Performance PerSPECtives

Waters 996 PDA Variable Pathlength Flow Cell:

Preparative Chromatography Library Matching and Peak Purity Analysis

Spectral analyses with the Waters 996 Photodiode Array Detector (PDA) and Millennium® 996 software are useful in preparative chromatography for determination of peak identification and peak homogeneity. The 996 PDA Variable Pathlength flow cell permits use of these spectral techniques at high concentration of analyte (see Performance PerSPECtives WPP20) because the detector linearity can be extended through pathlength reduction from 3 mm to 0.15 mm.

Figure 1 shows the absorbance of the same sample load with two different pathlength setting on the 996 PDA Variable Pathlength flow cell. At 3 mm, the detector is overloaded (peak is flat topped and skewed). At 0.5 mm pathlength the peak is in the linear range.

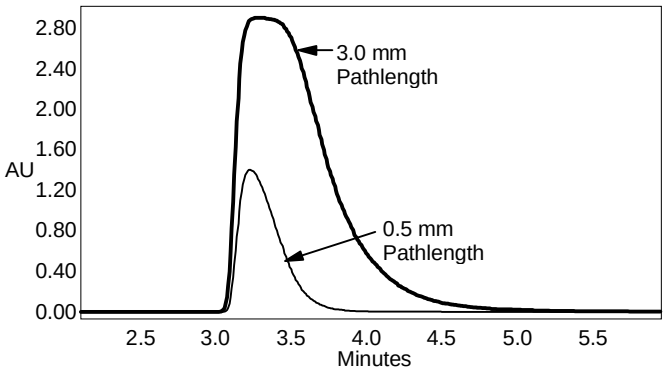

Figure 1. The same sample load of propylparaben was chromatographed on Symmetry® C18 with 30% acetonitrile - 70% water and monitored at 254 nm.

Waters 996 with the Variable Pathlength flow cell was set at 0.5 or 3.0 mm.

Working within the detector's linear range is required for good spectral analyses as well as for quantitation. If some of the wavelengths of a spectrum exceed the detector linear range, usually in the low UV, the spectrum will be distorted and therefore not a true representation of the compound under investigation (see Performance PerSPECtive WPP08). This may results in mis-identification of a peak when using library matching or an incorrect conclusion that a peak is not homogeneous.

Library matching and peak purity analysis were performed on the two peaks in Figure 1. The need for working within the detector's linear range for good spectral data is shown.

Waters

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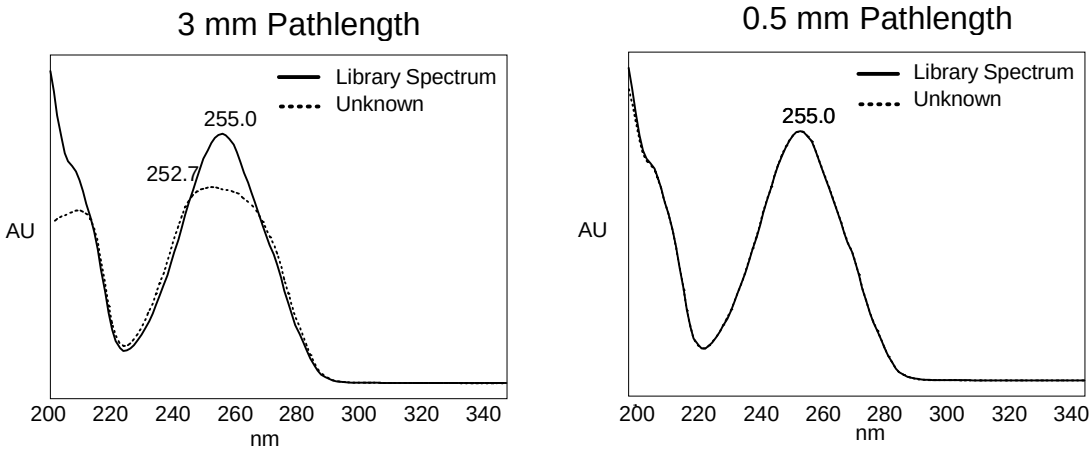
Waters 996 PDA Variable Pathlength Flow Cell - Spectral Analyses

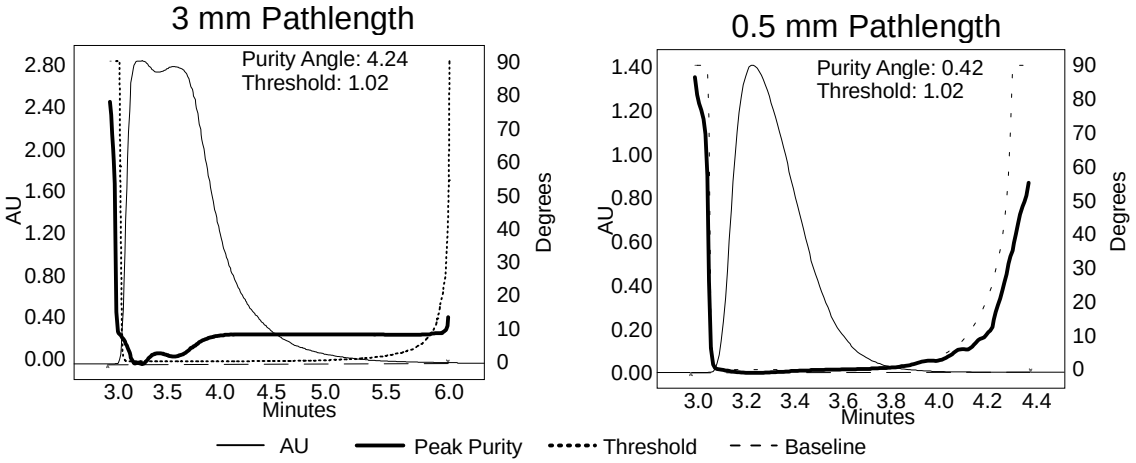
Library matching of the same concentration of unknown were done with a 3 mm or a 0.5 mm pathlength. When the absorbance was too high with the 3 mm pathlength (Figure 2, left panel), the library match was not good as shown by the mismatch of the overlaid spectra. When the 0.5 mm pathlength was used, the unknown spectrum is almost a perfect overlay with the library reference spectrum.

Figure 2: Library Match



Peak purity analysis to determine peak homogeneity is shown in Figure 3. The propylparaben that was chromatographed was chemically pure. When the absorbance is too high, the peak purity plot is distorted because some of the wavelengths in the spectrum are distorted (out of the linear range - left panel). When the 0.5 mm pathlength was used the peak purity plot for the chemically pure compound appeared as expected.

Figure 3: Peak Purity



With the Variable Pathlength flow cell in preparative chromatography, additional information about the chromatographic peak, peak identification and peak homogeneity can be obtained from spectral data of the 996 PDA detector. This brings valuable analytical techniques to preparative chromatography.