Isocratic Separation of Analgesics Using a Thermal Gradient

Introduction

A selection consisting of eight over-the-counter analgesics was chosen not only to demonstrate the benefits of high temperature liquid chromatography (HTLC), but also to show the selectivity differences of a high temperature silica versus that of a graphitic carbon stationary phase when using thermal gradients. HPLC analysis at elevated temperatures reduces analysis time and improves resolution. Reduced viscosities at elevated temperatures result in significantly lower backpressure, allowing for higher flow rates and shorter analysis times. It also permits the use of smaller particle size packings to increase efficiency while operating at lower backpressure. Flatter van Deemter curves mean that operating at higher flow rates does not sacrifice efficiency. Solvent properties also change as the temperature is increased. Hydrogen bonding interactions in water are reduced at higher temperatures, making water less polar so that it behaves more like an organic solvent as the temperature is elevated. This suggests that many solvent gradients can be replaced with temperature gradients so that samples requiring complex solvent gradients can be separated isocratically. This note shows that a mixture typically separated using a solvent gradient can be analyzed by using isocratic conditions and a simple thermal gradient. Both columns show very different selectivites for the analytes under thermal gradient conditions, further showing the versatility of the technique.

Table 1 :
HTLC Conditions for Analysis of Analgesics on Selerity Blaze C_8

Column:	Blaze C_8 100 x 4.6 mm, 3 µm
Mobile Phase:	40:60 Acetonitrile:Water with 0.1% TFA
Flow:	1.5 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

Experimental Conditions

Conditions are summarized in Table 1 and Table 2. A Milton Roy CM4000 pump, Alltech vacuum degasser, Thermo Separations UV2000 variable wavelength detector and Alcott autosampler were used in conjunction with a Selerity Technologies Series 8000 programmable HTLC oven.

Results and Discussion

Figure 1 shows the separation of over-the-counter analgesics on a Selerity Blaze C_8 polydentate silica column with an isocratic mobile phase and a temperature gradient. Baseline resolution of seven compounds is achieved in about nine minutes. Phenacetin was originally added to this mixture, but it co-eluted with caffeine and was removed. Figure 2 shows the separation of analgesics on a Thermo Hypersil-Keystone Hypercarb® column using a thermal gradient from 125°C to 200°C. Note that the increased temperature permitted a flow rate of 4.0 mL/min. In this case, aspirin and salicylamide co-eluted, but phenacetin and caffeine are well separated.

Table 2:
HTLC Conditions for Analysis of Analgesics on Hypercarb® Column

Column:	Thermo Hypersil-Keystone Hypercarb® 100 x 4.6 mm, 7 µm
Mobile Phase:	35:65 Acetonitrile:Water with 0.1% TFA
Flow:	4.0 mL/min
Detection:	UV @ 220 nm
Injection:	5 µL
Temperature:	Thermal gradient from 125°C to 200°C at 30°/min, hold five min

