MS/MS interpretation in identification of unknowns

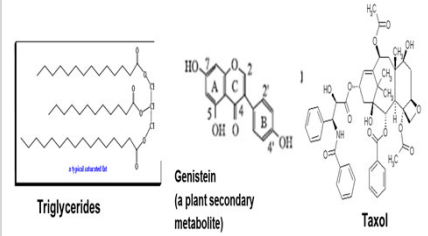
Jeevan Prasain, PhD
jprasain@uab.edu
6-2612

1

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

2

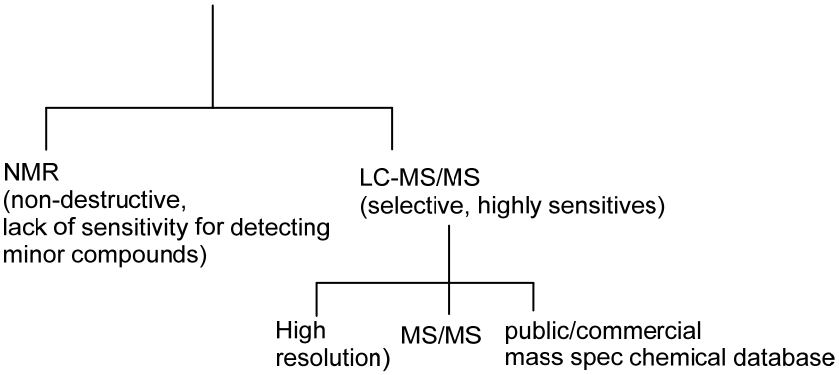


Small molecules are important!!

- 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)

3

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)

4

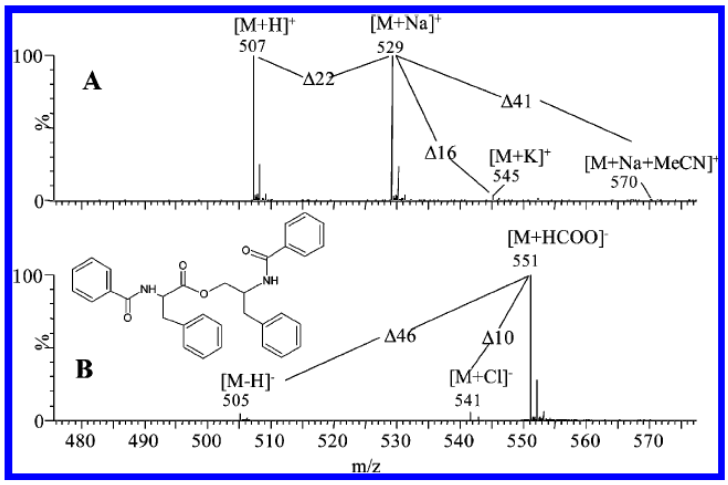
Keys to identifying unknown structures (putative/definitive) by mass spectrometry

