Agilent HPLC IonoSpher A Columns

Data Sheet

Warning

Agilent IonoSpher A 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 A columns are packed with a silica-based strong anion exchange material, containing quaternary amine functional groups. It is designed specifically for the analysis of organic and inorganic anions with conventional HPLC equipment.

2. Column conditioning

Before starting up the analysis, the column must be conditioned properly. A column not properly conditioned may give rise to problems, such as poor performance, changing the separation, etc.

To condition this type of column, rinse with deionized water, then equilibrate with the eluent of choice.

Every column has been tested before shipment and has been conditioned already. So, in case of first use, rinsing with water is not necessary.

3. Eluent

The eluents recommended for this type of column are low conducting, or UV-absorbing buffers, like phthalate buffer, with a pH ranging from 2.5 to 6.5.

Never use salicylate buffers, as the salycilate decomposition products will alter the stationary phase properties. Addition of ammonium nitrate (1 mM) may improve the peak shape, without significantly influencing retention, detection or quantitative results.

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 Anion 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 Anion 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 A 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 anion determination with a phthalate buffer as eluent, several detection methods can be used:

- Refractive index detection
- Conductivity detection
- Indirect UV detection

Phthalate buffers serve very well for all three detection methods because of their high refractive index, relatively low conductivity and UV absorbance properties. The absorbance wavelength choice depends on the ions determined.

11. System peak

When using the IonoSpher A column, system peaks may occur. One usually elutes with the solvent front, the second elutes around the sulphate peak. Direction (negative or positive peak) depends on the pH of the sample, positions depend on the pH of the mobile phase. Lowering the pH will shift the system peak to the front.

In our experience, a pH between 3.8-4.3 is optimal.

12. 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.)

13. Possible operations to correct contamination

13.1. 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.

13.2. 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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