Note d'application

Effective Use of Temperature Control in Compound Isolation

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Abstract

All chemical processes are temperature sensitive. As the temperature rises, viscosity and pressure decrease, retention decreases, and the selectivity of the separation changes. While temperature can effectively improve a separation, it is seldom used at the preparative scale. Lack of effective temperature control results in preparative chromatograms that are not comparable to the analytical separation, making compound isolation more difficult. This work illustrates an effective solution for controlling temperature of large columns at high flow rates.

Introduction

All chemical processes are temperature sensitive and chromatography illustrates this point quite dramatically. Generally, as the temperature rises, viscosity and pressure decrease, retention decreases, and the selectivity of the separation may change. Temperature control in analytical chromatography is routine, but temperature is seldom controlled or used as a parameter for manipulating chromatography at the preparative scale. The reason for this is two-fold. First, large diameter columns cannot be effectively heated from the outside. Second, high flow rate separations actually occur at the temperature of the incoming solvent. Electric blankets and column ovens, while satisfactory for small scale chromatography, cannot heat a large column uniformly from its inlet to its outlet or across the diameter of the column. A temperature gradient also forms from the wall to the center of the column, which impacts the chromatography. The variable temperature inside the column results in the preparative separation not being comparable to the analytical separation. This work illustrates an effective solution for controlling temperature of large columns at high flow rates.

Experimental

- Measure time for system pressure stabilization at RT
- Measure time for system pressure stabilization at 60 °C
- Determine optimal configuration for preheating solvent
- Ensure scalability with temperature control

Instrumentation

LC system:	Waters 2525 Binary Gradient Module, 2767 Sample Manager, Column Fluidics Organizer, 2996 Photodiode Array Detector, ZQ Mass Spectrometer, Water Bath
Column:	XBridge Prep OBD C_{18} Column 19 x 150 mm, 5 μm (Part Number 186002979)
Flow rate:	25 mL/min
Mobile phase A:	0.1% TFA in water
Mobile phase B:	0.08% TFA in acetonitrile

Results and Discussion

Pressure stabilization at room temperature

The observed system pressure changes only as a result of the change in column pressure and in the viscosity of the solvent upon heating. As the solvent is heated, the viscosity decreases and the column pressure decreases. The pressure of the system without the column is constant. The column pressure can then be calculated using the following equation:

Column pressure = system pressure with column – system pressure without column

Therefore, temperature is measured indirectly by noting the column pressure. A stable column pressure indicates that the column is at equilibrium. This does not demonstrate, however, that the temperature is uniform throughout the column.

Run 100% water through the column at room temperature. Record the observed pressure and the length of time required for the pressure to stabilize. Replace the column with a union and repeat the experiment to establish the pressure attributed to the system. It takes approximately one minute to achieve stable pressure at 25 mL/min. The column pressure is ~1100 psi. Results are shown in Figure 1.

