Ion fragmentation of small molecules in mass spectrometry

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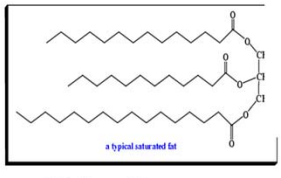
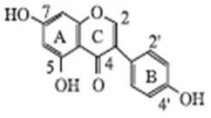
Small molecules are important!!

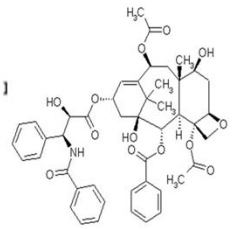
- 89% of all known drugs and 50% of all drugs are derived from pre-existing metabolites.
- Small molecules are cofactors and signalling molecules to 1000's of proteins.
- 100,000 (lipidome)



Triglycerides



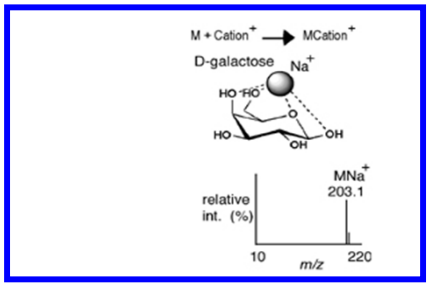
Genistein
(a plant secondary metabolite)



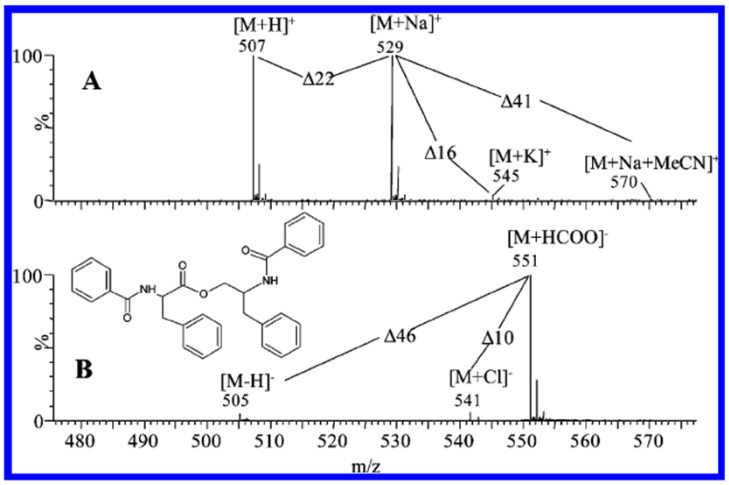
Taxol

Nomenclature: the main names and acronyms used in mass spectrometry

- **Molecular ion:** **Ion formed by addition or the removal of one or several electrons to or from the sample molecules- Electron Impact (EI-MS). $M + e^- \rightarrow M^{+\bullet} + 2e^-$**
- **Adduct Ion:** **Ion formed through interaction of two species and containing all the atoms of one of them plus one or several atoms of them (e.g. alkali, ammonium).**

