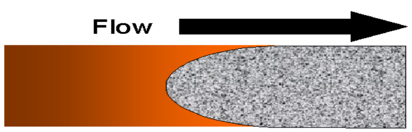
PREHEATING REQUIREMENTS IN HIGH TEMPERATURE LIQUID CHROMATOGRAPHY (HTLC)

INTRODUCTION

Preheating of the mobile phase has been shown to be an important parameter in attaining maximum performance in high temperature liquid chromatography (HTLC). Peak broadening occurs when cold mobile phase enters the column inlet and is warmed from the column wall during a chromatographic run. Mobile phase flow profile and diffusion characteristics are strongly influenced by the relationship between temperature and viscosity, and non-uniformities lead to band broadening. Mobile phase preheating has previously been accomplished with varied lengths and diameters of metal tubing in a heated zone leading to the column. The tubing length requirements for adequate preheating can be pronounced at high mobile phase flow rates. Selerity Technologies has developed a highly responsive low-volume, low-mass solvent preheater for use in high temperature liquid chromatography (HTLC). Effective solvent preheating with columns of different diameters, under isothermal and fast temperature gradient conditions, has been accomplished.

When solvent that is not preheated passes through a heated column, the packing in the center is cooled by the entering solvent. Solvent along the wall is heated by the oven faster than the interior solvent. Consequently, the viscosity along the wall is less than that at the center of the column, resulting in the solvent moving faster along the wall than at the center. The chromatographic result is a significant broadening of the peak and loss of resolution. This phenomenon is known as thermal mismatch.

Without Preheating



Representation of a column with solvent that is not preheated. Cool solvent gradually heats up as it moves through the column with the most rapid heat-up occurring near the walls.

With Preheating

Representation of a column with preheated solvent entering the column at the same temperature as the oven air. The solvent front is generally flat with perhaps some minor trailing at the wall. Peaks are now sharper and resolution is greatly improved.

UNIQUE PREHEATER DESIGN

A preheater designed to keep the amount of tubing required for preheating the mobile phase low, and without invading the fluid path was developed (see Figure 1). A 1/16" OD stainless steel tube was wrapped with electrically insulated nichrome wire. The heated zone is then thermally insulated. About 5 mm downstream from this heater, a small thermocouple is attached directly to the tubing and connected using an electrically isolated circuit. The thermocouple is insulated to shield it from interferences from the surrounding environment. This ensures that the probe is reading the tubing wall temperature which accurately follows that of the fluid down its core. The entire assembly is then housed in a stainless steel sheath. The entire heater weighs less than 2 grams (including the weight of the tubing), and has a volume of less than 1 µL.

1. **From pump**
2. **Heated zone**
3. **Thermocouple sensor**
4. **To column**

FIGURE 1: Design of the Selerity Technologies mobile phase preheater

Preheating of the mobile phase to the desired set point is accomplished by heating the tubing wall. Constant feedback from the thermocouple provides accurate information for the amount of heat needed to keep the mobile phase at the desired set point. Heat is input with voltages from 60 millivolts to 22 volts in 4000 increments under continuous microprocessor control. The demand is only 6 volts for 1 ml/min mobile phase flows heated to 150°C. The temperature differential between the heater and the fluid may be very large and will be dependent on the mobile phase flow rate and its heat capacity. This large spread allows for very rapid heat transfer rates into the moving fluid. It should be noted that while the heater itself reaches temperatures higher than the control set point, at no time does the mobile phase fluid within the tubing approach such values. Since all heat input and sensing occurs on the outside of the tubing, there is no risk of ignition of the fluid components by this non-invasive assembly.

At low flow rates typical with microbore columns, it has been found that very short lengths of tubing within an air oven were sufficient to bring the mobile phase to the temperature of the separation column. For this case, the heater assembly senses that the fluid is already at the desired temperature and no power is applied to the auxiliary heater. Only when the tubing sensor detects a temperature differential is heater power applied. Thus, a single device is suitable to accommodate controlled heating for a wide range of flows and fluids over a large range of temperatures. Because the heater is controlled through feedback from the temperature sensor, this device automatically compensates for the heating requirements of fluids over a wide range of heat capacities at different flow rates. Figure 2 shows the linear transfer of heat into the mobile phase flow path and Figure 3 shows the transfer of heat through the tubing wall.

