

MS/MS interpretation in identification of unknowns

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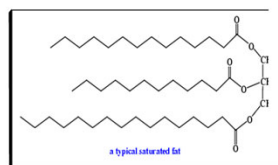
Outline

- **Introduction**
- **How to interpret LC-MS and MS/MS data.**
- **Identification of some conjugated metabolites.**
- **Conclusions**

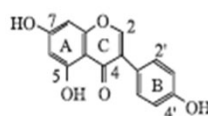
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Small molecules are important!!

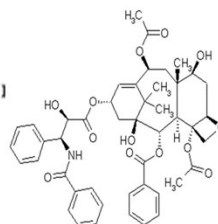
- 89% of all known drugs and 50% of all drugs are derived from pre-existing metabolites.
- Small molecules are cofactors and signalling molecules to 1000's of proteins.
- 100,000 (lipidome)



Triglycerides



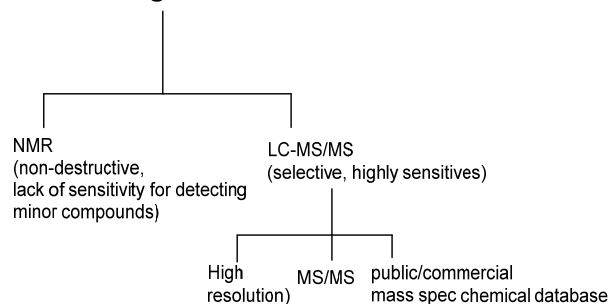
Genistein
(a plant secondary metabolite)



Taxol

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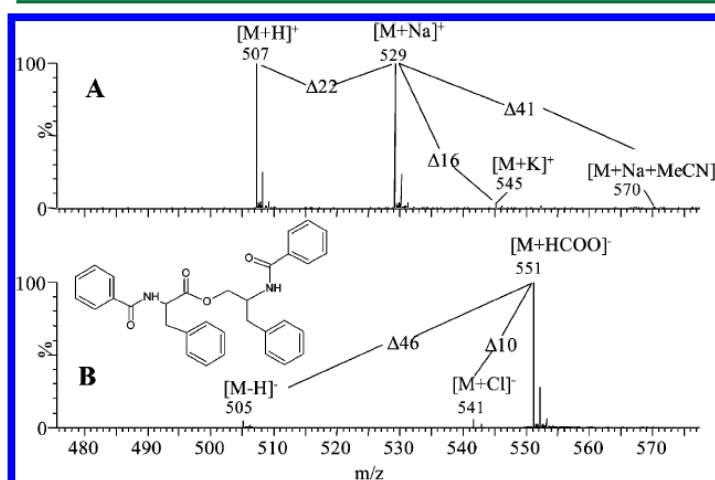
Keys to identifying unknown structures (putative/definitive) by mass spectrometry

- 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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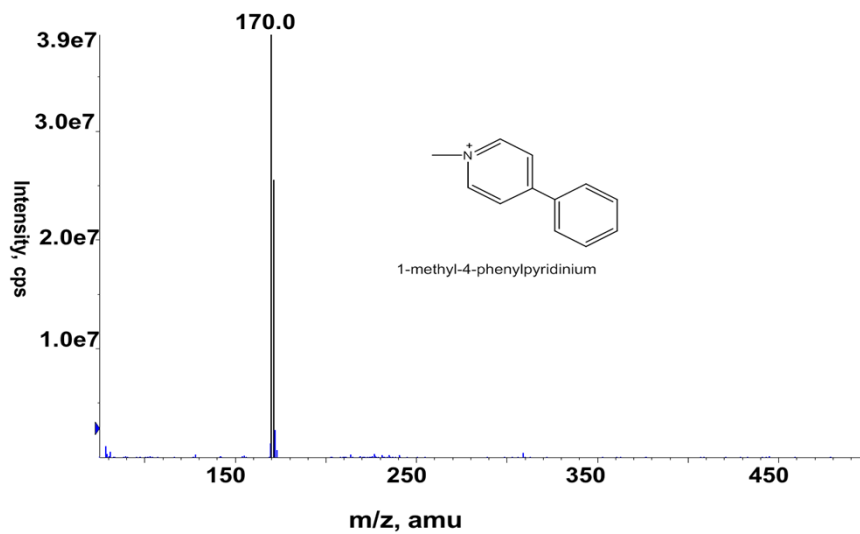
Adduct formation in +/-ve ion modes



