Improved HPLC Separations at Elevated Temperatures Using a New Stand-alone Preheater

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Abstract

Recent advances in instrumentation and column technology have made it possible to perform HPLC separations at temperatures up to 200°C. Increasing the temperature in HPLC yields a reduction in analysis time, improved efficiencies and better peak shapes. The mobile phase must be adequately preheated to avoid thermal mismatch band-broadening (1). This band-broadening can occur at temperatures lower than 50°C. Most traditional block column heaters on the market have very limited or no accommodations for preheating the mobile phase. A new stand-alone mobile phase preheater has been developed for use with existing column heaters to eliminate thermal mismatch. This device works independently of the existing column heater, is completely non-invasive, and adds no dead volume to the HPLC system. It works with any column heater. Several applications performed at elevated temperatures - with and without mobile phase preheating - will be presented to demonstrate the importance of proper preheating of the mobile phase.

Introduction

The need for adequate preheating of the mobile phase in elevated temperature liquid chromatography has been described by many authors. Most means of adding energy to the mobile phase have been through passive means – extended lengths of tubing in intimate contact with blocks, fluids, or circulating air heated to the operating temperature. The length requirements can be quite large for thermal equilibration, particularly at high mobile phase flow rates(1). Several researchers have described the use of active preheaters, wherein considerably shorter thermal equilibration tubing lengths can be used between the injector and column(2).

Horvath(3) described the use of tubing coiled around a mandrel heated and controlled by a GC injector module. Wolcott(4) attached a heater to the outside capillary tubing wall and sensed the temperature of the mobile phase downstream through a small thermocouple installed in a PEEK tee. This sensor provided feedback to the controller, which regulated the amount of power delivered to the heater according to its setpoint. Marin(5) described the use of a small preheater with temperature sensing downstream on the external wall of the tubing, allowing a short connection between injector and column, and with the advantage of being non-invasive and adding no dead volume.

