

Improving Chromatography and Developing Faster HPLC Methods with a Stand-alone Mobile Phase Preheater

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Introduction

Decreasing analysis time in the modern HPLC laboratory through a combination of increasing mobile phase flow rate, decreasing particle size, and shortening columns does little good if it comes at the expense of resolution. Most techniques for increasing analysis speed quickly lead to an increase in system pressure. While new instrumentation is available allowing operation at higher pressures, the increased flow rates lead to viscous heating effects that can begin to compromise resolution.[1]

The power generated within the column is a function of the pressure drop across it and the mobile phase flow rate. Narrow-bore columns are less prone to production of resolution destroying radial temperature gradients than those with 4.6 mm and larger inner diameter because flow rates, and thus power generated, are proportionally less for the same linear velocity, as is their radius.

Frictional heating within the column bed tends to increase the flow rate through its center relative to that at the walls.[2,3] This physical phenomenon produces band spreading, increasing peak width and producing fronting and tailing.

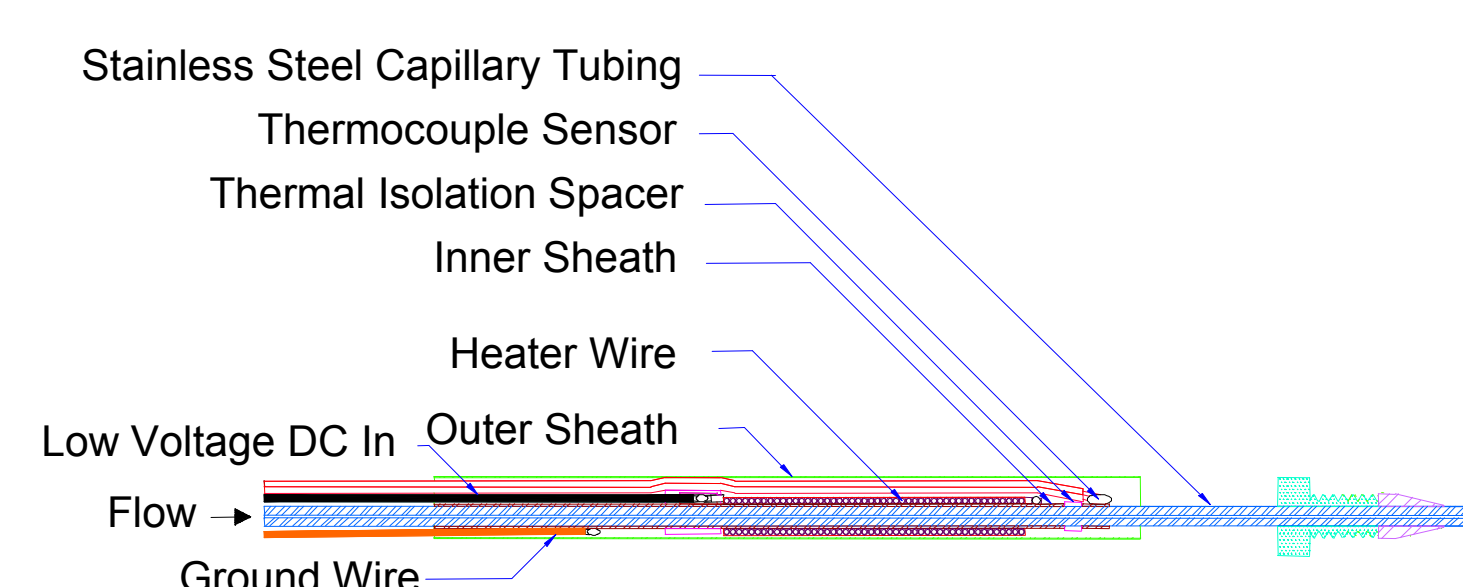
Increasing the temperature in an HPLC separation yields a reduction in mobile phase viscosity, allowing higher mobile phase velocities at lower pressures, producing less frictional heating, increased analyte diffusion rates, reduced analysis time, reduced peak widths and better peak asymmetry.[4] For all of these benefits to be realized, temperature control of the mobile phase through preheating may be the most significant parameter.[5,6]

Experimental

A Selerity Technologies Caloratherm™ Mobile Phase Temperature Controller was used in this study.

