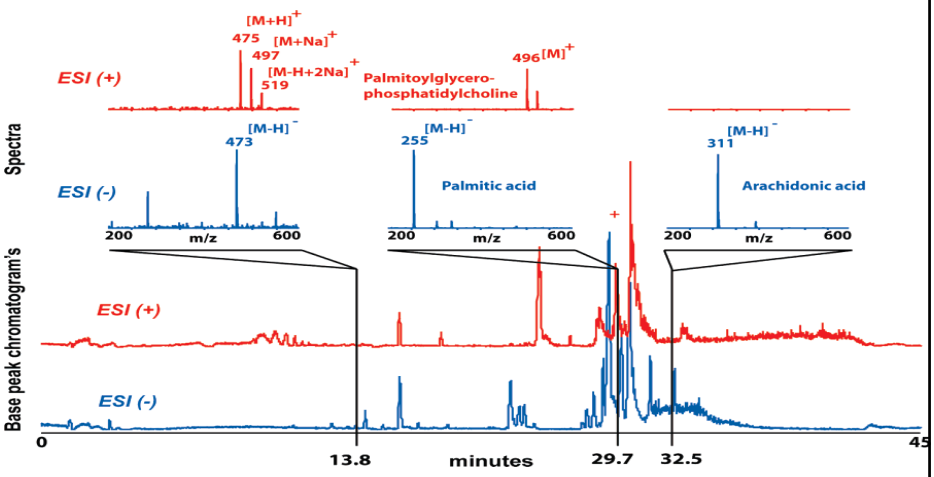
Increasing metabolite coverage using +ve and –ve ion mode



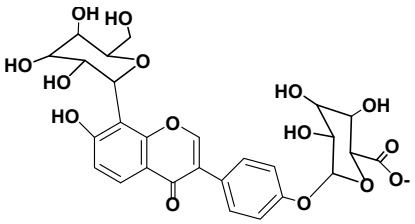
Representative Q1 scans of a methanolic extract of human blood serum

Source: Nordstrom et al. Analytical Chemistry, 2007

Interpreting MS/MS spectra

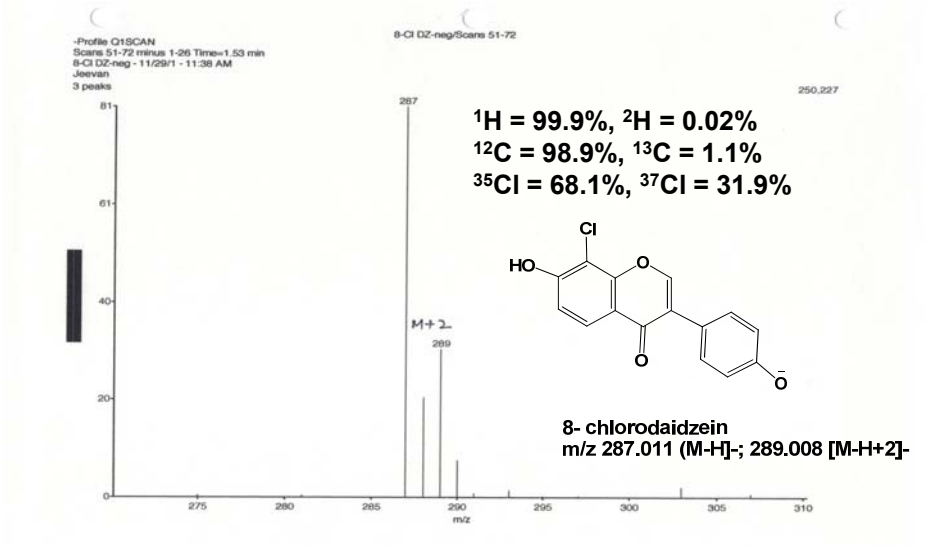
- **Likely sites of protonation or deprotonation.**
- **Likely leaving group.**
- **Literature study**