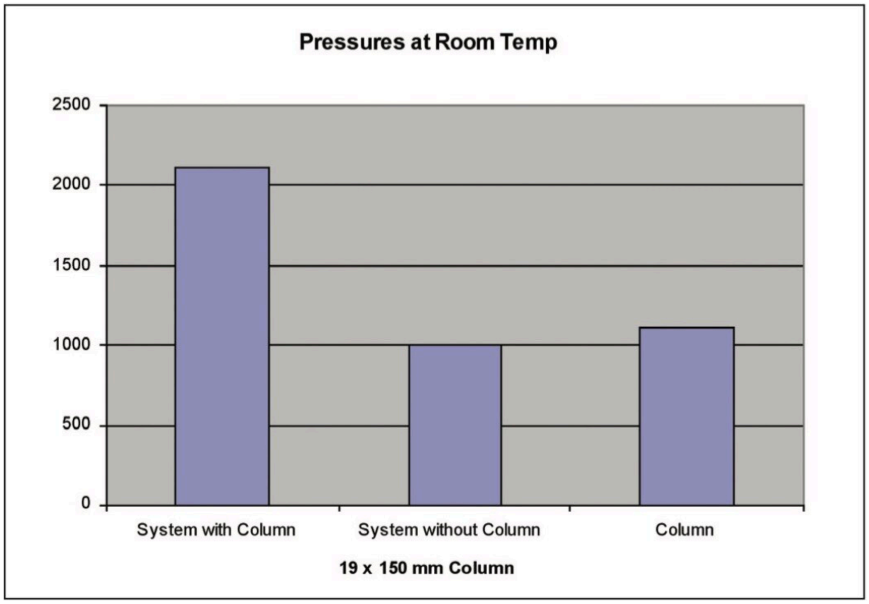

Figure 1. System pressure comparisons at room temperature.

Proposed solution for temperature control

After submerging the column in an equilibrated water bath at 60 °C, as shown in Figure 2, it took approximately two minutes for the pressure to stabilize. A 19 x 150 mm column has a volume of about 36 mL and the flow rate is maintained at 25 mL/min, so this amount of time for pressure stabilization is quite reasonable.

