# Ion fragmentation of small molecules in mass spectrometry

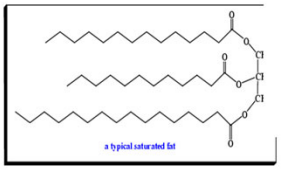
**Jeevan Prasain**

jprasain@uab.edu

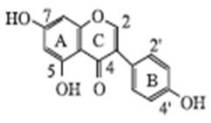
**6-2612**

# Small molecules are important!!

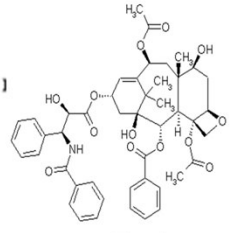
- 89% of all known drugs and 50% of all drugs are derived from pre-existing metabolites.
- Small molecules are cofactors and signalling molecules to 1000's of proteins.
- 100,000 (lipidome)




Triglycerides



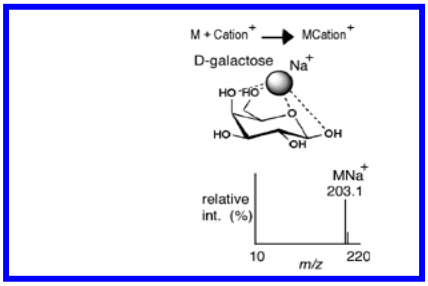

Genistein
(a plant secondary metabolite)



