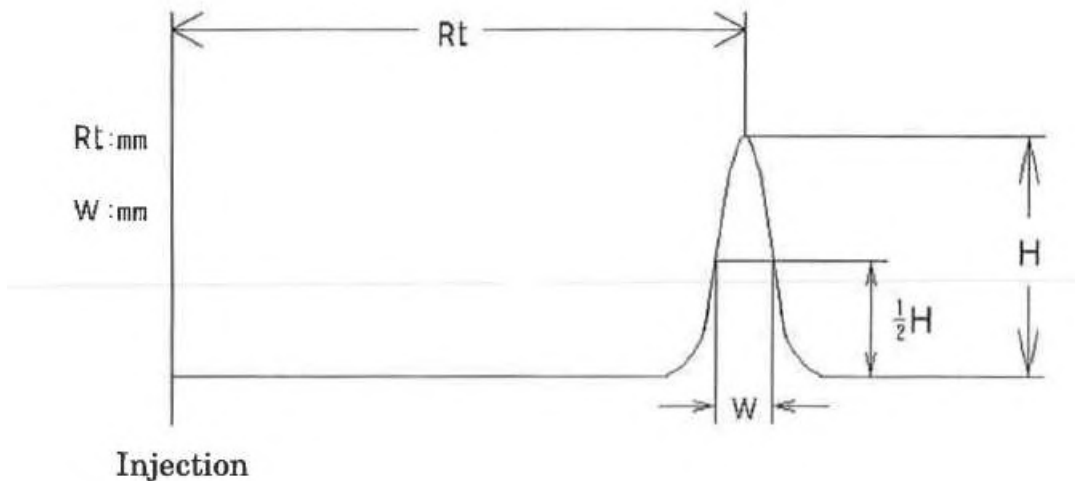
The column performance can be tested by calculating the plate number under the following conditions.

- 1) Specimen: Nitric acid ions (10ppm).
- 2) Injection volume: 30  $\mu$ L.
- 3) Mobile phase: Aqueous 2.5mM Phthalic acid (pH 4.0).
- 4) Flow rate: 1.5mL/min.
- 5) Column temperature: 40°C.
- 6) Detector: Shodex conductivity detector CD-5, 3 $\mu$ S/cmFS.
- 7) Calculation formula:  $NTP=5.54 \times (Rt/W)^2$

where NTP: Number of theoretical plate

Rt: Retention time

W: Peak half width



## 10. 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 remodeled by anyone other than person or firm designated by Showa Denko K.K.

(3) If the Shodex Column is resold by the user without giving prior written notice to Showa Denko K.K.

(4) 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 Shodex Column 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.