Ion fragmentation of small molecules in mass spectrometry

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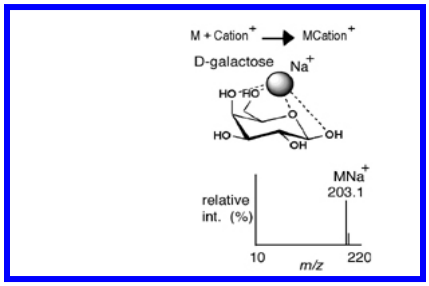
6-2612

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).



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.**

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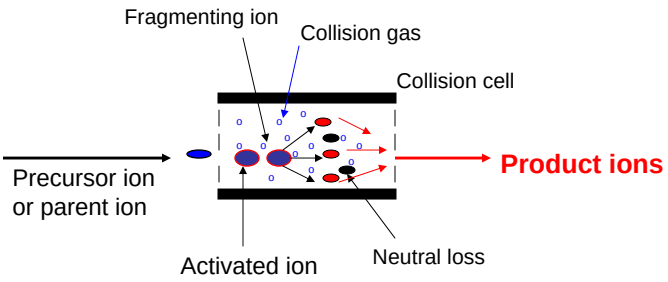
What is Collision Induced Dissociation (CID) or Collisionally Activated Dissociation (CAD) ?



Schematic of CID fragmentation

Other activation processes:
PSD (post source-decay)
ECD (electron capture dissociation)
SID (surface-induced dissociation

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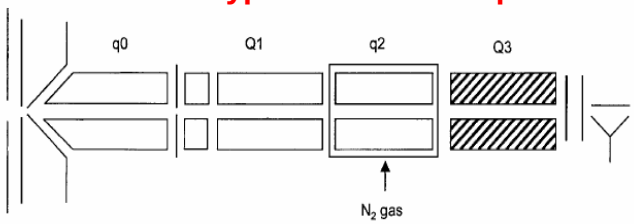
Various types of MS/MS experiments



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

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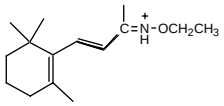
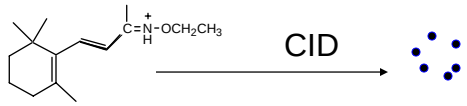
Steps involved in fragmentation

Step # 1: Creation of ions

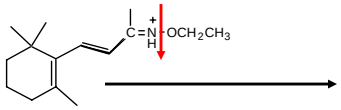
ESI



Step # 2: Add energy of activation



Step # 3: Charge directed fragmentation



Fragment ion + neutral molecule

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Applications of MS/MS

- Identification and characterization drug metabolites
- Authentification and profiling of chemical components in a crude mixture
- Substructure analysis of unknown components
- Quantification of analytes in biological samples

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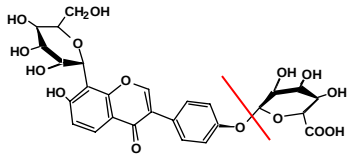
Ways to approach predicting MS/MS

- Likely sites of protonation or deprotonation.
- Likely leaving group.
- Mobility of protons
- 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

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

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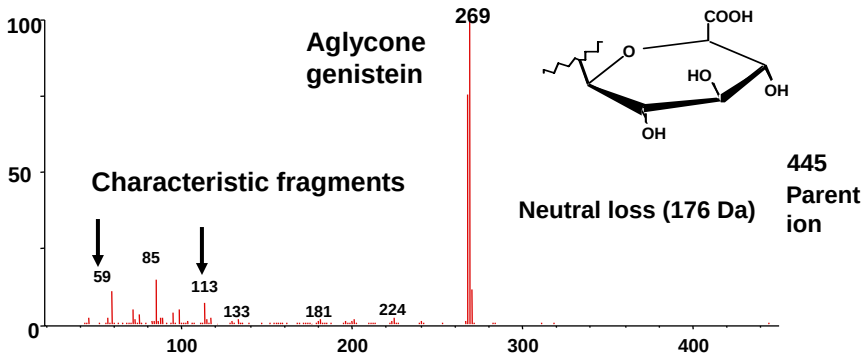
What fragment ions are characteristics for glucuronide conjugates?

