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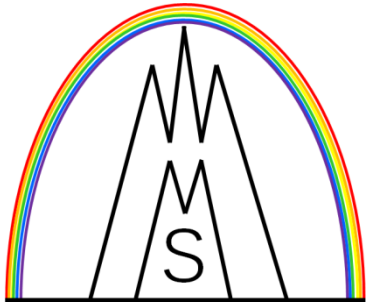

Understanding Mass Spectra of Small Molecules

Josef Cvačka

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ESI, ACN/H2O
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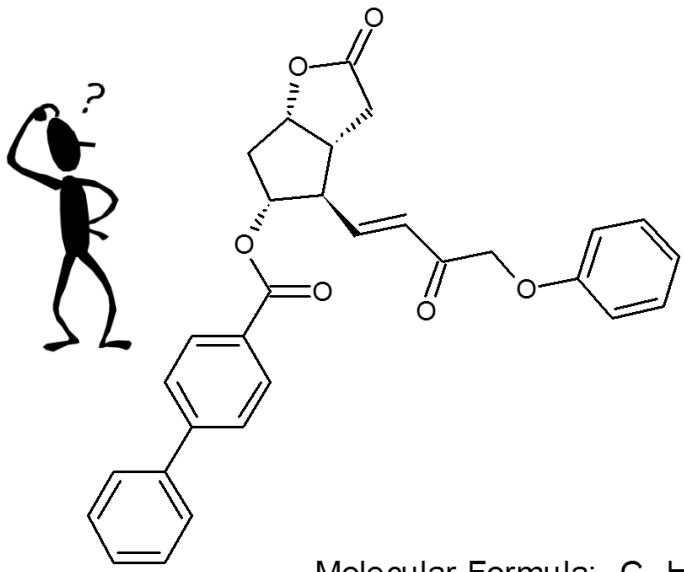
T: ITMS + c ESI Full ms [150.00-600.00]



Molecular Formula: $C_{30}H_{26}O_6$
Monoisotopic Mass: 482.172939 Da

General interpretation procedure for mass spectra

1/ Identification of signals that are not related to the analyte

2/ Determination of the molecular weight – looking for molecular ions $M^{+\bullet}$, molecular adducts $[M + H]^+$, $[M + Na]^+$, $[M + Cl]^-$, deprotonated molecules $[M - H]^-$, dimers and multiply charged ions

3/ Identification of the elements which can be present: inspection of isotope cluster, application of nitrogen rule

4/ Determination of the elemental formula from exact mass measurement

5/ Searching the spectrum against libraries looking for at least a similar spectrum

6/ Solving fragmentation spectra (requires knowledge of fragmentation mechanisms and empirical rules)

