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Back to Basics

*Gradient Retention Factor, K**

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What is Gradient Retention Factor, *k**?

In isocratic separations, analyte retention time can be normalised to retention factor, *k*, which allows a direct comparison between columns with different dimensions. This is possible due to the constant mobile phase composition used in isocratic methods.

For gradient chromatography, however, there is a change in mobile phase composition, which prohibits the use of *k*. The front of the peak is moving slower than the tail of the peak, with the increasing organic composition, which creates a focused, narrow peak. Gradients are typically used to separate a mixture with a wide range of polarity which would be impractical to separate using isocratic conditions. For a reminder of the different uses and types of gradient profiles typically used, see "Back to Basics: Gradient Anatomy".

Gradient retention factor, *k**, also known as average *k*, can be used to describe the chromatographic conditions (Equation 1). *k** requires a range of $2 < k^* < 10$, similar to that of retention factor, *k*. If *k** is below 2, there is potentially insufficient interaction between the analyte and the stationary phase, whilst above 10, the run times can be excessive, which can decrease laboratory productivity and increase solvent consumption.

$$k^* = \frac{t_G F}{\Delta\phi V_m S} \qquad \textit{Eq. 1}$$

Where t_G is the gradient run time, *F* is the flow rate, $\Delta\phi$ is the %B range displayed as a decimal, V_m is the column void volume and *S* is a constant based on compound molecular weight.

Parameters which affect *k**

A developed method will possess the desired selectivity profile, where all peaks are resolved. This is dependent on a number of factors, including:

- Column dimensions (V_m)
  - Column length impacts on efficiency, which impacts on resolution.
  - The V_m can be calculated experimentally using equation 2, or roughly estimated using equation 3 or 4.

$$V_m = t_0 \times F \qquad \textit{Eq. 2}$$

$$V_m \approx 0.5 \times L \times (d_c)^2 \qquad \textit{Eq. 3}$$

$$V_m \approx \pi \times \left(\frac{d_c}{2}\right)^2 \times L \times \frac{\varepsilon_t}{1000} \qquad \textit{Eq. 4}$$

Where t_0 is void time, *L* is column length, d_c is column internal diameter and εt is the porosity of the particle.

- Gradient time (t_G)
  - t_G is the time at which the gradient composition is changing.
  - If t_G increases, k^* increases, so does average resolution (R_s). However, it is possible for selectivity to change with increased k^*.
- Flow rate (F)
  - Flow rate affects retention and chromatographic performance.
- %Organic range ($\Delta\phi$)
  - The gradient range is represented as a decimal, thus if the gradient is between 10-90%B, the range is 80% which is represented as 0.8.
  - It is important to ensure peaks are eluting on the gradient, and not eluting too early or after the gradient finishes. An appropriate gradient range is therefore required.
- Molecular weight (S)
  - In general, for small molecules with a molecular weight below 200, the constant is 5. This value is more critical for macromolecules such as proteins, peptides and synthetic polymers. Values of S can be approximated using Equation 5.

$$S \approx 0.25(molecular\ weight)^{1/2}$$

Example

It is possible that the method may need to be translated to different column formats or decrease the run time. A good starting point for maintaining selectivity, is to maintain k^*.

(a) A method was developed on a 3 µm, 100 x 3.0 mm column, with an experimentally determined V_m of 0.45 mL and flow rate of 0.43 mL/min. The gradient went from 5-95%B over 10 minutes, and was re-equilibrated at the starting conditions for 10 column volumes.

$$k^* = \frac{10 \times 0.43}{0.9 \times 0.45 \times 5}$$

$$k^* = 2.15$$

The peaks elute in the last five minutes of the gradient (Figure 1a), thus there is scope to reduce the run time. However, if one parameter in the k^* equation is changed, another parameter must be altered. In this case, if run time is shortened, so too must the $\Delta\phi$ range.

(b) The same column and flow rate will be used. However, the gradient time is now 5 minutes. k^* needs to remain constant, therefore the equation can be rearranged to calculate $\Delta\phi$ (Equation 6).

$$\Delta\phi = \frac{t_G F}{k^* V_m S} \qquad \textit{Eq. 6}$$

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$$\Delta\phi = \frac{5 \times 0.43}{2.15 \times 0.45 \times 5}$$

$$\Delta\phi = 0.44 \text{ OR } 44\%$$

Although the calculation suggests a %B range of 44%, it is sensible to round that to 45% with limited effect on the *k**, therefore the range should be 50-95%B over 5 minutes (Figure 1b).

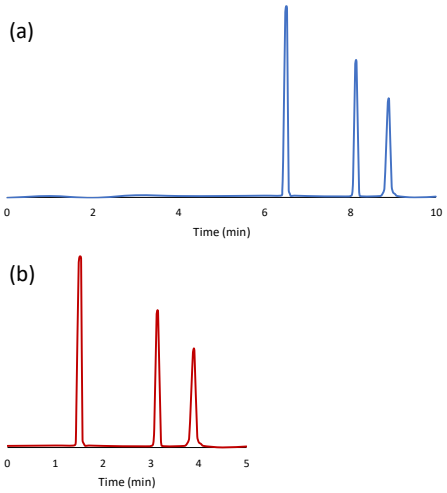

Figure 1 *Illustration of maintaining k* when decreasing the gradient time.*

Remember

- Increase *k** by increasing the gradient time, increasing flow rate, decreasing the column length and decreasing the gradient range. By changing one parameter, the corresponding parameter needs to change.
- Stationary phase manufacturer should ensure that a given ligand chemistry is transferrable between different particle sizes. Particle size may be decreased to increase efficiency which will increase resolution (See "Back to Basics: Explaining Resolution"). However, it is therefore important that each particle size exhibits the same chromatographic profile.
- There are analytes which are sensitive to pressure (e.g. decreasing particle size). These compounds can increase or decrease in retention with changes in pressure. This could be particularly problematic for transferring methods between HPLC and UHPLC conditions.
- Transferring methods between LC with different dwell volumes can also affect retentivity of compounds. It is therefore important to translate your method for different dwell volumes. There are various programs available online to assist with translating methods, but it is important to measure the dwell volume of the two system. There are also some LC's, such as the Nexera-i and LC-40 series which can mimic different flow paths to increase or decrease dwell volume.

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