MS/MS interpretation in identification of unknowns

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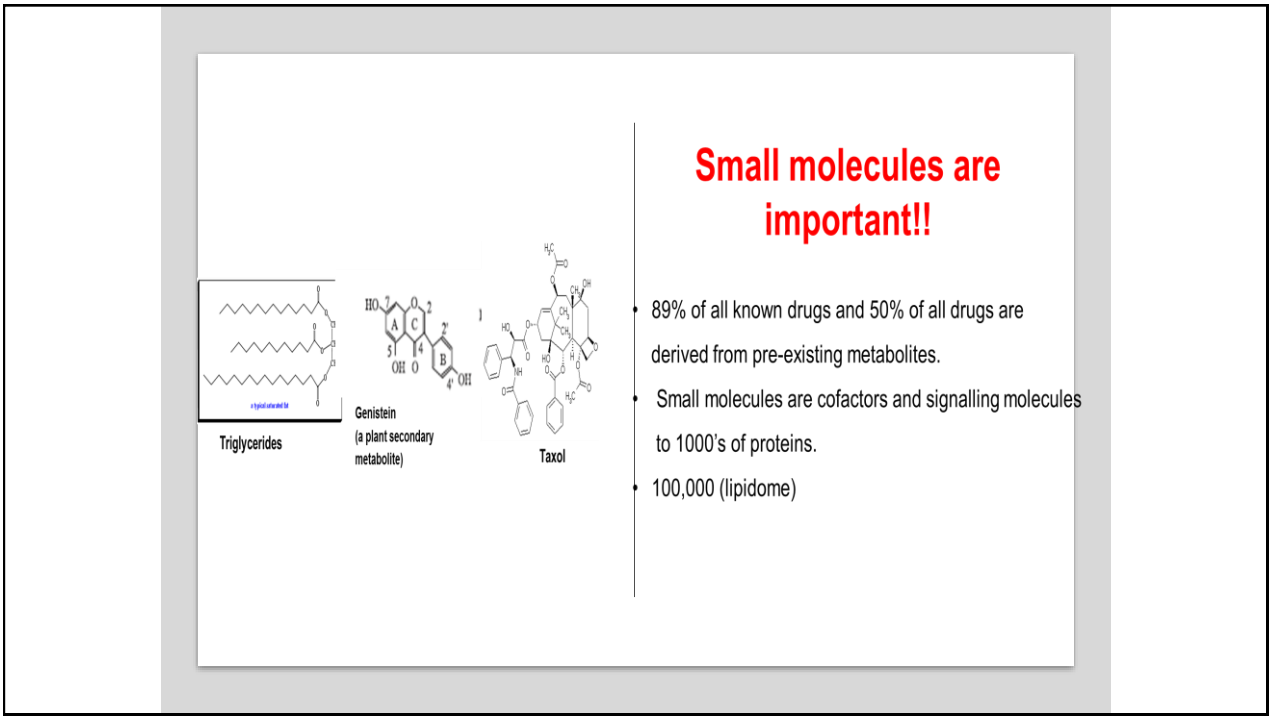
Outline

- Introduction
- How to interpret LC-MS and MS/MS data
- Identification of some conjugated metabolites
- Sensitivity and selectivity enhancement through derivatization
- Identification of tryptophane metabolites



Triglycerides

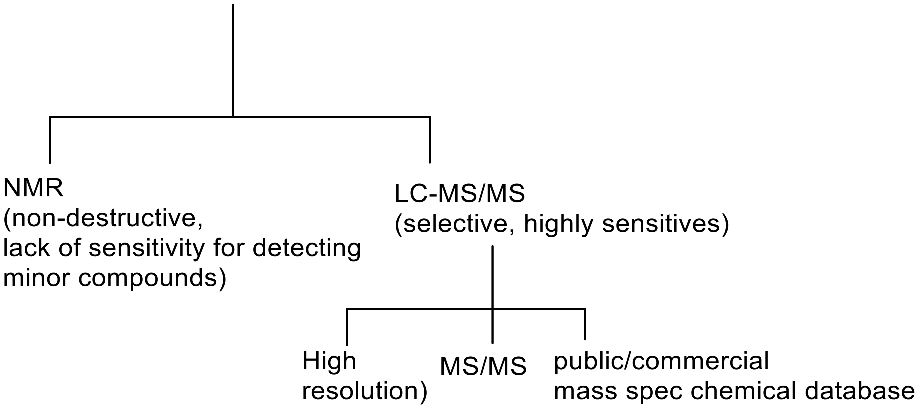
Genistein
(a plant secondary metabolite)

Taxol

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)

Knowing the unknowns



NMR
(non-destructive,
lack of sensitivity for detecting minor compounds)

LC-MS/MS
(selective, highly sensitives)

High resolution)

MS/MS

public/commercial mass spec chemical database

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).