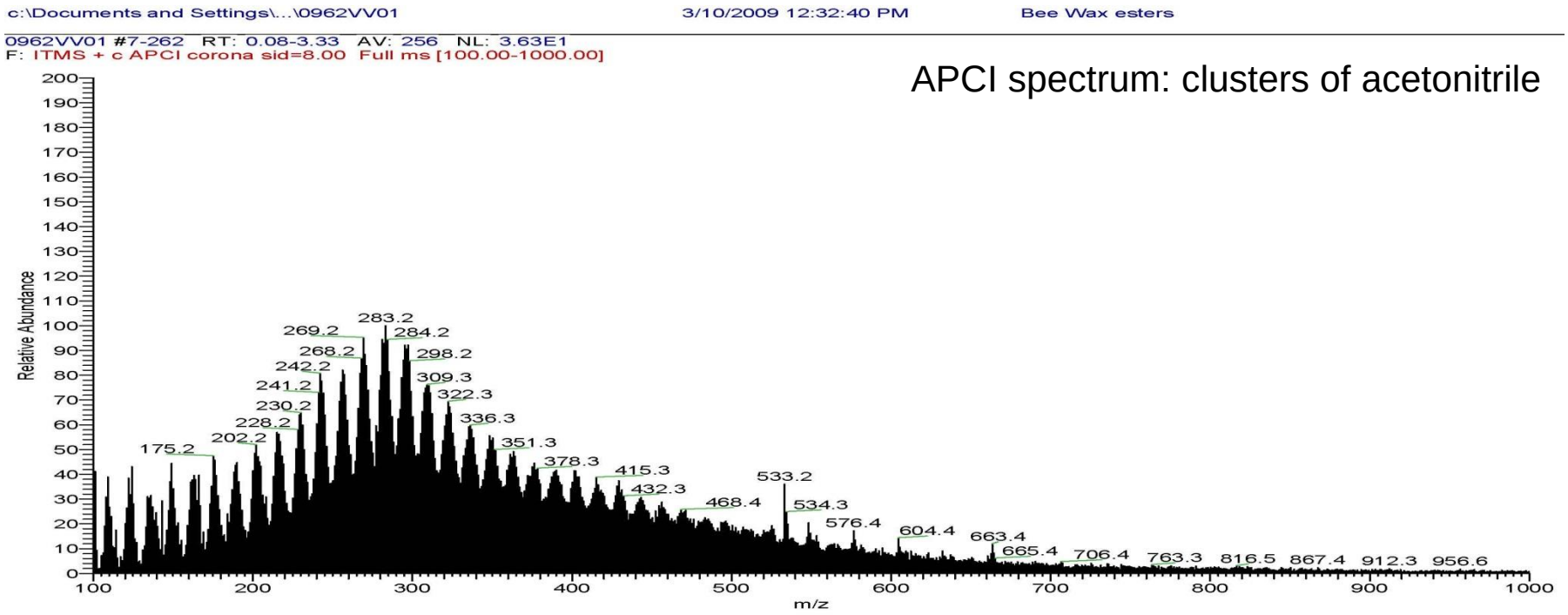
1.

Ions, which are not related to the analyte

Background ions, contaminants

Mass spectra often contain signals which are not related to the analyte:

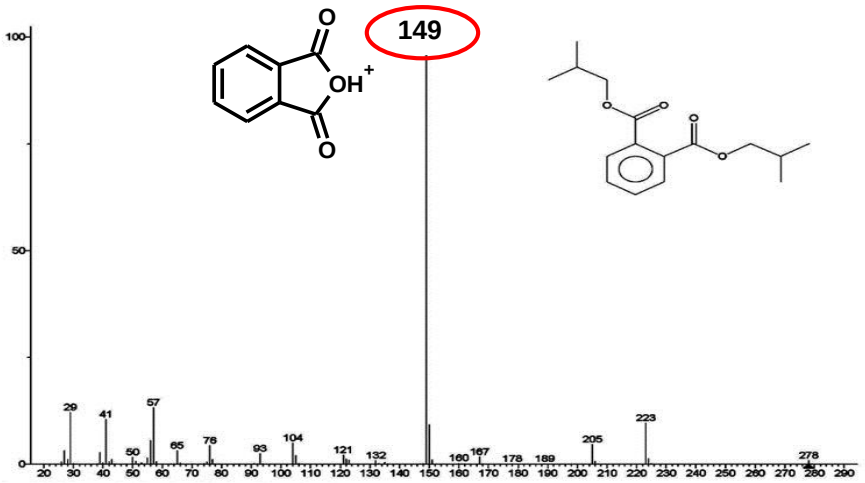
- impurities (from sample handling, solvents, previous injections)
- column bleeding peaks (GC/MS)
- solvent cluster ions
- matrix ions (MALDI)