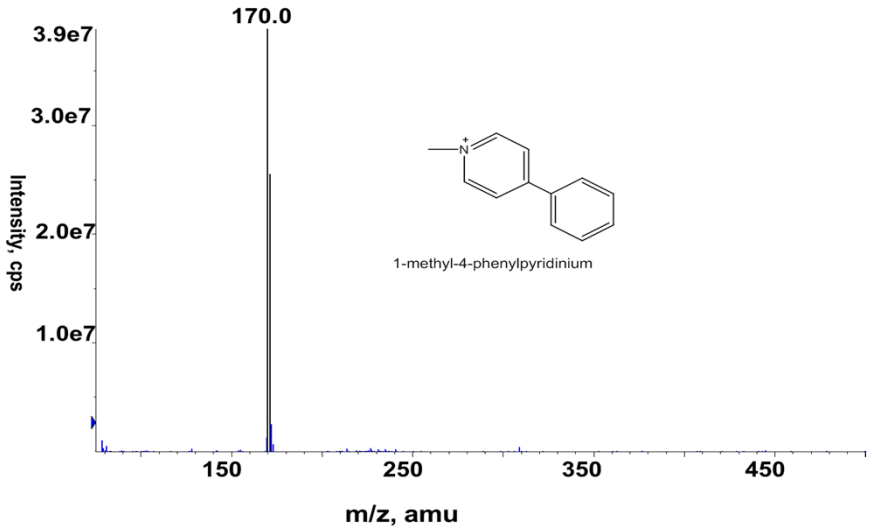


Adduct formation in +/–ve ion modes

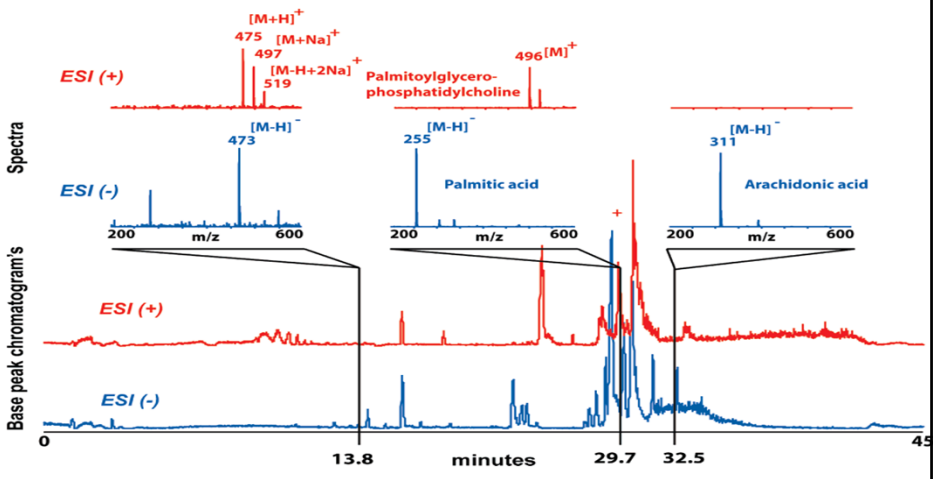


Nielsen et al., J Nat Prod. 2011

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



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

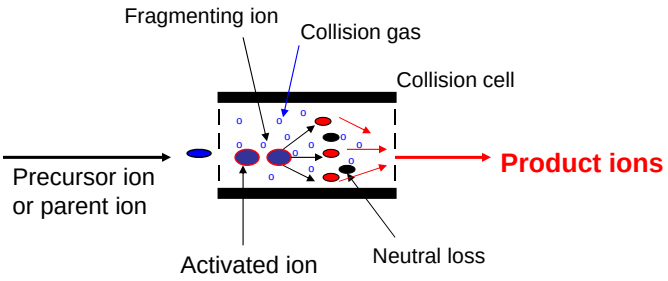
Contd..

- **Pseudomolecular ion:** Ion originating from the analyte molecule by abstraction of a proton [M-H]- or addition of proton [M+H]+
- **Tandem mass spectrometry (Cooks, 1976): MS/MS (McLafferty, 1978), tandem in space or time**
- **Precursor ion/parent ion:** Ions undergoing fragmentation.
- **Product ion/daughter ion:** Ions resulting from parent/precursor ions.
- **Neutral loss:** Fragments lost as neutral molecules
- **In positive ionization mode**, a trace of formic acid is often added to aid protonation of the sample molecules; in **negative ionization mode** a trace of ammonia solution or a volatile amine is added to aid deprotonation of the sample molecules. Proteins and peptides are usually analysed under positive ionization conditions and polyphenols and acids under negative ionization conditions. In all cases, the *m/z* scale must be calibrated.

Isotopic distribution and MS

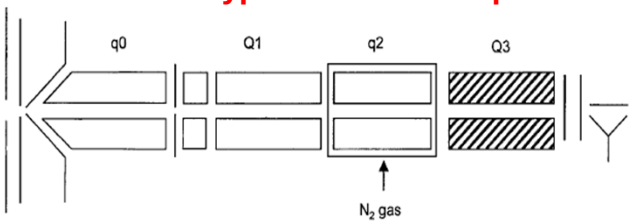
- 1H = 99.9%, 2H = 0.02%
- 12C = 98.9%, 13C = 1.1%
- 35Cl = 68.1%, 37Cl = 31.9%
- Monoisotopic mass - the mass of the most abundant isotope
- Average mass- the abundance weighted mas of all isotopic components.