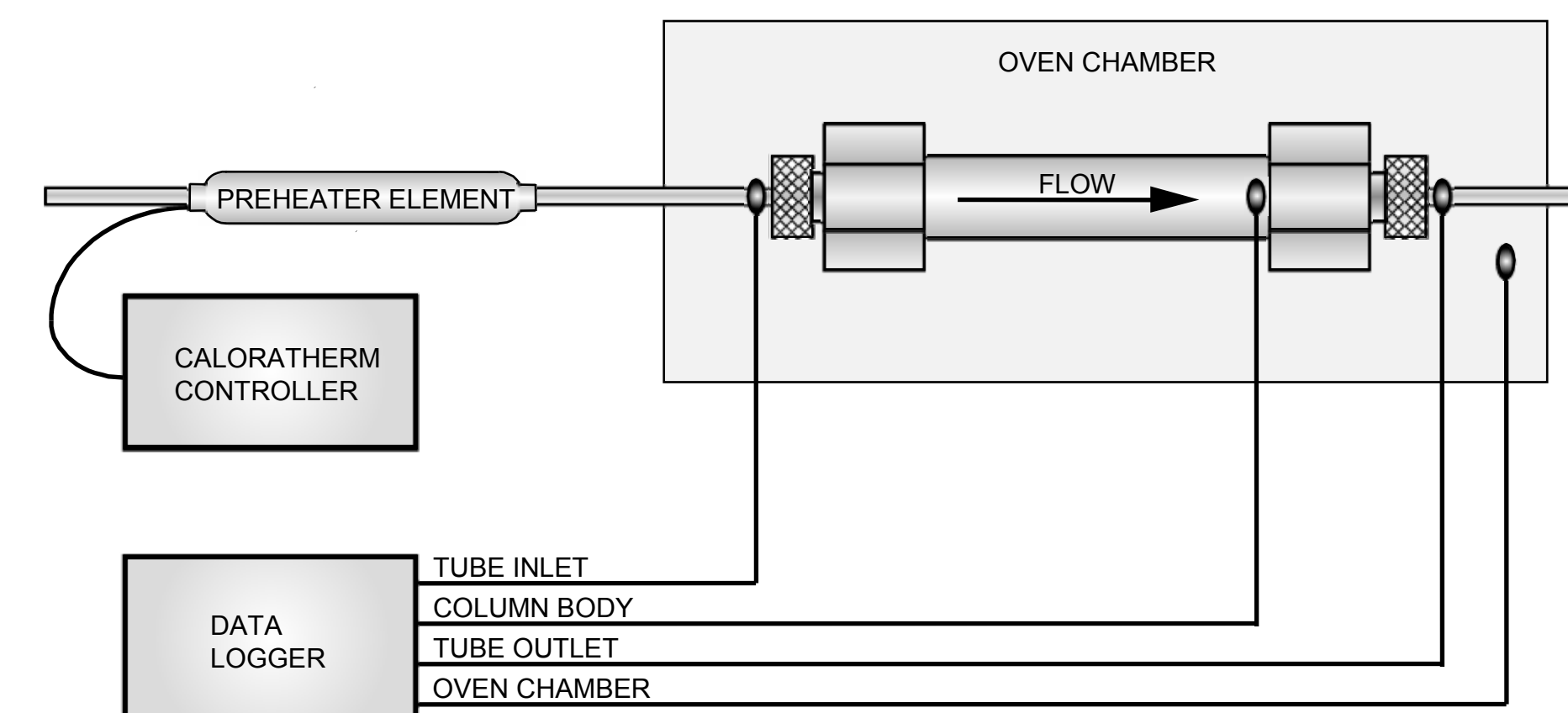


Preheating was accomplished with a sheathed heater element under microprocessor control and a non-invasive downstream thermocouple sensor providing feedback as shown below:



Experimental (cont.)