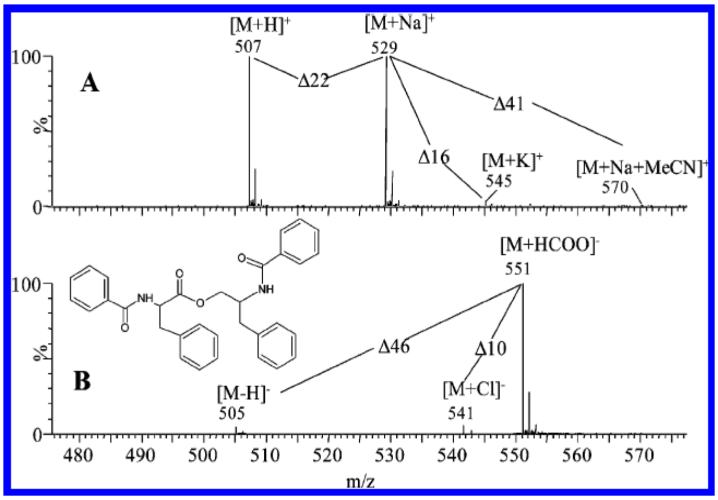
Unknown unknown- not previously cited (Little et al., 2011)

Keys to identifying unknown structures (putative or 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)

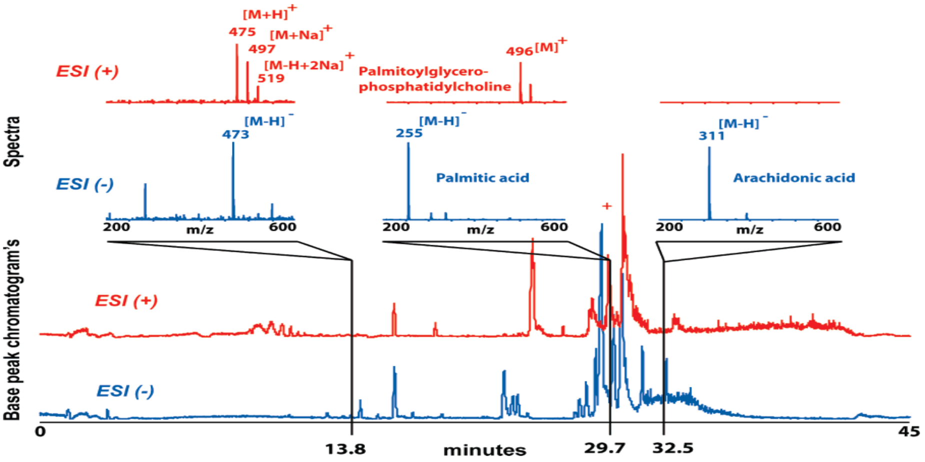
Moco et al. Trends in Analytical Chemistry, 2007

Not every ion represents individual metabolite:
Adduct formation in +/–ve ion modes



Nielsen et al., J Nat Prod. 2011

Increasing metabolite coverage using +ve and –ve ion modes



Representative Q1 scans of a methanolic extract of human blood serum

Source: Nordstrom et al. Analytical Chemistry, 2007

Isotopic distribution and MS

$^{1}H = 99.9\%$, $^{2}H = 0.015\%$

$^{12}C = 98.9\%$, $^{13}C = 1.1\%$

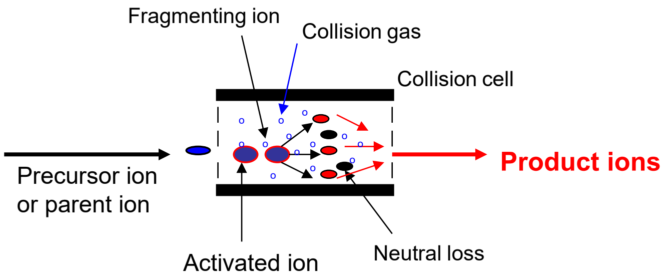
$^{35}Cl = 75.7\%$, $^{37}Cl = 24.2\%$

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

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



Schematic of CID fragmentation

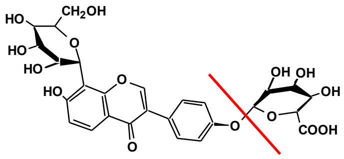
Applications of MS/MS

- 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 - e.g., neonatal screening, steroids in athletes, etc.
- Environment - e.g., dioxins in fish..
- Geological - e.g., oil compositions…