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

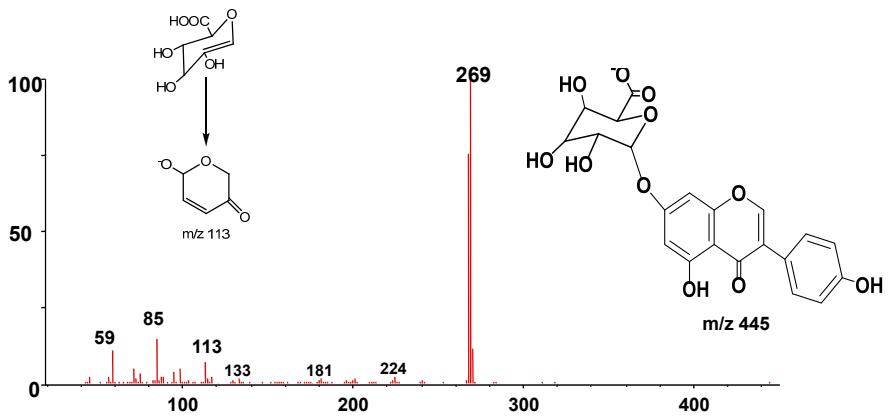
Isotopic pattern and intensity of ions indicates the number of carbons and hetero atoms in the molecular ion



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

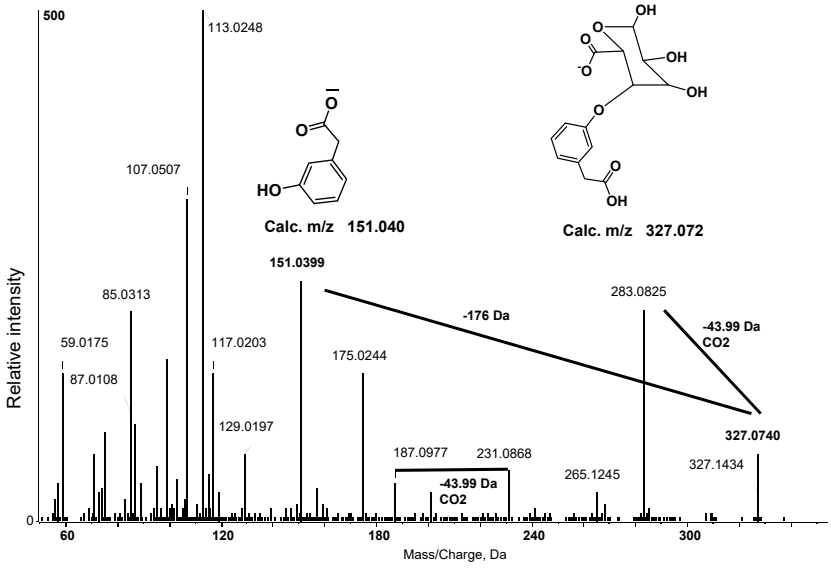
What fragment ions are characteristics for glucuronide conjugates?

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

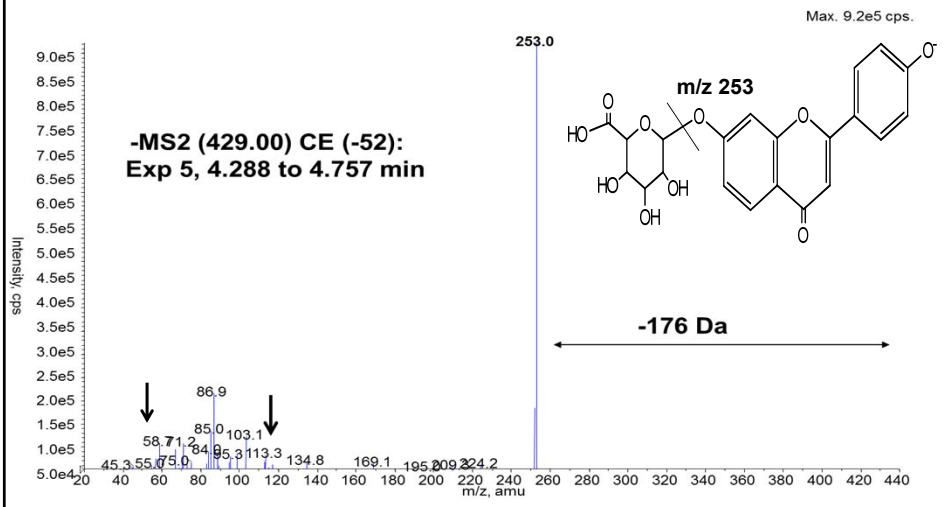


Glucosides/glucuronides conjugates are easily cleaved off by higher potential at orifice