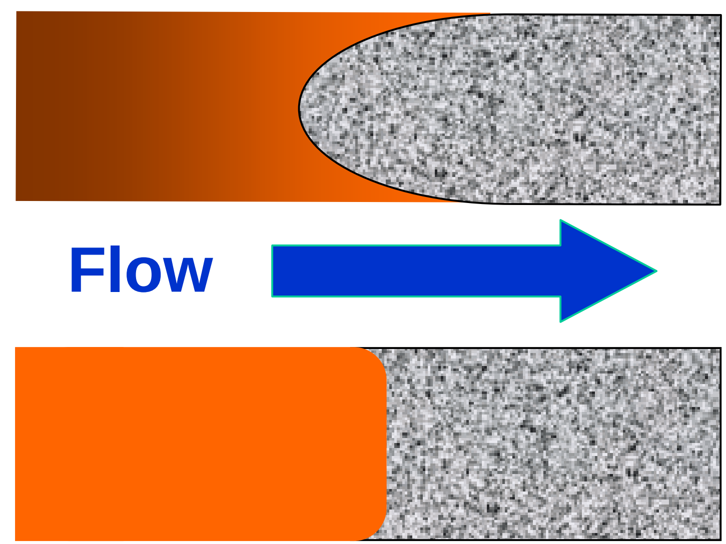
Thermal Mis-match Effects

No Preheating

Parabolic flow caused by mobile phase heating up faster along column wall

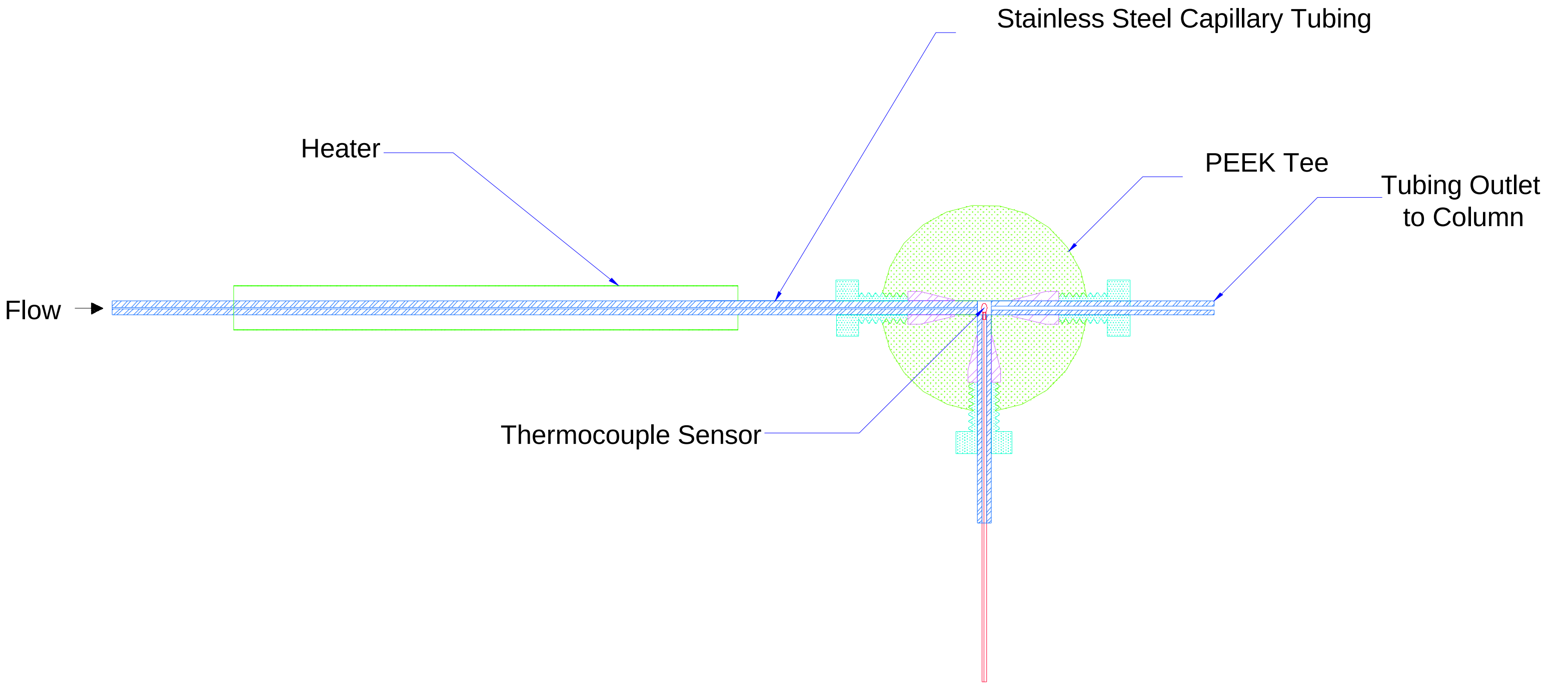
With Preheating

Mobile phase at column temperature eliminates parabolic flow



Not only is flow not uniform with thermal mis-match, but analyte retention gradients are also present, giving misshaped peaks.

