Fast Separations: Higher Throughput and Reduced Costs

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Today's chemists are being asked to increase productivity and reduce their laboratory costs. In the area of high-performance liquid chromatography (HPLC), one way of achieving these requests is to reduce the overall runtime of a separation. We have developed new columns in 20 mm lengths packed with a variety of stationary phases that offer excellent peak shapes, reproducibility and fast separations for high sample throughput. We have used these columns to scale separations from a 150 mm length to a 20 mm length column.

Traditional Approaches to Fast Separations

Using current HPLC technology, we know that by optimizing the column length, particle size and the flow-rate/temperature of the separation, we can achieve faster separations. We can reduce the length of the column to shorten our runtime and also give us lower backpressures. However, the efficiency of the column is directly proportional to the length of the column. Therefore, we might lose efficiency by reducing the length of the column. We can gain back some efficiency by using a smaller diameter particle. However, with smaller particles, we can also observe higher backpressures. We can also increase the flow-rate to make our runtime shorter, but we might lose some of our resolution and our backpressures might be outside of limits of the HPLC system. By increasing the temperature, we can also speed up a separation and reduce the viscosity of the mobile phase, thereby reducing the backpressure. However, many silica-based particles cannot be run above 45 °C without suffering from short lifetimes. Taking all of this into consideration, we must find the right balance of particle size, column length and flow-rate/temperature that gives us the fastest separation with the necessary resolution.

Column Hardware

Waters designed Intelligent Speed (*IS*™) 20 mm length columns. This careful design of the endfittings and frits is a crucial factor in reducing bandspread and voids in your column. A column format offers better peak shapes and consistent results—and allows you to do fast chromatography. The new Intelligent Speed (*IS*™) columns offered by Waters are available in 2.1, 3.0, 3.9, and 4.6 mm internal diameters in the XTerra®, Symmetry® and Atlantis™ dC_{18} product lines.

Theory Behind Fast Gradient Separations

In order to talk about fast gradient separations, we need to review the principles behind measuring the performance of a gradient separation. We can use the concept of peak capacity to measure the separation power of a particular gradient on a given column.[1,2]

The peak capacity is defined as follows:

$$P = 1 + \frac{t_g}{w} \qquad [1]$$

where tg is the gradient run time and w is the peak width. By making various substitutions of chromatographic relationships, we obtain a mathematical relationship that we can use to assess the gradient performance as a function of the gradient duration, column length, particle size, linear velocity and diffusivity of the analyte.[1,2] For gradient times of under 5 min run on 4.6 × 20 mm *IS*™, 3.5 µm columns, we found that a flow-rate of 3 mL/min results in optimized peak capacities. Therefore, our separations will be run at this flow-rate.

Scaling Separations for Increased Throughput and Reduced Costs

We developed a separation for 6 analytes on a 4.6 × 150 mm, 5 mm XTerra® MS C_{18} column. The separation is a gradient separation in 20 min with a 25 min total cycle time as shown in Figure 1(a). In order to scale the separation to a 20 mm length column, we used Equation 2 to scale the gradient time:

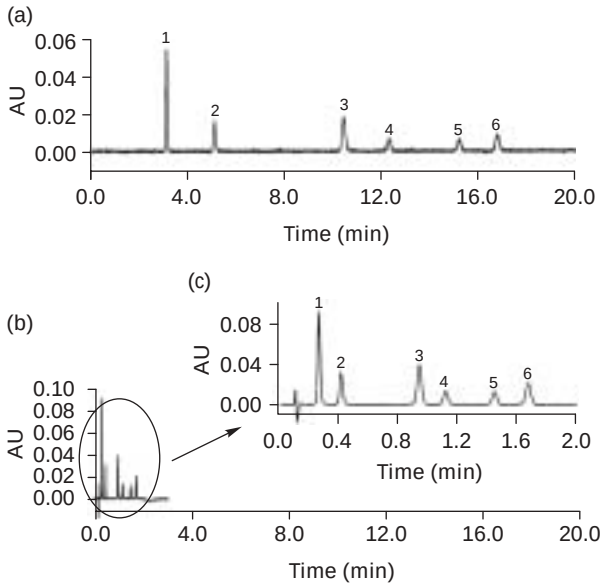
$$\frac{L_2}{L_1} \times t_{g1} = t_{g2} \qquad [2]$$

L_1 is the length of the longer column, L_2 is the length of the shorter column, t_{g1} is the gradient time on the long column, and t_{g2} is the new gradient time on the short column. To run the same separation on a 4.6 × 20 mm IS™, 3.5 mm column, we calculate a new gradient time of 2.7 min. We ran that separation at 3 mL/min and then further reduced the gradient time to 2 min for a total cycle time of 3 min. This separation is shown in Figure 2(b). This is an 8.3-fold reduction in the cycle time from the separation on the longer column. In terms of quantifying cost reductions, this means we can analyse 8 times as many samples in a given time period, freeing up systems and analyst time for other projects. Additionally, even at a flow-rate of 3 mL/min, the short cycle times also allow for less solvent to be consumed and disposed for a given laboratory.

Conclusions

Fast separations that increase sample throughput and reduce laboratory costs can be achieved using the new 20 mm length Intelligent Speed (*IS*™) columns. These columns are available in

Figure 1: Example of a scaled down separation. (a) Separation of 6 analytes on a 4.6 × 150 mm, 5 mm, XTerra® MS C18 column. Total cycle time of 25 min. (b) Scaled down separation on a 4.6 × 20 mm IS™, 3.5 mm, XTerra® MS C18 column. Total cycle time of 3 min. Sample: 1 = Caffeine, 2 = Aniline, 3 = N-Methylaniline, 4 = 2-Ethylaniline, 5 = 4-Nitroanisole, and 6 = N-N-Dimethylaniline. Mobile phase consisted of A: water, B: acetonitrile, and C: 100 mM ammonium bicarbonate buffer, pH 10. Gradient separation conditions were 80% A, 10% B, and 10% C to 50% A, 40% B, and 10% C over 20 min, with 5 min re-equilibration time, at a flow-rate of 1.4 mL/min, and over 2 min, with a 1 min re-equilibration time, at a flow-rate of 3 mL/min, on the 150 mm and 20 mm length columns, respectively.



several of Waters' chemistries, particle sizes and column diameters. Separations originally run on long columns can be scaled down and run on the *IS*™ columns with runtimes of under 5 min. New methods can also be developed on the columns, saving time in method development.

References

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