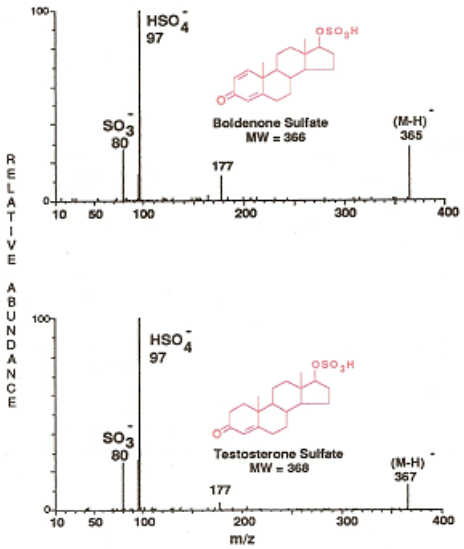
The neutral loss of 176 Da is an indicative of glucuronide metabolites



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



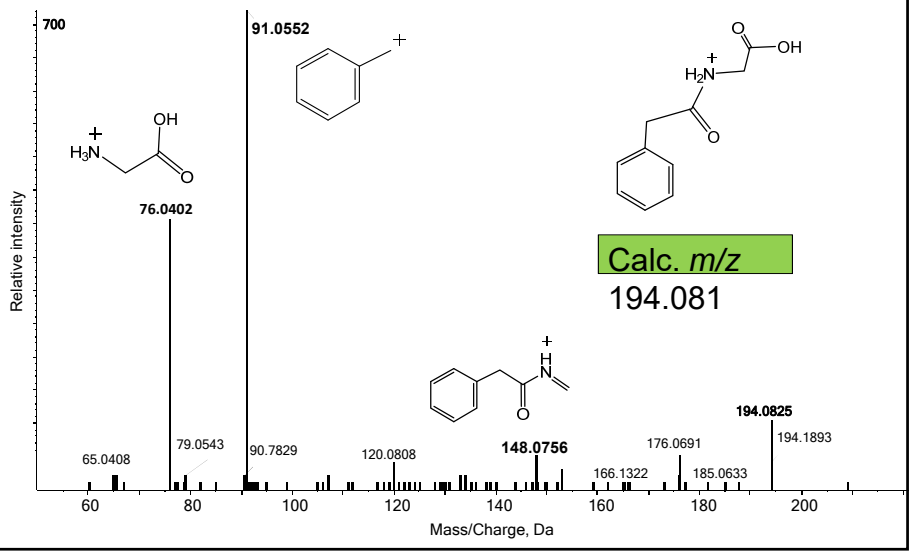
What happens with aliphatic sulfates in MS/MS?



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

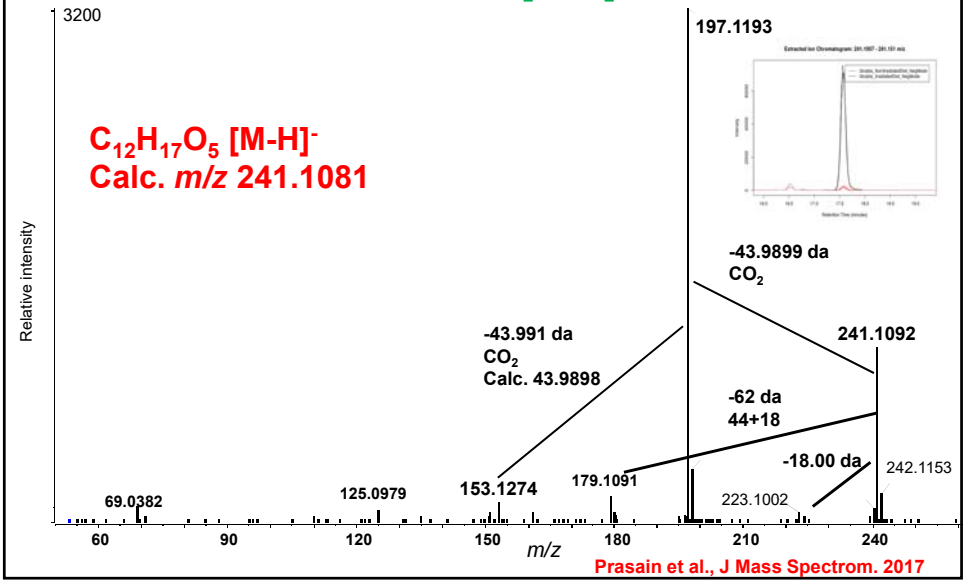
Source: Weidolf et al. Biomed. and Environ. Mass Spec. 1988