Interpreting CID induced 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

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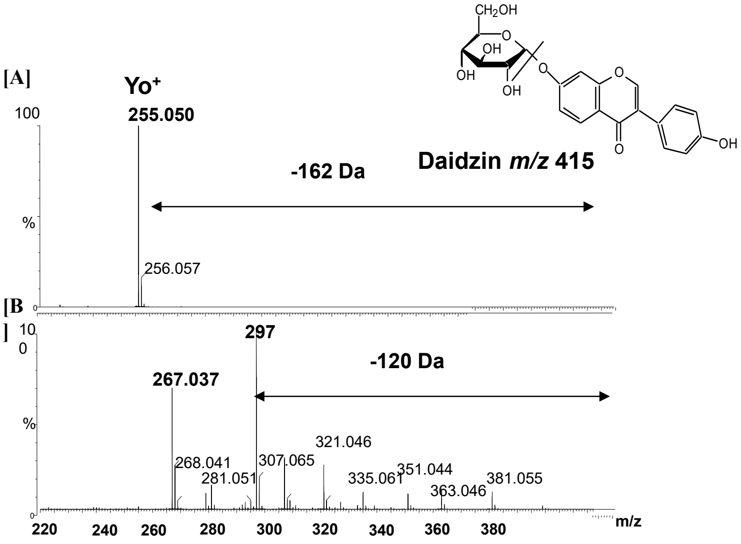
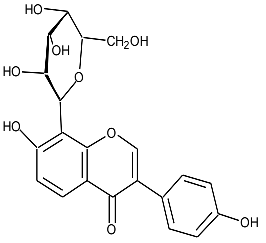
O- and C-glucosides fragment differently in ESI-MS/MS



