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アプリケーションノート

Combining Elevated Temperature and UPLC to Achieve Ultra-High Efficiency Separations

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Abstract

This application note demonstrate a 2-fold reduction in UPLC analysis time while maintaining resolution by running at 90 °C rather than at 60 °C and also shows a 100,000 plate UPLC separation for extreme resolution.

Introduction

Developing chromatographic assays that yield higher resolution or higher throughput results can greatly improve productivity in the analytical laboratory. The pressure capabilities of Waters ACQUITY UltraPerformance LC (UPLC) System allow small particle packed columns (<2 µm) to be run at their optimal linear velocities to achieve the maximum resolution in the shortest analysis time.[1] Further improvements can be realized by combining the separation capabilities of UPLC with elevated temperature.[2,3] Increasing the temperature of a separation has several positive effects, including analyte diffusivity and reduced viscosity.

As temperature is increased, the diffusion of the analyte both into and out of the chromatographic pores is faster. Reducing diffusion time minimizes band-broadening. This benefit, however, is contingent upon operating at the optimal linear velocity, which will be higher. For example, an increase in temperature from ambient to 90 °C results in nearly a 4-fold increase in diffusivity, which equates to a 4-fold decrease in analysis time while maintaining the resolution of the separation.[1]

Reduced solvent viscosity (and therefore system backpressures) allow the use of longer columns packed with small particles, and the ability to run at higher flow rates. This produces higher resolution separations with shorter analysis times. The new capabilities of the ACQUITY UPLC System offer the means to combine higher pressures and elevated temperature. It is therefore possible to link multiple columns together in series to achieve high plate count separations and unparalleled chromatographic throughput and performance.

In this note, we demonstrate a 2-fold reduction in UPLC analysis time while maintaining resolution by running at 90 °C rather than at 60 °C. We also show a 100,000 plate UPLC separation for extreme resolution.

Experimental

To investigate reduced run times, a mixture of neutral test probes was analyzed on an ACQUITY UPLC BEH C_{18} 2.1 x 150 mm, 1.7 µm Column at 60, 75, and 90 °C. The neutral probes were thiourea (void

marker), toluene, heptanophenone, octanophenone, and amylbenzene. Efficiency was reported for heptanophenone. The system flow rate was increased with increasing temperature to maintain constant backpressure and constant efficiency.

To maximize resolution, a second experiment was conducted. ACQUITY UPLC BEH C_{18} 2.1 x 150 mm, 1.7 µm Columns were linked, in series, to form 300 mm and 450 mm packed bed lengths in order to exploit the extended temperature range capabilities of the ACQUITY UPLC Column Heater/Cooler-equipped system. Each series of columns was run close to its optimal linear velocity. The neutral test probes mixture, as listed above, was also used for this experiment.

Waters ACQUITY UPLC System with the ACQUITY UPLC Column Heater/Cooler.

LC Conditions

LC system:	ACQUITY UPLC System with the ACQUITY UPLC Column Heater/Cooler and the ACQUITY UPLC Tunable UV (TUV) Detector
Data software:	Empower Software, Build 2154

Column:	ACQUITY UPLC BEH C_{18} Column, 2.1 x 150 mm, 1.7 µm
Mobile phases:	A: Water B: Acetonitrile
Isocratic elution:	70% B
Flow rate:	Variable, as indicated
Injection volume:	0.7, 1.3, and 2.0 µL (scaled for column volume)
Sample conc:	20 µg/mL
Temperature:	Variable, as indicated
Detection:	UV @ 220 nm
Sampling rate:	40 Hz
Time constant:	0

Results and Discussion

Improving Throughput with Elevated Temperature

As temperature is increased, the optimal flow rate, or more precisely linear velocity, is defined by maximum separation efficiency. Separation efficiency is constant at the optimum flow.[1] Increasing the column temperature will allow for equivalent resolution within a shortened time frame.[4,5] To run a separation at its optimal flow rate when increasing temperature, it is practical to adjust the flow rate to maintain a constant column backpressure. This holds the separation efficiency constant.[1] This strategy was applied when monitoring the performance of a 2.1 x 150 mm, 1.7 µm UPLC column at 60, 75, and 90 °C. As depicted in Figure 1, the flow rate was increased with increasing temperature to keep the total system pressure constant. This allowed a 2-fold reduction in analysis time, while maintaining a constant efficiency of 35,500 plates.

