MS/MS interpretation in identification of unknowns

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Outline

- Introduction
- How to interpret LC-MS and MS/MS data.
- Identification of some conjugated metabolites.
- Sensitivity enhancement through derivatization
- Dereplication of natural products, sub-structure analysis of unknowns

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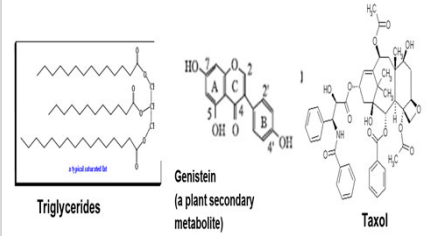

Small molecules are important!!

- 89% of all known drugs and 50% of all drugs are derived from pre-existing metabolites.
- Small molecules are cofactors and signalling molecules to 1000's of proteins.
- 100,000 (lipidome)

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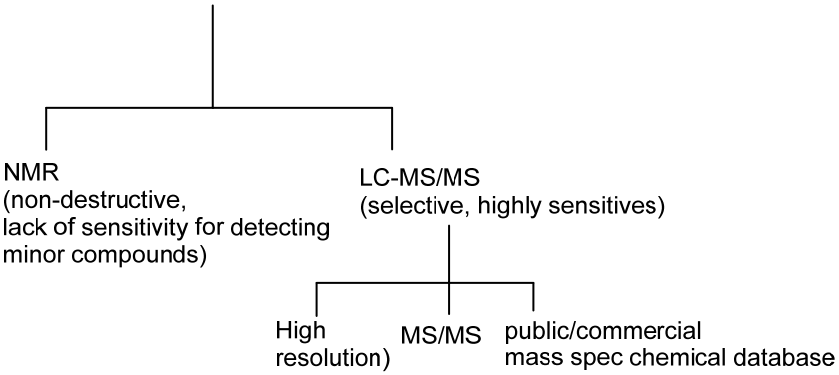
Knowing the unknowns



An unknown compound is a small molecule that can reproducibly be detected and quantified in a metabolomics experiment, but whose chemical identification has not been elucidated yet (Krumsiek et al., 2012).

Unknown unknown- not previously cited (Little et al., 2011)

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Keys to identifying unknown structures (putative/definitive) by mass spectrometry

- Knowing the precursor ion
- Retention time of metabolites in LC
- Accurate mass
- Isotope distribution
- Nitrogen rule
- Fragmentation pattern of a precursor ion
- Product/precursor ion intensity ratio
- Comparison with authentic standards (definitive)

Moco et al. Trends in Analytical Chemistry, 2007

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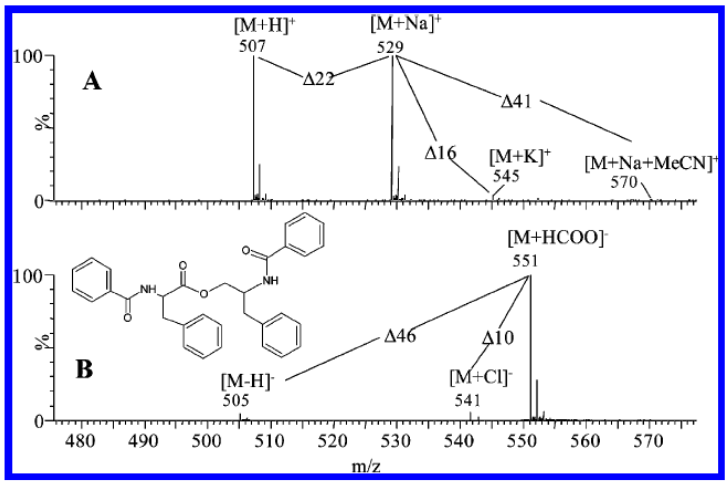
Adduct formation in +/–ve ion modes