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



Schematic of CID fragmentation

Various types of MS/MS experiments



Mode of operation	Q1	q2	Q3
Q1 Scan	Resolving (Scan)	RF-only	RF-only
Q3 Scan	RF-only	RF-only	Resolving (Scan)
Product Ion Scan (PI)	Resolving (Fixed)	Fragment	Resolving (Scan)
Precursor Ion Scan (PC)	Resolving (Scan)	Fragment	Resolving (Fixed)
Neutral Loss Scan (NL)	Resolving (Scan)	Fragment	Resolving (Scan Offset)
Selected Reaction Monitoring mode (SRM)	Resolving (Fixed)	Fragment	Resolving (Fixed)

Enhanced Q3 Single MS (EMS)	RF-only	No frag	Trap/scan
Enhanced Product Ion (EPI)	Resolving (Fixed)	Fragment	Trap/scan
MS^3	Resolving (Fixed)	Fragment	Isolation/frag trap/scan
Time delayed fragmentation (TDF)	Resolving (Fixed)	Trap/No frag	Frag/trap/scan
Enhanced Resolution Q3 Single MS (ER)	RF-only	No frag	Trap/scan
Enhanced Multiply Charged (EMC)	RF-only	No frag	Trap/scan

Figure 1. Schematic of QqLIT (Q TRAP, AB/MDS, Sciex) and description of the various triple quadrupole and trap operation modes.

Hopfgartner etal. J. Mass Spectrom, 2004

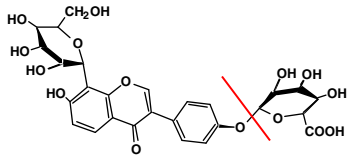
Applications of MS/MS

- **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…**

Interpreting MS/MS spectra

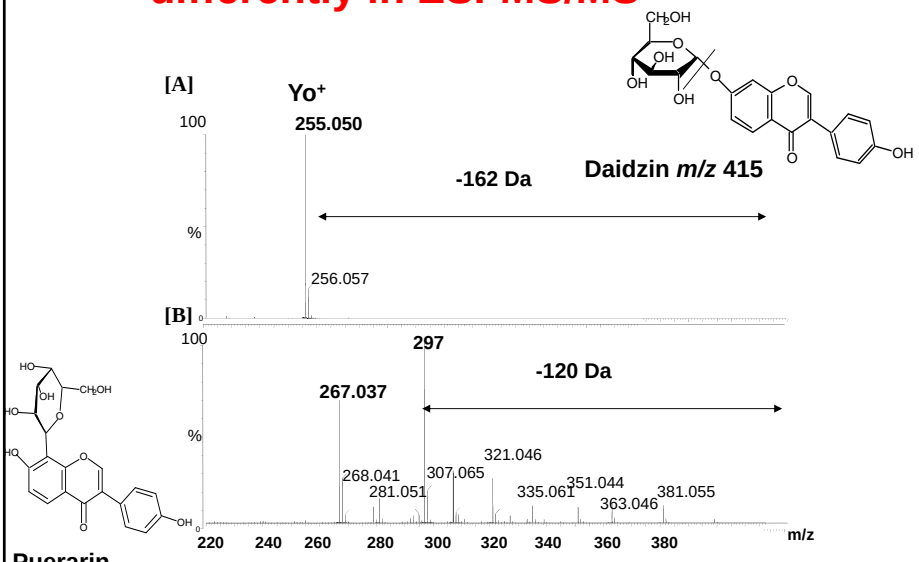
- **Likely sites of protonation or deprotonation.**
- **Likely leaving group.**
- **Literature study**