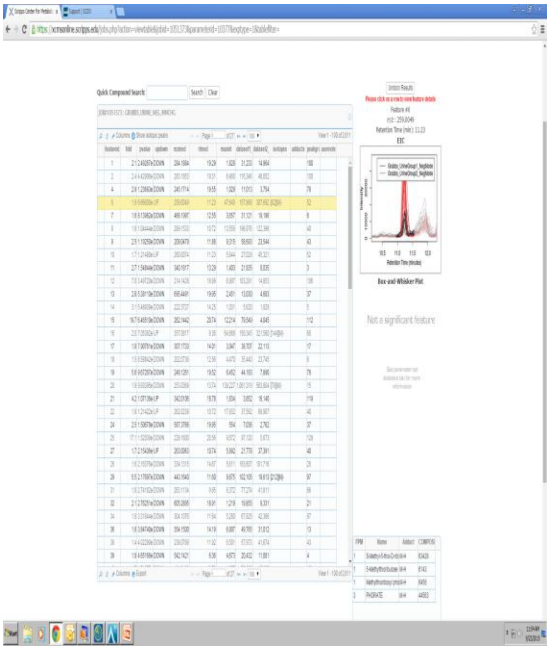
Daidzin *m/z* 415



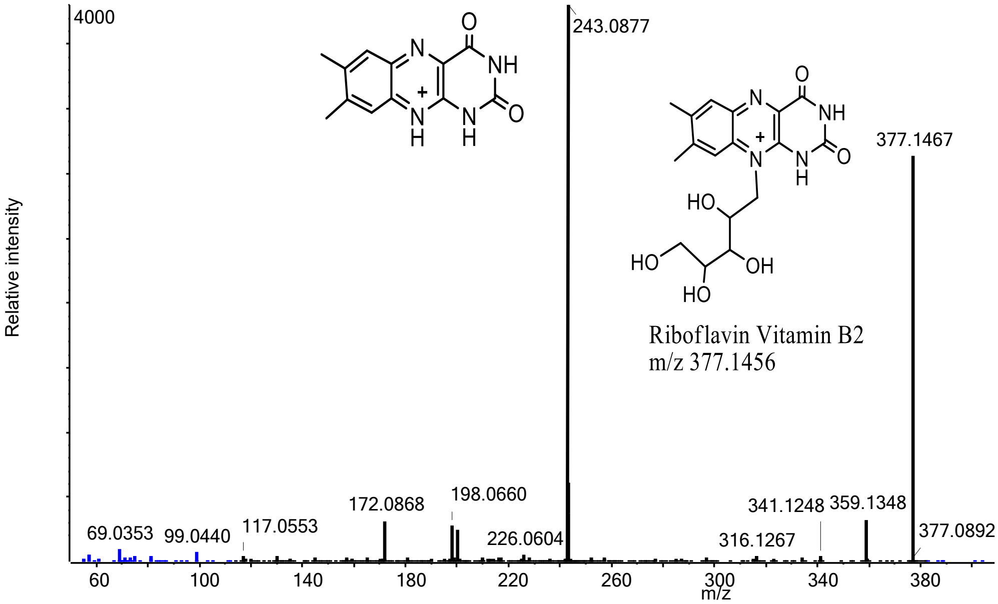
Puerarin
***m/z* 415**

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

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



Detection of Vitamin B2 (riboflavin) as urinary metabolite

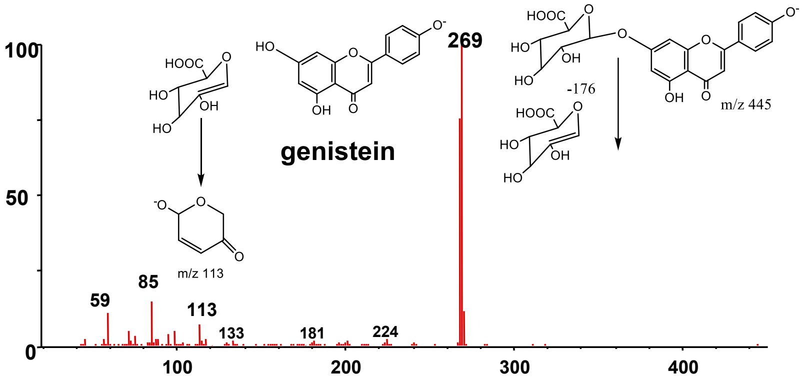


Riboflavin Vitamin B2
m/z 377.1456

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

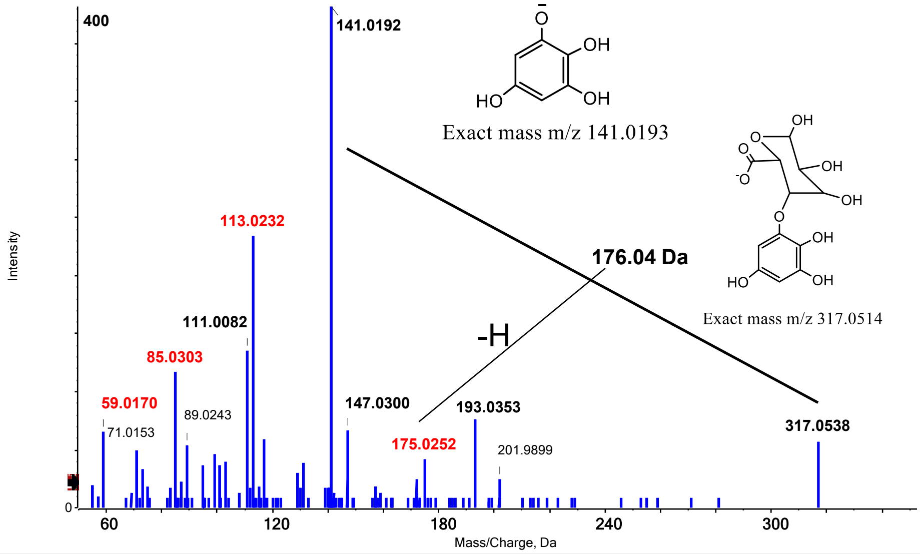
What fragment ions are characteristic for glucuronide conjugates?

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

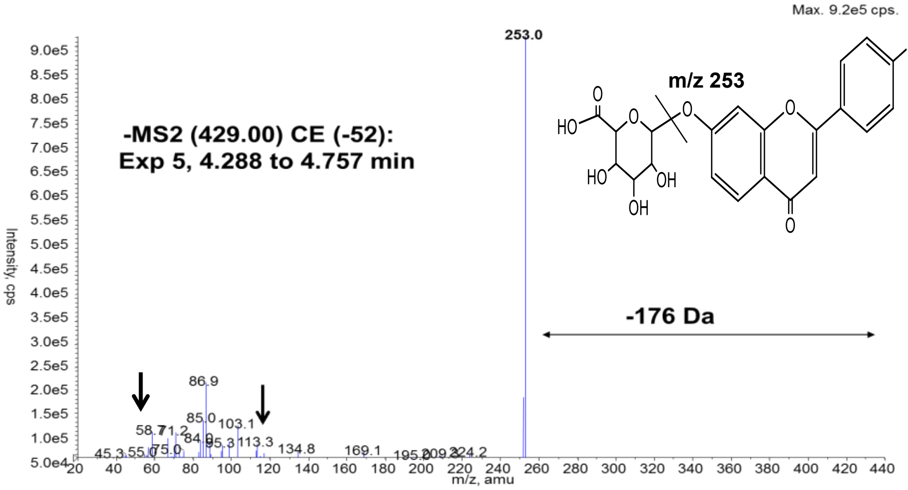


Glucosides/glucuronides conjugates are easily cleaved off by higher potential at orifice