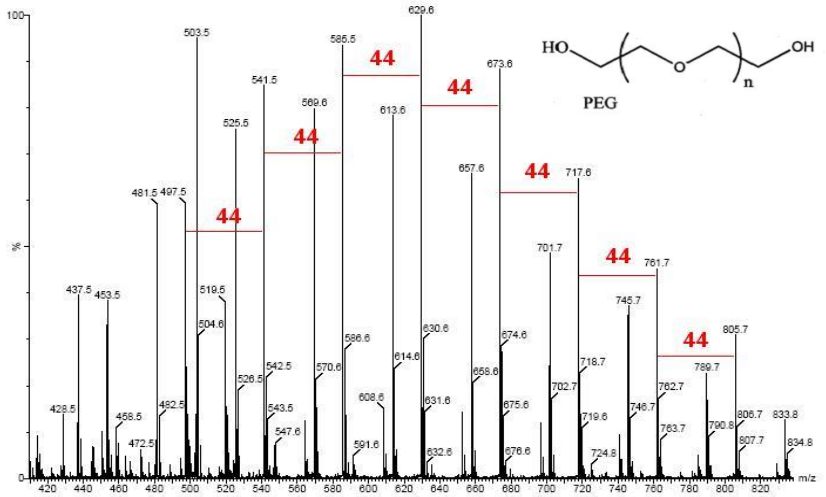
APCI spectrum: clusters of acetonitrile

Background ions, contaminants

Phthalates: common plasticizers,
from laboratory plastics
m/z 149, 279, 301, 391, 413 ...



Polyethylenglycols:
from laboratory plastics, gloves,
skin lotion
peak difference 44 u



MeOH wash of laboratory gloves

Background ions, contaminants

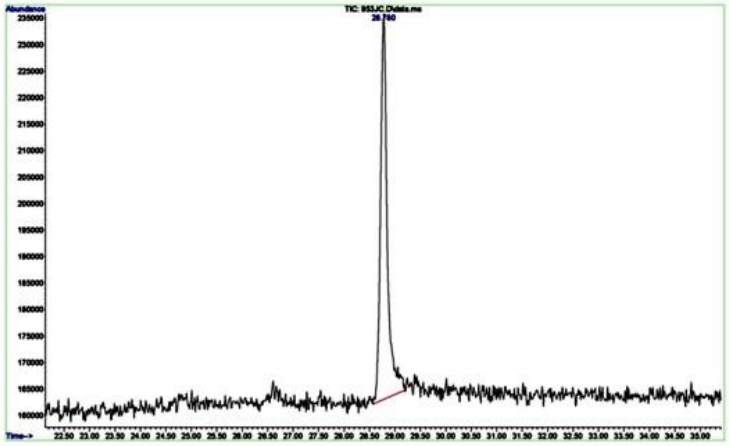
Free databases of common contaminants:

