Waters® Alliance™ LC/MS System

LC/MS Methods Development: Faster Gradient Chromatography by Optimizing the Chromatography (Part 1)

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Column Dimensions

Flow Rates

Gradient Optimization

Key Words:
High Throughput, cycle time, fast gradients, Alliance HT System, Symmetry® columns

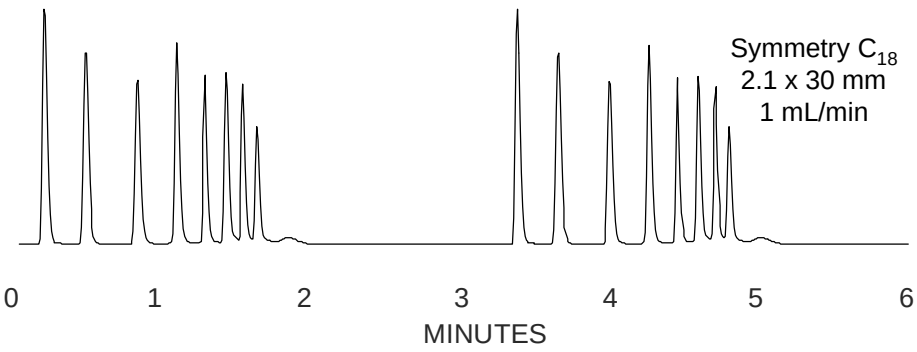
Why is faster gradient chromatography required?

- High throughput applications - screening combinatorial libraries
- Shorter chromatographic run times
- More samples per day, per week
- More efficient laboratory

How can this be accomplished?

- Reduce column length and diameter
- Increase flow rate above the recommended
- Optimize the gradient separation

Result is faster cycle time, (2 cycles in 6 minutes)



Optimizing gradient chromatography for fast throughput may include changing the column from an analytical column (4.5 or 3.9 mm I.d.) to a narrowbore column (2.1 mm). The advantages include higher sensitivity and lower detection limits, and saving of solvents. New columns, e.g. Waters Symmetry columns, are more efficient, allowing the use of shorter columns. The run time can also be shortened by making the gradient steeper, for example, increase the slope from 2%/min to 8%/min. Decreasing the length of the column and the gradient time proportionately also shortens the run time. As the column length decreases, the backpressure decreases. It is then possible to increase the flow rate and shorten the gradient time in proportion to further decrease the run time. Two minute run times can be achieved. For more information refer to Part 2 of this Applications Note and J.B. Li and J. Morawski, LC-GC 16 (5), 468-476, 1998.

Chromatographic conditions: Waters Alliance System; Symmetry C_{18} columns; Mobile phases, A = water, B = methanol; Gradient 40-100% B; Flow rate for the 3.9 mm id and 2.1 mm id columns were 1 mL/min and 0.29 mL/min, respectively. The sample was acetone and C2-C8 alkylphenones.