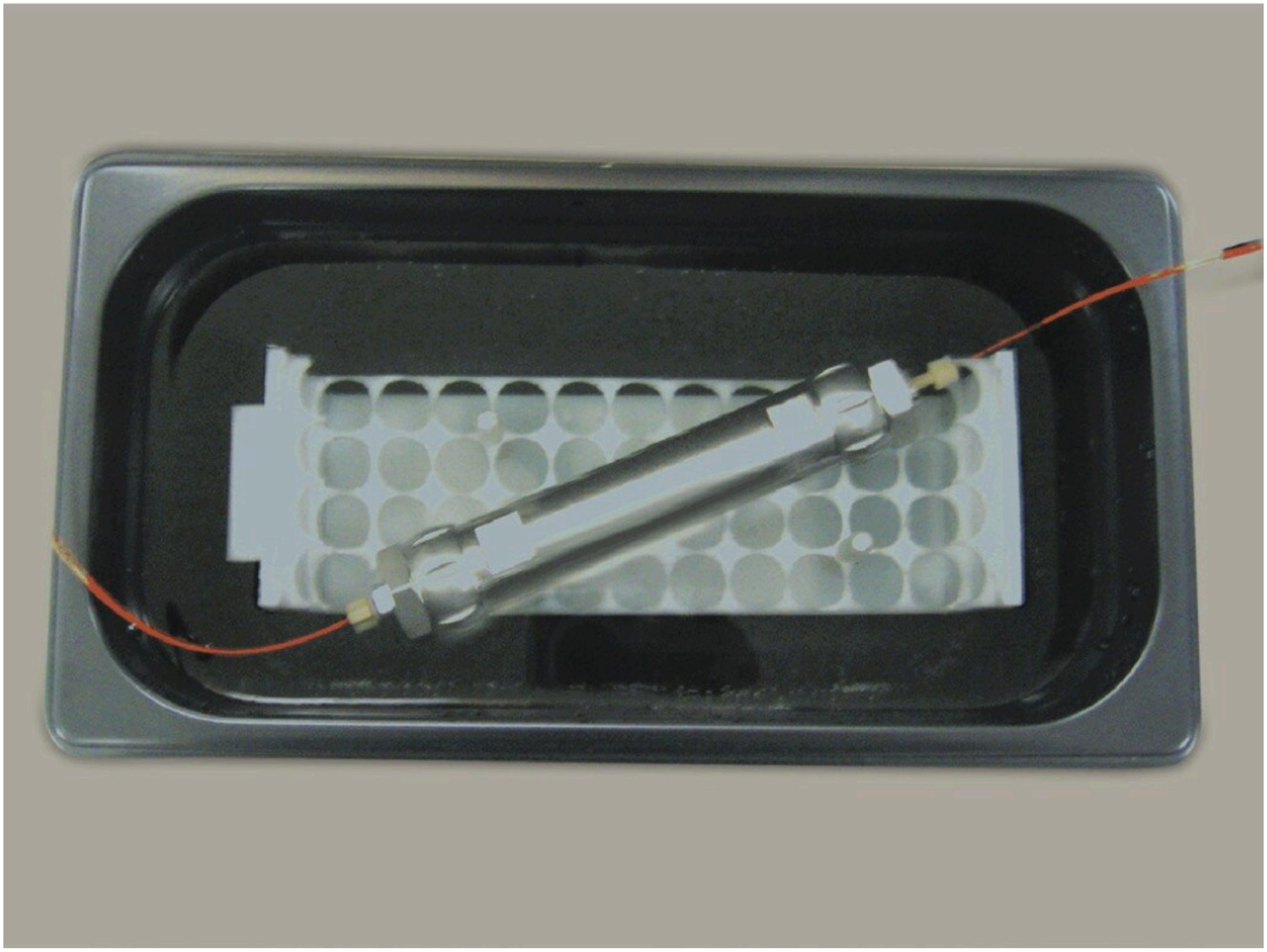

Figure 2. Approximately 2 min is needed to achieve stable pressure at 60 °C with the column submerged in an equilibrated water.

As shown in Figure 3, a 5 mL loop plumbed at the head of the column serves as a solvent preheater. Continuously introducing the solvent at 60 °C brings the column to equilibrium internally while the water bath stabilizes the external column environment. In this case, it takes about 2.5 minutes for the column to come to stable pressure due to the extra volume contributed by the 5 mL loop and because the column is coming to equilibrium. Adding a second 5 mL loop for extended solvent preheating does not change the amount of time for the pressure to stabilize; therefore, one 5 mL loop is sufficient for heating the column uniformly. As shown in Figure 4, the 1100 psi attributed to the column at room temperature drops to 650 psi by putting the column into the water bath. The pressure drops another 100 psi when the solvent is heated before reaching the column. A 100 psi reflects a change in viscosity of about 15%. An additional 5 mL solvent preheating loop has no effect on the column or system pressure.

