Column Performance Prediction and Comparison

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Abstract

Column performance (speed of analysis and separation power) are a function of the column dimensions, the particle size and extra-column effects. A calculator has been created that allows to calculate the column performance as a function of the operating parameters in isocratic and gradient chromatography.

For isocratic chromatography, it takes into account the column design, particle size, analyte, mobile phase composition and operating temperature.

For gradient chromatography, it adds the gradient operating conditions to the set of parameters used for isocratic chromatography.

Theory

1. Isocratic chromatography:

The column plate count and the theoretical plate height are calculated from the van-Deemter equation, with a knowledge of the average particle diameter.

For this calculation, it is necessary to know the diffusion coefficient. This value can be obtained from the molecular weight of the sample, the temperature, and the viscosity of the mobile phase via the Wilke-Chang equation. The relationship between the viscosity of the mobile phase and its composition and the temperature has been measured by Carr for the typical reversed-phase solvent compositions.

2. Gradient chromatography:

In addition to the parameters used in isocratic chromatography, the peak width is calculated from the gradient conditions using the assumption of a linear gradient. The ultimate output for gradient chromatography is the peak capacity.

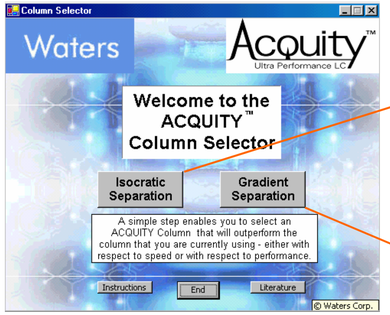
Equations

$$N = \frac{L}{H} \qquad H = A \cdot d_p + \frac{B \cdot D_m}{u} + C \cdot \frac{d_p^2}{D_m} \cdot u$$ Neue, HPLC Columns

$$\frac{F \cdot \eta \cdot L}{\pi \cdot r^2 \cdot \Delta p} = \frac{1}{180} \cdot \frac{\varepsilon_i^3}{(1-\varepsilon)^2} \cdot d_p^2$$ Kozeny-Carman

$$D_m = 7.4 \cdot 10^{-8} \cdot \frac{T \cdot \sqrt{\Psi_2 \cdot M_2}}{\eta \cdot V_1^{0.6}}$$ Wilke-Chang

$$P = 1 + \frac{\sqrt{N}}{4} \cdot \frac{B \cdot \Delta c}{B \cdot \Delta c \cdot \frac{t_0}{t_g} + 1}$$ Neue, J. Sep. Sci.

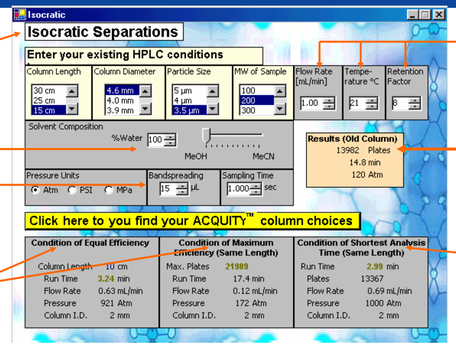


Data input in the yellow section

Select solvent composition (water / methanol / acetonitrile) here

Values for extra-column dispersion or time constant / sampling time issues

Predicted results for UPLC columns shown here

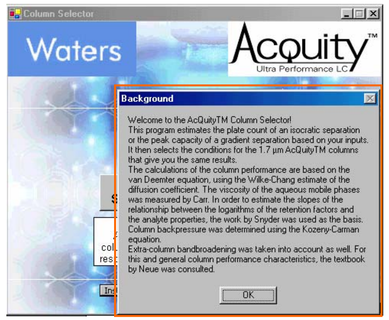


Input area for flow rate, temperature and retention factor

Predicted **isocratic** performance of the selected column shown here

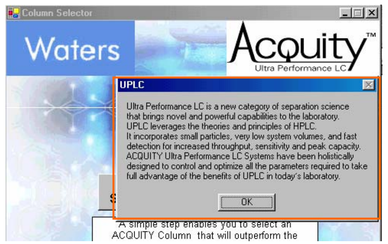
Predicted results for UPLC columns shown here

Background Information



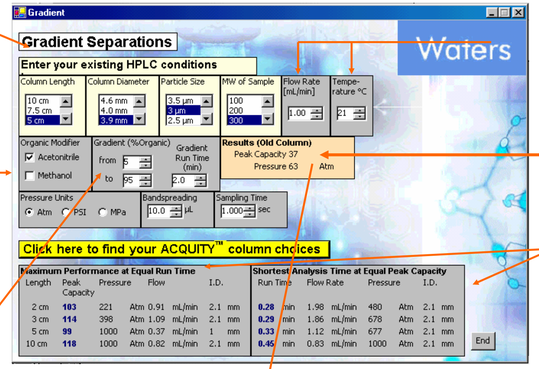
Data input in the yellow section here:

Viscosity of Solvent Mixtures as a Function of Temperature:
Empirical Equation Following J. Li and P. W. Carr, Anal. Chem. 69 (1997), 2530-2536 and H. Colin, J. C. Diez-Masa, T. Czaykowska, I. Miedziac, G. Guiochon, J. Chromatogr. 167 (1978), 41-65



Dependence of the Slope of the Relationship between Retention and Solvent Composition on the MW of the Analyte (for Acetonitrile):

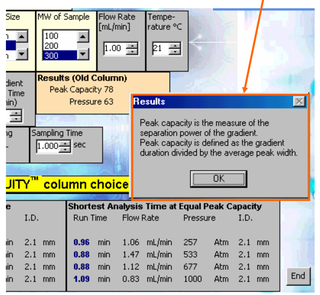
$$B = \frac{MW^{0.69}}{4.41}$$



Input area for flow rate and temperature

Predicted **gradient** performance of the selected column shown here

Predicted results for UPLC columns shown here:
Left: performance at equal run time
Right: shortest analysis time at equal separation power



Additional information:

Peak Capacity is the best measure of separation performance under gradient conditions. It is also by necessity an approximation, since not all the peaks in a chromatogram will have the same molecular weight, diffusion coefficient and interaction with the stationary phase.

The approximations used here are not suitable for very flat gradients with very low molecular weight samples, but are quite useful for the range of operating conditions in the pharmaceutical industry and for analytes with the typical molecular weights of a modern pharmaceutical as well as for peptide samples. Excellent predictions have been obtained (see on the right above).

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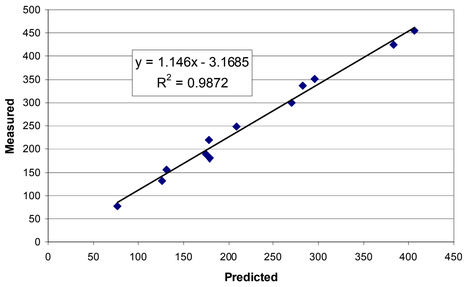
Measured vs. Predicted Peak Capacity for Peptide Separations



An excellent prediction pattern for a set of peptide separations under different reversed-phase conditions was achieved. The pattern shown above includes variation of the column length, the particle size, and the gradient execution (gradient time, column length, flow rate).

Conclusion

- A new tool has been developed that allows the prediction of the column performance in both isocratic and gradient separations from scratch. It takes into account the analyte, the column, the mobile phase, the temperature and other operating conditions such as the flow rate and extra-column bandspreading.
- An excellent correlation between predictions and measured results has been demonstrated for a complex task such as the gradient separation of peptide samples.
- The tool can be used to select optimal chromatographic conditions independent of the surface chemistry of a packing.

References