Chromatography Solutions

Knowledge note #0002

Retention Factor, k

INTRODUCTION

The retention factor (k, also commonly referred to as capacity factor), provides a measure of retention of an analyte by the stationary phase. For practical purposes, k can be thought of as the number of column volumes of mobile phase required to elute an analyte. For a set of defined analytical conditions (i.e. mobile phase composition, stationary phase and temperature), k for a given analyte is independent of flow rate and column geometry and is therefore a convenient tool for comparing analyte retention on different LC systems.

RETENTION FACTOR

Retention factor (k), is measured according to equation 1, where t_R is the retention time of the analyte of interest and t_0 is the column dead time.

$$k = \frac{t_R - t_0}{t_0} \quad (1)$$

The column dead time (t_0) is defined as the time taken to flush one column volume of mobile phase through the column. The dead time can be measured experimentally by observing the first disturbance in the baseline after sample injection or by injecting a non-retained analyte such as uracil or water (Figure 1).

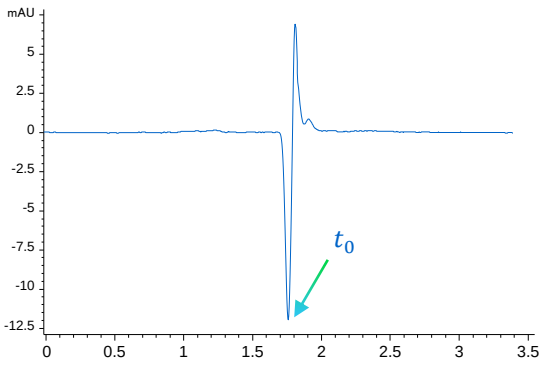

Figure 1: Water injection at 254 nm.

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t_0 can also be estimated according to equation 2, where V_M is the void volume of the column in mL and F is the flow rate in mL/min.

$$t_0 = \frac{V_M}{F} \quad (2)$$

V_M can be calculated using Equation 3, where d_c is the internal diameter of the column in cm, L is the length in cm and ε is the porosity of the phase (see AKN0015). For Avantor® ACE® fully-porous particles the porosity is 0.63 and for Avantor® ACE® UltraCore particles it is 0.55.

$$V_M = \pi \times \left(\frac{d_c}{2}\right)^2 \times L \times \varepsilon \quad (3)$$

For isocratic chromatography, a useful rule of thumb is to aim for 2 < k < 10. A value of less than 2 can provide a separation which lacks robustness and insufficient time to interact with the stationary phase, whilst values over 10 lead to long analysis times. If some sample components give long retention times then gradient chromatography should be considered.

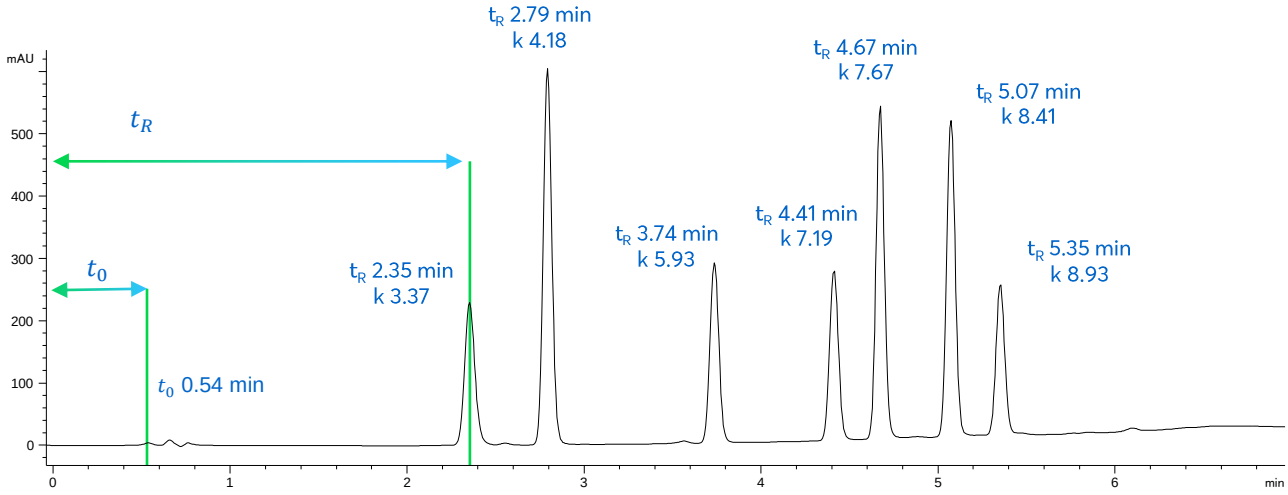

Figure 2: Typical chromatogram with retention time and retention factors.
ACE 5 C18, 50 x 4.6 mm, A: H_2O, B: MeCN, 10-90% B in 5 minutes, 1 mL/min, 60 °C.