Review Article

Fundamental Chromatographic Parameters

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ABSTRACT

HPLC plays a vital role in product assessment, research and environmental monitoring. HPLC is suited to separate higher molecular weight compounds in order to give quantitative and qualitative information. HPLC separatory systems, chromatographic separations are characterized by the resolution, retention time of analyte peak, selectivity and efficiency (plate number). HPLC separations are effected with liquid mobile phases following through a column packed with a solid stationary phase. This article briefly describes the theory and equations behind many of the concepts that drive chromatography in pellucid and simple way of essential chromatography concepts such as efficiency, retention factor, selectivity, resolution, pressure, Van Deemter curves and gradient equation.

Keywords: Efficiency, retention factor, selectivity, resolution, chromatographic parameters.

INTRODUCTION

HPLC is just the premier technique for trace analysis of organic and inorganic compounds. If I think of all the work which has being done in pharma/biochem, environmental, forensics/toxicology, industrial and food safety all these things are done routinely and rapidly by HPLC.

Essential chromatographic parameters

We start with fundamentals of performance:

1. Efficiency
2. Retention factor
3. Selectivity
4. Resolution
5. Pressure

These are all key to understand how to optimize results and successfully develop methods.

We also explore a few more complex concepts:

1. Van Deemter curves
2. The gradient equation

These two topics are also important for method development.

Efficiency (N)

Column efficiency is used to compare the performance of different columns. It is probably the most frequently cited parameter of column performance and is expressed as the theoretical plate number, N.

Efficiency Retention time Peak width at base

$$N = 16 (t_R/W_t)^2$$

Equation 1a: Efficiency equation

Retention time Peak width at half height

$$N = 5.54 (t_R/W_{1/2})^2$$

Equation 1b: Alternate equation for calculating efficiency

Columns with high plate numbers are more efficient. A column with a high N will have a narrower peak at a given retention time than a column with a lower N number.

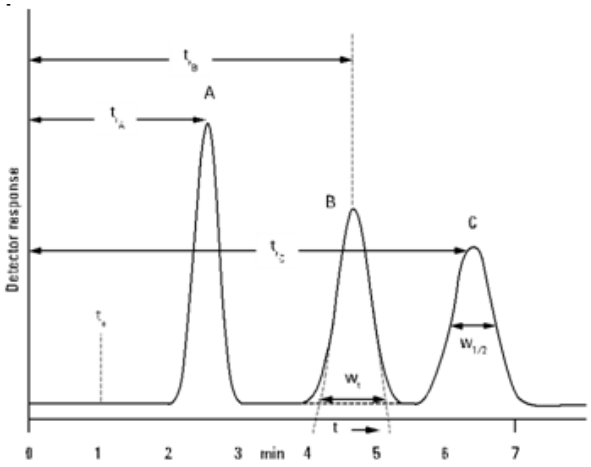


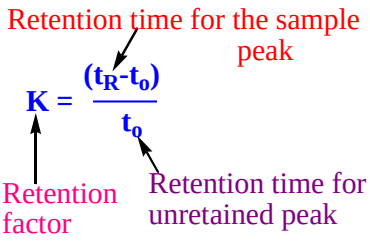
Figure 1: Chromatographic illustration of efficiency, retention factor and resolution

If we measure the distance t_w here (**Figure 1**), by drawing tangent lines to approximate the four - sigma peak width, we can measure the theoretical plates for peak B, using **Equation 1a**, $N = 16 (t_R/t_W)^2$. Sometimes the four - sigma peak width is difficult to measure (e.g., with a noisy baseline), so an alternate equation (**Equation 1b**) involves measuring the peak width at half - height ($w_{1/2}$): $N = 5.54 (t_R/w_{1/2})^2$.

High column efficiency is beneficial since less selectivity is required to completely resolve narrow peaks. Column efficiency is affected by column parameters (diameter, length, particle size), the type of eluent (especially its viscosity), and flow rate or average linear velocity. Efficiency is also affected by the compound and its retention. When comparing columns, the number of theoretical plates per meter (N/m) is often used. However, the same chromatographic temperature conditions and peak retention (k) are required for the comparision to be valid. On stationary phases where α is small, more efficient columns are beneficial.

