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The Effect of Elevated Column Operating Temperatures on Chromatographic Performance

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief discusses the effects on column performance when operating temperatures are increased.

Introduction

High Temperature Column Operation

Many of today’s chromatographic challenges require the implementation of a high resolution chromatography system to separate tens to hundreds of components (e.g., pesticide analysis and environmental water monitoring). This task has traditionally been achieved by using both long columns (250 mm), and long analysis times (30—60 minutes). Today, the combination of sub-2 µm porous particles and correspondingly optimized LC systems with low delay volumes has enabled a new level of chromatographic performance to be achieved. When employing these small particles, column backpressure increases with the inverse square of the particle size, thus requiring a mechanically hardened instrument to deal with these increased system backpressure (10,000—15,000 psi), such as the Waters ACQUITY UltraPerformance LC (UPLC) System. Alternatively, using elevated column temperatures has been promoted as an option to lower backpressure for non-specialized LC instrumentation. In this application brief, we will discuss the effects on column performance when operating temperatures are increased.

Experimental

LC Conditions

LC system:	ACQUITY UPLC System
Columns:	ACQUITY UPLC BEH C_{18}, 2.1 x 150 mm, 1.7 µm XBridge BEH C_{18}, 2.1 x 100 mm, 3.5 µm
Flow rate:	0.45 mL/min

Injection volume:	5 µL
Column temp.:	90 °C
Isocratic:	70:30 Water:Acetonitrile
Gradient:	5%–95% B over 10 minutes, where A = 0.1% Formic acid in water, and B = Acetonitrile
Curve:	Linear

A series of probe compounds was used to evaluate isocratic column performance at various operating temperatures using a XBridge C_{18} 2.1 x 100 mm, 3.5 µm Column. From the data obtained and displayed in Figure 1, we can see that as the column temperature is raised, the optimal linear velocity moves to a higher value. Note that the highest obtainable efficiency does not change with temperature.

