Ion fragmentation in mass spectrometry

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4-7117 6-2612

Lecture goals

- Value of fragmentation in determining structure
- How peptides fragment
  - Interpreting the tandem mass spectrum
- Automating identification of peptides from their fragment ions
  - pros and cons
- Controlling fragmentation
  - Choice of ionization and fragmentation methods

Why ion fragmentation provides useful information

- **Compounds can have the same empirical formula, i.e., the same molecular weight or *m/z*, but be different chemically.**
- **Breaking them into parts (fragmenting them) helps to identify what they are.**
- **Each of the following gives the same $[M+2H]^{2+}$ ion**
  - **NH_2VFAQHLK-COOH NH_2VAFQHLK-COOH**
  - **NH_2VFQHALK-COOH NH_2VHLAFQK-COOH**
- **In proteomics we want to distinguish these peptides**

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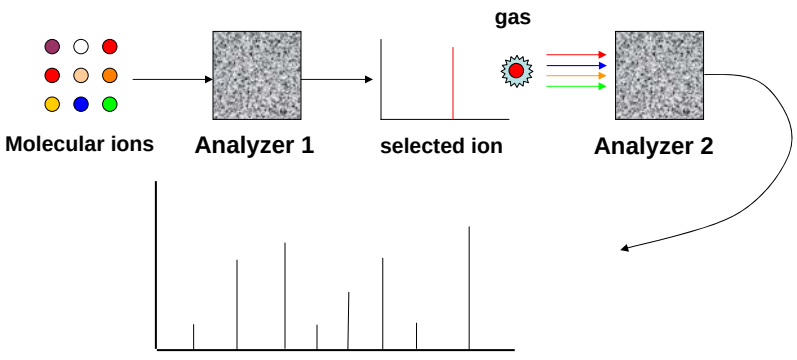
What is MS-MS (tandem mass spectrometry)?



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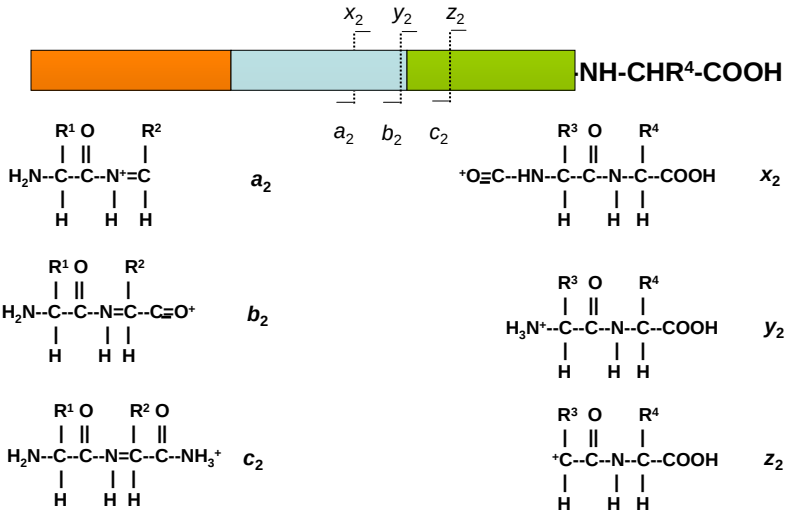
Fragmenting a peptide



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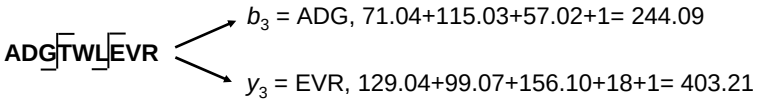
Calculating expected b- and y-ion fragments

Alanine	71.037	Leucine	113.084
Arginine	156.101	Lysine	128.094
Asparagine	114.043	Methionine	131.040
Aspartic acid	115.027	Phenylalanine	147.068
Cysteine	103.009	Proline	97.053
Glutamic acid	129.043	Serine	87.032
Glutamine	128.058	Threonine	101.048
Glycine	57.021	Tryptophan	186.079
Histidine	137.059	Tyrosine	163.063
Isoleucine	113.084	Valine	99.068

b_n = [residue masses + 1]
y_n = [residue masses + H_2O + 1]



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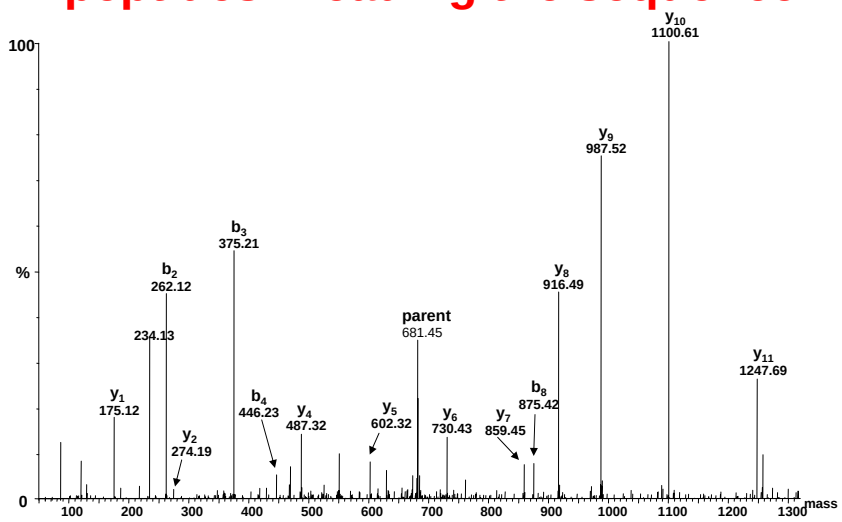
Identification of daughter ions and peptides - reading the sequence