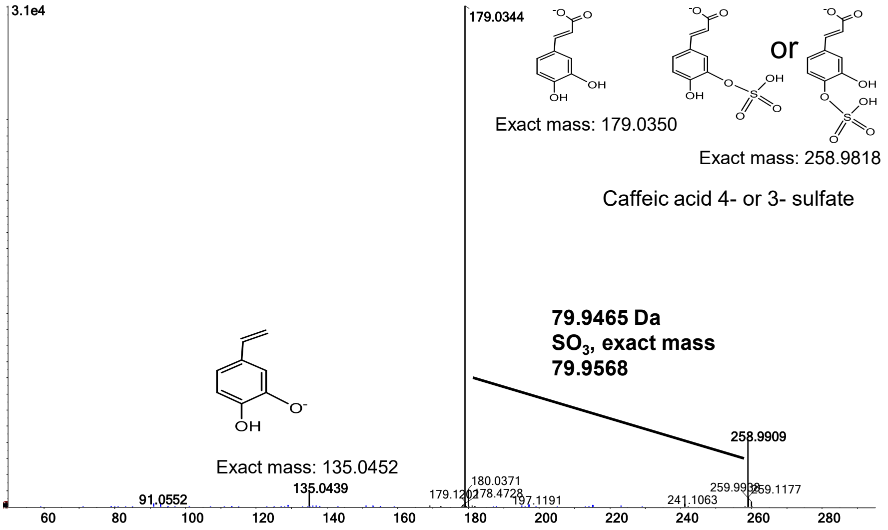
Putative identification- a glucuronide conjugate of tetrahydroxybenzene



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



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



Exact mass: 179.0350

Exact mass: 258.9818

Caffeic acid 4- or 3- sulfate

79.9465 Da
SO_3, exact mass
79.9568

Exact mass: 135.0452

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**

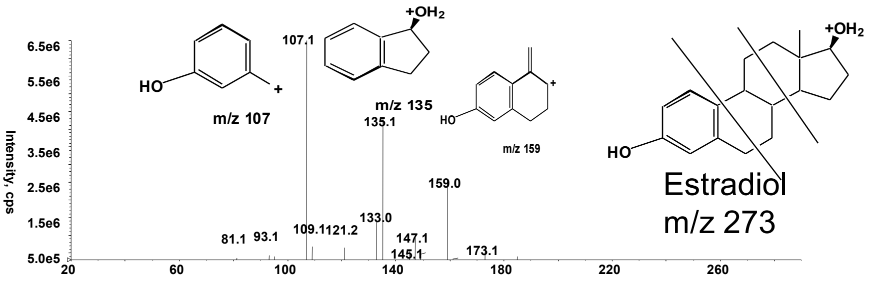
Characteristic neutral loss and precursor Ion scans for conjugated metabolites

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

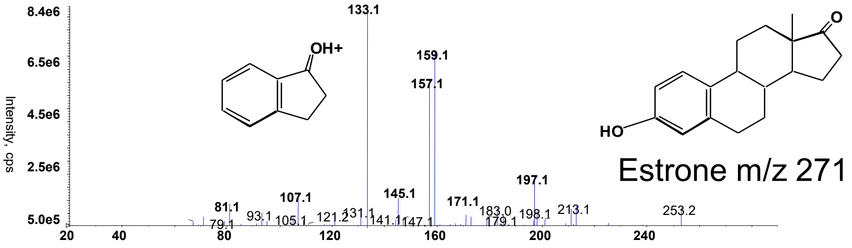
NL = neutral loss.

