Hypericin Analysis by LC/MS

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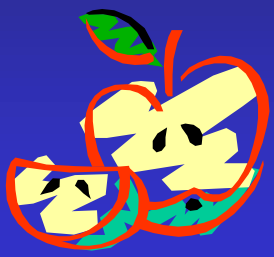
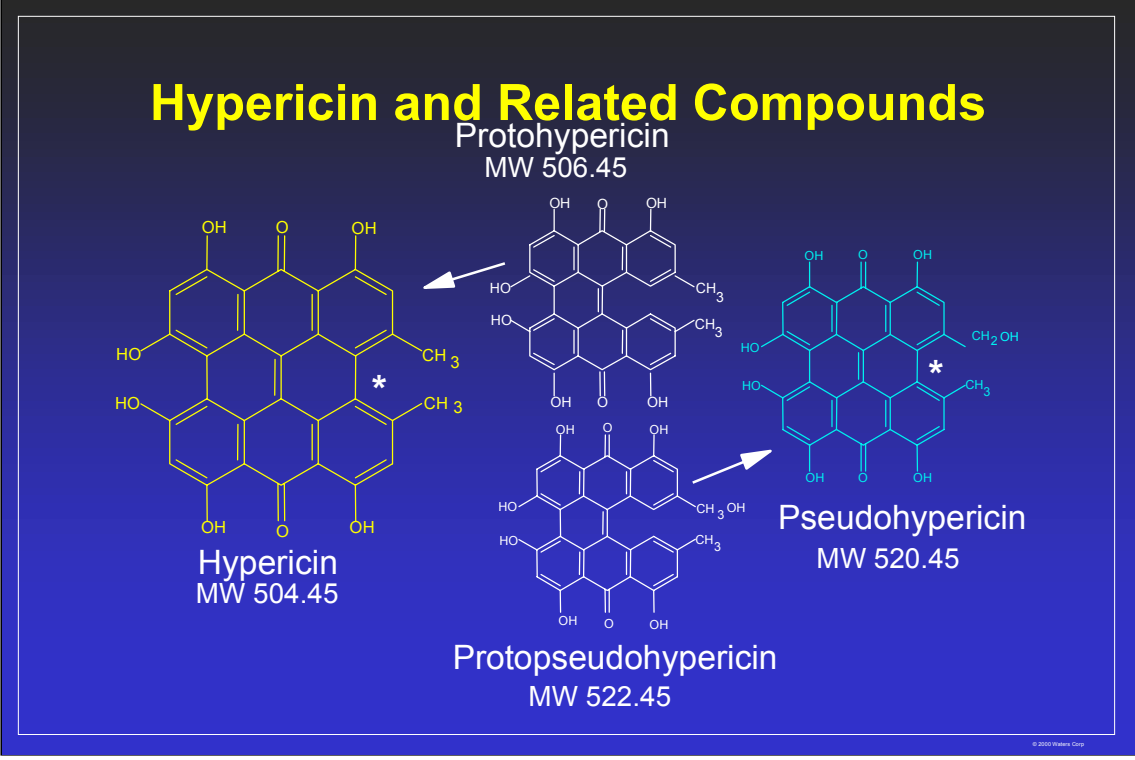
LC/MS
Waters Corporation

LC•GC , Vol 17, No. 6, June 1999



Introduction to hypericin analysis by LC/MS



The structures of the compounds of interest in the plant extract are shown in this slide. Note the closing of the ring E on the right-hand side of hypericin and pseudohypericin. This makes them more hydrophobic which effects their elution order in reversed-phase separations.

The protohypericin and protopseudohypericin are the biosynthetic precursors that are easily converted to hypericin and psuedohypericin.

