RediSep® Prep Column Care and Use Guide

TELEDYNE ISCO
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Chromatography Technical Note
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Overview

Columns packed with microparticulate silica packing materials require special care in handling in order to maintain performance and to ensure a long life. The purpose of this Technical Note is to give instruction on how to best care for these columns.

About the Columns

Our columns are constructed of 316 stainless steel tubing and 316 stainless-steel zero dead volume end fittings and frits. Sizes range from 4.6 mm ID to 50 mm ID.

Included with each column is a chromatogram describing the test conditions. Upon receipt, check to be sure that nothing has been damaged in shipment.

Installing the Column

The column should be installed so as to produce the least amount of void volume between the injection valve and the flow cell. 316 stainless steel tubing with an OD of $^{1}/_{16}$" should be used for all connections prior to the column. Tubing with an ID of 0.030" or less cut to the shortest practical length should be used for connections after the injection valve.

☑ ***Note***

Note the flow direction on the column label and connect the column accordingly.

Guard Columns

Guard columns or pre-columns, packed with the same type of packing material as in the primary column, should be used when injecting crude or dirty samples.

☑ ***Note***

Sample filtration is always recommended, even when using a guard column.

Sample components that may adhere to the packing material or clog the column will be caught by the guard column, thus prolonging the life of the primary column. Guard columns do not normally affect the results of a chromatographic separation, but after repeated injections of a crude sample, signs of degradation (such as loss of efficiency) may begin to appear. The guard column or cartridge should then be replaced.

If a pre-column or guard cartridge column is used, install it between the injection valve and the primary column inlet using a minimum of connection tubing. Most guard columns also have a designated flow direction.

Mobile Phase Requirements

Water should be deionized and further purified through a cartridge system containing ion exchange resin and activated charcoal for organic removal. Filter water through a 0.2 or 0.45 micron filter before use. Use fresh water daily, as growth of microorganisms can occur even in relatively pure water. HPLC grade water can also be purchased.

Organic solvents used for HPLC must be at least spectrophotometric grade and filtered through a 0.45 micron (or finer) filter. Since UV detection is very common with HPLC systems, a low UV cut off is desirable.

When preparing aqueous buffers, carefully weigh the salt and dissolve completely in water. Buffer pH should be in the range of 2 - 8. A pH above or below this range can dissolve silica or cleave active sites. When mixing buffers with organic solvents, test to be sure the buffer is completely miscible with the solvent before pumping. Too high an organic content can cause precipitation of the salt, which will clog or damage the entire system. **Filtration of buffers is highly recommended.** Even analytical grade salts contain impurities which can interfere with chromatography.

Column Equilibration

Teledyne ISCO C18, C18Aq, and C8 columns are shipped filled with 80/20 methanol/water. Silica columns, are filled with isopropyl alcohol. All columns need to be conditioned with appropriate solvents before the mobile phase can be introduced.

If the mobile phase is compatible with the solvent on the column, ie. acetonitrile with methanol, it can be directly pumped onto the column with no adverse effects. The column does need to be conditioned however, with at least 5 column volumes of the mobile phase before a sample can be injected. For example, a 4.6 x 150 mm analytical column has a volume of 2.5 ml. At a flow rate of 1 ml/min, 12 minutes are required to pump 5 column volumes. Some solvents require longer equilibration times; a stable baseline and reproducible retention times are signs that the column is sufficiently conditioned.

If the mobile phase is incompatible with the solvent on the column, i.e. aqueous salt buffers with methanol, extra precaution is required to assure a problem-free solvent changeover. The following sequences should be used for solvent changeover. At least 3 column volumes of the intermediate solvent in the sequence must be pumped through the column, followed by 5-10 volumes of the final solvent.

Solvent changeover sequences:

- Methanol to water to aqueous salt buffers
- Isopropyl alcohol to water to aqueous salt buffers
- Methanol to isopropyl alcohol to hexane, heptane, etc.