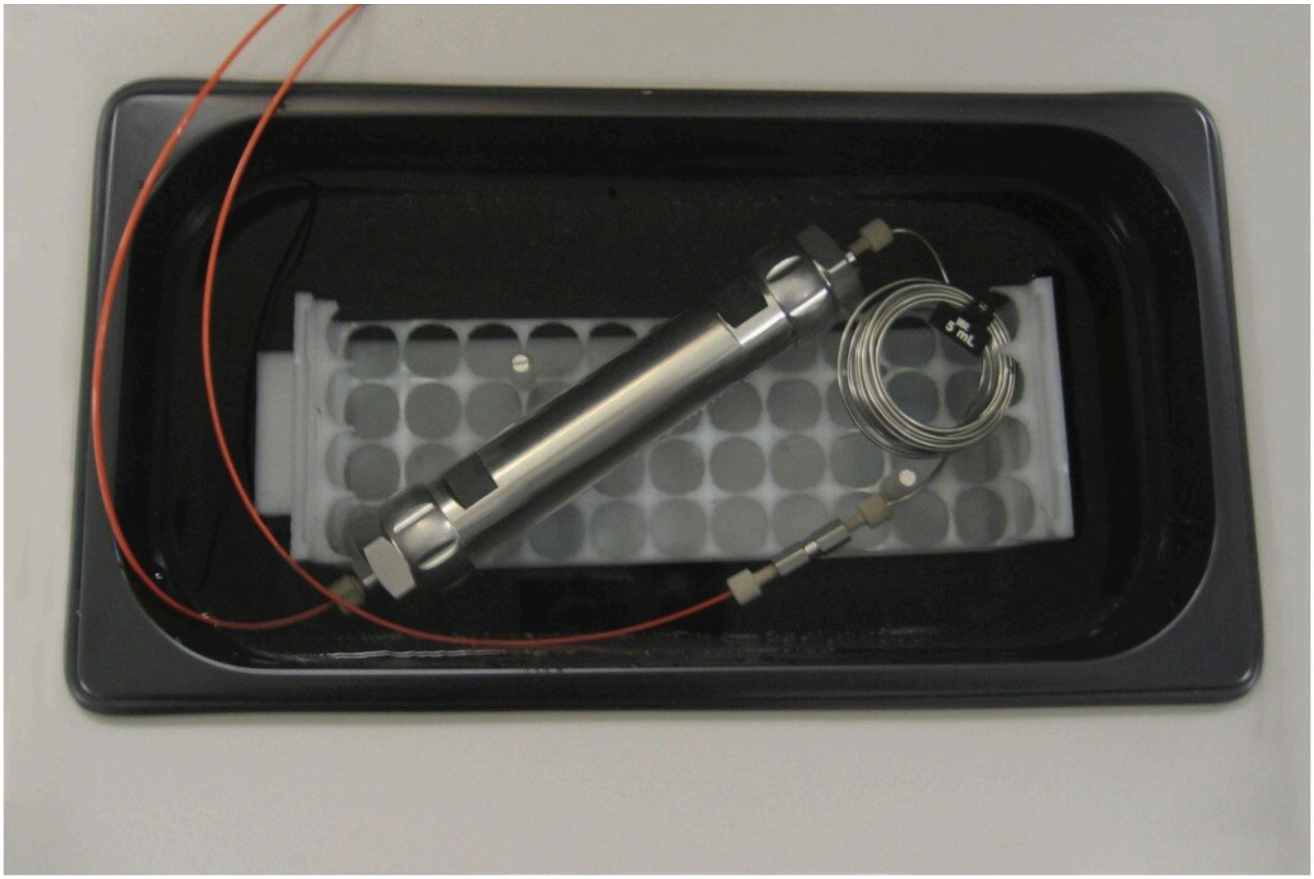

Figure 3. A 5 mL loop plumbed at the head of the column and submerged in the water bath acts as a solvent preheater. Approximately 2.5 min are required to achieve stable pressure at 60 °C.

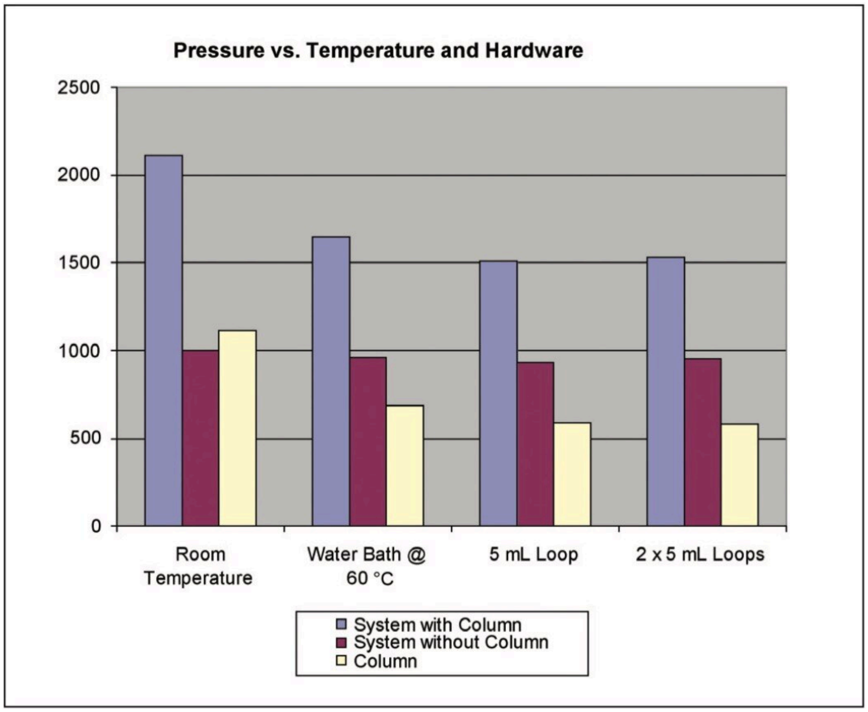

Figure 4. System and column pressure comparison at room temperature and at 60 °C.

