Performance PerSPECtives

Waters® Alliance® / 2996 PDA / Millennium®32: **Composition Analysis of Natural Product Extracts**

Providing "peak purity" information. Natural product extracts usually contain compounds with similar structures, spectra, and chromatographic behavior. Peak retention time comparisons alone may not provide sufficient information for correct identification and quantitation of marker compounds.

First, the purity of the peak must be established. Otherwise, reliable identification of the analyte by comparing its UV spectrum to a library of UV spectra of pure analyte standards may be compromised.

To estimate the purity of a peak, Waters Millennium^{32} Photodiode Array (PDA) option compares extracted spectra across the entire peak to the spectrum at the apex of the peak. Using patented Spectral Contrast Technology based on vector analysis, a "Purity Angle" is derived together with a "Purity Threshold" value. A reported Purity Angle that is greater than the Purity Threshold indicates that the peak is not spectrally pure, strongly indicating the presence of co-eluting contaminant(s). A typical example is the evaluation of the six kavalactone marker compounds in Kava Kava extract, a popular nutraceutical used as a sedative and muscle relaxant (Figure 1).

Providing "peak identification." Once peak purity has been established, the same Spectral Contrast Technology can be used to compare the analyte peak apex spectrum to spectra of individual standards stored in a user generated library, acquired from the analysis of pure standards. A calculated " Match Angle" value that is less than the "Match Threshold" increases the confidence in correct peak identification (Figure 1).

Figure 1: Peak Purity and Library Match information obtained from Waters 996 PDA and Millennium^{32} Software

	Name	RT	Purity1 Angle	Purity1 Threshold	PDA Match1 Angle	PDA Match1 Threshold
1	Kavalactones					
2	Methysticin	10.777	**0.445**	0.211	**1.058**	1.015
3	DiHydroMethysticin	11.342	**0.296**	0.210	0.207	1.021
4	Kavain	12.058	**2.386**	0.218	**1.774**	1.005
5	DiHydroKavain	13.100	**1.546**	0.209		
6	DesMethyoxyYangonin	16.397	0.127	0.208	0.469	1.027
7	Yangonin	17.345	0.095	0.206	0.136	1.030

In this example, the first 4 analytes give peak purity angles greater than the threshold indicating potential coelution with an unknown component. Analogously, the library match angles for Methysticin and Kavain are greater than the library threshold, supporting potential coelution.

Kavain shows the greatest difference. As the difference between angle and threshold increases, the confidence in accurate identification and quantitation decreases.

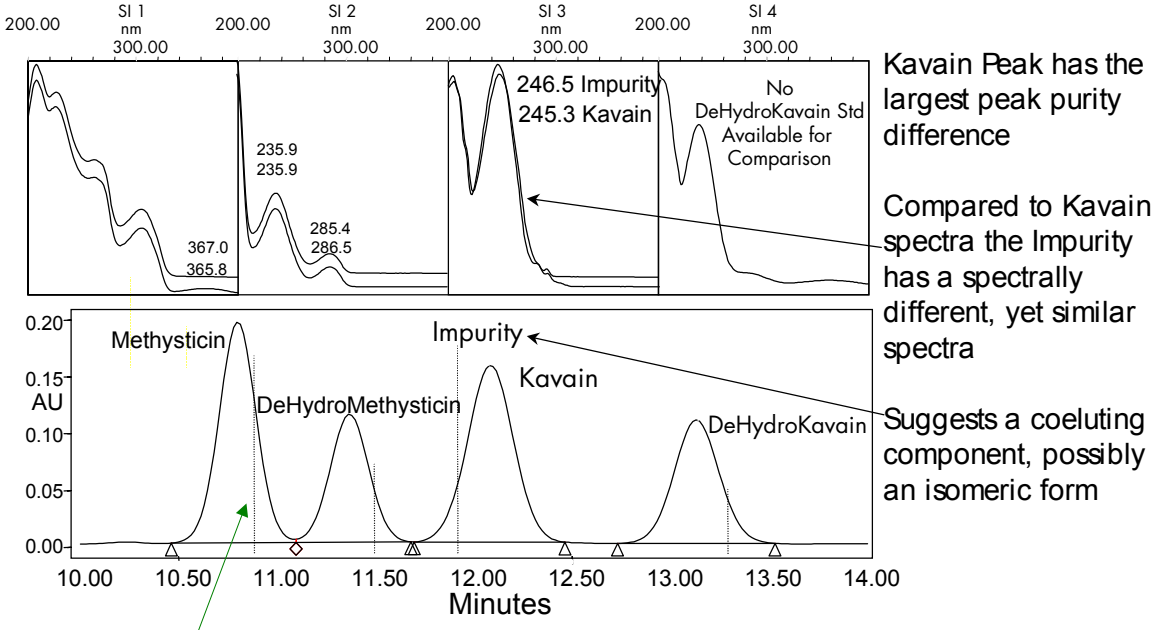
Examining the nature of the coeluting impurities. Referring to the chromatography of the extract, note that the kavain peak in Figure 2 appears symmetrical. Using single wavelength UV detection, it would be considered to be a pure peak. However, extraction of the PDA data indicates the presence of spectral impurities. There is a coelution that would bias the kavain quantitation.

Methysticin, DeHydroMethysticin, and DeHydroKavain also show potential coelutions affecting their quantitation. This is to be expected when analyzing complex natural product extracts.

Millennium[32] PDA software determines the point of maximum spectral difference (Figure 2), and displays the spectrum at this point as well as library spectra of the standard. With Kavain, the difference in lambda max between the 2 spectra is only 1.2 nm, a routine capability of the Waters 2996 or 996 photodiode array detectors. Although very small, this difference is potentially very significant.

This data helps the chemist to understand and evaluate the variability of various natural products, and aid in the development of an optimized analysis method. In situations where the purchase price of raw material or extracts is based on marker compound content, any biased quantitation will affect label claim accuracy, with financial and credibility consequences.

Figure 2: High spectral resolution of Waters 996 PDA distinguishes very subtle spectral differences compared to spectra of library standards



Summary:

• HPLC is an important technique for analyzing natural product extracts.

• Waters 2996 Photodiode Array Detectors with Millennium[32] software enables reliable determination of peak purity, higher confidence identification, and differentiation of spectral differences as small as 1.2 nm.