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

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

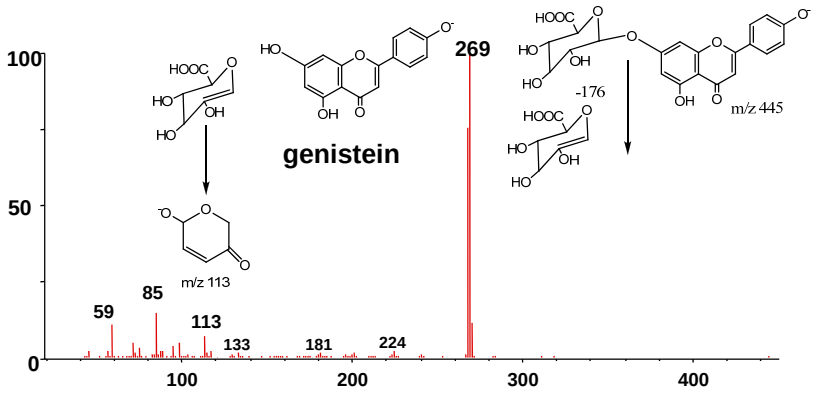


Prasain et al. J. Agric. Food Chem. 2003

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

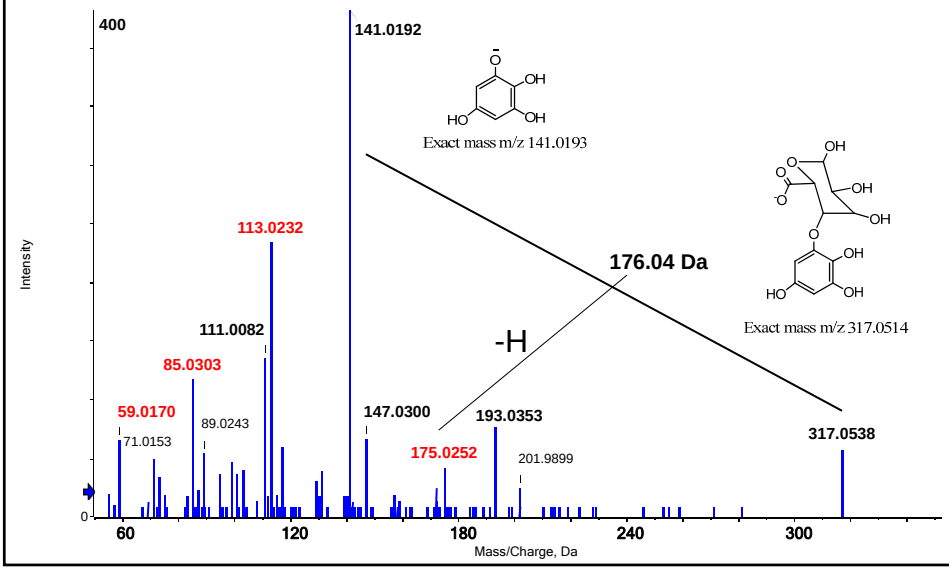
What fragment ions are characteristics for glucuronide conjugates?

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

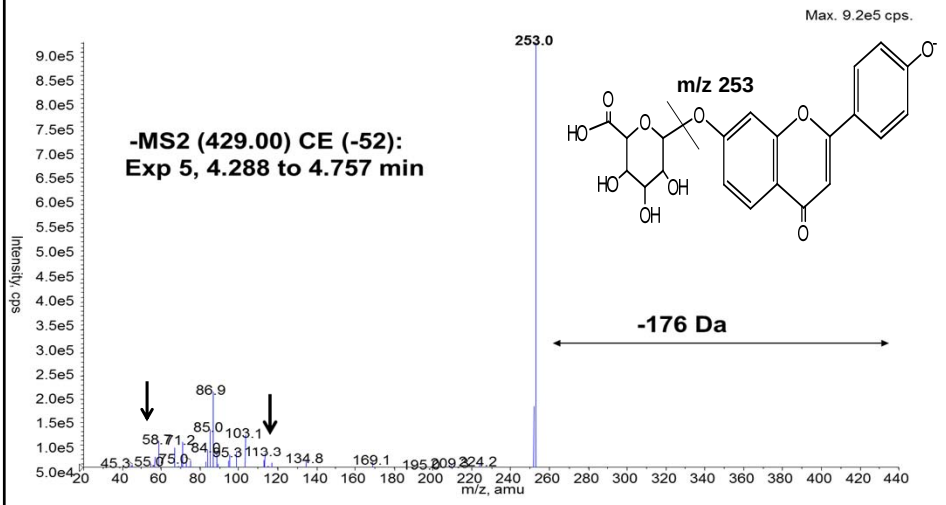


Glucosides/glucuronides conjugates are easily cleaved off by higher potential at orifice