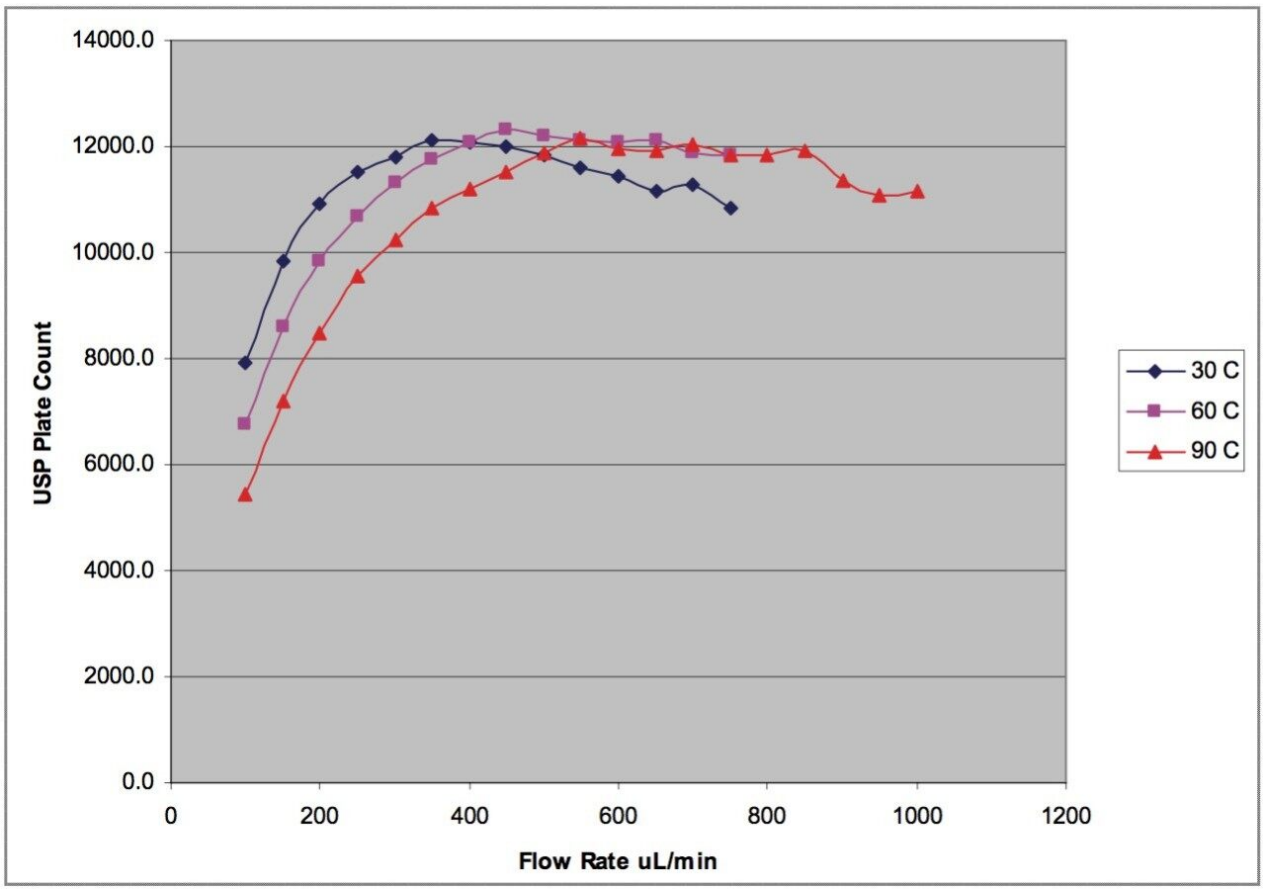

Figure 1. van Deemter plots for a 3.5 µm XBridge column at 30 °C, 60 °C, and 90 °C.

The need to increase the mobile phase flow rate as the column temperature is raised eliminates the potential lower pressure benefits of reduced viscosity at higher temperature.

Results and Discussion

Increased Speed of Analysis

Plates Per Unit Time

One of the major benefits of operating at higher temperatures is that the mobile phase flow rate can be increased, allowing a gradient separation to be carried out in less time. In the example in Figure 2, we can see the effects of increasing the operating temperature from 35 °C to 65 °C to 90 °C on an ACQUITY UPLC C_{18} 2.1 x 150 mm, 1.7 µm Column. The column flow rate was increased from 0.45 mL/min at 30 °C, to 0.65 mL/min at 60 °C, and 1 mL/min at 90 °C, thus maintaining the backpressure at 11,000 psi. The chromatographic resolution between the peaks remains unchanged, but the gradient time is reduced from 11 minutes to 5 minutes.