Hypericin Analysis Chromatographic Conditions

Column: Waters Symmetry C_8, 2.1 mm x 150 mm

Mobile Phase: A = 100mM TEA•Acetate, pH 7
B = Methanol
C = Acetonitrile

Gradient: Linear over 15 minutes
30:39:31 to 10:50:40

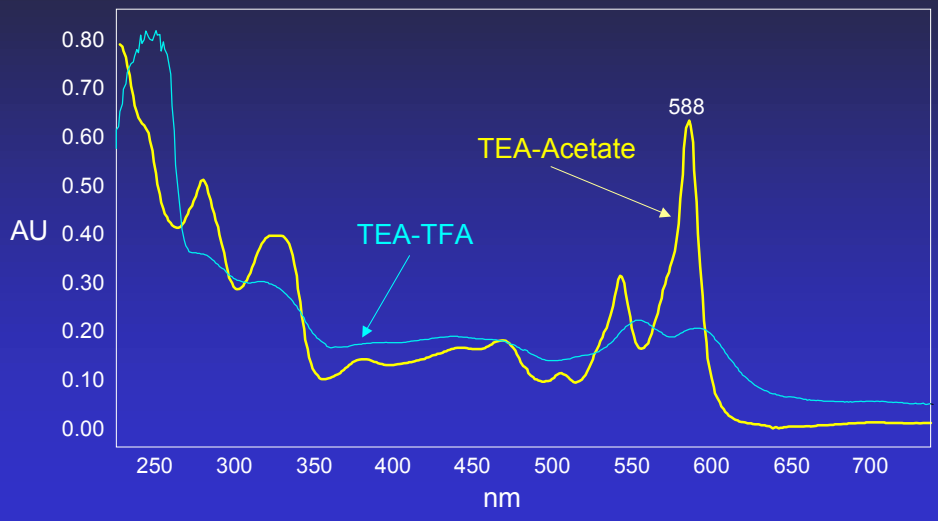
Flow Rate: 300 µL/min

Detection: PDA at 588 nm

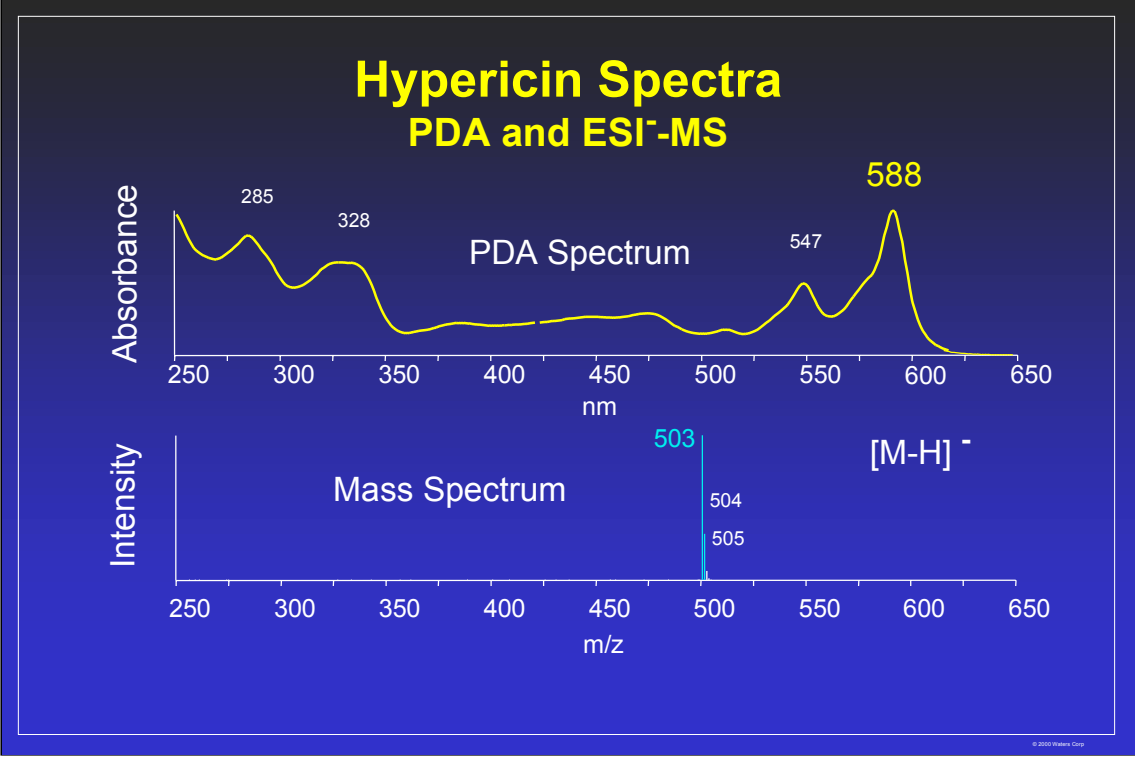
Mass Spec: Negative Electrospray Ionization (ESI^-)

Here are the optimized chromatoghraphic conditions for hypericin analysis. Both PDA and MS detectors were used.