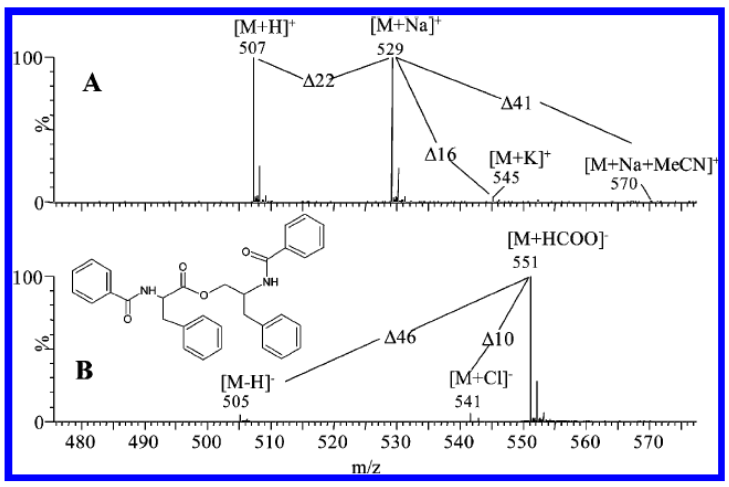

Taxol

# 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).

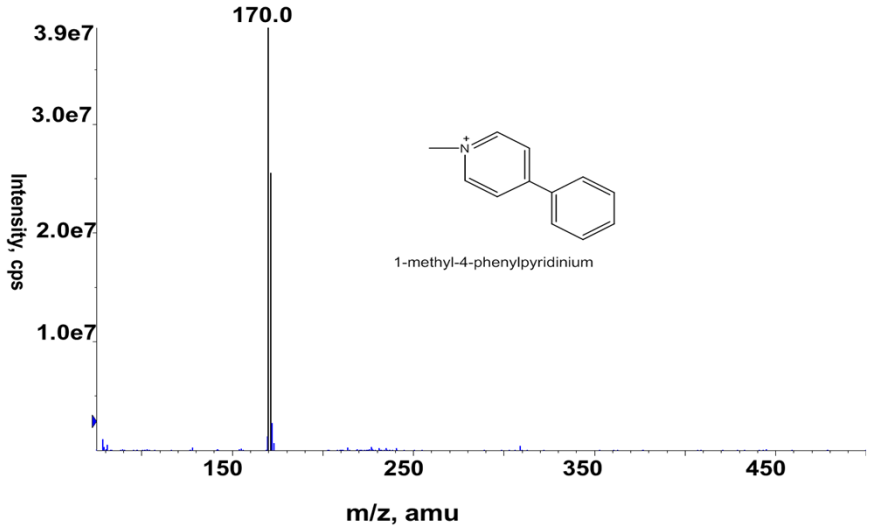



# Adduct formation in +/–ve ion modes

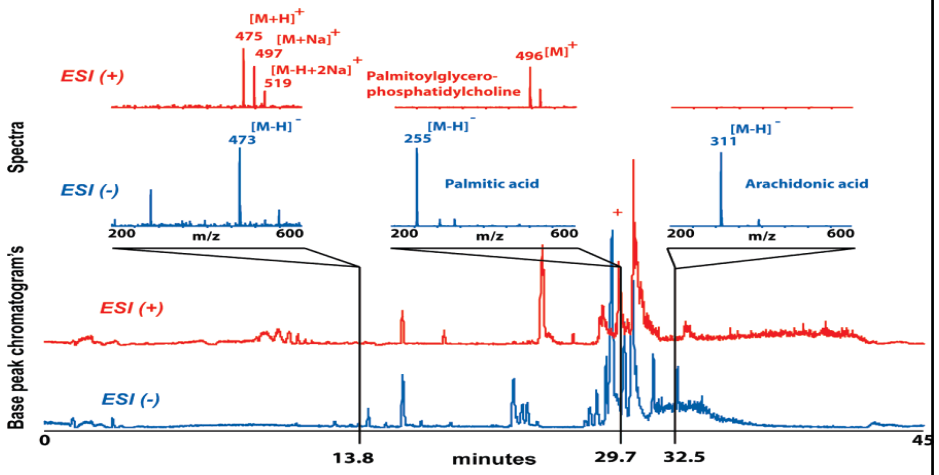



Nielsen et al., J Nat Prod. 2011

## 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

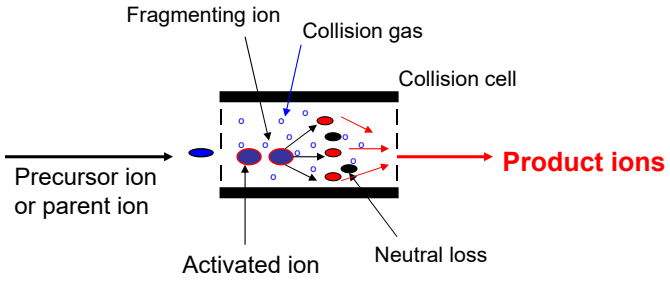
## Contd..

- **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

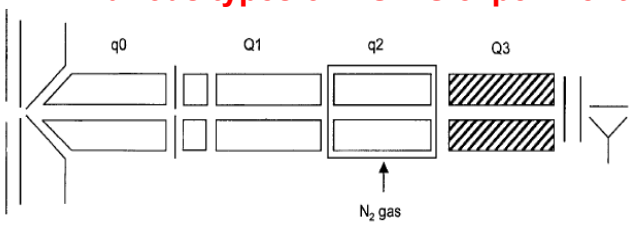
- 1H = 99.9%, 2H = 0.02%
- 12C = 98.9%, 13C = 1.1%
- 35Cl = 68.1%, 37Cl = 31.9%
- Monoisotopic mass - the mass of the most abundant isotope
- Average mass- the abundance weighted mas of all isotopic components.

## 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

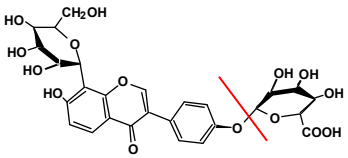
## Applications of MS/MS

- **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

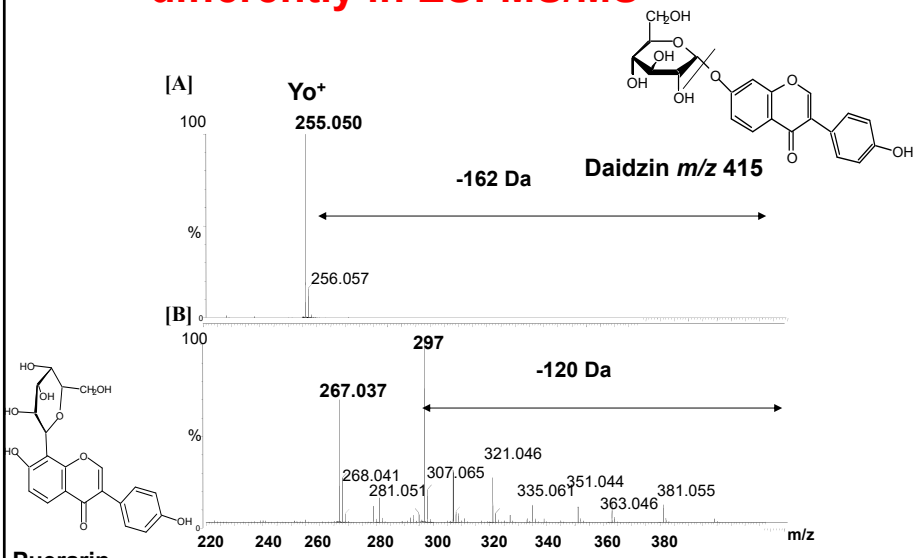
- **Likely sites of protonation or deprotonation.**
- **Likely leaving group.**
- **Literature study**