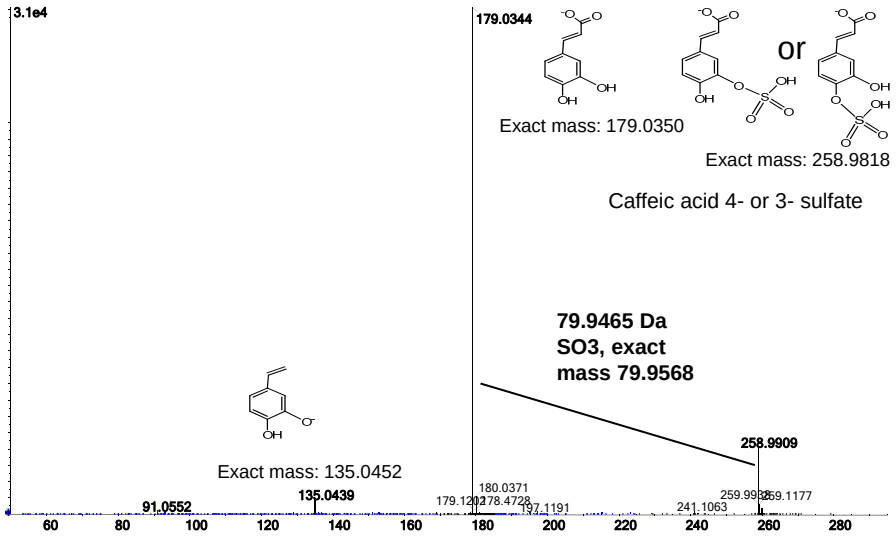
Putative identification- a glucuronide conjugate of tetrahydroxybenzene



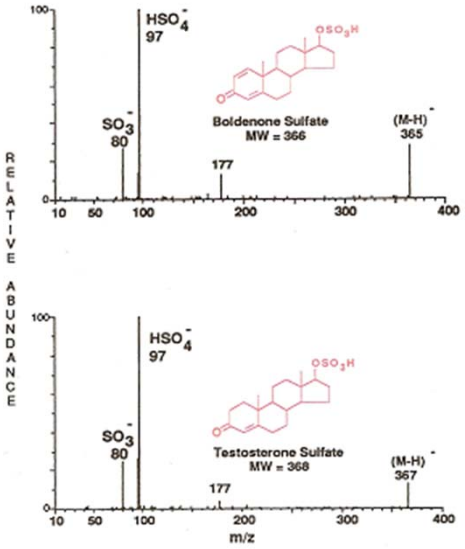
MSMS of m/z 429 indicate that it may be daidzein glucuronide



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

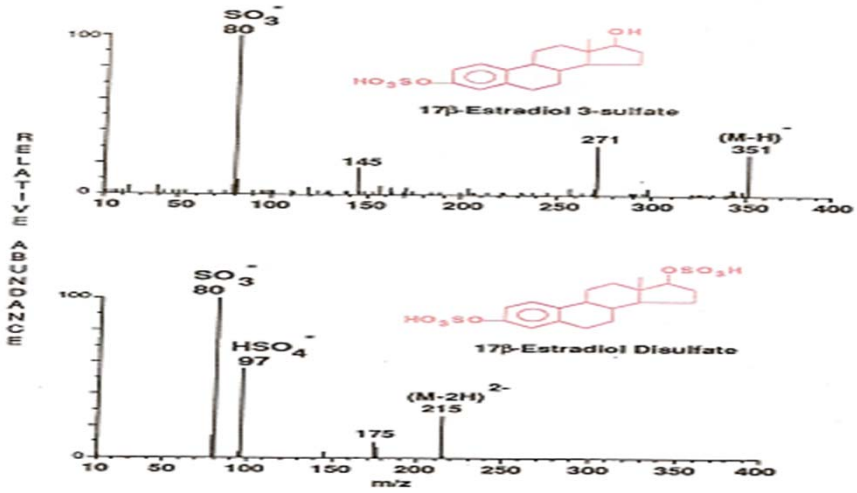


What happens with aliphatic sulfates in MS/MS?



