



FAST SEPARATION OF AROMATIC HYDROCARBONS BY HIGH TEMPERATURE LIQUID CHROMATOGRAPHY (HTLC) USING TEMPERATURE PROGRAMMING

INTRODUCTION

Performing HPLC at elevated temperatures provides several advantages to the analyst. Reduced viscosities at elevated temperatures result in significantly lower backpressure, allowing for increased flow rate which reduces analysis time. Higher column efficiencies due to better diffusion rates can improve resolution and sensitivity. 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 and improve a separation. This work exhibits the separation of ten aromatic compounds at three different isothermal temperatures followed by obtaining the optimal method by use of a temperature gradient.

EXPERIMENTAL CONDITIONS

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

TABLE 1 : HTLC CONDITIONS FOR ANALYSIS OF AROMATIC HYDROCARBONS	
COLUMN:	ZIRCHROM PBD 100 x 4.6 MM, 3 µM
MOBILE PHASE:	35:65 ACETONITRILE:WATER
FLOW:	2.0 ML/MIN
DETECTION:	UV @ 254 NM
INJECTION:	5 µL

RESULTS AND DISCUSSION

Figure 1 shows the separation of the ten component mixture isothermally at 50°C, 100°C and 150°C. Isothermally at 50°C, all ten aromatic compounds are separated, but three late eluting peaks (phenanthrene, anthracene, and chrysene) are broad and would be difficult to quantitate. Chrysene elutes at 22 minutes with very poor efficiency and symmetry. All ten compounds are not completely resolved at the higher temperatures. At 100°C ethylbenzene and naphthalene co-elute, and chrysene still has poor peak shape. At 150°C the run is completed in 2 minutes, with good efficiency on the chrysene peak, but resolution of the other peaks is severely compromised. Using a temperature gradient (Figure 2), the run time was reduced to seven minutes and the peak shapes of phenanthrene, anthracene, and chrysene are much improved. Re-equilibration time between runs was five minutes. Method development time was greatly reduced compared to running solvent gradients – all of the work in this application note took a few hours, due largely to fast temperature re-equilibration times and the ease of changing temperature rather than solvent composition.

CONCLUSION

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. In the separation of ten aromatic hydrocarbons, isothermal runs were not sufficient to resolve all ten components with good peak shape and efficiency. A temperature gradient separated all ten components in seven minutes with excellent peak shape and resolution.



FIGURE 1 : Chromatograms showing the separation of ten aromatic hydrocarbons isothermally at 50°C, 100°C and 150°C