Kostiainen et al., 2003

Analysis of steroids by MS/MS

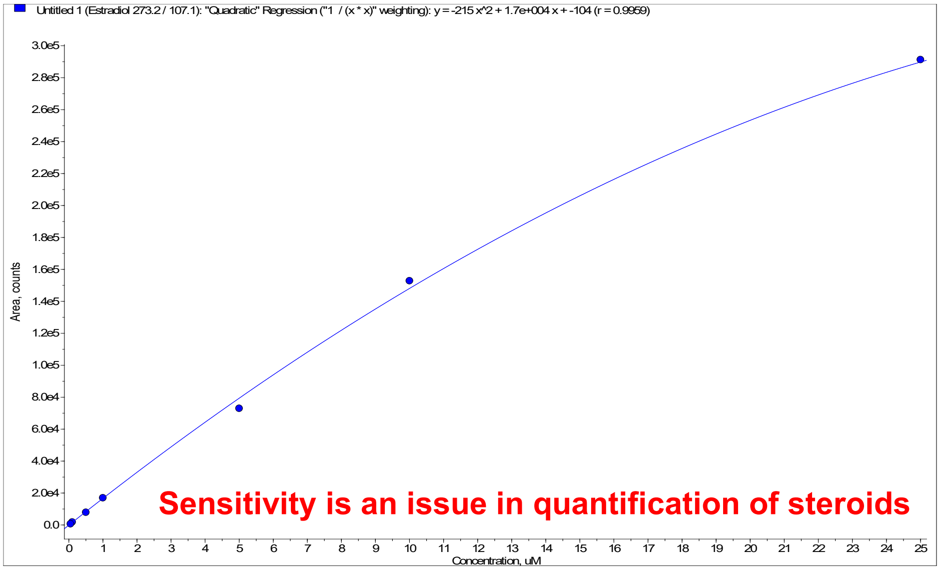


Estradiol m/z 273



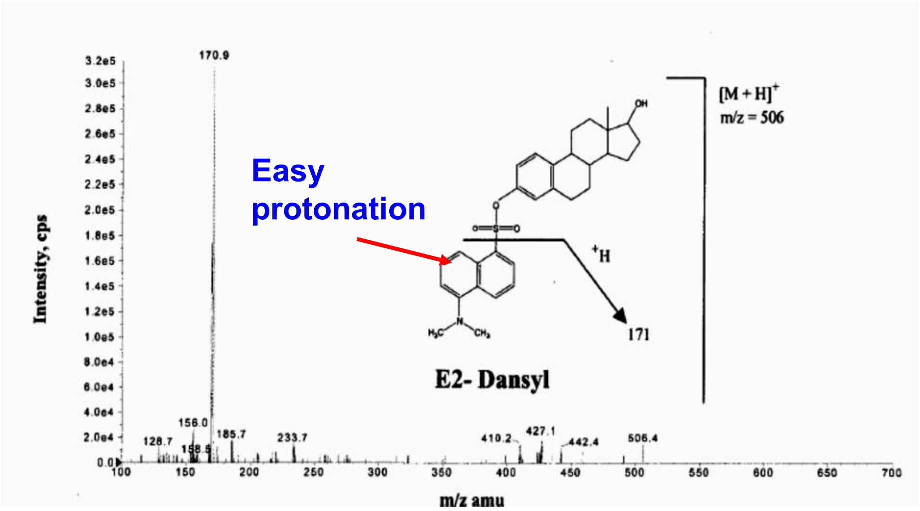
Estrone m/z 271

Estradiol Standard Curve 0.05 – 25 µM
r = 0.9959



Sensitivity is an issue in quantification of steroids

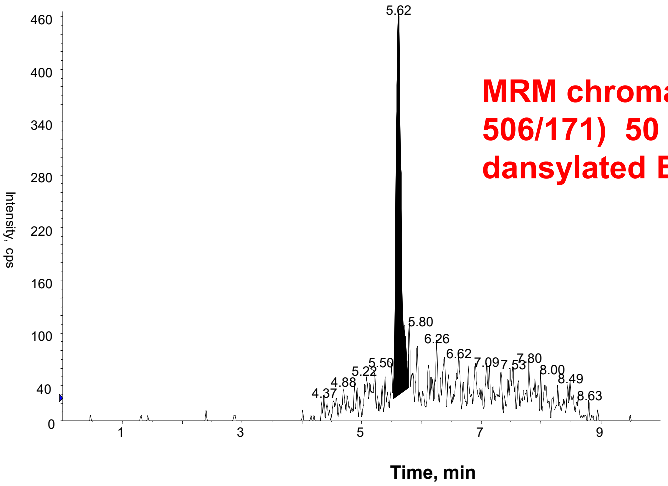
Derivatization of estradiol with dansyl chloride leads to the formation of E_2-dansyl (*m/z* 506)



Easy protonation

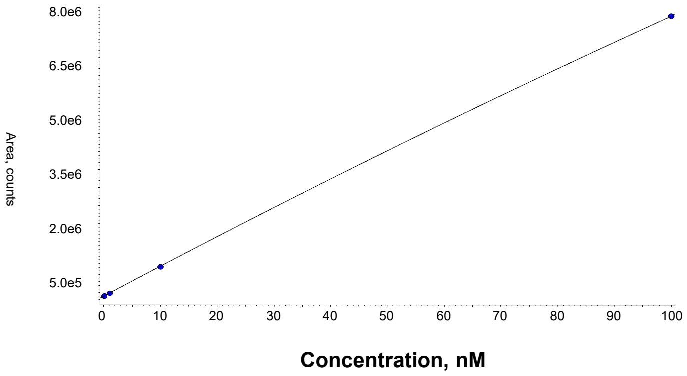
Source: Nelson et al. Clinical Chemistry, 2004