Active Preheater with Invasive Sensing



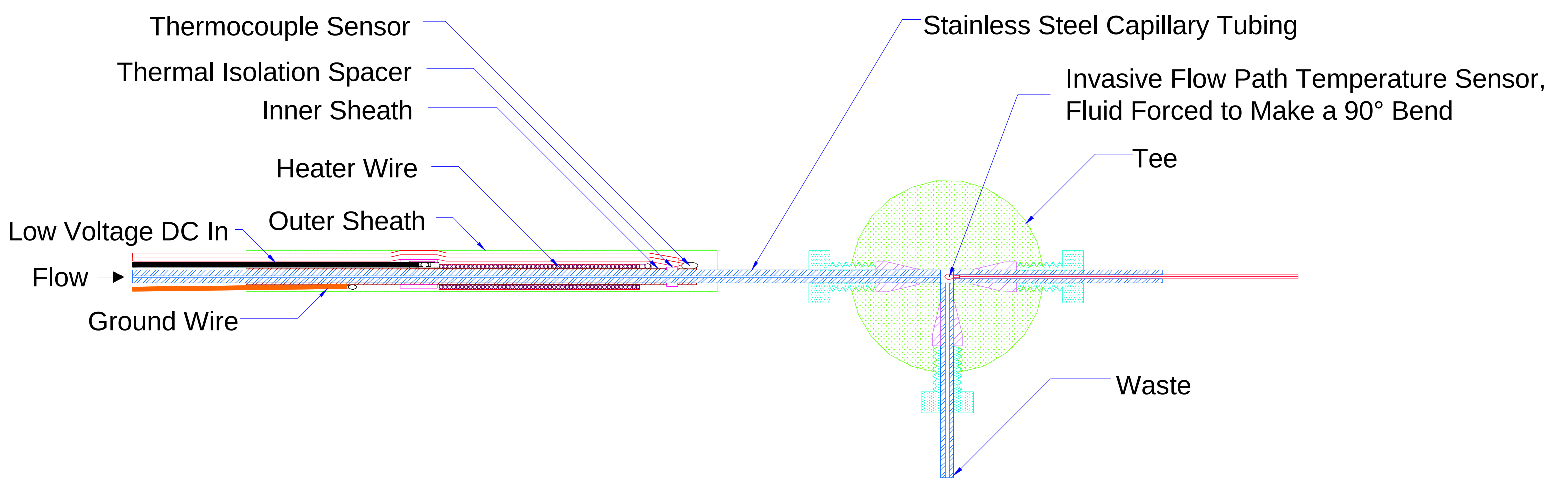
Invasive sensing with feedback control to a heater attached to the stainless steel tube leading to the column (Ref 3).

Experimental

An Eppendorf CH-30 Block Column Heater was used in most of the studies, with a Lab Alliance Model 500 Pump and Lab Alliance UV Series 1500 Detector. A Dionex GPM-2 Pump, VDM-2 Detector, and ACI controller were also used. A prototype Caloratherm™ Active Mobile Phase Preheater with external slide-on heater/sensor was used for mobile phase temperature sensing and heating.

Selerity Caloratherm^{TM} Mobile Phase Temperature Controller

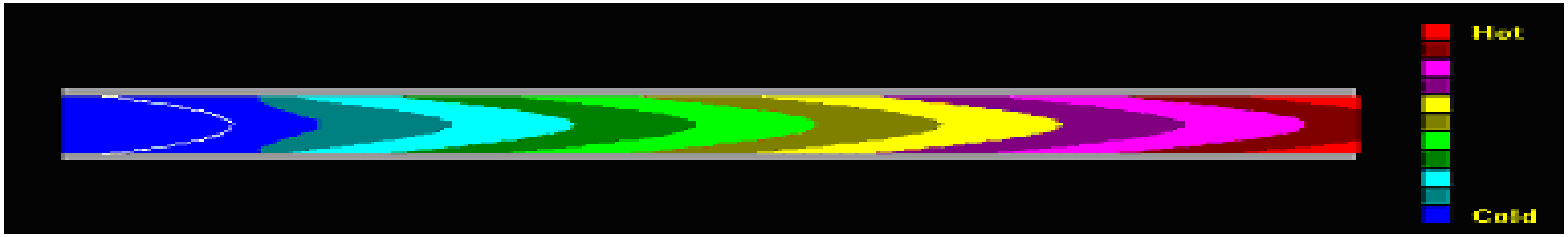
Experimental Setup for Temperature Monitoring With the Caloratherm™ Preheater Under Flow Conditions