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



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

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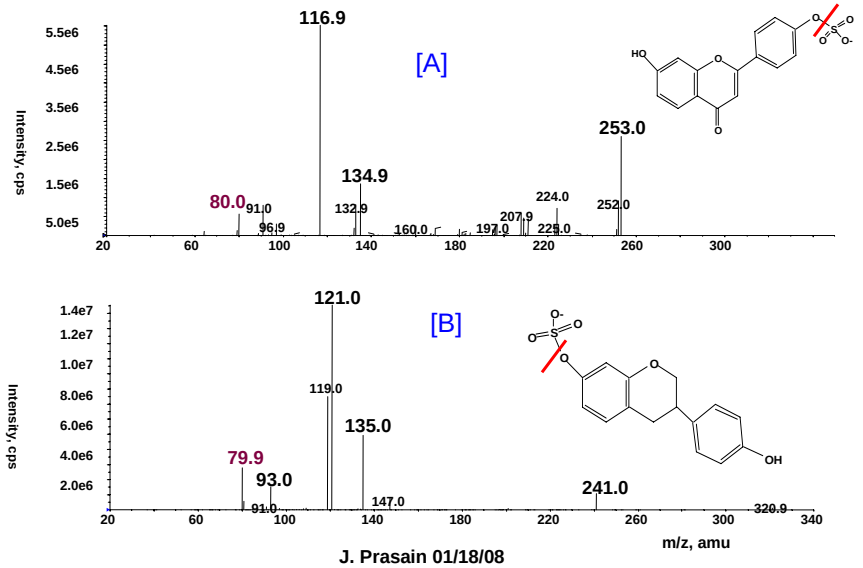
The loss of 80 Da from the parent ion and the presence of *m/z* 80 in the product ion spectra are the indicative of sulfate conjugates of phenolic compounds like daidzein [A] and equol [B]



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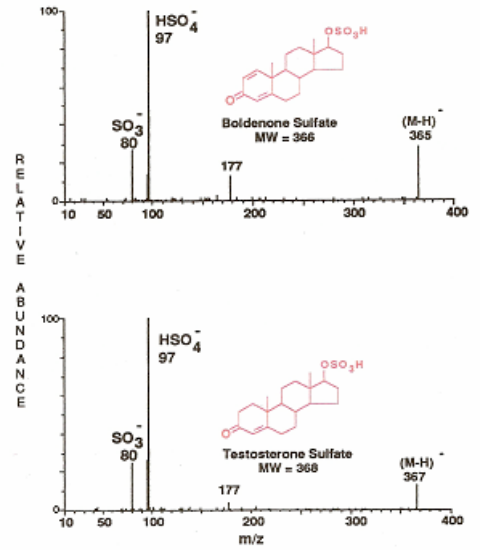
What happens with aliphatic sulfates in MS/MS?



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

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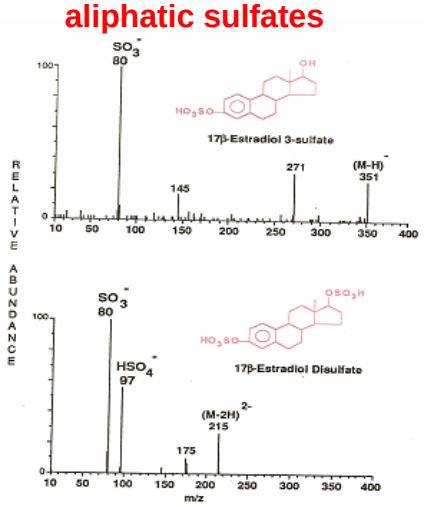
The absence of the *m/z* 97 fragment with the base peak *m/z* 80 makes the distinction between aromatic and aliphatic sulfates



Source: Weidolf et al. Biomed. and Environ. Mass Spec. 15, 283-289, 1988

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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

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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	neg	Precursor of m/z 124
N-acetylcysteins	neg	NL 129 amu