Nielsen et al., J Nat Prod. 2011

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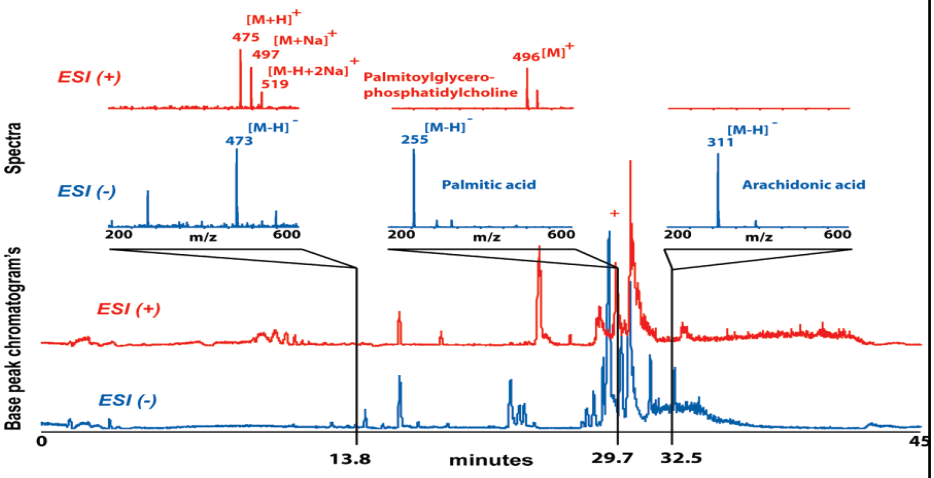
Increasing metabolite coverage using +ve and –ve ion mode



Representative Q1 scans of a methanolic extract of human blood serum

Source: Nordstrom et al. Analytical Chemistry, 2007

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Isotopic distribution and MS

1H = 99.9%, 2H = 0.015%

12C = 98.9%, 13C = 1.1%

35Cl = 75.7%, 37Cl = 24.2%

Monoisotopic mass - the mass of the most abundant isotope

Average mass- the abundance weighted mas of all isotopic components.

https://www2.chemistry.msu.edu/faculty/reusch/OrgPage/mass.htm

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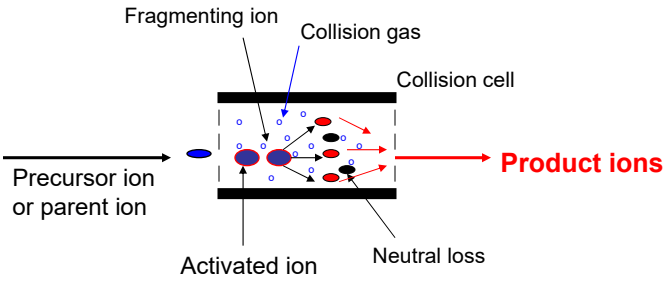
What is Collision Induced Dissociation (CID) or Collisionally Activated Dissociation (CAD) ?



Schematic of CID fragmentation

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Applications of MS/MS

- **Pharmaceuticals- Identification and quantification of drug metabolites, PK/PD**
- **Academic/biotechnology- analysis of protein/peptides, authentification and profiling of chemical components in a crude mixture, substructure analysis of unknown components**
- **Clinical- eg. neonatal screening, steroids in athletes etc.**
- **Environment- eg. dioxins in fish..**
- **Geological- eg. oil compositions…**

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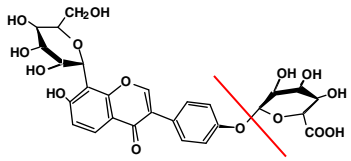
Interpreting MS/MS spectra

- Likely sites of protonation or deprotonation.
- Likely leaving group after cleavage (C-C vs C-O bond)
- Relative abundance of fragment ions
- Neutral molecules are lost



Where are the sites of deprotonation/protonation? What is the most likely leaving group in this molecule?

Fragmentation always follows the basic rules of chemistry- resonance stabilization