- 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

5

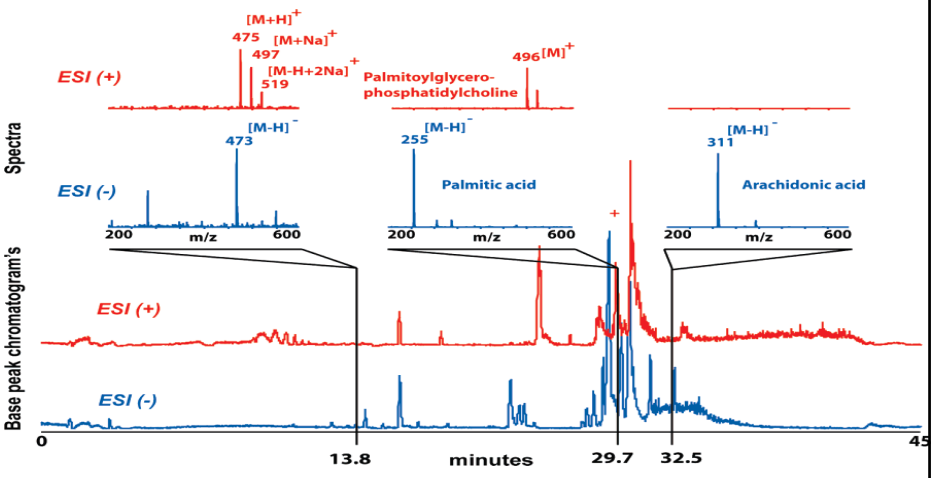
Adduct formation in +/–ve ion modes



Nielsen et al., J Nat Prod. 2011

6

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

7

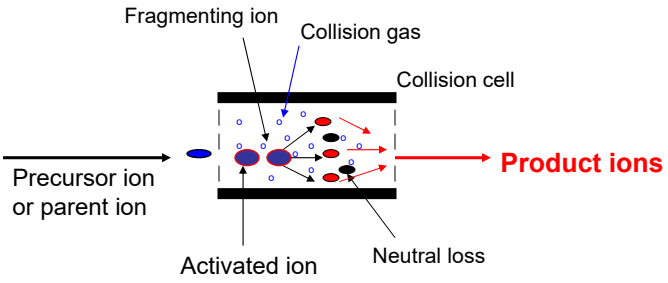
Isotopic distribution and MS

- 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

8

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



Schematic of CID fragmentation

9

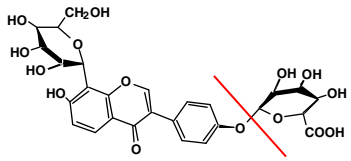
Applications of MS/MS

- **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…**

10

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

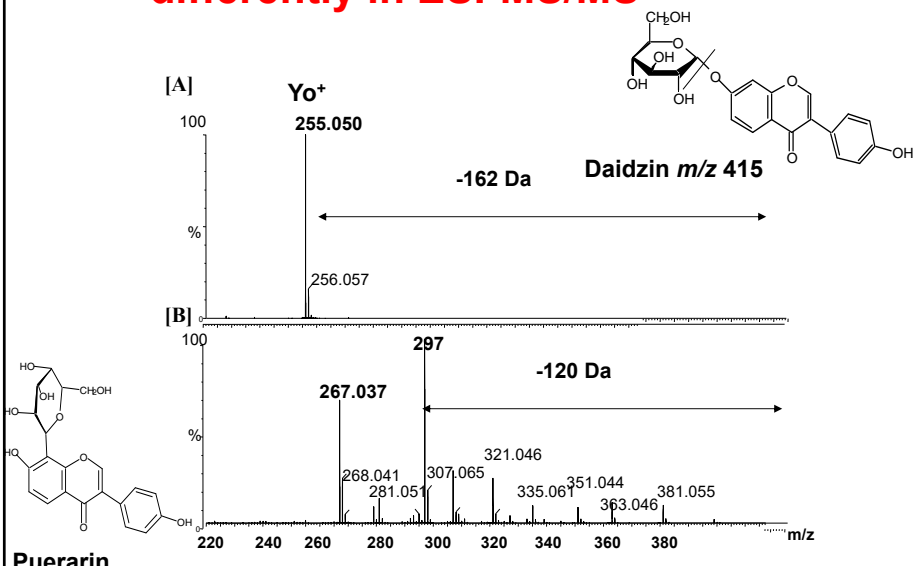


Where are the sites of deprotonation/protonation? What is the most likely leaving group in this molecule?

Fragmentation always follows the basic rules of chemistry- resonance stabilization

11

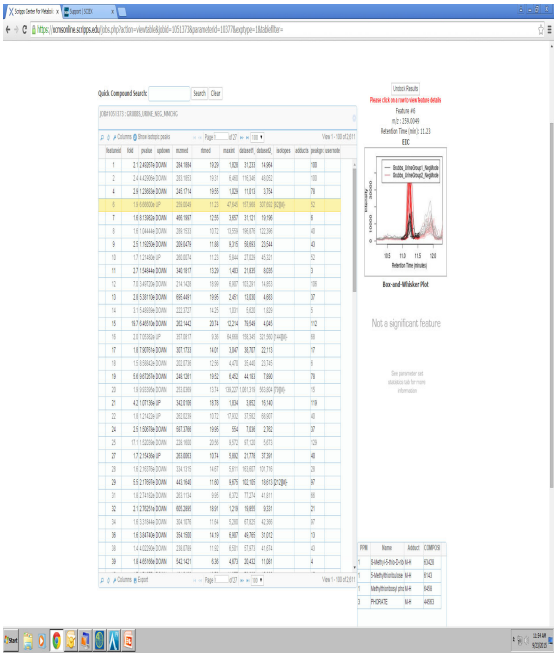
O- and C-glucosides fragment differently in ESI-MS/MS



