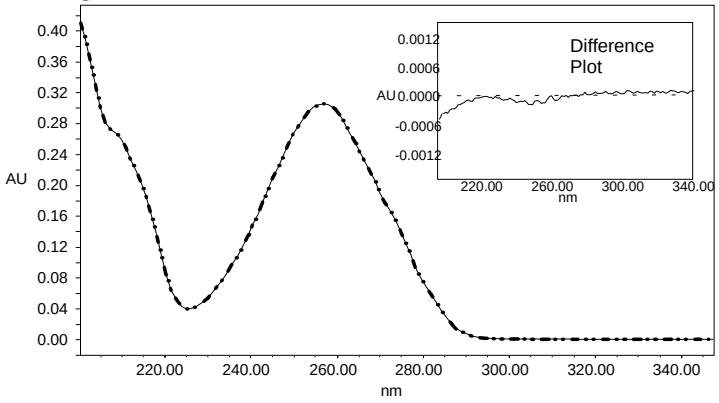
Performance PerSPECtives

Peak Identification with Waters 996 Photodiode Detector (PDA)

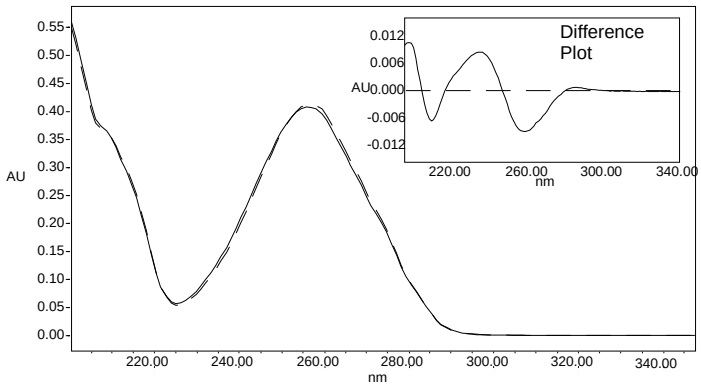
Library Matching

Peak identification with PDA is done by comparing a spectrum from an unknown peak to a spectrum from a user created library. The best match of spectra is the closest to the correct identification. There are three ways of doing the comparison:

1. Overlay spectra and make a visual determination. The lower figure may look like a good match to you, but not to others. This is a subjective test.
2. Evaluation with spectral differences between the reference spectrum and the unknown spectrum using Millennium® PDA software. The difference plots are shown in the inserts. Larger differences (lower figure) suggest that the unknown is a different compound than the reference or that it is a mixture.
3. Use of Spectral Contrast™ algorithms in the Millennium PDA software to calculate a numerical value. This is called the Match Angle, and it mathematically defines the closeness of the match. A value close to zero is a good match.



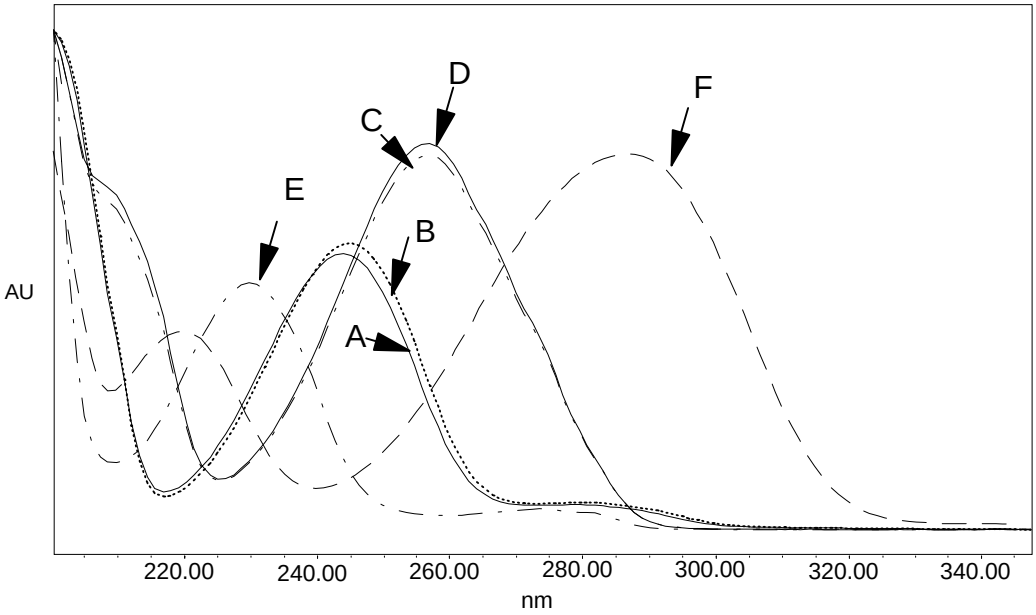
- **Match Angle = 0.063**
- Good match
- Peak ID = ethylparaben



- **Match Angle =1.432**
- Best match = ethylparaben
- Differences indicate either a different compound or a mixture.

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

How different are spectra?



Library matching is used for peak identification. UV/vis spectra of the unknowns are match against reference spectra in a user built library. This figure has six spectra overlaid to show how similar or different they can be. The table has the numerical value generated by comparing the spectrum of propiophenone (A) with the other spectra. When matched to itself, there is a perfect match, 0.00. When matched to acetophenone, a related compound minus a CH2 group the differences is significant, 3.202. Differences as small as 0.05 can be measured with confidence. As the spectral shapes change more, the Match Angle value increases (maximum is 90). Notice the two other related compounds, methyl and ethylparaben are only different from each other by 0.25 degrees. This ability to distinguish between spectra is very powerful. However, **it requires 1.2 nm optical resolution and low baseline noise (±1.5 x 10-5 AU) that is available with the Waters 996, as well as the sophisticated software algorithms of Millennium.**

Spectrum	Match Spectrum Name	Match Angle
A	Propiophenone	0.000
B	Acetophenone	3.202
C	Ethylparaben	37.906
D	Methylparaben	38.151
E	Benzoic acid	44.472
F	Ethyl-p-aminobenzoate	67.583