The peak capacity is defined as follows:

\[ P = 1 + \frac{t_g}{w} \]  \hspace{1cm} [1]

where \( t_g \) 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 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 C18 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:

\[ \frac{L_2}{L_1} \times t_{g1} = t_{g2} \]  \hspace{1cm} [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...
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