Characterization of Plant Extract Isoflavonoids Using LC-PDA-Mass Spec

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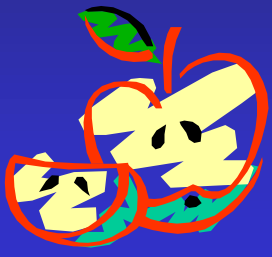


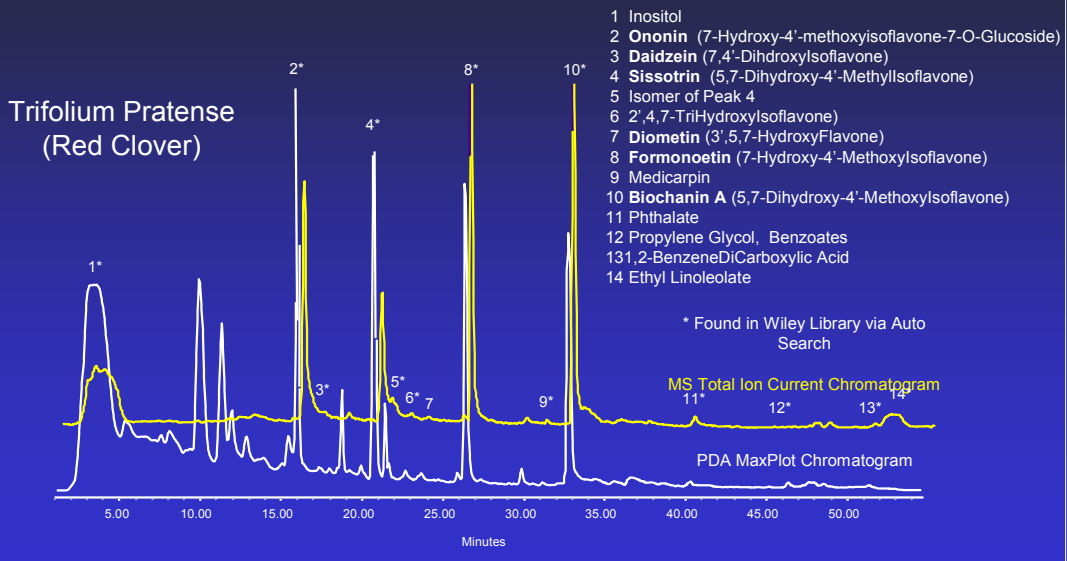
Introduction slide to the analysis of isoflavonoids in red clover using the Waters Integrity LC/MS System.

Natural Product Analysis
Red Clover Isoflavonoid Characterization
Chromatographic Approach

Column: Waters Symmetry C_8, 3mm x 150mm
Mobile Phase: AcCN / Water Linear Gradient
15/85 to 36/64 over 40 minutes
Flow Rate: 400 µL/min
Detector 1: PDA Scan from 200 to 600 nm
Detector 2: Mass Spec, Electron Ionization
Scan from50 to 500 m/z

Here are the optimized chromatographic conditions for the analysis. Both PDA and MS detection are employed.

Natural Product Analysis
Red Clover Isoflavonid Characterization

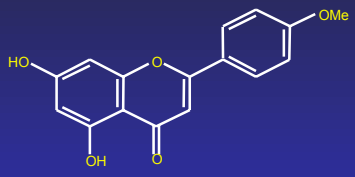


The chromatographic separation of red clover extract. The PDA plot (lower chromatogram) and mass spec TIC (upper chromatogram) is shown here. Note that all of the starred compounds were identified using the commercially available Wiley Library.

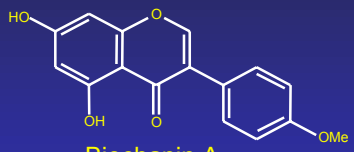
Some Common Flavonoids -
their derivatives and secondary metabolites