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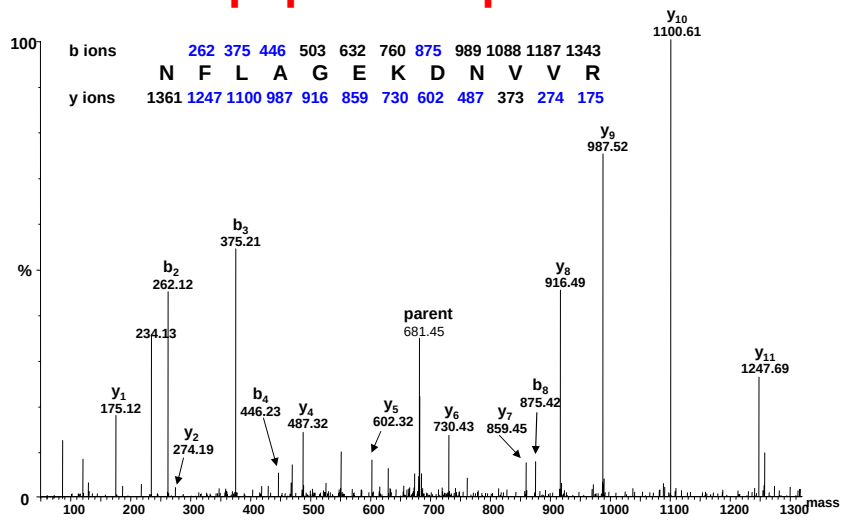
Identification of daughter ions and peptide sequence



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What's in a peptide MSMS spectrum?

- **In most cases, some, but rarely all, of the theoretic *b*- and *y*-ions are observed**
- **Besides *b*- and *y*-ions, other types of fragmentation can occur to form a_n and x_n ions, as well as also losing CO, NH_3 and H_2O groups**
- **Internal cleavage reactions can occur at acidic (Asp - Glu) residue sites**

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Other ions observed in peptide fragmentation

Immonium and Related Ions												
	87.06	120.08	86.10	---	---	102.05	84.08 101.11 129.10	88.04	87.06	72.08	72.08	70.07 87.09 100.09 112.09
N-terminal ions												
a-NH_3 ions	---	217.10	330.18	401.22	458.24	587.28	715.38	830.40	944.45	1043.52	1142.58	---
a ions	---	234.12	347.21	418.24	475.27	604.31	732.40	847.43	961.47	1060.54	1159.61	---
b-NH_3 ions	---	245.09	358.18	429.21	486.23	615.28	743.37	858.40	972.44	1071.51	1170.58	---
b-H_2O ions	---	---	---	---	---	614.29	742.39	857.42	971.46	1070.53	1169.59	---
b ions	---	262.12	375.20	446.24	503.26	632.30	760.40	875.43	989.47	1088.54	1187.61	---
	1	2	3	4	5	6	7	8	9	10	11	12
H -	N	F	L	A	G	E	K	D	N	V	V	R
y ions	---	1247.67	1100.61	987.52	916.48	859.46	730.42	602.33	487.30	373.26	274.19	175.12
y-NH_3 ions	---	1230.65	1083.58	970.50	899.46	842.44	713.39	585.30	470.27	356.23	257.16	158.09
y-H_2O ions	---	1229.66	1082.60	969.51	898.47	841.45	712.41	584.32-	---	---	--	---

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Towards automated MSMS sequencing

- **The 2D-LC-ESI-MSMS method (MuDPIT) generates 50,000+ MSMS spectra for each sample**
- **If it takes 15 min to hand interpret one MS-MS spectrum, then it would take 12,500 hours to complete the analysis. For someone working 8 hours/day and a five-day week, this would be about 6 years!**
- **Using SEQUEST and MASCOT, methods were developed to use computer-driven approaches to analyze MSMS data**

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Issues in MS-MS experiment

- **At any one moment, several peptides may be co-eluting**
- **Data-dependent operation:**
  - **The most intense peptide molecular ion is selected first (must exceed an initial threshold value)**
  - **A 2-3 Da window is used (to maximize the signal)**
  - **The ion must be in 2^+ or 3^+ state**
  - **Since the ion trap scan of the fragment ions takes ~ 1 sec, only the most intense ions will be measured**
  - **However, can use an exclusion list on a subsequent run to study minor ions**

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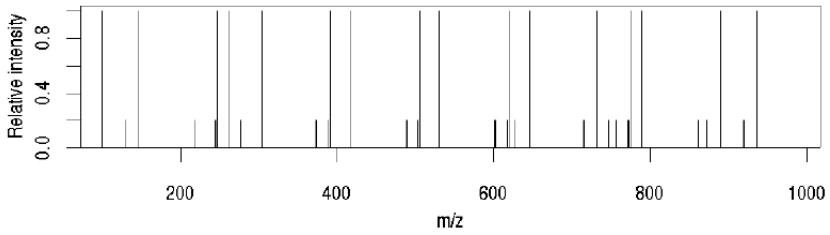
The SEQUEST approach

- **Each observed MSMS spectrum has a corresponding molecular ion $[M+nH]^{n+}$. For ion trap data, ions are selected from the known or virtual proteome that are within 1 Da. These are then "fragmented" *in silico* to produce *b*- and *y*-ions and less abundant fragment ions.**



- **The cross correlations of the observed MSMS spectrum to each of the virtual MSMS spectra are calculated. The peptides are scored and the one having the highest score is deemed to be identified.**

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What has SEQUEST provided to proteomics?

- **Initially, it seemed an awful lot! Typically, the "identified" proteins covered most of known biochemistry, so they satisfied everybody**
- **But the method obviously has limitations. There is redundancy - each protein yields multiple peptides**
- **The number of unique proteins was much less than the observed peptides**
- **Critically, it was missing controls**

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SEQUEST sequencing

- Use of SEQUEST requires considerable computing power - if there are 500 possible peptides to compare, then examination of 50,000+ spectra would require 25 million correlations
- Data analysis is typically carried out using computer clusters to accelerate the analysis

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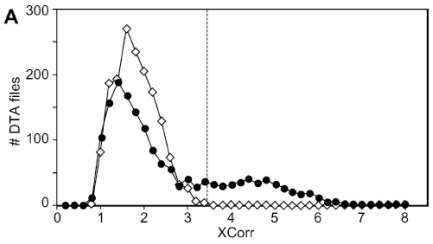
More haste, less speed?