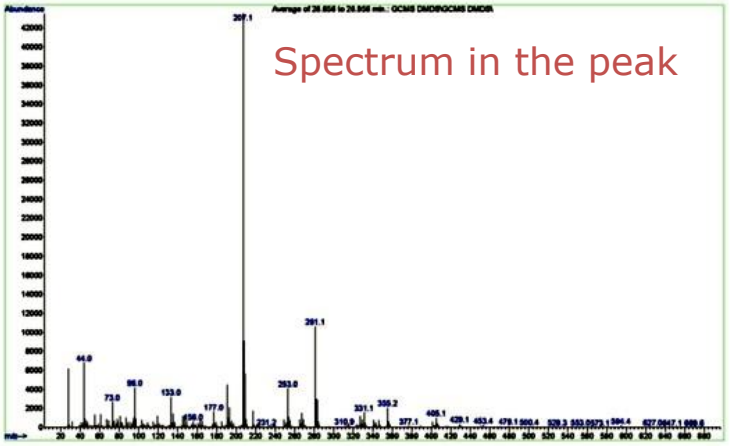
Mass spectrometry Contaminant Database

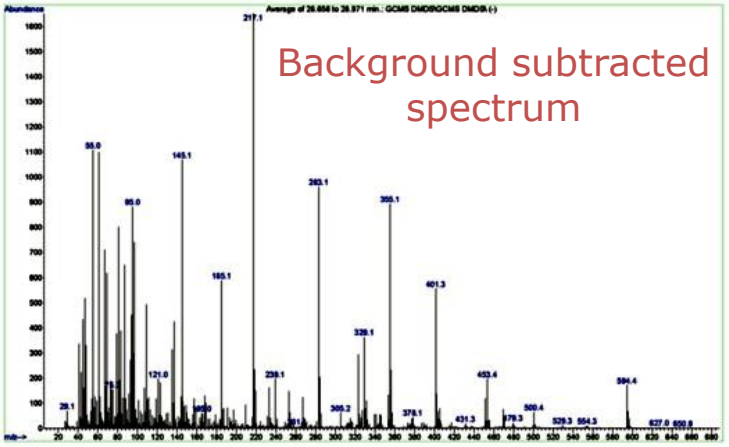
http://www.maconda.bham.ac.uk/index.php

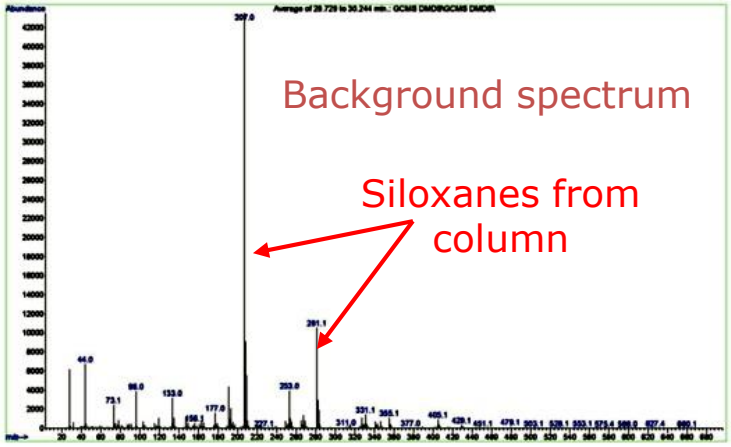
List of tables and databases: ***Common background contaminant ions and adducts.xls***, *http://sea.rice.edu/*