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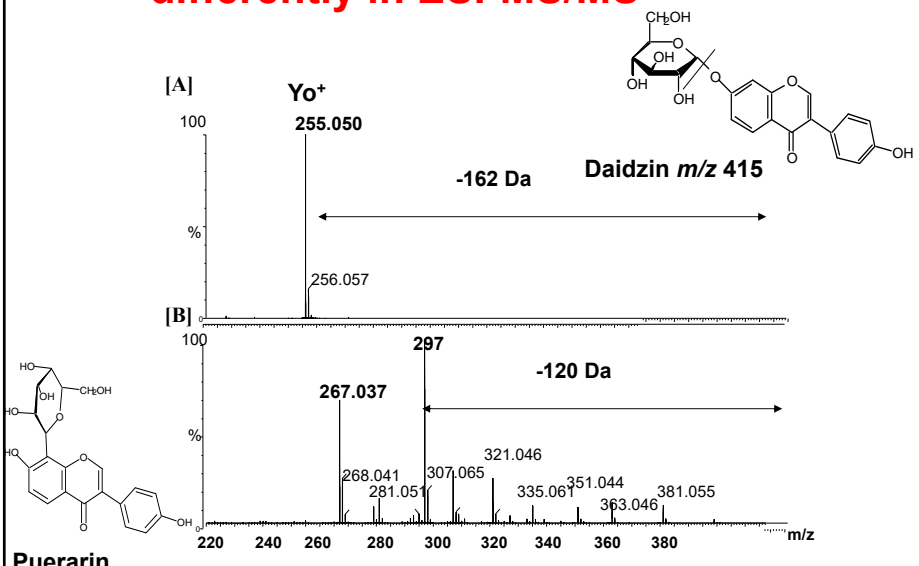
O- and C-glucosides fragment differently in ESI-MS/MS



Prasain et al. J. Agric. Food Chem. 2003

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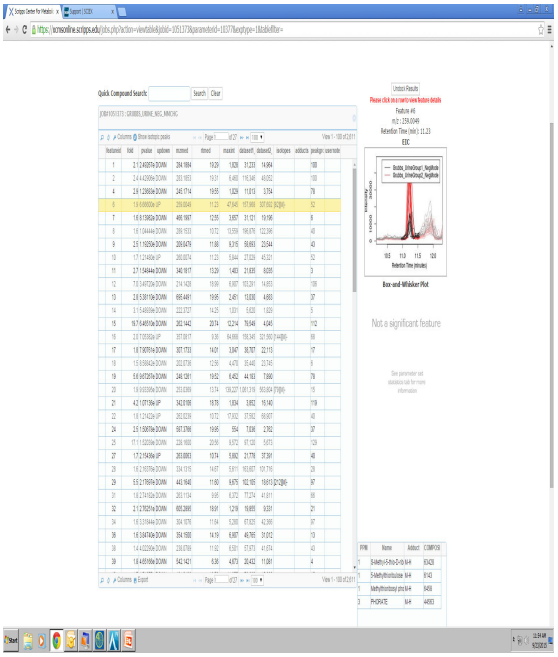
- **XCMSonline screen shot showing list of potential**
- **metabolites, with p-values and fold changes**



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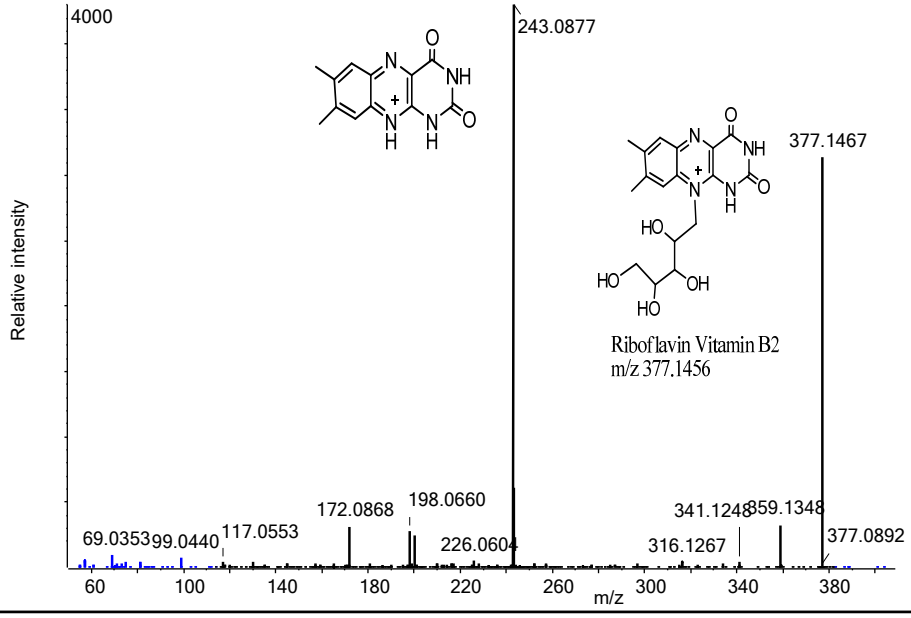
Detection of Vitamin B2 (riboflavin) as urinary metabolite