Figure 5 shows a comparison of the system equilibration time as it relates to temperature. A column at room temperature takes about a minute to equilibrate to stable pressure; whereas, increasing the temperature of the column with solvent preheating requires the longest equilibration time to ensure equilibrium conditions within the column interior.

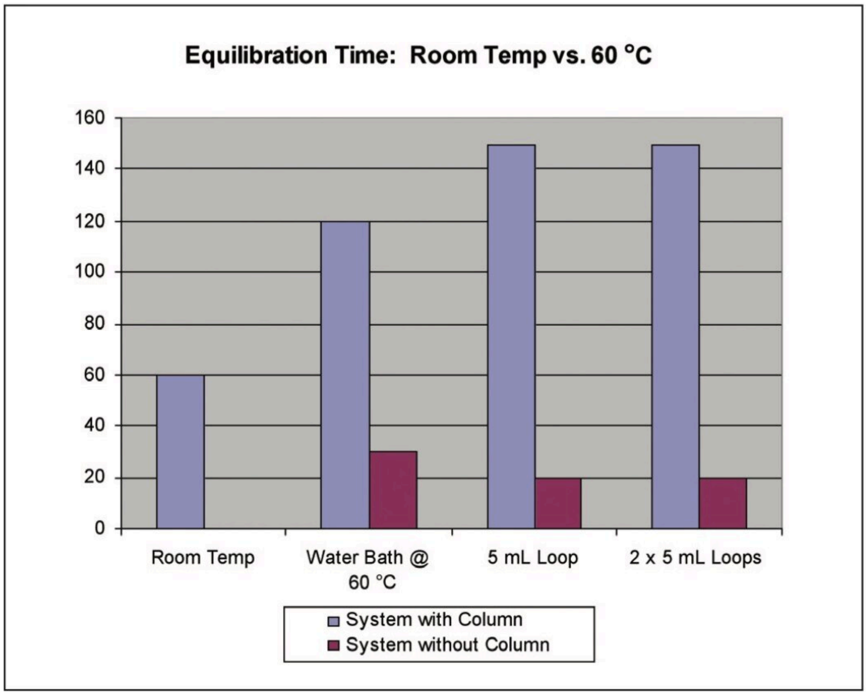

Figure 5. Equilibration time at room temperature and at 60 °C.

Scalability of temperature control

For those instances when pilot scale chromatography will also be performed, the solvent should have the same amount of preheating before entering the analytical column as the solvent does before entering the preparative column. This ensures that the chromatography at both scales will be identical, assuming that the separation is otherwise scaled properly.

To calculate how long the mobile phase is heated at the prep scale, multiply the volume of the preheater loop by the flow rate. A 5 mL loop on a system running at a flow rate of 25 mL/min provides 0.2 min or 12 seconds of residence time for the solvent in the preheater loop. Scaling from 25 mL/min down to 1.46 mL/min, the flow rate at which the pilot scale chromatography will be conducted, is a 17-fold reduction in flow. By proportion, a 12-second residence time for the prep scale calculates to 0.7 second heating time for the pilot scale. At a flow rate of 1.46 mL/min, the volume of the preheating loop needs to be about 17 µL. A 0.010|| I.D. tubing has a volume of about 0.4 µL per cm. Therefore, the length of the tubing required to make the pilot scale preheating loop is 42.5 cm. Make the small scale preheating loop with stainless steel tubing to maximize heat transfer. Install the preheating loop at the head of the analytical column and place both the loop and the column in the water bath.

The amount of band broadening attributed to a solvent preheating loop is negligible because the loop is made with narrow inside diameter tubing. Furthermore, temperature control is used most often with gradient methods which reconstitute the sample at the head of the column.

