

Waters Alliance® HPLC System

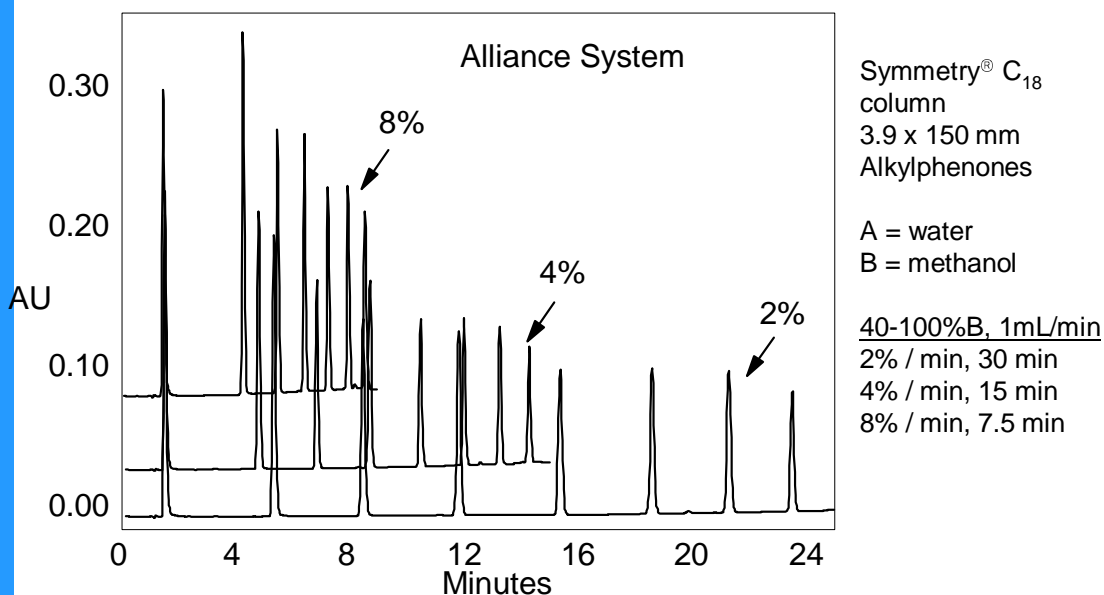
Faster Gradient Chromatography

Historically, analysts have chosen not to use gradient chromatography for routine assays due to extended run and reequilibration times and/or poor retention time precision. The Waters Alliance system has excellent retention time reproducibility (see Performance PerSPECTive WWP201 and 202). In this Performance PerSPECTive several strategies for faster gradient chromatography will be shown.

A simple chromatographic gradient separation is used to illustrate these three approaches - steeper gradient slopes, shorter columns, faster flow rates. The sample mixture is acetone and C2-C8 alkylphenones. The separation is accomplished using a linear gradient with A=water and B=methanol from 40-100%B. The HPLC consisted of a Waters Alliance Separations Module, Waters 996 Photodiode Array Detector, and Millennium® Chromatography Manager software with Waters Symmetry® columns.

Gradient Slope

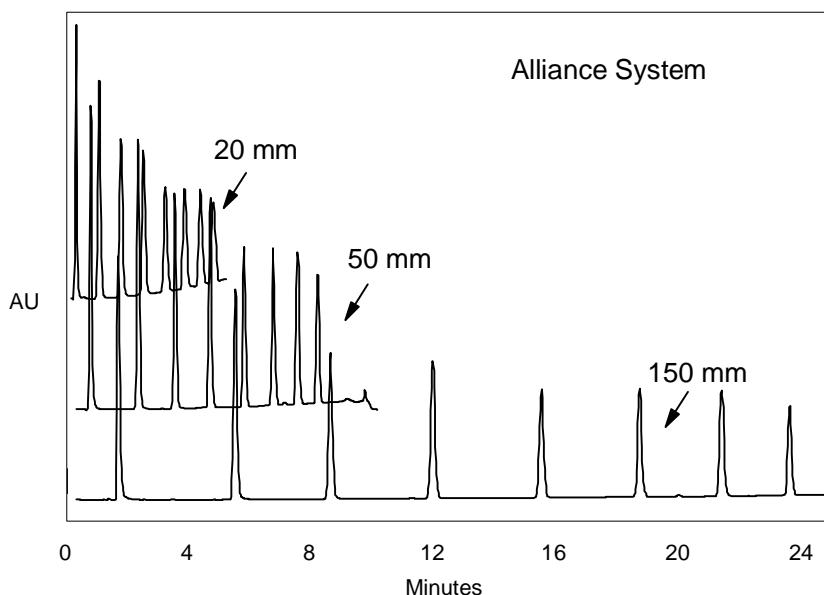
Do you lose chromatographic resolution if the gradient slope is steeper? Yes. However, for faster gradients use the steepest gradient that will maintain baseline peak separation. In this example, the separation run time is reduced from 25 minutes to 9 minutes by influencing the gradient slope.