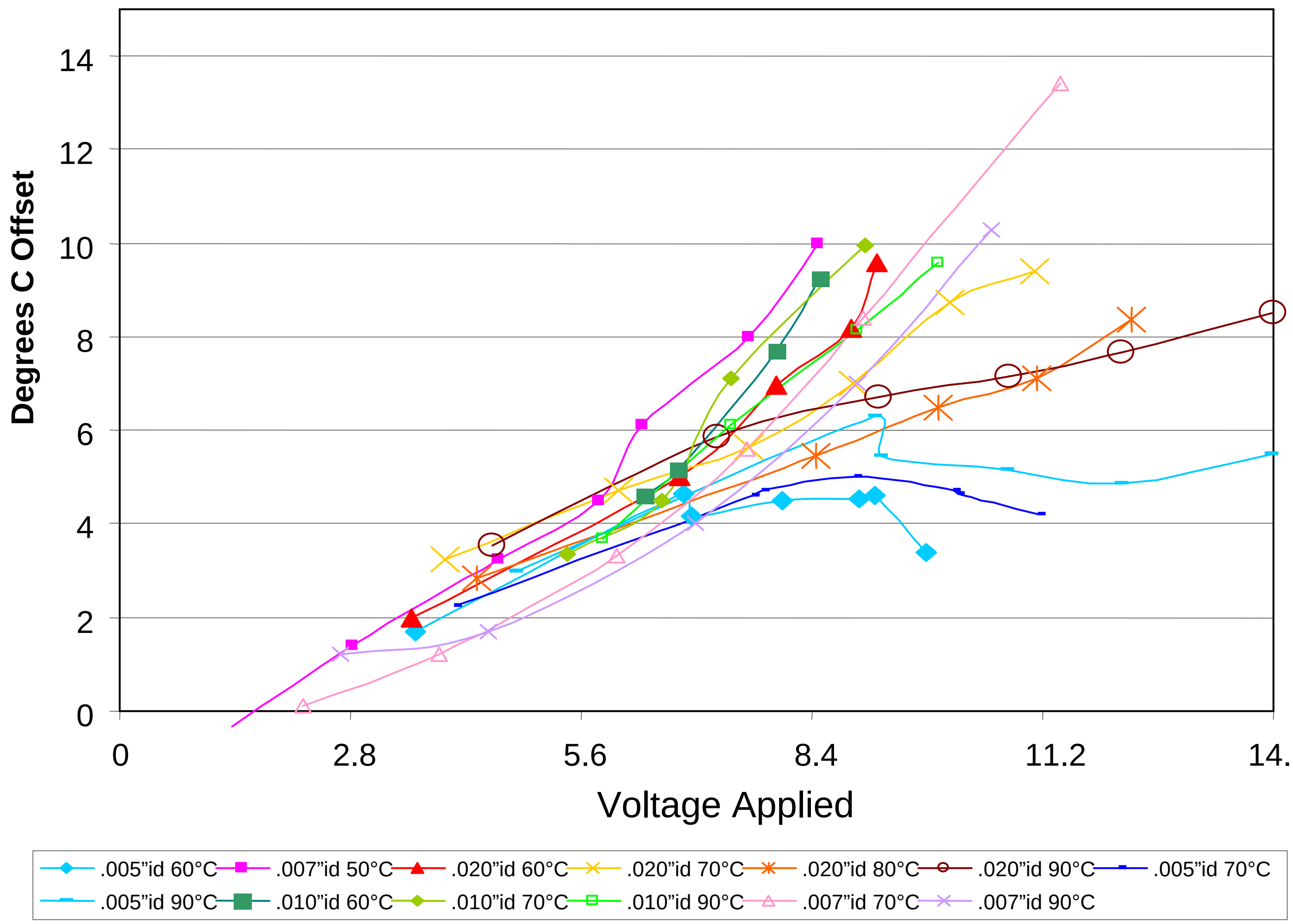
-- Not to Scale --

With the temperature monitoring setup on the preceding page, a variety of measurements were taken with various flow rates of water in 1/16” o.d. stainless steel tubing covering several internal diameters. All thermocouples were calibrated before use. The voltage delivered to the heater element and the offset between the Caloratherm-sensed temperature and the measured values inside the fluid path were plotted. A trend of increasing offset was observed as a function of the power applied to the heater, with less dependence on the tubing internal diameter.

The literature describes a slip-flow phenomenon with microchannel heat exchangers where in the case of constant heat flux into the fluid, a region of low viscosity near the tubing wall is generated, affecting the friction factor and flow profile. The result is a parabolic temperature distribution represented below within the preheating zone, even with tubing as small as 0.005” in internal diameter (6-8).



