Fast High Temperature Isothermal Solvent Gradients are OK - But Thermal Gradients are Better!

Introduction

There are numerous advantages to increasing the temperature of HPLC separations. Faster runs often result by merely raising the temperature and leaving other parameters untouched. However, the most powerful use of elevated temperature in HPLC is temperature programming. A temperature gradient can be used instead of a solvent gradient to decrease retention, improve a separation and shorten analysis time. This work shows a selection of aromatic hydrocarbons separated isothermally using a fast solvent gradient and a better, faster separation achieved isocratically by using a thermal gradient.

Experimental Conditions

Conditions are summarized in Table 1. Isothermal runs at 50°C, 75°C, and 100°C and a temperature gradient from 50 °C to 150°C at 20 degrees/minute were conducted. The seven component aromatic mix consisted of: uracil, benzene, toluene, ethylbenzene, naphthalene, tetrahydronaphthalene, phenanthrene. A Knauer isocratic pump, degasser, and variable wavelength UV detector were used in conjunction with a Selerity Technologies Series 8000 programmable HTLC oven.

Table 1 :
HTLC Conditions for Analysis of Aromatic Hydrocarbons

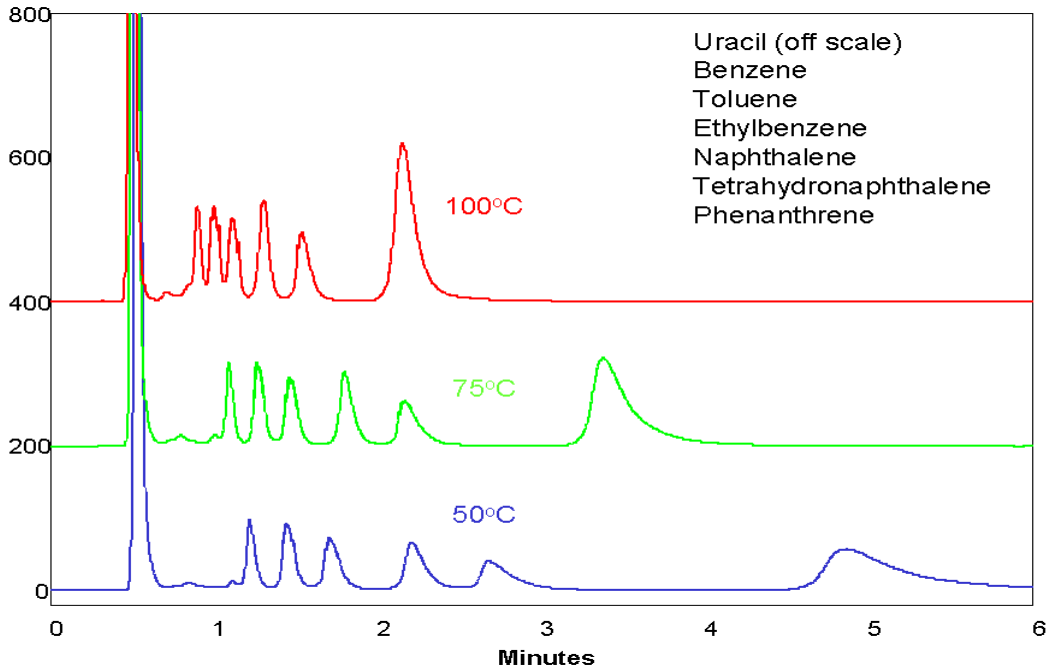
Column:	Hamilton PRP-1®, 100 x 2.0 mm, 3 µm
Mobile Phase:	75:25 Acetonitrile:Water
Flow:	0.5 mL/min
Detection:	UV @ 254 nm
Injection:	5 µL
Solvent Gradient:	75% to 90% acetonitrile over five minutes, hold two minutes
Temperature Gradient:	50 to 150°C at 20°/min, hold two min