Aliphatic and aromatic sulfate conjugates behave differently in MS/MS, aliphatic typically show *m/z* 97 (HSO4-) and *m/z* 80 (SO3-.)

The absence of the *m/z* 97 fragment with the base peak *m/z* 80 makes the distinction between aromatic and aliphatic sulfates



Source: Weidolf et al. Biomed. and Environ. Mass Spec. 1988

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

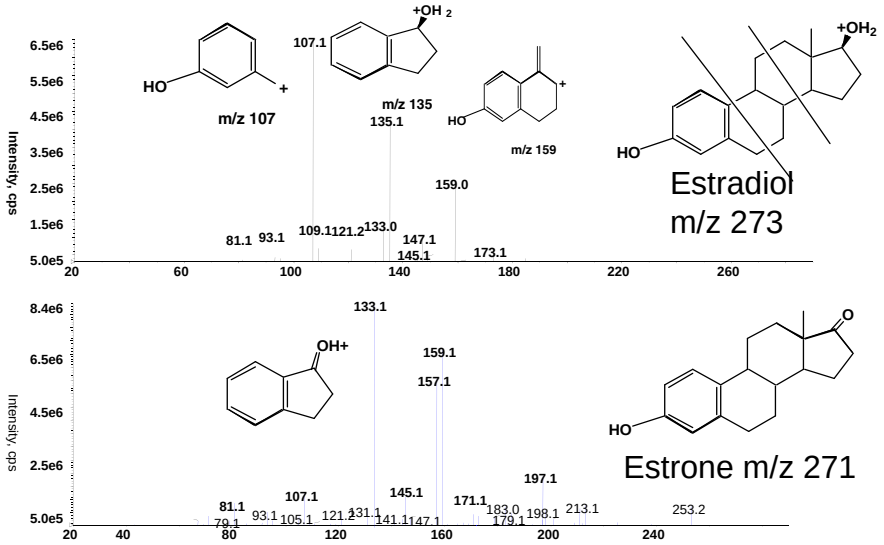
Characteristic fragmentation of drug conjugates by MS/MS

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

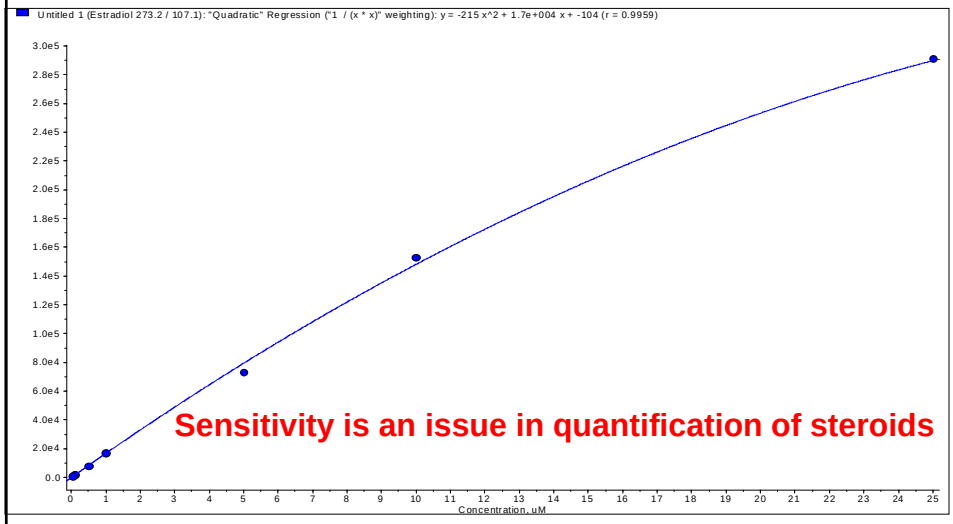
NL = neutral loss.