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

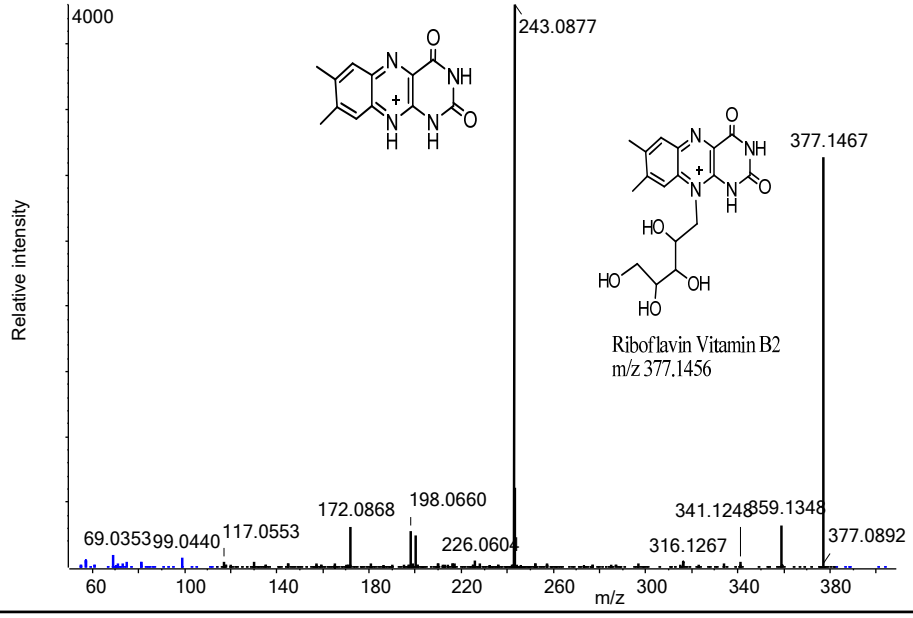
12

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



13

Detection of Vitamin B2 (riboflavin) as urinary metabolite



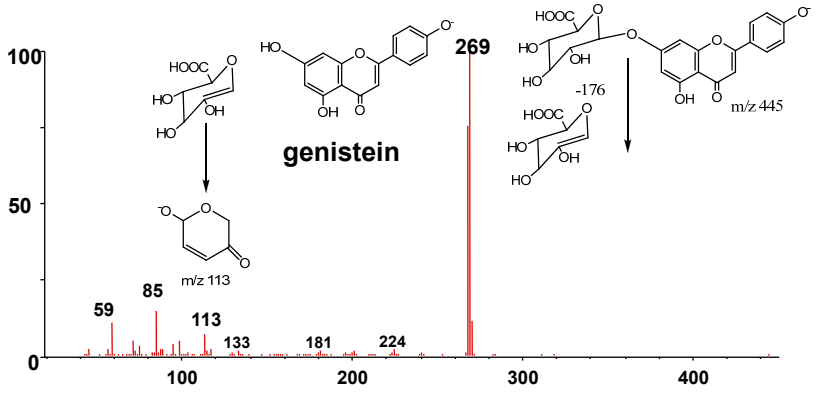
14

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

15

What fragment ions are characteristics for glucuronide conjugates?

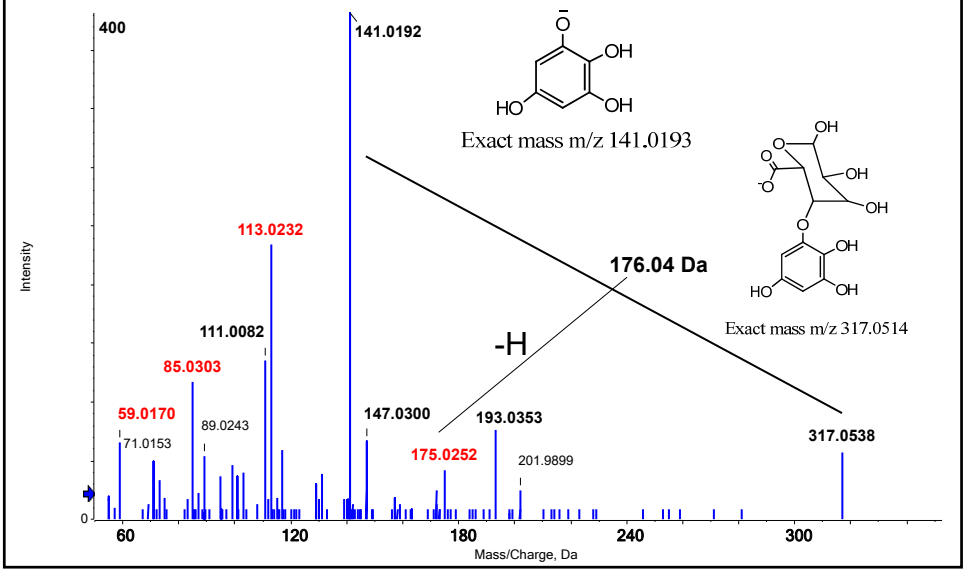
Product ion spectrum of genistein glucuronide in ESI-MS/MS