Background subtraction





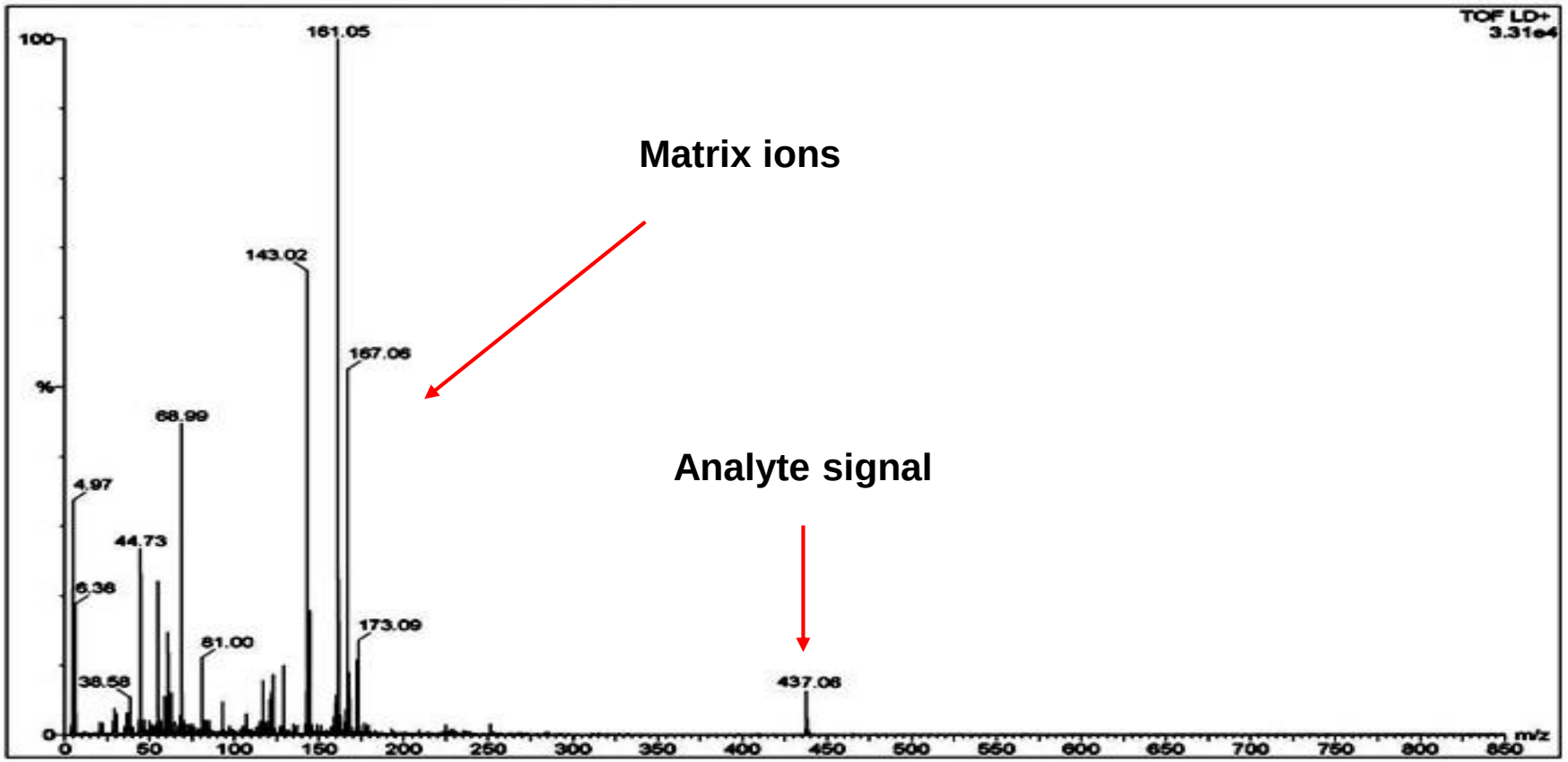




Matrix ions in MALDI spectra

- Clusters, fragments, adducts of the matrix ions in the low mass range; very intense

- MALDI spectra are usually recorded starting form ~*m/z* 500



2.

Determination of the molecular weight

Determination of molecular weight

Identification of molecular ion or molecular adduct

I. The electron ionization

$M + e^- \rightarrow M^{+\bullet} + 2\ e^-$

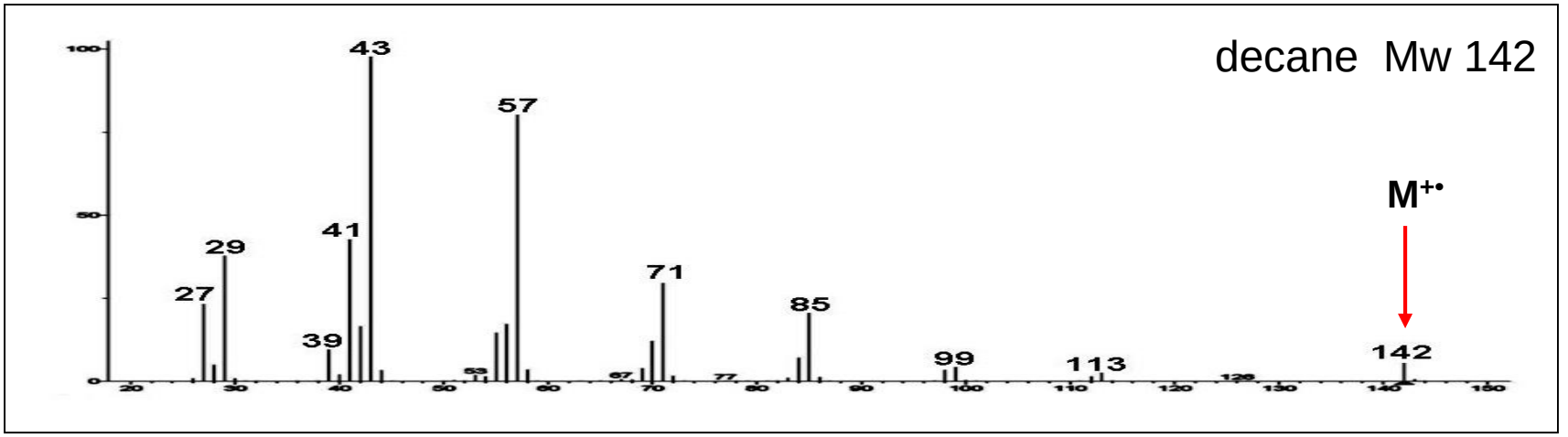
Molecular ion ($M^{+\bullet}$) is a radical cation (odd number of electrons). The *m/z* corresponds to the mass of the analyte.