- Post analysis, the masses of the peptides triggering MS-MS are used to create a set of virtual peptides with masses within ± 1 Da
- Predicted MS-MS are compared to the observed and the best fit is reported as a hit
- The abundance of these hits are plotted in the figure as closed circles

Resing et al. (2004)



However, if the sequences of the peptides within ± 1 Da are reversed *in silico* and their predicted MS-MS compared to the observed spectra, a similar histogram is obtained (open circles), but without the right side tail

A forced fit to a set of data will always come up with a match, but not necessarily the truth

A reversed sequence has its problems as a control

Normal sequence

b ions		262	375	446	503	632	760	875	989	1088	1187	1343
	N	F	L	A	G	E	K	D	N	V	V	R
y ions	1361	1247	1100	987	916	859	730	602	487	373	274	175

Reversed sequence

b ions		256	355	469	584	712	841	898	969	1082	1229	1343
	R	V	V	N	D	K	E	G	A	L	F	N
y ions	1361	1205	1106	1007	893	778	650	521	464	393	280	133

So, $b_{2corr} = y_{2reverse} - 18$ and $y_{2corr} = b_{2reverse} + 18$

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How to improve MUDPIT

- Reproducible column engineering
  - Tandem columns, each built to separate, but high specifications
  - Columns on a chip
- More careful selection of the parent ion
  - Accurate measurement of the peptide's mass will eliminate many false peptides
  - Accurate measurement of peptide fragments' masses
- Greater stringency in assessing score cutoff

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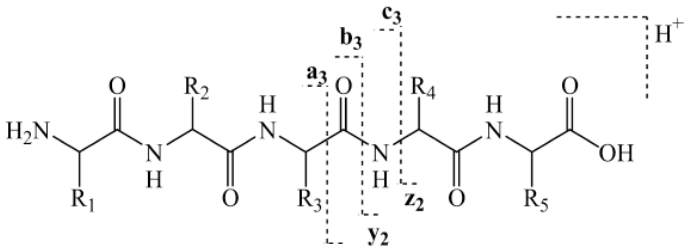
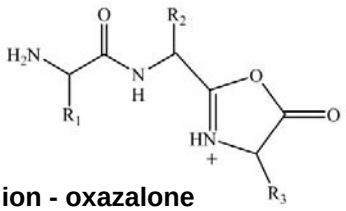
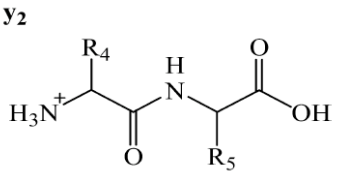
Let's take a closer look at fragmentation







b_3 ion - oxazalone
no b_1 ion

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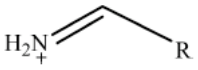
Wysocki et al. 2005

Other amino acid fragment ions

m/z values of common immonium ions

Immonium ion (*m/z*)	Amino acid residue	Major (M) or minor (m) peak
60.04	S	M
70.07	R or P	M
72.08	V	M
73.00	R	m
74.06	T	M
84.08	K or Q	M
86.1	I or L	M
87.09	N or R	M
88.04	D	M
100.09	R	m
101.11	K or Q	M
102.06	E	M
104.05	M	M
110.07	H	M
112.09	R	M
120.08	F	M
126.06	P	M
129.1	K or Q	m
136.08	Y	M
138.07	H	m
159.09	W	M



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Wysocki et al. 2005

Detecting posttranslational modifications by MS

- A key issue is that the energy of ionization or the collisional process should not exceed the dissociational energy of the PTM
- MALDI-TOF MS with a N_2 laser causes fragmentation of a nitrated tyrosine residue
  - Use ESI to make the molecular ion
  - Go to another wavelength
- O-glucosyl groups fragment more easily than the peptide to which they are attached
  - Use electron capture dissociation

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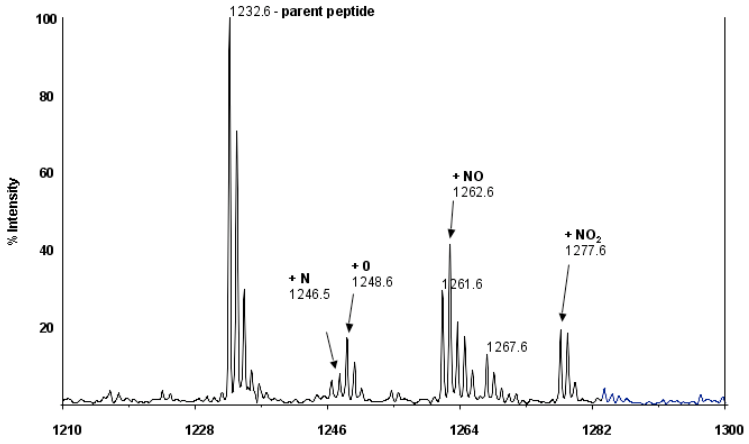
Fragmentation of nitrated peptides in MALDI-TOF experiment



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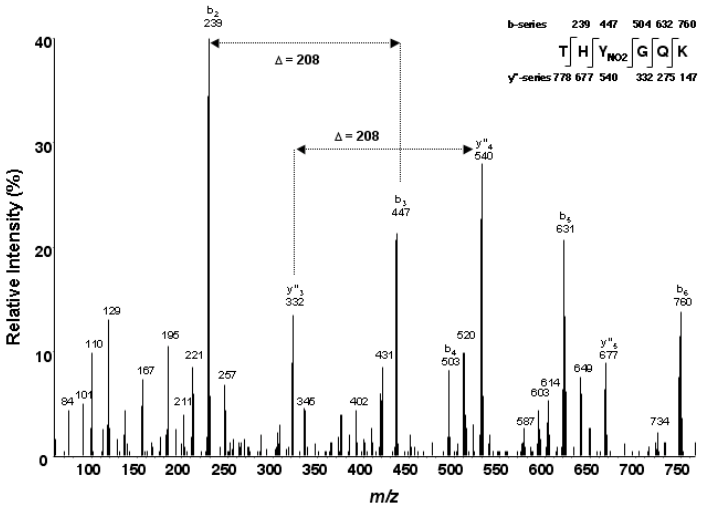
ESI-tandem MS of a nitrated peptide