Nielsen et al., J Nat Prod. 2011

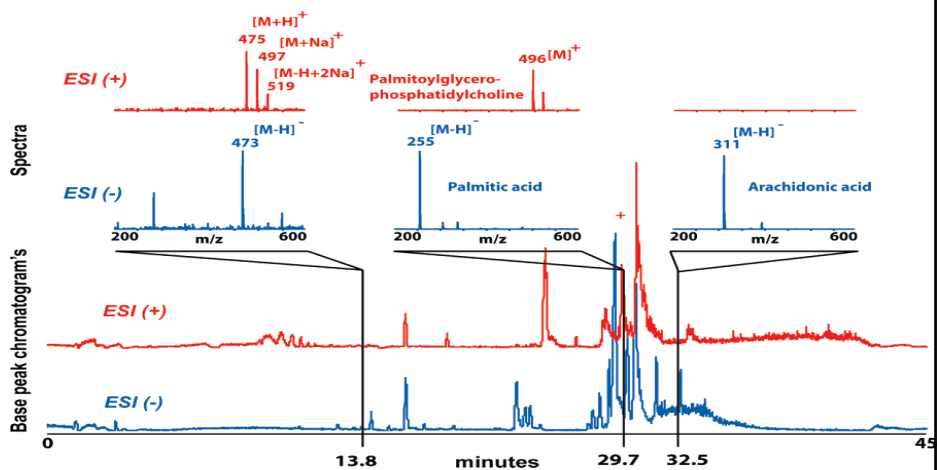
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Molecules with inherent positive charge- molecular weight and m/z are same



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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

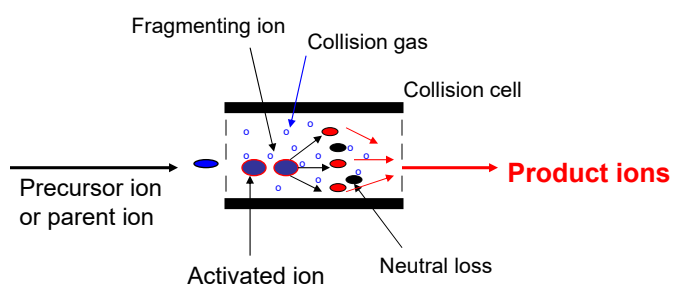
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Isotopic distribution and MS

- $1\text{H} = 99.9\%$, $2\text{H} = 0.015\%$
- $12\text{C} = 98.9\%$, $13\text{C} = 1.1\%$
- $35\text{Cl} = 67.4\%$, $37\text{Cl} = 32.6\%$
- **Monoisotopic mass** - the mass of the most abundant isotope
- **Average mass**- the abundance weighted mass of all isotopic components.

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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

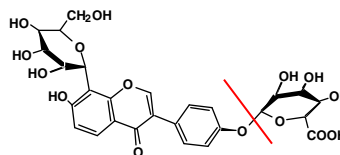
- **Pharmaceuticals**- Identification and quantification of drug metabolites, PK/PD
- **Academic/biotechnology**- analysis of protein/peptides, authentication and profiling of chemical components in a crude mixture, substructure analysis of unknown components
- **Clinical**- eg. neonatal screening, steroids 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.
- Literature study

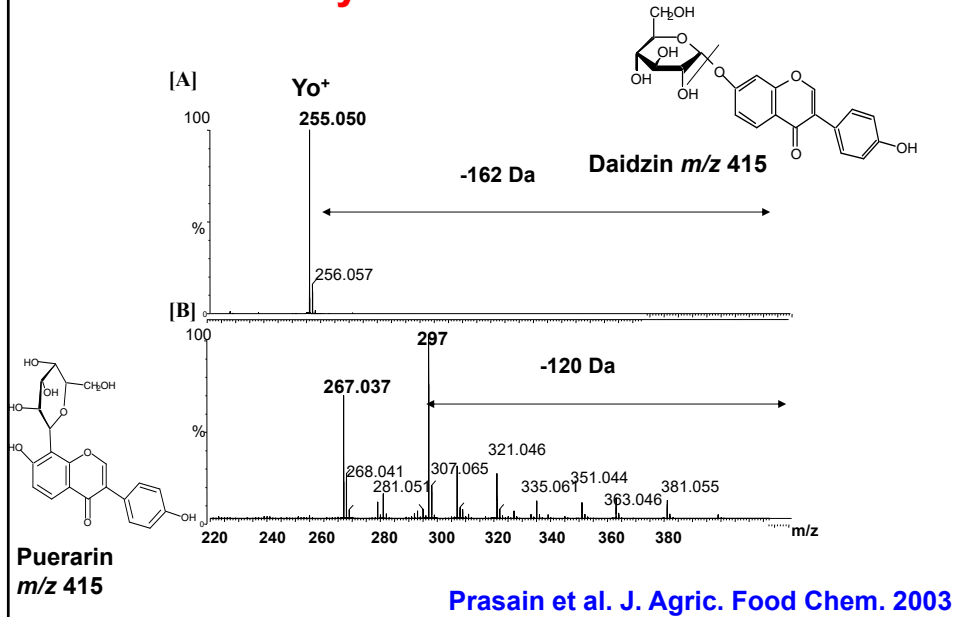
Where are the sites of deprotonation/protonation?
What is the most likely leaving group in this molecule?



