Agilent HPLC ChromSpher B Columns

Data Sheet

Warning

Agilent ChromSpher B columns are packed with a derivatized silica material. Introduction of basic solvents (pH > 7) or acidic solvents (pH < 2) into the column may damage the column. You should thoroughly familiarize yourself with the contents of this manual before using your column. Improper use will invalidate the warranty.

Please take careful note of section 7 "injection volume and concentration".

1. Introduction

ChromSpher B columns are packed with silica-based material, which is designed specifically for the analysis of polar solutes, especially amines and other basic compounds.

2. Column conditioning

Before starting up the analysis, the column must be conditioned properly. A column not properly conditioned may cause problems like poor performance, changing separation, etc. To condition this type of column, first rinse with acetonitrile or methanol (10 – 200 mL). Then, equilibrate with the eluent of choice. Every column has been tested before shipment and has been pre-conditioned. Therefore, in case of first use, rinsing with water is not necessary.

If mobile phase additives are used (like buffers or ion-pair reagents), it is advisable to perform an intermediate flush with a mobile phase of the right composition, but without these additions. The intermediate flush should be done first with a low flow, then later at a normal flow.

3. Eluent

Eluents recommended for this type of column are mixtures of acetonitrile and aqueous phosphate buffers (pH 2.5 – 4.0). Solutes of an extreme adsorbtivity may elute as tailing peaks. In this case small concentrations of triethyl amine can be added to the eluent to improve peak shape. For most substances, however, this is not necessary.

Never use buffers with a pH lower than 2 or higher than 7, as they 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 up 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	4.5	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 RP guard column. This column is packed with material similar to that used in the analytical 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 RP High Efficiency (10 x 3.0 mm) or ChromGuard High Capacity (50 x 3.0 mm) columns.

7. Injection volume and concentration

The loadability of ChromSpher B is much lower than that of standard RP materials. If the columns are overloaded it will result in tailing (usually not fronting as with standard phases) and efficiency loss.

Column dimensions L x i.d.	Maximum sample volume	Maximum sample mass
250 x 2.0 mm	± 10 µL	± 0.2 µg
200 x 3.0 mm	± 15 µL	± 0.4 µg
250 x 4.6 mm	± 50 µL	± 1 µg
250 x 10.0 mm	± 250 µL	± 5 µg

8. Temperature

ChromSpher B columns should be used with a column thermostat. Reproducibility of analysis on this type of material depends on temperature control to a large extent. The optimal temperature depends on the specific application. Temperature influences the eluent viscosity. Always adjust the flow rate in order to keep the pressure below 200 bar when ChromSep glass columns are used.

9. Storage

Before storing these columns it is advisable to rinse them with water, followed by rinsing with methanol or acetronitrile. Never store these columns while they are filled with buffers, or other salt-containing eluents. Storage solvents should contain at least 20% organic modifier to prevent bacterial growth.

10. Possible causes of performance loss

- Extra column band broadening. When using columns with small i.d.s or short lengths, external band broadening may be pronounced. Make sure the tubing length and tubing internal diameter are kept to a minimum. Check whether injection volume and detector cell volume are appropriate for the column volume.
- Insufficient equilibration time with starting eluent.
- Improper column temperature.
- Improper modifier concentration.
- Bed compression. Excessive eluent flow rate has been used. Invert the column and use it at a lower flow rate.

11. Performance loss and/or high backpressure

Particulate accumulation on the frit or resin bed (together with backpressure increase). If column backpressure is high, disconnect the column from the injector and run the pumps to verify that backpressure is due to the column and not the pumping system or tubing. Track down the source of the particulates (sample, eluent, system).

Invert the column and flush out the particulates in the reversed flow direction. If this doesn't solve the problem, replace the inlet frit or screen.

Microbial growth in eluent. Use fresh eluent. Invert the column and try to rinse out the contaminants in reversed flow direction. Replace inlet frit/screen.

Contamination with proteins, fats, oils, etc. Regenerate the column (see section 12).

12. Regeneration

To regenerate the column:

1. First invert the column.
2. Rinse the column at ±40% of the optimum flow rate, for about 45-60 min with solvent in the following order: water – methanol – isopropanol – dichloromethane – isopropanol – methanol – water.
3. Invert the column to the original position and equilibrate with the analytical eluent.

Note: When using another rinsing method: always start with water to remove buffers and ensure that consecutive eluents are mixable.

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