Performance PerSPECtives

Waters® CapLC™ System

Enhanced Sensitivity by Design:

Sensitive detection of mass limited samples:

Given the increased need to analyze mass limited samples, capillary columns (defined as having an internal diameter of 0.1 to 1 mm) have replaced traditional analytical columns in many HPLC and LC/MS applications. The Waters Performance PerSPECtive entitled: "Waters CapLC System: Reproducible Capillary Separations by Design" (WPP 400) discussed the importance of solvent delivery design in providing a new level of unsurpassed retention time reproducibility previously not seen with traditional spilt-flow capillary systems. This Performance PerSPECtive discusses how the patented "light-guided flow cell technology" contained in the Waters CapLC System photodiode array detector improves upon absorbance detection limitations seen with many commercially available Capillary LC systems.

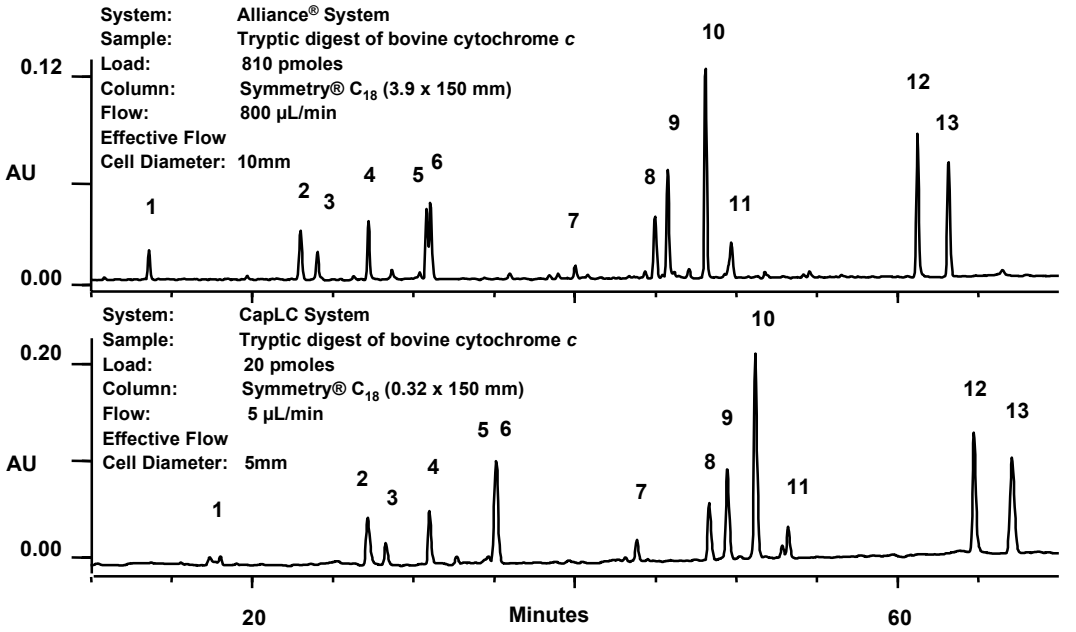
Use of capillary columns to increase detection sensitivity:

The mass sensitivity increase that is possible by using capillary columns occurs because compounds separated on columns of reduced internal diameter elute in lower peak volumes. This increase in elution concentration translates into increased detection sensitivity. Under ideal conditions, the theoretical increase in sensitivity is calculated by comparing the diameter of the analytical column to that of the capillary column (see Figure 1). As shown, a reduction in the internal column diameter from 3.9 mm to 0.32 mm results in a theoretical mass sensitivity increase of 149X. This gain assumes that 1) the capillary column contains the same number of theoretical plates as the analytical column, 2) the capillary chromatograph maintains the separation efficiency obtained during the analytical scale chromatography, and 3) the detector flow cells for both separations are of equivalent length. Figure 2 shows the gain in sensitivity possible by transferring a separation from an analytical to a capillary column. The separation performed on the Waters CapLC System yielded almost twice the response using approximately 40 times less sample. (Note: Different absorbance scales shown in Figure 2 top vs. bottom.)