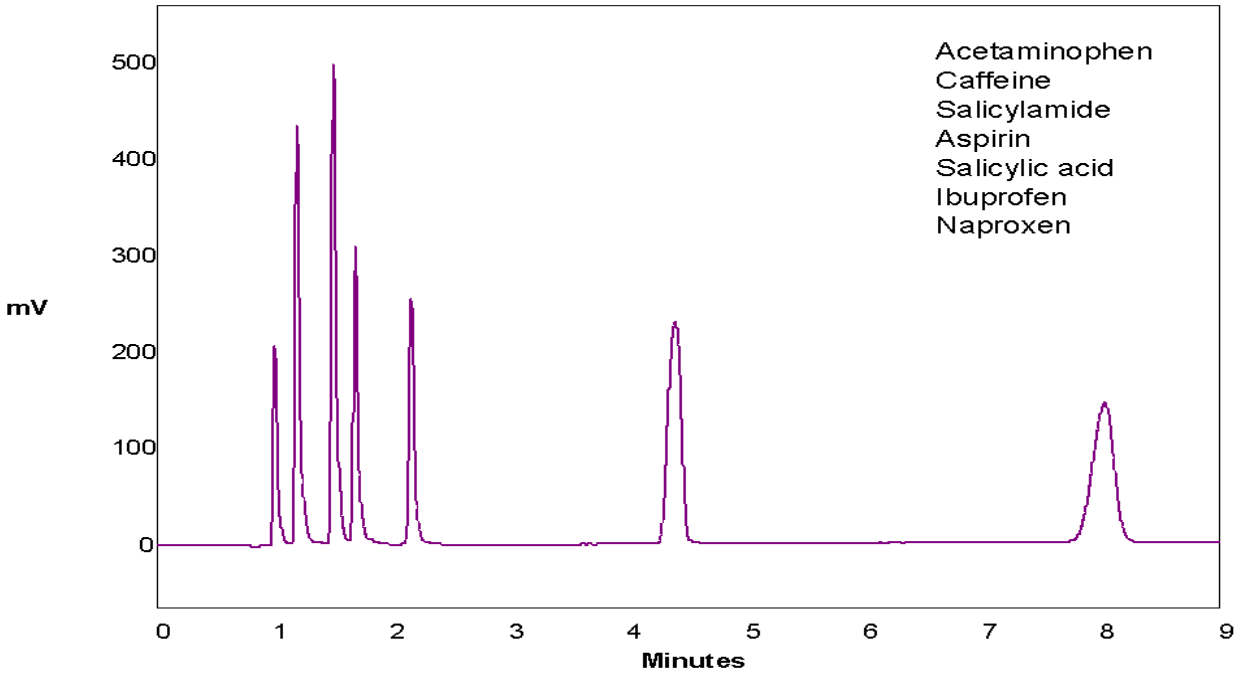

FIGURE 1 : *Separation of over-the-counter analgesics using a Selerity Blaze C_8 column and a thermal gradient from 50°C to 100°C.*

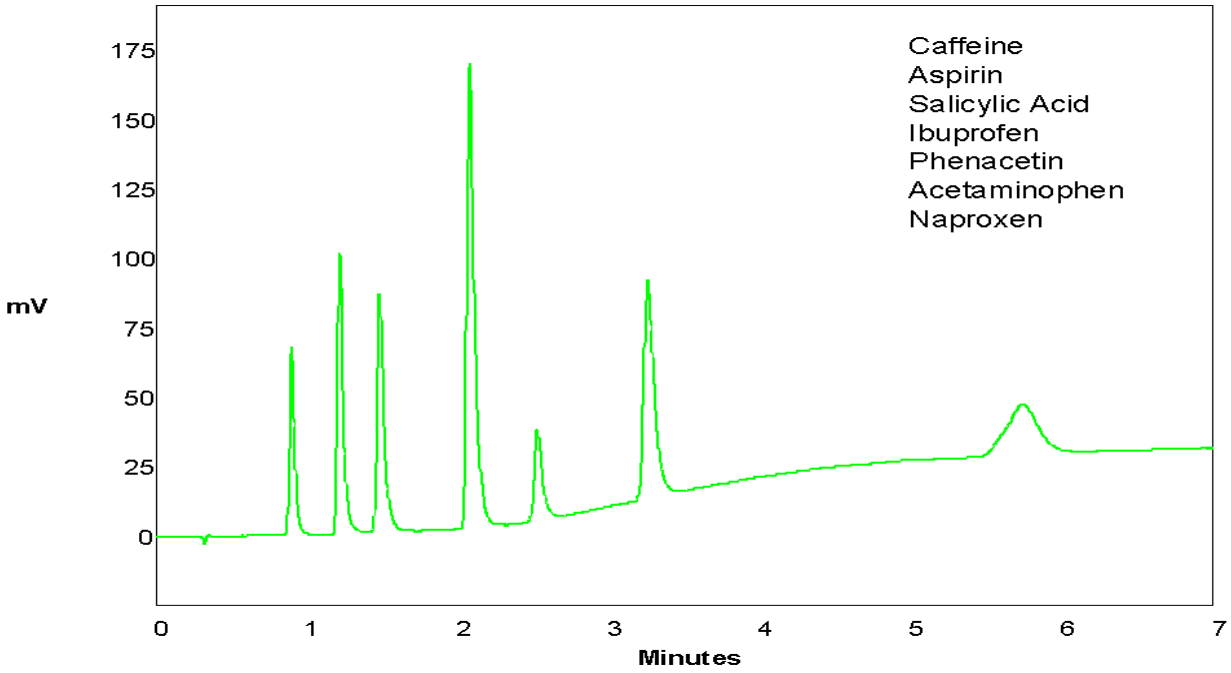

FIGURE 2: *Separation of over-the-counter analgesics using a Hypercarb® column and a thermal gradient from 125°C to 200°C.*