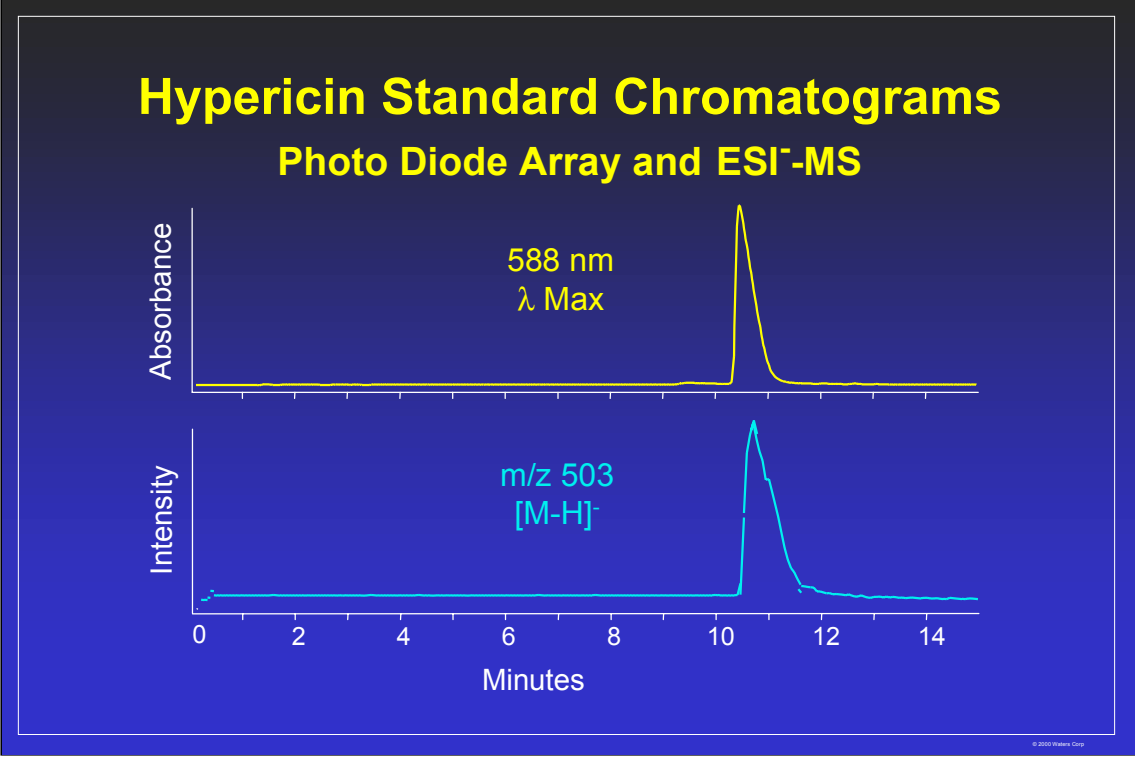
Hypericin UV/Vis Spectra
TEA-Acetate vs. TriFluoroAcetate (TFA)



