Guidelines for using Orochem HPLC and UHPLC Columns

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

Certificate of Analysis

Each Orochem HPLC or UHPLC column is testedbefore packaging. The column test chromatogram, which is your Certificate of Analysis (COA), is included and contains the column description, test conditions, column performance data, and the column serial number. Please retain this Certificate of Analysis. Should there be any questions about the column, please include the column name, type, and serial number when corresponding.

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 the components in the chromatogram illustrate the cability of the column to separate various compounds and may be use to compare similar columns.

General Operating Conditions

The flow direction of the mobile phase is denoted by the 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 dimensions. Typical flow rates are from 0.5 – 2.5 mL/min.

Samples to be injected onto the column should be free from 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 2-8. Operating the column at the extremes of pressure, temperature, and pH could shorten column life.

Improving Chromatographic Reproducibility

The column performs best in a temperature controlled instrument, like a column heater. Likewise, the column performs best when the mobile phase(s) and the HPLC instrument are at a controlled temperature.

A reproducible procedure should be followedwhen preparing mobile phases. For solvent blends, carefullymeasure each component separately and then blend them. Gradient reproducibility problems could indicate dissolved air in the solvent lines or a mechanical problem with the gradient mixer of the instrument. Mobile phases should be degassed and filtered through a 0.2-micron membrane filter before use. An inline mobile phase degasser is recommended for best performance of the column.

Allow the column to establish a stable baseline before injecting samples. Establishing a stable baseline is especially important for gradients analyses.

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Mobile Phases

Certified HPLC grade solventsare recommended for the best performance of the column. 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 each column. The mobile phase may be exchangedfor any compatible solvent, provided the replacement solvent is miscible with the shipped mobile phase. If the mobile phases are not miscible, then an intermediate solvent that is miscible with both mobile phases is recommended. The intermediate solvent should be run until a stable baseline is obtained. The proper solvent exchange procedure is especially necessary to avoid precipitation of buffer salts that can potentially plug the column.

Mobile phase selection depends on the column type, e.g.reverse phase, normal phase, ion exchange, or HILIC. The public literature contains many examples of suitable solvents and mobile phase modifiers. Specific 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 might irreversibly damage the bonded phase.Typical modifiers for eluting acidic samples are acetic acid and trifluoroacetic acid (TFA) at a typical use level is 0.1%.

Amino columns are known to react with aldehydes and ketones. Therefore, aldehyde and ketone mobile phasesare not recommended for Orochem amino phase. Caution should also be taken with aldehyde and ketone containing samples.

Column Fouling and Plugging

A change in peak shape, such as peak broadening, the appearance of shoulders, or peak splitting, could indicate column deterioration by fouling. Changes in retention times, decreased resolution, or an increase in backpressure may also indicate column fouling by sample contaminants. If the deterioration is due to fouling, a simple column flushing with mobile phase might restore its performance.In some cases, it may be necessary to reverse the flow direction of the column (back flushing) to remove fouling and particulates. If simple mobile phase flushing or reverse flow does not restore performance, stronger cleaning may be required.

Column Cleaning and Regeneration

Column cleaning and regeneration sequences for Orochem columns are specified in the table below. The table includes reverse phase, normal phase, and ion exchange phase products.Use the table as a guide for all Orochem silica-based columns.

Before cleaning or regenerating the HPLC column, remove the detector from the flow path.Do this bysending the outlet of the column directly to a suitable solvent waste container.

Column cleaning or regeneration varies depending on the column type.Bufferremoval is done as the first step by flushing with 10-20 column volumes of solvent, typically water. Cleaning or regenerationfollows by flushing with 10-20 column volumes of each cleaning solvents. Each solvent has a specific purpose. The progression of solvents must be miscible with the previous solvent. Increasing the column temperature to 40-50^{o}C can often improve cleaning effectiveness. Strong basic solvents of pH 8 and above are not recommended for silica-based columns.

For tough regenerations of ion-exchange phases, phosphate buffer as high as500 mM may be used. For protein samples, a 0.1% trifluoroacetic acid solution, followed by flushing with water, may be used. THF and acetone, followed by flushing with water, may be used for even tougher ion exchange samples.

If none of the techniques in this guide restores the column to acceptable performance, then the column should be replaced. Contact Orochem Technologies, Inc. to order a new column.

Column Storage

Buffers, acids, or bases should be completely removed from the column before storage by flushing with the appropriate buffer free solvent or mobile phase. For long-term storage, it is recommended to store reversed phase columns in acetonitrile, methanol, or the shipping solvent. Normal phase columns should be stored in heptane, hexane or the shipping solvent. Ion exchange columns should be stored in the shipping solvent. Shipping solvents are shown on the Certificate of Analysis.

The column should be tightly capped using column plugs. Storing the column in the original box can help protect it from damage. Documenting the storage solvent can be helped when the column is put back into service.

Orochem Silica Based Column Flushing Table

- Flush with 10-20 column volumes of each solvent. See text for appropriate modifications.

Sequence	Reverse Phase Columns e.g., C18, C8, C30, Phenyl	Normal Phase Columns e.g., Silica, Diol, CN, Amino	Silica Based Ion Exchange e.g. SCX, SAX, WCX, WAX
1	Water	Heptane or hexane	Water
2	Methanol	Ethyl acetate or THF	50 mM Phosphate Buffer or 100 mM Phosphate Buffer
3	Methylene chloride or chloroform	Methanol	Water or Methanol
4	Tetrahydrofuran (THF)	Chloroform or dichloromethane	10% Acetic Acid or 0.1M EDTA sodium salt
5	Hexane or heptane	Heptane or hexane	Water (Acetone or THF depending on the history)
6	Ethanol or acetone ***(Do not use acetone with amino columns)***	Mobile phase	Methanol or chloroform
7	Water		Water or Mobile phase
8	Mobile Phase		

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

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