Effect of temperature in compound isolation

Temperature control is most often used to ensure reproducible separations. In isolation and purification it is also valuable for those separations where sample solubility is less than ideal, and in those cases where the mobile phase viscosity is high, resulting in higher system pressure. As shown in Figure 6, a compound mixture with poor resolution at 40 °C has better resolution at 60 °C.

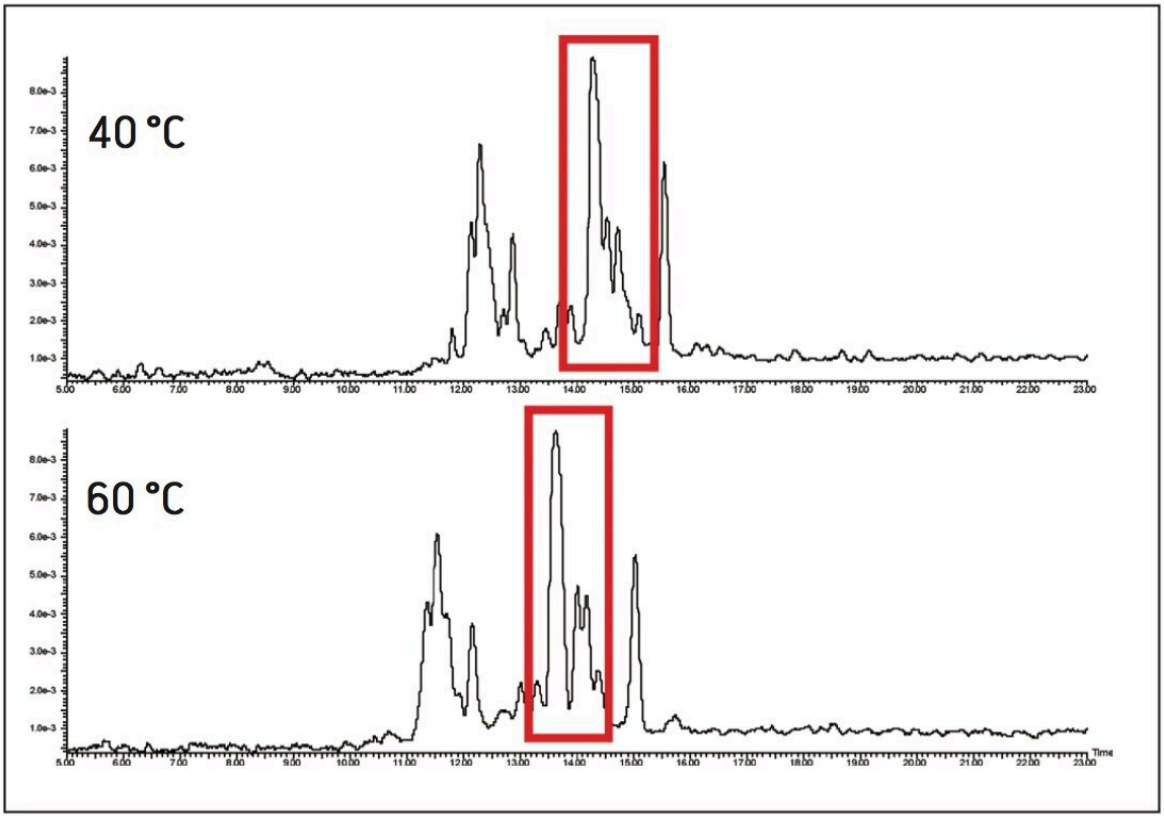

Figure 6. Temperature effect on sample mixture with poor resolution at 40 °C and better resolution at 60 °C.

Some samples give better separations at lower temperature, as shown in Figure 7. These examples illustrate the effectiveness of temperature as a method development tool. An optimized separation makes purification easier and leads to higher product recovery.