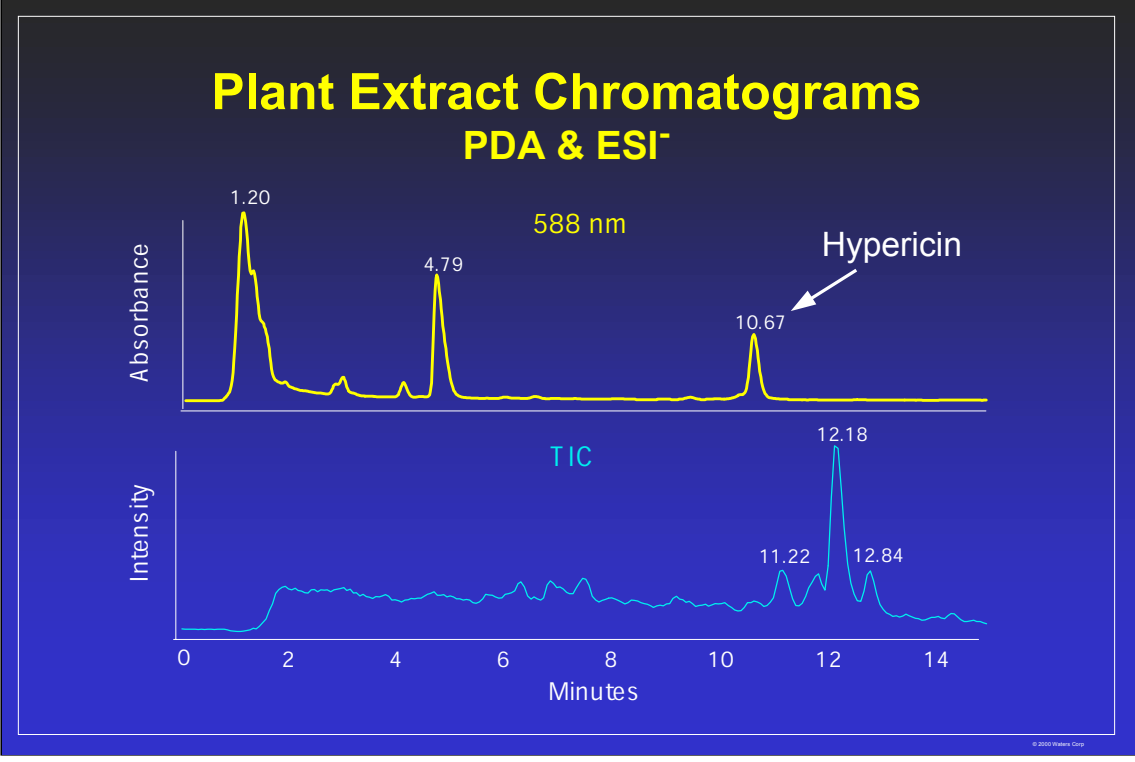
The absorbance spectra of hypericin in trifluoroacetic acid and in triethylammonium acetate are shown in this slide. The unique UV-VIS spectrum of naphthodianthrones has much stronger absorbance at 588 nm in triethylamine acetate than in trifluoroactic acid. This buffer also was conducive to good ionization by electrospray of hypericin and the other compounds in negative mode.