Among the annotated list of compounds by Metlin - phenylacetylglycine's validation by MS/MS interpretation



Many metabolites, unidentified by the Metlin database

A medium chain dicarboxylic fatty acid with *m/z* 241.109 [M-H]⁻



Prasain et al., J Mass Spectrom. 2017

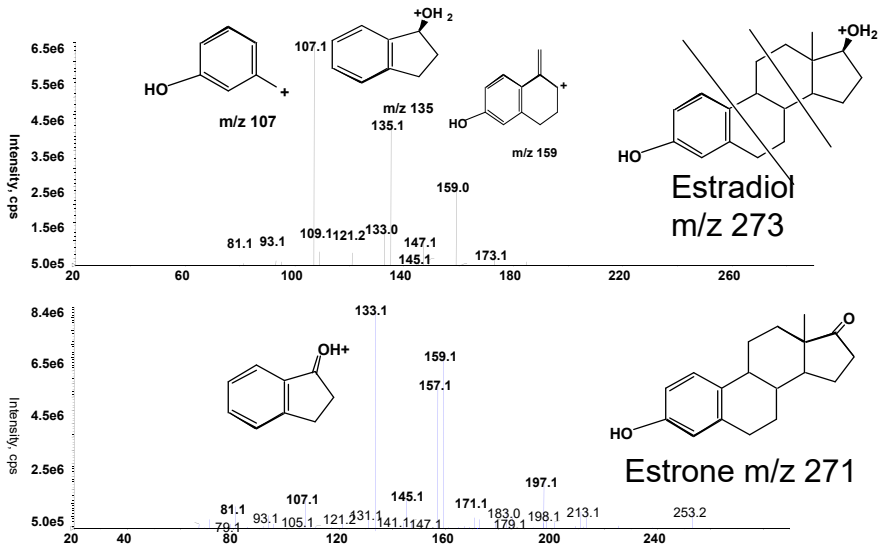
Characteristic fragmentation of drug conjugates by MS/MS

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

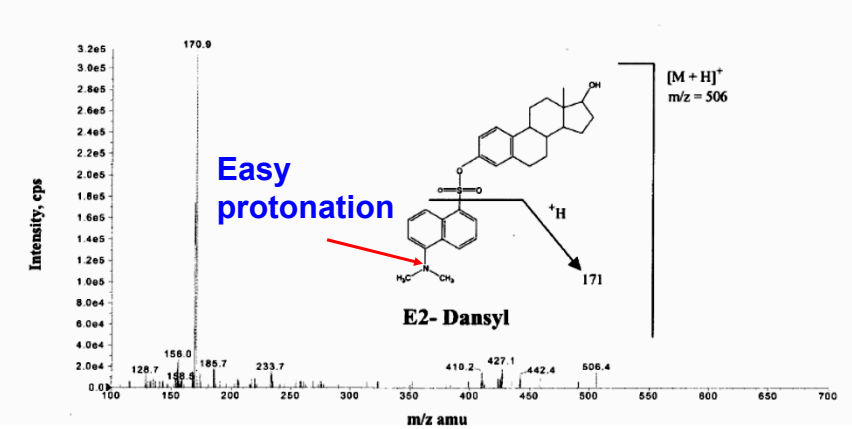
NL = neutral loss.

