

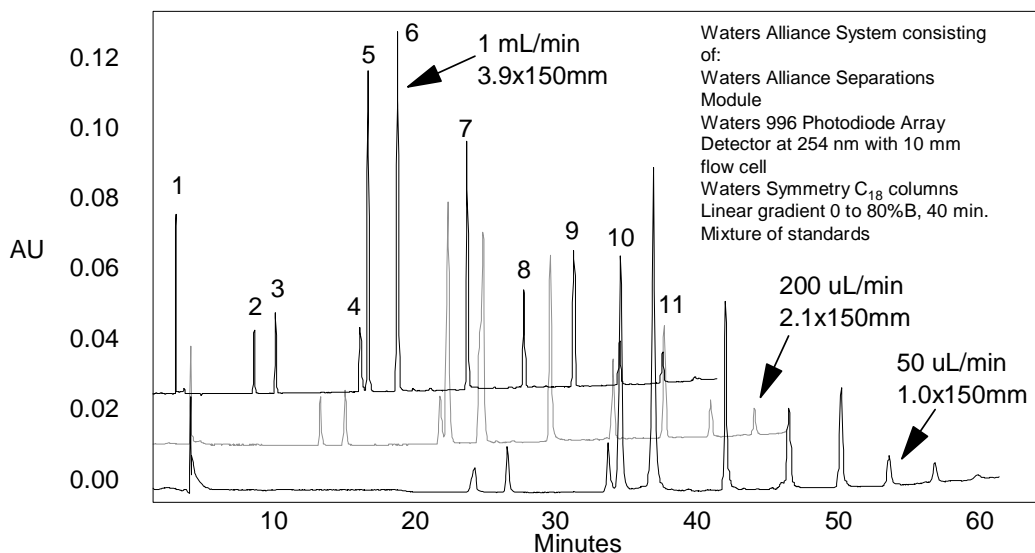
# Waters Alliance® System

## Gradient Chromatography

### GRADIENT SEPARATIONS

Gradient chromatography provides the means of resolving complex mixtures of compounds through variations in the solvent strength (organic or ionic). This is done by changing the proportion of two or more eluents during the separation process. Properly delivered gradients require a complex interaction of events which challenge instrument design. The solvent management system of the Waters Alliance produces precise and accurate gradients that are appropriate for micobore, narrow bore and traditional analytical HPLC flow rates (50  $\mu$ L/min to 1 mL/min).

In the figure below, the separation of standards was performed at different flow rates with various diameter Waters Symmetry® C<sub>18</sub> columns, using a linear gradient.



The retained peaks elute later as the flow rate is decreased because the system volume is fixed. **The Waters Alliance system volume is low (<650  $\mu$ L)**, but it is sufficient to delay the gradient at 50 and 200  $\mu$ L/min by a significant number of minutes. **The resolution between the critical pair, peaks 4 and 5, is maintained at all flow rates even with the Waters 996 standard 10 mm flow cell.** This demonstrates that the design and performance of the Waters Alliance is suitable for micobore, narrow bore and analytical gradient chromatography with no instrument modifications, as well as versatile performance of Waters 996 PDA analytical flow cell.

## EXPERIMENTAL CONDITIONS

Linear gradients from 0 to 80% acetonitrile in 40 minutes were followed by a hold at 80% before returning to initial conditions and reequilibration. The hold and reequilibration times were dependent on column volume, see table below.

Symmetry C18 Column Parameter	Microbore	Narrowbore	Analytical
Column dimensions	1.0 x 150 mm	2.1 x 150 mm	3.9 x 150 mm
Column volume	0.12 mL	0.47 mL	1.8 mL
Flow rate	50 $\mu$ L/min	200 $\mu$ L/min	1.000 mL/min
Gradient slope	2%/min	2%/min	2%/min

## GRADIENT REPRODUCIBILITY

The design of the Alliance maintains the integrity of the most demanding separations while delivering superior gradient performance at all flow rates. Each panel below illustrates an overlay of six chromatograms. The retention time reproducibility is excellent at the three demonstrated flow rates.