Retention Factor (k)

Formerly referred to as capacity factor or k´ (k prime), the retention factor measures the period of time that the sample component resides in a stationary phase relative to the time it resides in the mobile phase. It is calculated from the retention time divided from the time for an unretained peak (t_0).



Equation 2: Retention factor equation

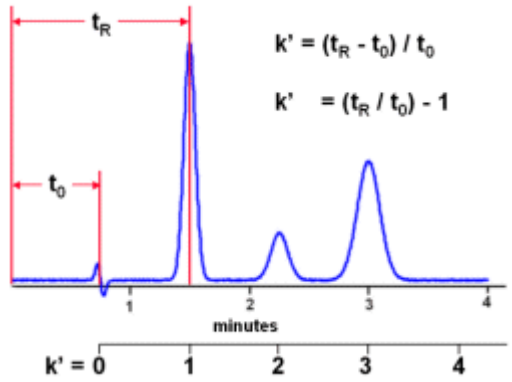
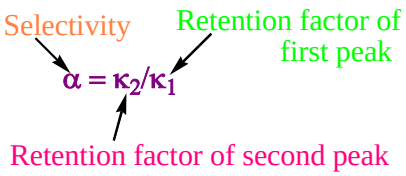


Figure 2: Chromatographic illustration of retention factor

Selectivity or separation factor (α)

The separation factor is a measure of the time or distance between the maxima of two peaks. If α = 1, the two peaks have the same retention time and co-elute.



Equation 3: Selectivity equation

Selectivity is defined as the ratio in capacity factors. In **Figure 1**, you will see that there is better selectivity between peaks A and B than between B and C. Calculations are provided to demonstrate.

Selectivity can be changed by changing the mobile phase constituents or changing the stationary phase. Temperature may also be a factor in adjusting selectivity.

Resolution (R_s)

Resolution describes the ability of a column to separate the peaks of interest, and so the higher the resolution, the easier it is to achieve baseline separation between two peaks. Resolution takes into consideration efficiency, selectivity and retention, as can be seen in **Equation 4**. One can improve resolution by improving any one of these parameters. Figure 3a shows the impact of efficiency, selectivity and retention on resolution. As a matter of fact selectivity may be the major effective tool for optimizing resolution.

$$Rs = \frac{\sqrt{N}}{4} \frac{(\alpha-1)}{\alpha} \frac{k}{(k+1)}$$

Equation 4: Resolution equation

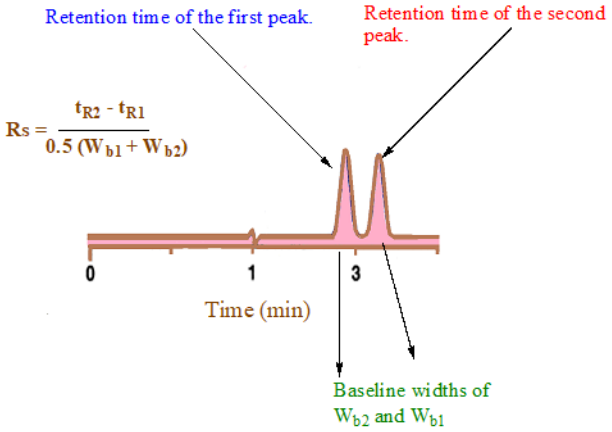


Figure 3: Chromatographic illustration of resolution

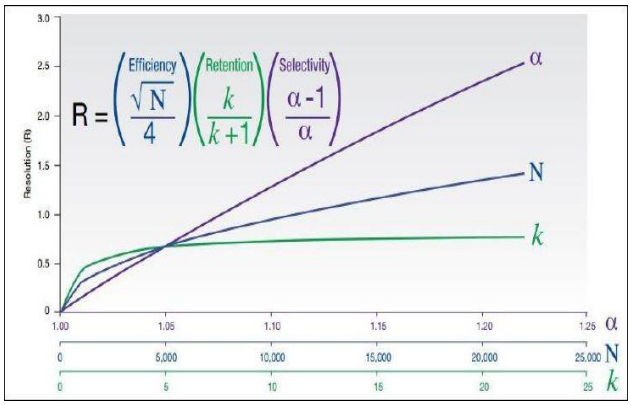


Figure 3a: Impact of selectivity, efficiency, and retention on resolution

In **Figure 4**, we see the different effects of each component on the separation process. All of these terms show a diminishing return. This means that the more you

try to work on something to improve the separation, the less effective it will become.