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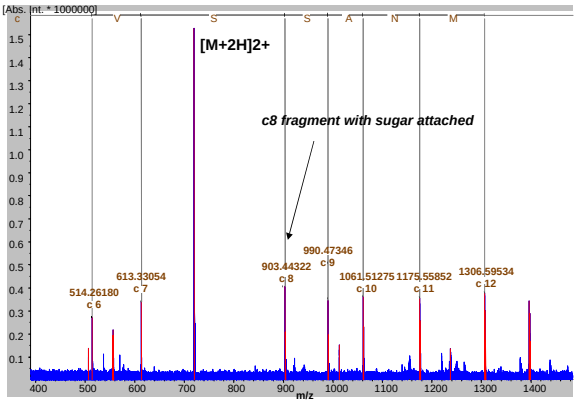
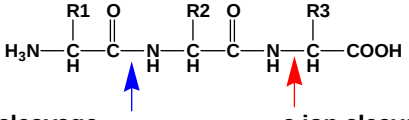
Sequencing O-GlcNAc peptides by ECD FT-ICR-MS

Casein kinase II - AGGSTPVSSANMMSG





b ion cleavage c ion cleavage

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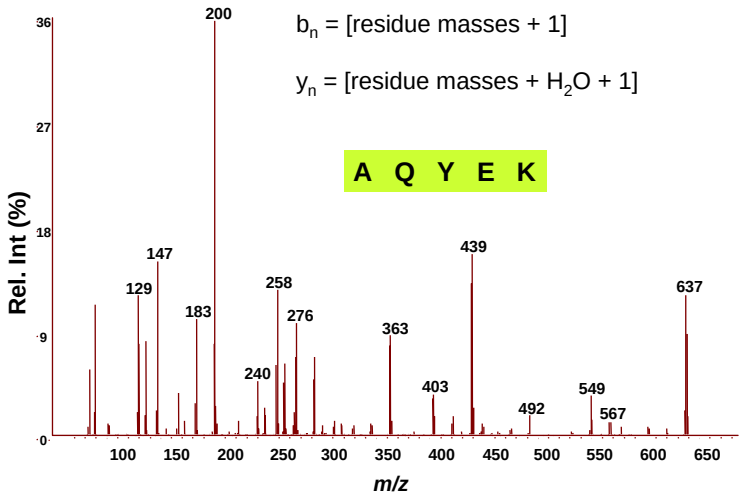
Fragment ions of a small 5-mer peptide

Homework - write down the masses of the b and y ions



b_n = [residue masses + 1]

y_n = [residue masses + H_2O + 1]

A Q Y E K

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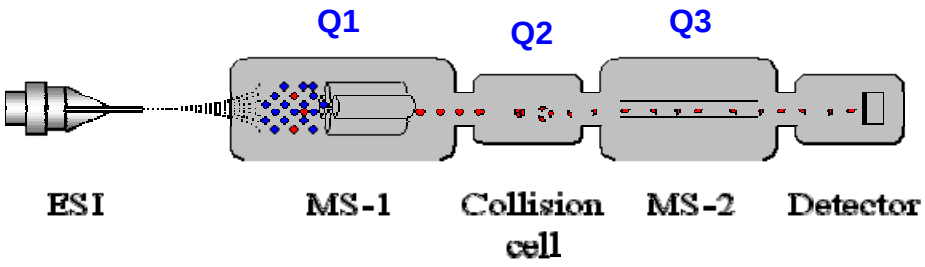
What is tandem mass spectrometry?

- Tandem mass spectrometry, abbreviated MS/MS, is any general method involving at least two stages of mass analyzers (one to pre-select an ion and the second to analyze fragments induced. This dual analysis can be dual in space, or dual in time. The most commonly used tandem mass spectrometry is the triple quadrupole.



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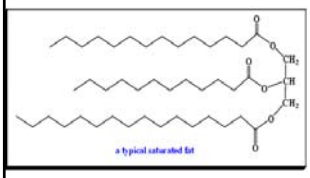
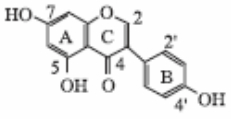
Metabolomics and small molecules

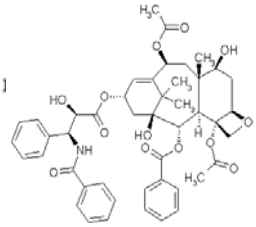
Metabolomics is an emerging "omics" science that determines the physiological status of a sample or tissue by comparing the concentration of small molecules (biochemicals) in a tissue or sample with a similar measurement in a control sample. Small molecules are a diverse group of natural and synthetic substances that generally have a low molecular weight (f. w. 150-3000).



Triglycerides



Genistein (a plant secondary metabolite)



Taxol

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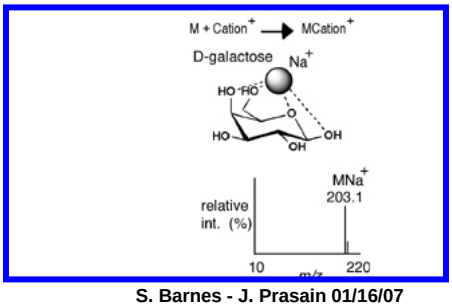
Nomenclature: the main names and acronyms used in MS/MS

- **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).**



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Contd..

- **Pseudomolecular ion:** Ion originating from the analyte molecule by abstraction of a proton [M-H]- or addition of proton [M+H]+
- **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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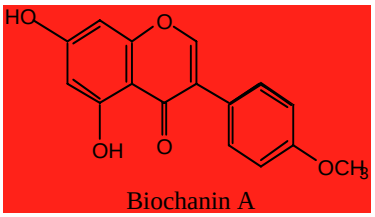
Ways to approach predicting MS/MS

- 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?