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Ion fragmentation for identification of phase II drug metabolites (glucuronide/sulfate conjugates)

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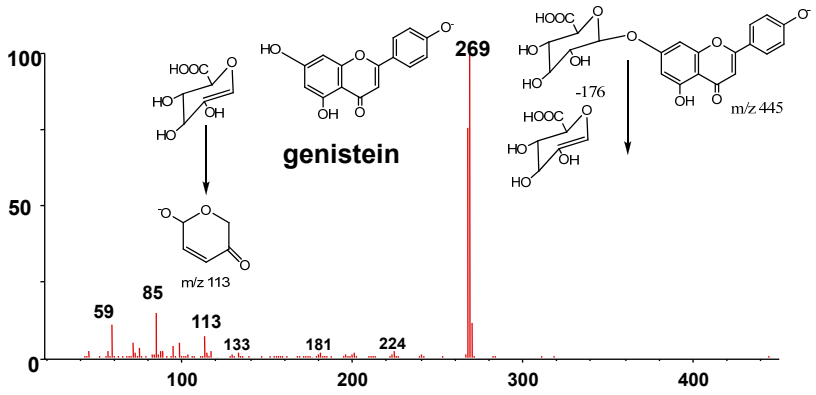
What fragment ions are characteristics for glucuronide conjugates?

Product ion spectrum of genistein glucuronide in ESI-MS/MS



Glucosides/glucuronides conjugates are easily cleaved off by higher potential at orifice

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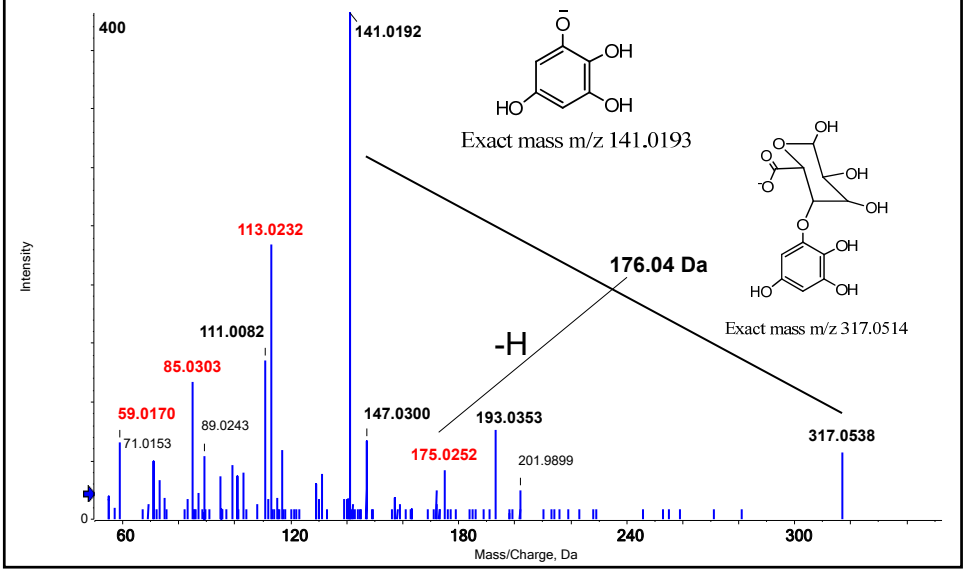
Putative identification- a glucuronide conjugate of tetrahydroxybenzene