Kostiainen et al., 2003

Analysis of steroids by MS/MS

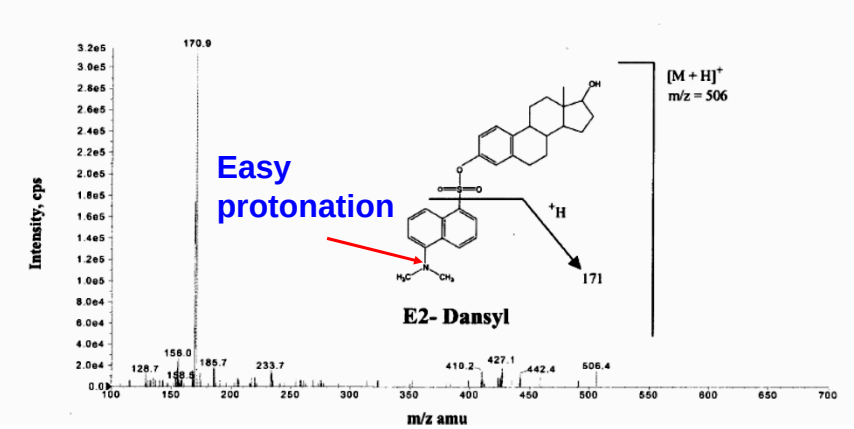


Estradiol Standard Curve 0.05 – 25 µM
r = 0.9959



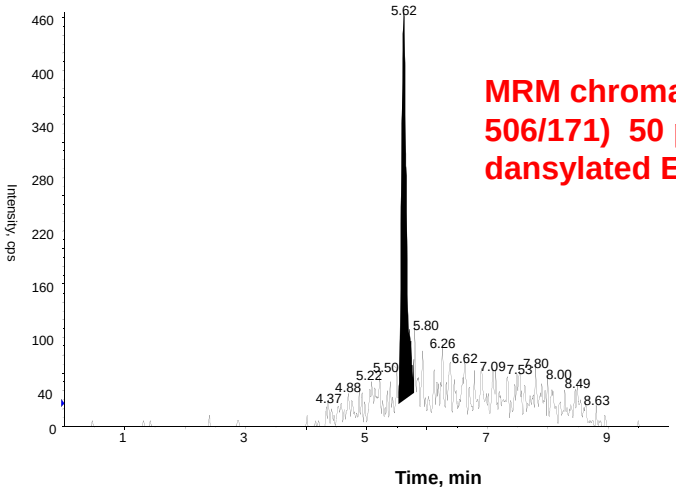
Sensitivity is an issue in quantification of steroids

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

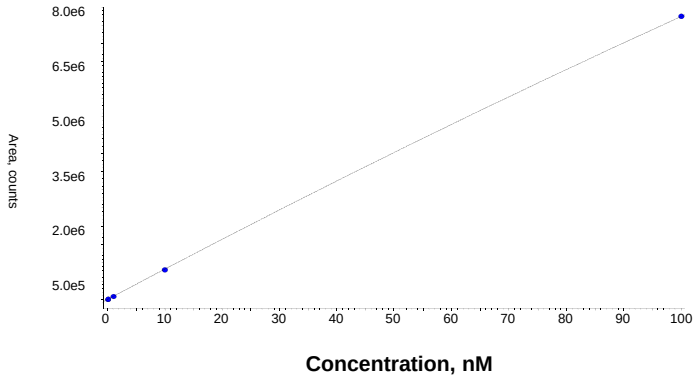


Source: Nelson et al. Clinical Chemistry, 2004

Derivatization tremendously helps increase sensitivity of E2



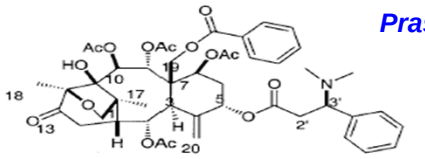
Calibration curve for dansylated E2 showing linearity from 0.005-100 nM concentration range (r = 0.999)



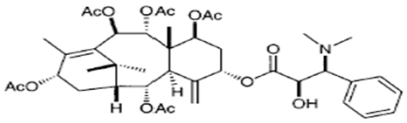
Substructure analysis in ESI-MS/MS (dereplication and partial identification of natural products)