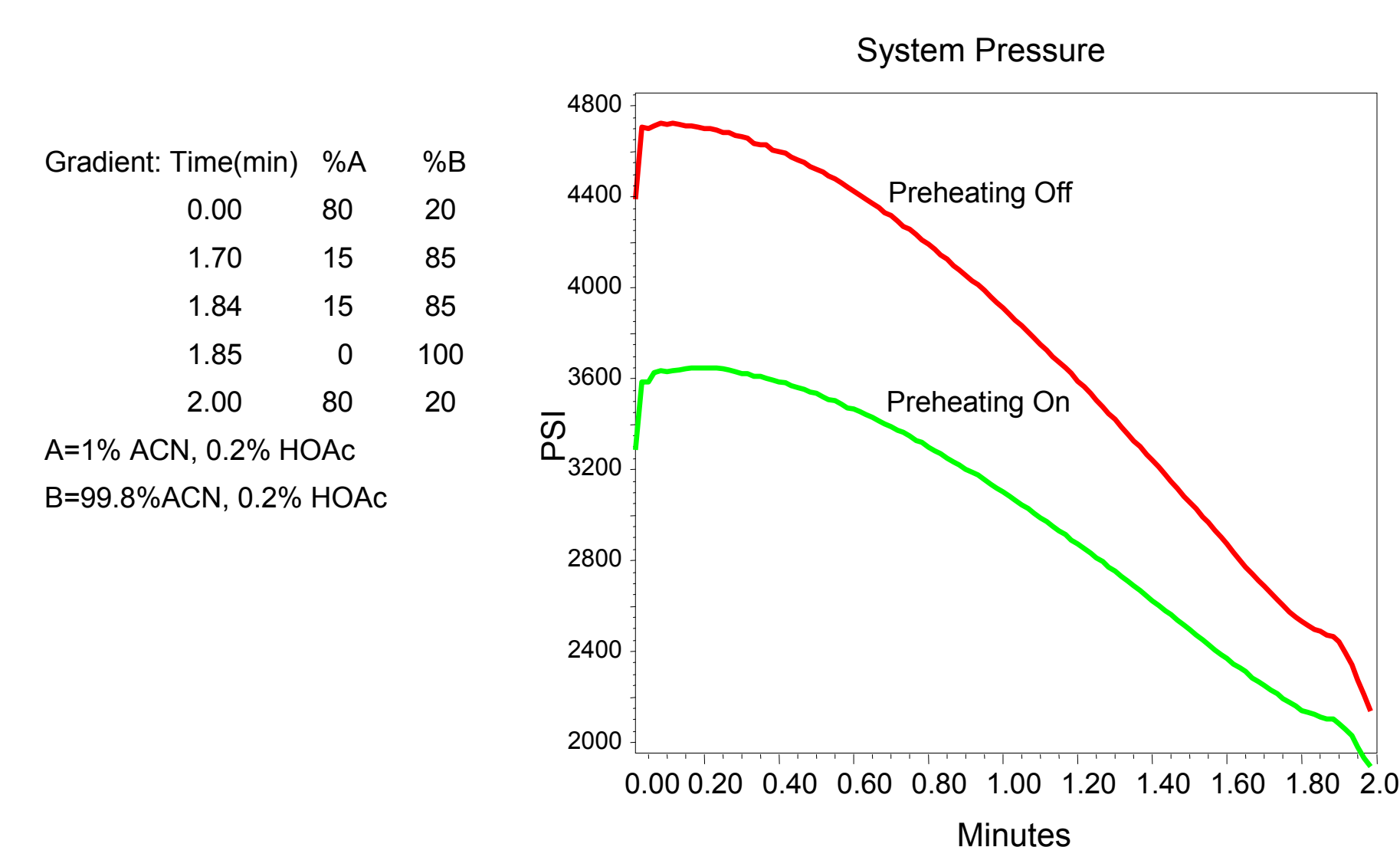
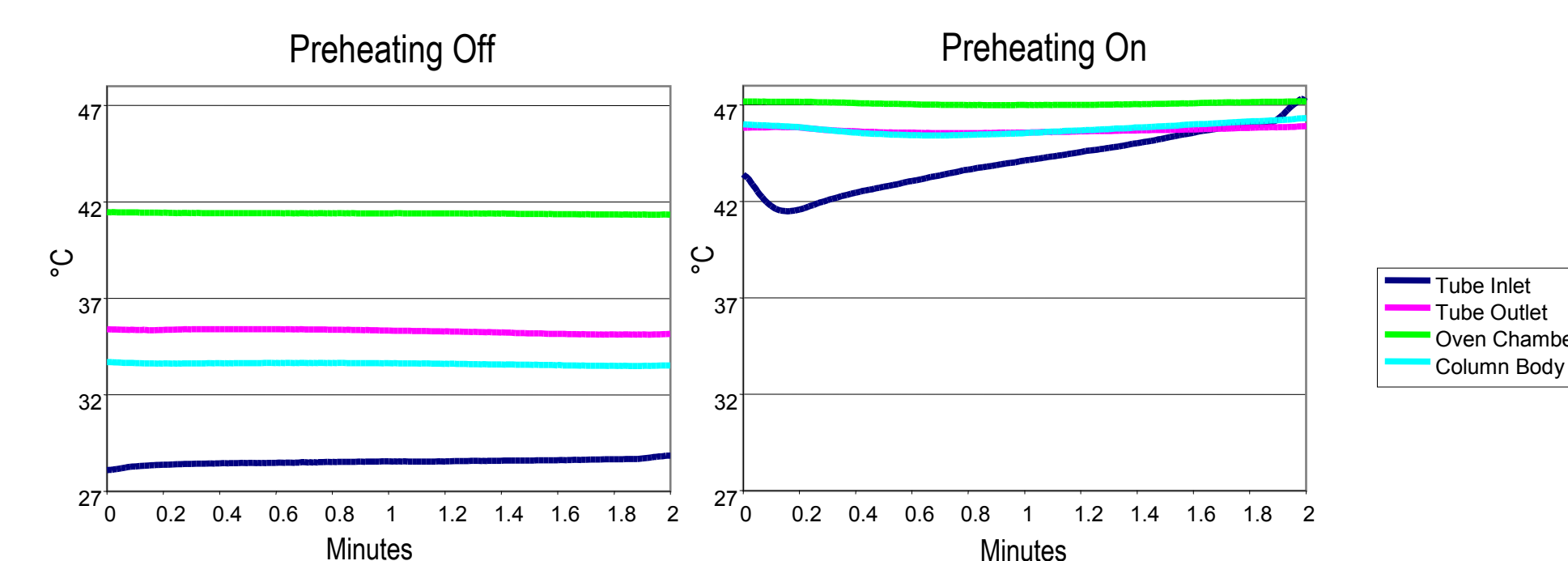
Temperatures were measured at various points near the column with calibrated thermistors and platinum RTDs and recorded directly into Excel through a Dataq Datalogger as shown below:



This allowed temperature measurements to be taken during fast gradient runs where mobile phase composition, viscosity, and heat capacity changed rapidly.

Results and Discussion

Temperature profiles for an Inertsil C8 4.6x50mm, 3µm column with a sheath heater at 45°C, Caloratherm preheater (delivering 7-10 watts when on), mobile phase flow 5mL/min are shown below.

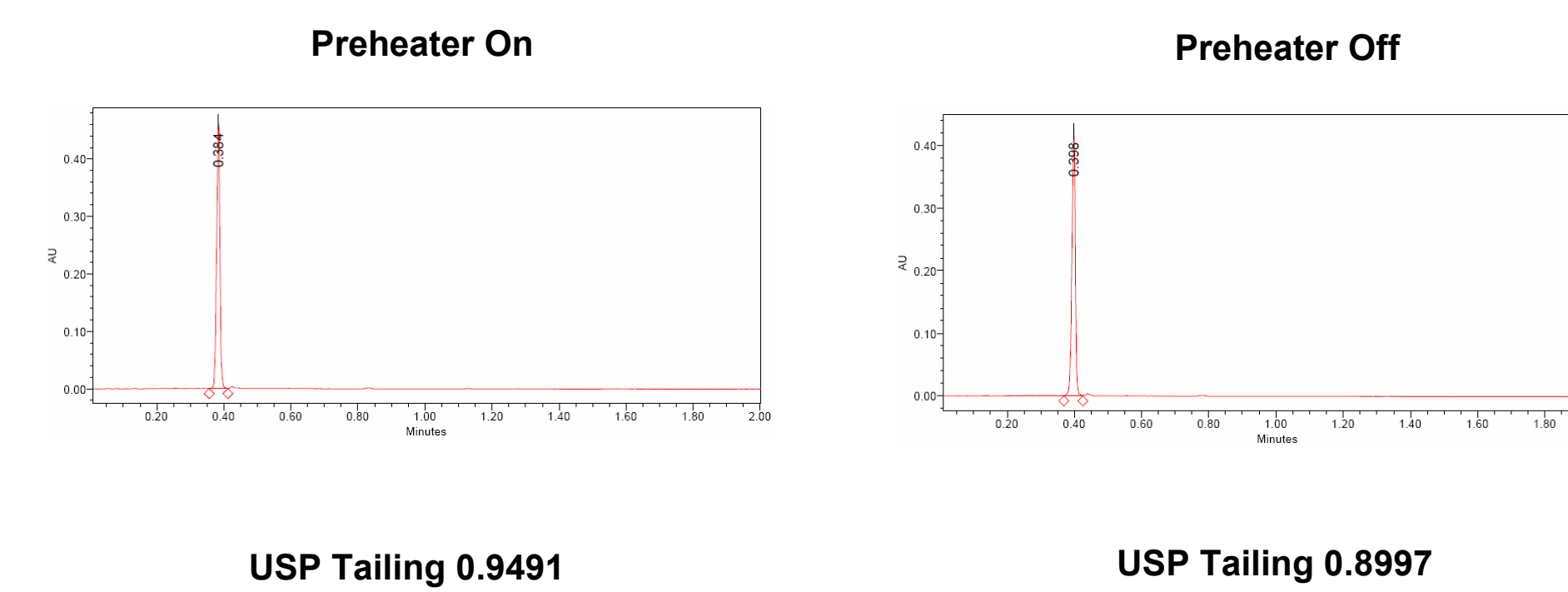


System pressure shows the largest response to mobile phase preheating in this example. Without preheating, operation is very close to the 5,000 psi limit of this Alliance system, where slight perturbations due to injection transients can cause shutdown.