Fragmentation always follows the basic rules of chemistry

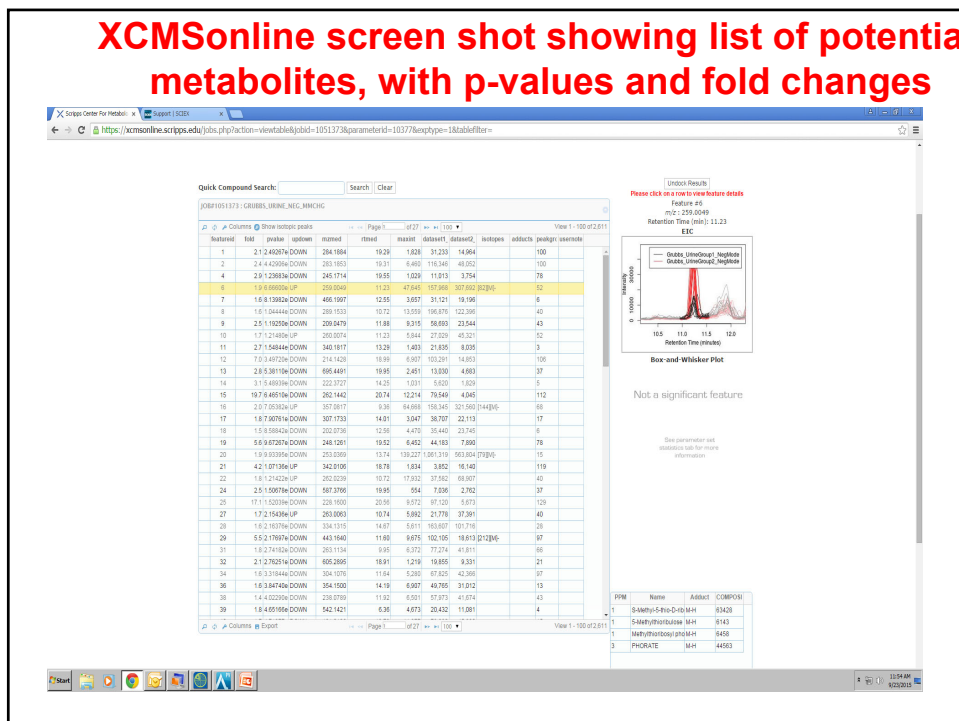
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O- and C-glucosides fragment differently in ESI-MS/MS



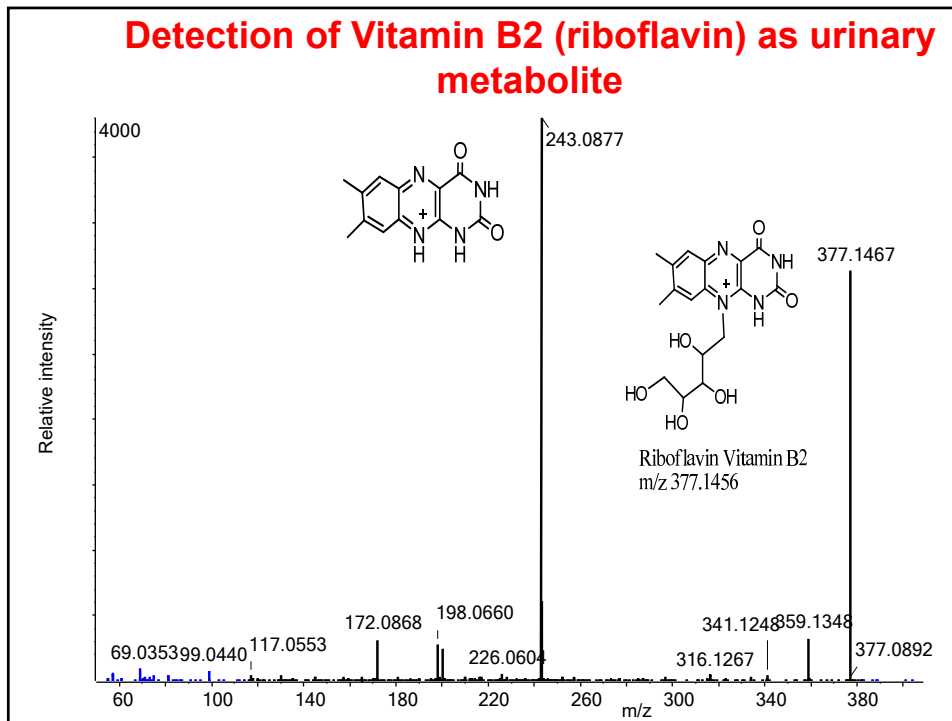
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XCMSOnline screen shot showing list of potential metabolites, with p-values and fold changes



