Column Care and Use - Silica based phases

Column Usage and Column Care

The proper care of a preparative HPLC column is extremely important for the lifetime of the column and, consequently, for the quality of your preparative HPLC separation. The following pages will give you some guidelines for the use, cleaning and storage of preparative 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 preparative 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 preparative 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 vendor's product information first before using 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

The maximum pressure is dependent on the diameter of the column (16 and 20 mm ID 400bar, 30 mm ID 300 bar, 50 mm ID 200 bar). However, pressure shocks to the column should be avoided. Pressure shocks can lead to channelling 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 preparative HPLC columns

- Silica based columns should be stored in an aprotic solvent. The best solvent for storage of RP packings (C18, C8, C4, C1, C30, CN, NH_2 and Phenyl) is acetonitrile/water. The water content should not be greater than 50%. The best solvent for storage of NP packings (Silica, Diol, Nitro, Cyano and Amino) is hexan/isopropanol 90:10 (v/v).
- Caution! Even for short-term storage, flush out all buffer solution from the column to prevent algal growth. 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]	recommended Flow Rate [ml/min]	Equilibration Time [min]	max. pressure [bar]
250 x 16 mm	35	12	58	400
120 x 16 mm	17	12	28	400
60 x 16 mm	9	12	15	400
250 x 20 mm	55	19	58	400
150 x 20 mm	33	19	35	400
250 x 30 mm	132	45	58	300
150 x 30 mm	79	45	35	300
250 x 50 mm	344	118	58	200
150 x 50 mm	206	118	35	200

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 Isopropanol
- 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, Cyano 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

Compressing of the column bed of axial compressing columns

With axial compressible columns it is possible to lower the column head stamp down on the column bed to close a developed dead volume. Please follow the enclosed instructions.

If there are any further questions do not hesitate to contact us:

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