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

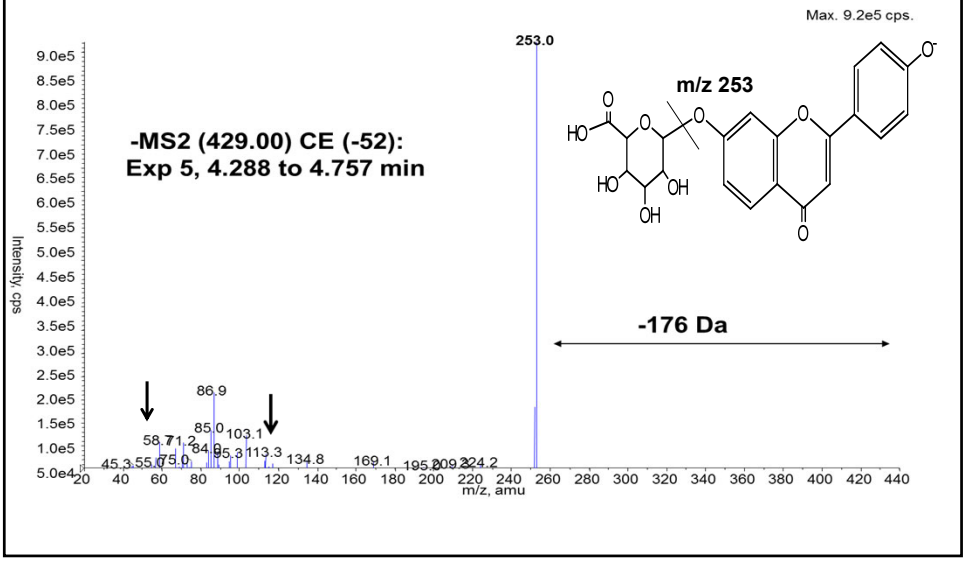
16

Putative identification- a glucuronide conjugate of tetrahydroxybenzene



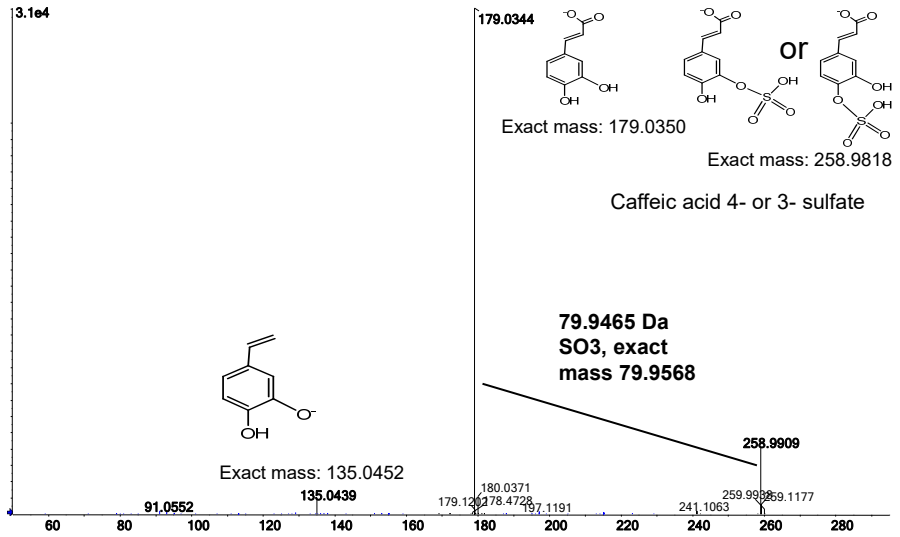
17

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



18

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



19

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

20

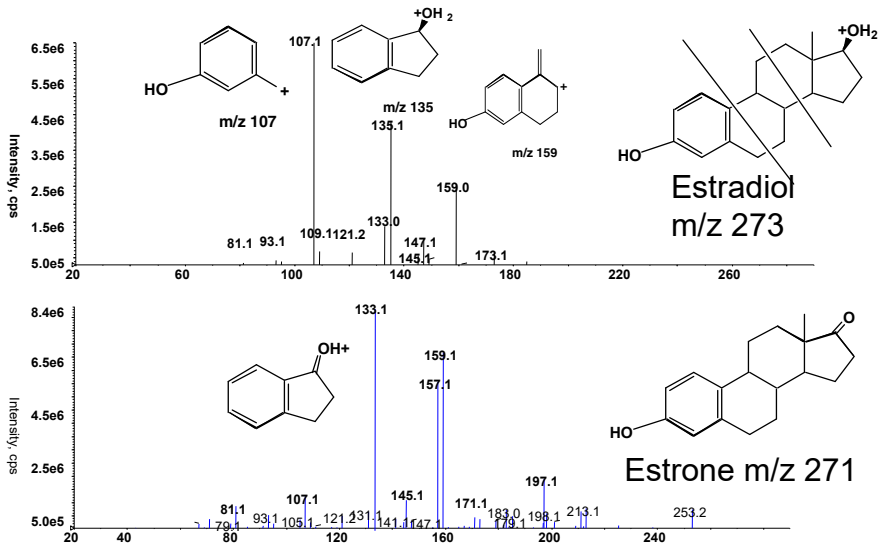
Characteristic neutral loss and precursor Ion scans for conjugated metabolites

