# Science Together

## Column Care and Use - Silica based phases

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 page will give you some guidelines for the use, cleaning and storage of HPLC and UHPLC columns. These guidelines will depend on the nature of the chromatographic support and on the surface chemistry of the corresponding stationary phase. Each KNAUER column is individually packed and tested to ensure reliable performance. The enclosed test certificate includes a test chromatogram and specific column data concerning performance. The serial number of your column is noted on the column certificate as well as on the column label. Please retain this information. To ensure that your column provides you with reliable chromatography results, please adhere to the guidelines below.

### Column Installation

**Additional information for integrated guardcolumns: Before installation, make sure the guard column holder is sufficiently tightened. A Loose column holder can lead to increased dead volume and peak broadening.**

Please handle the column with care, every drop or shock to the column can damage the packed column bed. The column is shipped with PEEK end plugs. Please loosen and remove the plugs before installation. Flush all capillaries with compatible eluent before use with the column. When the column is shipped, it contains the solvent listed on the column test certificate (the column is also safely stored in this solvent.) Be sure that your mobile phase is compatible with this storage solvent. If not, flush the column with an intermediate solvent which is compatible with both solvents. We recommend isopropanol. The flow direction is given by an arrow on the column label. Firstly, connect the column only at the injector, flush the system and column at low flow rates and gradually increase the flow rate up to the optimum value. Finally, after about 10 mins, connect the column to your detector. This procedure helps to avoid air bubbles from being introduced into the flow cell. Before starting any analysis, check for leak tightness by observing the backpressure or using a flow control unit.

### 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 HPLC columns can be used outside that pH range. New bonding chemistry allows for operating at pH 1 to 12 with some stationary phases. However, you should check vendor's product information first before using silica based columns outside the pH range of 2 to 8.

### Mechanical stability

Stationary phases based on silica with a pore size of < 200 A are mechanically very stable. Stationary phases with particle sizes of 5 µm or larger can be used routinely at up to 40 MPa (6,000 psi) without any problem. HPLC Plus phases with particle sizes of 3 µm can be used at up to 60 MPa (8,700 psi). UHPLC columns with particle sizes of 2 µm or smaller and inner column diameter of 2 mm can be used at up to 100 MPa (14,500 psi). It is always recommended to work below the maximum allowed pressure range to guarantee a longer column lifetime. 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. For stationary phases with pore sizes > 200 A the maximum pressure can be lower. Please contact the column manufacturer to be sure.

### 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

- Silica based columns should be stored in an aprotic solvent. The best solvent for storage of RP packings (C18, C8, C4, C1, C30, CN and Phenyl) is acetonitrile/water (50:50 v/v). The water content should not be greater than 50%. The best solvent for storage of NP packings (Silica, Diol, Nitro, Cyano and Amino) is hexane/isopropanol 90:10 (v/v). The best solvent for storage of columns used in HILIC mode (HILIC, Amino, and Silica) is acetonitrile/water (90:10 v/v) or acetonitrile/5 mM ammonium acetate, pH 5,3 (90:10 v/v). Acetonitrile content should always be greater than 90 %.
- 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 10-20 column volumes to make sure the column is equilibrated.

| Column dimension (length x ID) | Column volume [ml] | Typical flow rate [ml/min] | Equilibration time [min] |
|---|---|---|---|
| 250 x 8.0 mm | 8.54 | 4.0 | 43 |
| 250 x 4.6 mm | 2.82 | 1.0 | 56 |
| 150 x 4.6 mm | 1.69 | 1.0 | 34 |
| 250 x 4.0 mm | 2.14 | 1.0 | 43 |
| 150 x 4.0 mm | 1.28 | 1.0 | 26 |
| 150 x 3.0 mm | 0.72 | 0.6 | 24 |
| 100 x 3.0 mm | 0.48 | 0.6 | 16 |
| 100 x 2.0 mm | 0.21 | 0.5 | 9 |
| 50 x 2.0 mm | 0.11 | 0.5 | 5 |

### Regeneration of a column

We recommend regenerating a column if a change in peak form, retention time, resolution or an increase in backpressure is observed. If the system pressure begins to rise, remove the column and check the system to find whether the pressure increase is being caused by the system or the column.

Pressure increase caused by system: flush system, exchange eluent filters, frits and/or blocked capillaries. Pressure increase caused by column: backflush the column carefully to remove particle buildup from the inlet frit (connect the column outlet to the pump/injector and flush). *Do not connect the column to the detector.*

If the column still has a high backpressure, flush the column according to the following regeneration scheme.

| **Regeneration scheme for RP columns** (C18, C8, C4, C1, C30, CN and Phenyl stationary phases) | **Regeneration scheme for NP columns** (Silica, Diol, Nitro, Cyano and Amino stationary phases) | **Regeneration scheme for columns used in HILIC mode** (HILIC and Silica stationary phases) |
|---|---|---|
| 20 column volumes water | 20 column volumes heptane | 20 column volumes water |
| 20 column volumes acetonitrile | 5 column volumes isopropanol | 30 column volumes 0.5 M ammonium acetate |
| 5 column volumes isopropanol | 20 column volumes acetonitrile | 30 column volumes water |
| 20 column volumes heptane | 20 column volumes water | 20 column volumes acetonitrile/water (50:50 v/v) |
| 5 column volumes isopropanol | 20 column volumes acetonitrile | 20 column volumes acetonitrile |
| 20 column volumes acetonitrile | 5 column volumes isopropanol | 20 column volumes acetonitrile/water (50:50 v/v) |
| | 20 column volumes heptane | |

After the regeneration procedure, re-equilibrate the column with the mobile phase before analyses.

**Because every HPLC system is unique, especially regarding the dwell volume, your results may vary from those obtained in our laboratory. Please don't hesitate to call our column specialists to assist you in optimizing your separation. Failure to follow these precautions may void the column warranty. Technical data are subject to change without notice.**
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If there are any further questions do not hesitate to contact us:

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