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Detection of Vitamin B2 (riboflavin) as urinary metabolite



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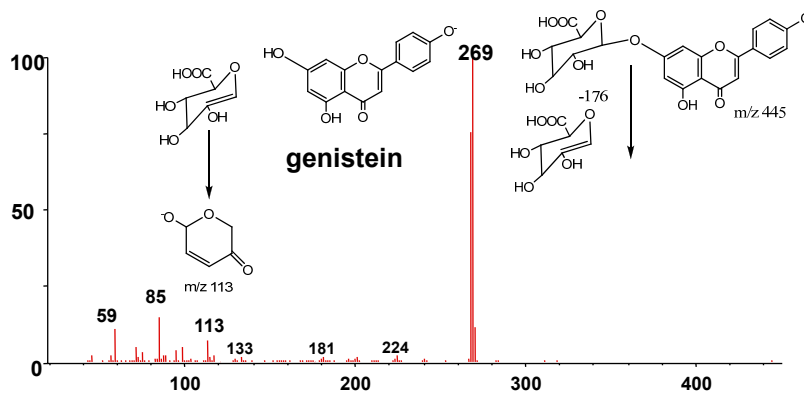
Many unknown metabolites, which are not in the Metlin database- What to do?

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

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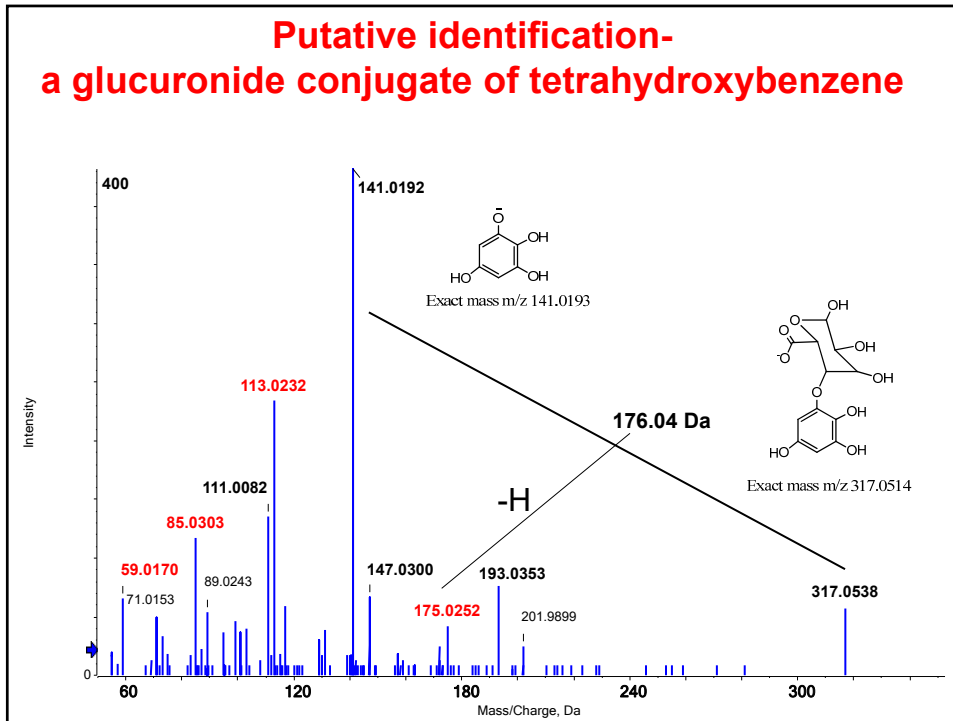
What fragment ions are characteristic for glucuronide conjugates? Product ion spectrum of genistein glucuronide in ESI-MS/MS