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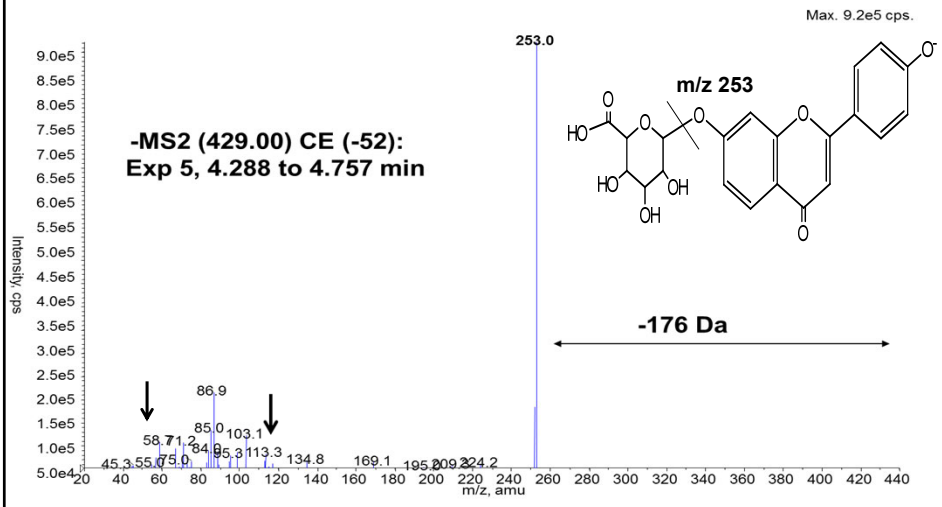
MSMS of m/z 429 indicate that it may be daidzein glucuronide



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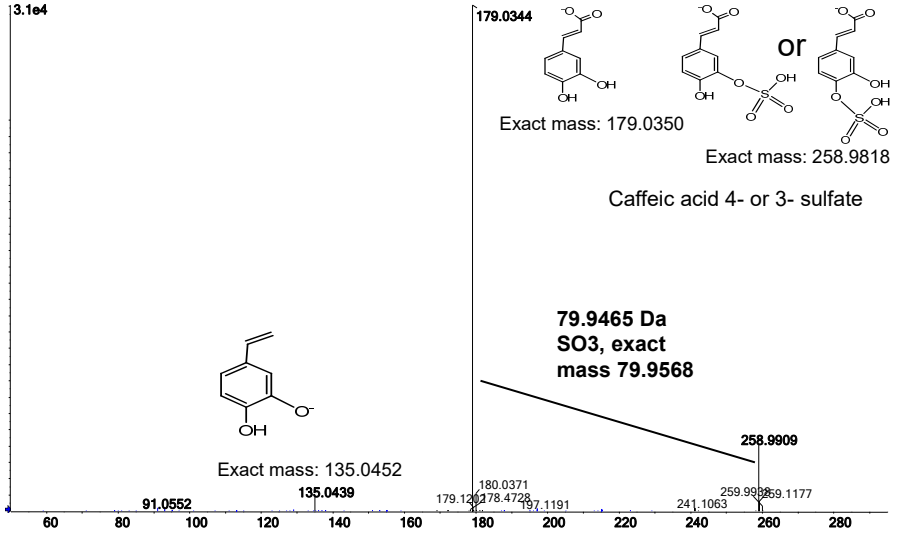
High resolution accurate MS/MS help identify sulfated conjugates in unknowns



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Change in mass is associated with possible metabolic reaction

Metabolic rxn	Change in mass
Methylation	14
Demethylation	-14
Hydroxylation	16
Acetylation	42
Epoxidation	16
Desulfuration	-32
Decarboxylation	-44
Hydration	18
Dehydration	-18

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Characteristic neutral loss and precursor Ion scans for conjugated metabolites

Conjugate	Ionization mode	Scan
Glucuronides	pos/neg	NL 176 amu
Hexose sugar	pos/neg	NL 162 amu
Pentose sugar	pos/neg	NL 132 amu
Phenolic sulphate	pos	NL 80 amu
Phosphate	neg	Precursor of m/z 79
Aryl-GSH	pos	NL 275 amu
Aliphatic-GSH	pos	NL 129
taurines	Pos	Precursor of m/z 126
N-acetylcysteins	neg	NL 129 amu