Biochanin A

Fragmentation always follows the basic rules of chemistry

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Applications of MS/MS

- **Identification, characterization and authentification of chemical components in a crude mixture**
- **Substructure analysis of unknown components**
- **Quantification of analytes in biological samples**

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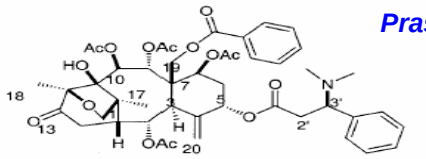
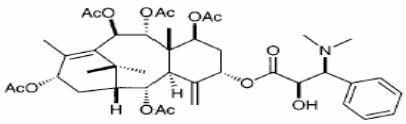
Profiling taxoids metabolites in *T. Wallichiana* extract

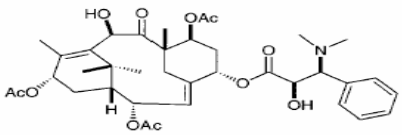
Prasain et al. Anal Chem, 2001



[1] MW=861



[2] MW=769



[3] MW=683

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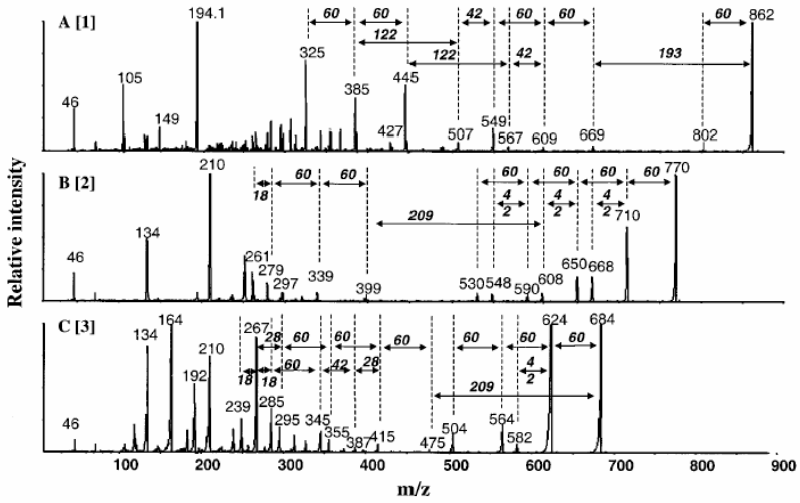
ESI-MS/MS spectra of taxoids (1-3). Peaks *m/z* 194 and 210 represent the intact alkaloid side chain.



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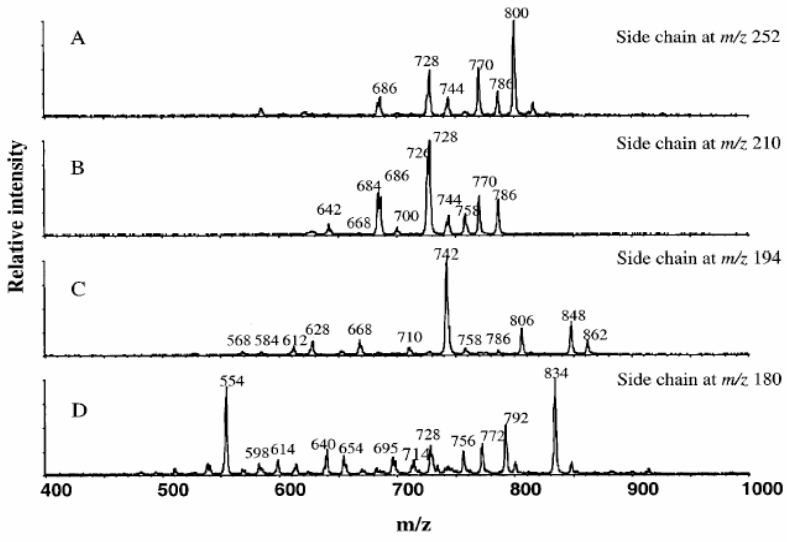
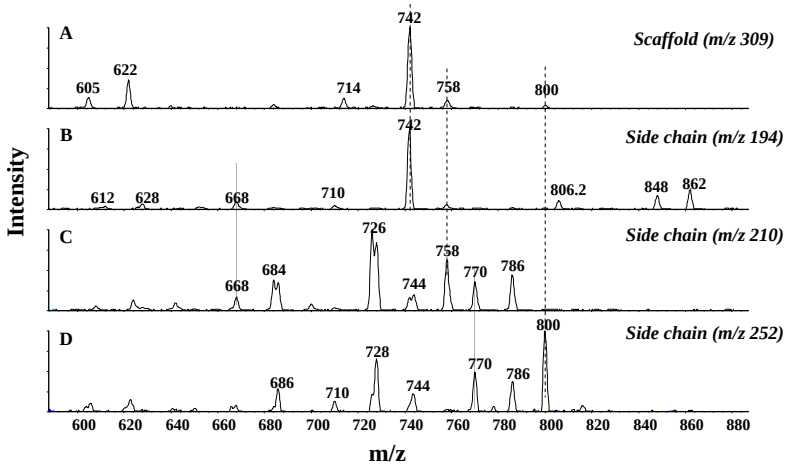
MS/MS precursor-scan spectra of typical alkaloid side chains to identify the basic taxoids compounds in an ethyl acetate extract of *T. wallichiana*.



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

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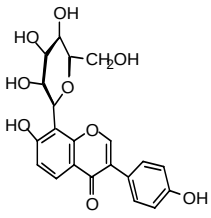
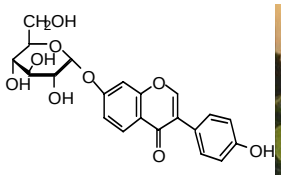
MS/MS may help distinguish isomer structures

The Kudzu as a source of isoflavones



Puerarin, mol. wt. 416



Daidzin, mol. wt. 416

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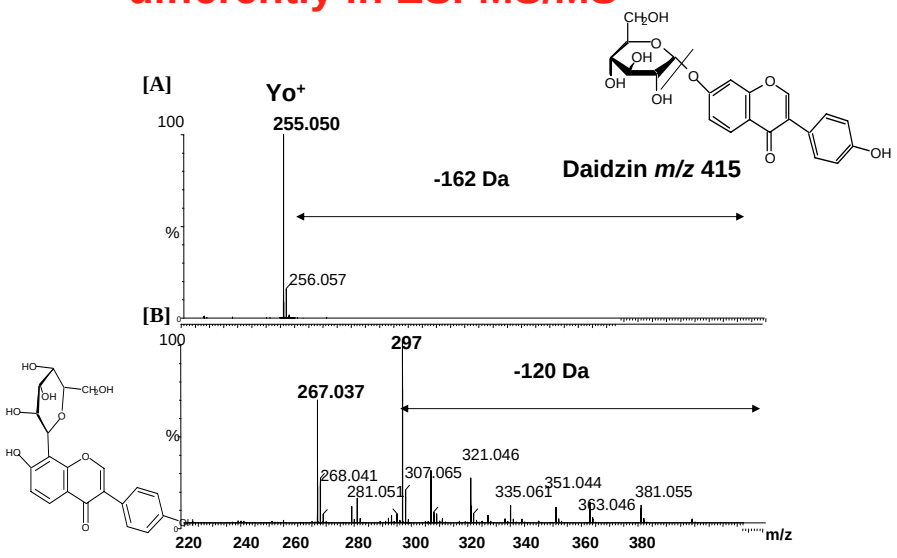
O- and C-glucosides fragment differently in ESI-MS/MS



