Using Temperature as a Variable to Alter Selectivity in HPLC Separations

Introduction

Selectivity changes can be observed in HPLC separations with changes in mobile phase composition, switching to a different organic modifier in the mobile phase (replacing methanol with acetonitrile), or changing the pH of the mobile phase. Most chromatographers do not realize that analysis temperature can also alter selectivity for many analytes. Hydrogen bonding interactions are weakened as temperature is increased. These changes affect the hydration sphere around the analyte and the partitioning of the analyte between the stationary and mobile phases. Temperature is one of the easiest variables to change in method development and there are now instruments and columns that permit separations to be performed at temperatures up to 200°C in isothermal or temperature programmed mode. This technical note demonstrates an application where temperature induced selectivity changes were observed.

Experimental

Chromatographic conditions are summarized in Table 1. The sample was a mixture of phenylurea pesticides consisting of Fenuron, Metoxuron, Chlortoluron, Diuoron, Isoproturon, Linuron, and Chloroxuron. The Polaratherm Total Temperature Controller was used with an Agilent 1100 HPLC system.

Results

Figure 1 shows the separation of the phenylurea pesticides performed at three different temperatures. Notice that at 40°C, peaks four and five (Diuoron and Isoproturon) coelute. At 60°C, the two components are nearly baseline resolved, and at 80°C, the two components are baseline resolved, and are still well resolved from the other components in the test mixture. The only variable that was changed is the analysis temperature.

Conclusions

Temperature is a variable that can be used to alter selectivity in HPLC separations. For another example of selectivity changes with temperature, see Application Note 811.

Acknowledgement

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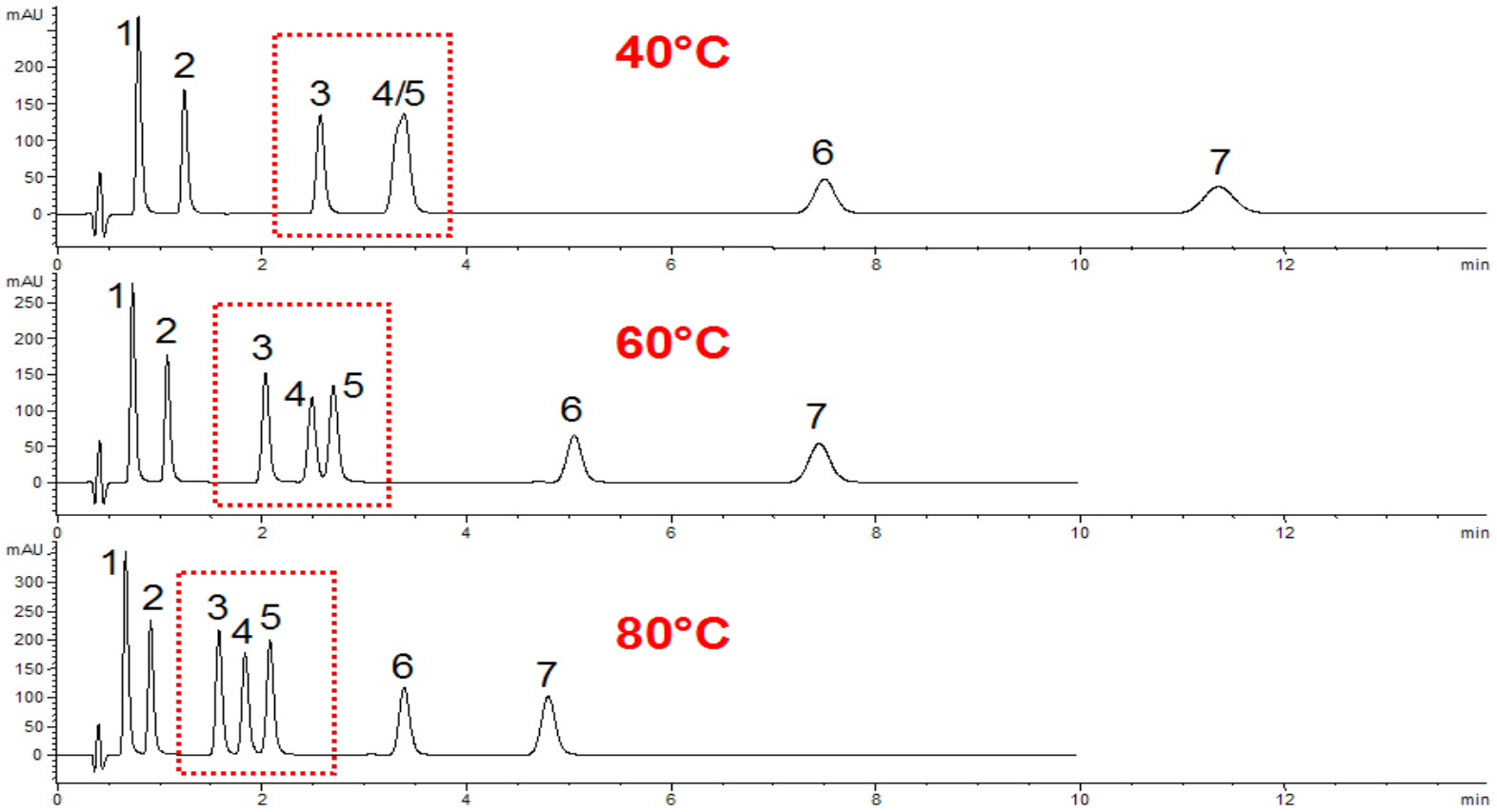
Table 1
HPLC conditions for Phenyurea pesticides

Columns:	Zorbax StableBond C18,50 x 2.1 mm, 1.8 µm
Mobile Phase:	30:70 Acetonitrile:Water, isocratic
Flow:	0.35 mL/min
Detection:	UV @ 245 nm
Injection:	1.0 uL
Sample:	Standard solution Phenylurea pesticides (100 ppm each)
Temperature:	40°C, 60°C and 80°C



1. Fenuron, 2. Metoxuron, 3. Chlortoluron, 4. Diuoron,
5. Isoproturon, 6. Linuron, 7. Chloroxuron

Figure 1. *The separation of the phenylurea pesticides performed at three different temperatures. Notice that at 40°C, peaks four and five (Diuoron and Isoproturon) coelute. At 60°C, the two components are nearly baseline resolved, and at 80°C, the two components are baseline resolved, and are still well resolved from the other components in the test mixture.*