Caloratherm™ Offsets with Water



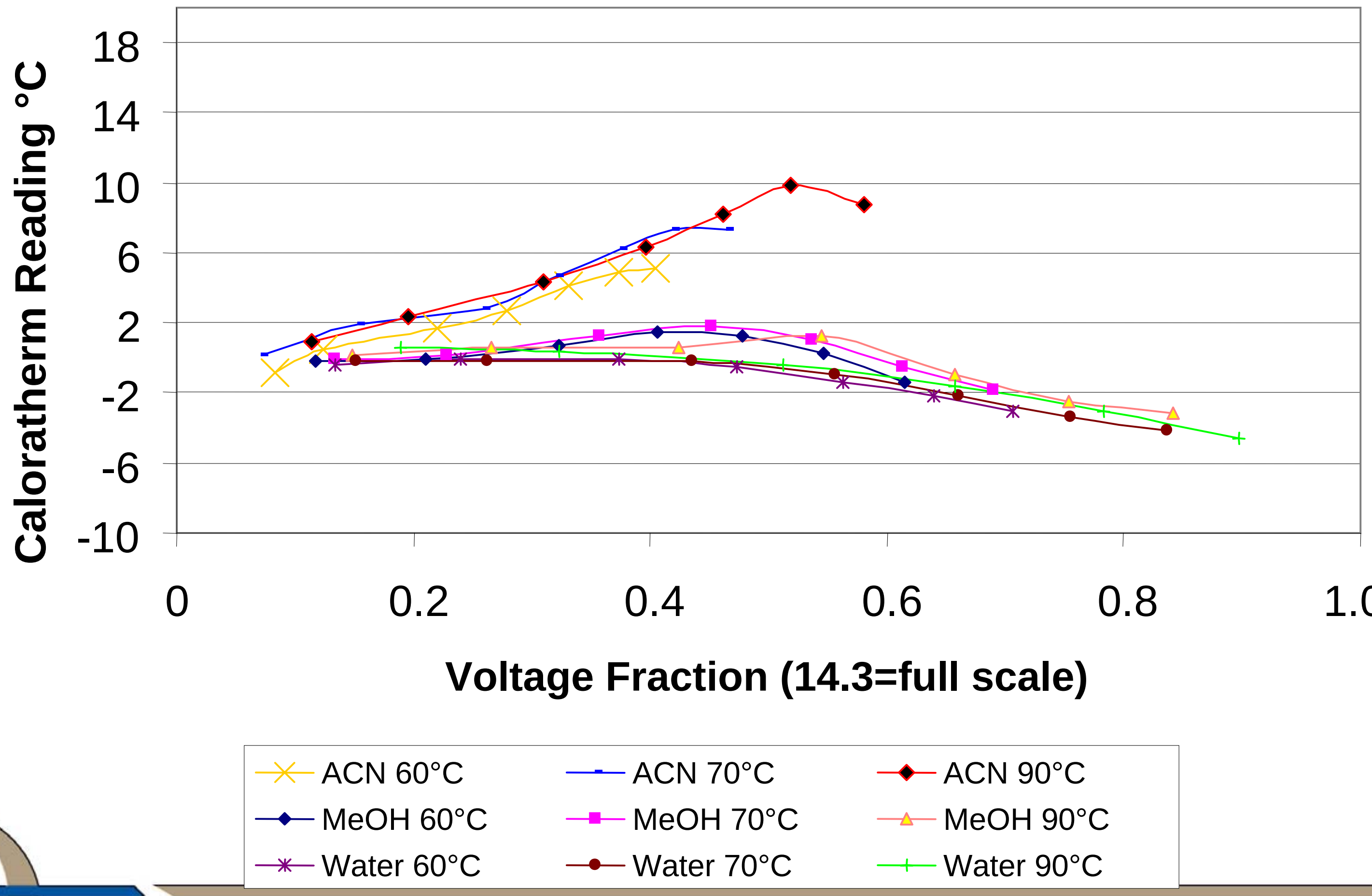
Flow conditions and fluids within tubing typically used in HPLC give rise to Reynolds numbers less than 2000, which results in laminar flow. Heat transfer from the vicinity of the tubing wall into the core is thus dependent on convective effects. Classical theories on heat transfer within pipes do not always follow experimental observations in microchannels (6-8). Both higher and lower friction factors and Nusselt numbers have been reported compared to theoretical predictions in microchannel laminar flow. Suggested reasons for these deviations are surface roughness effects, entrance effects, electric double-layer effects, nonconstant fluid properties, two- and three dimensional transport effects, and slip flow.