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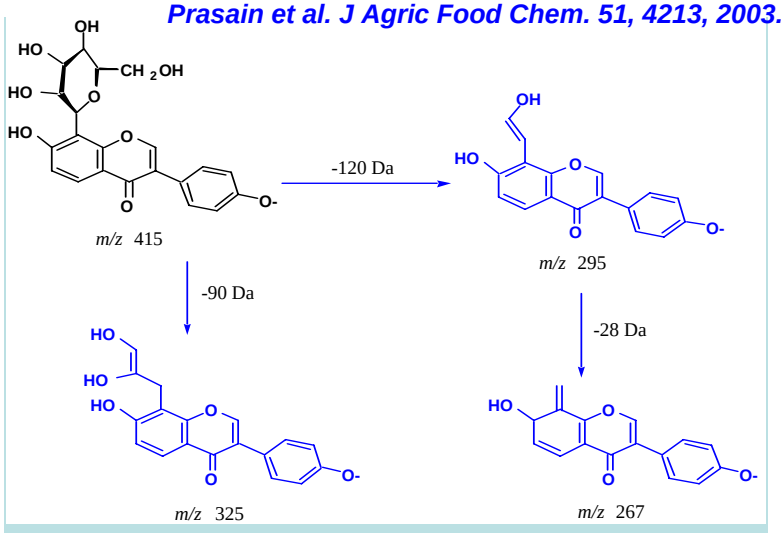
Prasain et al. J. Agric. Food Chem. 2003

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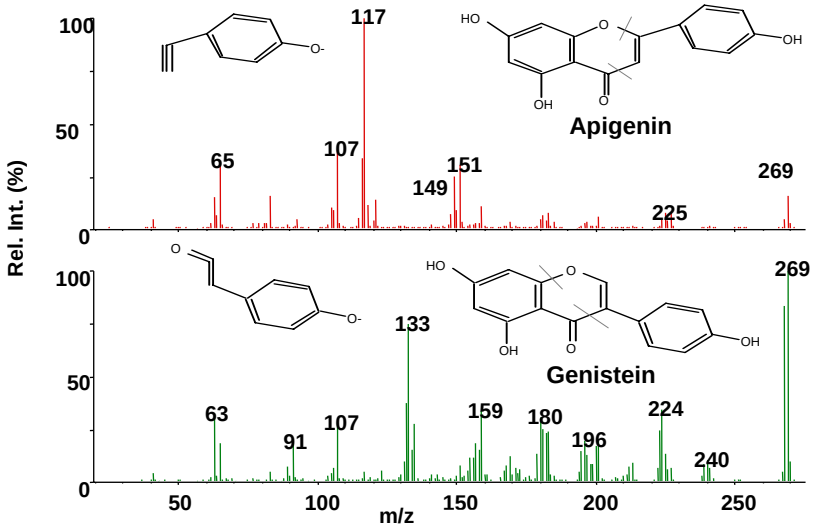
Isomers like genistein and apigenin are readily separated by tandem mass spectrometry



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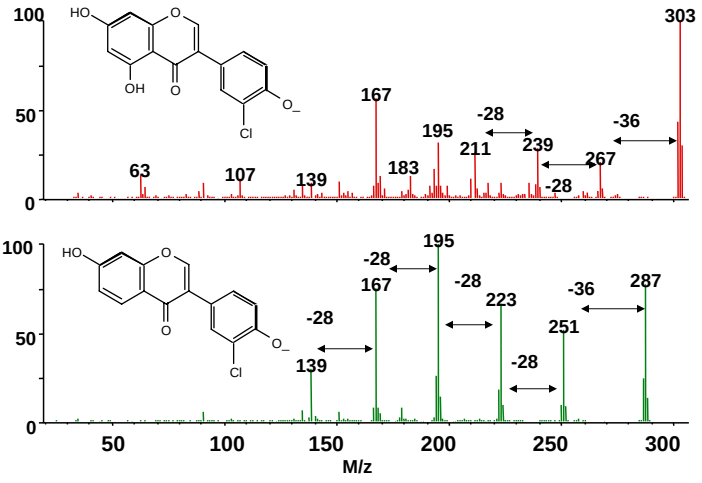
HCl loss (36 Da) is diagnostic for 3'-chloro derivative of genistein and daidzein in ESI-MS/MS



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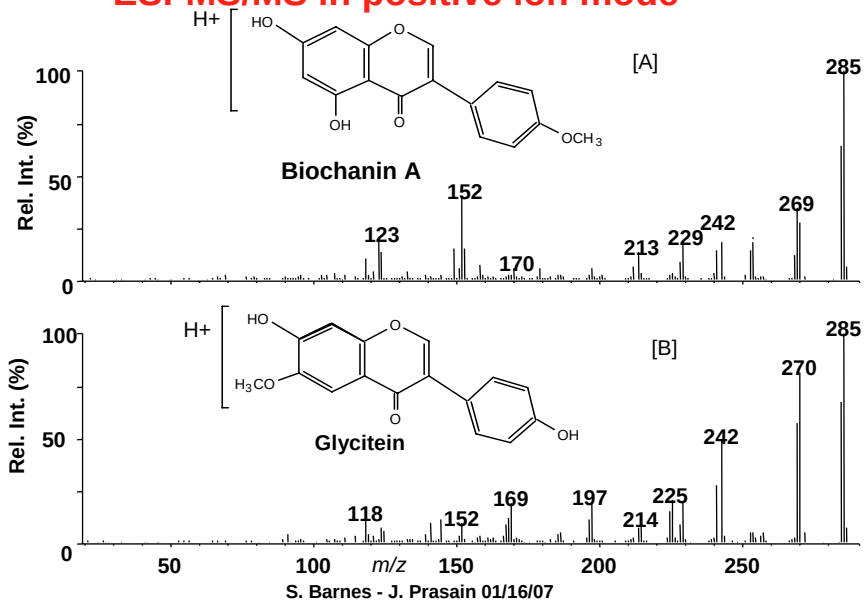
Biochanin A and glycitein can be distinguished by ESI-MS/MS in positive ion mode