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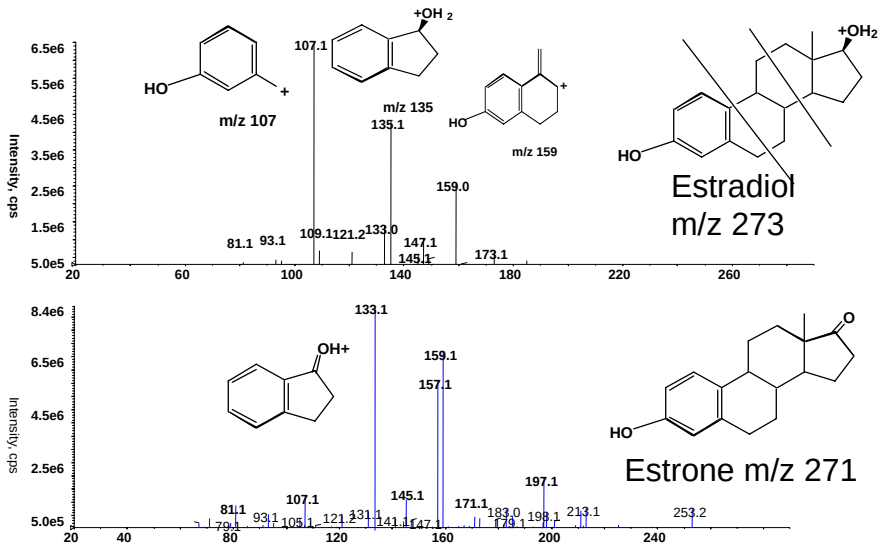
NL = neutral loss.

How steroids get fragmented in MS/MS?



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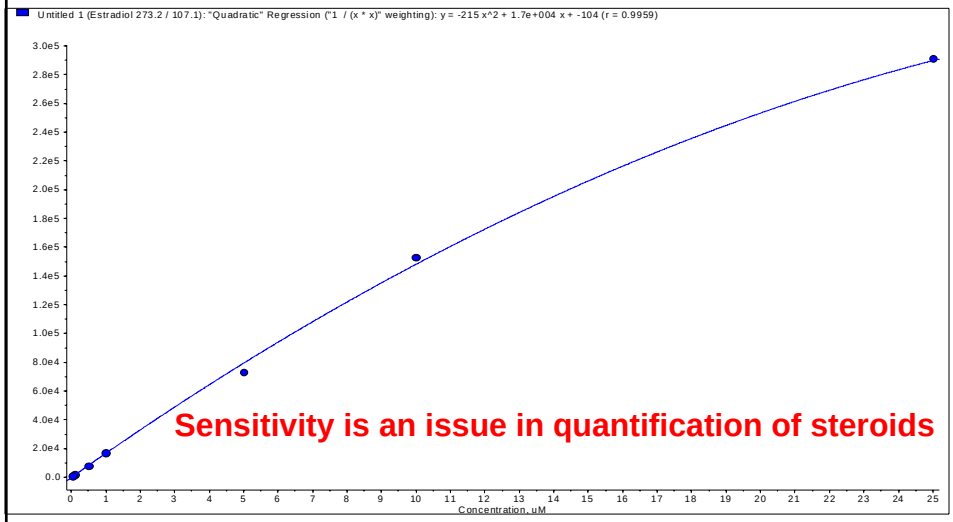
Estradiol Standard Curve 0.05 – 25 µM
r = 0.9959



Sensitivity is an issue in quantification of steroids

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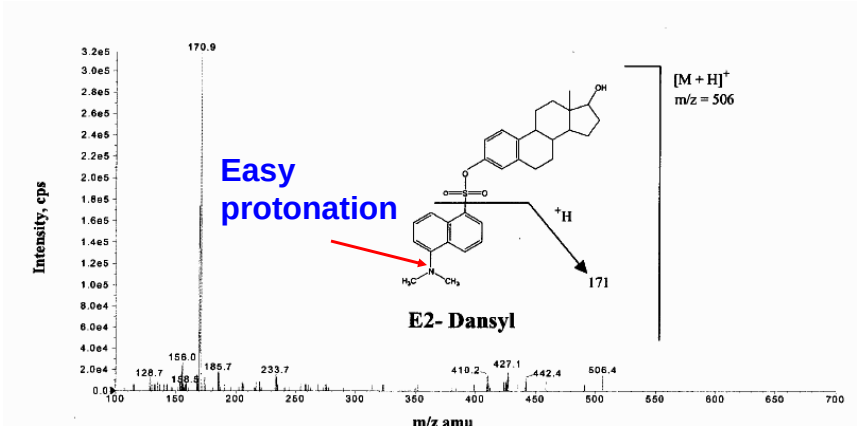
Derivatization of estradiol with dansyl chloride leads to the formation of E_2-dansyl (*m/z* 506)