**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

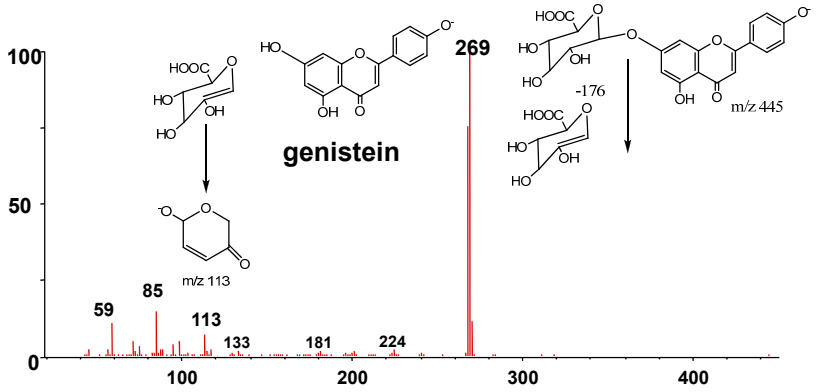



Prasain et al. J. Agric. Food Chem. 2003

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

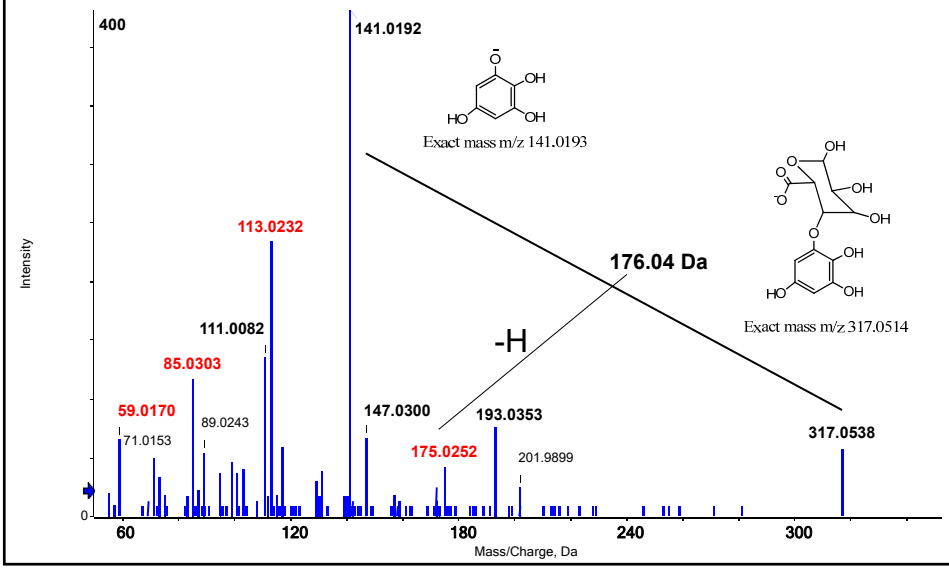
# What fragment ions are characteristics for glucuronide conjugates?

