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Euroline

KNAUER Column Care and Use

1. Column Care and Use

1.1 Silica based phases

Column Usage and Column Care

The proper care of an HPLC column is extremely important for the lifetime of the column and, consequently, for the quality of your HPLC analysis. The following pages will give you some guidelines for the use, cleaning and storage of HPLC columns. These guidelines will depend on the nature of the chromatographic support (silica, polymers or others) and on the surface chemistry of the corresponding stationary phase.

General guidelines

Silica is the ideal support for HPLC columns. It offers good mechanical stability, excellent physicochemical surface properties, a wide range of bonding chemistry and is compatible with a broad range of organic solvents. However, the following points are extremely important when working with silica based HPLC columns.

pH stability

In general silica based HPLC columns are stable within a pH range of 2 to 8. When measuring pH, the measurement should be done in the aqueous media before mixing the eluent with organic solvents. This will give a more accurate and consistent measurement of pH than taking a measurement in a mixed aqueous/organic media. Some modern HPLC columns can be used outside that pH range. New bonding chemistry allows for operating as low as pH 1 with some stationary phases. However, you should check vendors product information first before using a silica based column outside the pH range of 2 to 8. Stationary phases based on ultra pure silica gel can also be used at a pH as high as 11, depending on the chemical nature of the modifier used in the mobile phase. Large bases (such as pyrolidine) are not able to attack the surface of the silica and, therefore, can be used as mobile phase modifiers when higher pH values are required. If you are working at pH values above 8 using small bases as the modifier (such as ammonia), we highly recommend using stationary phases based on polymers or zirconium dioxide.

Mechanical stability

Stationary phases based on silica are mechanically very stable, and well-packed columns can be used at more than 40 MPa (6000 psi) without any problem. However, pressure shocks to the column should be avoided. Pressure shocks can lead to channeling in the bed column, which may result in peak splitting in the corresponding chromatogram.

Mobile phases (eluents)

Silica based stationary phases are compatible with all organic solvents in the above mentioned pH range. For best results, the highest quality solvents available, such as HPLC grade solvents, should be used. Also, all prepared buffers should be filtered through a 0.45 µm filter before using them in your HPLC system. Always keep in mind that your column will collect any particulate material that enters the flow stream.
The use of non-pure solvents in HPLC causes irreversible adsorption of impurities on the column head. These impurities block adsorption sites, change the selectivity of the column and eventually lead to peak splitting in the chromatogram. In gradient elution, they cause so-called "ghost peaks". "Ghost peaks" are peaks that always appear at the same position in the chromatogram. Their origin is not the sample, but the impurities from the solvents or solvent additives. Therefore, it is highly recommended to run a gradient without injecting a sample at the beginning of each method to determine if ghost peaks will be a problem.
To avoid irreversible adsorption at the head of the column, you should always use a precolumn. The use of a precolumn increases the lifetime of a column dramatically. In addition to that, a precolumn can filter particulate material coming from pump seals or injection rotors. An alternative to a precolumn is an in-line filter. These filters are placed between the column and the injector and newer versions can be mounted directly on columns.
These filters are great for removing particulate material from the eluent, but they will not take the place of precolumns by removing organic impurities that may irreversibly adsorb to the column.

Proper storage of silica based HPLC columns

- For short-term storage, i.e. overnight, columns can be stored in the eluent.
- For middle term storage, i.e. 2 days or over the weekend, columns should be flushed with pure water to prevent algal growth.
- For long term storage, silica based columns should be stored in an aprotic solvent. The water content should not be greater than 50%. The best solvent for storage is acetonitrile.
- Caution! Make sure that all buffers are washed out of the column before exchanging aqueous mobile phases by organic solvents. Buffer salts are not soluble in acetonitrile and can block capillary tubing and the column.

Equilibration time

The equilibration time of a column depends on the column dimensions. In general, a column is equilibrated after 20 column volumes are flushed through it. The equilibration times for the most important column dimensions are summarized in the following table.

You can reduce the equilibration time by simply increasing the flow rate. However, make sure to flush the column with at least 20 column volumes to make sure the column is equilibrated.

