



## SEPARATION OF ANALGESICS-ANALYSIS TIME REDUCTION BY USING ACTIVE PREHEATING

### INTRODUCTION

HPLC at elevated temperatures can reduce analysis time and provide better separations. Preheating of the mobile phase is essential for good chromatography at elevated temperatures. Without preheating, room temperature mobile phase enters the heated column and produces thermal mismatch band broadening (please see Technical Note 803 for additional information). When the mobile phase is heated to the column temperature, band broadening is eliminated and good chromatography with sharp peaks is obtained.

The column oven on many conventional HPLC systems are capable of temperatures of 80 to 100°C. Most column heaters provide no preheating at all. Some manufacturers offer limited preheating in the form of a heat exchanger, which is usually tubing encased in an aluminum block. These preheaters add dead volume and do not sufficiently heat the mobile phase at the flow rates attained during high temperature HPLC analysis. The Caloratherm Mobile phase preheater efficiently preheats the mobile phase and eliminates thermal mismatch when added to an existing HPLC column heater.

Table 1:  
Conditions for Analysis of Analgesics with and without Caloratherm

Column:	Selerity Blaze <sub>200</sub> C <sub>18</sub> , 3 $\mu$ m, 100 x 4.6 mm
Mobile Phase:	30:70 Acetonitrile:Water with 0.1% TFA
Flow Rate:	1.0 or 2.0 mL/min
Detection:	UV at 220 nm
Temperature:	35, 50 and 70°C
Injection:	5 $\mu$ L

### EXPERIMENTAL CONDITIONS

Separation conditions are summarized in Table 1. A Dionex pump and UV detector, and an Eppendorf block column heater were used with a Selerity Technologies Caloratherm dynamic mobile phase preheater.

Thermal mismatch band broadening is evident at temperatures as low as 35°C. Figure 1 shows the separation of a five analgesics at 35°C, 50°C and 70°C with and without preheating. The chromatogram clearly shows a reduction in analysis time and sharper peaks when the Caloratherm mobile phase preheater was used. Although the separation looks acceptable without preheating, peak width and run time were reduced when dynamic mobile phase preheating was employed.

### RESULTS AND DISCUSSION

Increasing the temperature from 35°C to 70°C and properly preheating the mobile phase reduced the run time by 24% with no loss of separation quality. Increasing the flow rate to 2.0 mL/min reduced the run time further. This was possible because of the reduction in mobile phase viscosity at elevated temperature that results in lower system back pressure. Figure 2 shows the improvement in the separation by increasing the temperature and flow rate.

### CONCLUSIONS

Thermal mismatch band broadening was evident at temperatures as low as 35°C. Significant increases in throughput by reducing analysis time were achieved with small changes to an existing HPLC method by changing the temperature. A modest increase in temperature and flow rate resulted in a 2.5-fold reduction in analysis time with no loss of resolution. Small changes using temperature can provide big increases in sample throughput.



Figure 1: Separation of a five analgesics at 35°C, 50°C and 70°C with and without preheating. Elution order: acetaminophen, caffeine, salicylamide, aspirin, salicylic acid.

