We all know that HPLC columns have a finite lifetime and even after good clean-up and storage they will reach the end of their lifetime. However, there are certain measures that you can follow to get the maximum life out of your column.

All our HPLC columns are shipped to you in a shipping solvent. For reversed phase columns this usually consists of a certain composition of organic (acetonitrile or methanol) and water. For such columns, before installation, you can flush the solvent lines with your mobile phase, Install the new column, condition the column with your mobile phase and you are good to go with the analysis.

For those columns that run in both reversed and normal phase conditions, you need to pay attention to the shipping solvent and confirm that your mobile phase solvents are miscible/compatible with the shipping solvents. If not, you need to use solvent switching procedures.

Once your HPLC column is ready to use, you can -help maintain its integrity by installing an inline filter, trap column, and a guard column. These will help keep contaminants from damaging your HPLC analytical column. However, when contamination does build up, there are several ways to combat it.

Cleaning HPLC Columns

After using the column, it is always recommended to clean the column before storing it. The cleaning procedure typically involves using an isocratic/gradient wash that is closest to the last solvent system on the column, but replacing the buffer with HPLC grade water. This will ensure that the buffer components are removed from the column. You may then increase the percent organic in the wash to further remove any hydrophobic impurities.

Reverse Flushing HPLC Columns

If you notice that there is a deterioration of peak shape coupled with increase in backpressure, it is recommended to reverse flush the column. Before reverse flushing it is important to ensure that your mobile phase or in-column solvent is miscible with your cleaning solvents and that your flow rates are no more than half the typical recommended flow rate for the column. For detailed procedures, follow the instructions mentioned in our column care guides. You can determine your column volume using the following equation: V = pr2L where V is the column volume in mL, r is the column radius in cm, and L is the column length in cm.

- When it comes to the proper cleaning of your reversed phase HPLC column after use, first change your mobile phase to 95% HPLC grade water and 5% Acetonitrile, then flush with about 10 column volumes of this through the HPLC column at half the flow rate. This will remove any buffer that has been left in the HPLC column. You may then move step-by-step as needed, to increase organic content to about 95% Acetonitrile and 5% Water

- After reverse flushing, connect the column in the forward direction and condition with the regular mobile phase before using.

Note that while reverse flushing the column, disconnect the column from the detector. Learn how to properly reverse flush your HPLC column using these tips.

HPLC Column Storage

It is also important to store the column properly after usage, especially of it going to be for a long time. It is critical that you do not store your HPLC column while it still contains any buffers or ion-pairing agents. To ensure this, flush five column volumes of your mobile phase without buffer through the HPLC column. For columns that have ion-pair reagents, extended washing might be required to completely remove them from the column. After washing, store the reversed phase columns in acetonitrile/ water (65:35 v/v), methanol can be used in place of acetonitrile, normal phase columns in 100% Hexane or IPA, ion-exchange columns in 100% methanol, and HILIC columns in acetonitrile/ water (80:20 v/v)

If you have any questions or are seeking technical assistance with your HPLC analysis or HPLC method development and troubleshooting , Phenomenex offers a free, 24/7, online Technical Support service – **Chat Now**.

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