NL = neutral loss. Kostiainen et al., 2003

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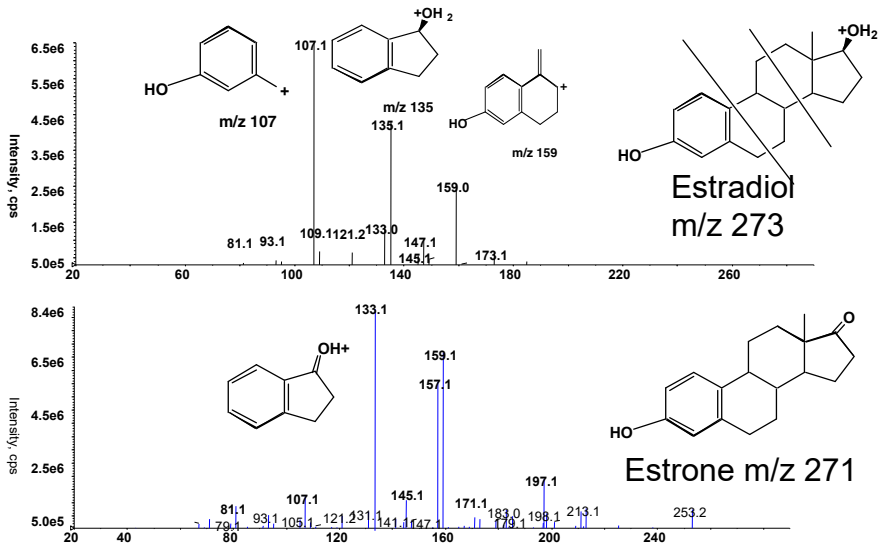
Analysis of steroids by MS/MS



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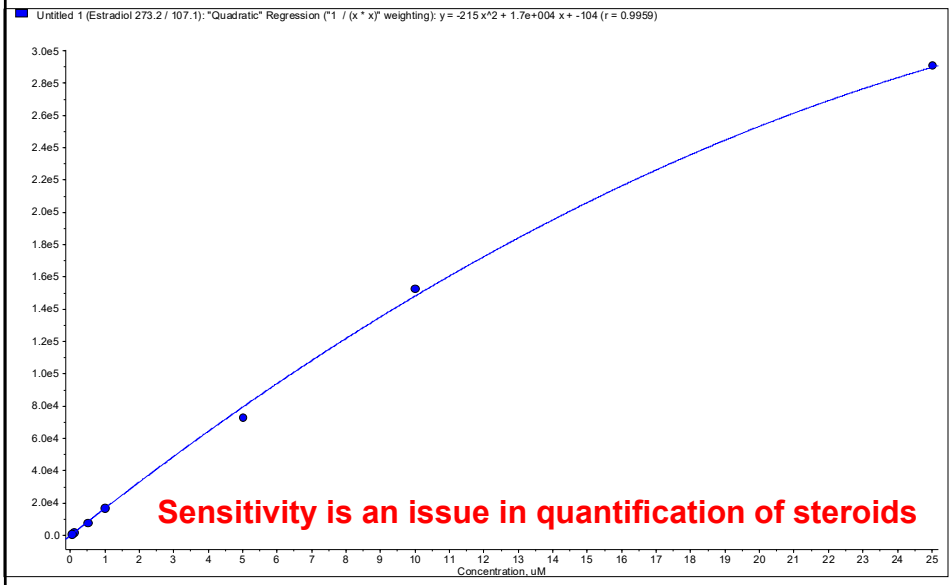
Estradiol Standard Curve 0.05 – 25 µM
r = 0.9959



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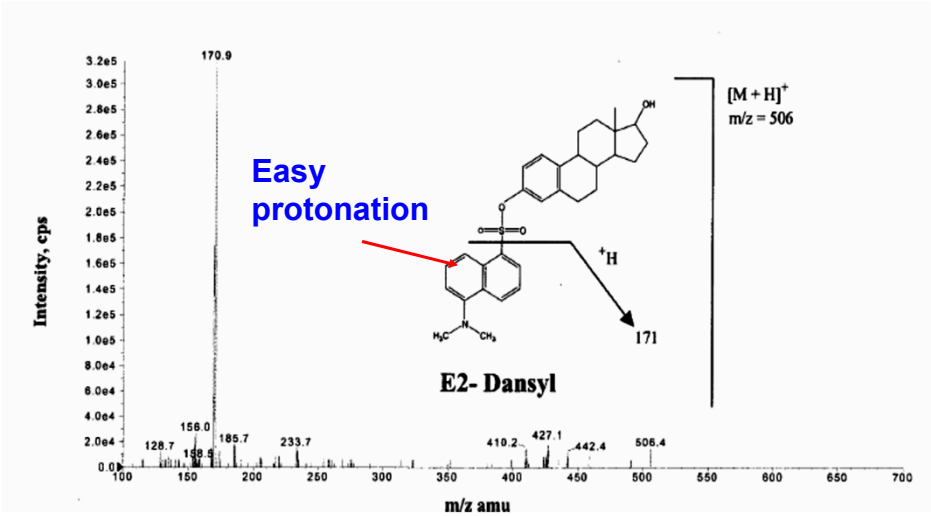
Derivatization of estradiol with dansyl chloride leads to the formation of E_2-dansyl (*m/z* 506)