If you double the column length, you will obtain more theoretical plates, but your separation will take twice as long; you will only get a square root of 2 or 1.4 improvement in the resolution.

A value of 1 is the minimum for a measurable separation to occur and to allow adequate quantitation. A value of 0.6 is required to discern a valley between two equal - height peaks. Values of 1.7 or greater generally are desirable for rugged methods. A value of 1.6 is considered to be a baseline separation and ensures the most accurate quantitative result.

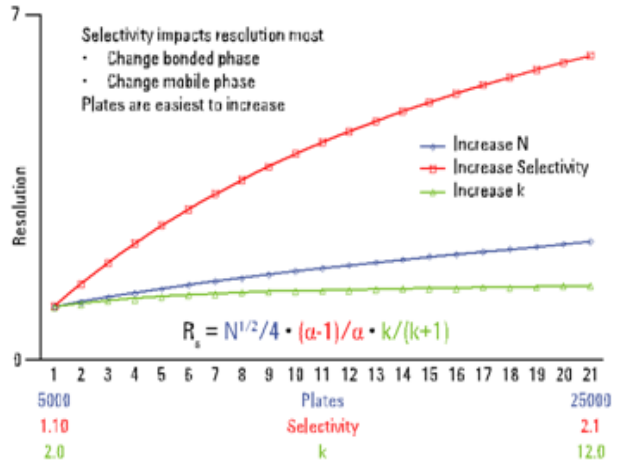


Figure 4: Resolution as a function of selectivity, column efficiency or retention

Tailing factor and Asymmetry factor

If the peak to be quantified is asymmetric, a calculation of the asymmetry would also be useful in controlling or characterizing the chromatographic system. Peak asymmetry arises from a number of factors. The increase in the peak asymmetry is responsible for a decrease in chromatographic resolution, and precision. The peak asymmetry can be calculated by using formula:

Tailing factor

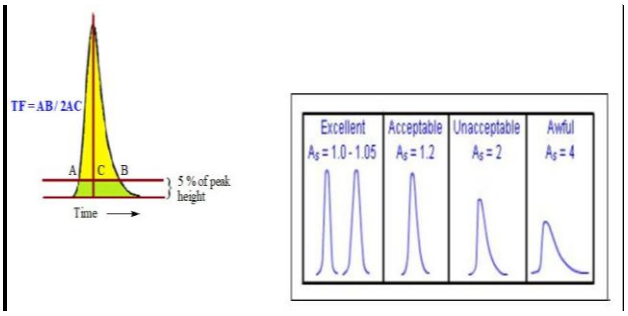


Figure 5a: Chromatographic illustration of tailing factor

Where, TF is tailing factor (measured at 5 % peak height)
B is distance from the point at the peak midpoint to the tailing edge

A is distance from the leading edge of peak midpoint to the midpoint

Asymmetry factor

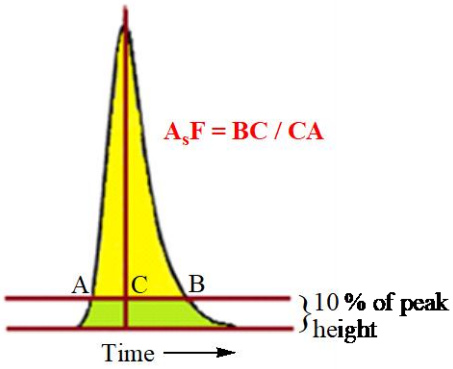


Figure 5b: Chromatographic illustration of asymmetry factor

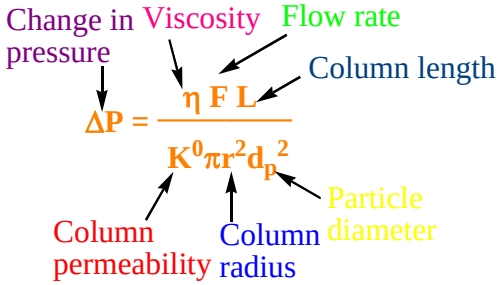
Where, A_sF is peak asymmetry factor

B is distance from the point at the peak midpoint to the tailing edge

A is distance from the leading edge of peak midpoint to the midpoint

Pressure

The Pressure equation (**Equation 5**) identifies five key factors that affect system pressure: solvent viscosity (h), flow rate (F), column length (L), column radius (r) and particle diameter (d_p). It is a good idea to familiarize yourself with the pressure equation to understand these key contributors to system pressure.



