Increasing Resolution and Efficiency by Increasing Temperature

Introduction

Most HPLC methods are developed to optimize resolution and plate count, therefore, cromatographers are always looking for new ways to gain higher plate counts from their HPLC separations. Some of the ways to achieve higher efficiencies are to use smaller particle size column packings and longer columns. The penalty of smaller particle sizes and longer columns is a significant increase in back pressure. Some have tried to solve this pressure problem by developing specialty HPLC instrumentation that is capable of operating at pressures up to 15,000 psi (1,034 bar). An easier way of eliminating the pressure penalty is to increase the analysis temperature which decreases the viscosity of the mobile phase. Figure 1 shows that the back pressure decreases 40% when the analysis temperature is increased from 30°C to 80°C. This allows smaller particle sizes and longer columns to be used with existing HPLC equipment that is rated to perform at 6,000 psi (413 bar). This technical note demonstrates how increasing the analysis temperature allowed the use of multiple columns for tremendous increases in chromatographic efficiency.

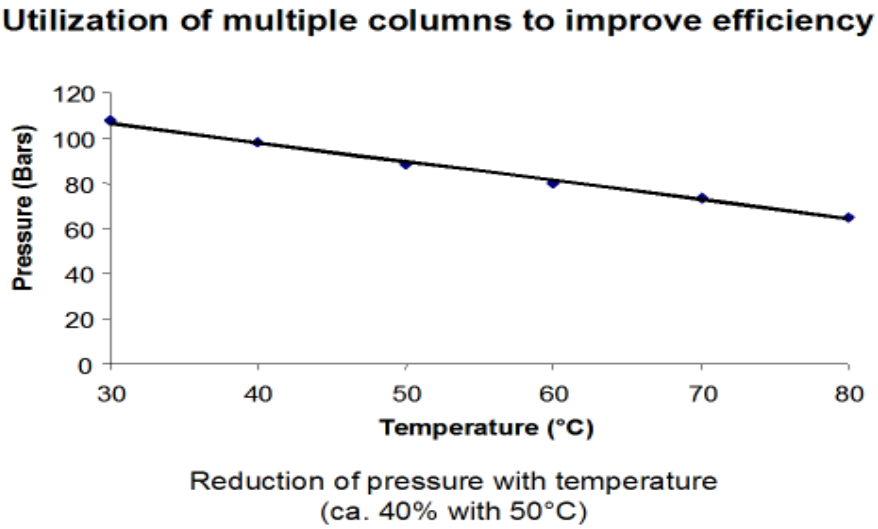

Figure 1. *Effect of temperature on back pressure. There is a 40% reduction in back pressure by increasing the temperature from 30°C to 80°C.*

Experimental

HPLC conditions are detailed in Table 1. The sample mixture consisted of uracil, caffeine, pyridine, phenol, aniline, benzene and toluene. The Selerity Polaratherm Total Temperature Controller was used with an Agilent 1100 HPLC system to perform the analyses. In this experiment, separations were performed under the same set of conditions. Columns were added in series to increase resolution and efficiency. Three separations were performed, the first with one analytical column, the next with four columns, and the last with eight columns coupled together. In addition, 3.5 um particles were used which provides increased efficiency when compared to the standard 5 um particles.

Table 1
HPLC conditions for high resolution separation

Columns:	Zorbax StableBond C18,3.0 mm ID, 3.5 µm
Mobile Phase:	40:60 Acetonitrile:Water, isocratic
Flow:	0.40 mL/min
Detection:	UV @ 254 nm
Temperature:	80°C

Results

Figure 2 shows the separations obtained when one, four and eight columns were used for the analysis at 80°C. Table 2 shows the theoretical plates/meter for each peak for one, four and eight columns. Even though the back pressure increased as more columns were connected in series, even with eight columns the pressure remained less than 6,000 psi (413 bar). As observed in Figure 2, peaks that are not separated at all with only one column are completely baseline resolved when additional columns are added. Figure 3 shows the linear relationship of the increase in theoretical plates to column length.

Table 1 Theoretical Plates Obtained with One, Four and Eight Columns			
Analyte	Plates/meter One Column	Plates/meter Four Columns	Plates/meter Eight Columns
Caffeine	5,000	41,000	46,000
Pyridine	6,000	35,000	56,000
Phenol	6,000	43,000	68,000
Aniline	6,000	43,000	78,000
Benzene	10,000	43,000	79,000
Toluene	10,000	42,000	81,000