Our observations suggest that sensing within 0.375” of the heated zone on the tubing exterior wall gives a high temperature reading that has a strong dependence on heat flux rates. A correction factor was built into the Caloratherm control algorithm that changes the control reading according to the equation:

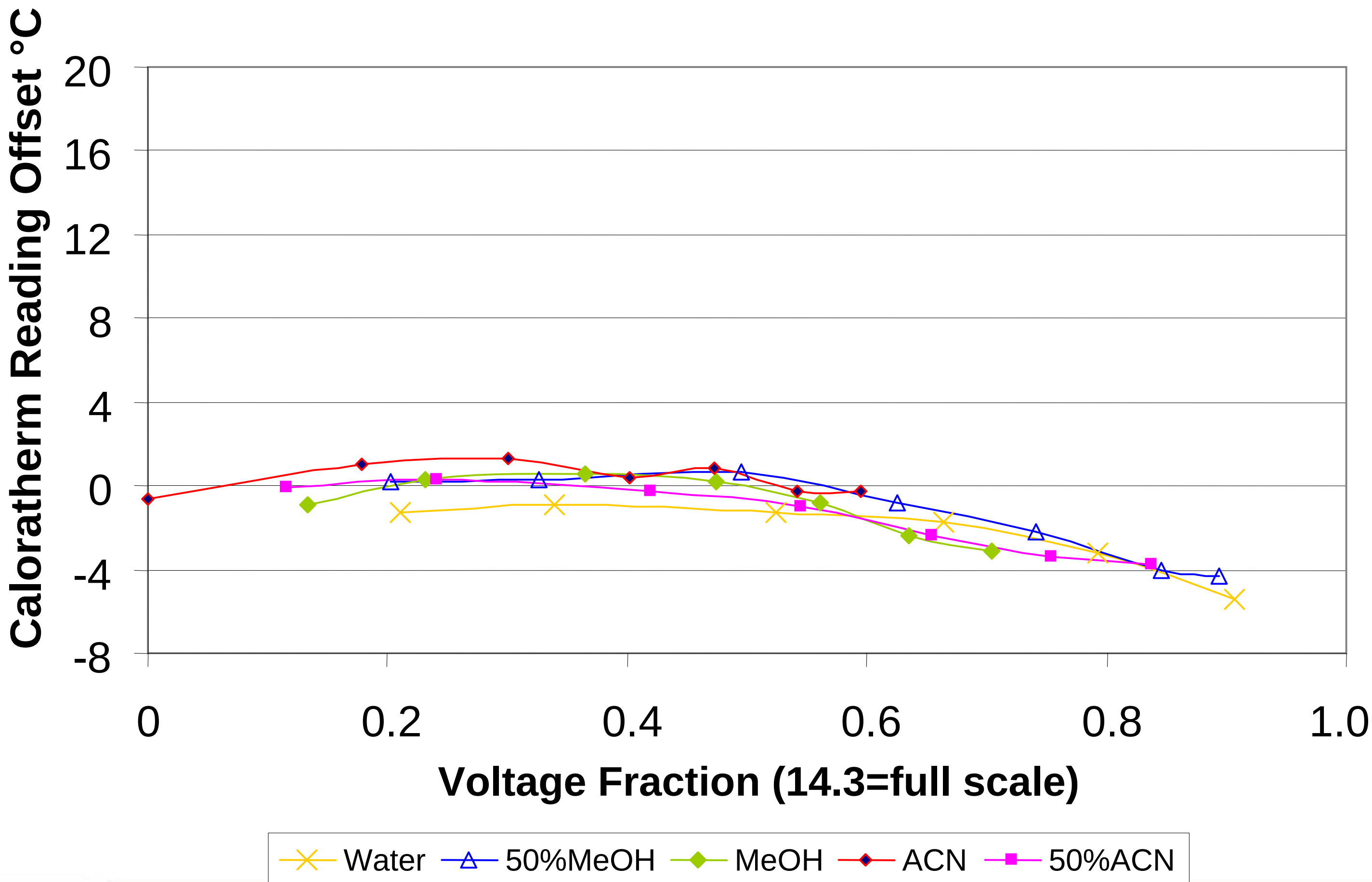
$$-10 * (\text{Voltage Fraction}) + 1 = (\text{Actual Temperature in °C})$$

The Voltage Fraction is the percentage of full scale voltage(14.3V) divided by 100. With this factor in place, the following two plots were generated.