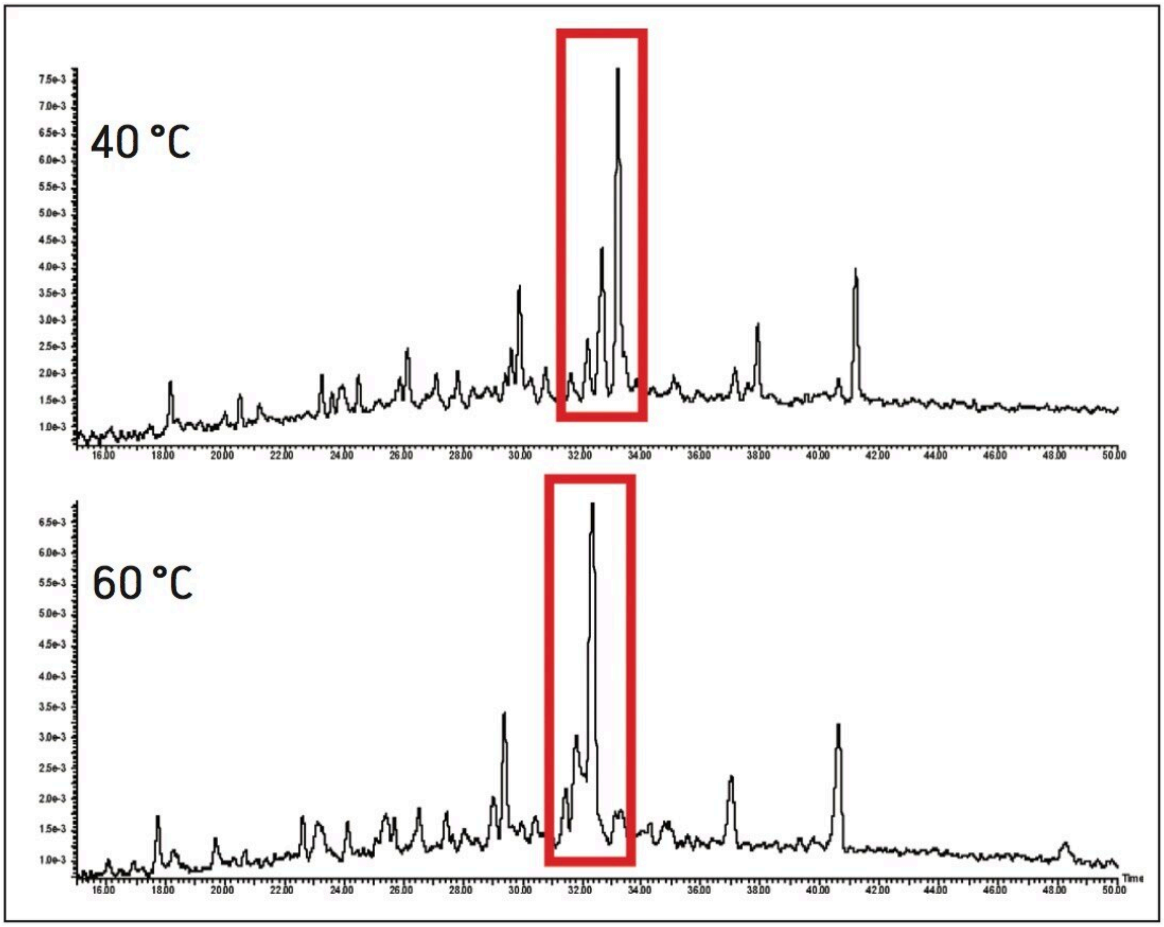

Figure 7. Temperature effect on sample mixture with poor resolution at 60 °C and better resolution at 40 °C.

Conclusion

- High flow rate separations occur at the temperature of the incoming solvent.
- Large diameter columns can be effectively heated by using a solvent preheating loop.
- Additional benefits to using higher temperatures include increasing the solubility of hydrophobic samples and

improving chromatographic peak shape.

- Improved peak shape leads to higher product purity and recovery, making compound isolation faster and more efficient.

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2545 Binary Gradient Module <https://www.waters.com/534320>

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