Column Length

Traditional column lengths for most chromatography have been 150 or 250 mm. Over the years the packing materials have improved with smaller particles and better packing techniques providing more efficiency of separation. Why not try shorter columns?

Using shorter columns is an easy methods transfer. If the HPLC system has a low system volume, the gradient shape will not be distorted and the relative retention times will be similar. As can be seen, the run time can be shortened from 25 to 6 minutes simply through shortening the column.

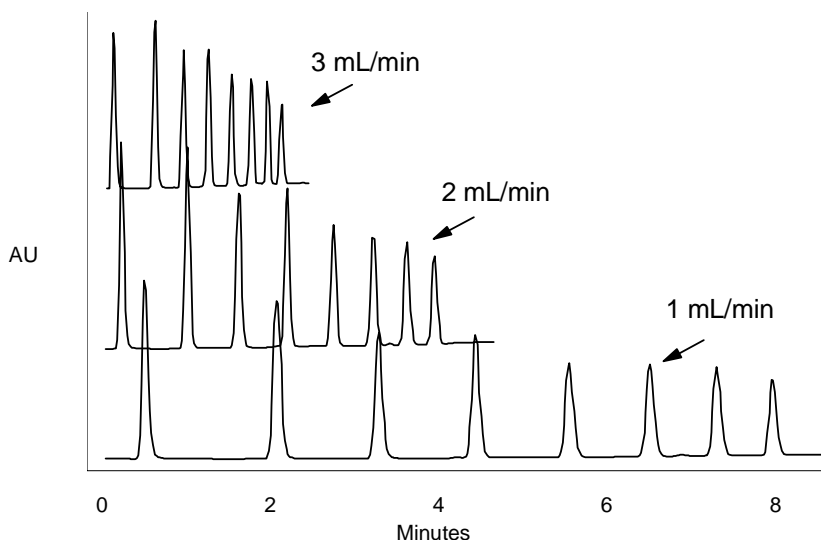


Symmetry® C₁₈ Column
Acetone + Alkylphenones

A = water
B = methanol

40-100%B, 1 mL/min
3.9 x 150 mm, 30 min
gradient
3.9 x 50 mm, 10 min
gradient
3.9 x 20 mm, 4 min
gradient

A secondary benefit of using shorter columns is a reduced backpressure. Backpressure is proportional to the column length. This feature permits another way to achieve faster gradient chromatography by increasing the flow rate. As shown below, the run time on a 3.9x50 mm column with increased flow rates can be as low as 3 minutes.



Symmetry® C₁₈
3.9x50 mm column
Acetone + Alkylphenones

A = water
B = methanol

40-100%B
9 min, 1mL/min
4.5 min, 2 mL/min
3 min, 3 mL/min

Fast gradient chromatography is an example of performance by design. To achieve the ten fold reduction on run time illustrated here, the HPLC system must be fully integrated, like the Alliance family of HPLC systems. Performance requirements are excellent gradient proportioning, solvent mixing, flow rate control, and low system volume. In addition, a variety of column dimensions with the same packing, such as Waters Symmetry® C₁₈ family simplifies method transfer to faster gradient chromatography.