Caloratherm^{TM} Offsets Using Compensation, 0.010" ID Tubing



Caloratherm^{TM} Offsets Using Compensation, 0.005” ID Tubing, 90°C



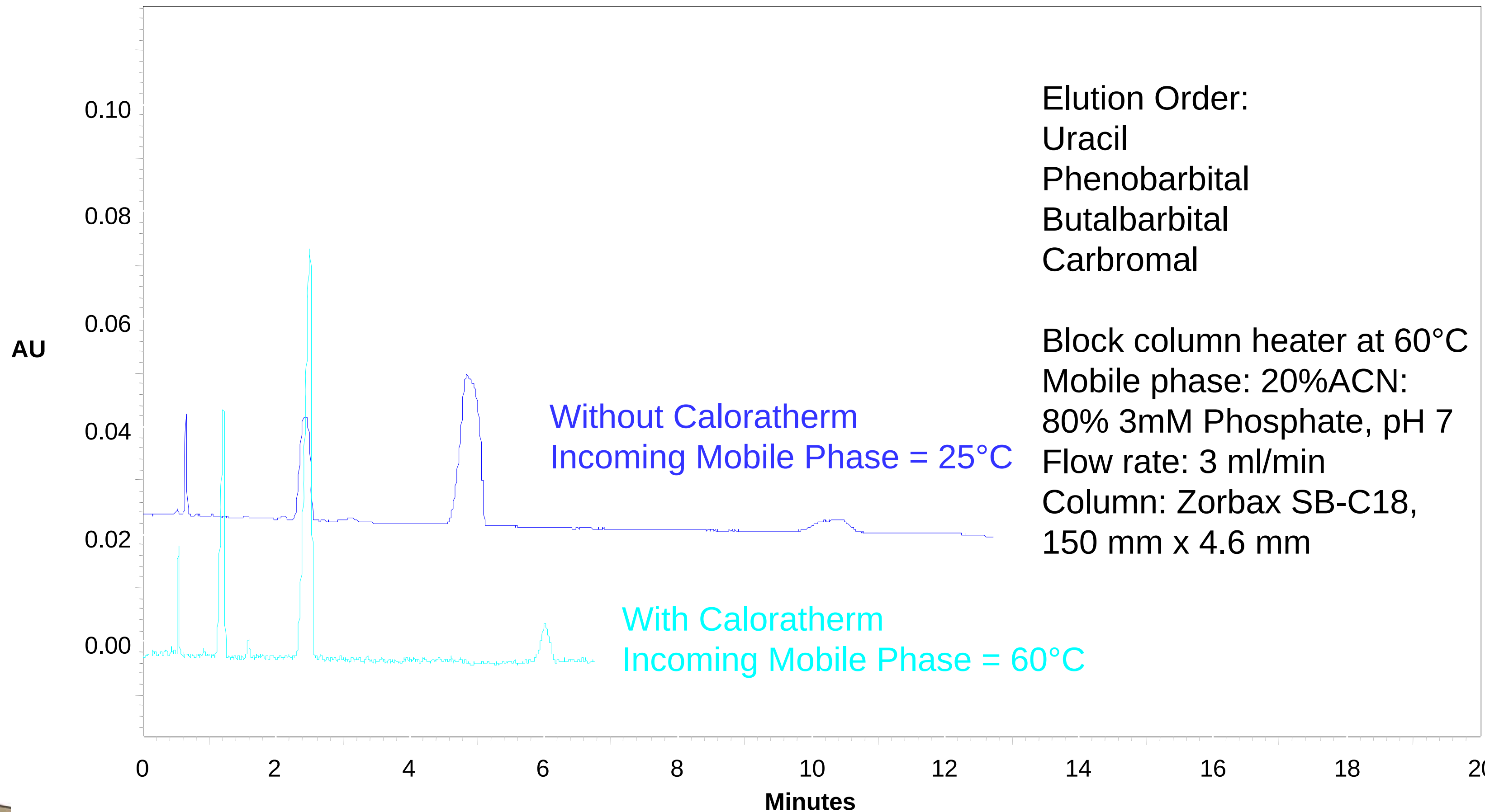
It should be noted that other preheater/sensor configurations would require a different compensation formula for accurate control.