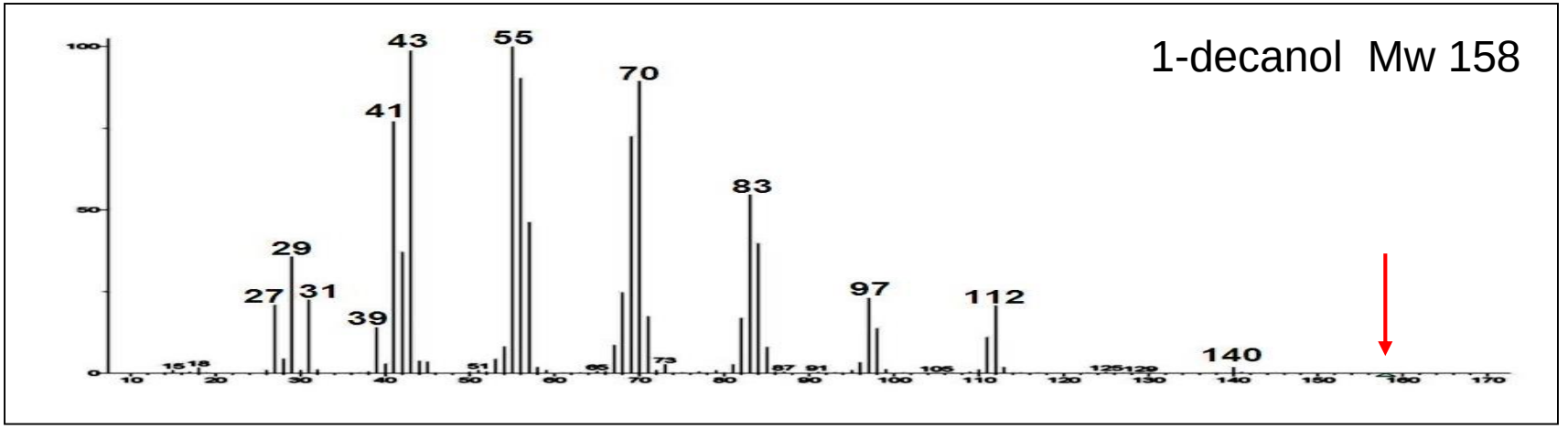
Identification of the molecular ion in EI spectra

1/ molecular ion may not be present
2/ if present, it must have the highest *m/z* value
3/ the molecular ion provides logical neutral losses