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

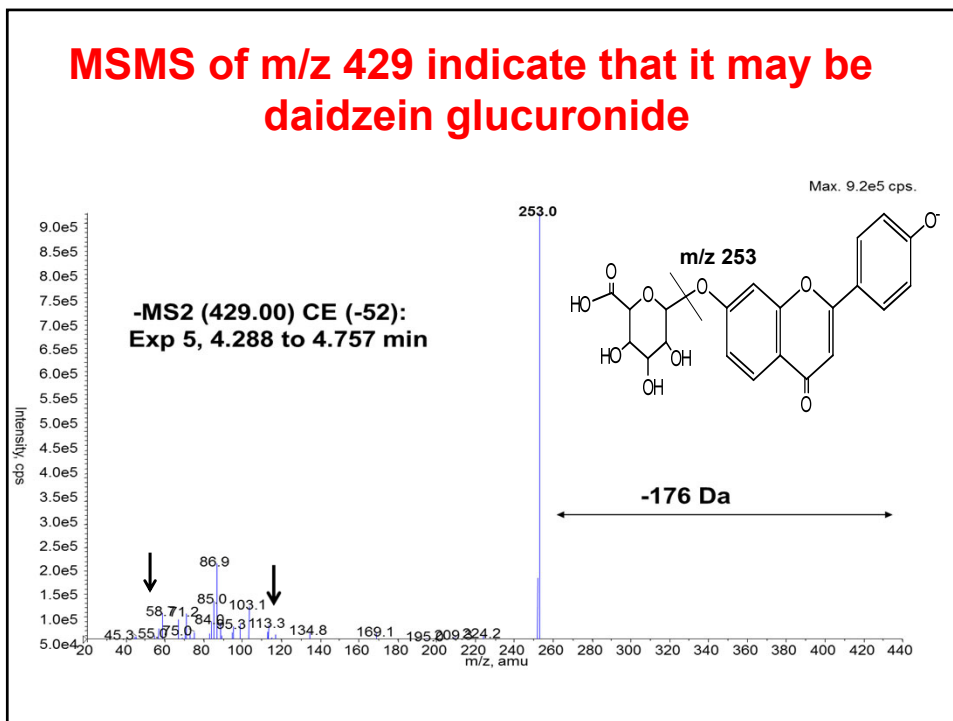
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Putative identification- a glucuronide conjugate of tetrahydroxybenzene



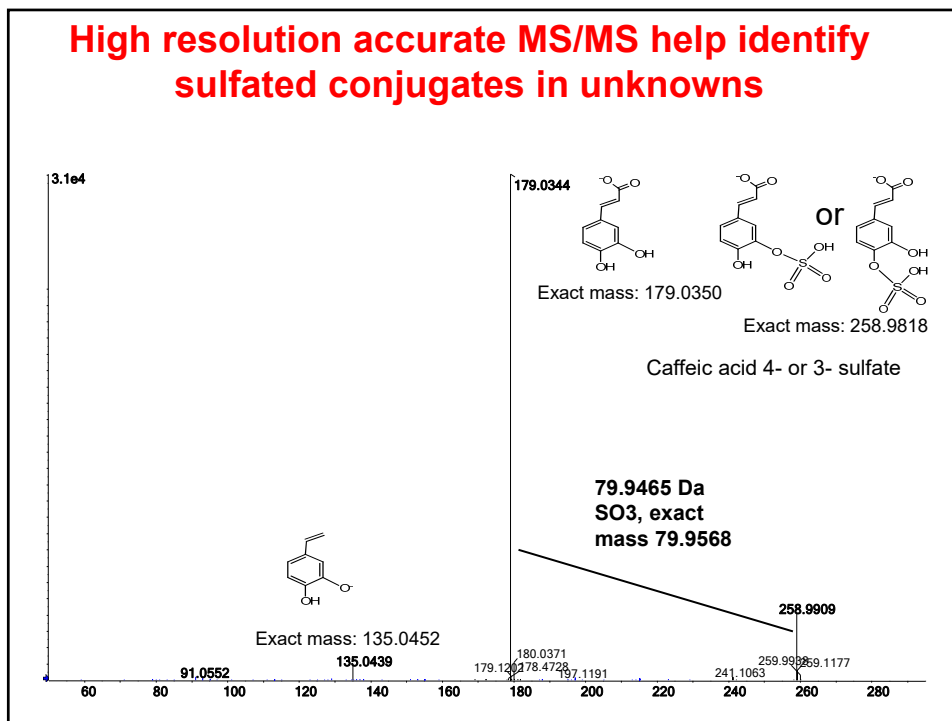
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MSMS of m/z 429 indicate that it may be daidzein glucuronide