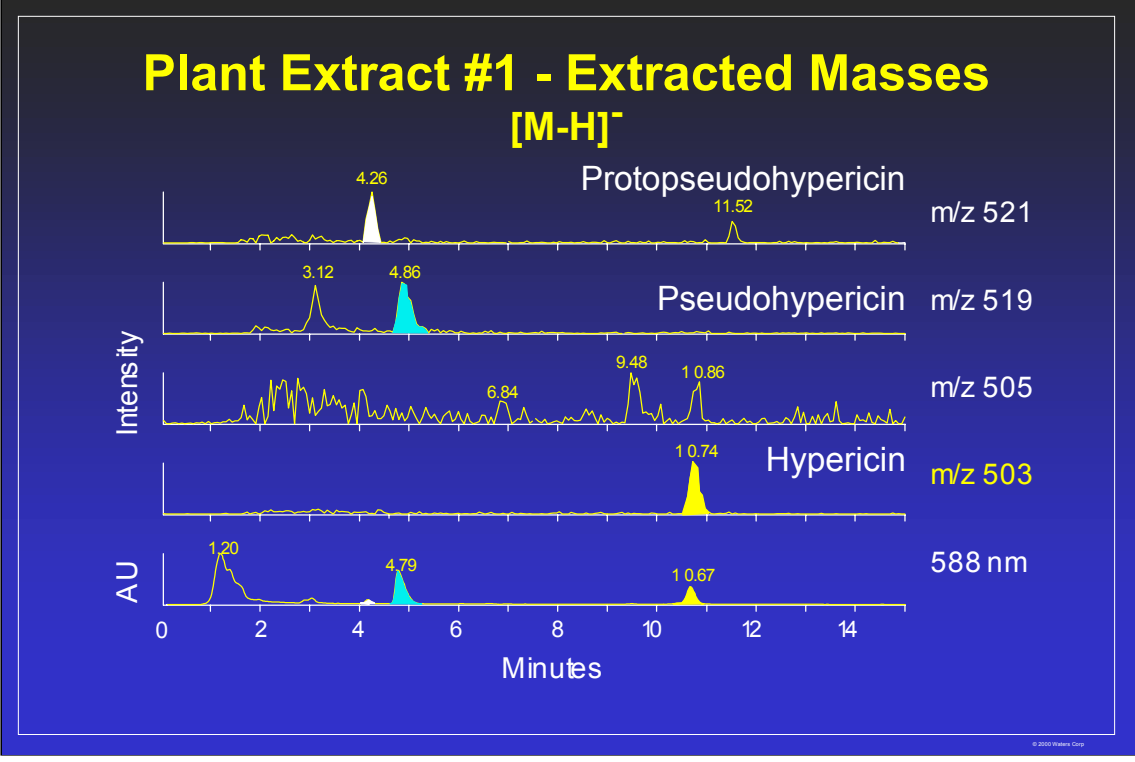
A comparison of the UV-VIS spectrum and mass spectrum of the hypericin peak.



Extracted chromatograms from the separation of hypericin standard monitored simultaneously by PDA detection at 588 nm and MS detection at m/z 503.

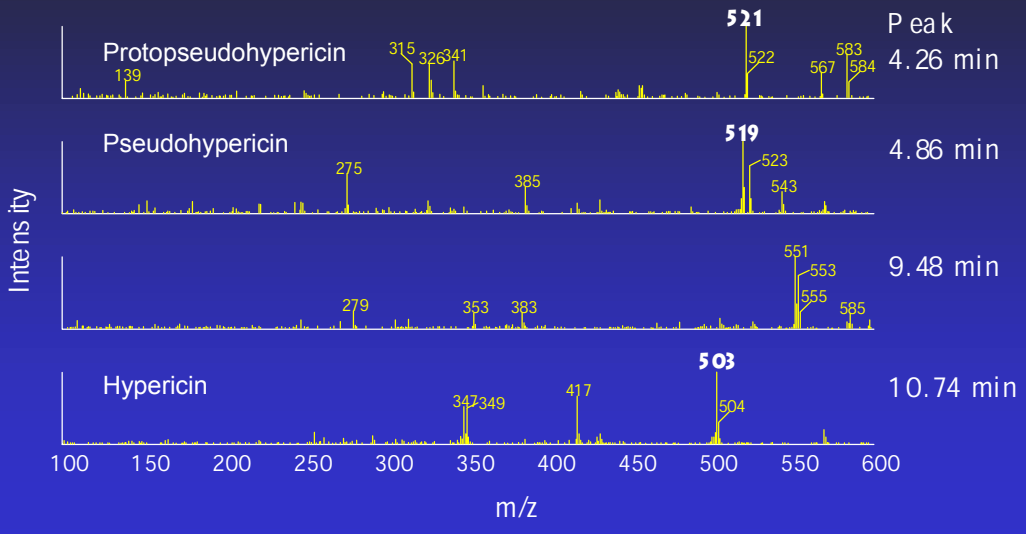


The chromatograms of the plant extract of sample 1 obtained by monitoring aborbance at 588 nm (upper chromatogram) and the total ion current (lower chromatogram). The peak at 10.67 minutes is hypericin.

