Performance PerSPECtives

Solvent Composition Differences

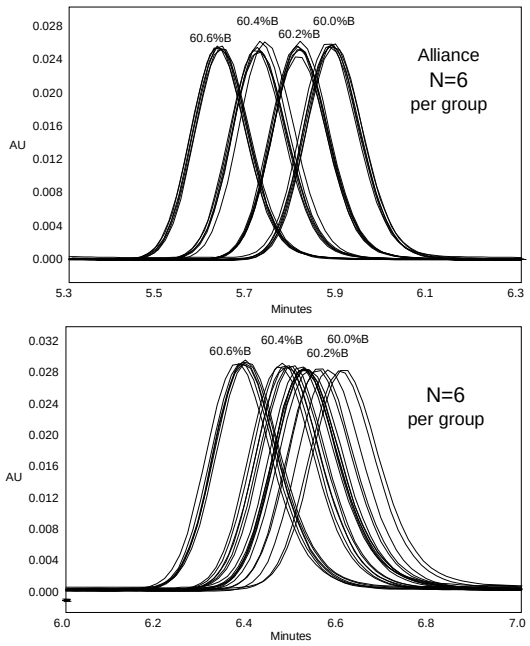
Effects on Peak Retention Time and Area

Accurate mobile phase composition is required for good HPLC separations. Peak identification and quantitation is primarily done by injecting standards. The identification is made by comparing the retention times of the unknown peaks with those of standards. Similarly, analyte concentrations are calculated from the peak areas by comparison to known standards. It is well known that changes in mobile phase will cause peak retention times to shift. What is not always considered is that changes in retention time will also cause changes in peak area. This Performance PerSPECtive will discuss the relationship between mobile phase composition, retention time and peak area.

Effects of Composition on Retention Time

Figure 1 upper panel shows the effect of varying the percentage of methanol in 0.2% increments from 60 to 60.6%. With Waters Alliance/PDA System (upper panel), the retention times for this isocratic separation with automated solvent blending are very reproducible when compared to a competitors traditional system (Figure 1 lower panel and WPP201 and 203).

FIGURE 1



Waters
Alliance™ System
Waters 2690, 996, and Millennium®

A = Water
B= Methanol
1 mL/min

Symmetry® C_{18} 3.9x150 mm. 30C
PQ Mixture, peak #4

Competitors Traditional
HPLC gradient system

A = Water
B= Methanol
1 mL/min

Symmetry® C_{18} 3.9x150mm. 30C
PQ Mixture, peak #4

As expected, the retention time of butyrophenone decreases as the methanol concentration is increased. When solvents can be blended accurately and reproducibly, and the flow rate is reproducible, retention times are very reproducible and controllable as in the case of the Alliance system. However, if control of flow rate and/or solvent composition are not as precise, such as with traditional systems, the result is peak retention time variability.

Retention time variability, as shown in the lower panel, was generated with small changes in the %A and %B and is not predictable due to system variability. However, similar variability in retention time can occur with premixed mobile phases when there is preferential evaporative loss of one solvent, or batch to batch inaccuracies.

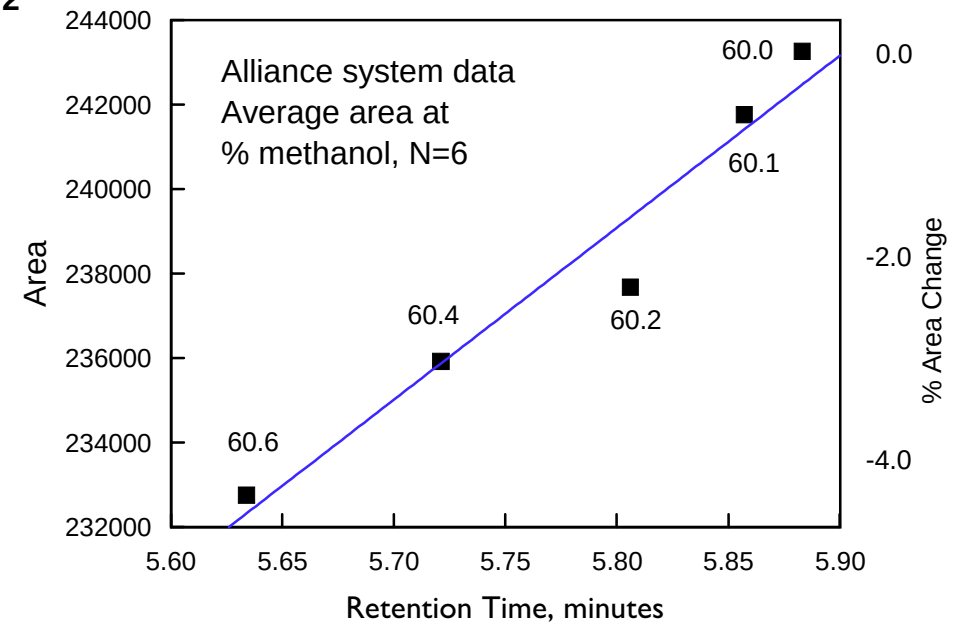
Waters

Waters Corporation 34 Maple Street Milford, MA 01757 508 478-2000

Effect of Composition on Peak Area

In Figure 2, area counts for the peaks in the upper panel of Figure 1 were graphed verus retention time. The Y-axis on the left is area and the Y-axis on the right is % change in area. The values next to the data points are percentages of methanol.

FIGURE 2



The peak area changes with variable retention times. In this case 0.6% increase in methanol concentration caused a 0.25 minute shift in retention time which leads to a 4.3% change in area count! Although the differences in the percentage of methanol is not great, and the peak identification will probably be correct, the quantitation is greatly affected.

Pharmaceutical industry standard operating procedures call for system suitability standards to be run before a batch of unknowns. Variability in peak areas are typically required to be less than 1 or 2% RSD. When the system suitability of area does not pass, the injector is generally blamed. However, Figure 2 (and Performance Perspective WPP19) show solvent management is equally important.

Therefore, to achieve the best quantitation, solvent delivery must be managed to provide the most accurate and precise composition and flow; and the sample injection must be reproducible. The Waters 2690 Separations Module provides excellent solvent and sample management to increase the accuracy and precision of analytical results, in both peak area and retention time.