Derivatization tremendously helps increase sensitivity of E_2



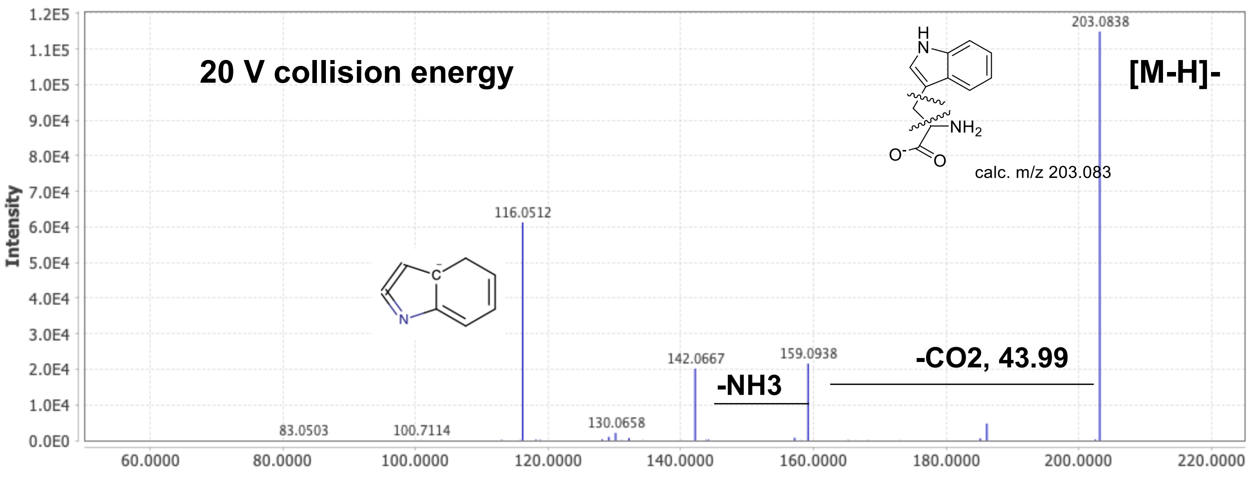
MRM chromatogram (*m/z* 506/171) 50 picomole dansylated E_2

Calibration curve for dansylated E_2 showing linearity from 0.005-100 nM concentration range (r = 0.999)



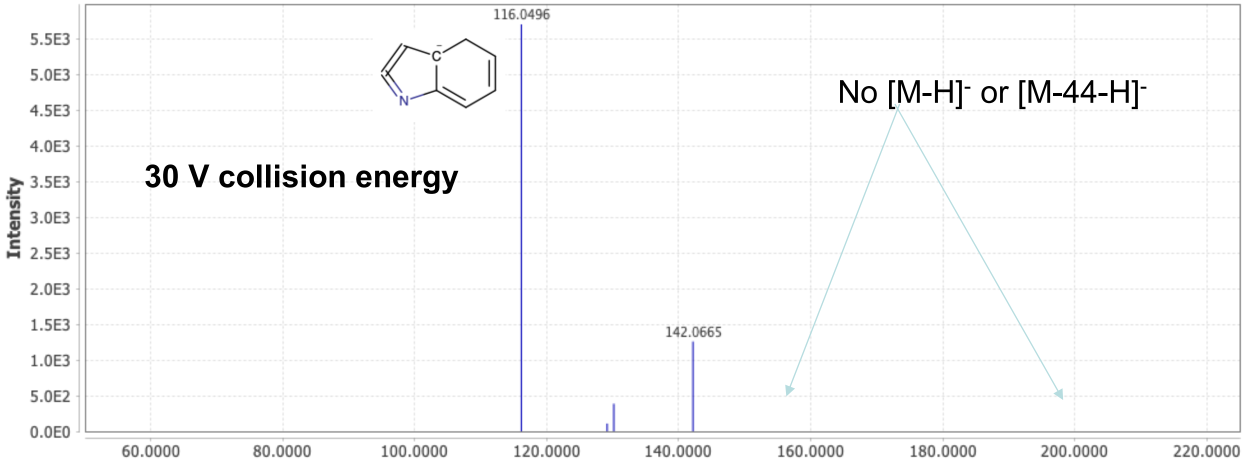
LC-MS/MS analysis of tryptophane metabolites

Negative MS/MS spectrum of tryptophan



0-7 min 2-98% linear ACN gradient in 0.1% FA: Duty cycle (250 msec) – 100 msec 50-1000 m/z TOF scan; top 30 five msec MSMS