1. Nichrome wire wrapped around stainless steel tube
2. Mobile phase flow through stainless steel tube

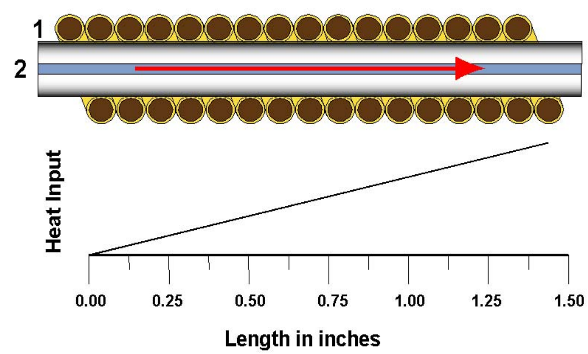
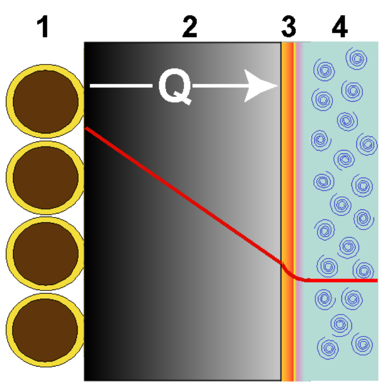


Figure 2: Heat transfer into the fluid path in a linear fashion over the length of the nichrome wire wrapped section (drawing not to scale).



1. Nichrome wire
2. Outer tubing wall
3. Transition zone
4. Mobile phase

Figure 3: Heat flux (and temperature) transfer through the stainless steel tubing into the mobile phase. At the inner tubing wall a very thin transition zone is apparent with the bulk fluid reaching a constant uniform temperature a short distance later. The spirals represent turbulence within the mobile phase that assists in rapidly reaching a uniform state. The inner diameter of the tubing is only 0.005" to 0.010" (drawing not to scale).

Temperature Programming

This low mass preheater is able to respond quickly for temperature programming applications. Moving air inside the oven across the preheater quickly cools the assembly at the conclusion of an analysis, allowing for fast re-equilibration at the end of a run. Temperature program rates in excess of 10°C/sec are possible at full power over a range of flows. Attaching the temperature probe in close proximity to the heater element itself provides for safety in the case of no mobile phase flow, a situation that could conceivably arise in a number of situations during chromatographic runs. In this scenario, heat is conducted through the metal tubing wall into the sensor probe, producing a response that controls energy input into the heater element and prevents catastrophic overheating.

Separation of a mixture of sedatives on a ZirChrom PBD column at 80°C was performed with the preheater off and then repeated with it turned on (see Figure 2). The misshapen peaks with the preheater turned off are typical of thermal mismatch broadening effects.

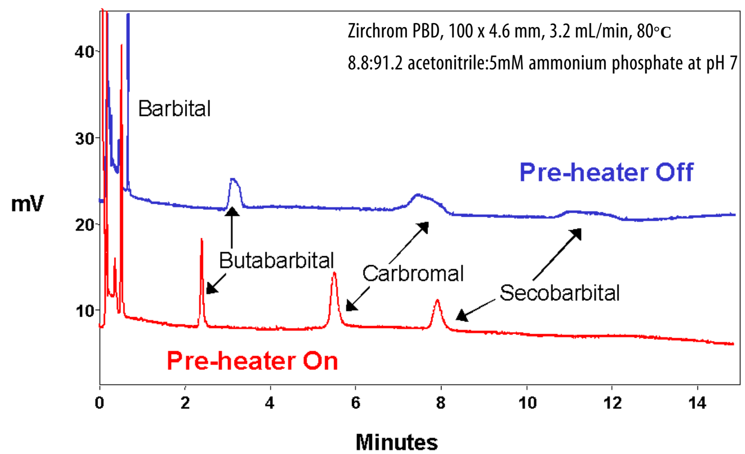


Figure 4: Comparison of the separation of a mixture of sedatives with and without preheating.

Conclusion

A simple low-mass, non-invasive preheater for use in high temperature liquid chromatography has been developed by Selerity Technologies, Inc. In a short length with a volume of less than 1 μL, mobile phases can be controllably heated to over 200°C over a wide range of flows. Fast heating and rapid cool-down at the conclusion of temperature programmed runs are possible, which is essential for fast analytical methods at high sample throughput rates. Patent applications have been filed relative to the new technologies presented here.