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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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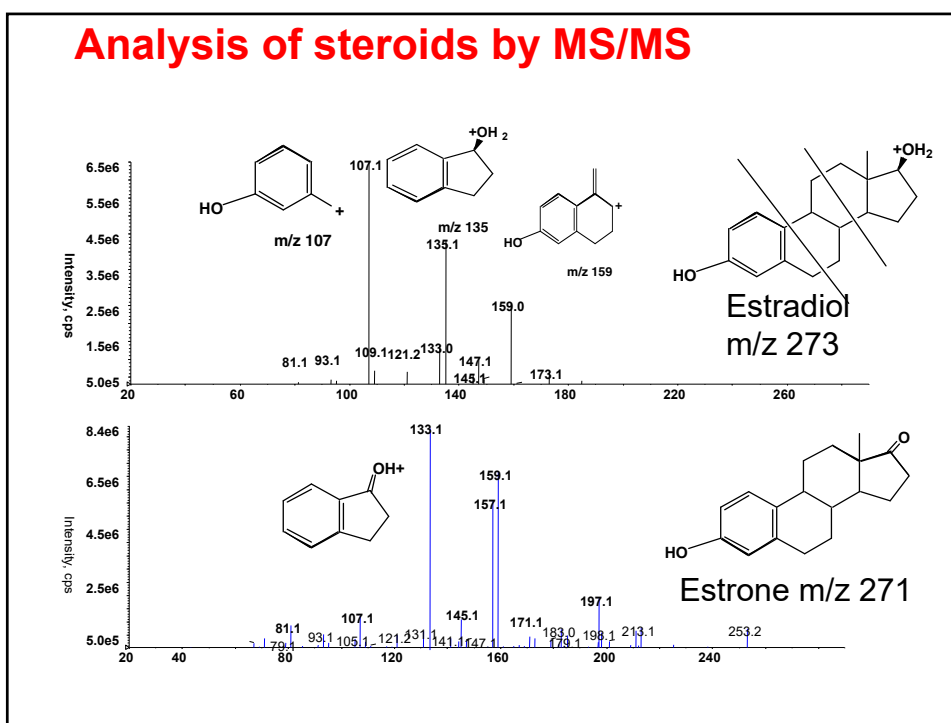
Characteristic fragmentation of drug conjugates by MS/MS

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

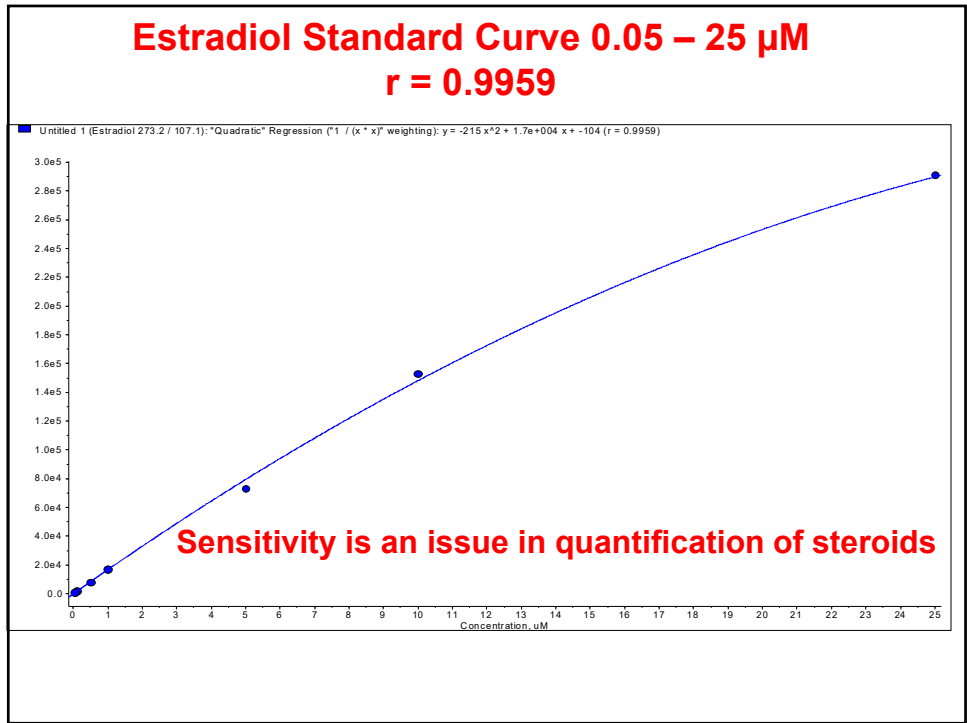
NL = neutral loss. **Kostiainen et al., 2003**