Source: Nelson et al. Clinical Chemistry, 2004

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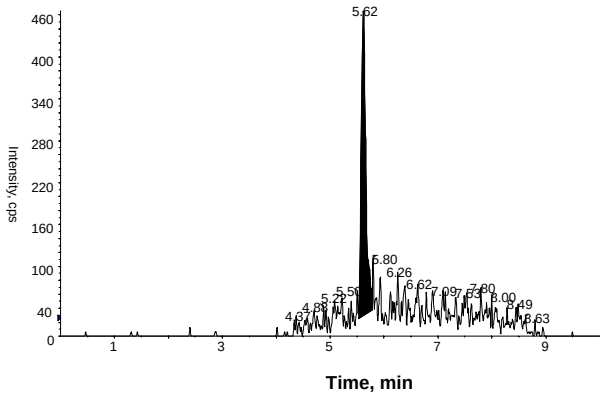
Does derivatization help increase sensitivity?

Representative MRM chromatogram (mass transition 506/171) obtained from 50 picomole concentration of dansylated E2



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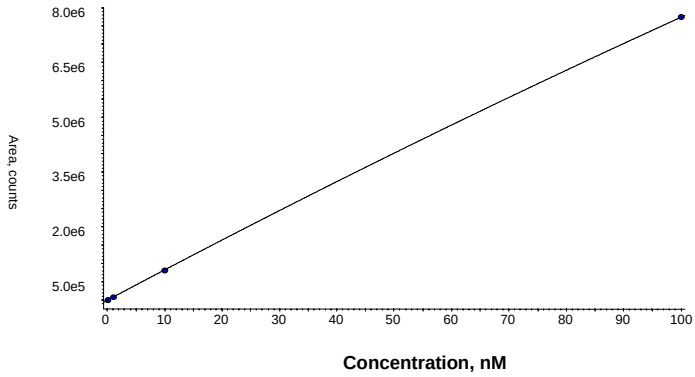
Calibration curve for dansylated E2 showing linearity from 50 picomole to 100 nanomole concentration range (r = 0.999)



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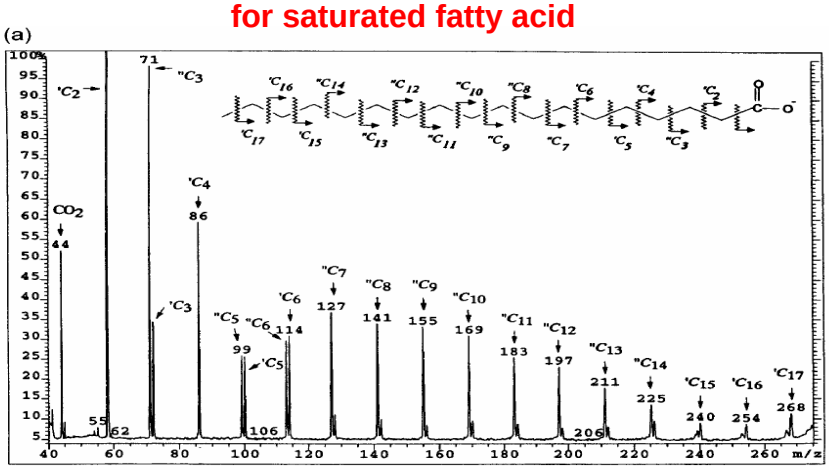
ES-MS/MS of stearic acid [M-H]- *m/z* 283. A series of ions due to the loss of CH_2 (14 Da) is characteristic for saturated fatty acid



Source: Griffiths W, Mass Spectrometry Review, 2003, 22, 81-152.

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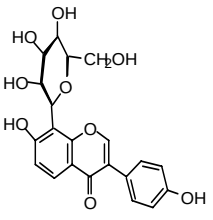
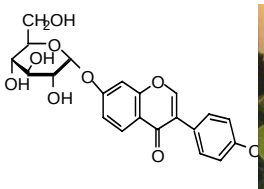
Can MS/MS analysis help distinguish isoflavone glucosides?

