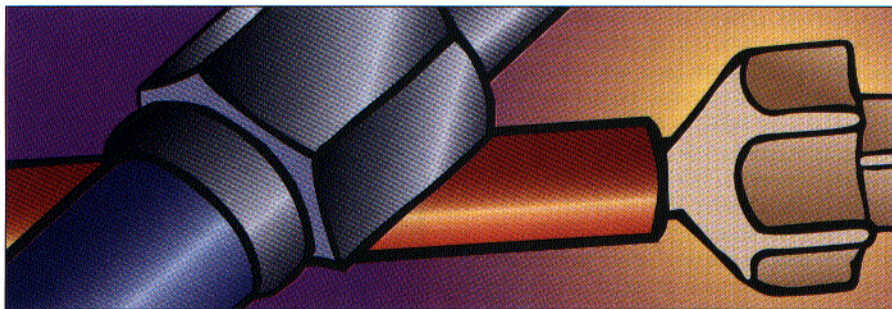


LC Troubleshooting



Useful or Useless? — Part I

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Is it an old wives' tale or something to enhance a method?

This month's "LC Troubleshooting" column is the first of a two-part series that examines a number of practices that exist in liquid chromatography (LC) methods. The topics were chosen from common questions asked in short courses and submitted to me by readers. Some of these practices are holdovers from the early days of LC, when columns were more unstable; however, others make sense to use in any system configuration.

SATURATOR COLUMN

Q: It is a policy in our laboratory that each LC system has a saturator column installed for use with every method. It seems to me that this procedure is unnecessary and, in some cases, actually may be counterproductive. Can you clarify the use of these columns?

A: A saturator column, sometimes called a *precolumn*, is placed between the pump and the autosampler to pretreat the mobile phase when the mobile phase is likely to cause premature column failure. Most silica-based LC columns are stable at pH levels as high as 8. When the pH exceeds 8, the silica dissolves with obvious adverse effects on the column. Newer columns and special bonded phases impart column stability to much higher pH values than historically seen. Densely bonded stationary phases, special stationary-phase chemistry, and extensive endcapping all contribute to better stability at high pH. For example, some manufacturers publish applications at pH 9 or higher (1). Analysts can use polymeric packing particles or inorganic packings such as zirconium as alternatives for column stability at high pH.

When operating with high-pH mobile phases, some workers use a saturator column to help extend the life of the analytical column. A saturator column is just an old column of the same type as the analytical column placed between the pump and the autosampler. To minimize additional back pressure, analysts typically use a 15-cm or shorter saturator column. The principle is that the mobile phase will attack the saturator column first, pretreating the mobile phase so it will be less aggressive on the analytical column. Indeed, chromatographers observe that the packing in the saturator column dissolves over time, and most users claim extended analytical column life. As the packing dissolves in the saturator column, the particles become smaller, and eventually some particles leak through the 2- μ m porosity frit at the end of the saturator column. It is important to install a 0.5- μ m in-line filter between the saturator column and the autosampler to trap these fine particles or they can enter the autosampler, where they can abrade the injection valve rotor seal.

Should you use a saturator column? Using a saturator column at pH levels lower than 8 provides no advantage — the silica is stable under those conditions. In fact, use of a saturator column at low pH is a liability. Higher back pressure caused by the saturator column will force analysts to use lower flow rates to maintain a reasonable system pressure. Lower flow rates, in turn, mean longer run times and, thus, increased analytical costs. Another liability of the saturator column is the chance that partially dissolved fine particles of silica will block tubing downstream or damage the injector. At pH levels higher than 8, analysts can support a better argument for using a satu-

rator column, but with the more pH-stable silica packings and nonsilica stationary phases, I can see little justification for the saturator column at high pH, either.

IN-LINE FILTERS

Q: When should I use an in-line filter in my LC system? If I use a guard column, isn't an in-line filter just redundant?

A: The in-line filter is a frit mounted in a holder and used to trap particulate matter in the mobile phase. In the ideal LC system, the mobile phase and samples will be filtered, so no additional filtration is needed. Unfortunately, the mobile phase seldom is free of particulate matter by the time it reaches the column. Dust from the laboratory settles in the mobile-phase reservoir, and it isn't always stopped by the inlet line frit. Many workers don't filter every sample — the added work and expense just isn't worth the trouble. Sample filtration requires an additional step to validate when a method is developed. Finally, some particulate matter can come from within the system. For example, wear on pump seals and injector rotors can put small particles of polymeric material in the mobile phase.

No matter what the source of particles in the mobile phase, the frit at the head of the column usually traps them. When the column frit is blocked, peak distortion and high system pressures can result. Changing the column frit risks disturbing the column packing and, thus, ruining the column. Analysts can use an in-line filter slightly downstream from the injector as a more cost-effective solution. A 0.5- μ m frit is used in the filter. This frit traps any material that normally would end up on the 2- μ m column frit. The frit in the in-line filter is easier to change than the column frit — requiring just a couple of minutes — and does not risk damaging the column.

I strongly recommend the use of an in-line filter on every LC system, immediately after the injector, even if a guard column is used. The filter is one of the least expensive insurance policies that you can purchase for your system. I would hesitate using an in-line filter only with an extremely low-dead-volume LC system in which extracolumn volume must be minimized.

GUARD COLUMNS

Q: None of our LC methods use guard columns. From what I read, it seems to me that a guard column would pay for itself very quickly by extending the useful life of the analytical column. Shouldn't we routinely use a guard column?

A: At first glance, the guard column does seem to be an essential part of the system. The guard column acts as a filter in at least three

ways. First, the frit at the head of the guard column usually matches the one at the head of the analytical column — 2- μ m porosity. Thus, any particles that might be caught at the head of the main column will be caught on the guard column instead. But if you use an in-line filter, it has done this job already and with a smaller porosity frit.