Kostiainen et al., 2003

Analysis of steroids by MS/MS

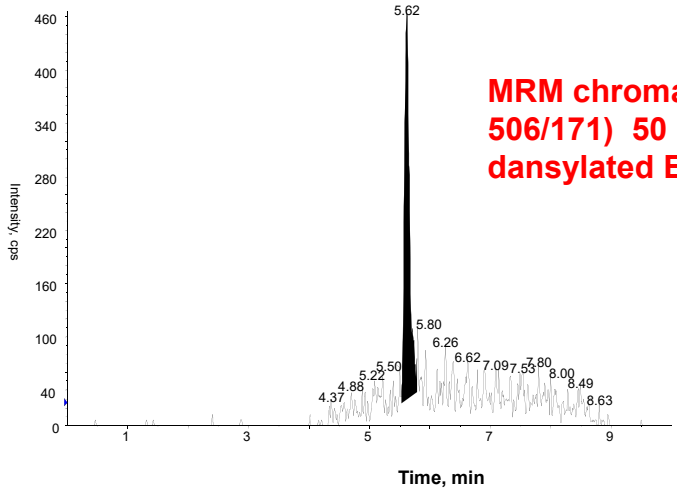


Derivatization of estradiol with dansyl chloride leads to the formation of E_2-dansyl (*m/z* 506)



Source: Nelson et al. Clinical Chemistry, 2004

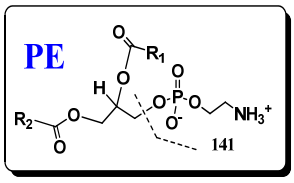
Derivatization tremendously helps increase sensitivity of E2



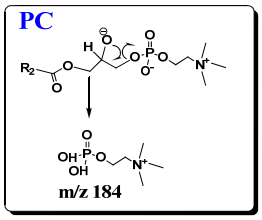
MRM chromatogram (m/z 506/171) 50 picomole dansylated E2

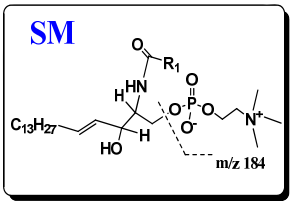
MS based-Lipidomics

Profiling phospholipids and sphingosines in a complex mixture using MS/MS

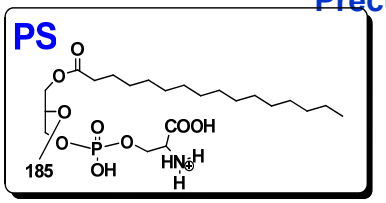


PE
Neutral Loss scan 141



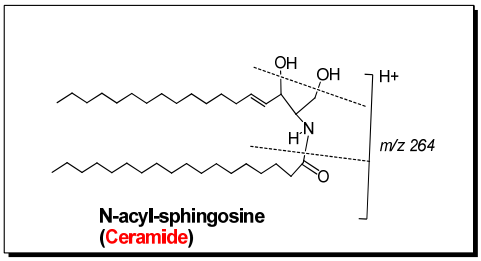


PC & SM
Precursor ion scan 184

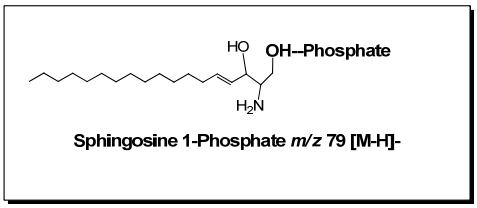


PS
Neutral Loss scan 185

How to profile sphingolipids in a complex mixture using MS/MS?



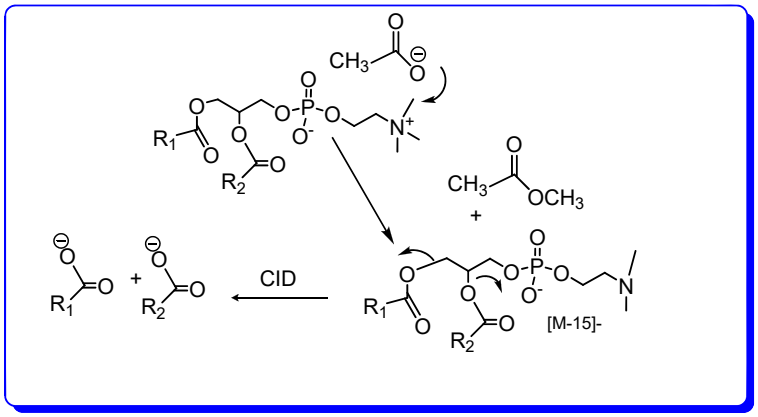
N-acyl-sphingosine
(Ceramide)



