Troubleshooting

LC Columns — The Top-10 List

Want to improve column life? Here are some ideas.

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Recently, someone asked me for some tips for the care and use of liquid chromatography (LC) columns, and I quickly listed 10. Feeling a little bit like David Letterman, I decided to prepare my own top-10 list. Several of the tips are essential and some are optional. For that reason, and because every reader is likely to prioritize them differently, I have deviated from Letterman's top-10 countdown format and listed the tips in no particular order.

1 Dedicate Columns

It is a common assumption that column selectivity for a given brand and model or part number is the same from column to column. This assumption is good for new columns, but it may be false after the column has been used for real samples. Columns can change in many subtle ways during use. Under low-pH conditions, some columns lose their endcapping. Many samples contain non-chromatographically mobile materials that stick at the head of the column, and other samples can contain strong bases that bind firmly to free silanol groups on the column packing. The list goes on and on. Because each LC method has somewhat different conditions and is used for a different sample type, the column changes that occur are unique to that method. After a column has been used in routine applications, it may no longer be equivalent to a nominally identical new column. If this same column is used for another LC method, subtle (or not so subtle) differences in the separation often can be observed. The best way to minimize such unexpected changes is to dedicate a column to a specific method. Furthermore, most workers find that they will use fewer columns over an extended time period if they dedicate columns rather than rotate them through several different methods until they are no longer useful.

Is it a hard-and-fast rule that a column should never be used for more than one method? Of course not. If a column was used for a short time in exploratory work with relatively clean samples, then there usually is no reason that the column should be discarded. However, if you develop a new method on a column with some use history, it is a good idea to test the separation on a new column of the same brand and model number before proceeding. I've seen numerous cases in which chromatographers have developed methods on a single column only to find that it is not representative of new columns of the same brand and model because of exposure to other sample types early in its use history. Chromatographers might have more justification to use expensive columns — such as chiral columns, which cost three to four times more than standard analytical columns — for multiple methods. If you take this route, be careful not to invest too much time and energy in a column that might not be equivalent to a new column.

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An alternative to dedicating columns is to start a new method development project with a new or like-new column. For a robust method, it is imperative that the column used in development can be duplicated with another new column. The safest way to ensure this outcome is to begin each method development project with a new column. Used columns can be useful for column screening, but as soon as a specific column brand and model is chosen, replace the used column with a new one.

2 Use In-line Filters

One of the least expensive ways to extend column life is to use an in-line filter between the autosampler

and the column. In my laboratory, the in-line filter is a standard part of every system, even if a guard column is in use. One of the most common reasons for failure of LC columns is development of excessive pressure. Most often, a pressure increase results from a buildup of particulate matter on the inlet frit of the column. In the old days, most columns came with a spare frit or

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two, and chromatographers routinely replaced the frit at the column inlet when the pressure began to rise. Most manufacturers no longer supply spare frits, and many workers are reluctant to remove the column-inlet fitting because doing so can disturb the column packing and degrade or ruin a column. An easy way around that problem is to use an in-line frit. A frit with a porosity of 0.5 µm is a typical choice because it is less porous than the 2-µm frit at the head of the column usually used with 3.5–5.0 µm diameter particles (3-µm-particle columns typically use 0.5-µm frits). In-line frit holders are designed for easy access; experienced users can shut down a system, change the frit, and resume operation in 10 min or less. If you don't use in-line frits already, I strongly suggest that you add one to each LC system.

3 Flush Columns Regularly

Unless you inject only very pure samples, unwanted materials will build up on the column after time. One of my colleagues refers to these materials as CRUD — chromatographically retained undesirable debris. Sometimes materials build up on the column and are not eluted under the method conditions but slowly change the characteristics of the column. In other cases, strongly retained compounds can be eluted in later chromatograms and result in extraneous peaks. If detector response to these materials is poor or if the retention times are very long, a rolling or bumpy baseline may result.

A simple fix for most of these problems is to flush the column regularly with a strong solvent. For isocratic separations, flushing is most convenient at the end of each batch of samples. If a buffered mobile phase is used, it is a good idea to replace the buffer with water so that a water–organic solvent mobile phase is used to flush out any residual buffer. Then go to 100% organic solvent. If you skip the water–organic solvent step, you might precipitate the buffer in the system, especially if acetonitrile is used as the organic solvent. Usually 10–20 column volumes (for example, 25 mL) of solvent is sufficient to flush the column. One easy way to incorporate the flush is to switch to strong solvent conditions at the end of the series of runs just before shutting down the system.

If gradient elution is being used, a stronger solvent flush is built into every run. This flush could be sufficient to keep the column clean, but it is a good idea to flush the column with 100% organic solvent before shutting down to remove any strongly retained materials.

What about backflushing? If the CRUD is strongly retained, it is likely to be held near the column inlet. In such cases, reversing the column before flushing will mean that the material will need to be moved only 1–2 cm instead of all the way through the column in the normal direction. Most silica-based columns can be reversed without detrimental consequences; if you are in doubt, consult the care and use instructions for the column.

4 Use Guard Columns

A guard column is simply a short column (for example, 10-mm long) that is packed with the same type of packing as the analytical column and placed upstream from the main column. If used properly, a guard column will protect the main column from degradation by catching any strongly retained or aggressive materials before they reach it. But guard columns can cause problems, as well. They often are poorly packed, so even though they may add 10% to the length of the column, the plate number of the combined columns can be less than the guard column alone. If the guard column is not changed before its capacity is exceeded, it can spill its collected contaminants onto the analytical column. Finally, if the system is flushed with a strong solvent while the guard column is installed, much of the CRUD collected by the guard column can flush onto the analytical column, thus negating the benefits of the guard column.