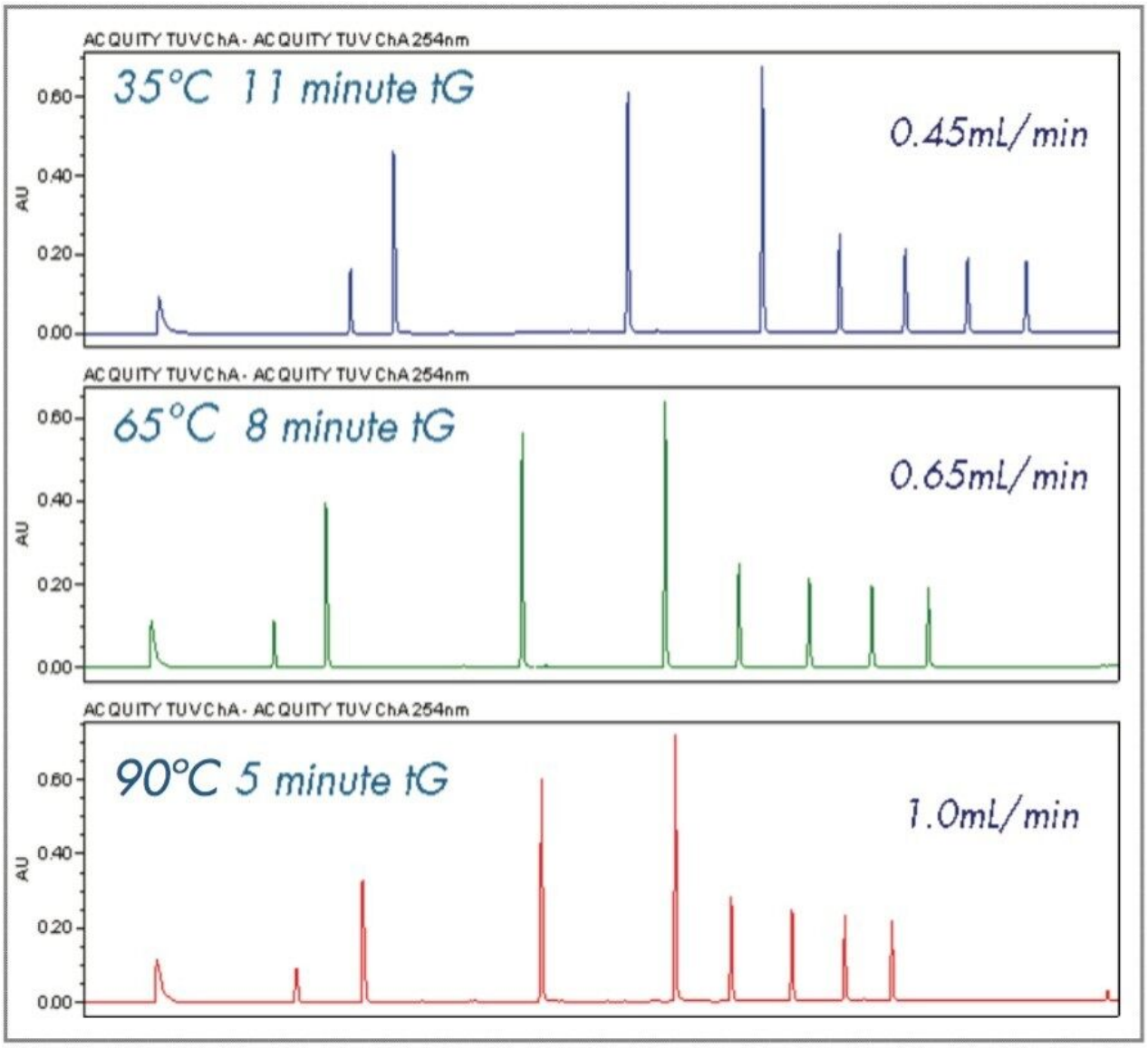

Figure 2. Increasing throughput by increasing temperature when analyzing a homologous chromatographic test mix.

The peak capacities of the individual separations were determined to be 283 at 35 °C, 284 at 65 °C, and 276 at 90 °C. As the column temperature is raised, the number of plates generated per unit time increases, leading to faster analysis.

Effect of Temperature on Elution Order

Managing Selectivity

In Figure 3, we observe that increasing column temperatures reduces retention of the analytes at a different rate. This effect on selectivity may not be an issue when analyzing a homologous series of compounds as shown in Figure 2, but this is rarely the case in "real-world" analyses. The effect of

temperature on the order of elution can be confounding during method development as the order of peak elution often changes. This point is illustrated by the two pairs of peaks in a pharmaceutical sample: 1 and 2, which move from being resolved at 30 °C to co-eluting at 90 °C, and peaks 5, 6 and 7, which reverse their order of elution when moving from 30 to 90 °C. Thus, if we were transferring a method from a low temperature HPLC separation to a high temperature separation, we could not simply transfer and expect to keep the same resolution. In fact, it may well be necessary to completely redevelop the methodology.