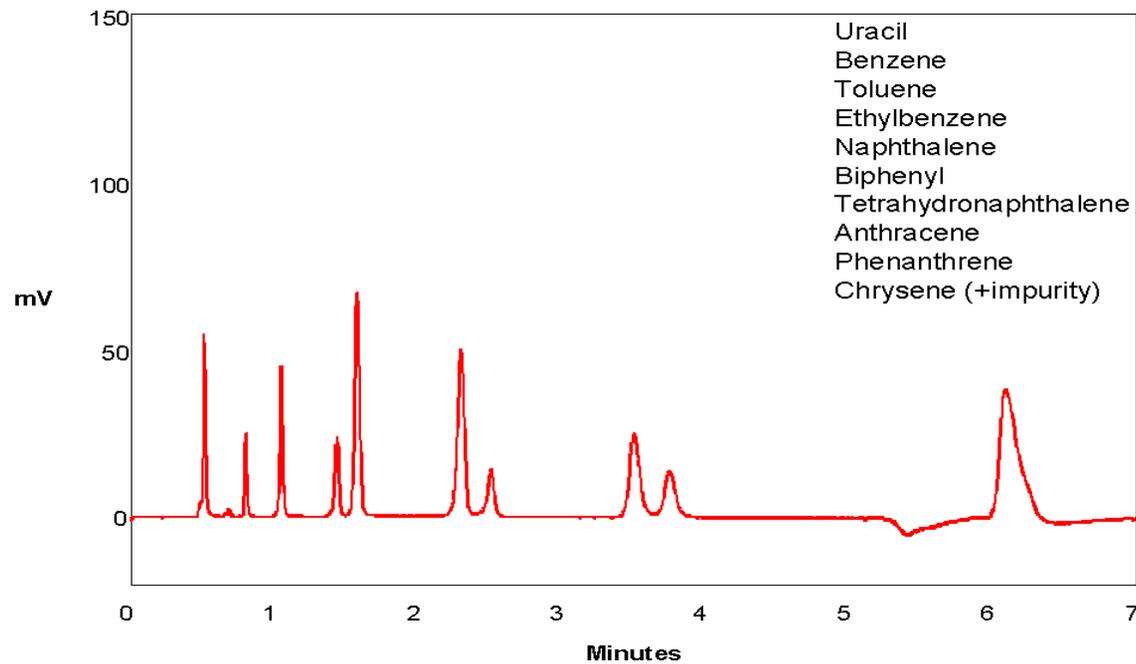
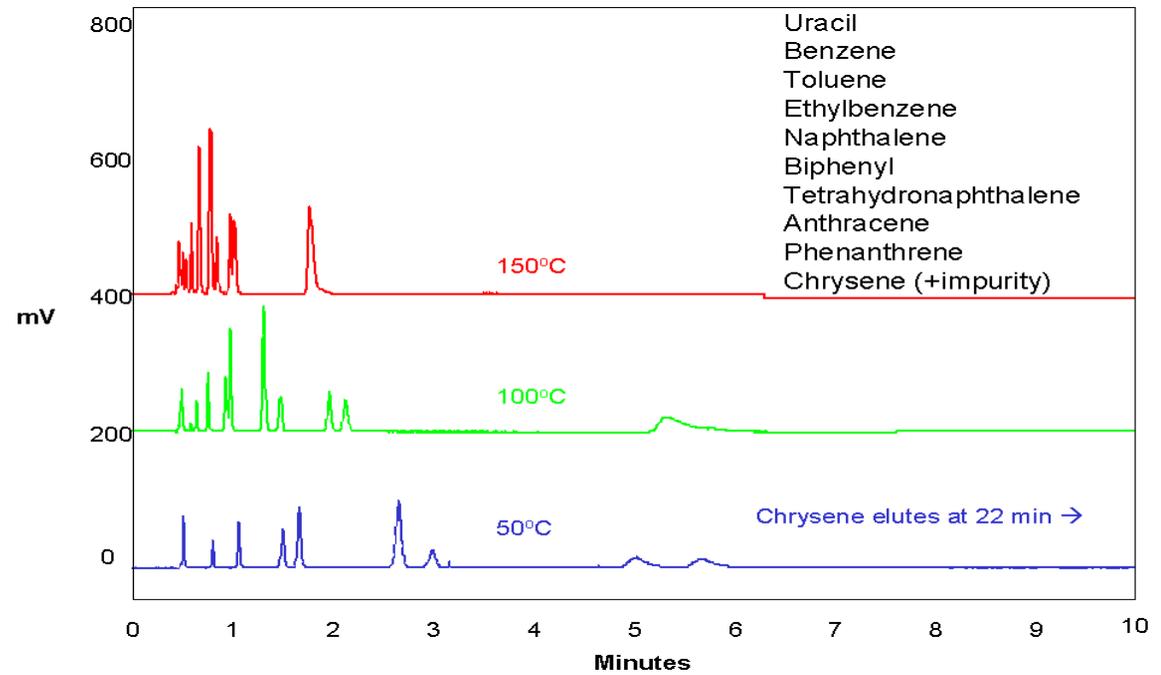


FIGURE 2: Chromatogram showing the separation of ten aromatic hydrocarbons using a temperature gradient from 50°C to 150°C at 20°C/min, holding at 150°C for two minutes.