MASS DETECTION FOR CHROMATOGRAPHERS: BENEFITS FOR THE ANALYSIS OF HERBAL MEDICINES

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HIGHLIGHTS

- **Many botanicals (including medicinal herbs) contain compounds of interest that have poor UV chromophores.**
- **UV detection method at low wavelength suffers from poor specificity and may not be appropriate for routine use**
- **Mass detection provides alternative specificity for such compounds and can help QC labs develop more robust and reliable methods for routine testing.**

BACKGROUND

Feverfew (*Tanacetum parthenium*) is a medicinal herb that has long enjoyed traditional use as a remedy for migraine headache pain. The aerial parts of Feverfew (flowers, leaves and stems) contain parthenolide (a sesquiterpene lactone) which is believed to be responsible for the plant's biological activity. Dried feverfew aerial parts should contain approximately 0.2-0.5 wt.% parthenolide and adults are recommended a dose of 0.2-0.6 mg parthenolide daily for migraine prophylaxis[1,2]. As a result of these recommendations manufacturers of feverfew herbal supplements require robust and accurate methods capable of detecting and quantifying low levels of parthenolide in feverfew ingredients and finished products.

HPLC with UV detection is widely used in the supplement industry for the analysis of plant materials and herbal supplement products. While many naturally occurring compounds have strong natural chromophores there are other classes of compounds (such as sesquiterpene lactones) which lack a chromophore and demonstrate poor UV absorbance. Regardless, many labs continue to use UV detection for the measurement of poor UV absorbers even though such methods suffer from low sensitivity and poor specificity.

When low UV wavelengths (200 - 220 nm) are used for measurement, a UV detector will respond to nearly all compounds present in a sample and offers limited specificity for a specific compound of interest. While this may not be an issue for testing highly-purified materials, it is often unsuitable for the analysis of complex mixtures (such as whole herbal extracts and multi-ingredient herbal products) where numerous other compounds will absorb at that wavelength.

Sample cleanup (such as SPE) and rigorous method development could both potentially be employed to minimize the effect of interferences and to ensure accurate detection, however these options require development time and cost. Additionally, the chemical composition of plants have a tendency to change over time (due to factors such as growing conditions, geographic location, plant maturity, post-harvest processing, etc.) and methods which are developed for one particular source of material may fail to work appropriately when applied to a different source of the same material.

A better option for developing more robust methods is to use a detection technique which is more specific to the analyte of interest (e.g., mass detection) than low-wavelength UV detection. In this poster we examine how mass detection with the ACQUITY QDa Mass Detector can be used to more reliably detect parthenolide in Feverfew raw materials and finished products.

METHODS

SAMPLE PREPARATION

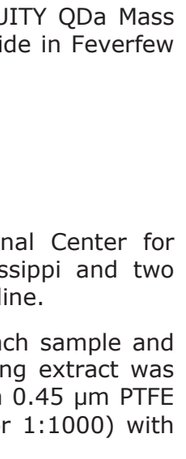
Feverfew raw materials were provided by the National Center for Natural Products Research at the University of Mississippi and two multi-ingredient herbal supplements were purchased online.

10mL of Acetonitrile (ACN) was added to 500mg of each sample and the solution was sonicated for 30 minutes; the resulting extract was allowed to settle and the supernatant filtered through a 0.45 µm PTFE syringe filter; each solution was then diluted (1:100 or 1:1000) with ACN prior to analysis.

A stock standard solution of parthenolide was prepared by dissolving 2.5 mg parthenolide in 10 mL ACN; individual calibration standards were prepared by diluting the stock standard with additional ACN.