⚠ **WARNING**

DO NOT use an aqueous mobile phase with silica columns since water is adsorbed strongly by the packing and causes surface deactivation.

Maximum Operating Pressures

Maximum operating pressure varies based on column diameter. For an analytical column of 4.6 mm ID, a maximum pressure of 420 bar (6,000 psi) is appropriate. The maximum operating pressure recommendation for 20 mm ID and larger is 240 bar (3,500 psi). Running above these pressures could result in shorter column life.

Testing the Column Before Use

After installation, the column should be tested under the conditions described in the test chromatogram. Results such as peak retention time, peak area, and efficiency should be reproducible, and similar to that of the test chromatogram. It is essential that the test conditions be identical: test results will obviously be affected by differences in flow rates or solvent concentration.

Peak efficiency gives an indication of column performance and can be used to detect column degradation. Periodic testing of the column under the conditions of the test chromatogram will allow early detection of column degradation.

Sample Preparation

It is always best to dissolve the sample in the same solvent or buffer that will be flowing through the column at the time of injection. If different solvents are used to dissolve the sample, undesirable results may occur. When the sample is dissolved in a solvent that is immiscible with the mobile phase, an emulsion is created which can interfere with the separation, the baseline, or both. If the sample is insoluble in the mobile phase and for this reason is dissolved in another solvent, precipitation can occur when the sample is injected. Dissolving the sample in a stronger solvent than the mobile phase can cause unusual peak shapes, front tailing, and in extreme cases can cause shouldering or double peaks.

Always filter samples before injecting, and filter crude samples twice as an extra precaution. Do not inject samples containing obvious particulates which can clog the column. If components are not dissolved in the sample, they will not be dissolved on the column. Results are usually not reproducible when poor sample preparation techniques are employed.

Sample Injection

Appropriate injection volumes are also important to good results. Overloading a column with sample can cause unusual peak shapes such as those mentioned above. Injection volumes 5 microliters are recommended for fast LC columns (5, 10, 15 cm x 4.6 mm ID); 10 to 50 microliter injection volumes are recommended for analytical sized columns. Semi-prep or prep columns are required for extremely large samples (greater than 100 microliters). Using a weaker solvent strength than the initial mobile phase for dissolution of the sample may allow for larger injection volumes than recommended here.

Sample volumes may be measured with a syringe or by the sample loop. On an analytical scale HPLC system, always inject more sample into the loop than is capacity to assure good rinsing and filling. Due to human error, it is more difficult to obtain reproducible results when measuring sample volumes with a syringe. We recommend using the loop to measure the sample volume whenever possible. On a preparative scale HPLC, overfilling the loop will result in the loss of sample. Partial loop fill or loading by the dilute sample load pump are preferred. See tech notes TN43 and TN44 for more details on minimum and maximum injection volume.

Column Maintenance

After prolonged use of a column, especially under rigorous conditions such as injection of crude samples or continually running at low or high pH, the column will begin to show signs of deterioration. This is to be expected with any column regardless of type or manufacturer. Columns have a finite life.

Common signs of deterioration include tailing peaks, shouldered or double peaks, decrease in efficiency, severe changes in resolution or selectivity, or a significant increase in system back pressure. Column regeneration may help to resolve some of these problems; however, if deterioration is extensive and regeneration does not help, a new column should be installed.

Most commonly, column deterioration results from con-tamination by sample components or other impurities. Column washing from a weak to a strong solvent should be employed if the column begins to show signs of deterioration. A recommended procedure for reverse phase columns is to wash with pure methanol followed by methylene chloride. This should remove any strongly retained materials from the column. For normal phase columns, hexane followed by tetrahydrofuran is recommended.

Column Storage

DO NOT store columns under water, buffers, or under low polarity organic solvents. Recommended storage solvents are methanol, isopropyl alcohol, or acetonitrile. After using buffers, rinse the entire system (pumps, flow cell, etc.) with water so as to not precipitate salt and cause serious clogging. After a thorough water wash, rinse the system with methanol or other recommended storage solvent.

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