A second use of a guard column is to act as a chemical filter. Strongly retained materials that can foul the main column are removed by the guard column instead. In my mind, this practice is the only legitimate use for a guard column. But it also raises several questions. Why not spend more effort at sample cleanup so that your samples don't contain garbage that fouls the column? As noted below, the cost of the column may be very small in relation to sample preparation, so use of a guard column may be more cost-effective than extensive sample cleanup.

How long does the guard column last? This question is perennial, and the answer varies, depending on the application. About the only way I know to determine guard column life is to look at the separation with and without the guard column. If it is worse with the guard installed, the guard column is worn out. Most users replace guard columns based on time (for example, once a week) or number of injections rather than try to establish a system-suitability requirement for the guard column.

How is the guard column cleaned? Most users ignore this question and just flush the guard column along with the analytical column at the end of a series of runs. This practice can be counterproductive because the strongly retained materials from the guard column are flushed onto the main column. This procedure defeats the purpose of the guard column's intended use — preventing contaminants from reaching the analytical column. The proper way to flush the system is to remove the outlet end of the guard column and flush it to waste before flushing the analytical column. This procedure is not conducive to automated flushing in the middle of the night at the end of a series of runs. Another alternative is to throw the guard column away before flushing the system.

Finally, the guard column can act as a saturator column, dissolving over time to protect the main column. This strategy is not always good because a void can form in the guard column and can compromise the chromatography. If a saturator column is required, analysts should mount it upstream from the injector as described above.

So you can see that the use of a guard column requires much thought. In my laboratory guard columns are used only to protect very expensive columns. For example, a chiral column may cost four times as much as a traditional reversed-phase column. When using samples of biological origin such as plasma that can foul the column quickly, we often use a guard column to help protect our investment. For more traditional applications, we rarely

use a guard column. The choice is yours — I don't feel there is economic justification in guard column use for most applications.

FILLING VOIDS

Q: When a column develops a void, I hate to throw it away. What is the best way to fill the void?

A: For most applications, filling the void at the head of the column doesn't make very good economic sense. Let's examine a few of the factors. Column voids form for three primary reasons. First, if the particles are exposed to excessive pressure shock, they can break and settle in the column, creating a void. Second, something in the mobile phase or sample can dissolve the packing in the column, leaving a void. Finally, if the column is not packed well, it can settle and form a void. The first two causes are most common today. In both of these cases, fracturing or dissolution, the surface chemistry of the packing changes. Even if the void is filled successfully, the chemical changes are not restored, so the likelihood of recovering the same separation chemistry is limited. The third cause, settling, is a historic problem that modern columns have eliminated for the most part. Column settling happened commonly 15–20 years ago, when perhaps 10% of the columns failed during shipping. When this kind of a void formed, no change in the chemistry occurred, just a hole in the column, so filling the void often restored the column to normal service.

Another factor to consider before embarking on a void-filling project is the economic impact of the column on the cost of analysis. For most pharmaceutical companies, it costs \$50–100 per sample for analysis. If a \$500 column is used (the high end of cost) and a worker can analyze 500 samples before the column must be discarded (the low end of use), it costs approximately \$1/sample for the column. This amount is less than 2% of the overall analytical cost. Where are the other major costs? Sample pretreatment easily can cost \$10/sample (\$2 per solid-phase extraction cartridge plus reagents, labor, and sometimes a \$70,000 robot to process the samples). Of course the majority of the cost is labor in sample handling, data processing, and report writing. So in the big scheme of things, it doesn't make much sense to try to extend the column life to save a fraction of a percent of cost.

When does filling a void make sense?

When the column cost is much higher, such as for chiral or other specialty columns, void filling can be justified. And, of course, when you are desperate — no backup column is available and the samples *have* to be analyzed *now*.

Traditional void filling involves removing some packing material from an old column of the same type, slurring it with methanol or acetonitrile, and filling the void using a spatula or pipette. The frit is replaced, the endfitter rinsed, and the column is placed back in service. The problem with this technique is that the fresh packing often settles, requiring

another round or two of void filling before the column is stable. Remember that this procedure fills only the hole in the column and may recover the loss in plate number because of reduced mixing at the head of the column. Filling the void does not necessarily restore the chemistry changes in the column that can result if the column packing dissolves or is crushed.

An alternative technique of void filling is more successful. In this technique, the void is filled as described above, but then the column is reversed before reinstallation. The workers describing this technique report that it does not have the problem of packing compression encountered with the traditional technique (2).

CONCLUSIONS

We can conclude that *it depends* is the appropriate answer for most of the questions posed this month. Saturator columns, for the most part, are a holdover from the days when LC columns were more unstable than they are today. New packing materials and bonded-phase chemistry have extended the pH range for modern columns, so saturator columns should be reserved for the few cases when they are really necessary. In-line filters are inexpensive insurance for LC systems, and I strongly recommend them for use in every application. However, in-line filters can add unwanted dead volume for applications of microbore or very small particle columns in which any volume outside the column adds band broadening. The use of a guard column is a matter of choice. In principle, the guard column should add life to the analytical column, but only if it is used properly. The guard column needs to be flushed separately from the analytical column and replaced before it spills trapped contaminants onto the main column. Finally, filling a column void generally is an unwise time investment. However, as with the other techniques discussed this month, filling the void makes sense to extend the life of an expensive column or to get you out of a jam when you don't have a backup column.

REFERENCES

- (1) L.R. Snyder, J.J. Kirkland, and J.L. Glajch, *Practical HPLC Method Development* (John Wiley & Sons, New York, 1997, 2nd ed.), pp. 193–205.
- (2) J. Vendrell and F.X. Aviles, *J. Chromatogr.* **356**, 420 (1986).

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