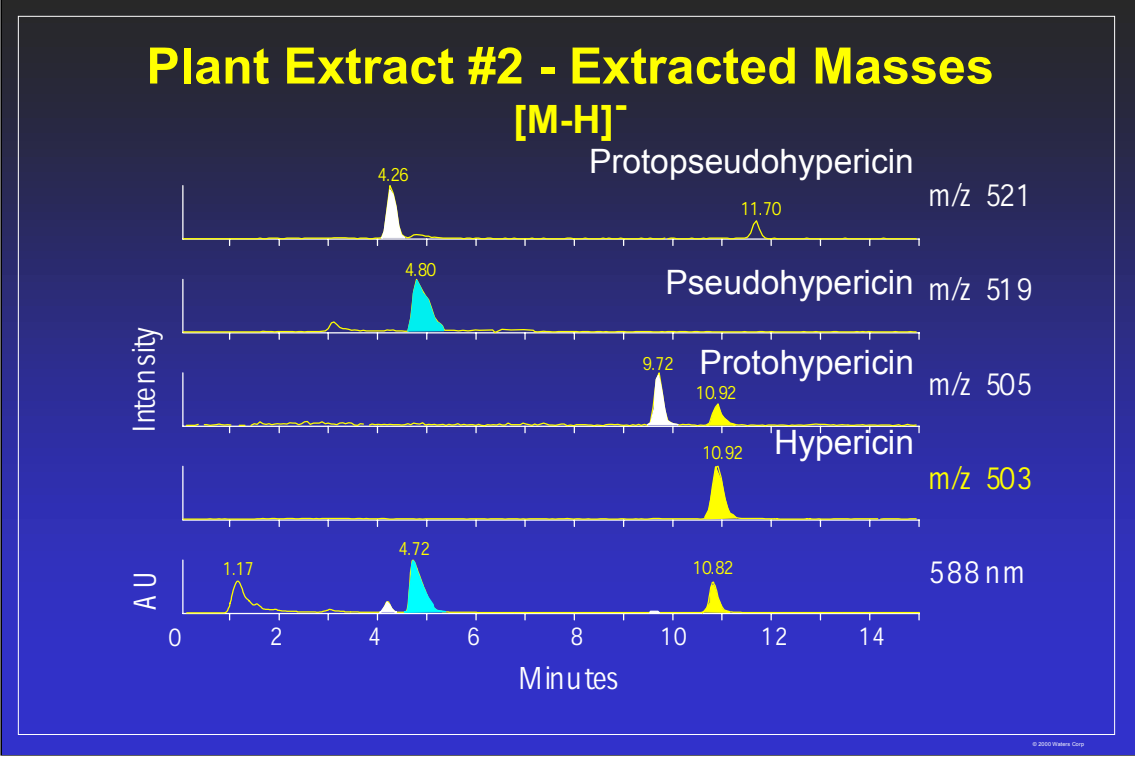


Shown here are the extracted mass chromatograms of the plant extract from sample 1 with the UV absorbance chromatogram on the bottom. Note that protohypericin was not detected in this sample.