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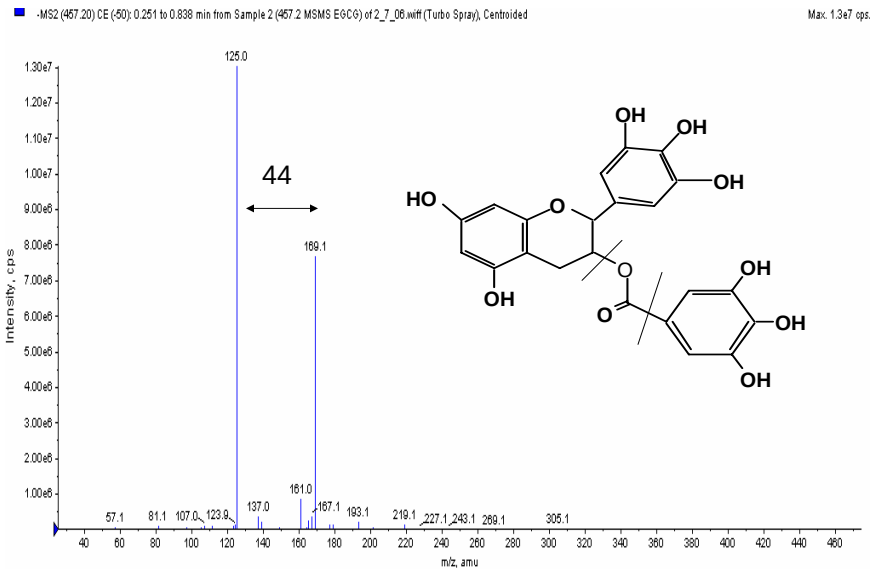
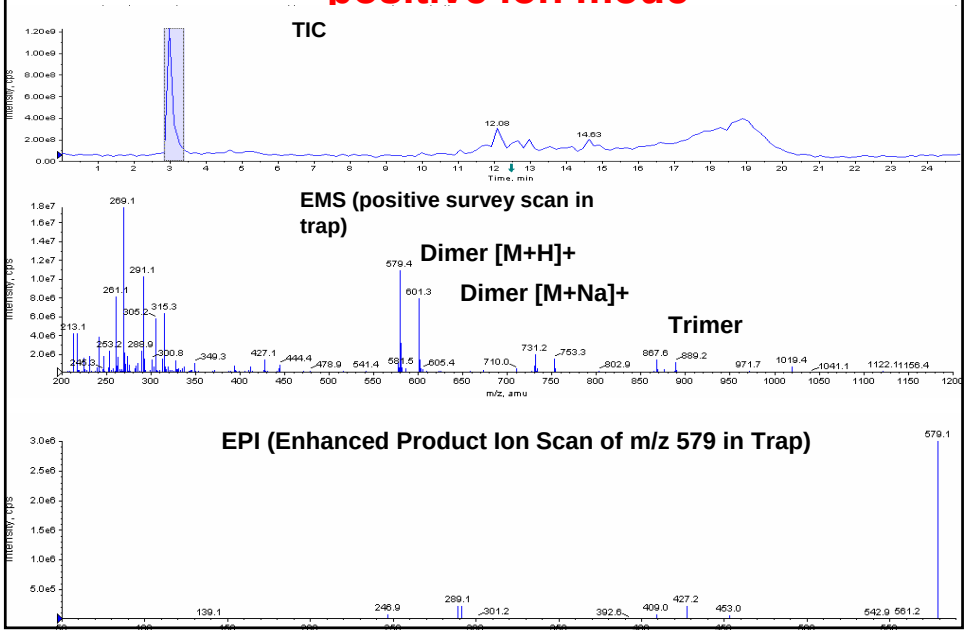
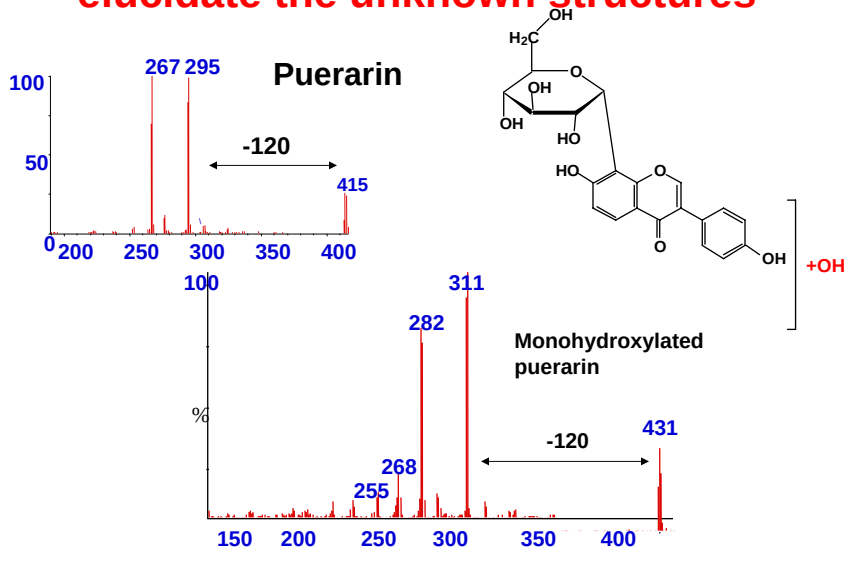
Intensity of product ions indicates their stability - ions bearing aromatic ring are more intense