LC CONDITIONS:

ACQUITY QDa mass detector



LC System:	ACQUITY UPLC H-Class
Software:	Empower 3 FR2
Detection:	ACQUITY PDA eλ Detector and ACQUITY QDa Mass Detector
Column:	Waters XBridge C18, 150 x 4.6mm, 5µm
Column Temp:	50°C
Sample Temp:	25°C
Inj. Volume:	10µL
Wavelength:	217nm
Mobile Phase A:	Water
Mobile Phase B:	Methanol

Time	Flow Rate	%A	%B
0.00	1.000	80	20
30.00	1.000	10	90
35.00	1.000	80	20
40.00	1.000	80	20

MS CONDITIONS:

Ionization Mode:	ESI+
Cone Voltage:	10V
Sampling Rate:	1 point/sec (Hz)
Mass Range:	100 – 600 Da
MS Experiment:	Single Ion Recording (SIR) @ m/z = 249

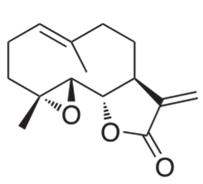


Figure 1 *– Feverfew herb and structure of parthenolide. Note the lack of UV chromophore.*

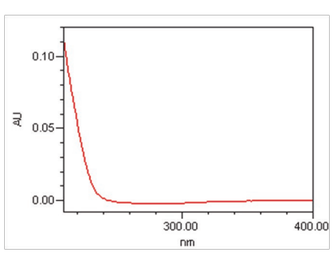


Figure 2 *– UV absorbance spectrum for parthenolide.*

RESULTS AND DISCUSSION

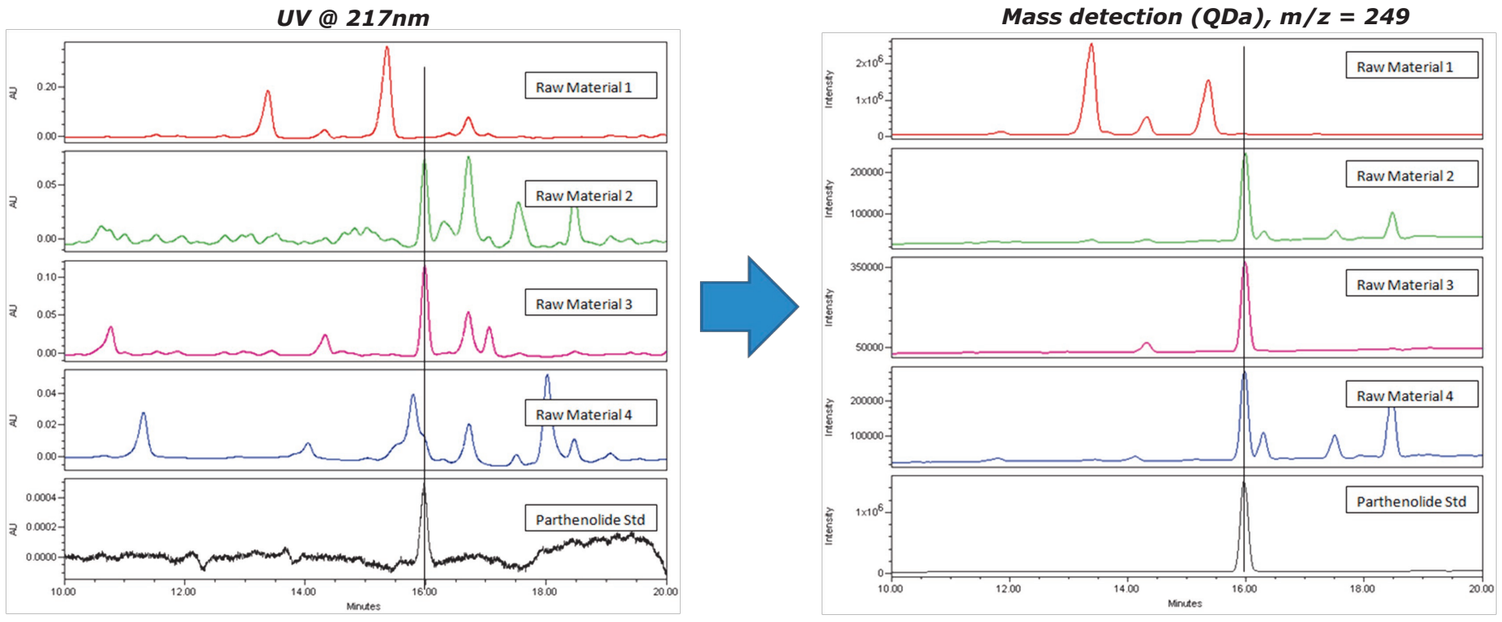


Figure 3a (left) *– UV chromatograms of the four feverfew raw material samples and the parthenolide reference standard. Note the naturally occurring interference in Raw Material 4 which prevents accurate detection of parthenolide – this is a common issue analysts must deal with when using non-specific methods.*