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

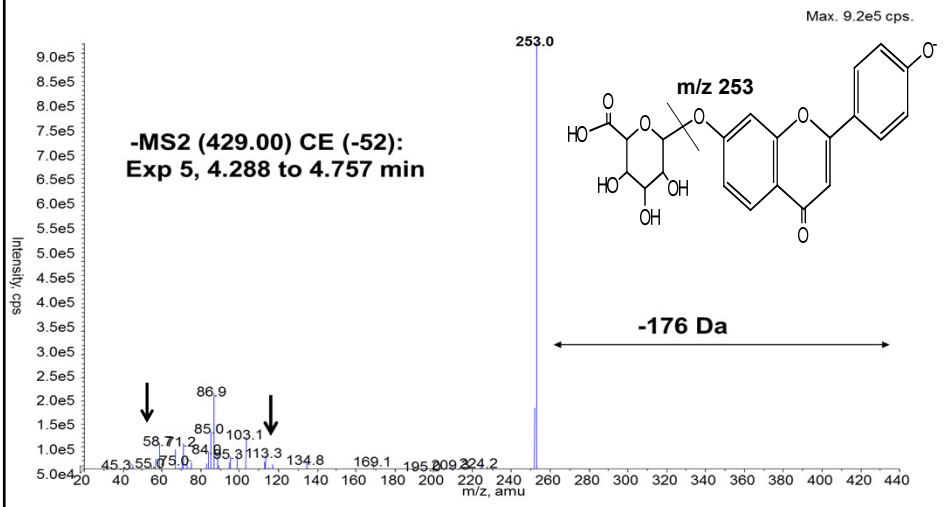



**Glucosides/glucuronides conjugates are easily cleaved off by higher potential at orifice**

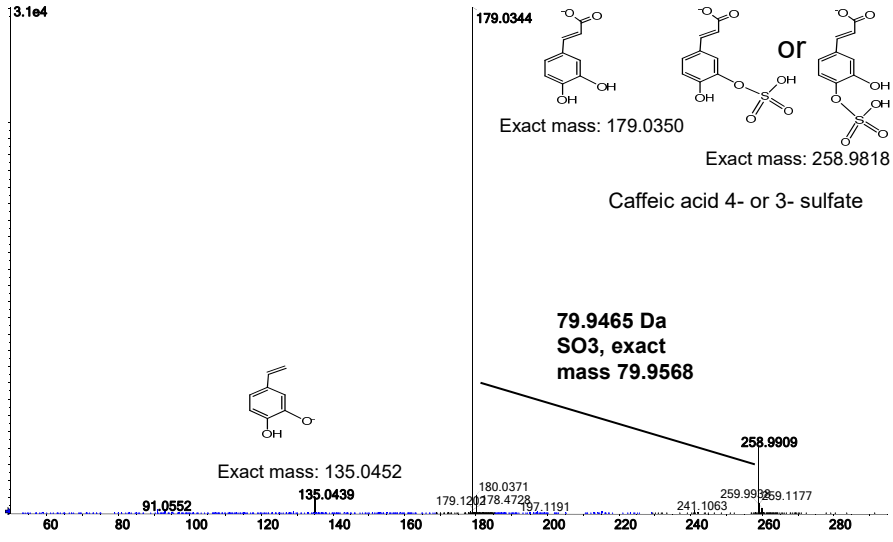
# Putative identification- a glucuronide conjugate of tetrahydroxybenzene



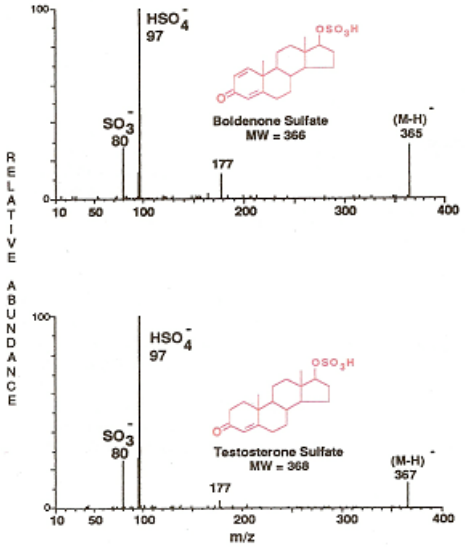
# MSMS of m/z 429 indicate that it may be daidzein glucuronide




