



Welcome to the November 2006 issue of our monthly newsletter. This month we have a Tech Tip on the key factors affecting resolution in HPLC.

Next month in the Christmas edition of the Newsletter, the focus will be on care and regeneration of GC capillary and packed columns.

On-going technical tips explained in the monthly Newsletter will cover both liquid and gas chromatography areas and will focus on areas which are pertinent to the practising chromatographer. The tips will be always focus on simple modifications you can make to further optimize your instrument.

[More about Gerard Sharp](#)

Tech Tip

Care of GC Capillary Columns

There are three main factors which will shorten the life of a capillary column. These are:

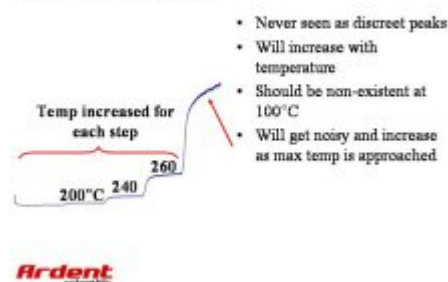
- Temperature
- Oxygen
- Sample

There are cases of capillary columns which have lasted longer than 10 years as long as the column has been treated well. The next few sections provide a few simple tips to prolong column life.

Low Temperature

The best practice with regards to temperature is not to use excessively high temperature if this is not required. The column must be ramped to a temperature which will elute all the components from the sample before the next injection. However, excessive temperature will lead to column bleed (as shown in this diagram) and this is caused by breakdown and loss of stationary phase. This is irreversible damage of the column phase. A consideration often overlooked with EPC / EFC (electronic pressure or flow control systems) is to use

Typical Bleed profile



a higher flow (higher inlet pressure) to push the unwanted components out of the column faster. This can reduce the maximum method temperature and reduce column deterioration.

Oxygen Free

Oxygen is the biggest enemy of capillary columns, and especially polar columns like a WAX column. Oxygen breaks down the stationary phase into smaller more volatile components and these components elute from the column causing a higher background signal - bleed. The easiest way to protect the column is to use a high capacity oxygen trap and preferably also an oxygen indicating trap (on the cylinder side of the carrier gas line).

Another important point, which often catches people out, is to purge all the oxygen from the column before heating. It is recommended to leave the column at room temperature and purge with oxygen-free carrier gas for at least ten minutes and then it is OK to heat the column.

Clean Samples

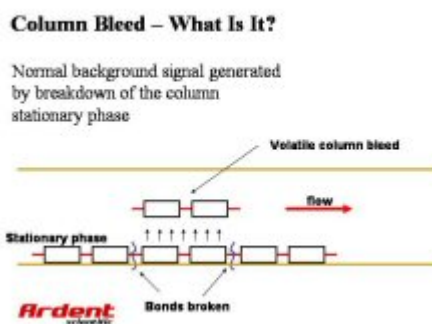
The best way to extend the life of the column is to inject clean samples i.e. samples containing only volatile components. Any non-volatile material will collect at the front end of the column and eventually lead to altered retention times and tailing peaks. The use of wool in the liner can protect the column by acting as a filter for the non-volatile material.

If injecting aqueous solutions, the pH of the column with polysiloxane based phases should be between 2-8. Alkaline samples especially will break down the stationary phase and produce bleed and eventually loss of resolution through loss of phase.

Excessive derivatizing agents can react with the stationary phase and change the selectivity of the column. This can lead to peak retention time movement. Derivatizing agents should be completely removed from the sample prior to injection.

Column Bleed

Column bleed is the breakdown of the stationary phase caused by oxygen and heat (and often the combination of both). It is seen as a higher background signal as shown in this slide. The stationary phase will break down equally along the whole length of the column and therefore the breakdown (volatile) products elute continuously from the phase. There is no chance for the breakdown products to group and so bleed is seen as a continuous signal rather than a peak of collection of peaks.



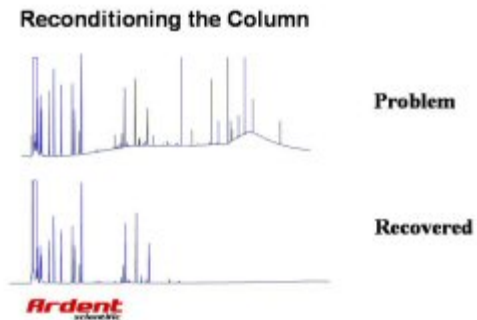
For GC-MS users, the most common ions associated with column bleed are 207 and 281 m/z ions. These ions are associated with cyclic siloxane structures which form as the polysiloxane chain backbites on itself.

Reconditioning the column

There are three ways to regenerate a column once the baseline bleed has become unacceptable or if peaks have begun to tail

- Baking
- Cutting the front section
- Rinsing

It is common practice to condition (bake) a column when the baseline bleed becomes unacceptably high or there are humps in the baseline as shown in the chromatogram in this diagram. All capillary columns will have a maximum operating temperature but rather than condition the column at this temperature, it is better practice to condition at 20°C above the method temperature as long as this is below the maximum allowable temperature of the column. For example, if the maximum temperature of the column is 320°C but the top temperature of your method is 250°C, then condition the column for one hour at 270°C. The column will last longer. The column will condition faster if the column direction is reversed - turn the column around. Sample residue at the front end of the column will now only have to travel a short distance to be completely removed.



Cutting between 0.5 - 1.0 metres from the front of the column will remove sample contamination if the baking hasn't been able to do this. A use of a guard column which is 5 metres of deactivated uncoated fused silica joined to the analytical column will save money and save the more expensive analytical column. Also, by cutting the guard column, retention times will stay the same as long as the new column length is adjusted in the software because there will be no loss of stationary phase.

Rinsing the column is the last option but can be very effective. There are commercial apparatus available for this. The column is rinsed with several column volumes worth of solvent (about 5mL for a 30m x 0.25mm column). The solvent should be the one that is used for samples or alternatively rinse with hexane, acetone and ethanol in this order. These solvents vary from non-polar through to polar and are effective.

Packed Columns

Packed columns are easier than capillary columns because sample contamination is the main cause of column deterioration and this collects on the first few centimeters of the column. Regenerating the column is simply a matter of removing the quartz wool and old packing by shaking this out of the column and replacing with fresh wool and packing. Ensure the new packing is free of voids by shaking continuously while filling the column.

Chromatography Training

The 2006 training program is almost complete but stayed tuned for next year's program with some new courses. There is still time for on site training in the lead-up to Christmas.

Click on the links below for further information and prices and the modules from each course can be customized and packaged for on-site courses e.g. a mixture of GC and HPLC training.

- [GC Beginner Course](#)
- [GC Intermediate Course](#)
- [GC-MS Beginner Course](#)
- [HPLC Beginner Course](#)

For more details and course objectives etc, [click here](#).



On-site Training

Alternatively, for three or more staff, on-site training can be a very cost-effective option. The benefit of on-site training is that the focus is your application on your instrument and the training modules can be customized to suit your needs. On-site courses are offered across Australia. [For more information click here](#)

Featured Products

[GC inlet liner recycling service](#)



Ardent Scientific offers a complete GC inlet liner recycling service including cleaning, re-packing with quartz wool (where applicable) and in-situ deactivation. Price is \$5 / liner (minimum of 30 liners) + \$15 freight.

[GC fixed wool inlet liners](#)



For the months of November and December, all deactivated fixed wool liners will be **50%** off usual price

View the full range of Ardent Scientific products [here](#)