Figure 3b (right) *– ACQUITY QDa SIR chromatograms of the same four feverfew raw material samples and the parthenolide reference standard. Note how much cleaner the chromatograms are and how the interference in Raw Material 4 is no longer an issue.*

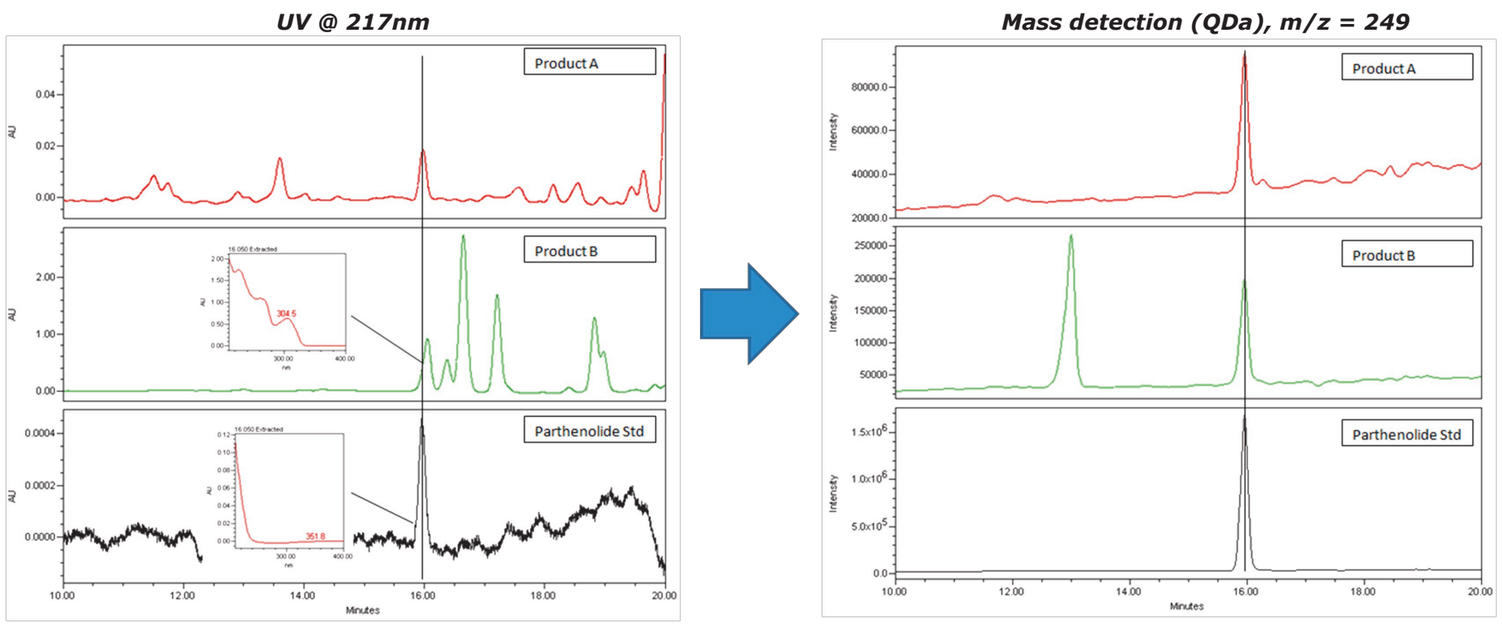


Figure 4a (left) *– UV chromatograms of Product A and Product B. Note the peak in Product B eluting at ~16.0 min which interferes with accurate identification of parthenolide in this sample. Examination of the UV spectra for the sample peak and reference standard confirm these are different compounds.*

Figure 4b (right) *– ACQUITY QDa SIR chromatograms of Product A and Product B. Note the clean response for parthenolide in each sample despite the interference present in Product B. This example illustrates the benefits of using more specific detection techniques*