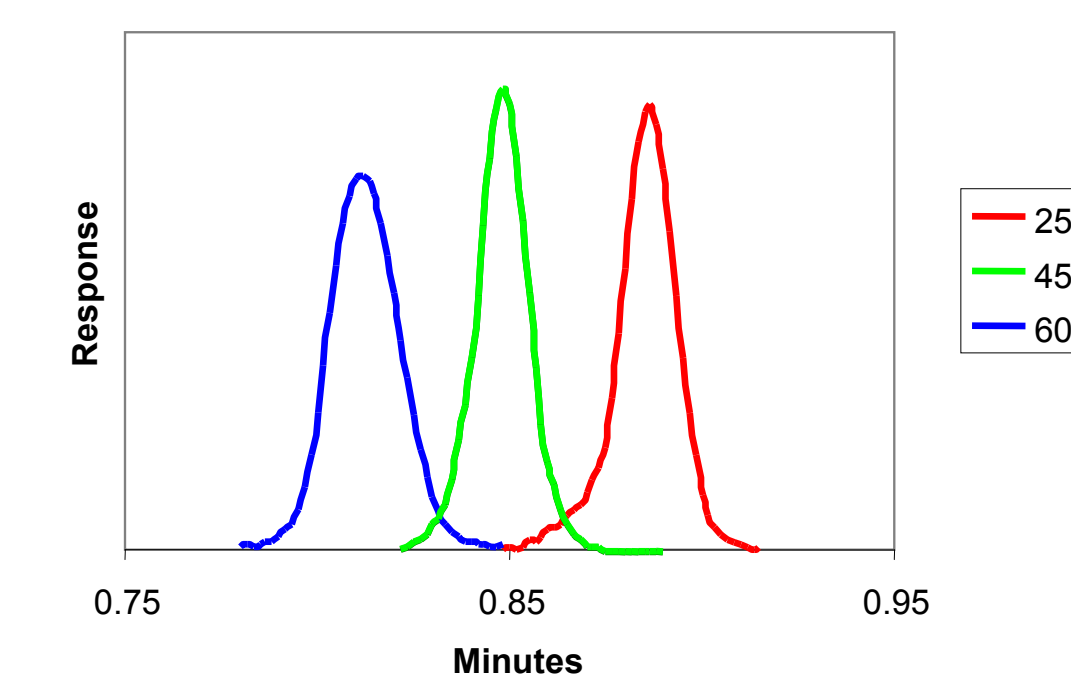
Note that system pressure follows the viscosity profile for the mobile phase solvent system, first increasing and then decreasing as the amount of acetonitrile goes up. Offsets in the mobile phase exit temperature relative to the inlet are apparent in these measurements and are due to energy input from viscous flow.

Chromatographic results using a PDA at 276nm for reserpine are shown. Aside from shorter retention when closer to the 45°C temperature with preheating,

more fronting is seen from the thermal mismatch produced when the preheater is off. This effective increase in peak width (or variance) reduces resolution.



Optimization of a separation at a particular high velocity requires selection of a corresponding temperature to balance the diffusion rates and their effect on the a, b, and c terms of the van Deemter equation. Elution of methionine enkephalin at a linear velocity of 7 mm/sec under similar conditions to the previous chromatograms shows the effect of temperature on peak elution and shape.

