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Liquid chromatography

How to prolong the lifetime of your HPLC column

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Introduction

Prolonging the lifetime of your analytical column is good business, as a longer lifetime means more injections per column and reduced cost of analysis per sample. Please don't fall for it, there is no such thing as free money. To achieve a longer lifetime requires concrete action, and those actions are what we will discuss in this white paper.

Preventative measures

Sample preparation

Sample preparation is the first step to evaluate when setting up a robust method that supports a longer column lifetime. Sample preparation (sample prep) is a broad term that covers various levels of work and protocols. We will briefly mention some here, but **much more information and additional tips** can be found on these techniques.

Filtration is one of the lowest levels of sample preparation and predominantly removes particulates of various sizes—depending on filter pore size—from the sample prior to injection. Without filtration, particulates will accumulate at the top of the HPLC column, causing high backpressure and possible irreversible adsorption to the packing material, and decreased column lifetime. The most common type of sample filtration product is a syringe filter. Protein precipitation plates (PPT plates) can also be categorized as a filter as they filter the precipitated sample after a protein crash. PPT plates often have a non-drip membrane that prevents the sample from exiting the well before full reaction with the organic solvent during a crash. The sample is filtered by applying vacuum on the filter plate with a 96 well plate manifold.

Thermo Scientific™ Target2™ Syringe Filters

Liquid-liquid extraction (LLE) and its lower solvent consumption and easy-to-use alternative **supported-liquid extraction** (SLE) are effective sample preparation methods that will provide you with the ability to extract your target analytes and remove unwanted matrix components (that could bind to the stationary phase and decrease the column lifetime). The techniques leverage the partitioning coefficients for the analytes to a certain solvent. This means that analytes are extracted in the solvent in which they most prefer to exist. The solvents used are traditionally immiscible, making it easy to separate and extract the solvent containing the target analytes. LLE is an old technique with high solvent consumption and has in later years been replaced by SLE, which is based on the same concept. The difference between the two is that in SLE a solid support of diatomaceous earth is used to hold the sample over a large surface area. This provides very efficient partitioning into the extraction solvent, and the solvent volume can be greatly reduced. However, the SLE does not preconcentrate samples as seen for SPE. LLE and SLE also require, in some instances, the use of toxic solvents and may be of less interest for some labs.

Solid-phase extraction (SPE) is a technique that can provide the most reliable results in terms of robustness and sensitivity. The technique consists of a series of steps with a catch and release of the target analytes upon a sorbent with a selective stationary phase. The sorbent catches the target analytes in a hydrophobic or ionic bonding mechanism, making it possible to wash the sorbent of matrix interferences. Next, the target analyte is released (and collected) by adjusting the pH or organic strength, effectively breaking the bonds between the analyte and the sorbent. SPE is available in various formats, such as µSPE or online SPE, to help the chemist attain the best possible results for their analysis, whether this is preconcentration and sensitivity or high throughput.

Some chemists think of sample prep as a costly affair in both time and money. However, put into the perspective of possible cost savings on the column side, the elevated robustness of analysis, and the higher sensitivity that can be achieved with the habit of preparing samples for chromatographic analysis, it is clear that sample preparation is both beneficial and strategically sound. At the end of the day, it is the individual chemist's professional choice whether sample preparation is needed or not, as it is the results of the analysis that will tell the story of the samples and not the materials used in the analysis.

Guard column and inline filters

There are easy measures that can be implemented to effectively increase the lifetime of the column instantly. Using a **guard column**, or at the very least an inline filter, will prevent some of the unwanted interferences introduced to the chromatographic system to get trapped before they reach the analytical column.

A guard column is a small/short column or cartridge packed with sorbent bonded with the same stationary phase as the main column. The guard column is used as a chemical filter as it will not only prevent particulates to enter the analytical column but also detain any chemical contaminants with a high affinity for the column phase. This is beneficial as the chemical contaminants could prevent the analytical column from effectively retaining the target analytes. A guard column is traditionally packed in the same or larger particle size materials as the main column and does not significantly affect the chromatography, as the guard column or cartridge are significantly shorter than the analytical column.

Thermo Scientific™ SOLA™ Solid Phase Extraction Cartridges

It is recommended to replace the guard column after every 200 injections on average, but the frequency may vary depending on applications and sample cleanliness. We will return to this topic shortly.