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

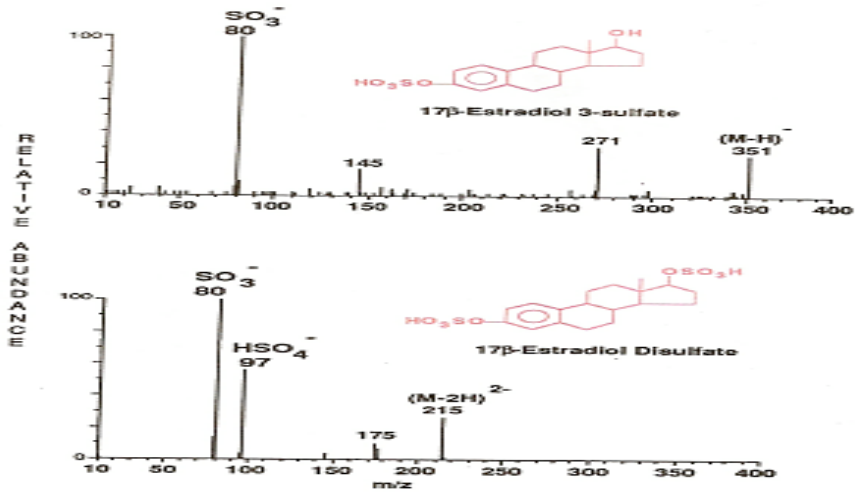


## What happens with aliphatic sulfates in MS/MS?




**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 |

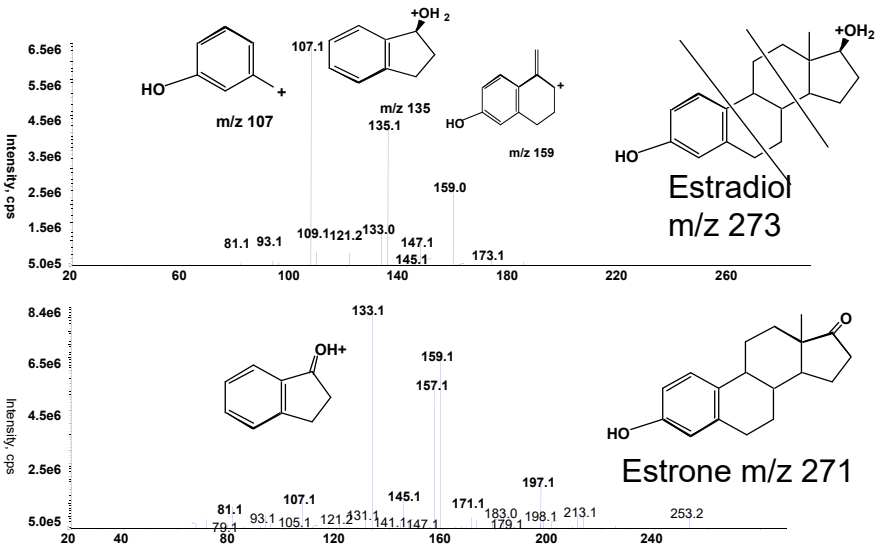
## Characteristic fragmentation of drug conjugates by MS/MS

| 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 |

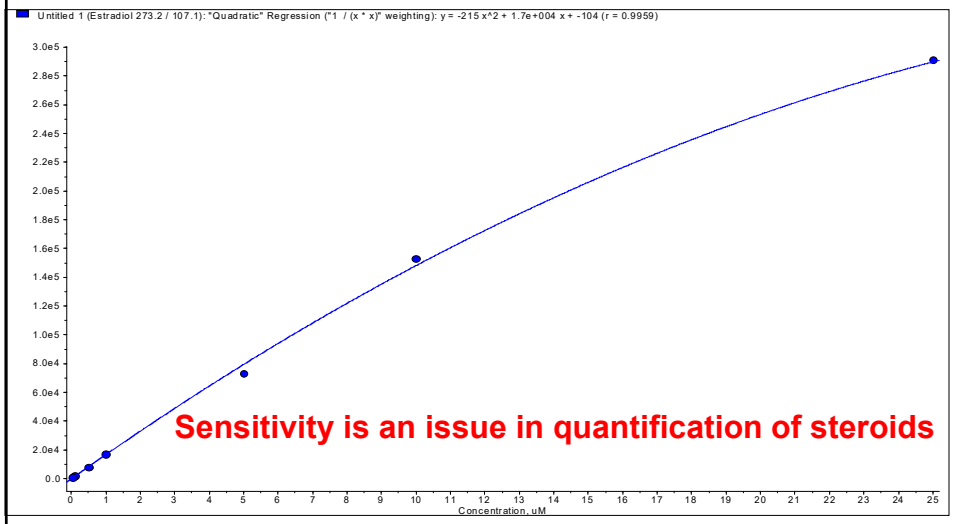
NL = neutral loss.