The Kudzu as a source of isoflavones



Puerarin, mol. wt. 416



Daidzin, mol. wt. 416

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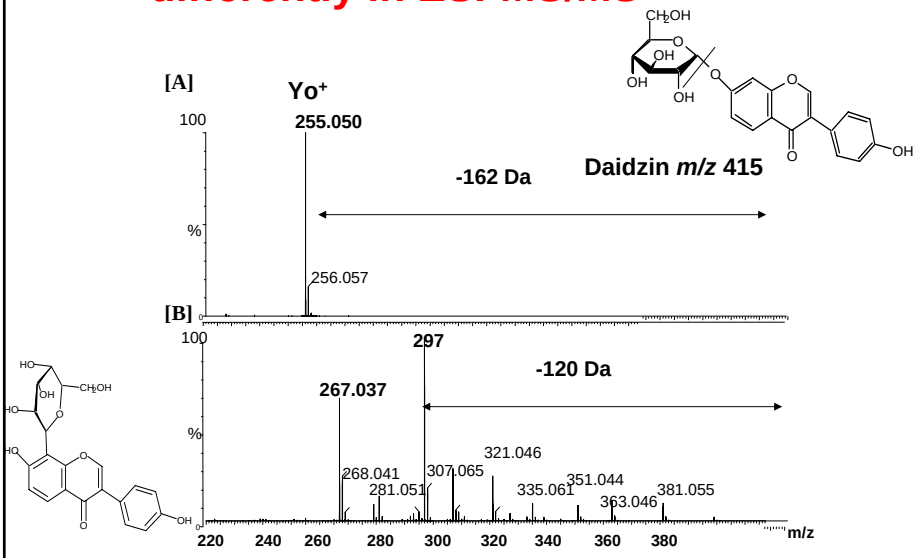
O- and C-glucosides fragment differently in ESI-MS/MS



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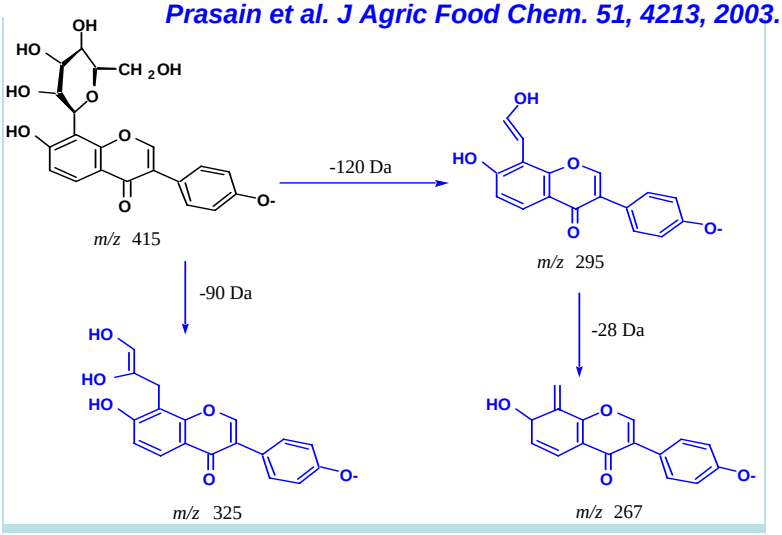
Prasain et al. J. Agric. Food Chem. 2003

Possible product ions of puerarin in ESI-MS/MS in negative ion mode

Prasain et al. J Agric Food Chem. 51, 4213, 2003.