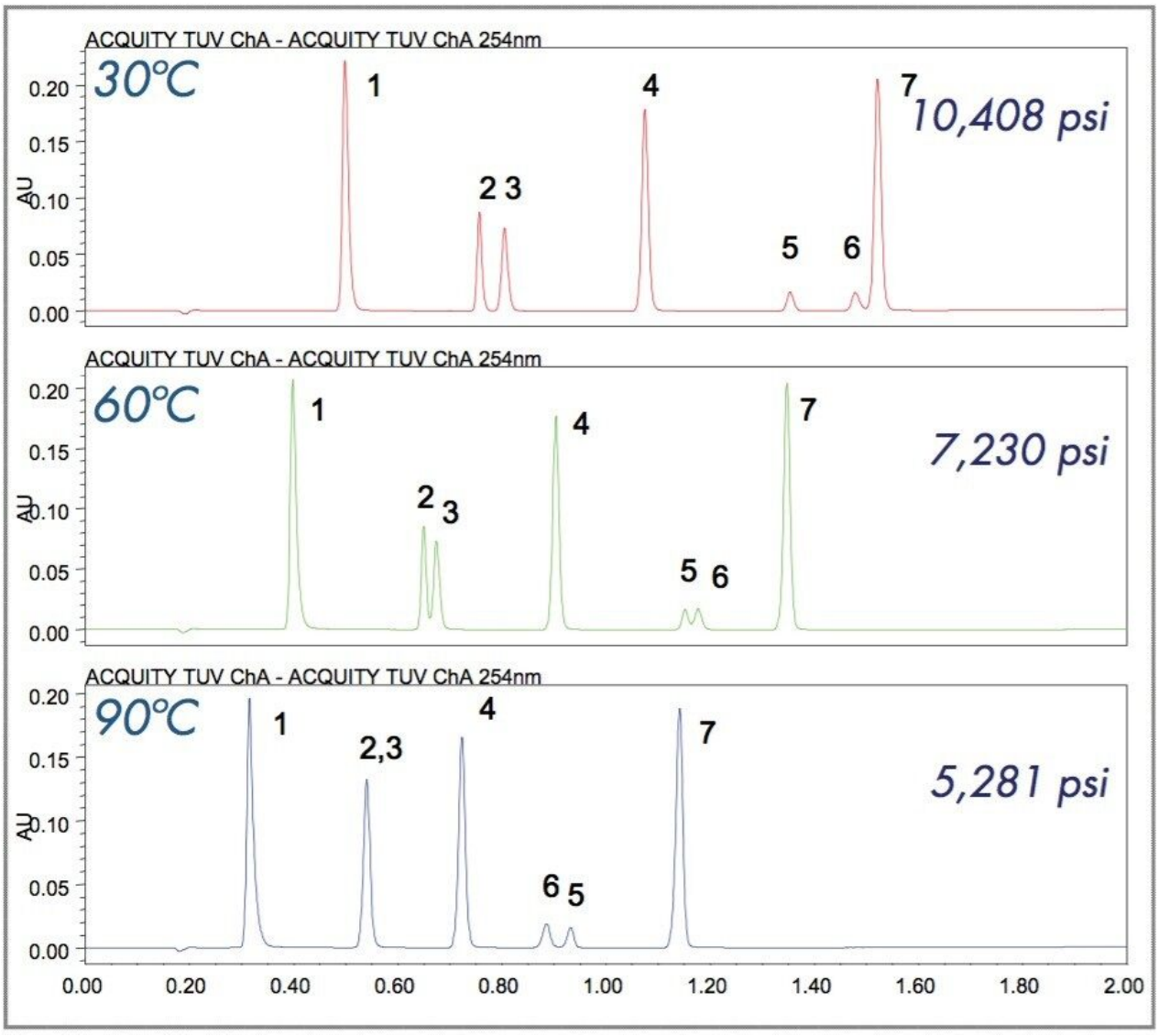

Figure 3. Increasing throughput by increasing temperature, with associated selectivity changes when analyzing a typical pharmaceutical sample.

For example, when a high temperature separation is used for purity screening, it may not be possible to predict the appropriate preparative methodology from the high temperature results.

In this scenario, if the target analyte was peak 6, then the 90 °C separation would be satisfactory for isolation. However, as we move back to a temperature at which preparative-scale separations can be performed, this is no longer the case. Peaks 5 and 6 have reversed order of elution and peaks 6 and 7 are now barely resolved.

Conclusion

The use of elevated temperatures can be very useful in liquid chromatography for performing faster separations, however, it does not increase the efficiency of the system in either isocratic or gradient mode. As column operating temperature increases the optimal mobile phase linear velocity increases, therefore the pressure benefits gained by reduced solvent viscosities are negated. Thus, it is necessary to have a chromatographic system that is designed for optimal performance at very high pressures (>10,000 psi) to fully exploit sub-2 µm particles, as with the ACQUITY UPLC System. The use of increased temperatures allows optimal ACQUITY UPLC System performance to be obtained in shorter time. As a caveat, however, when operating at higher temperatures analyte retention is not only reduced but the order of elution can change, so extra care must be taken when transferring HPLC methodologies to high temperature separations or when using these separations to predict or define preparative-scale gradients.

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