Fragmentation of basic taxoids from *T. Wallichiana* extract

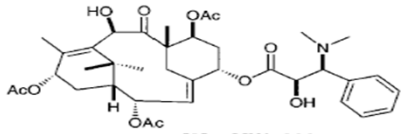
Prasain et al. Anal Chem, 2001



[1] MW=861

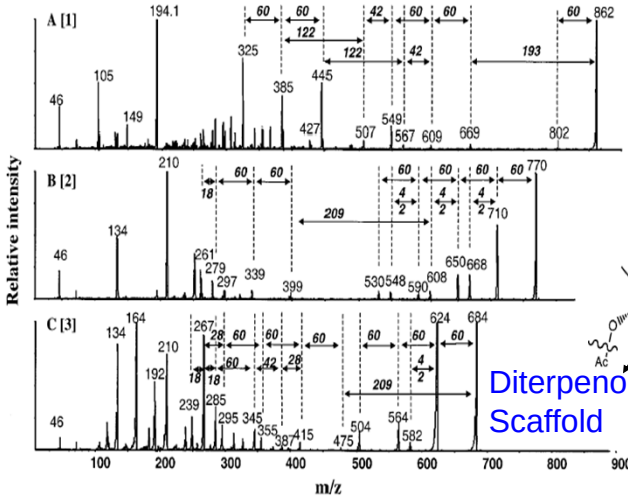


[2] MW=769

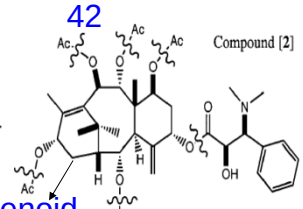


[3] MW=683

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



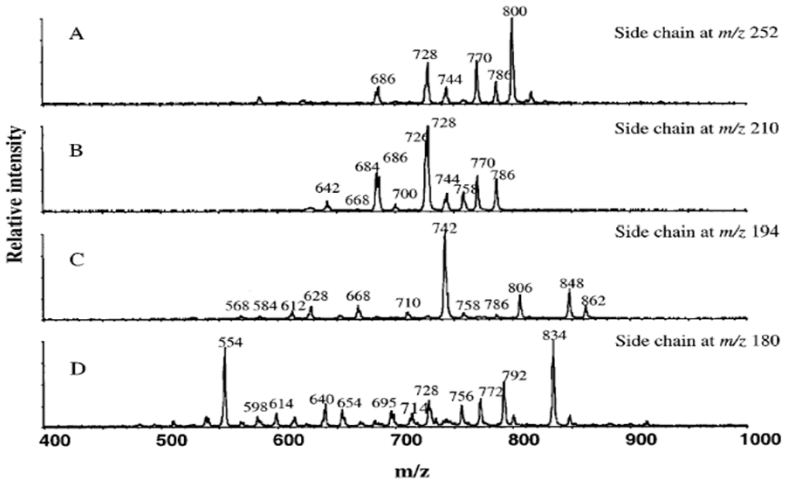
Loss of 60 or 42



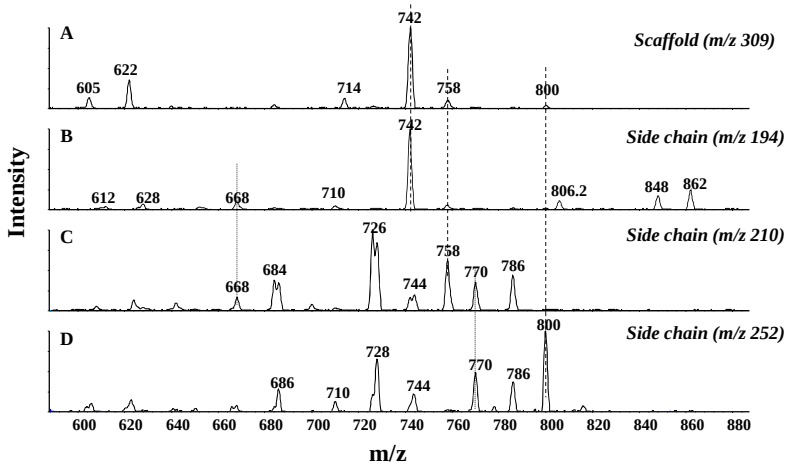
Diterpenoid Scaffold

Alkaloid Side chain m/z 210

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



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

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.**