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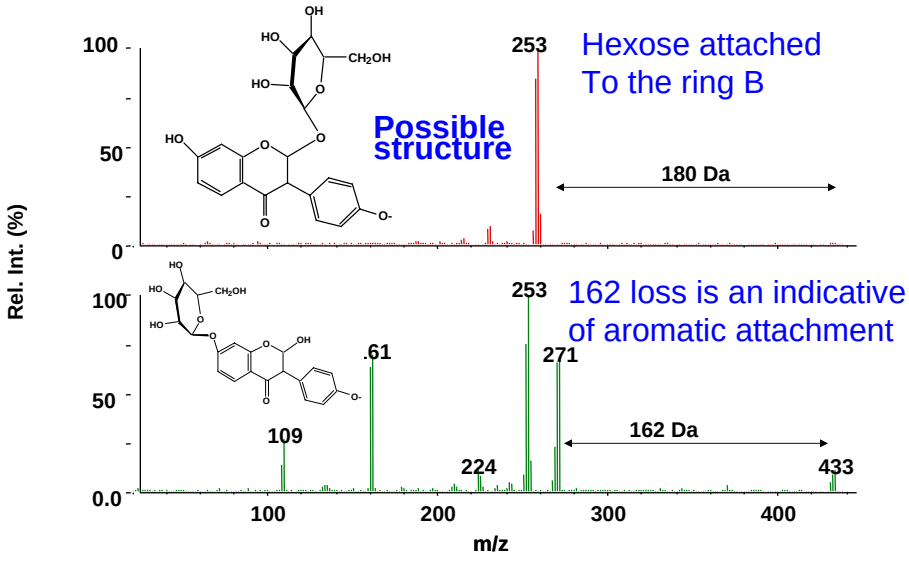
Neutral losses (162 and 180) is useful in deciding whether sugar is attached to an aromatic ring or not



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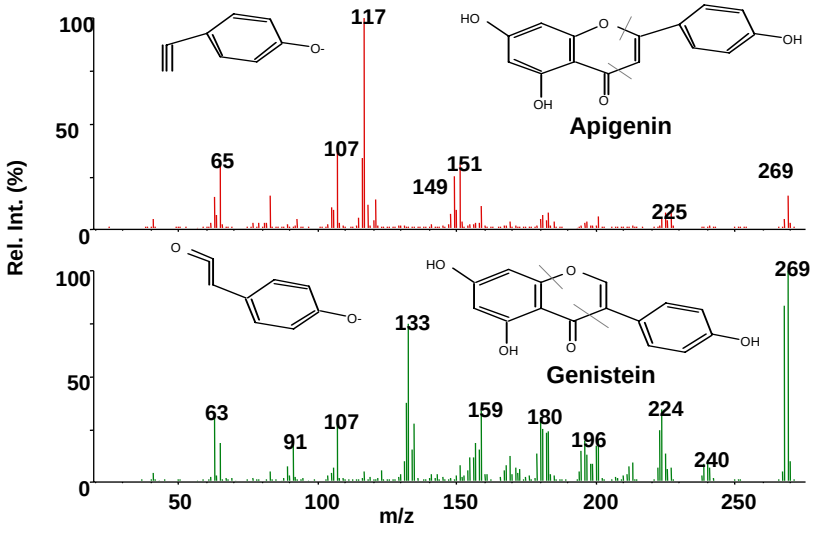
Isomers like genistein and apigenin are readily separated by tandem mass spectrometry



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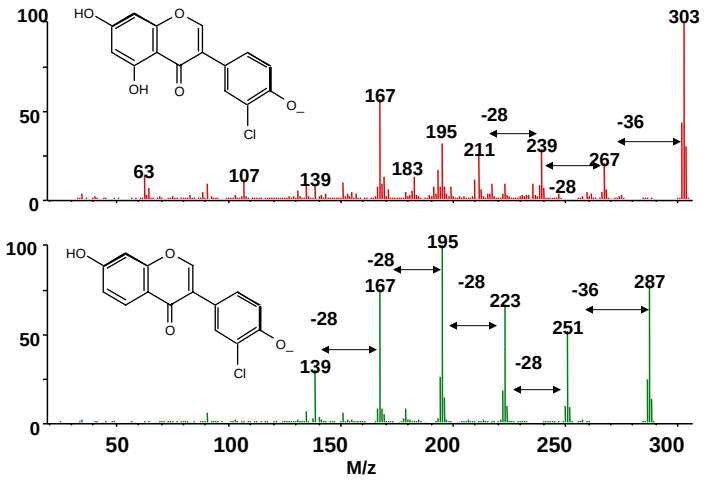
Neutral loss of HCl (36 Da) is diagnostic for 3'-chloro derivative of genistein and daidzein in ESI-MS/MS but not in 8-chloro derivatives