Kostiainen et al., 2003

# Analysis of steroids by MS/MS

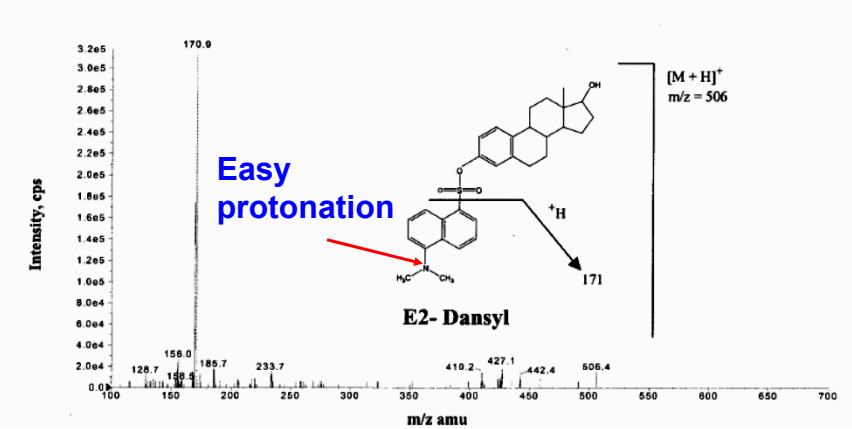



# Estradiol Standard Curve 0.05 – 25 µM
# r = 0.9959



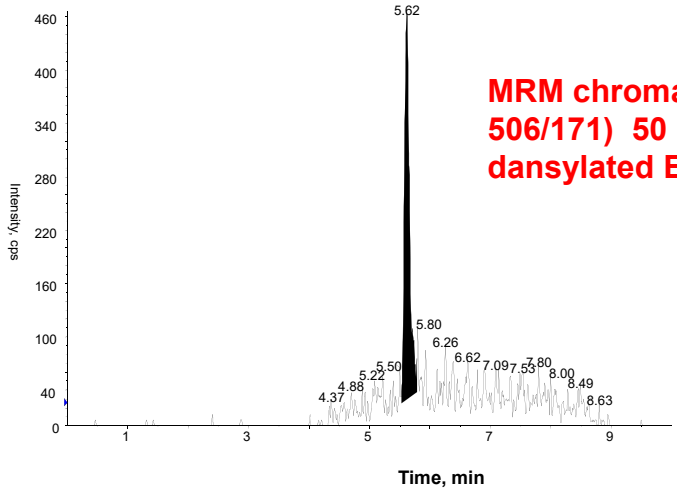

**Sensitivity is an issue in quantification of steroids**

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



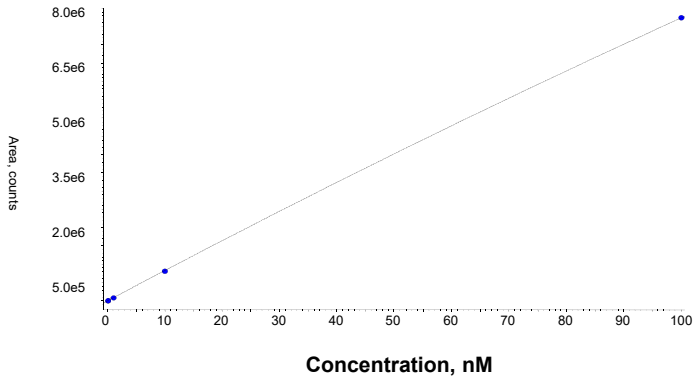

Source: Nelson et al. Clinical Chemistry, 2004

## Derivatization tremendously helps increase sensitivity of E2




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

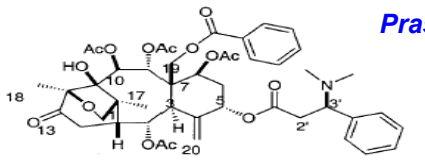
## Calibration curve for dansylated E2 showing linearity from 0.005-100 nM concentration range (r = 0.999)