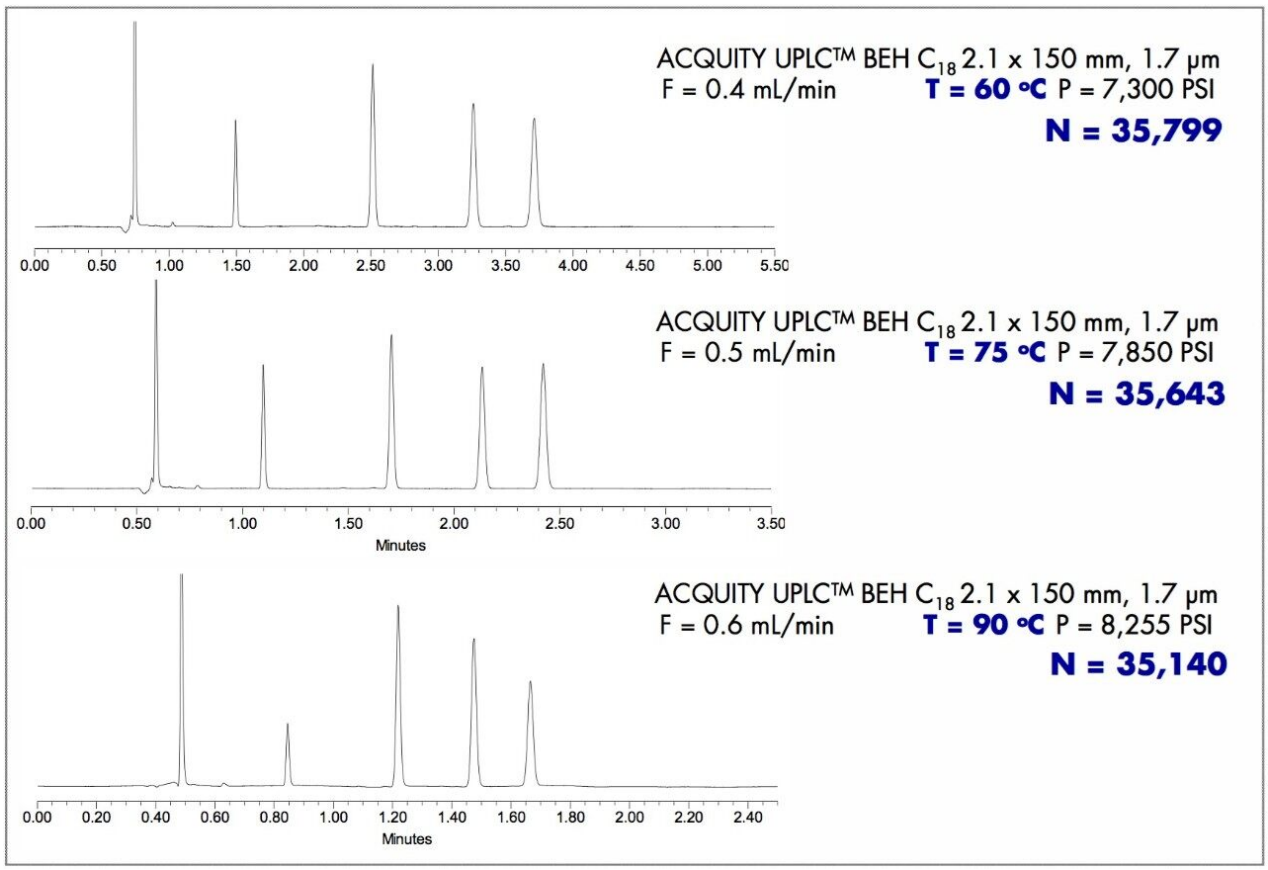

Figure 1. Constant efficiency with varied flow rates and column temperatures, reducing the UPLC analysis time 2-fold.

Maximizing Separation Power: Combining Elevated Temperature with UPLC

High resolution, rapid analysis can be achieved by employing UPLC at elevated temperatures.

To demonstrate this concept, 150 mm-length columns packed with 1.7 µm particles were linked together with low-volume connector tubing. Packed bed lengths of 300 mm and 450 mm were formed by joining two and three columns, respectively. As expected, these column combinations have very high backpressures. Therefore, column temperature was held constant at 90 °C to reduce viscosity. The flow rate was adjusted to achieve the highest plate count separation for each column length, while operating within the pressure capabilities of the ACQUITY UPLC System. As shown in Figure 2, efficiency increases with increasing column length. A single 150 mm column produces 35,000 plates while two linked columns (300 mm) produce 79,000 plates. Separation efficiency can be further increased by linking three columns (450 mm) to produce 109,000 plates. It is important to note the ability to achieve greater than 100,000 plates in less than 12 minutes.