Sphingosine 1-Phosphate *m/z* 79 [M-H]-

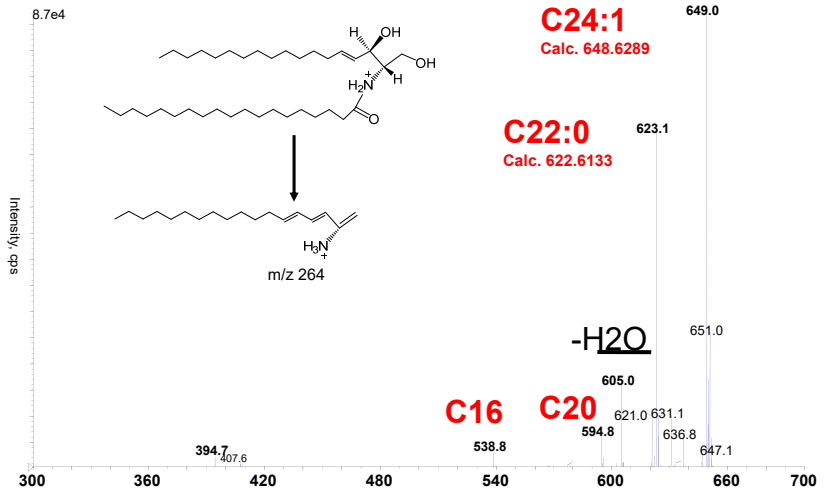
***m/z* 264 is a characteristic ion for all compounds containing a sphingosine backbone**

Phosphatidylcholine loses a methyl group to form a negatively charged, pseudomolecular ion

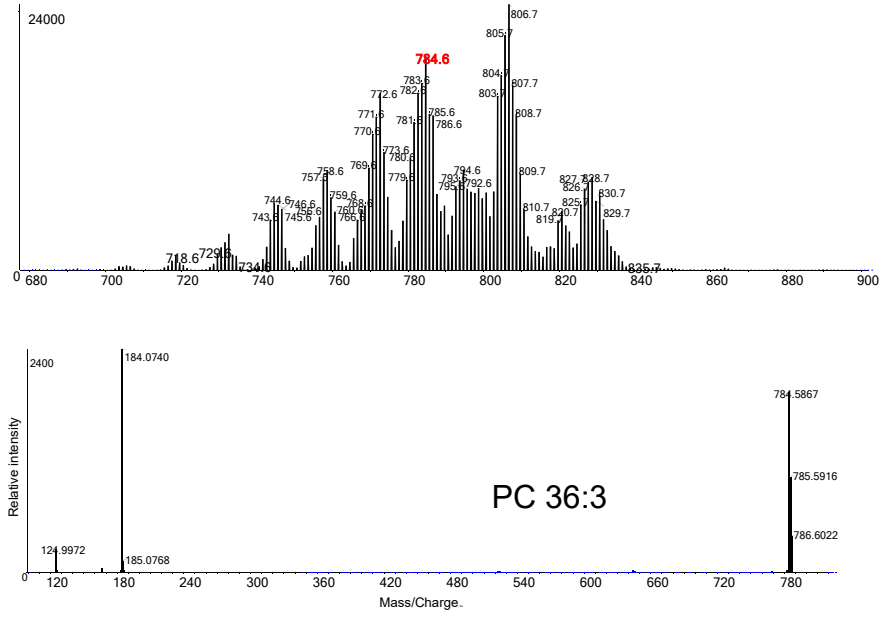


Phospholipids may undergo demethylation and then the loss of the fatty acyl groups from glycerophosphocholine backbone.

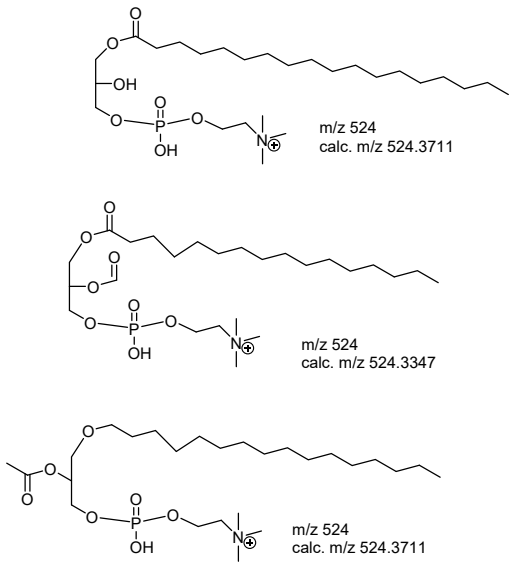
Precursor ion scan m/z 264 in +ve ion mode is specific to identify ceramides in a sample