Negative MS/MS spectrum of tryptophan

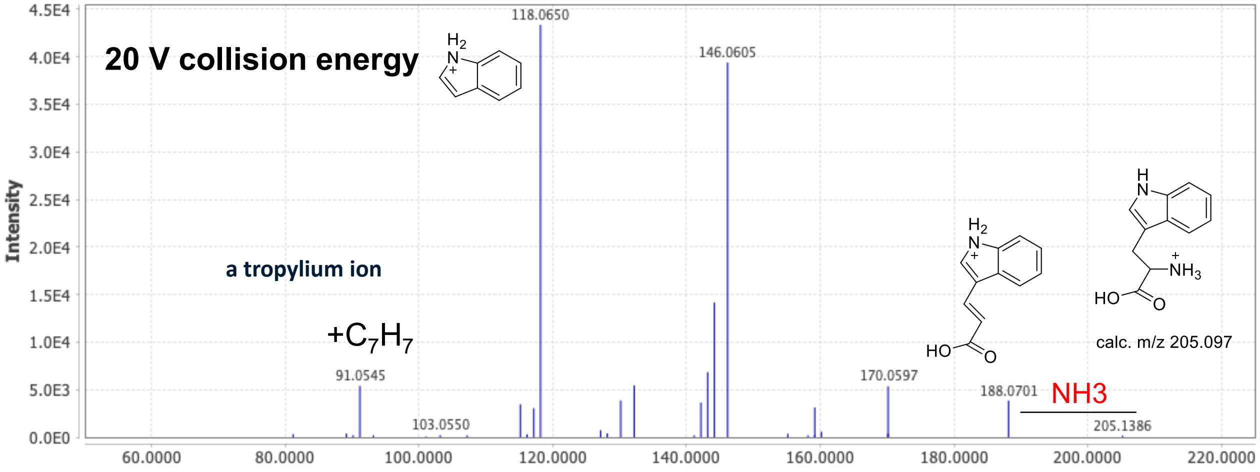


30 V collision energy

No [M-H]$^-$ or [M-44-H]$^-$

0-7 min 2-98% linear ACN gradient in 0.1% FA: Duty cycle (250 msec) – 100 msec 50-1000 m/z TOF scan; top 30 five msec MSMS

Positive ion MSMS spectrum of tryptophan



20 V collision energy

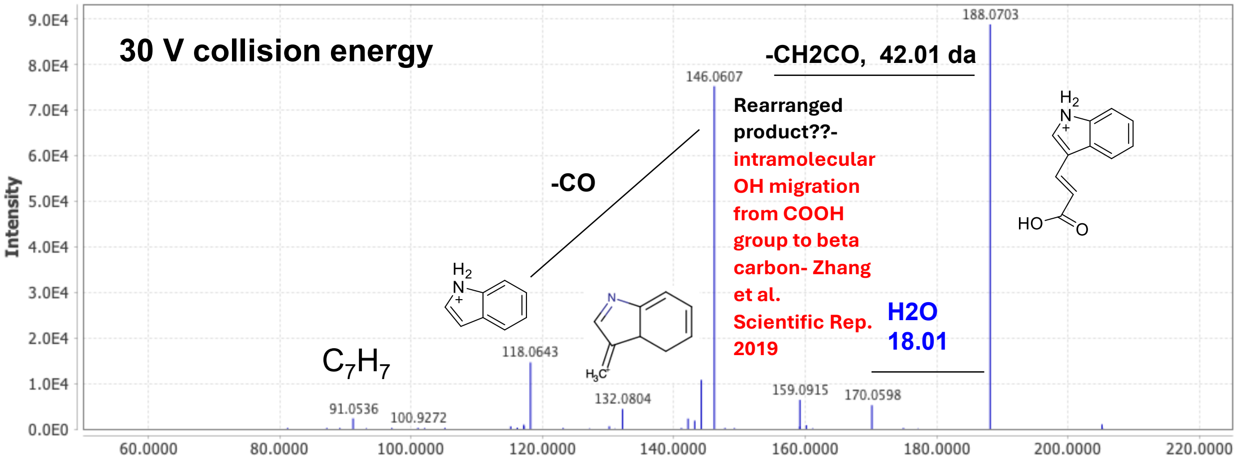
a tropylium ion

+C_7H_7

NH3

calc. m/z 205.097

Positive ion MSMS spectrum of tryptophan



30 V collision energy

-CH2CO, 42.01 da

-CO

C_7H_7

Rearranged product??- intramolecular OH migration from COOH group to beta carbon- Zhang et al. Scientific Rep. 2019

H2O 18.01

m/z 146.0607 not predicted computationally.

https://massbank.eu/MassBank/RecordDisplay?id=MSBNK-IPB_Halle-PB000416

References

1. **Electrospray Ionization Mass Spectrometry by Richard B. Cole..**
2. **Prasain J.K., Wang C.-C., Barnes S. Mass spectrometric analysis of flavonoids in biological samples. *Free Radical Biology & Medicine*, 37: 1324-1350, 2004.**
3. **William Griffiths. Tandem mass spectrometry in the study of fatty acids, bile acids and steroids. Mass Spectrometry Reviews, 2003;22:81-152.**
4. **Yi et al., Anal Bioanal Chem. 2006.**