



Agilent HPLC ChromSpher Lipids Columns Data Sheet

Warning

Agilent ChromSpher Lipids 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

ChromSpher Lipids columns are packed with a cation exchange resin in the Ag⁺ ionic form. It is specifically designed for the separation of triglycerides.

2. Column conditioning

The column has been pre-conditioned with the mobile phase mentioned on the test report. When you switch to another mobile phase be aware that it must be mixable with the mobile phase that is in the column.

3. Eluent

The eluents recommended for this type of column are dichloroethane, dichloromethane, acetone, acetonitrile, toluene and ethylacetate.

Normally the column should be used with non-aqueous, clean (peroxide-free) solvents, use of the column with aqueous or wet solvents is possible, but may permanently alter column performance.

Never use water-containing anions, which may precipitate with the silver ions.

Never use eluents or mobile phase additives, which might oxidize the silver ions to metal state.

Never use eluents or mobile phase additives, which contains acids. The acids will displace the silver ions and in this way the capacity (retentive character) decreases.

For optimum performance, eluents must be dried on molecular sieves prior to use to prevent deactivation of the stationary phase, and filtered through a 0.5 µm filter.

4. Flow and pressure

The optimal flow rate is 1.50 mL/min. Do not exceed a flow rate of 5.0 mL/min or a pressure of 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.

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. In particular, you should avoid introduction of particulate matter. This 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.

We recommend an Agilent Cation Exchange High Efficiency guard column (Part No. CP28089). Replacement of the guard column is required when increased column pressure and/or loss of performance is observed.

7. Injection volume

Generally use sample volumes in the 10-50 µL range. Injection of 100 µL or more may cause peaks to broaden or merge.



8. Temperature

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

In some cases, column performance has been reported to be temperature dependent. Thermostration and temperature optimization may be advantageous.

9. Storage

Columns can be stored in the eluent itself, without specific precautions. It is advisable to elute all injected compounds before storing the column.

10. Possible causes of performance loss

- Extra column band broadening. Make sure 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.
- 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 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 to 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 does not solve the problem, replace the inlet frit.

12. Regeneration

- To regenerate the column we advise the following procedure (no guarantee):
 - a. First invert the column
 - b. Rinse the column for about 45-60 min (flow rate 1.0 mL/min) with MeOH/ACN 1:1. During this rinsing procedure inject onto the column a solution of silver nitrate.
 - c. Invert the column to the original position and rinse with dry dichloromethane.
 - d. Equilibrate with the eluent.

13. Ordering information

Column	Dimensions	Part No.
ChromSpher	250 x 4.6 mm	CP28313

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