Performance PerSPECtives

Waters® 2487 Dual Wavelength Absorbance Detector

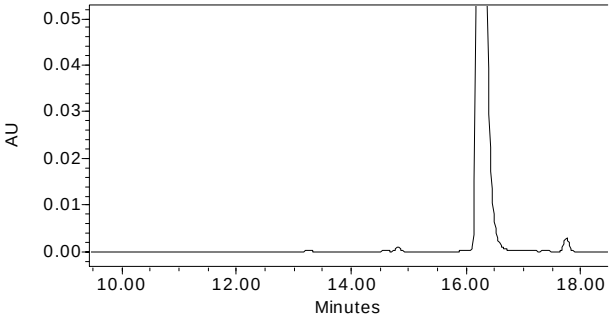
Superior Performance by Design: 2nd Order Filters

Theory and Practice Behind Second Order Filter Utilization:

Reflection gratings are used as the wavelength dispersion element in many HPLC absorbance detectors. In addition to the selected wavelength, all gratings reflect light from the source lamp at additional wavelengths which are known as 2nd, 3rd *etc.* order reflections. Because reflection gratings are *blazed*, very little energy appears in the higher order reflections, but erroneous absorbance can be measured if the 2nd order light is not removed. For example, when a wavelength of 508 nm is selected, a small portion of the lamp's emission at 254 nm is also reflected into the flow cell as second order radiation. The detector will respond to compounds which absorb at either 254 nm or 508 nm. Removing the 254 nm light is accomplished by placing a 2nd order filter before the flow cell when wavelengths greater than 360 nm are selected. Below 360 nm, 2nd order radiation does not exist since wavelengths below 180 nm are absorbed by oxygen in the light path. (It is necessary to remove air from the spectrophotometer to measure absorbance below 180 nm and this is known as *vacuum UV*). For wavelengths above 360 nm, a 2nd order filter which absorbs all light below 360 nm is required.

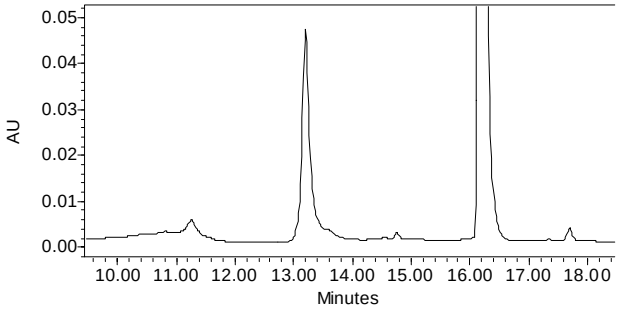
Shown below are chromatograms obtained at 508nm from the reversed-phase separation of commercially available Food Color and Egg Dye. (Note: The material contained water, propylene glycol, FD&C Red 40 and propylparaben.) In this study, the Waters 2487 detector was configured in two different modes. The chromatogram in Figure 1 was obtained with the second order filter engaged. Note the presence of only one major peak at 16.5 minutes. By comparison, an extra peak is seen at 13.4 minutes when the separation was monitored with the second order filter intentionally disabled during the separation (Figure 2). As will be demonstrated in Figure 3, these data clearly indicate how use of a second order filters prevents collection of erroneous data when separations are monitored in the visible spectrum.

Figure 1: Red Dye solution on Waters 2487 with Second Order Filter Activated



Column: Symmetry® C18 (4.6 x 150mm)
Sample: 7.5ngs of Red Food and Egg Dye
Eluent A: Water with PIC® A
Eluent B: Water/methanol (40:60) with PIC®A
Flow: 1.0 ml/min
Gradient: 0 - 100%B in 30 minutes
Temp: 30ºC
Detection: UV / Vis detector at 508nm
Second Order Filter Activated

