Performance PerSPECtives

Waters® 996 Photodiode Array Detector Spectral Resolution:

Analyze isomers with the unparalleled optical and spectral resolution of the Waters 996 Photodiode Array Detector

Discriminate between Spectrally Similar Compounds:

Photodiode array detectors can differentiate between HPLC resolved compounds that possess different chemical constituents. However, not all PDA detectors have the ability to spectrally resolve compounds that exist as chemical isomers. This Performance PerSPECtive demonstrates the ability of the Waters 996 PDA detector and Millennium®32 software to spectrally resolve this class of challenging compounds.

Figure 1 below shows the chemical structure of 2,4,6,8 -all E-3,7-dimethyl-9-[5-(2,4-diaminopyrimidine)]-tetraenoic acid ethyl ester as it exists in the all trans configuration. When exposed to light, this compound undergoes isomerization yielding seven species that can be resolved via reversed-phase HPLC (see Fig.2b). Of particular interest is the ability of the Waters 996 PDA detector, with 1.2nm optical resolution, to spectrally differentiate these isomers as indicated in the Millennium32 Spectral Index Plot (See Fig 2a).

Fig.1

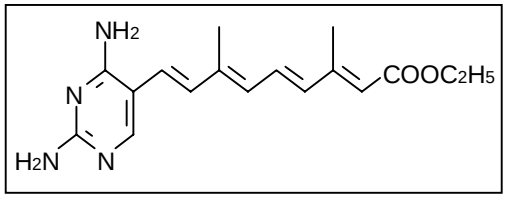


Fig.2a

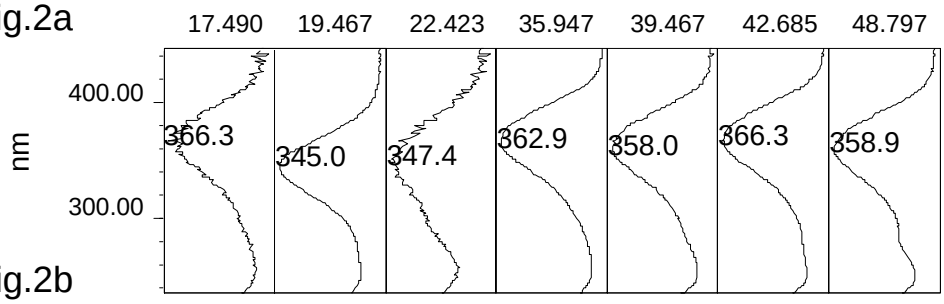
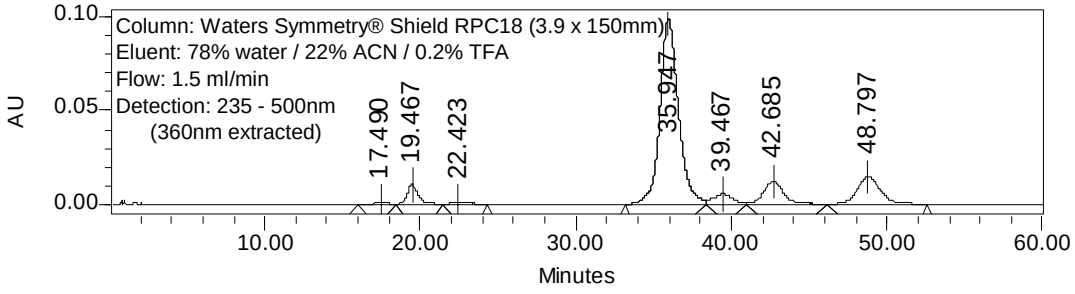


Fig.2b



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Millennium[32] Software for Peak Purity Determinations:

The ability of Waters 996 PDA detector and Millennium[32] software to detect spectral impurities within a "pure peak" is also demonstrated in this study. The symmetrical peak shape of the major component contained in the light-degraded sample (Fig. 2b. R.T.= 35.947) might suggest the presence of only one isomer. However, review of the Millennium[32] Purity Plot clearly indicates the presence of an impurity at the leading edge of the peak as shown in Figure 3. A Purity Angle of 1.030 degrees compared to a Purity Threshold of 0.240 degrees clearly indicates the presence of a spectrally dissimilar species. A search for a second, spectrally dissimilar compound contained within this peak yielded negative results (i.e., Purity Angle of 0.064 compared to a Purity Threshold of 0.241). By comparison, no spectral impurities were detected within the major peak of the material that had not been exposed to light-degrading conditions (data not shown).

Fig. 3

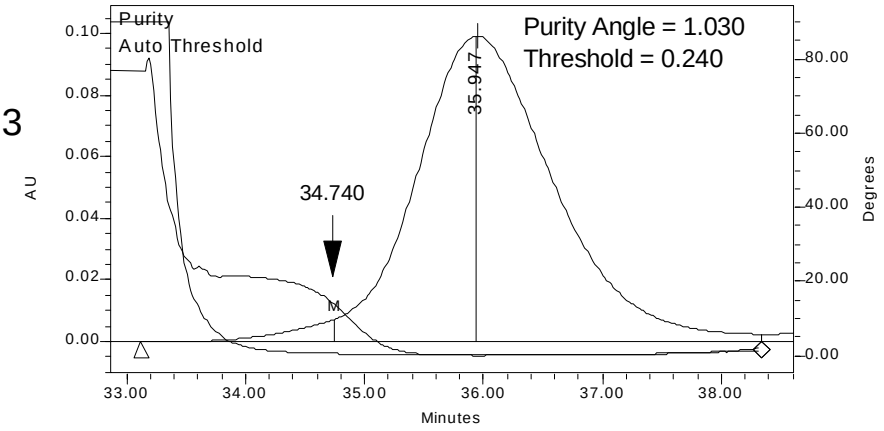
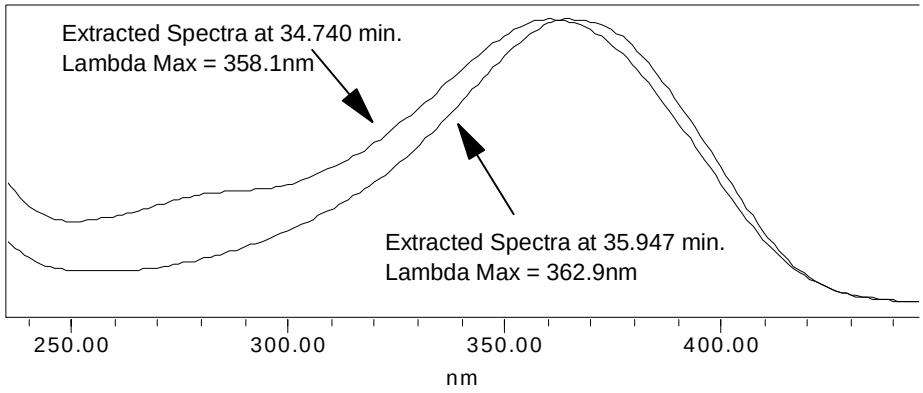


Figure 4 compares the apex spectrum of the major component peak (i.e., R.T.= 35.947) to the extracted spectrum taken at the point of maximum spectral dissimilarity (i.e., R.T. = 34.740). **The 1.2nm optical resolution that is available with the Waters 996 PDA detector, as well as the sophisticated data reduction methods of Millennium[32] software, enables this combined technology to clearly differentiate between these cis / trans isomers of identical molecular weight.**

Fig. 4



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