I'm not a strong proponent of guard columns, but they can be very effective if they are replaced regularly and flushed off-line from the main column. In some cases, a guard column can provide enough protection for the main column to reduce or eliminate the need for expensive and time-consuming sample cleanup steps.

5 Control Temperature

LC separations are sensitive to temperature. The general rule is that a 1 °C change in column temperature will change retention by 1–3%, which means that an LC system operating in a laboratory with a temperature that fluctuates 5 °C or more can show retention time shifts of 10% or more during the course of a run. This fluctuation can cause data-processing systems to miss or misidentify peaks in a run. In many cases, especially in gradient elution, changes in selectivity (relative peak spacing) are common when the column temperature changes. Temperature control also is essential for methods in which the equilibrium between the mobile and stationary phases is critical, such as in ion-pair chromatography. So a word to the wise: One of the easiest ways to improve retention and separation reproducibility is to control the column temperature. In my laboratory, workers don't like to risk the problems associated with column temperature changes, so every system is equipped with a column oven.

6 Avoid the Void (Volume, That Is)

The region of the chromatogram near the column void volume is an area fraught with problems. Unless the injection solvent is perfectly matched with the mobile phase, users will observe a baseline disturbance in the chromatogram. Whenever real samples are analyzed, the peak at the column dead time is likely to be off-scale, and it may take 1 min or more for the detector response to return to the baseline. When an LC system uses a mass spectrometer for detection, additional concerns arise about ion suppression, which more often than not occurs early in the run. All of these circumstances and more suggest that chromatographers will obtain a more reliable method if they select conditions so that the first peak of interest is not eluted with a retention factor of less than approximately two. A safe guideline for isocratic or gradient runs is to adjust conditions so that the retention of the first peak of interest is at least three times the column dead time.

7 Retire Early

One of the hardest concepts for many chromatographers to accept is that analytical columns should be considered disposable. In a business environment in which anything that costs more than $1000 is considered a capital item, how can we justify throwing away a $500 column before it has failed completely? Consider the context: A contract laboratory typically charges $50/sample or more, which suggests that this amount is less than the cost of in-house work for many companies. If a column is used for only 500 samples, the cost contribution of the column is 2% or less — certainly not a major portion of the cost.

How long should a column last? In my laboratory, analysts routinely inject 500–2000 samples before the column shows signs of failure. I strongly recommend discarding the column before it fails, because column failure can be so expensive. Just do the math. In the simplest of cases, a column might fail in the middle of a run, which would require the reinjection of the samples. A standard curve and quality control samples easily can amount to 10 injections or more in a batch of samples. At $50/sample (the low end of analysis charges), it costs more than the value of the column to run the standard curve. If sample preparation must be repeated, the costs of column failure will skyrocket. During method development, determine how many samples can be run before column failure, reduce this number by 10–20%, and replace the column after a predetermined number of samples, even if the results still look good. It isn't worth the risk of trying to stretch the column's usefulness for another batch of samples.

8 Ensure the Supply

Columns don't last forever, so sooner or later you'll have to find a replacement for the column you're currently using. You want to be able to purchase a column that gives the same separation characteristics as the one in use, but how do you guarantee that? Unfortunately, there are no guarantees, but you can improve your chances by testing more than one batch of packing material from the supplier during method development and validation. I like to stick with suppliers who have been making columns for years and will continue to be in business for many years to come. For some people this means sticking with the major brands. Others feel confident that smaller or specialty suppliers can provide the necessary continuity. Some companies require that every method be developed so that an equivalent column from another supplier is demonstrated to be satisfactory in case the primary vendor can no longer supply the column. In short, plan ahead. You'll be very sorry if you can't get a replacement column.

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9 Minimize Garbage

What is the easiest way to extend column life? Don't inject any samples. What is the next easiest way? Inject only clean samples. It is a very simple correlation: the cleaner the samples are, the longer the column will last. It's all about balancing the cost of sample cleanup with the cost of column replacement.

As an illustration, consider a drug sample in plasma. A simple protein precipitation cleanup can be achieved by adding acetonitrile to the plasma, vortexing, centrifuging, and injecting the supernatant. This sample is fairly dirty and could foul the analytical column in 100–500 injections, for a column cost of $1–5/sample. A much cleaner sample can be obtained by using a solid-phase extraction (SPE) procedure, which could yield column lifetimes of 1000–2000 injections ($0.25–$0.50/sample for the column). In the 96-well format, SPE cartridges cost $2 or more per well and could require an investment in a $70,000 robot to process the samples. Is the gain in column lifetime worth the added cost of sample preparation? Strictly from a cost standpoint, the precipitation technique is more economical, but many workers will choose the more involved cleanup because of higher method reliability. In any event, there is a tradeoff between sample cleanup costs and column life.

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10 Pure is Good

Although the focus on column contamination usually focuses on dirty samples, don't forget that the reagents also can contribute to column contamination. Be sure to use the best reagents available (generally high performance liquid chromatography [HPLC]–grade). I recommend that all mobile phases be filtered through 0.5-µm or smaller porosity filters before use unless they contain only HPLC-grade solvents and water, which typically are filtered to 0.2 µm already. As everyone who has encountered contaminated water in a gradient elution application knows, it takes only trace levels of contaminants to build up into extraneous chromatographic peaks.

Conclusions

If you follow my top-10 list, will your column problems be history? Nope. But if you consider each of these items and the effect each may have on your particular LC application, you are likely to have fewer problems in the future. Like so many things in life, it's all about balance. Every one of these items costs you something in time and effort; is it worth it for your application? Only you can decide, and your top-10 list might be different from mine.

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