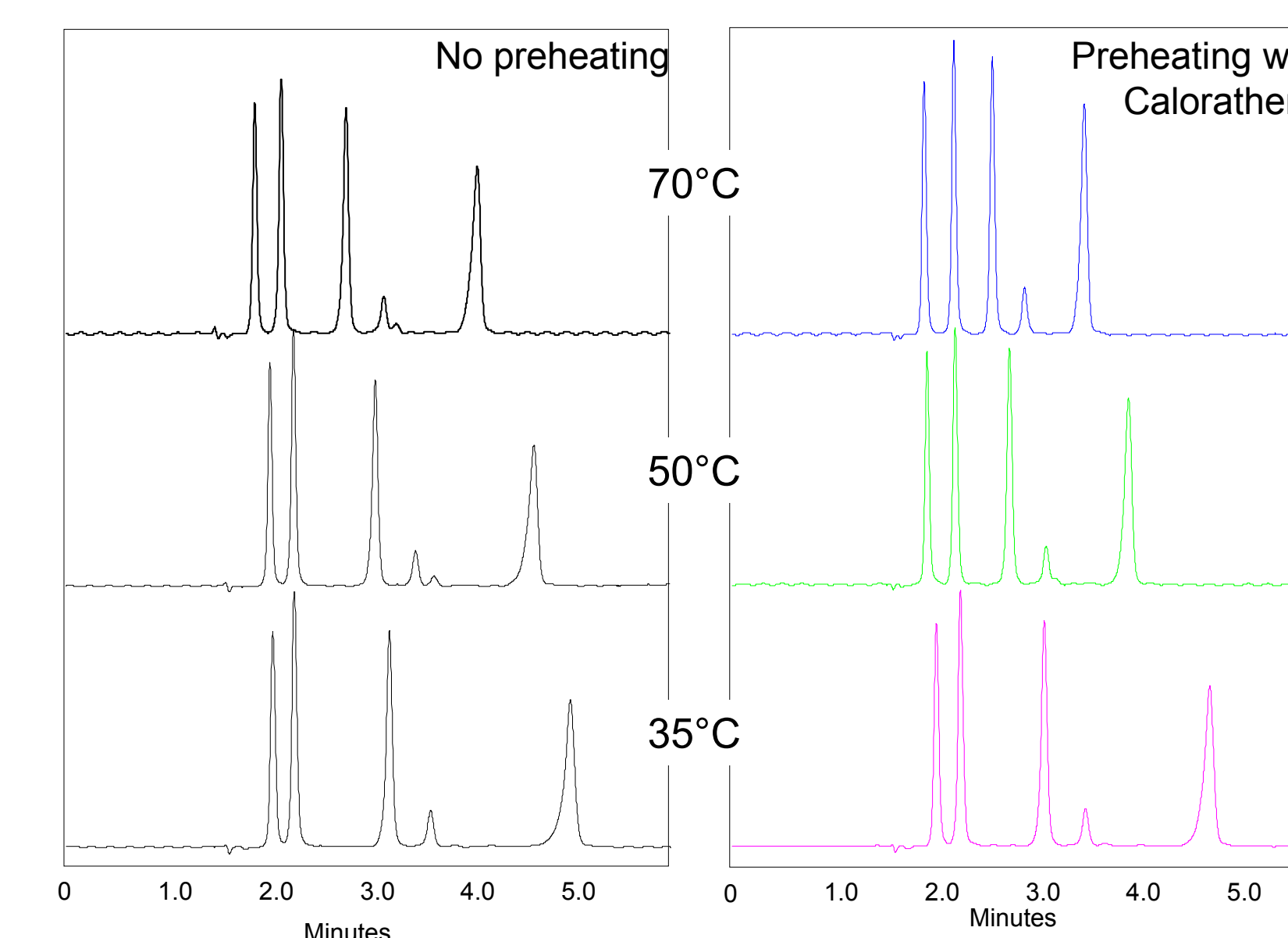


-Data courtesy of Mark J. Hayward, Lundbeck Research

In each case, mobile phase preheating was employed, but fronting is observed at low temperature, a narrow symmetrical peak at 45°C, and a symmetrical, but broader one at still higher temperature.

At traditional flow rates and velocities, preheating the mobile phase gives improved peak widths and geometry, even at modest temperatures:

