Chromatography Solutions

Knowledge note #0005

The Fundamental Resolution Equation and Impact of k, N and α.

INTRODUCTION

The fundamental resolution equation is one of the most useful equations in liquid chromatography and describes how changes in retention factor (k), the number of theoretical plates (N) and selectivity (α) affect the resolution obtained between two chromatographic peaks. This Knowledge Note introduces the resolution equation and describes how chromatographers can control and vary the three parameters that affect resolution.

THE RESOLUTION EQUATION

The most fundamental aim in liquid chromatography is to obtain adequate resolution between analyte peaks of interest, thus enabling their accurate determination and quantitation. In order to maximise resolution, it is necessary to understand the key factors that influence it and how chromatographic parameters can be adjusted to help resolve sample analytes. The fundamental resolution equation (equation 1) conveniently describes how resolution is impacted by a combination of three factors: the number of theoretical plates (N), retention factor (k) and selectivity (α).

There are several forms of the resolution equation; equation 1 represents a generalized form appropriate for this discussion.

Efficiency Selectivity Retention

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

N is a measure of the efficiency of the separation and can be increased by either increasing the column length or decreasing the particle size. It is important to remember that both of these options are accompanied by an increase in back pressure across the column and may require the use of expensive LC equipment (i.e. UHPLC).

k is a measure of analyte retention and is defined by equation 2, where t_R is the retention time of the analyte and t_0 is the column dead time.

$$k = \frac{t_R - t_0}{t_0} \quad (2)$$

Generally in reversed-phase, k can be increased by decreasing the elution strength of the mobile phase or increasing the hydrophobicity of the stationary phase.

The selectivity (α) of the separation is defined as the ratio of the retention factors of two adjacent peaks (equation 3) and is a measure of how well the two peaks are separated. Higher values of α indicate that the peaks are well separated and the value approaches 1 as the two peaks co-elute. The selectivity of a separation can be adjusted in several ways including changing the stationary phase, using a different organic modifier, changing the pH etc. and will be discussed in more detail in the next section.

$$\alpha = \frac{k_2}{k_1} \quad (3)$$

THE EFFECT OF N, k AND α ON RESOLUTION

By plotting resolution as a function of N, k and α according to equation 1, it is possible to understand the influence that each parameter has on the resolution of two analyte peaks (Figure 1).

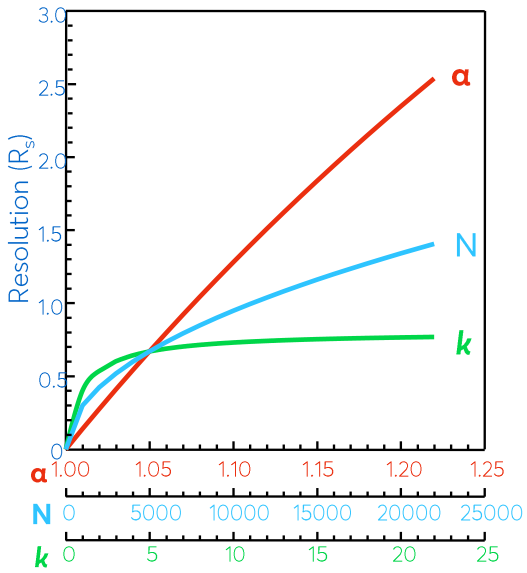

Figure 1: Plot of resolution as a function of N, k and α.

From this plot, it is apparent that for values of k between 0 and 5, significant gains in resolution can be achieved. However, the effect diminishes and beyond a value of 10, no further increases in resolution can be achieved by increasing analyte retention. For this reason, k values between ~5 to 10 are often regarded as optimal. Increasing the separation efficiency (N) continually increases the resolution, albeit with diminishing influence. In reality, increasing the column efficiency can be readily achieved by increasing column length or decreasing particle size, but is a trade-off against run-time and/or column back pressure. Doubling the column length also doubles the run time and back pressure across the column. Column backpressure is proportional to the inverse square of the particle size, therefore decreasing the particle size results in a large increase in back pressure. As a result, beyond a certain point, decreasing particle size requires an investment in UHPLC equipment or becomes unfeasible.