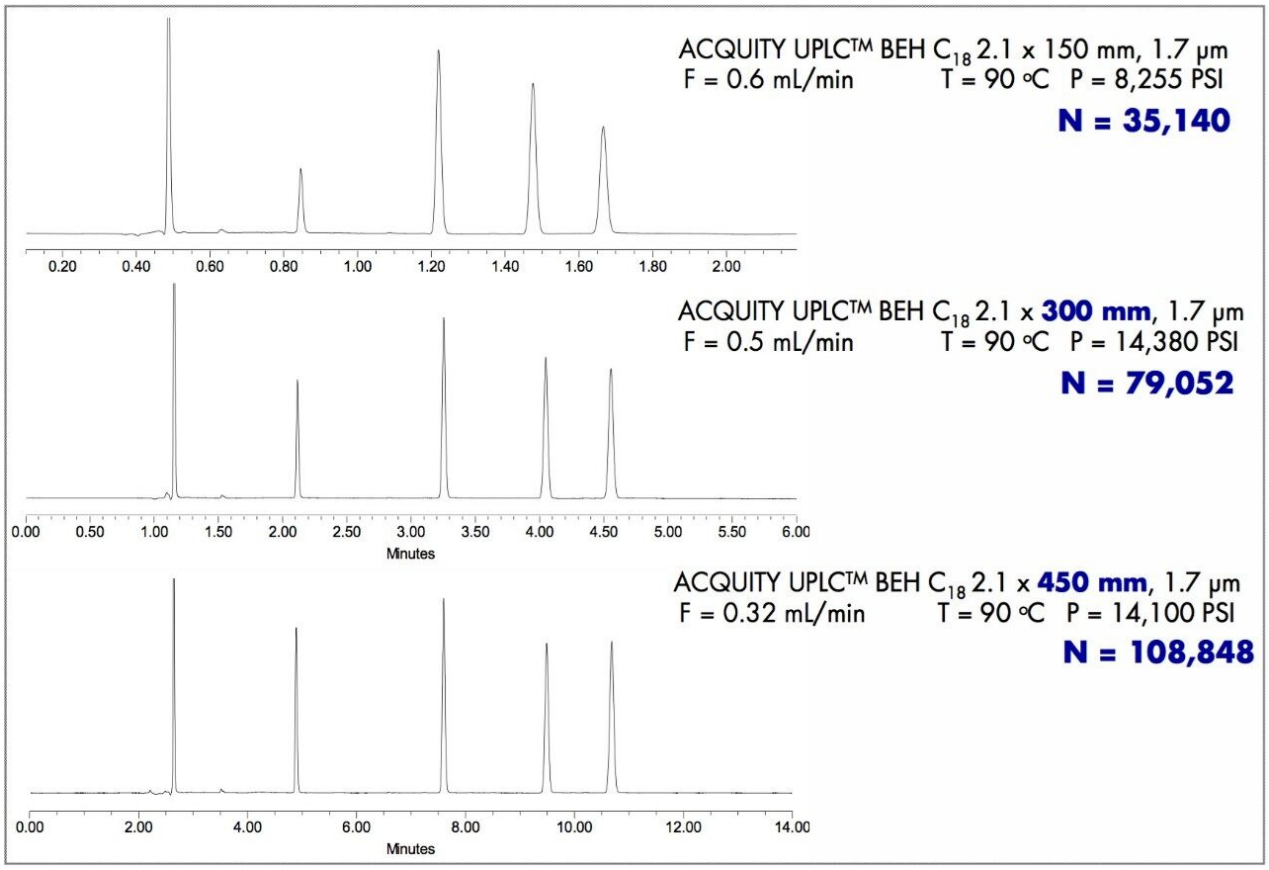

Figure 2. UPLC columns linked to achieve ultra-high efficiency separations.

Using such long columns packed with 1.7 µm particles, combined with elevated temperature, may prove extremely useful when developing separations for very complex mixtures.

Conclusion

By combining the resolving power of small particles, the improvement in mass transfer at elevated temperatures, and the extended pressure range of UltraPerformance LC, we have demonstrated the highest separation efficiency while achieving rapid analysis times. This technique can be employed to run ACQUITY UPLC BEH Columns at faster flow rates on a Column Heater/Cooler-equipped ACQUITY UPLC System to reduce analysis times, or to effectively use longer UPLC Columns to achieve ultra-high resolution separations for complex mixtures.

References

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720001800, August 2006