ESI⁻ Mass Spectra - Plant Extract #1

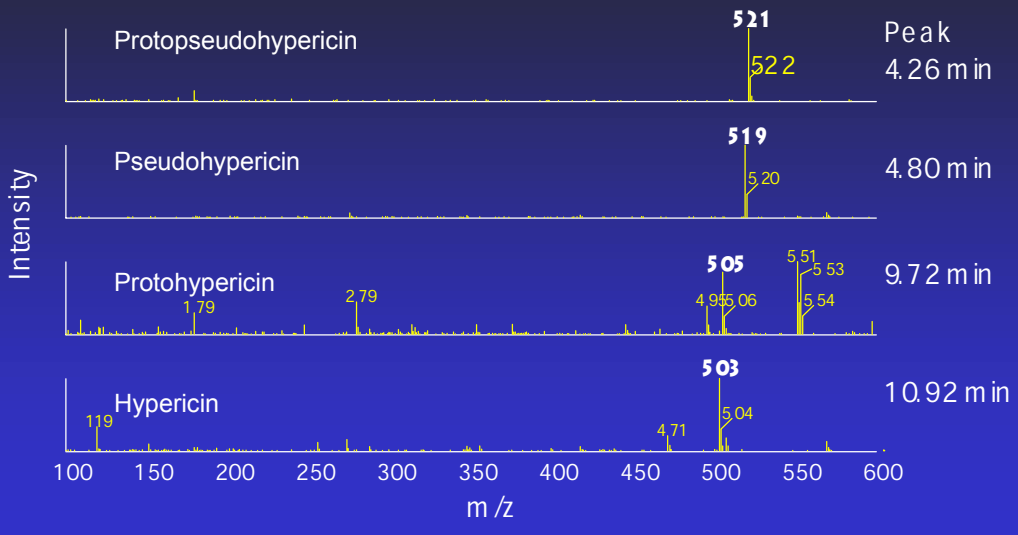


Shown in this slide are the mass spectra extracted from the chromatographic peaks in sample 1.

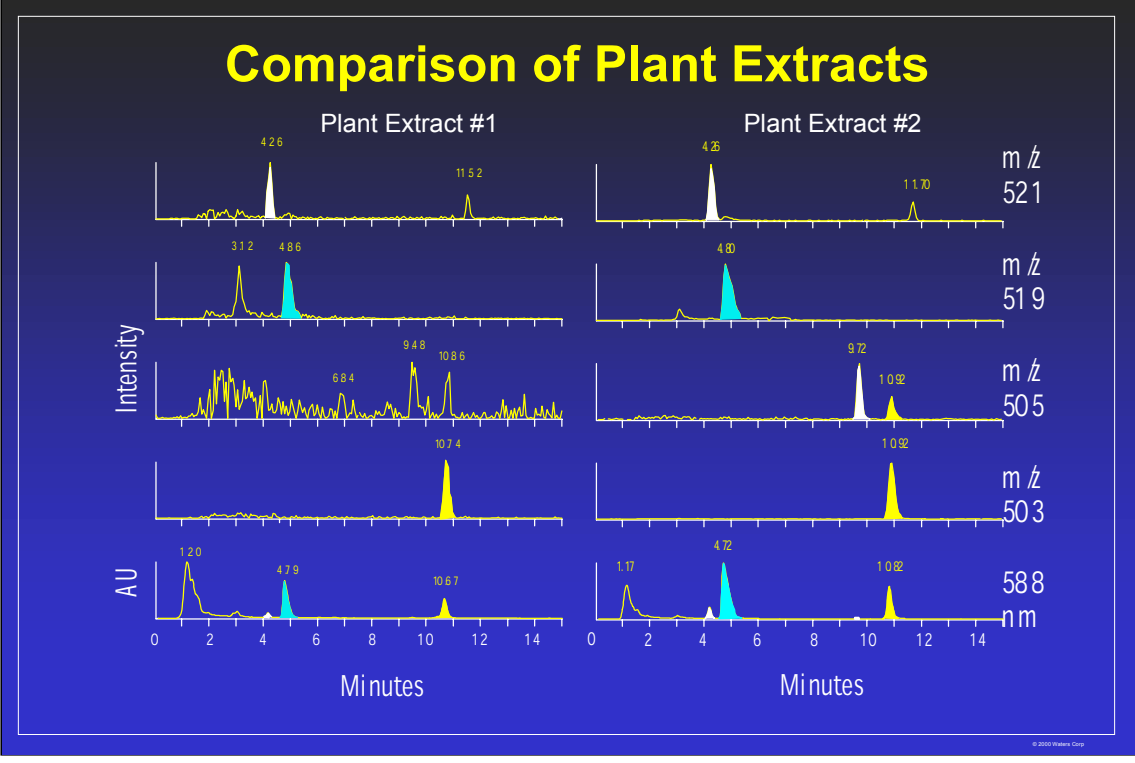


Shown here are the extracted mass chromatograms of the plant extract from sample 2 with the UV absorbance chromatogram on the bottom.