Source: Nelson et al. Clinical Chemistry, 2004

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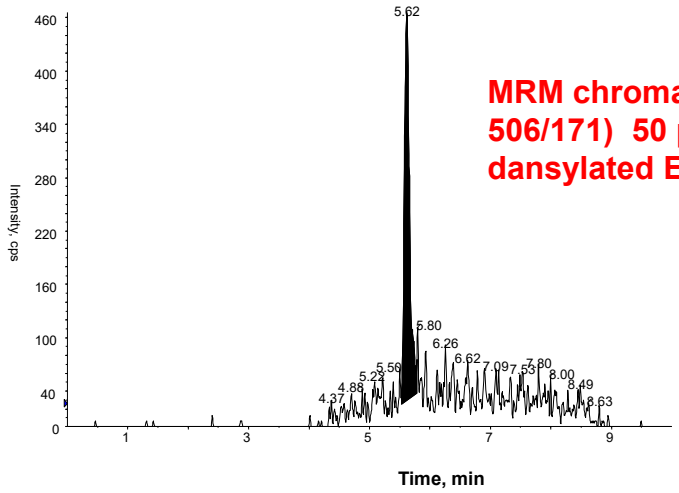
Derivatization tremendously helps increase sensitivity of E2



MRM chromatogram (m/z 506/171) 50 picomole dansylated E2

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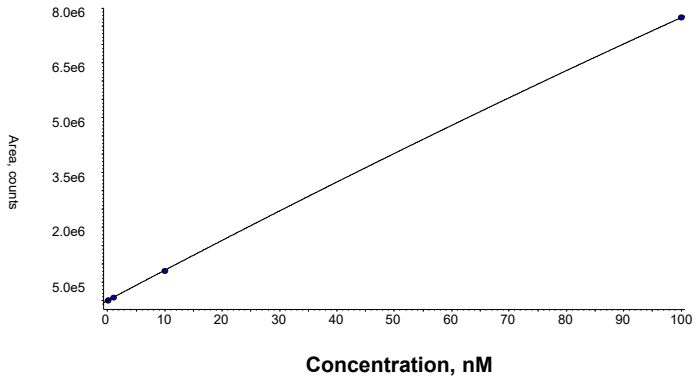
Calibration curve for dansylated E2 showing linearity from 0.005-100 nM concentration range (r = 0.999)



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Substructure analysis in ESI-MS/MS (dereplication and partial identification of natural products)

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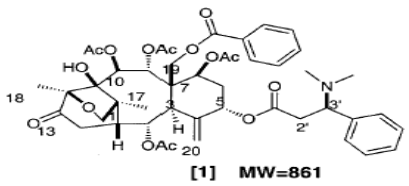
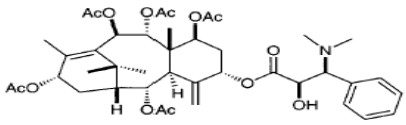
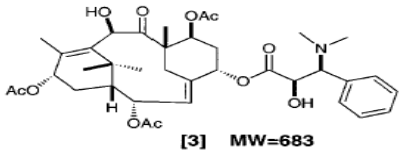
Fragmentation of basic taxoids from *T. Wallichiana* extract



[1] MW=861



[2] MW=769



[3] MW=683

Stefanowicz et al. Anal Chem, 2001

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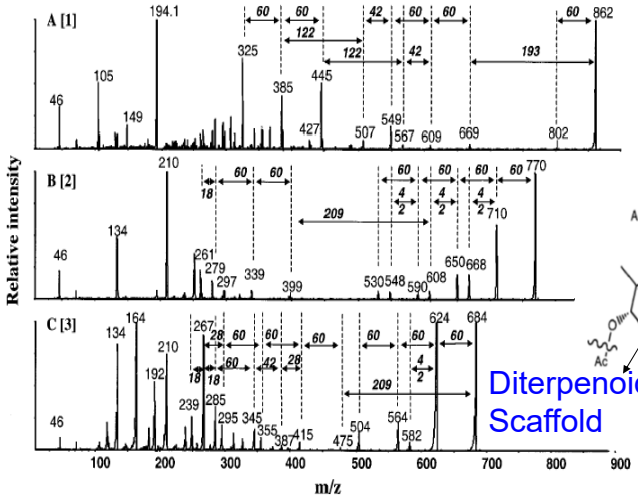
ESI-MS/MS spectra of taxoids (1-3). Peaks *m/z* 194 and 210 represent the intact alkaloid side chain.