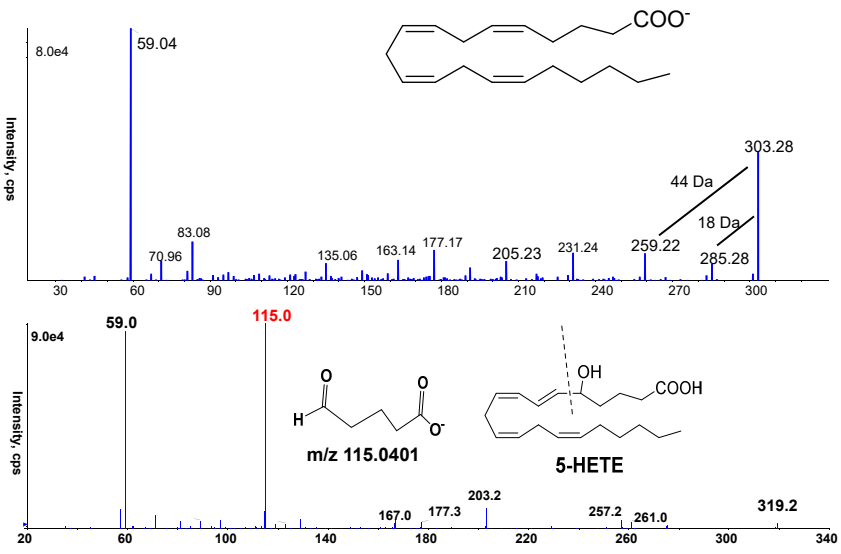
Precursor ion scan m/z 184.073 for PC/SM in a C. elegans lipid extract [A]; MS/MS of the precursor ion m/z 784 [B]



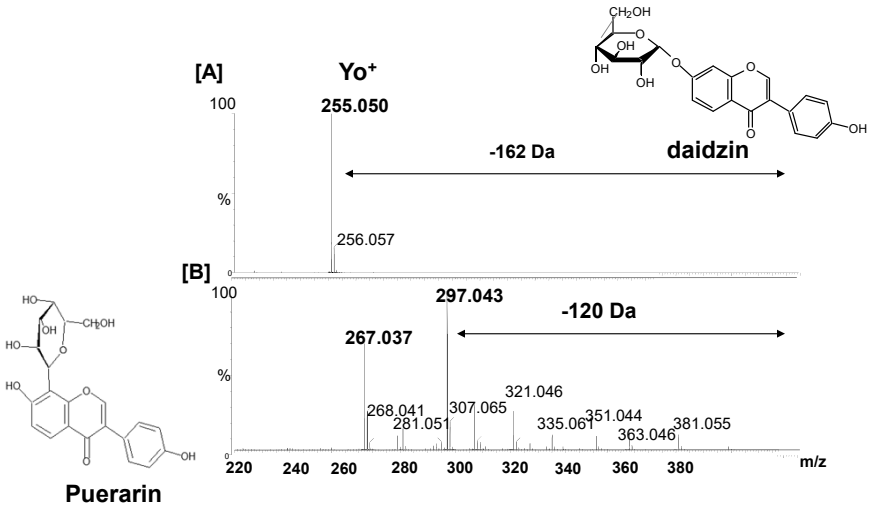
Several isobaric compounds- Identification by high resolution mass spectrometry



Product ion spectra of deprotonated arachidonic acid [AA] and its oxidation product 5-hydroxy-eicosatetraenoic acids [5-HETE]



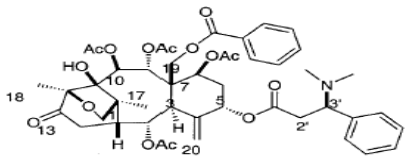
Structure determination: Accurate mass of a precursor ion is not enough, but MS/MS is



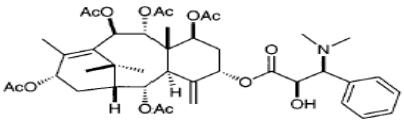
Prasain et al., J. Agric. Food Chem., 2003

