Waters® Alliance™ LC/MS System

Effect of Temperature on Column Pressure, Peak Retention Time and Peak Shape

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Column backpressure

Peak shape

Peak retention time

Key Words
Temperature, pressure, viscosity, peak shape, sensitivity

Background
To optimize chromatography for high throughput applications, the use of columns with 2-3 micron particles and higher than normal flow rates results in high pressures. Increasing temperature can reduce the backpressure. This application note illustrates some of the effects of temperature on column pressure, peak retention time and shape.

Column backpressure as a function of solvent composition
The two most common mobile phases for reverse phase chromatography are methanol-water and acetonitrile-water. The viscosity of the mobile phase changes with the composition. The increase in viscosity increases pressure. The maximum pressure can be significant for 50% methanol-water.

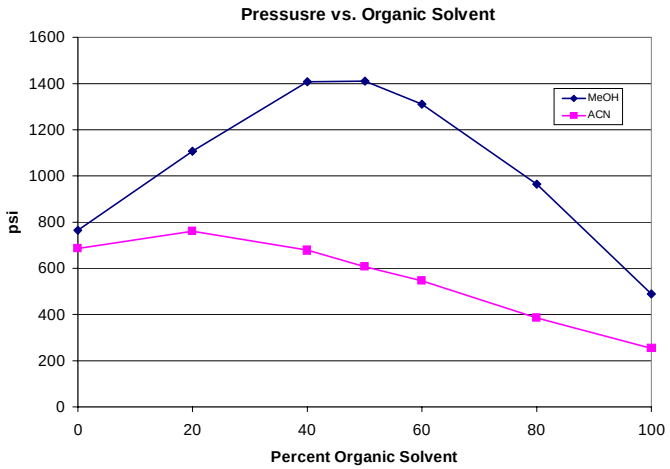

Figure 1. Pressure vs. Organic Solvent Composition

Column: Symmetry® C18, 2.1x 30mm
Flow rate: 0.3 mL/min
Temperature: Ambient (ca. 20° C)

Column backpressure as a function of flow rate and temperature

Pressure increases linearly with flow rate. The solvent viscosity determines the amount of pressure. This is shown in Figure 2. As the temperature increases, the viscosity decreases and the column backpressure will decrease. This is shown in Figure 3. The pressure can be reduced as much as 43% by raising the the temperature from 20 to 60°C. This allows higher flow rates to be used. In addition, the lower pressures can reduce wear on the HPLC instrumentation and can possibly increase column life.

Application to high throughput

Pressure is also linearly proportional to column length for the same column diameter and packing (data not shown). Therefore, for the fastest injection-to-injection cycle times, a method should use a short column, an elevated temperature and an increased flow rate. See LC/MS Applications Notes AMD21 and AMD22.