Figure 1: Calculated Theoretical Sensitivity Increase with Capillary LC

$$\text{Increase in theoretical analyte concentration assuming flow cells of equivalent diameter} = \frac{(\text{Analytical Column I.D.})^2}{(\text{Capillary Column I.D.})^2} \text{ or } \frac{(3.9\text{ mm})^2}{(0.32\text{ mm})^2} = 149\text{ X}$$

Figure 2: Sensitivity Comparison between an Analytical and a Capillary Separation



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What makes the Waters CapLC System photodiode array detector so sensitive?
The Waters CapLC System detector was specifically designed to maximize detection sensitivity while minimizing undesired band-broadening effects deleterious to capillary column separations. **This innovative design is based on the Waters 996 Photodiode Array Detector and uses a patented light-guided flow cell connected to the light source and the optics bench via fiber optic cabling** (Figure 3). This configuration is designed to produce low peak dispersion since the total volume of the flow cell is only 250 nL. High sensitivity and good detection linearity are also obtained because of an effective flow cell pathlength of 5 mm. The PDA can be connected serially to a mass spectrometer because the integrity of the analyte peak is maintained post-PDA due to the novel flow cell design. Having the PDA and MS connected serially provides more useful information per analysis. Figure 4 shows a series of peptide digest separations performed on the Waters CapLC System. The ability to detect as little as 2 pmol of material on-column attests to the effectiveness of the photodiode array detector design in the Waters CapLC System.

Figure 3: Light-guided PDA Flow Cell

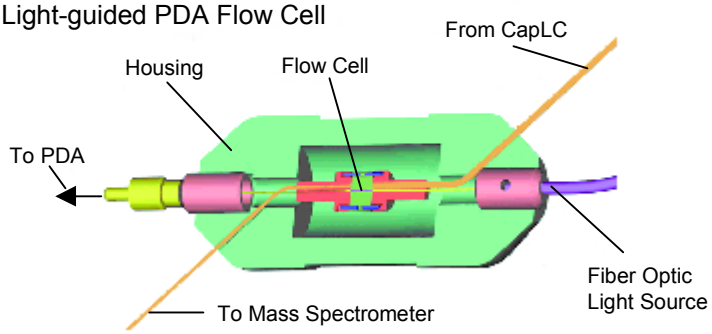
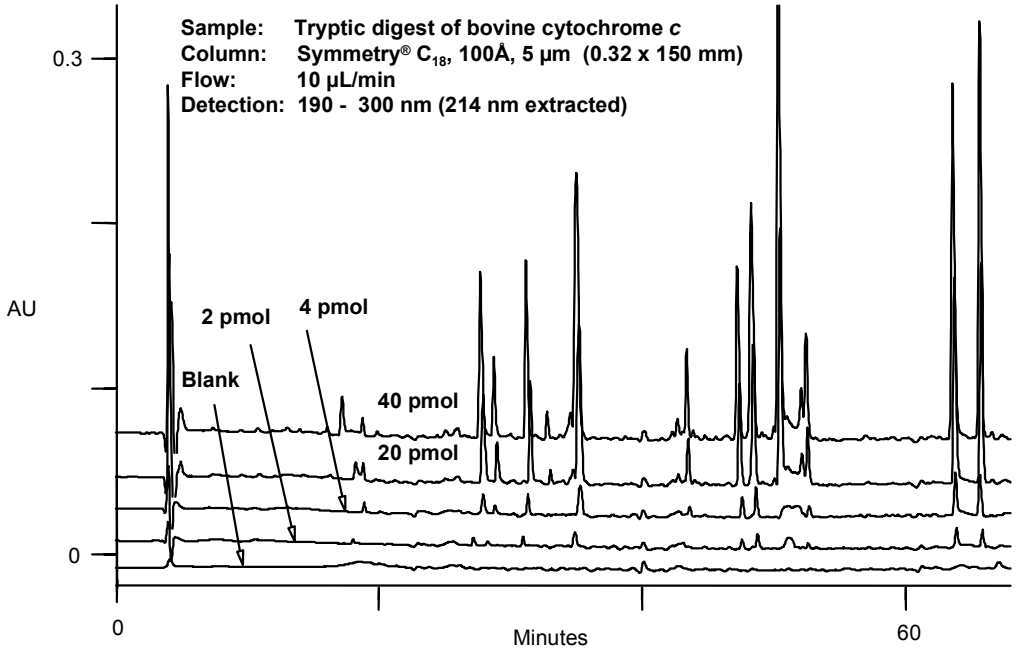


Figure 4: High Sensitivity Analysis of Peptide Separations on Waters CapLC System



Summary:

- High sensitivity detection of mass-limited samples has prompted scientists to consider using capillary columns for their chromatographic separations.
- The Waters CapLC System, using a novel flow cell design and patented light-guided technology enables scientists to obtain high quality photodiode array information from their capillary column separations.

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