Technical Note 805

Analyte Peak Focusing in High Temperature Liquid Chromatography by Employing Independent Preheater Control

Introduction

The importance of adequate mobile phase preheating in High Temperature Liquid Chromatography (HTLC) has been discussed in detail in Selerity Technologies' Tech Note 803. Selerity has designed a low mass, low volume preheater capable of fast response with analytical size columns during temperature programmed runs. This preheater is programmed independently of the column compartment of the Selerity Technologies Series 8000 HTLC Oven or the Series 9000 Total Temperature Controller. Recent work has indicated that analyte focusing is possible if the preheater is programmed differently than the column compartment during thermal gradient runs.

Experimental

A selection of over-the-counter analgesics was separated employing a thermal gradient. The analyses were performed using the same column, mobile phase and temperature program at three different flow rates using a Selerity Series 8000 HTLC Oven. Conditions are summarized in Table 1. At each flow rate, the preheater was programmed to the same temperature as the Series 8000, or programmed to lead or lag the oven temperature program by 10°C. The acetaminophen peak was carefully quantitated and information about the peak quality was recorded.

Table 1 :
HTLC Conditions for Analysis of Analgesics

Column:	Selerity Blaze C_8 100 x 4.6 mm, 3 µm
Mobile Phase:	40:60 Acetonitrile:Water with 0.1% TFA
Flow:	1.0, 2.0 and 4.0 mL/min
Detection:	UV @ 220 nm
Injection:	5 µL
Temperature:	50°C (hold one minute) ramp to 100°C at 30°/min, hold six min
Prehater Temperature:	programmed to track the Series 8000, and also lag or lead by 10°C

Results

Results are summarized in Table 2. Values for retention time, peak width and asymmetry were recorded for the acetaminophen peak. Peak width and asymmetry are the best markers for determining peak quality. The narrower the peak (the lower the peak width value) and the closer the asymmetry value is to one, the greater the efficiency of the peak. The best asymmetry values were found when the preheater lagged the thermal gradient of the column compartment by 10°C. Peak widths were similar for all preheater profiles.

Table 2:
Effect of Different Preheater Profiles on Peak Width and Asymmetry

Flow Rate (mL/min)	Preheater Temperature (°C)	Retention Time (min)	Peak Width (min)	Asymmetry
1.0	+10	7.99	0.243	1.423
1.0	0	8.00	0.239	1.443
1.0	-10	8.11	0.244	1.399
2.0	+10	4.49	0.109	1.727
2.0	0	4.63	0.115	1.575
2.0	-10	4.73	0.117	1.541
4.0	+10	2.84	0.061	1.621
4.0	0	3.03	0.065	1.505
4.0	-10	3.08	0.064	1.468

Conclusions

Analyte peak focusing for over-the-counter analgesics is possible by programming the mobile phase preheater to lag the temperature profile of the Series 8000 HTLC Oven by 10°C. Asymmetry improved when the preheater temperature lagged the thermal gradient program for the column when compared to programming the preheater at the same temperature as the column compartment. Programming the preheater to lead the temperature program produced higher asymmetry values, indicating a loss of peak quality.