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

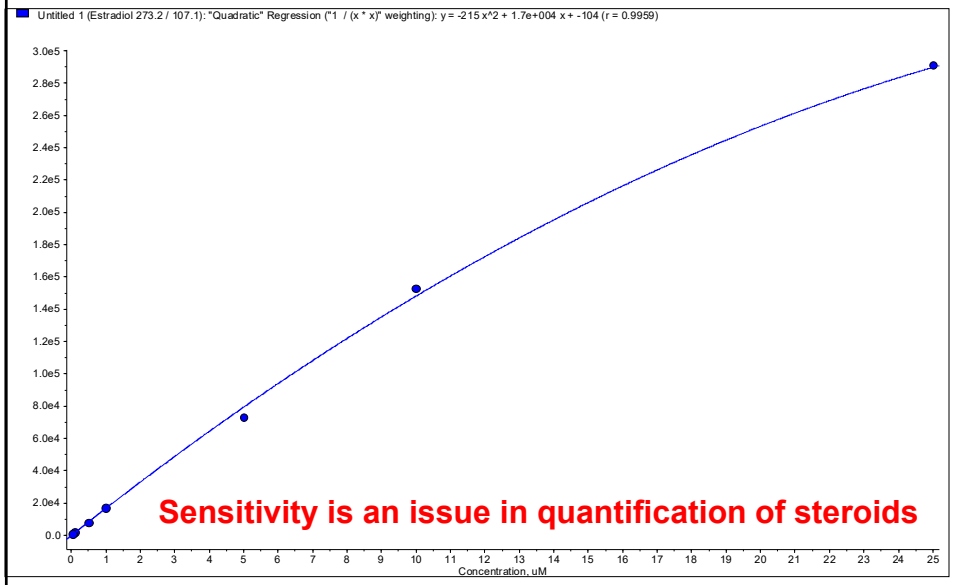
21

Analysis of steroids by MS/MS



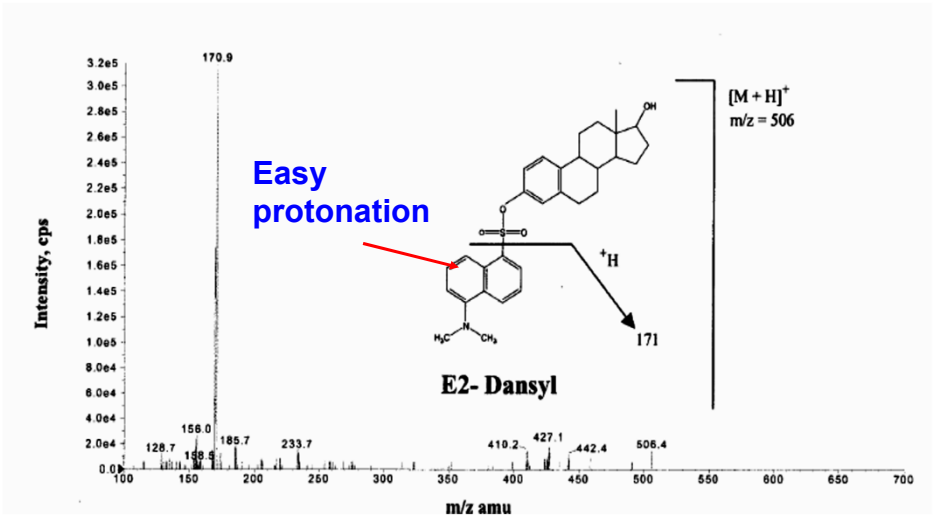
22

Estradiol Standard Curve 0.05 – 25 µM
r = 0.9959



23

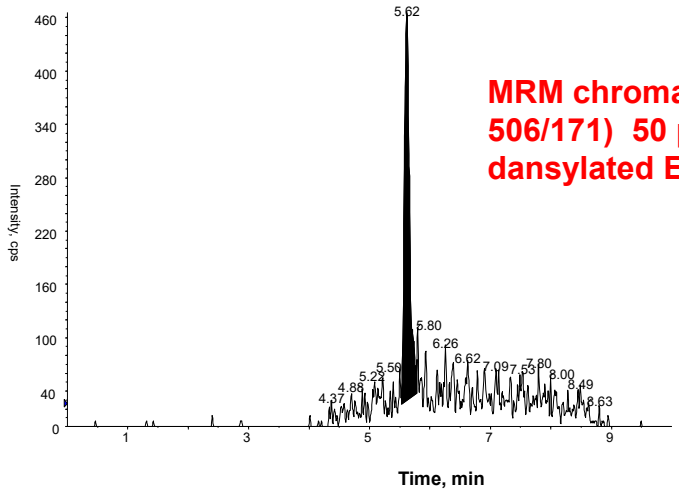
Derivatization of estradiol with dansyl chloride leads to the formation of E_2-dansyl (*m/z* 506)



Source: Nelson et al. Clinical Chemistry, 2004

24

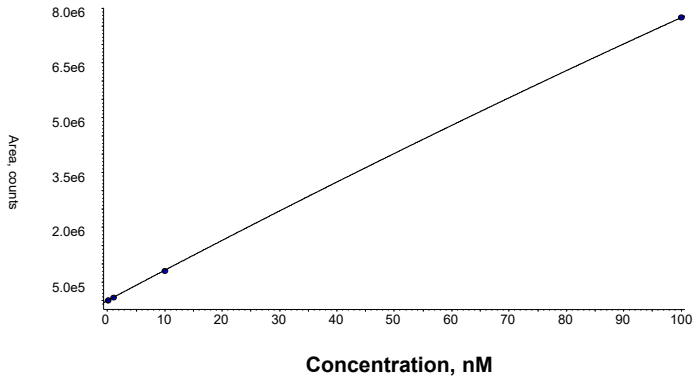
Derivatization tremendously helps increase sensitivity of E2



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

25

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

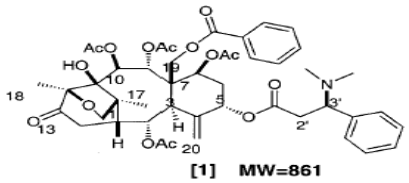


26

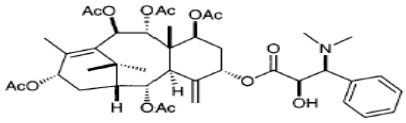
Substructure analysis in ESI-MS/MS (dereplication and partial identification of natural products)

27

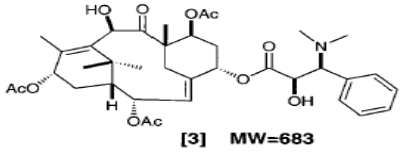
Fragmentation of basic taxoids from *T. Wallichiana* extract