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

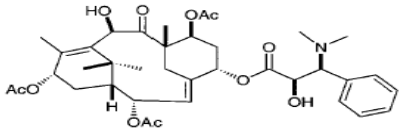
Fragmentation of basic taxoids from *T. Wallichiana* extract



[1] MW=861



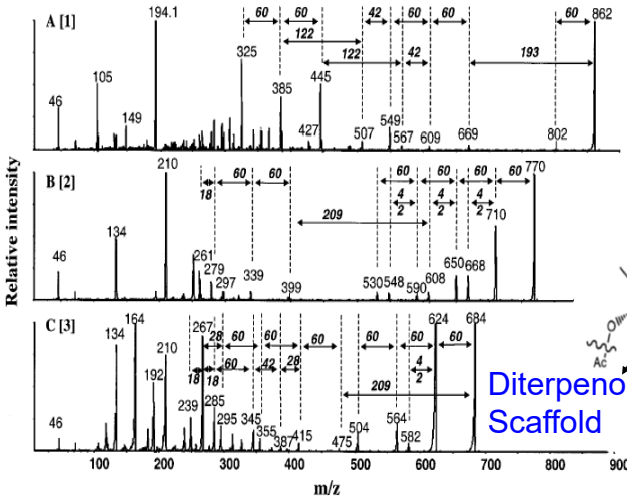
[2] MW=769



[3] MW=683

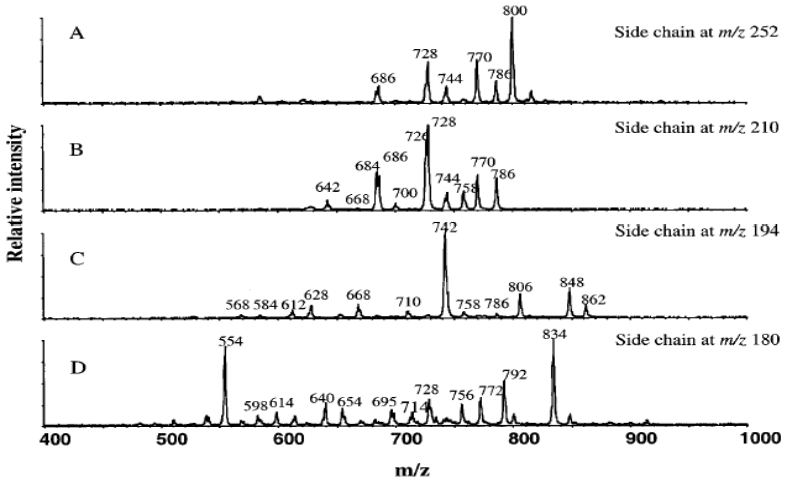
Source: Stefanowicz et al. Anal Chem, 2001

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



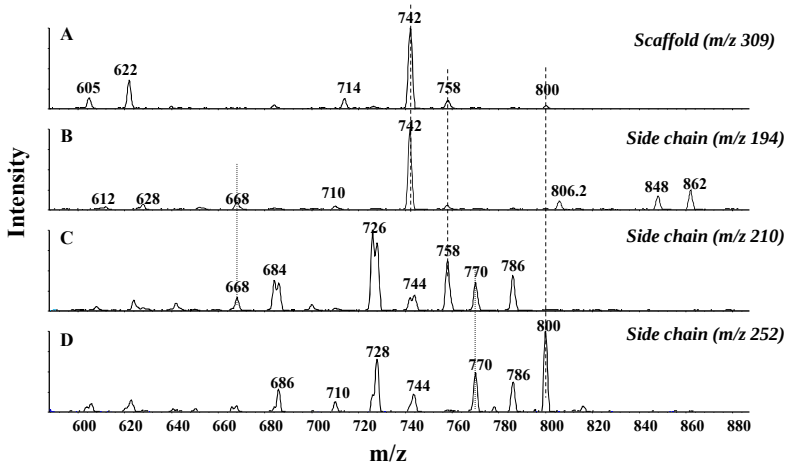
Stefanowicz et al. Anal Chem, 2001

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

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

Conclusions

- **Identifying unknown metabolites is a significant analytical challenge in metabolomics and it requires integrated analytical and bio-informative approaches.**
- **The use of high-resolution MS and MS^n provides important information to propose structures of fragment and precursor ions.**
- **Only an integrated approach can describes multitude of metabolites present in a biological sample.**