Ardent scientific | Chromatography Training & Products

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 optimise your instrument.

More about Gerard Sharp

Tech Tip

Factors Affecting Resolution in HPLC

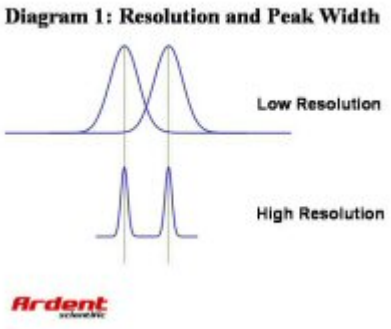
There are essentially three factors in chromatography which can be changed to gain better resolution. These are:

- Retention
- Selectivity
- Efficiency

Retention can be altered through a change in the mobile phase and the stationary phase. We can change selectivity by changing the nature of the column (stationary phase) and the solvent (mobile phase). Efficiency is a function of the column flow rate, particle size, column length and is measured by theoretical plates.

Defining Resolution

Firstly, we should define what we mean by resolution. Resolution is a function of the retention time difference of the two peaks and peak width. Resolution can be improved by either moving the peaks further apart or by decreasing the peak widths. This is graphically demonstrated by the two chromatograms shown in Diagram 1.

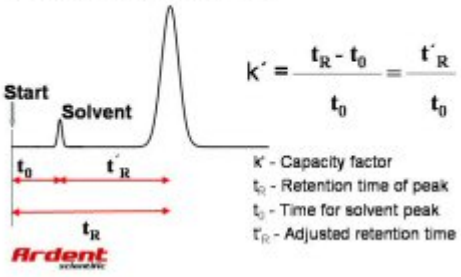


Changing Retention

The easiest way to change retention of a component is to change the mobile phase strength. For reversed phase separations, this is a matter of decreasing the amount of

organic modifier in the mobile phase. A decrease in the organic modifier by 10% will have a 2-3 factor increase in the retention factor (k'). The retention factor is defined in Diagram 2 and takes into account the time an unretained component takes to travel between the injector and the detector. It is a true measure of the retention of a component in the stationary phase. If the retention factor is less than 5, then changing the retention factor is the the first thing that should be tried as this can dramatically improve resolution. The relationship between Resolution and the retention factor is shown in Diagram 3. As can be seen, increasing retention is more important for the early eluting peaks with a smaller retention factor.

Diagram 2: Retention Factor - ideally between 1 and 5



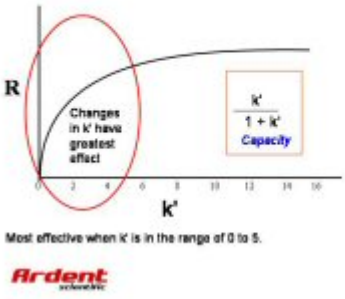
Changing Selectivity

Changing the mobile phase mix will also affect the selectivity (α)of the separation. this is not so predictable and mostly requires trial and error. As the mobile phase is changed the difference in the chemistry of the components in the mixture will result in different degrees of retention in the column and hence will aid separation.

Changing Efficiency

Efficiency is measured by theoretical plates (N) and the efficiency of the column can be found on test chromatograms provided with the new column. The higher the plate count, the better will be the separation. There are a few ways to increase the efficiency of the column and gain resolution.
Doubling the column length will double the plate count. Unfortunately the gain in resolution is only 40% (see equation below).

Diagram 3: Resolution vs Capacity



Decreasing the particle size will increase resolution. This is the trend of column producers. 20 years ago the common particle size was 10µm. Now it is 3.5-5µm and sub 2µm particles becoming more common. The trade-off with smaller particles is the resistance in the column is higher and more back pressure is generated. Manufacturers of HPLC equipment have addressed this by pumps and other components capable of handling the higher pressures.

The sub 2µm particles columns can also be used to provide a higher plate count per meter but packed in a shorter length column. Shorter columns means shorter run times. For example, a 150mm x 5µm particle size column provides about the same plates as a 75mm x 3.5µm particles size. The 75mm will elute the peaks in half the time without a loss in resolution.

Resolution Equation

Resolution: $\mathbf{R_s} = (1/4)[(\alpha-1)/\alpha](N)^{1/2}[k'/(1+k')]$

k' = the average of two peaks

For those more mathematically suited, the relationship between resolution and the various factors is described above.

Upcoming Chromatography Courses

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 customised 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 customised to suit your needs. On-site courses are offered across Australia. For more information click here

Featured Products

View the full range of Ardent Scientific products here

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 October and November, all deactivated fixed wool liners will be **50%** off usual price