Performance PerSPECtives

Waters® Alliance® / Millennium® 32:
Accurate Quantitation of Natural Product Extracts

HPLC provides tools to identify and quantitate natural product extracts. High performance liquid chromatography is especially well suited for analyzing marker compounds in natural product extracts. Traditionally, peak identity of chromatographically resolved peaks is established by comparing retention times between standards and unknowns using HPLC systems with high precision solvent delivery. The identified peaks can also be reliably quantitated using this same HPLC technology.

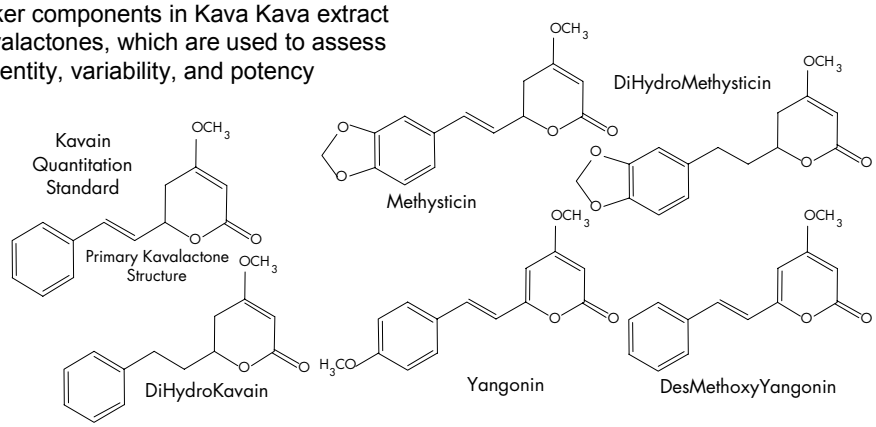
The challenge of controlling natural product extract quality. Herbal extracts are the starting materials for many popular nutraceutical products. The highly complex nature of these plant-derived ingredients presents a formidable challenge to incoming quality control operations. The most common approach is to analyze marker compounds, one or more of the prevalent compounds in these multicomponent extracts.

A typical example is the measurement of six kavalactones marker analytes in kava kava, a botanical root extract used as a sedative and muscle relaxant for conditions of nervous anxiety, stress, and restlessness[1]. Of the six, kavain is readily available as an inexpensive standard and is often used for quantitating the actual kavalactone content in the kava kava extract (Figure 1).

The other 5 kavalactones have limited availability and are expensive. An alternative means for quantitating these kavalactones is to determine their concentration based on kavain, then apply a Response Factor (RF) correction factor to account for the differences in spectral response.

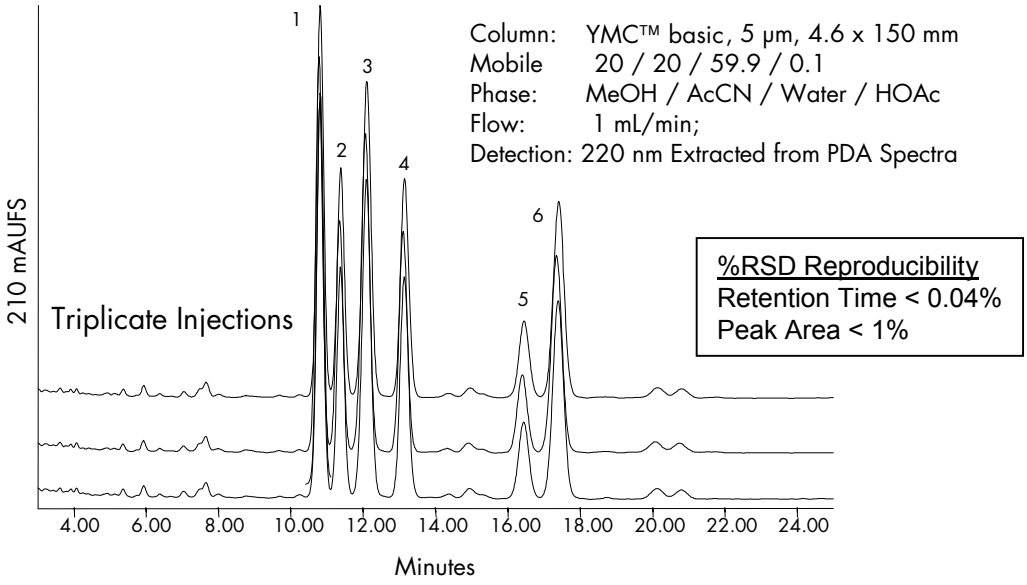
Figure 1: Structure of Kava Kava and other kavalactones.

The marker components in Kava Kava extract are 6 kavalactones, which are used to assess extract identity, variability, and potency



The kavalactone separation method, shown in Figure 2, was abstracted from a method described in the Institute for Nutraceutical Advancement[2]. Note the similarities in analyte structure, sometimes differing by a single double bond. This demonstrates the resolving potential of HPLC.

Figure 2: Waters Alliance System provides excellent Retention Time and Peak Area reproducibility for the analysis of a Kava Kava extract



		As Kavain	Corrected
1	Methysticin	= 6.12%	4.06%
2	DiHydroMethysticin	= 3.84%	6.35%
3	Kavain	= 6.08%	6.08%
4	DiHydroKavain	= 4.29%	7.29%
5	DesMethoxyYangonin	= 2.04%	1.85%
6	Yangonin	= 4.90%	4.30%
	Total Kavalactone	= 27.28%	29.93%

Kavain was used as the single calibration standard for all the kavalactones. Since the kavalactones have different spectra, their response relative to kavain is a function of the differences in their extinction coefficients. Using the Millennium[32] custom field capability[3], corrected response factors (CF) were employed for final quantitation.

Response factor correction factors can be derived from the ratio of the slope of the individual calibration curves using pure standard; ie, $CF = RF_{kavalactone} / RF_{Kavain}$. These are given in the Institute for Nutraceutical Advancement Method for kava kava analysis[2].

Kavalactone Correction Factors relative to Kavain:

Methysticin = 0.663 DeHydroMethysticin = 1.65 DeHydroKavain = 1.70
DesMethoxyYangonin = 0.904 Yangonin = 0.878

Millennium[32] custom field capability can also be used to sum the individual kavalactone amounts and report as a single number, in this case 29.93%. Extract label claim is 30% total kavalactone.

References:
1 The Complete German Commission E Monographs, American Botanical Council, Austin, TX, 1998, p156.
2 Institute for Nutraceutical Advancement, INAnetwork.com, Method for Kava Kava.
3 Waters Millennium[32] Software: Automate Custom Calculations Performance PerSPECtive (WPP65 / 720000281EN).

Summary:

- HPLC is an appropriate separations technique for analyzing complex natural product extracts.
- The flow and injection precision of Waters Alliance HPLC System insures reliable retention time identification and consistent quantitation of marker compounds.
- Millennium[32] software offers unique custom field, custom calculation, and custom report capabilities that help chemists get the right answer faster.