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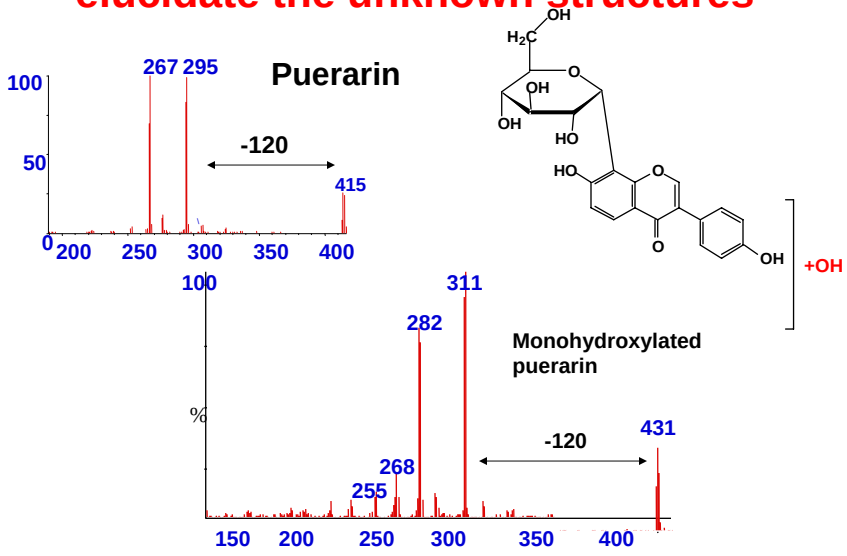
Comparison of product ions help elucidate the unknown structures



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Fragmentation of taxoids in ESI-MS/MS

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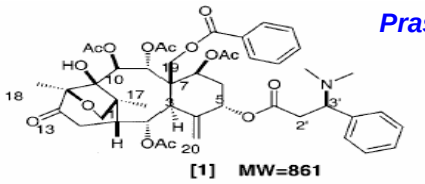
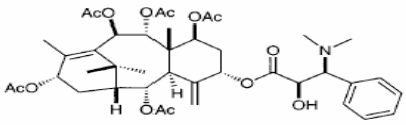
Fragmentation of basic taxoids from *T. Wallichiana* extract

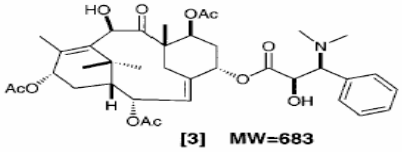
Prasain et al. Anal Chem, 2001



[1] MW=861



[2] MW=769



[3] MW=683

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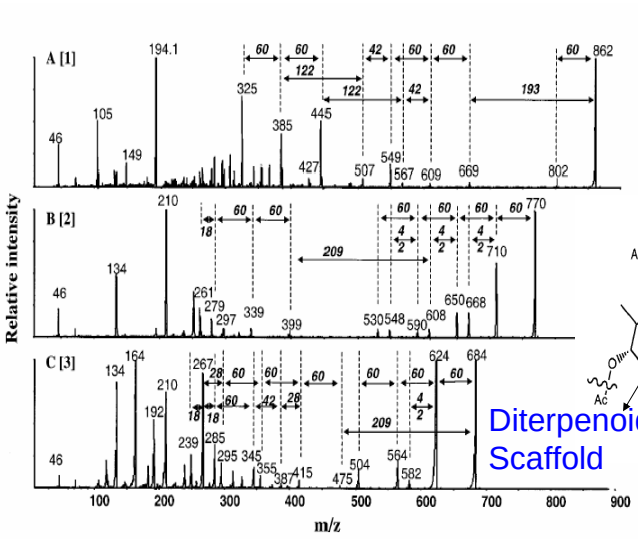
ESI-MS/MS spectra of taxoids (1-3). Peaks *m/z* 194 and 210 represent the intact alkaloid side chain.



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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 JK, Patel R, Kirk M, Wilson L, Botting N, Darley-Usmar VM, Barnes S. Mass spectrometric methods for the analysis of chlorinated and nitrated isoflavonoids: a novel class of biological metabolites. J Mass Spectrom. 2003;38:764-71.
4. William Griffiths. Tandem mass spectrometry in the study of fatty acids, bile acids and steroids. Mass Spectrometry Reviews, 2003;22:81-152.

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