MSVision

Dedicated to Mass Spectrometry

MS VISION TECH TIPPS SERIES
PART IV — HOW TO CLEAN YOUR LC COLUMNS

In this white paper we will talk about how to clean your LC column when you observe persistent leakage of contaminants. In LC-MS the columns for separation are usually the single most expensive consumable and proper treatment can significantly increase their lifetime.

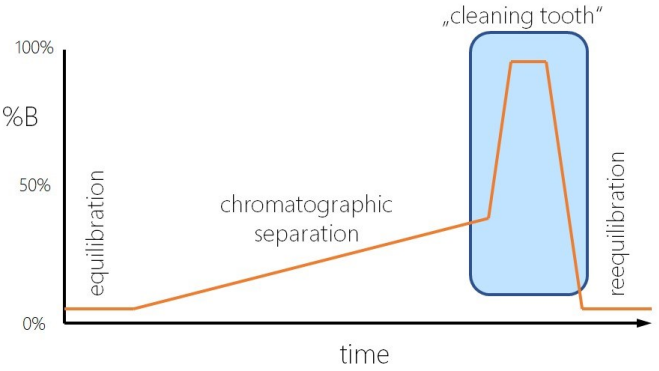
Let's start with what an LC column is. Typically a reversed phase LC column (or RP column) consists out of three parts: an outer metal housing, usually steel, and a two component filling consisting out of small porous silica balls which are covered on their (inner and outer) surface with a thin hydrophobic layer. Whereas the silica is basically building the framework to maintain the structure and increases the surface, the hydrophobic layer is where the magic happens.

These layers are created by chemical modification of the silica surface using silanes or silanoles. Attached to the silicium of the silanes is a carbon chain (C_4-C_{30}, most commonly C_{18}, sometimes also Phenyl or other specialties). As these silanes are usually bulky and can not modify every silicium atom on the silica surface (and should not, otherwise the surface would be extremely hydrophobic), nowadays commonly a second modification step, the endcapping is used. This can be hydrophilic (containing alcohol or amide residues) or hydrophobic (using alkyl residues) to finetune the column materials properties.

I do not want to get into further detail on LC column chemistry at this point, the important point is that we deal with chemical structures containing Si-O and Si-C bonds in this case. Of particular importance is the Si-O bond as that one is not very stable against basic solutions which is the reason why LC columns are commonly used in the acidic and not in the basic pH range. Over time, one would simply destroy or dissolve the silica material. But very acidic conditions are also not ideal for the column material, the alkyl chains can be removed from the silica over time as well.

So, silica based LC columns should ideally be operated in the range between pH 3 and 8. Due to that, material which is poorly soluble under these conditions can sometimes deposit on the column and bleed over time. The same thing can happen when the analytes are generally not well soluble in the used running buffers.

For the latter reason, the first good habit is to put a cleaning "tooth" at the end of the LC gradient where the percentage of buffer B is increased to create high solvent strength and to remove sticky analytes. After 1-2 minutes the %B can be reduced again and equilibration can take place:



The actual height of the cleaning tooth usually is set to around 95-98% B before it goes back to the starting concentration.

Remark: the reequilibration time looks rather short in the picture. Please note that for proper equilibration you will need 10-20 column volumes of solvent. If you calculate this properly, reequlibration of a 2mm ID/5cm column being operated at 0.8ml/min takes about 2-3 minutes. BUT: when you go to nanoLC with typically 75µm ID/15 cm column and 200nl/min flow rate, proper equilibration to starting conditions takes 30-45 minutes! Did you ever observe unstable gradients in proteomics? That might be the reason why! By the way, the same applies to HILIC, which also is very critical with regard to reequilibration.

Nevertheless, the more nasty your sample matrix is, the more contaminations will accumulate on your column. Therefore a golden rule to start with is that the sample has to be completely soluble in your buffers starting conditions. To test this, you can simply create a mock sample, mix it with your solvents and see what happens. The example below is from a troubleshooting at a customer site where unstable signal heights and frequent clogging of the LC injector valve during the analysis of a specific method was observed. We just diluted the sample (prepared in neat ethanol) in the starting solvent in a vial and observed what happened:

After a short while a precipitate formed, which likely was something extremely hydrophobic (similar to the analytes in this case, perhaps it was even the analytes) and turned the solution on the left opaque. Precipitation will then occur anywhere in your system between injection loop and column head as it is not formed spontaneously but with some delay. This may cause irreproducible peak heights (as you can hardly control the precipitation), peak broadening (because the starting point for the separation is not defined at the column head but anywhere from injector to column head), and if not completely dissolved during the LC gradient, sooner or later complete clogging of your system either at the injector or at the column head.

In another white paper we discuss ways to clean your system hardware, e.g. by injection of DMSO as a very strong solvent. Similar approaches can be used to clean your LC columns. First, disconnect the detector (UV, DAD, MS, whatever) from the LC column. Once the junk get's off the column, you do not want it to end up in your MS for sure! Then turn the column against the specified flow direction. We do this as the contaminations usually accumulate on the head of the column, so the path to bring them out is shortest when we turn it around. Make sure that the column has frits on both sides ("normal" LC columns do,

Self packed columns in most cases cannot be treated this way (but usually it's not worth the effort here anyway because of low costs)! Reduce the flow rate to ~25-50% of the typical flow rate you use (just to ensure not to strain the column to much).

Then clean the column with a solvent cascade such as (fresh and peroxide free!) THF > Isopropanole > Acetonitrile > 95%Water/5%ACN. You might additionally inject 20-100µl of (clean and fresh!) DMSO or DMF through the injection valve several times. Make sure all solvents are clean and of chromatographic quality, otherwise you might bring in new contaminations again. Use ~30-60 minutes per step to ensure enough column volumes have been flushed through and the previous solvent was properly exchanged (remember the remarks on the reequilibration! Same applies here at reduced flow rates). You can also let the solvent rest for some time in the column to allow contaminants to dissolve properly. After everything has been flushed through, turn the column back again and run a few blanks to properly requilibrate it and to make sure no "special" solvents remain in the column affecting your separations. If you have the chance to set up a standalone quarternary LC system for this you could even do it in an automated fashion over weekends.

If after this cleaning process there is still significant background it's probably time for new column. Before you use the cleaned column for your analyses, make sure it's surface is properly passivated again. Depending on your method it might be necessary to inject e.g. a couple of serum blanks/mock samples to create the column conditions you need for your method as ideally such a coating has been fully removed by the cleaning process as well.

Good luck with your cleaning exercise!