

# Waters Integrity System Applications

## Resolving Structurally Similar Components From Red Clover Extracts Using Both UV and MS Data

**Highlights:** Identification of the flavone diosmetin in a Red Clover plant extract in the presence of a co-eluting artifact; combined PDA and MS detection is utilized.

Phytochemicals extracted directly from plant sources have been used in various forms (dried, ethanolic tinctures, etc.) as therapeutics for centuries. More recently a number of products such as red clover (*trifolium pratense*) have been developed for use in concentrated form for anti-tumor and hormone replacement therapy due to their isoflavonoid content. Isoflavonoids have been determined in physiologic fluids to have cancer-preventative properties. In addition to the fundamental research interest, this may also account for the rise in OTC sales and the development of many of these products for use as dietary supplements. LC/MS is most well suited as a single technique for structural characterization when faced with the diversity of analysis found in natural products research. In the following example, the Integrity LC/MS System is used to identify diosmetin in the presence of a visible contaminant (coelution of an artifact peak) at the leading edge of the diosmetin peak.

### Sample Preparation:

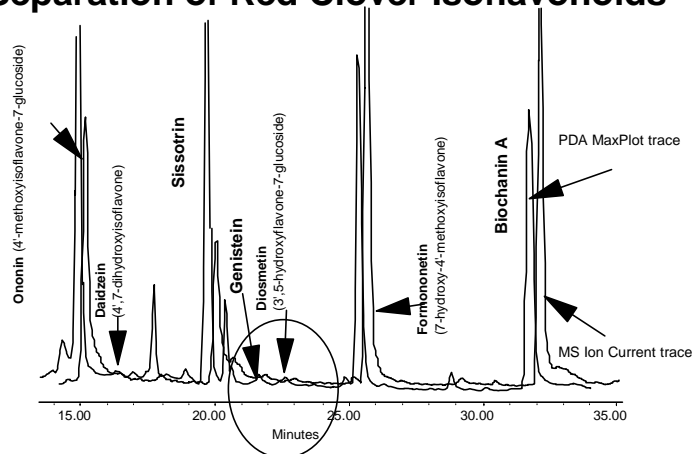
Extract 5g plant material in 70%EtOH, reflux 2H, 80 deg. C, filter, evaporate an aliquot to ca. 50% by volume

### Chromatographic Conditions:

Symmetry C8 column, 3 mm X 15 cm

A=Water:B=ACN, 85% A to 36% A at 45 minutes, 0.4 ml/min, PDA scan range: 220- 400 nm, 1.2 nm MS scan range: 50 - 500 Da

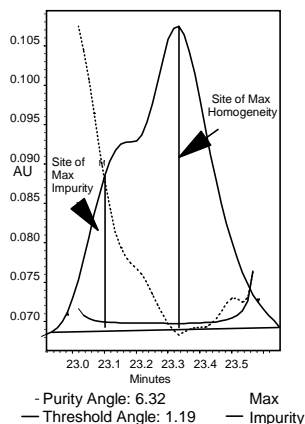
## Separation of Red Clover Isoflavonoids



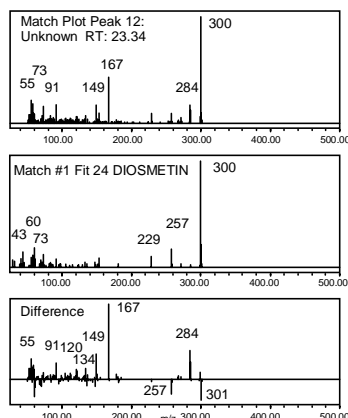
Determination of small components in a plant extract is simplified by the dual detector capabilities of the Integrity LC/MS System. Combined PDA and MS detection essentially yields a "third channel" of information to corroborate the identity of separated peaks. At left, the chromatograms overlaid from the PDA (UV) and the MS (TIC) are shown. A small peak near the limits of detection, tentatively identified as the flavone Diosmetin, produced only a "fit" value of 24 when a library search was performed. A relatively high contamination of the acquired Diosmetin spectrum versus the library reference spectrum was indicated.

## Combined PDA/MS Information

### Purity Analysis

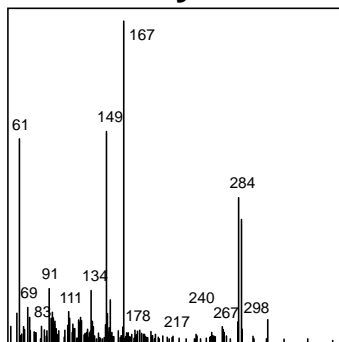


### Library Matching

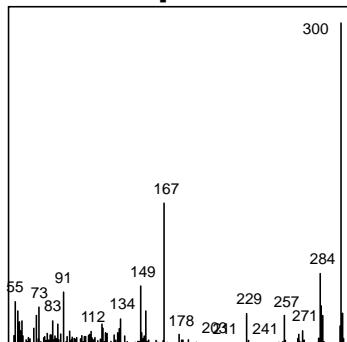


The comparative capability of the PDA and MS allows us to ascertain a visible contaminant, or coelution, of an artifact peak at the leading edge of the Diosmetin peak. PDA Peak Purity information indicates the presence and position of an impurity. The mass spec library search result is ambiguous since there is a high degree of difference between the sample spectrum and the library spectrum due to the coelution.

## Post-analysis Treatment of Spectral Data



Leading edge of peak #13



Main diosmetin peak

Using the PDA information as a guide, the source of contamination at the leading edge of the apparent Diosmetin peak can be subtracted from the main peak, and the result re-evaluated by the library search algorithm. The result is a higher degree of confidence that the identity of the peak is indeed Diosmetin. A comparison of the spectrum at the leading edge of the peak of interest to the spectrum of the main peak is shown at left.

## Improved Response for Peaks at the Limits of Detection

Hit	PBM Fit	% Cont	Spectrum Name	Formula	CAS No.
#1	24	43	diosmetin	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	--
#2	18	21	4-(3'-methylstyryl)naphtho...	C <sub>21</sub> H <sub>16</sub> S	107971-20-0

Following subtraction of leading edge spectrum:

Hit	PBM Fit	% Cont	Spectrum Name	Formula	CAS No.
#1	50	33	diosmetin	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	--
#2	35	25	diosmetin-7-glucoside	C <sub>22</sub> H <sub>22</sub> O <sub>11</sub>	20126-59-4

Here are the search results for a library match of Diosmetin before and after subtraction of a co-eluting artifact. The "fit" improves to an acceptable level as the contamination is decreased.

The range of analytical requirements demanded by natural products research makes good use of the inherent flexibility of the Integrity LC/MS System. Even at quite low signal-to-noise the increased confidence in results is significant when MS information is coupled with information from the PDA.