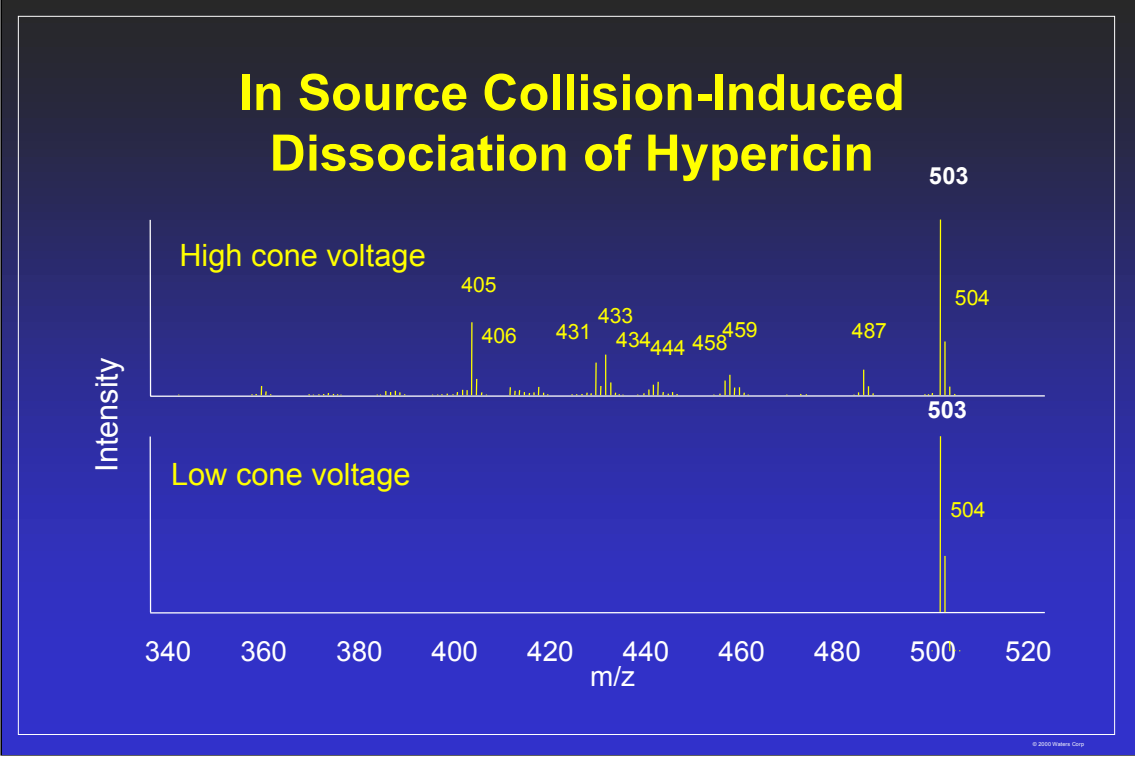
ESI$^-$ Mass Spectra - Plant Extract #2



Shown in this slide are the mass spectra extracted from the chromatographic peaks in sample 2.



Comparison of plant extracts 1 and 2 indicate that more of the hypericin compounds were solubilized from the plant powder of sample 2. The hypericin content in plants reportedly changes as a function of climate and season.



In-source Collision Induced Dissociation (CID) of hypericin in a single-quadrupole mass detector. Shown are the spectra at high (upper spectrum) and low (lower spectrum) cone voltages. The low cone voltage was optimized for sensitivity of the [M-H]- ion at M/Z 503.

Natural Product Analysis
Phytoestrogens-Isoflavonoids

- Electrospray, is a soft ionization, yielding a single $[M+H]^+$ or $[M-H]^-$ line
- Gives some fragmentation for structural identification, but no Libraries available to search
- Electron Ionization, Particle Beam, yields information rich spectra
- Can deduce molecular structure and libraries are available for Unknown Identification

A group of isoflavonoids commonly called phytoestrogens are found in red clover.

Analysis of plant extracts by LC/MS using an electrospray interface, which is a soft ionization technique, yields a single molecular ion with limited fragmentation.

The Thermabeam interface, which is an improved form of particle beam interface, is used to generate electron ionization (EI) spectra for LC/MS. Electron ionization, due to it's high ionization energy (about 70 eV), offers a reproducible fragmentation pattern of a molecule, which can be searched against a library for positive compound identification. This type of ionization is well suited for qualitative analysis where extensive structure-informative fragmentation is highly desirable.