Agilent HPLC IonoSpher C Columns

Data Sheet

Warning

Agilent IonoSpher C columns are packed with a derivatized silica material. Introduction of basic solvents (pH > 6.5) or acidic solvents (pH < 2.5) into the column may dissolve the silica material and damage the column. You should thoroughly familiarize yourself with the contents of this manual before using your column. Improper use will invalidate the warranty.

1. Introduction

Agilent IonoSpher C columns are packed with strong cation exchange silica material, containing sulfonate functional groups. It is designed specifically for the analysis of organic and inorganic cations with conventional HPLC equipment.

2. Column conditioning

Every column has been tested before shipment and has been pre-conditioned. The column is shipped in methanol. Flushing with 2-5 column volumes of deionized water is recommended before introducing eluent.

3. Eluent

The eluents recommended for this type of column are low conducting buffers like citric acid or tartaric acid buffers (or mixtures of these), with a pH ranging from 2.5 to 6.5 containing ethylenediamine as the cation eluent.

Never use salicylate buffers, as the salycilate decomposition products will alter the stationary phase properties. Eluents must be degassed prior to use to prevent detection and pumping problems, and filtered through a 0.5 µm filter.

Always check your eluents for microbial growth before starting up the system, otherwise your column may get clogged and backpressure will rise to unacceptable levels.

4. Flow and pressure

Column internal diameter (mm)	Flow (mL/min)	
	Optimum	Maximum
2.0	0.2	1.0*
3.0	0.4	2.0*
4.6	1.0	4.0*
10.0	4.7	18.0*

*Note: Maximum pressure:
for SS columns 300 bar (30 Mpa, 4500 psi)
for glass columns 200 bar (20 Mpa, 3000 psi)

An increase or decrease of flow rate must always take place in small steps, to prevent packing bed disturbances.

If you want to replace the column reduce the flow to zero and wait until no eluent is coming out of the column (2 minutes).

Removing the column without reducing the pressure will damage the column. High column pressures nearly always result from improper use of the column. Use of a guard column (see section 6) will usually prevent contaminants from accumulating on the analytical column.

5. Sample treatment

The key to long column life is proper treatment of samples prior to injection. Avoid the introduction of compounds whose hydrophobicity/polarity differs strongly from that of the mobile phase into the column by either mobile phases or samples. In particular, you should avoid introduction of particulate matter. These will ultimately cause an increase in operating pressure and may be difficult or impossible to remove.

6. Guard columns

Guard columns should always be used because sample and eluent contamination can result in excessive column pressures and altered selectivity.

For ChromSep columns we recommend a ChromSep Cation Exchange guard column. Replacement of the guard column is required when increased column pressure and/or loss of performance is observed.

For conventional SS columns we advise Chromguard Cation Exchange High Efficiency (10 x 3.0 mm) columns or High Capacity (50 x 3.0 mm) columns.

7. Injection volume and concentration

Column dimensions L x i.d.	Maximum sample volume
250 x 2.0 mm	± 10 µL
200 x 3.0 mm	± 15 µL
250 x 4.6 mm	± 50 µL
250 x 10.0 mm	± 250 µL

Higher sample volumes can be injected when the sample solvent has a lower elution strength than the eluent (on column sample pre-concentration).

8. Temperature

IonoSpher C columns can be used at ambient temperature. In order to prolong column stability, it is not advisable to exceed a temperature of
40 °C.

9. Storage

Before storing these columns, it is advisable to rinse them with deionized water, followed by rinsing with methanol.

Never store these columns while they are filled with buffers, or other salt-containing eluents.

10. Detection sensitivity

For cation determination, conductivity detection is advised, using the low conducting eluents mentioned before.

11. Possible causes of performance loss

1. Extra column band broadening. Ensure that the tubing length and tubing i.d. are kept to a minimum.
2. Insufficient equilibration time with eluent.
3. Improper pH or ionic strength of eluent.
4. Improper eluent anion present. Prepare fresh eluent.
5. Stationary phase contamination.

High column pressure accompanies resolution loss.

- Particulate accumulation on frit or packing bed.
  - a. Sample origin → filter or centrifuge samples
  - b. Eluent origin → filter eluents, enclose eluent reservoirs
  - c. System origin → flush all lines and pumps; install in-line system filter
- Accumulation of proteinaceous material
  - a. Microbial growth in samples
  - b. Microbial growth in eluent

Normal column pressure accompanies performance loss.

- Organic contamination
  - a. Fats, oils, lipids in sample & stationary phase surface becomes coated
  - b. Non-specific organics from sample or improperly prepared eluents
  - c. Non-specific organics introduced into eluents after preparation (e.g. from atmosphere, during transfer, etc.)

12. Possible operations to correct contamination

12.1. Prepare fresh eluent
In many cases, performance loss is traced to eluent contamination. Therefore, prepare fresh eluent and flush all liquid lines before using the column; eluents should be filtered through 0.2 to 0.4 µm membranes prior to use.

12.2. Regeneration
To regenerate the column:

- First invert the column
- Rinse with 30 mL 1 M ammonium nitrate at 0.4 mL/min
- Rinse with 30 mL methanol/water (40:60 v/v) at 0.4 mL/min
- Rinse with 30 mL iso-propanol at 0.4 mL/min
- Rinse with 30 mL water at 0.4 mL/min
- Rinse with 30 mL of eluent at 0.4 mL/min
- Invert the column to the original position
- Equilibrate with the eluent

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