Stefanowicz et al. Anal Chem, 2001

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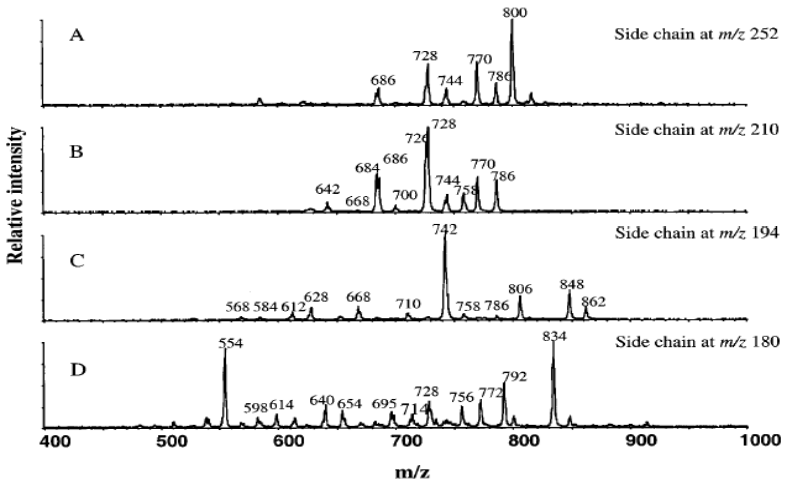
MS/MS precursor-scan spectra of typical alkaloid side chains to identify the basic taxoids compounds in an ethyl acetate extract of *T. wallichiana*.



Stefanowicz et al. Anal Chem, 2001

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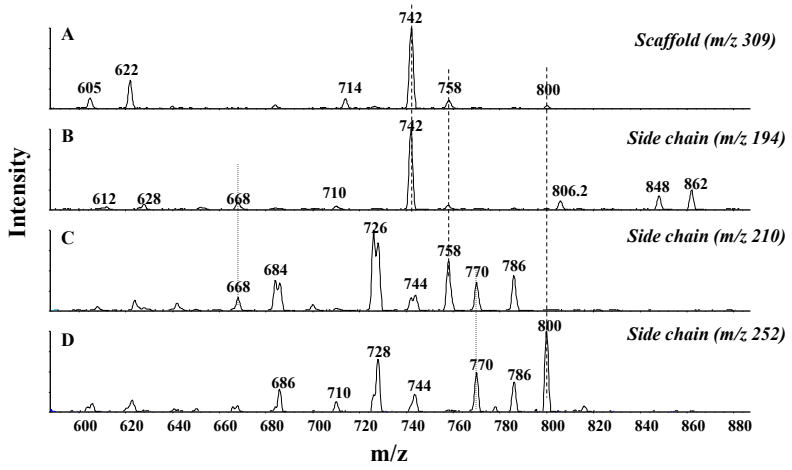
Comparison of precursor scan spectra obtained from the scaffold *m/z* 309 and side chain *m/z* 194, 210 and 252



Taxoids with scaffold *m/z* 309 and alkaloid side chains are shown by dashed lines

Stefanowicz et al. Anal Chem, 2001

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References

1. Electrospray Ionization Mass Spectrometry by Richard B. Cole.
2. Stefanowicz P, Prasain JK, Yeboah KF, Konishi Y. Detection and partial structure elucidation of basic taxoids from *Taxus wallichiana* by electrospray ionization tandem mass spectrometry. Anal Chem. 2001;73:3583-9.
3. Prasain J.K., Wang C.-C., Barnes S. Mass spectrometric analysis of flavonoids in biological samples. *Free Radical Biology & Medicine*, 37: 1324-1350, 2004.
4. William Griffiths. Tandem mass spectrometry in the study of fatty acids, bile acids and steroids. Mass Spectrometry Reviews, 2003;22:81-152.
5. Yi et al., Anal Bioanal Chem. 2006.

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