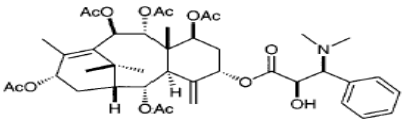

## Substructure analysis in ESI-MS/MS (dereplication and partial identification of natural products)

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

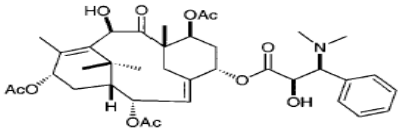
*Prasain et al. Anal Chem, 2001*



[1] MW=861

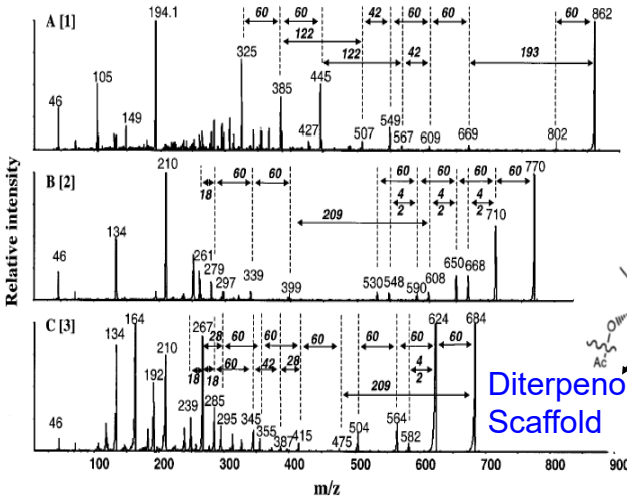


[2] MW=769

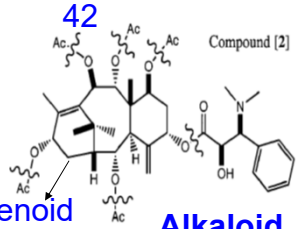


[3] MW=683

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



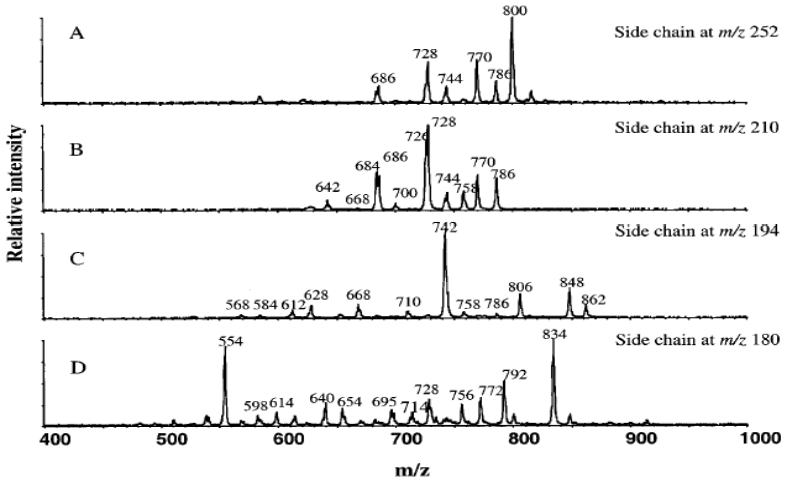

Loss of 60 or 42



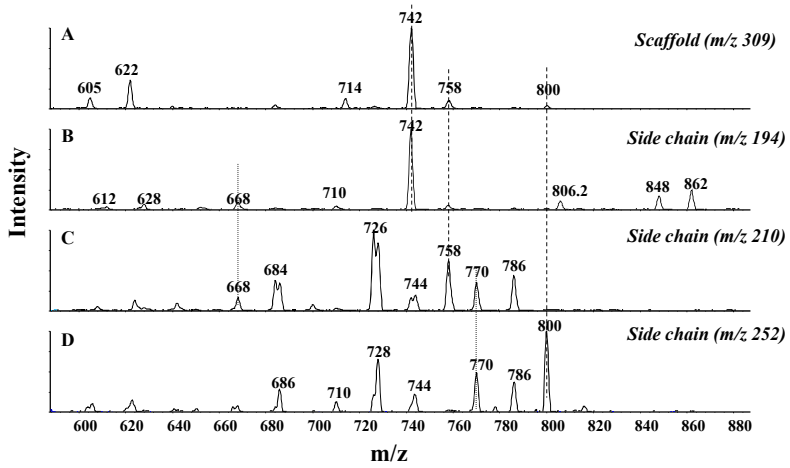

Diterpenoid Scaffold

**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.