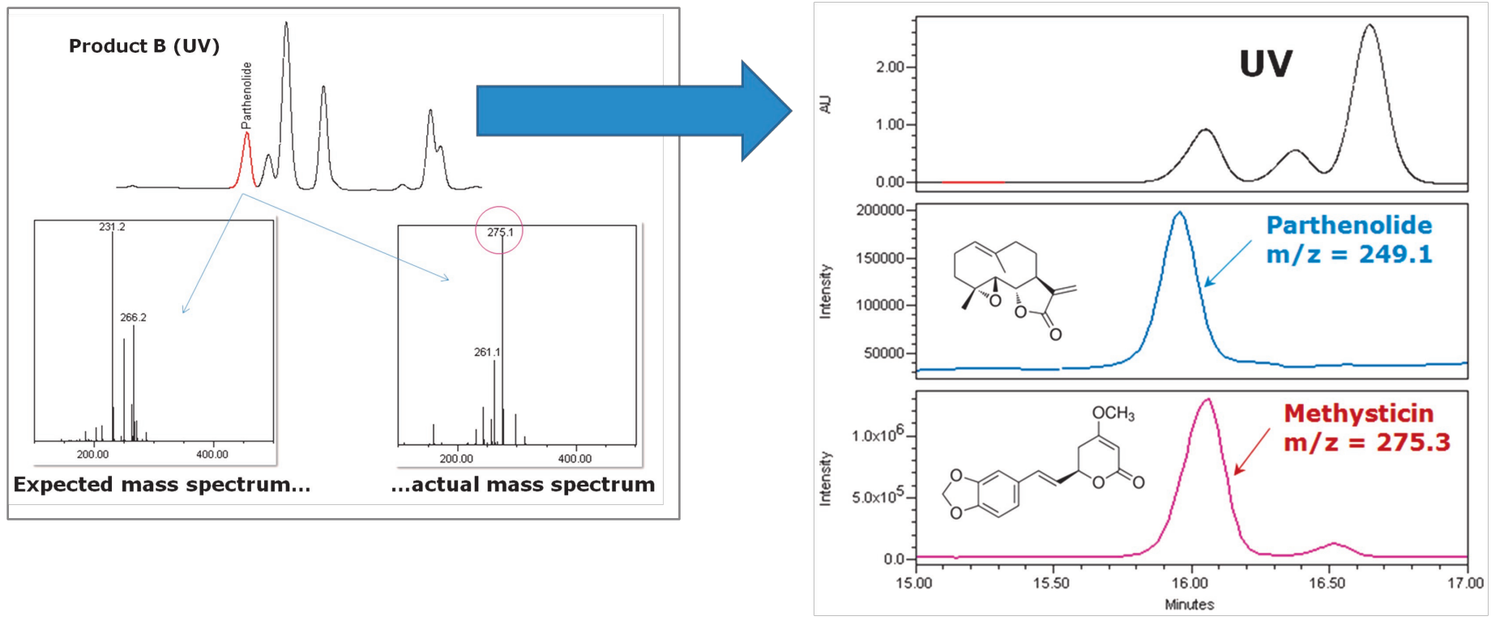


Figure 5a (left) *– Comparison of the mass spectrum of the peak at ~16.0 min in Product B vs. the mass spectrum of parthenolide. The large mass peak at 275 represents methysticin, one of the primary components of Kava Kava root.*

Figure 5b (right) *– While parthenolide and methysticin nearly co-elute and can not be accurately detected using UV, the compounds are easily resolved using mass detection and can be detected and quantified separately.*

CONCLUSIONS

- **Mass detection with the ACQUITY QDa provides improved specificity and sensitivity for parthenolide as compared to traditional low-UV wavelength detection.**
- **Parthenolide content was easily determined in both raw materials and multi-ingredient herbal supplements despite known interferences in Raw Material 4 and Product B.**
- **Mass detection may provide better results and more robust methods for the detection and quantification of other weakly UV absorbing compounds commonly found in natural products (sugars, saponins, terpenes, iridoids, etc.)**

REFERENCES

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