Guidelines for Using Orochem HPLC and UHPLC Columns

This instruction sheet should be read in its entirety before using your Orochem HPLC or UHPLC column.

These Guidelines are designed to help you achieve the best performance and longest column life from your Orochem HPLC or UHPLC column. Please retain the PEEK endcaps supplied with the column and use them to recap your column when it is not connected to your chromatograph.

Certificate of Analysis

Each Orochem HPLC or UHPLC column is tested prior to packaging. The actual column test chromatogram, which is your Certificate of Analysis, is included and contains the column description, test conditions, column performance data, and the column serial number. Please retain this Certificate of Analysis as it contains important information about your column. Should you have any questions about your column, please include the column name, type, and serial number in your correspondence.

The first peak in the chromatogram defines the void volume (dead volume) of the column. The last peak is used to determine the column efficiency and the peak asymmetry. All of the components in the chromatogram illustrate the column's ability to separate various types of compounds and are used to compare similar types of columns.

General Operating Conditions

The flow direction of the mobile phase is noted by an arrow on the column. The use of a guard column is recommended. Typical flow rates for each column depend on particle size, mobile phase, and column configuration (dimensions).

Samples to be injected onto the column should be free from any particulates or components that could react with the column's stationary phase. Samples should be filtered through a 0.2 micron filter before injection onto the column.

Unless stated otherwise, the maximum column pressure is 400 bar (5800 psi), the maximum column operating temperature is 60^{o}C, and the recommended pH range is between 2 and 8. Operating the column at the extremes of pressure, temperature, and pH will shorten column life.

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Improving Chromatographic Reproducibility

The column should be placed in a temperature controlled environment (column heater). If possible, the mobile phase(s) and the HPLC system components should be kept at a constant temperature.

Mobile phases should be carefully and reproducibly prepared. For solvent blends, measure out each component separately and then blend the components. Gradient reproducibility problems could indicate dissolved air in the solvent lines or a mechanical problem with the gradient mixer. Mobile phases should be degassed and filtered through a 0.2 micron membrane filter before use. An inline mobile phase degasser is recommended.

Allow the column to line out completely before injecting samples. This is especially the case if gradients are being run.

Mobile Phases

We recommend that only solvents specially prepared for use with HPLC be used. All solvents should be degassed and filtered through a 0.2 micron membrane filter before use.

Each column is shipped in a specific mobile phase that is listed on the Certificate of Analysis provided with the column. The mobile phase is also an example of a suitable mobile phase for that type of column. The mobile phase may be changed to any compatible solvent; however, make sure that the new mobile phase is miscible with the shipped mobile phase. If the mobile phases are not miscible, then you need to use an intermediate solvent that is miscible with both mobile phases. The intermediate solvent should be run at least until a stable baseline is obtained. This is critical if the new mobile phase contains a buffer, since the shipping solvent could cause the buffer to precipitate leading to column plugging.

Typical mobile phases depend on the column type (reverse phase, normal phase, or HILIC). The literature contains many examples of suitable solvents and mobile phase modifiers and examples can be found at www.orochem.com. Typical modifiers for eluting basic samples are triethylamine (TEA), diethylamine (DEA), ethanolamine, and similar bases. A typical use level is 0.1%. ***Strongly basic modifiers should be avoided since they may damage the silica gel.*** Typical modifiers for eluting acidic samples are acetic acid and trifluoroacetic acid (TFA). A typical use level is 0.1%.

Special Notes

Amino columns can react with aldehydes and ketones; hence these compounds should be avoided as mobile phases, solvents for the samples, or as components in the mobile phase.

Column Cleaning and Regeneration

A change in peak shape (peak broadening, the appearance of shoulders, peak splitting), changes in retention time, decreased resolution, or an increase in backpressure may indicate column contamination (fouling). If the polarity of the contaminate is known, then the appropriate flushing solvent may be used directly as long as it is miscible with the current mobile phase. Any buffers should be removed prior to column flushing.

NOTE: When cleaning or regenerating any HPLC column first remove the detector from the flow path. It is best to connect a piece of tubing to the outlet of the column and direct the flow to a suitable solvent waste container.

If the solvent flushing above does not restore the column, it may be necessary to use stronger cleaning and regeneration techniques. Column cleaning or regeneration varies depending on the column type. First, flush any buffers from the column. In general, to clean any column, flush the column with a solvent that is stronger than the mobile phase being used. Use at least 20 times the volume of the column of each cleaning solvent. Progressively stronger solvents can be used, if necessary, but each solvent must be miscible with the previous solvent. When returning the column to the original mobile phase, each solvent must be miscible with the previous solvent. Solvents that might react with or damage the column must be avoided; for example, strong bases.

The table below shows typical flushing sequences for reverse phase and normal phase columns; however, the best sequence depends on the contaminates to be removed. Increasing the column temperature to 40 or 50^{o}C will generally improve cleaning effectiveness. ***NOTE: These flushing sequences are <u>NOT</u> for Orochem's chiral columns.***

Typical Column Flushing Sequences

Sequence	Reverse Phase Columns e.g., C18, C8, C30, Phenyl	Normal Phase Columns e.g., Silica, Diol, CN, Amino
1	Water (for polar contaminates)	Heptane or hexane (non-polar contaminates)
2	Methanol	Ethyl acetate or THF
3	Methylene chloride or chloroform	Methanol
4	Tetrahydrofuran (THF)	Chloroform or dichloromethane
5	Hexane or heptane (for non-polar contaminates)	Heptane or hexane
6	Ethanol or acetone ***(DO NOT USE ACETONE WITH AMINO COLUMNS)***	Mobile Phase
7	Water	
8	Mobile Phase	

In some cases, it may be necessary to reverse the column (connect the pump to the column outlet) to flush it backwards, particularly if particles or some impurity have accumulated at the top of the column. This is acceptable and should not damage the column.

If none of these techniques restores the column to acceptable performance, then the column should be replaced.

Column Storage

Buffers, acids, or bases should be completely flushed from the column before storage. For short-term storage, it is usually sufficient to remove the buffer, acid, or base by flushing the column with the same mobile phase without the buffer, acid, or base. For long-term storage, it is recommended to store a reversed phase column in acetonitrile or methanol. A normal phase column should be stored in a non-polar solvent such as heptane or hexane.

The column should be tightly capped using column plugs and a note included stating the storage solvent used. Storing the column in the box in which the column was packaged will help protect the column from being bumped.

For questions about the use of Orochem columns or any problems, please contact ***Orochem Technologies, Inc.*** *at 1-630-210-8300.*