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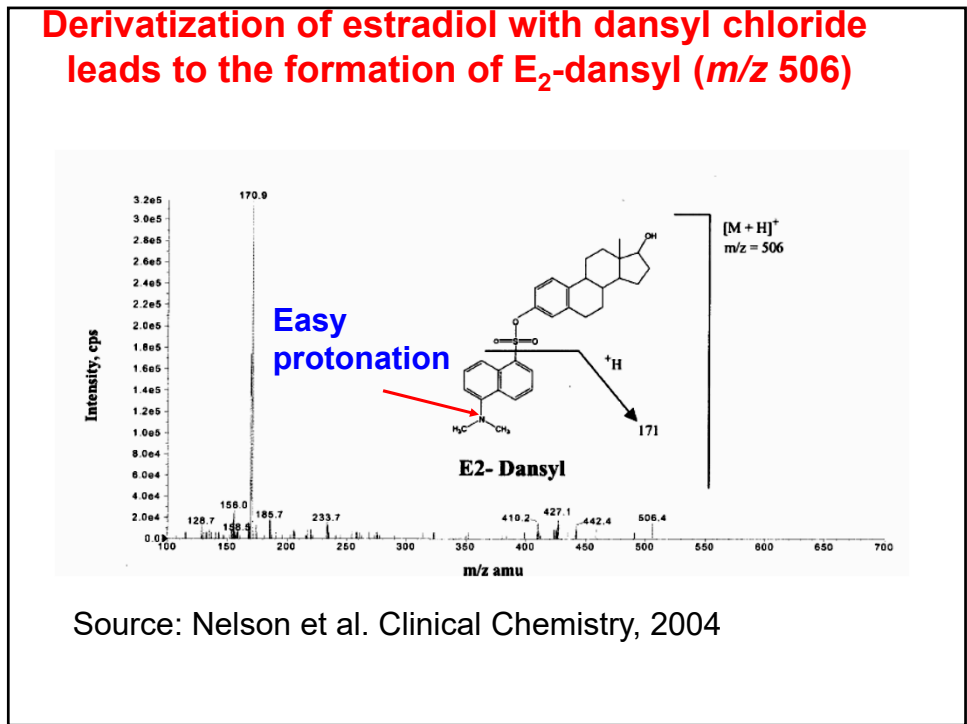
Analysis of steroids by MS/MS



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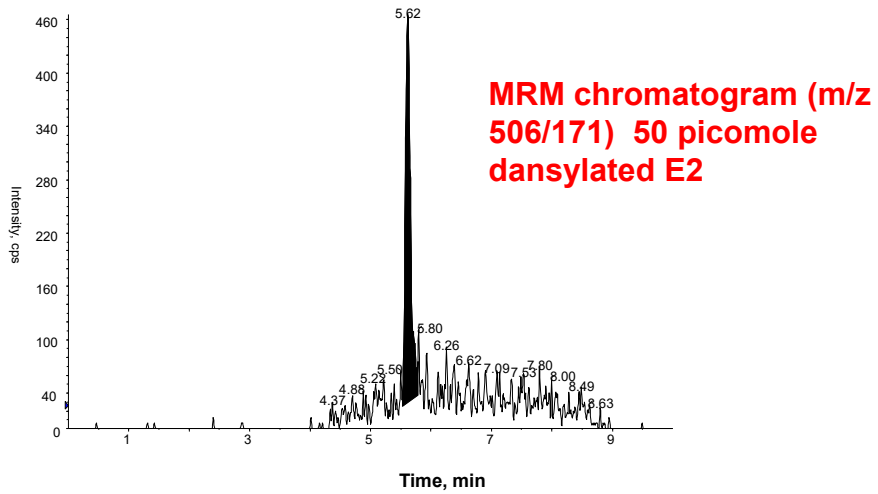


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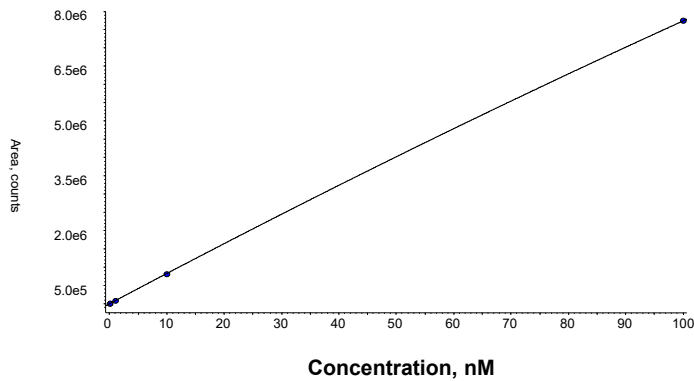
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Derivatization tremendously helps increase sensitivity of E2



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

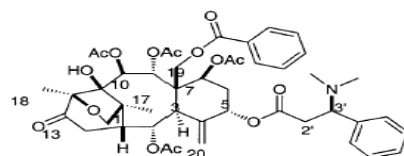


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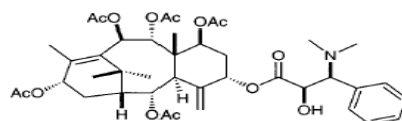
**Substructure analysis in ESI-MS/MS
(dereplication and partial identification
of natural products)**