Flavones

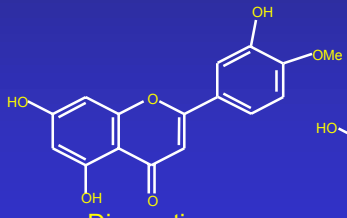
Isoflavones



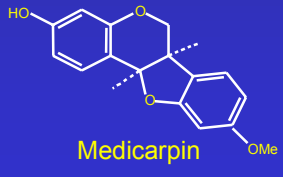
Acacetin



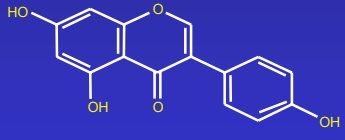
Biochanin A



Diosmetin



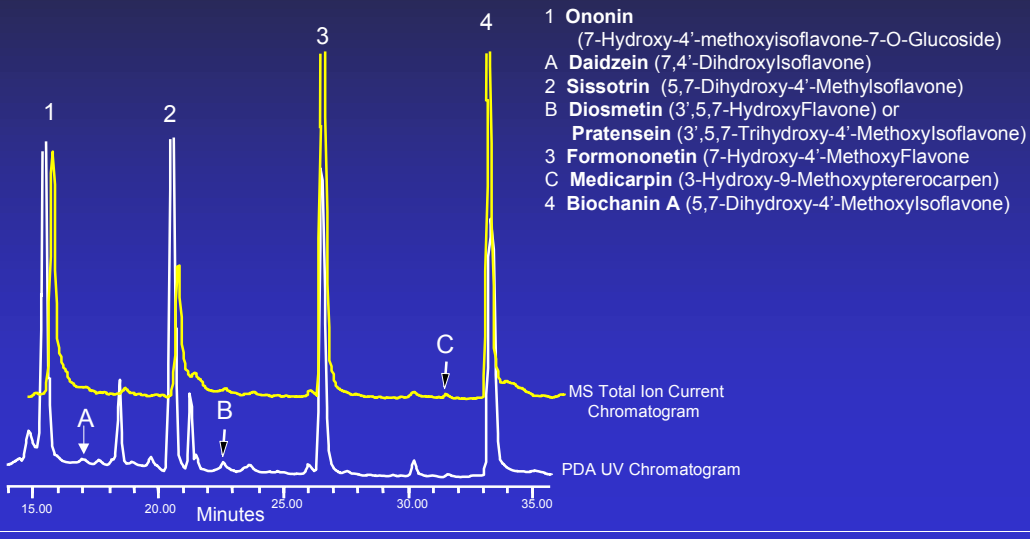
Medicarpin



Genistein

Shown here are some of the structures of the flavones and isoflavones examined in this study

Natural Product Analysis
Red Clover Isoflavonoid Characterization

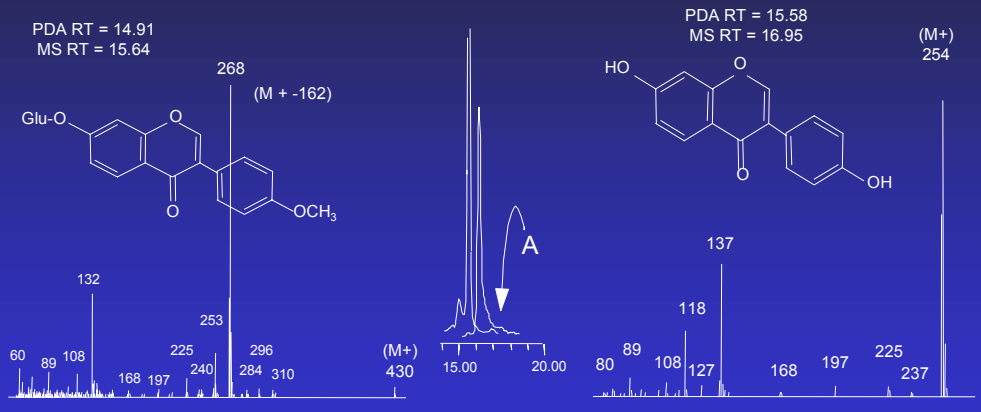


Identification of daidzein, diodmetin, and medicarpin in the red clover extract from the west coast.