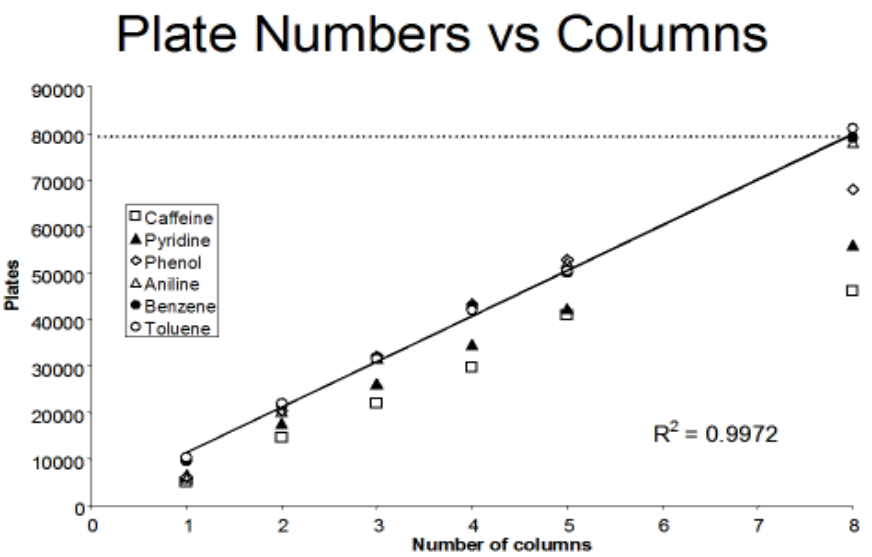

Figure 3. *Plot showing the linear relationship between theoretical plates and column length.*

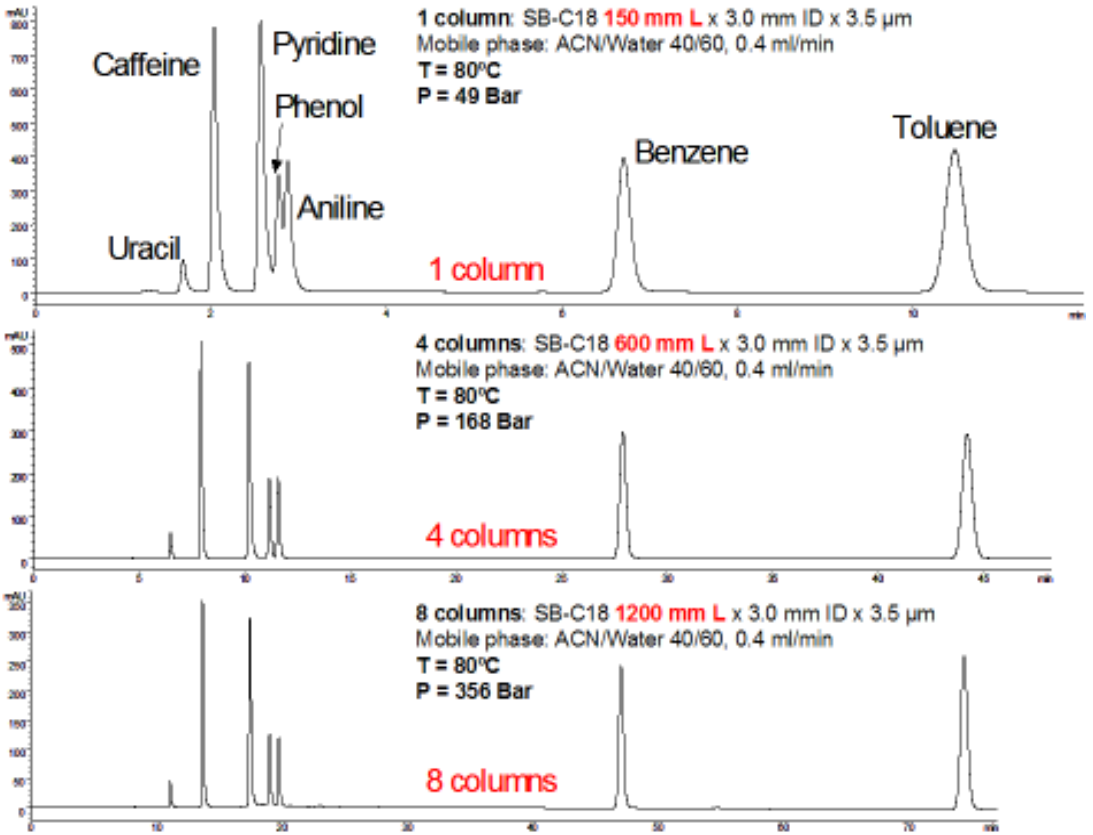

Figure 2. *The separation of standard mix using one, four and eight columns. Resolution and efficiency are greatly increased by coupling columns together. Back pressure is still within normal limits, even with eight columns*

Conclusions

Increasing temperature can provide dramatic increases in efficiency and resolution by allowing the use of multiple columns and smaller sized particle packings without a using special high pressure HPLC equipment.

Acknowledgement

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