Inline filters are porous frits, placed in the HPLC fluidic path, and do not possess the ability to retain chemical contaminants, but they will remove any particulates over a certain size depending on the filter pore size. When faced with no guard column option, or if prevented due to legislations or method validations, an inline filter potentially can be used. These filters mostly consist of the same materials as the column hardware and will improve the robustness and lifetime of the column.

Washing and storage

Cleanliness is the key to life—quite literally. The cleaner you keep your instrument and columns, the more you prolong their lifetimes. Regular washes of the column after or between samples can greatly increase the longevity of your column. At a bare minimum, all columns should be washed before storage and stored in the recommended long-term solvent.

As an example, let's consider how to wash a silica-based, reversed-phase column before storage. First, wash away any buffers by running 10 column volumes with a mix of organic and pure water (e.g., running at your method's starting conditions without the buffer). The importance of removing buffers from the column, especially before storage, is that the buffer itself can degrade the column over time, either by precipitating onto the column or by hydrolyzing the phase and degrading the column's ability to retain analytes. After removing the buffer, it is also beneficial to ramp up and wash the column at 90% organic methanol (MeOH) or acetonitrile (ACN) and hold this percentage for 10 column volumes. This will help to wash out any contaminants that have not been purged from the column. Optimum storage for a C18 column lies around 70% organic mobile phase. After the wash, these conditions should be achieved before disconnecting the column and storing it safely in a box or on a shelf. All columns have a manual (column care guide) that specifies cleaning and storage conditions. Please make sure you follow the correct guidelines.

For analysis with highly sticky analytes, samples with challenging sample matrix, or matrices with a high level of contaminants, it may be ideal to consider a wash between each sequence of sample separations. This can be done by simply running a washing program similar to that described above for cleaning a column. For very dirty samples with little or no sample prep, there may be a benefit to include a washing sequence after each sample. This can be done by either holding the end of the gradient (highest organic solvent content) for a longer period or potentially by introducing stronger organic solvents to help purge any contaminants from the system before introducing the next sample onto the column. These requirements will vary greatly from method to method. If in doubt, contact your local Thermo Fisher Scientific representative for more detailed guidance.

Correctional measures

When working with chromatography, column degradation and column death is inevitable. There is no such thing as a column that will operate into eternity. When degradation or chromatographic challenges occur, having a system, or operational plan, in place that helps you discover the cause of that degradation will save your lab time, as you will quickly be able to assess what further action is required to get your analysis up and running again.

Some best practices are shared here to help you create your own system, or operational plan, and gain a stronger knowledge about your method. As chromatographic challenges occur, it is good practice to investigate what has happened to take corrective action as well as incorporate into future methods. Adding these investigation strategies early in the method development implementation can follow the method through its lifetime.

Investigation

In any investigation, a point of reference is needed. Traditionally in chemistry, we think of a calibration curve, or an internal standard, as a point of reference for measuring concentration. When we are investigating what is wrong with our column, we are less interested in concentration but can benefit from repetitive standard chromatograms. This means that if we have run a standard at the start of every sequence, when a peak starts to tail or broaden, we will know how much degradation has happened over time, or at what point the degradation started.

A log can also be kept with the instrument, noting all samples that are introduced to the system. Using the standard chromatogram as a reference point can help identify which samples may have introduced the contaminants to the column. Simply by running a standard sample and keeping a journal of samples introduced to the system, much of the groundwork for investigations can be done quickly.

Adjustments

It is always important to act on new knowledge and aspire to change our habits for the better. This also means that any new knowledge discovered by any investigation performed on the column, instrument, or method is implemented to prevent further challenges.

A good example of this is when to change a guard column. As vendors, we recommend that guard columns are changed at a certain frequency—every 200 injections. As sample cleanliness may vary from method to method, the frequency of changing a guard column may also vary. It is, thus, good practice to start a new method with a new guard column and exchange the guard column at an estimated number of injections. Then, if an investigation shows rapid broadening of peaks, adjust the frequency of the guard column exchange accordingly.

In summary

How to extend column lifetime:

- Use sample preparation when needed
- Use guard columns and inline filters
- Wash and store columns properly

Monitor column performance regularly and adjust the operational plan as needed.

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