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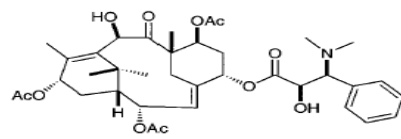
**Fragmentation of basic taxoids from *T.
Wallichiana* extract**



[1] MW=861



[2] MW=769

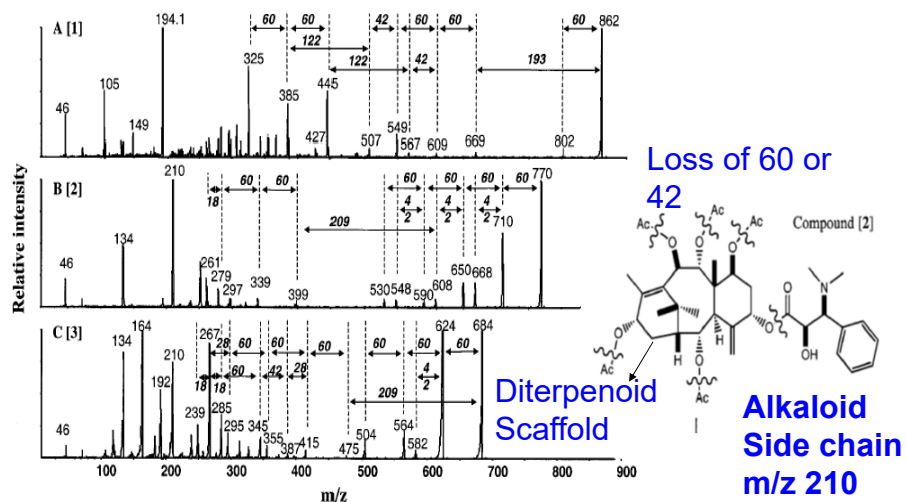


[3] MW=683

Stefanowicz et al. Anal Chem, 2001

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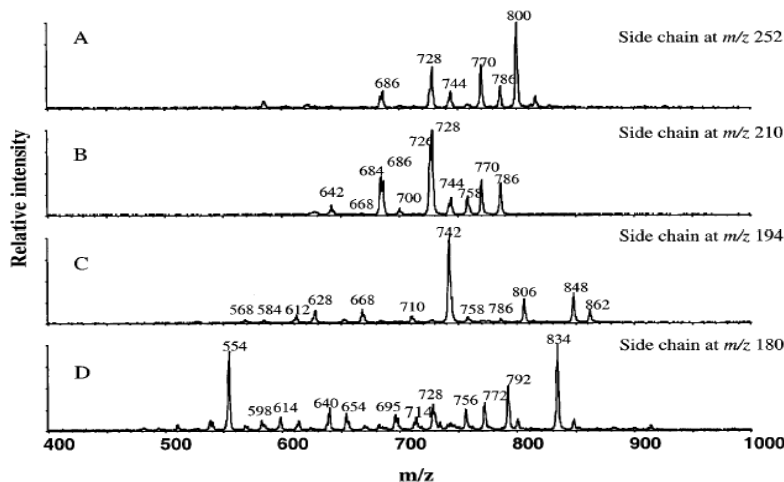
ESI-MS/MS spectra of taxoids (1-3). Peaks m/z 194 and 210 represent the intact alkaloid side chain.



Stefanowicz et al. Anal Chem, 2001

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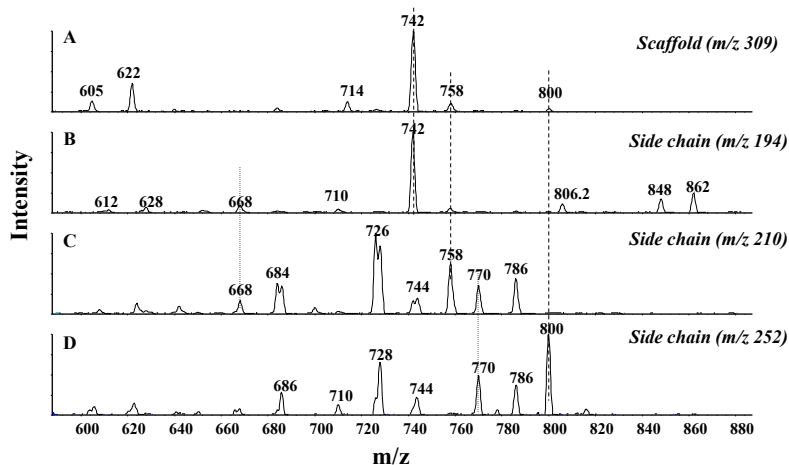
MS/MS precursor-scan spectra of typical alkaloid side chains to identify the basic taxoids compounds in an ethyl acetate extract of *T. wallichiana*.



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. Electro spray 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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