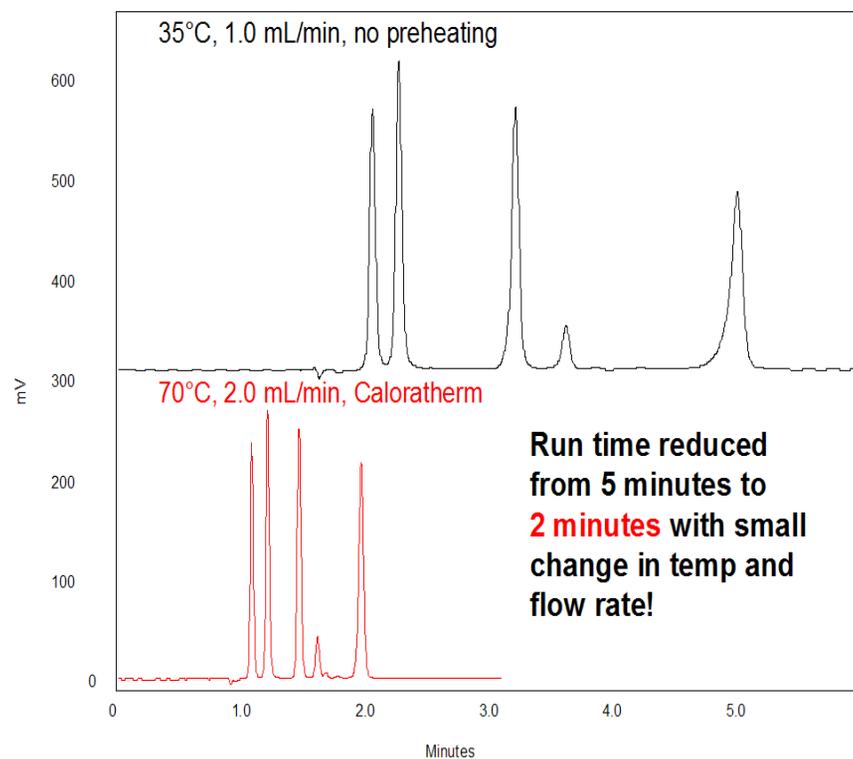
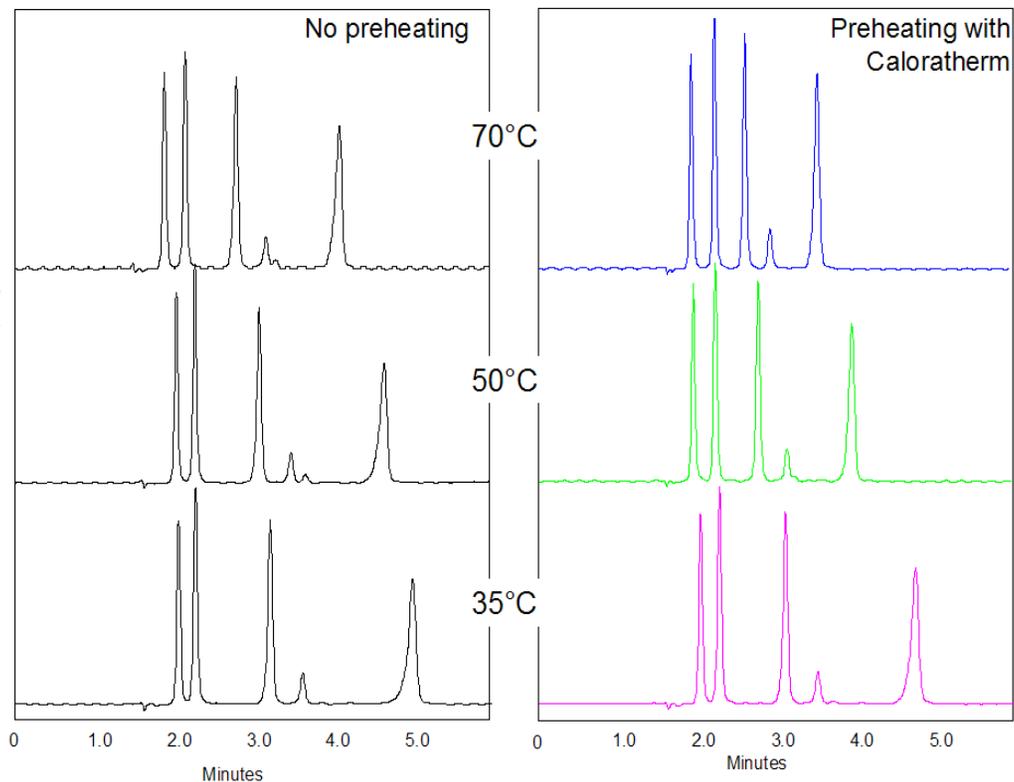


Figure 2: Improvement in the separation of analgesics by increasing the temperature and flow rate using the Caloratherm.