Results and Discussion

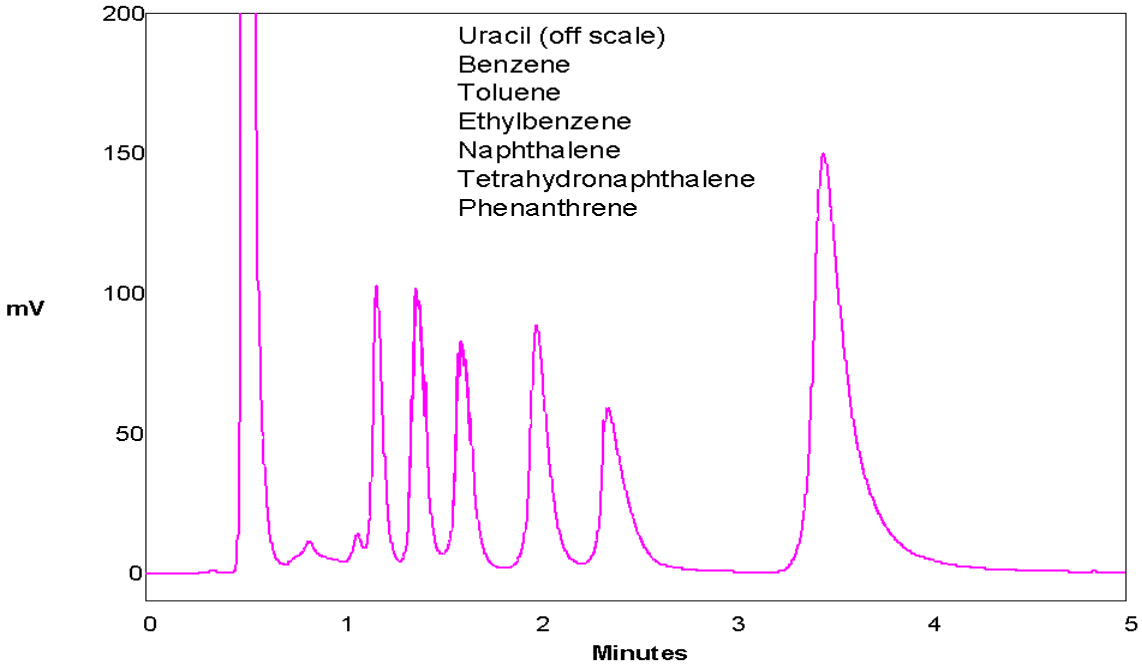
Figure 1 (see next page) shows the separation of the seven component mixture isothermally at 50°C, 75°C and 100°C. Isothermally at 50°C, all seven aromatic compounds are separated, but the last three peaks show bad tailing. The peak shape is improved and the run time is shorter at 100°C, but there is still tailing on the tetrahydronaphthalene and phenanthrene peak. All seven compounds are not completely resolved at 150°C, but the peak shape of the phenanthrene peak is much improved. Using a temperature gradient (Figure 2 next page), the run time was reduced to four minutes and the peak shapes of all seven components are acceptable. Clearly, the best separation was obtained using the thermal gradient with an isocratic mobile phase.

Conclusions

The use of a temperature gradient in HPLC can be used in place of a solvent gradient to decrease retention and improve peak shape of late-eluting aromatic compounds. This method has proven superior to running a fast solvent gradient at an elevated temperature. In the separation of seven aromatic hydrocarbons, isothermal runs were not sufficient to resolve all seven components with good peak shape and efficiency. A temperature gradient separated all seven components in four minutes with excellent peak shape and resolution.



Chromatograms showing the separation of seven aromatic hydrocarbons isothermally at 50°C, 75°C and 100°C using a fast solvent gradient.



Chromatogram showing the separation of seven aromatic hydrocarbons using a temperature gradient from 50°C to 150°C at 20°C/min, holding at 150°C for two minutes.