Natural Product Analysis

Red Clover Isoflavonoid Characterization

Identification of Close-Eluting Peaks by MS



Ononin
(7-Hydroxy-4'-Methoxyisoflavone-7-O-Glucoside)
$C_{22}H_{22}O_9$ MW = 430.46

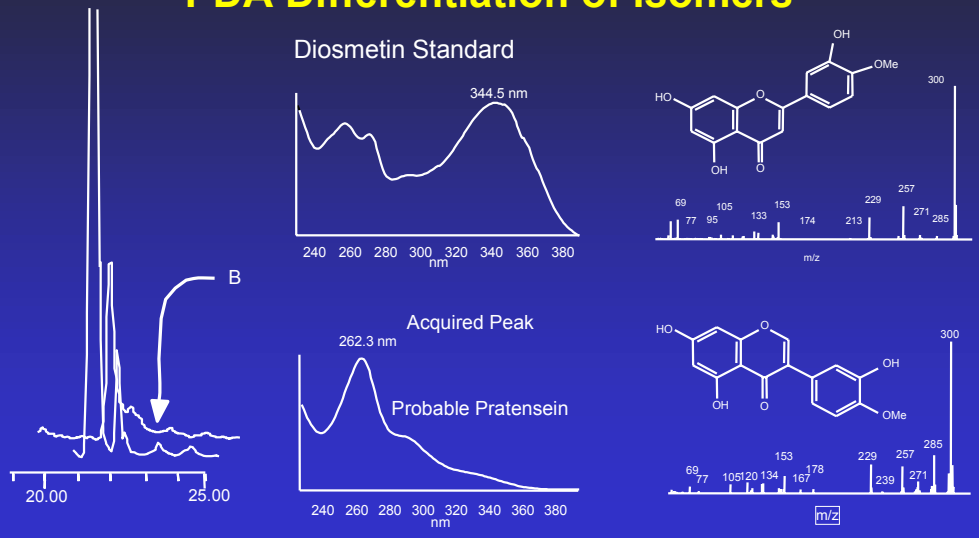
Daidzein
(4',7-DiHydroxyIsoflavone)
$C_{15}H_{10}O_4$ MW = 254.23

A close up view of the separation shows a very small peak (A) identified as the aglycone daidzein at the trailing edge of the glycoside ononin. The mass spectra for each are significantly different in this case, which allows direct identification by library match from a single experiment. The molecular ion of the large peak (ononin) at m/z 430 is followed by the loss of glucose (M^+-162) forming the base peak of the spectrum. The smaller peak (daidzein) produces the molecular ion at m/z 254 as the base peak of the spectrum.

Natural Product Analysis

Red Clover Isoflavonid Characterization

PDA Differentiation of Isomers



Comparison of the acquired peak with the UV spectrum of diosmetin standard, indicates that the peak is probably pratensein. Both diosmetin and pratensein have a formula weight of 300.27.

Natural Product and Nutraceutical Futures

- Increased use of Mass Spectrometry to compliment Photo Diode Array detection for Product and Component characterization
- Need for Validated Analytical Methods for Product Target Compounds
- Need for Standardization among Suppliers and Manufacturers for product consistency
- More FDA Regulation in the Future?

Waters foresees increased use of HPLC and LC/MS for the analysis of nutraceuticals.

More validated analytical methods are required.

Suppliers and manufacturers need to follow the same quality assurance procedures to assure product consistency.

Will the FDA regulate the nutraceutical industry more closely in the future?

Successful Analyses

Application	Sample Prep	Column	Technology
Sugars	C_{18} Sep-Pak	Carbohydrate	HPLC/RI
EED's	Oasis HLB	SymmetryShield RP_{18} & RP_8	HPLC/PDA LC/MS
PAH's	Oasis HLB	LiChrosphere PAH	HPLC/PDA & Fluorescence
Carbamates	Oasis HLB	Carbamate	HPLC/PCFD LC/MS
Pesticides	Oasis HLB	Symmetry C_{18}	LC/MS
PolymerAdditives	Org. Extract	Symmetry C_{18}	LC/MS
Nutraceuticals	Org. Extract	Symmetry C_8	LC/MS

In summary, this seminar has demonstrated successful analyses for a number of applications utilizing a variety of Waters products and technologies.

Agricultural Chemicals
Residues in Food & Water

Fine Chemicals
Pesticides
Polymer Additives

Functional Foods & Dietary Supplements
Nutrients
Nutraceuticals

Environmental Contaminants
Endocrine Disruptors

Conclusion: Waters offers solutions to a wide range of analytical challenges in the industrial market place.