Equation 5: Pressure equation

As noted in the formula, even a small decrease in the particle size (d_p) has a significant impact on back pressure.

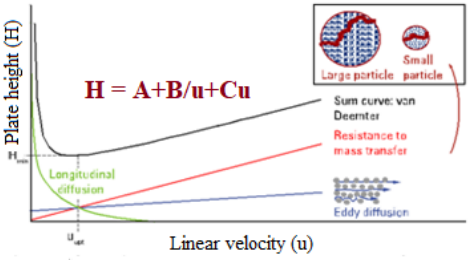
Van Deemter Curves

The Van Deemter equation evaluates efficiency (expressed as H, see **Equation 6**) as a function of linear velocity (u) or flow rate. The H - called plate height, or height of a theoretical plate is determined by dividing the column length (L) by the calculated number of theoretical plates. The goal is to get a small plate height. We can do this most effectively with smaller particle columns, optimum linear velocities and low viscosity mobile phase. As particle size decreases, the optimum linear velocity increases.

$$H = A + B/u + C\,u$$

$$H = L / N$$

Equation 6: Van Deemter equation



The smaller the plate height, the higher the plate number and the greater the chromatographic resolution

Figure 6: Illustration of the Van Deemter equation

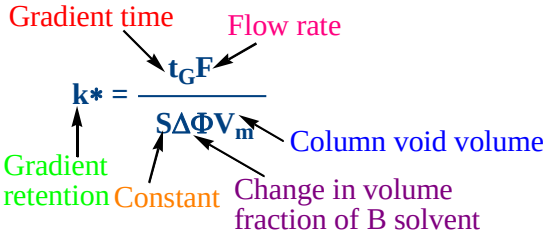
We often plot Van Deemter curves to evaluate the performance of different columns, and to understand the optimum linear velocity (u_{opt}) for a method.

The gradient equation

Whenever your sample has a wide variety of components present, it can be difficult to separate all of the components in a reasonable time using isocratic elution (e.g., constant mobile phase composition). Gradient elution is a process to increase the mobile phase strength as a function of time, resulting in faster analysis, better peak shape and quantitation. With gradient elution, peak widths are typically more narrow and of constant width.

The gradient equation (**Equation 7**) shows key variables that affect your analysis, and may cause issues with your chromatography if you don't account for them. The equation shows how the retention factor is influenced by flow rate (F), gradient time (t_G), gradient range (DF), and column volume (V_M). It is important to remember that in order to keep the retention factor constant, changes in the denominator need to be offset by proportional changes in the numerator, and vice versa.

Increasing the retention factor k (or k*, in a gradient) is an easy way to increase resolution, but as shown in **Figure 4**, it is not as effective as increasing efficiency or selectivity. If the retention factor is increased by increasing gradient time, you will have a longer run time, as **Equation 7** shows.



Equation 7: Gradient equation

In the gradient equation, S is a constant and is dependent on the size of the molecule being separated. For small molecules, the value of S is about 4 to 6. For peptides and proteins, S lies between 10 and 1,000.

These days, it is common to change the dimension of the column, either to something shorter (e.g., for higher throughput) or with a narrower internal diameter (e.g. for mass spectrometric detection). Any decrease in column volume must be offset by a proportional decrease in gradient time (t_G) or flow rate (F). Any change in the gradient compositional range (DF), using the same column, needs to be adjusted by a proportional change in gradient time (t_G) or flow rate (F) if you want to maintain the same gradient slope and k* value.

CONCLUSION

An essential role of chromatography is the quality control of the quality of drugs controlling the raw materials, finished drugs ensuring the safety of the people we are so dependant in the world today on synthetic chemicals made by chemists. So HPLC is the best separation technique for the quantitative trace analysis of toxic chemical impurities. We all remember the feeling we had in college as we learned math, wondering how it would actually come into practical use. Students, lecturers and scientists have to learn more math than many professionals. One should understand the above said concepts which will help you to troubleshoot and get the best results if you encounter problems in HPLC.

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