Column Dimension	Column Volume [ml] *	Flow Rate [ml/min]	Equilibration Time [min]
250 x 4.6 mm	2.91	1.00	58
150 x 4.6 mm	1.74	1.00	35
100 x 4.6 mm	1.16	1.00	23
50 x 4.6 mm	0.58	1.00	12
250 x 4.0 mm	2.20	1.00	44
125 x 4.0 mm	1.10	1.00	22
250 x 2.0 mm	0.55	0.25	44
150 x 2.0 mm	0.33	0.25	26
50 x 2.0 mm	0.11	0.25	9

Regeneration of a column

Impurities from the sample or mobile phase can adsorb to the head of a column and cause changes in selectivity or peak splitting. Often these "dirty columns" can be regenerated by applying the following protocols:

Regeneration of RP packings

C18, C8, C4, C1, C30, CN and Phenyl stationary phases:

- Flush the column with 20 column volumes of water
- Flush the column with 20 column volumes of acetonitrile
- Flush the column with 5 column volumes of Iiopropanol
- Flush the column with 20 column volumes of heptane
- Flush the column with 5 column volumes of isopropanol
- Flush the column with 20 column volumes of acetonitrile

Regeneration of NP packings

Silica, Diol, Nitro and Amino stationary phases:

- Flush the column with 20 column volumes of heptane
- Flush the column with 5 column volumes of isopropanol
- Flush the column with 20 column volumes of acetonitrile
- Flush the column with 20 column volumes of water
- Flush the column with 20 column volumes of acetonitrile
- Flush the column with 5 column volumes of isopropanol
- Flush the column with 20 column volumes of heptane

Regeneration of Ion Exchange Packings

Anion and Cation exchange (WCX, SCX, WAX and SAX):

- Flush the column with 20 column volumes of the same eluent, but double the buffer concentration
- Follow the regeneration protocol for RP packings (see above)
- Flush the column with 20 column volumes of water
- Equilibrate the column with the original conditions

1.2 Polymer based Phases

General guidelines

Polymer based stationary phases show a higher pH stability but a lower mechanical stability compared to silica based columns. Also polymer based packings are not compatible with all organic solvents. They will swell or shrink in some organic solvents. Unfortunately, the pressure stability and solvent compatibility are not only different for the different types of polymers, they are also different from manufacturer to manufacturer. Therefore, no general rules for the care of polymer based materials can be given. Always read the instructions for the use of those columns. In case of doubt, please contact the corresponding manufacturer. Nevertheless, general guidelines do exist for the storage and regeneration of the different types of polymer supports.

Storage guidelines for polymer based columns

- *Hydrophobic unmodified polystyrene-divinylbenzene (PS-DVB, e.g. Hamilton PRP-1):*
These types of columns can be stored for long periods analogously to silica based columns, that is, in an aprotic solvent. The best solvent for storage of these columns is acetonitrile.

- *Polymer based ion exchangers (e.g. Eurokat H, CarbEx H-Form or Hamilton PRP- X 100):*
These types of columns must not be treated with organic solvents in high concentrations. For storage we recommend a 0.01% aqueous sodium azide solution. Storing these columns at 4°C (i.e. refrigeration) also helps to prevent algal rowth.

Regeneration of polymer materials

When a column exhibits a change in selectivity or always shows a double peak at a particular retention time, the cause is frequently the strong adsorption of a matrix component. Generally these types of contaminated columns can be regenerated by one of the methods given below. Specific regeneration methods must be used for different types of polymer columns. Appropriate regeneration methods for specific polymer based columns are described below.

Hamilton PRP-1, PRP-3, PRP-Infinity
- Rinse the column with 20 column volumes of water
- · Run a linear gradient from 100% water to 100% acetonitrile
- Repeat the procedure 3 times

Hamilton PRP X-100
- Rinse the column with 20 column volumes of water
- Rinse the column with 20 column volumes of methanol with 1% 6 N nitric acid

Hamilton PRP X-200, PRP X-300
- Rinse the column with 20 column volumes of water
- Inject 100 µl of 1 N nitric acid, 5 times consecutively

Hamilton RCX-10
- Rinse the column with 20 column volumes of a 0.1 N sodium hydroxide solution

Hamilton RCX-30
- Rinse the column with 60 column volumes of a 0.1 N sodium hydroxide solution

Eurokat H, CarbEx H-Form, Hamilton HC-75 Hydrogen Form
- Rinse the column overnight with 0.1 N sulphuric acid at 60 °C, using a flow rate of 0.1 ml/min

Eurokat Ca, CarbEx Ca-Form, Hamilton HC-75 Calcium Form, Hamilton HC-40 Calcium Form
- Rinse the column overnight with a 1% calcium nitrate solution at 60 °C, using a flow rate of 0.1 ml/min

Eurokat Pb, CarbEx Pb-Form, Hamilton HC-75 Lead Form
- Rinse the column overnight with a 1% lead nitrate solution at 60 °C, using a flow rate of 0.1 ml/min