Figure 2: Red Dye solution on Waters 2487 with Second Order Filter Disabled



Column: Symmetry® C18 (4.6 x 150mm)
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Eluent A: Water with PIC® A
Eluent B: Water/methanol (40:60) with PIC® A
Flow: 1.0 ml/min
Gradient: 0 - 100%B in 30 minutes
Temp: 30ºC
Detection: UV/ VIS detector at 508nm
Second Order Filter Disabled

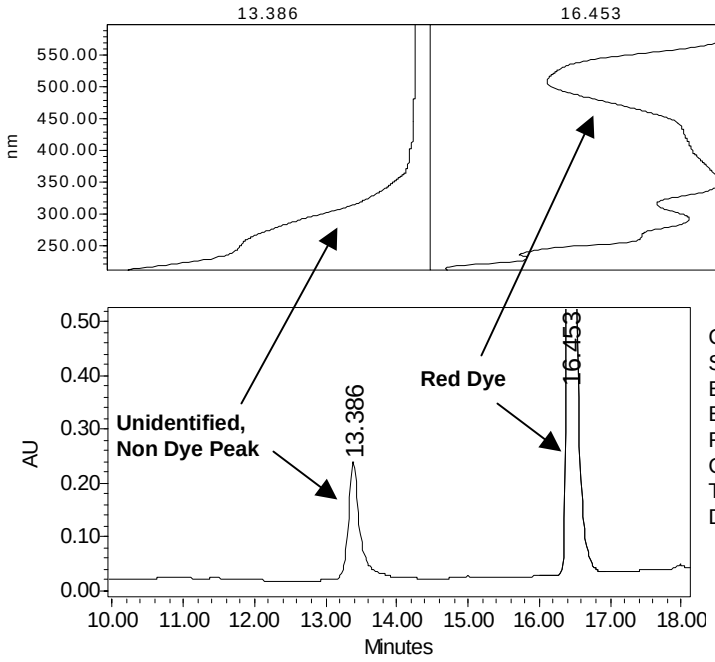
Waters

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

Confirmation of Peaks using Waters 996 Photodiode Array Detector

The same Red Dye containing solution shown in Figs. 1 & 2 was chromatographed using the Waters 996 photodiode array detector. Spectra from 210 - 800nm were collected at 1.2nm resolution and the extracted chromatogram at the Maximum Absorbance (i.e., MaxPlot) was analyzed (See Figure 3: bottom). Using MaxPlot, two peaks were detected. Spectral analysis from each peak apex (i.e., 13.386 and 16.453 minute peak) indicated that only the later, 16.453 eluting peak contained material that absorbed at a wavelength greater than 350nm (See Figure 3: Top). The unidentified peak at 13.386 minutes did not contain any visible wavelength absorbing material (i.e., red dye). Rather, this material absorbed at 254nm which was one half that of the 508nm wavelength monitored for the separation shown in Figure 2. **In combination, these data clearly demonstrate the value of using a second order filter for the analysis, in the visible wavelength range, of samples that contain both UV and VIS absorbing constituents.**

Figure 3: HPLC Analysis of Red Food and Egg Dye using Waters 996 Photodiode Array Detector



Column: Symmetry® C18 (4.6 x 150mm)
Sample: 7.5ngs of Red Food and Egg Dye
Eluent A: Water with PIC® A
Eluent B: Water/methanol (40:60) with PIC® A
Flow: 1.0 ml/min
Gradient: 0 - 100%B in 30 minutes
Temp: 30°C
Detection: 210 - 800nm (1.2nm resolution)
MaxPlot Extracted

Summary:

- The Waters 2487 detector, with built-in second order filter, eliminates the erroneous detection of UV absorbing peaks when only the analysis of visible absorbing compounds is desired.

- The Waters 2487 second order filter is automatically enabled when a single data channel is collected at wavelengths greater than 360nm.

- These data clearly demonstrate the value of using a second order filter for the analysis of complex samples in the visible wavelength range, that contain both UV and VIS absorbing constituents.

Special thanks to Jon Mulvany and Dick Andrews for their assistance in this study.