[1] MW=861



[2] MW=769

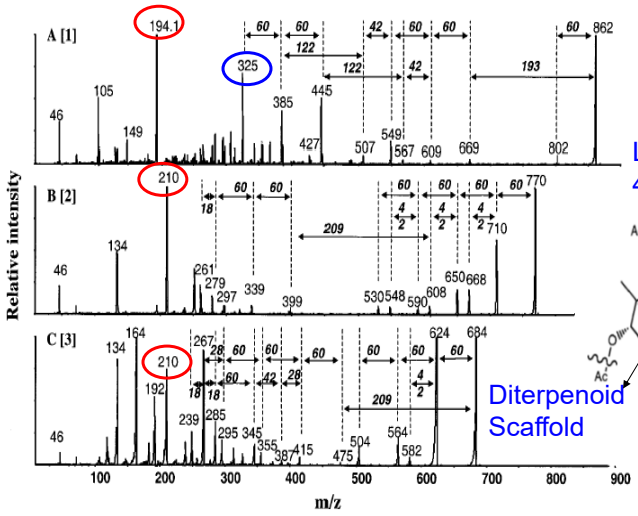


[3] MW=683

Stefanowicz et al. Anal Chem, 2001

28

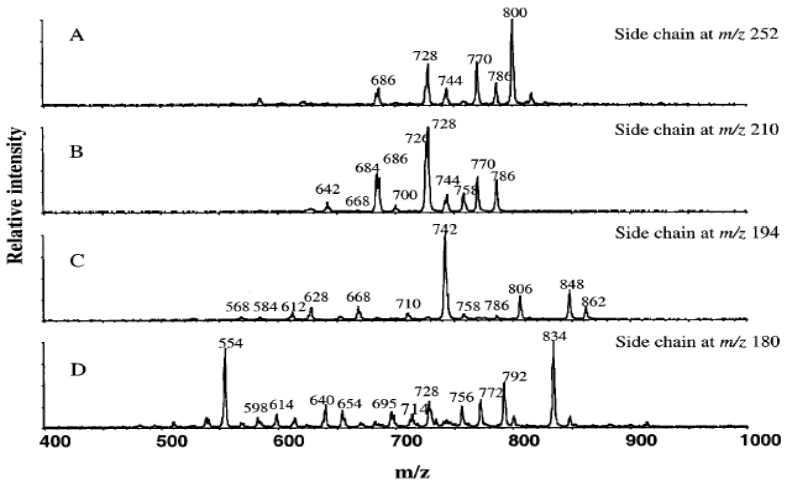
ESI-MS/MS spectra of taxoids (1-3). Peaks *m/z* 194 and 210 represent the intact alkaloid side chain.



Stefanowicz et al. Anal Chem, 2001

29

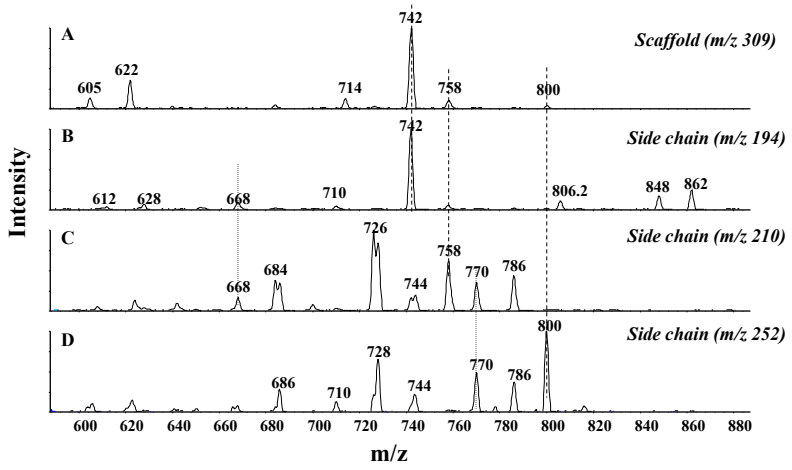
MS/MS precursor-scan spectra of typical alkaloid side chains to identify the basic taxoids compounds in an ethyl acetate extract of *T. wallichiana*.



Stefanowicz et al. Anal Chem, 2001

30

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

31

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.

32