Metal ion adducts are common in the positive ion mode



Comparison of product ions help elucidate the unknown structures



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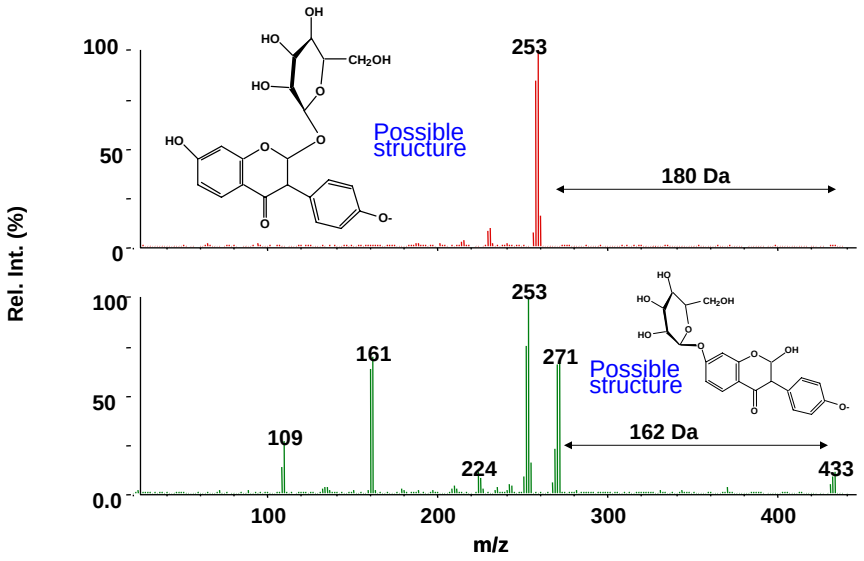
Tandem mass spectrometry can provide crucial information on structure of unknown glycosides



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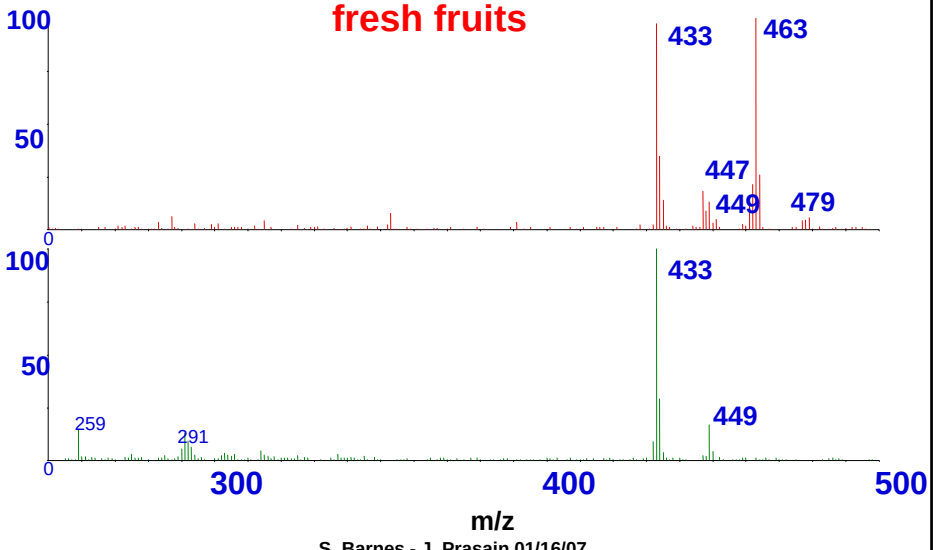
Ions obtained from neutral loss scan of 162 [A] and 132 [B] In the EtOAc extract of cranberry fresh fruits



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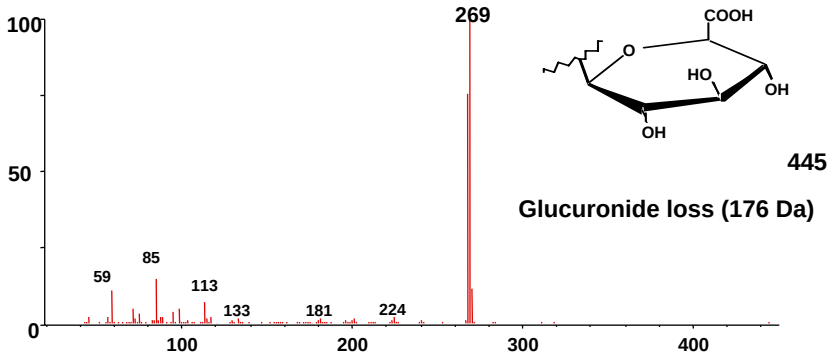
Product ion spectrum of genistein glucuronide in ESI-MS/MS



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

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Change in mass is associated with possible metabolic reactions

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

Conjugat	Ionization mo	Scan
Glucuronides	pos/neg	NL 176 amu
Hexose sugar	pos/neg	NL 162 amu
Pentose sugar	pos/neg	NL 132 amu
Phenolic sulp	pos	NL 80 amu
Phosphate	neg	Precursor of m/z 79
Aryl-GSH	pos	NL 275 amu
Aliphatic-GSH	pos	NL 129
taurines	ps	Precursor of m/z 126
N-acetylcyste	neg	NL 129 amu

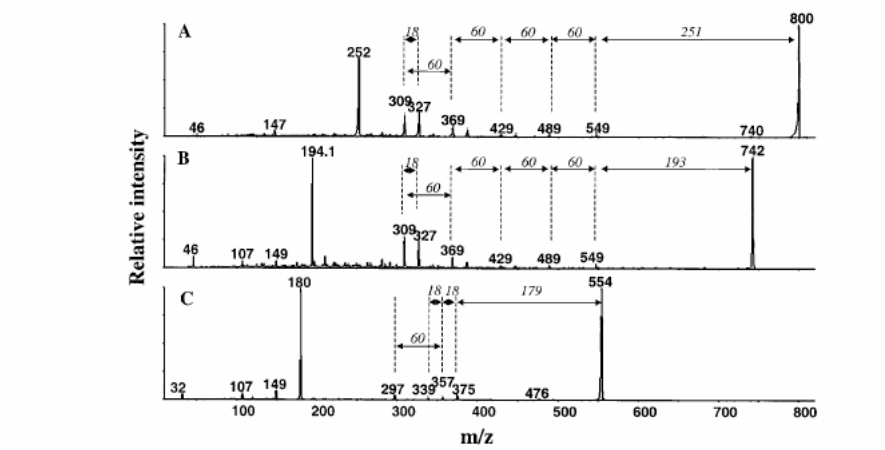
NL = neutral loss.

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.

Question-?

What structural information do you get from the MS/MS Spectra shown below.?



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