Clearly, the α term is the most powerful in the resolution equation and has the greatest influence on analyte resolution. It is therefore recommended that during method development, as long as analytes are sufficiently well retained, efforts are focussed on fully exploring and optimising the selectivity of the separation. Selectivity is dictated by the chemical interactions of the analytes with the mobile phase and stationary phase and there are a number of chromatographic parameters that can be varied to adjust the separation selectivity. Table 1 summarises the key parameters that are readily tuneable by the chromatographer, along with an indication of their relative influence.

Column stationary phase and organic modifier selection (e.g. acetonitrile or methanol) are the two most influential parameters for altering separation selectivity. Method development strategies that focus on optimising the stationary phase and mobile phase are therefore highly recommended (e.g. six-column screening strategies). Figure 2 shows an example of how selectivity can be substantially altered by changing the stationary phase chemistry, resulting in a large increase in resolution. For further details, please refer to AKN0008 and AKN0013.

Table 1. Parameters which affect selectivity and approximate relative influence.

Isocratic Separations		Gradient Separations
- Column stationary phase - pH (ionisable analytes only) - Organic modifier type - % Organic modifier - Buffer selection - Column temperature - Buffer concentration	MOST influential LEAST influential	All parameters for isocratic separations PLUS: - Gradient steepness - Dwell volume - Column dimensions

[1] Adapted from reference 1.

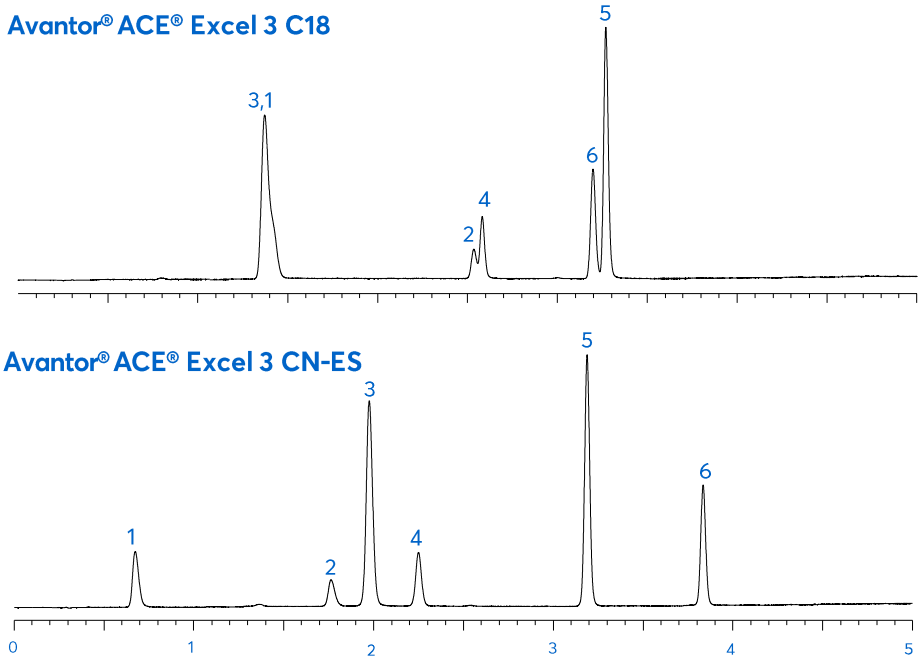

Figure 2: Effect of changing the column stationary phase chemistry on the selectivity of a gradient separation. Both columns are 50 x 2.1mm, for experimental details, see ACE Application Note AN2450.

CONCLUSION

The fundamental resolution equation describes how resolution is affected by changes in N, k and α. From these relationships, it is apparent that selectivity is the most powerful factor affecting analyte resolution and exploring the "selectivity space" of a separation is a valuable and effective approach to method development. Separation selectivity can be readily adjusted using a variety of parameters, in particular, stationary phase chemistry and choice of organic modifier.

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