Even with the compensation factors employed, 100% acetonitrile in the larger 0.010" id tubing showed a significant offset at mid-range power settings. The traces show data from flows as high as 6 ml/min, and the low voltages required to heat the mobile phase to the target temperature are indicative of acetonitrile's low heat capacity and density. Water is normally added in varying amounts to acetonitrile for chromatographic separations, however, which increases these parameters.

The effect of preheating on peak shape and retention is evident on the following page. In most cases, the use of effective mobile phase preheating produces shorter retention and higher efficiencies compared to mis-matched conditions.

The actual temperatures of analyses with two columns at varied flow rates are shown next in the same block heater, both with and without Caloratherm preheating of the mobile phase. A huge temperature differential between the inlet and outlet of the column was evident when active preheating was not employed. Analyte retention behavior confirmed a lower actual average separation temperature when active preheating was not used, as elution times were close to room temperature values.

Separation of Sedatives at 60°C



Actual tubing wall temperatures measured at the inlet and outlet of the column

			Preheater On		Preheater Off	
Column Dimensions	Oven Temperature	Flow Rate ml/min	Inlet Temp.	Outlet Temp.	Inlet Temp.	Outlet Temp.
50 x 7.8 mm, 3µm	60.0	1.0	60.0	61.0	32.5	50.6
	60.0	3.0	60.0	60.1	26.9	42.2
	60.0	5.0	60.0	61.0	26.9	40.0
	70.0	1.0	70.0	69.7	35.8	56.5
	70.0	3.0	70.0	70.7	30.3	46.6
	70.0	5.0	70.0	72.0	28.1	41.3
150 x 4.6 mm, 3µm	60.0	1.0	60.0	57.2	36.4	55.0
	60.0	2.0	60.0	64.5	32.6	53.1
	70.0	1.0	70.0	73.3	35.8	61.8
	70.0	2.0	70.0	73.8	31.7	58.3

Conclusions

A slip-on active mobile phase preheater has been shown to improve peak shape and efficiency in HPLC at elevated temperatures

An algorithm was developed for the Caloratherm design based on heat flux rates which compensates for the lack of radial equilibration when temperature sensing is performed on the tubing wall 0.375” downstream from the heated zone

Some aspects of this work are covered under
US and International Patents Pending

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References

[1] P. Carr et al, *Anal. Chem*., **72** (2000) 1253-1262.

[2] B.A. Jones, *J. Liq. Chromatogr. Rel. Techniq*. **27** (2004) 1331-1352.

[3] H. Chen and C. Horvath, *Analytical Methods and Instrumentation* **1** (1993) 213-222.

[4] R.G. Wolcott, J.W. Dolan, L.R. Snyder, S.R. Bakalyar, M.A. Arnold, and J.A. Nichols, *J. Chromatogr. A* **869** (2000) 211-230.

[5] S.J. Marin, B.A. Jones, W.D. Felix and J. Clark, *J. Chromatogr. A* **1030** (2004) 255–262.

[6] Björn Palm, *Microscale Thermophysical Engineering*, **5** (2001) 155–175.

[7] X. F. Peng and G. P. Peterson, *Exp. Thermal Fluid Sci.,* **12** (1996) 98–104.

[8] J. M. Cuta, C. E. McDonald, and A. Shekarriz, ASME PID-Vol. 2/HTD-Vol. 338, Advances in Energy Efficiency, Heat/Mass Transfer Enhancement, (1996) 17–23.