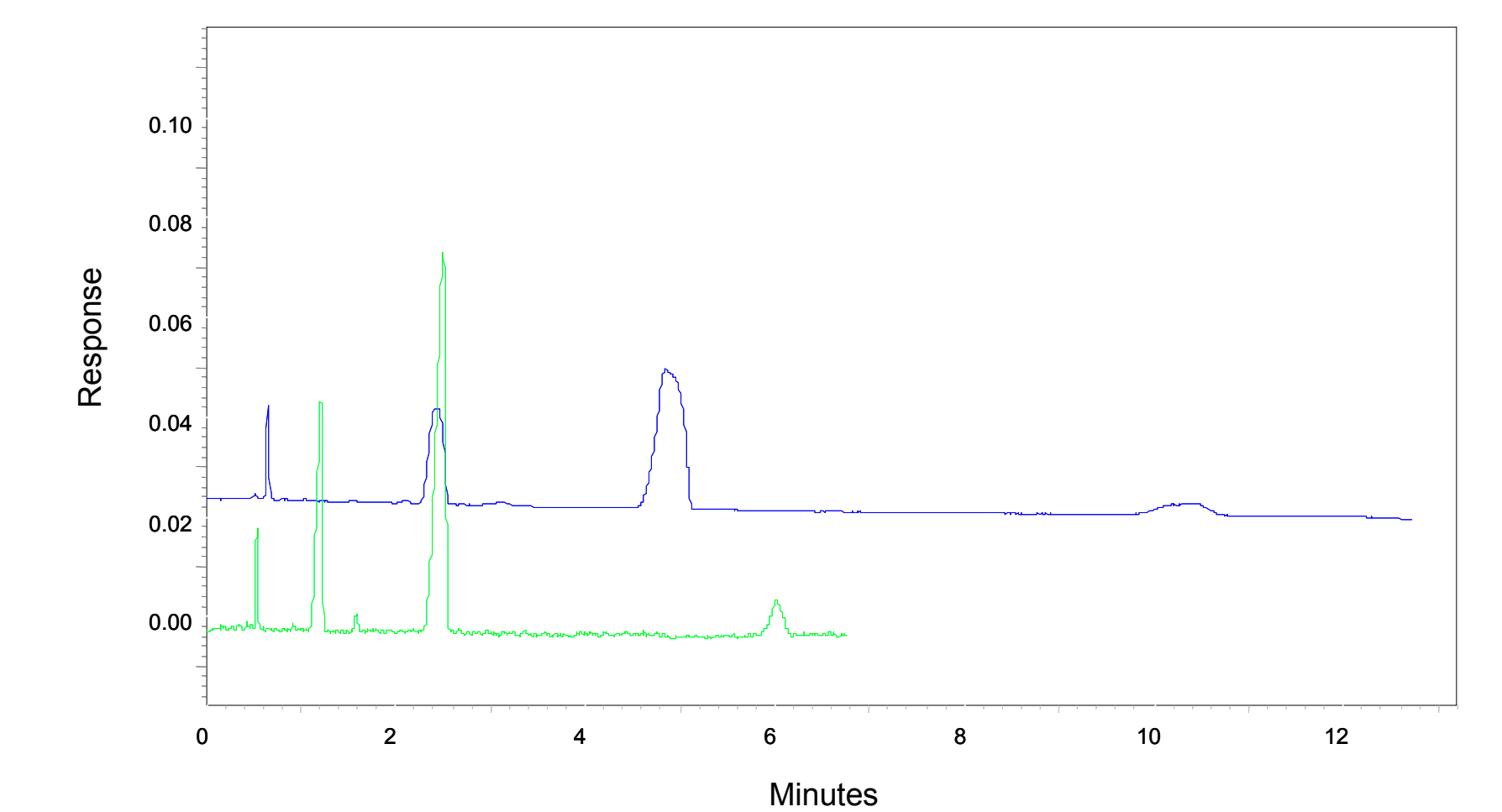
Comparison of analgesics with and without preheating at three temperatures



Elution Order:
Acetaminophen
Caffeine
Salicylamide
Aspirin
Salicylic acid

Column: Selerity Blaze 200 C₁₈,
3 µm, 100 x 4.6 mm
Mobile Phase: 30% acetonitrile with
0.1%TFA
Flow Rate: 1.0 mL/min
Detection: UV 220 nm

The separation of sedatives below used a block heater set at 60°C, with the blue trace collected without active preheating, and the green chromatogram with preheating of the mobile phase. Analytes eluting with higher k' often show more peak width variance under conditions with radial thermal gradients than less retained compounds.



Elution Order:
Uracil
Phenobarbital
Butalbarbital
Carbromal

Column: Zorbax SB-C18,
3 µm, 150 x 4.6 mm
Mobile Phase: 20% acetonitrile 3mM
phosphate, pH 7
Flow Rate: 3.0 mL/min
Detection: UV 220 nm

Conclusions

Active mobile phase preheating facilitates the use of higher velocities for faster chromatography by reducing thermal gradients and enhancing resolution, reducing system pressures, and allowing for targeted flow-based temperature optimization.

Mobile phase preheating can be accomplished non-invasively with short sheathed heaters under microprocessor control using existing connection tubing between the injector and column in conjunction with a variety of column heater formats.

References

- [1] André de Villiers, Henk Lauer, Roman Szucs, Stuart Goodall and Pat Sandra, "Influence of frictional heating on temperature gradients in ultra-high-pressure liquid chromatography on 2.1 mm I.D. columns", *J. Chromatogr. A*, **1113** (2006) 84-91.
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- [4] B. Yan, J. Zhao, J.S. Brown, J. Blackwell, and P.W. Carr, "High temperature ultrafast liquid chromatography", *Anal. Chem.*, **72** (2000) 1253-1262.
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Contact information

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