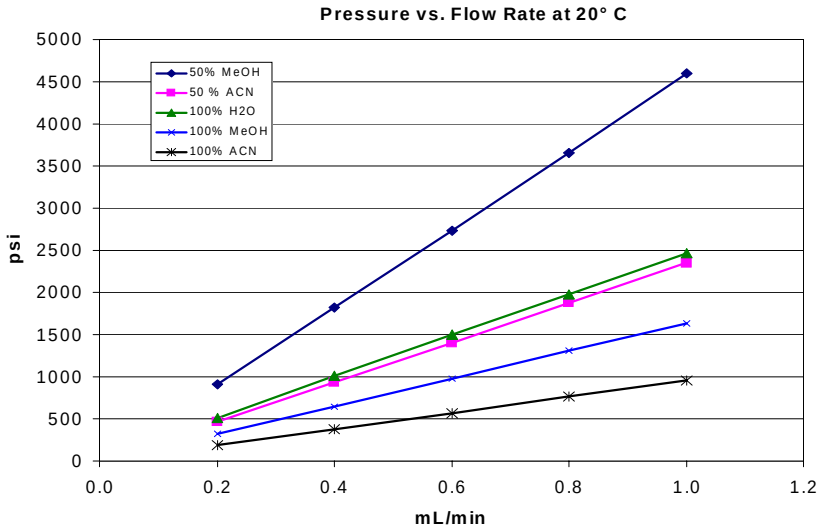

Figure 2. Pressure vs. Flow Rate

Column: Symmetry® C18, 2.1x 30mm
Flow rate: 0.2 to 1.0 mL/min
Temperature: 20° C

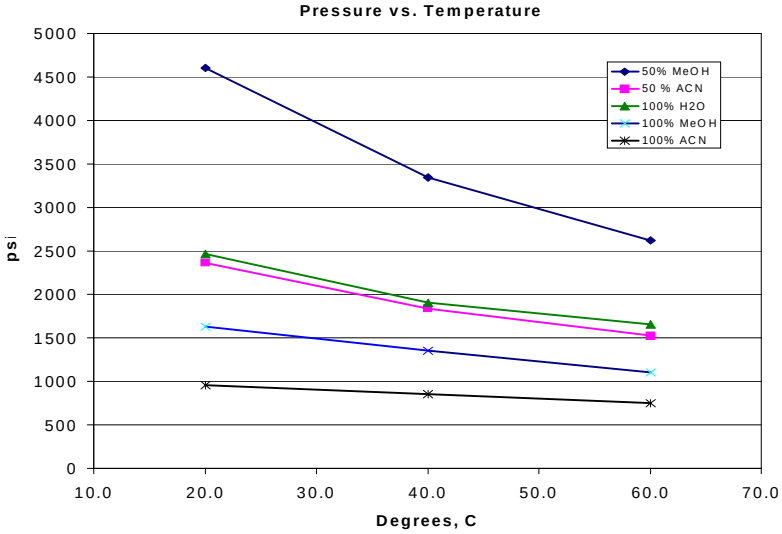

Figure 3. Pressure vs. Temperature

Column: Symmetry® C18, 2.1x 30mm
Flow rate: 1.0 mL/min
Temperature: 20°C to 60°C

Effect of temperature on peak retention time and peak shape

As the temperature is increased, the peak retention times are shortened. Chromatography is a series of equilibrium reactions where the analytes are either dissolved in the mobile phase or adsorbed to the stationary phase of the column. The higher the temperature, the faster the exchange of the analytes between the mobile phase and the stationary phase. This is seen in Figure 4. A simple isocratic separation show that a 30% reduction in retention time is the result of elevating the temperature to 50°C.

The overlay of Peak 4 of the 25° C and 50° C chromatograms shows the peak height is greater and the peak is narrower. The results is an increase in sensitivity of approximately 15%.

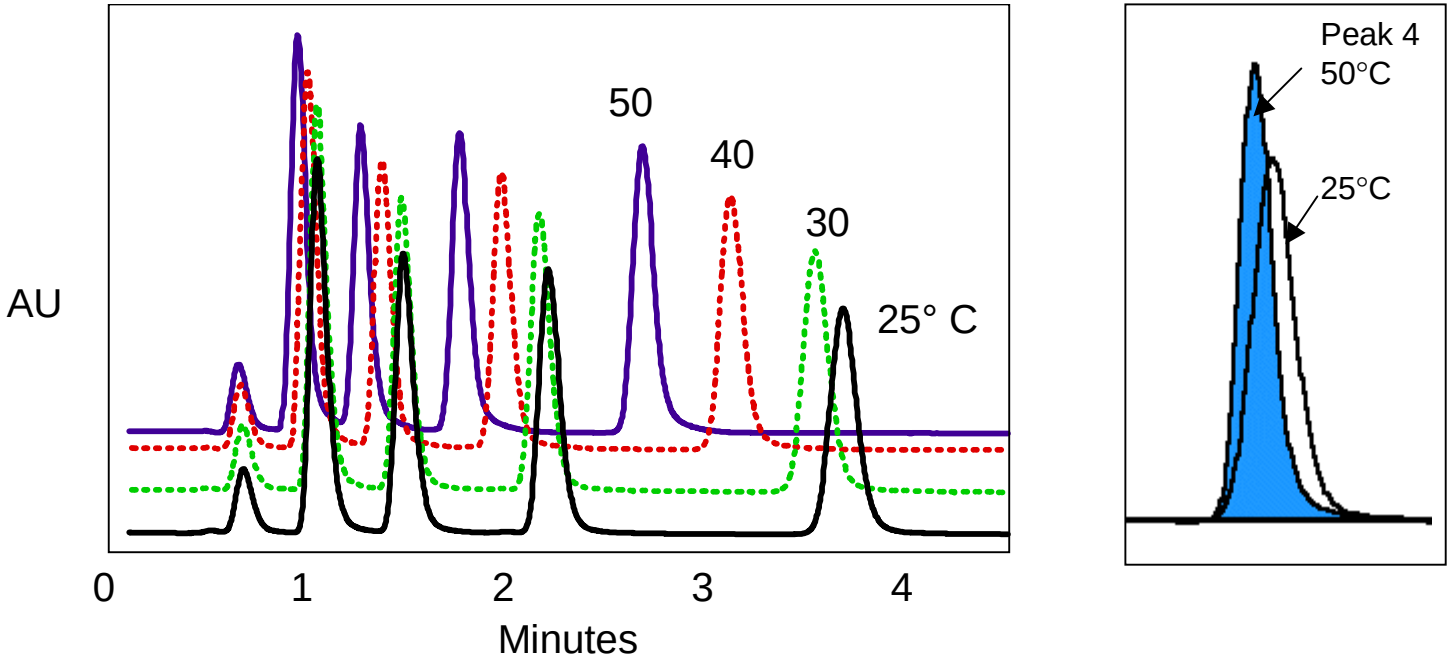

Figure 4. Effect of temperature on retention time and peak shape

Isocratic separation of acetone a C2-C4 alkylphenones in 60:40 methanol-water on a Symmetry® C_{18} 2.1x30mm column