Determination of molecular weight

I. Electron ionization



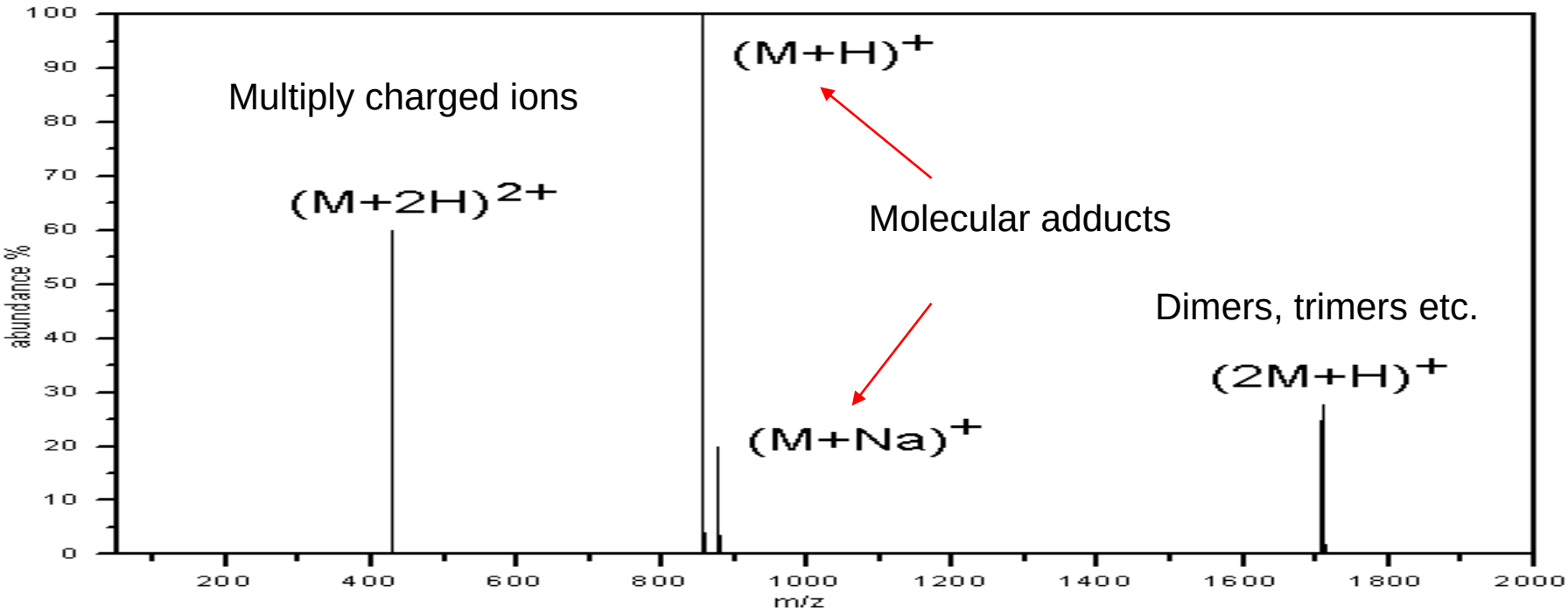


Determination of molecular weight

II. Soft ionization techniques (ESI, APCI, MALDI)

Molecular adducts (**$[M+H]^+$, $[M+Na]^+$**) or deprotonated molecules (**$[M-H]^-$**)

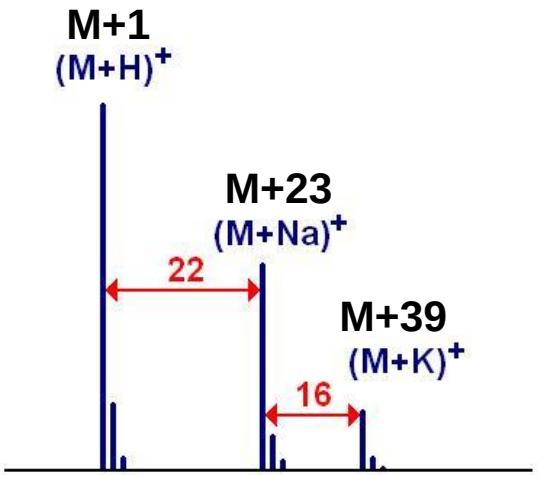
Molecular adduct is an ion with even number of electrons and may not be the most abundant ion in the spectrum.

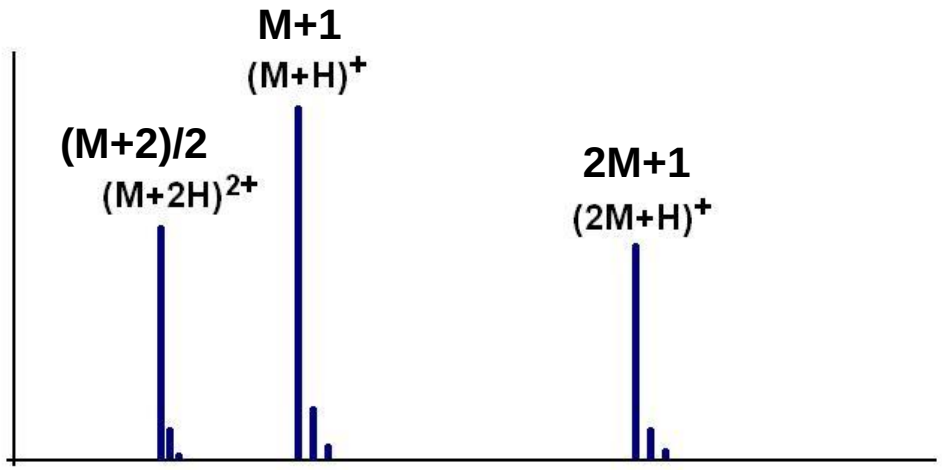


Determination of molecular weight

II. Soft ionization techniques (ESI, APCI, MALDI)

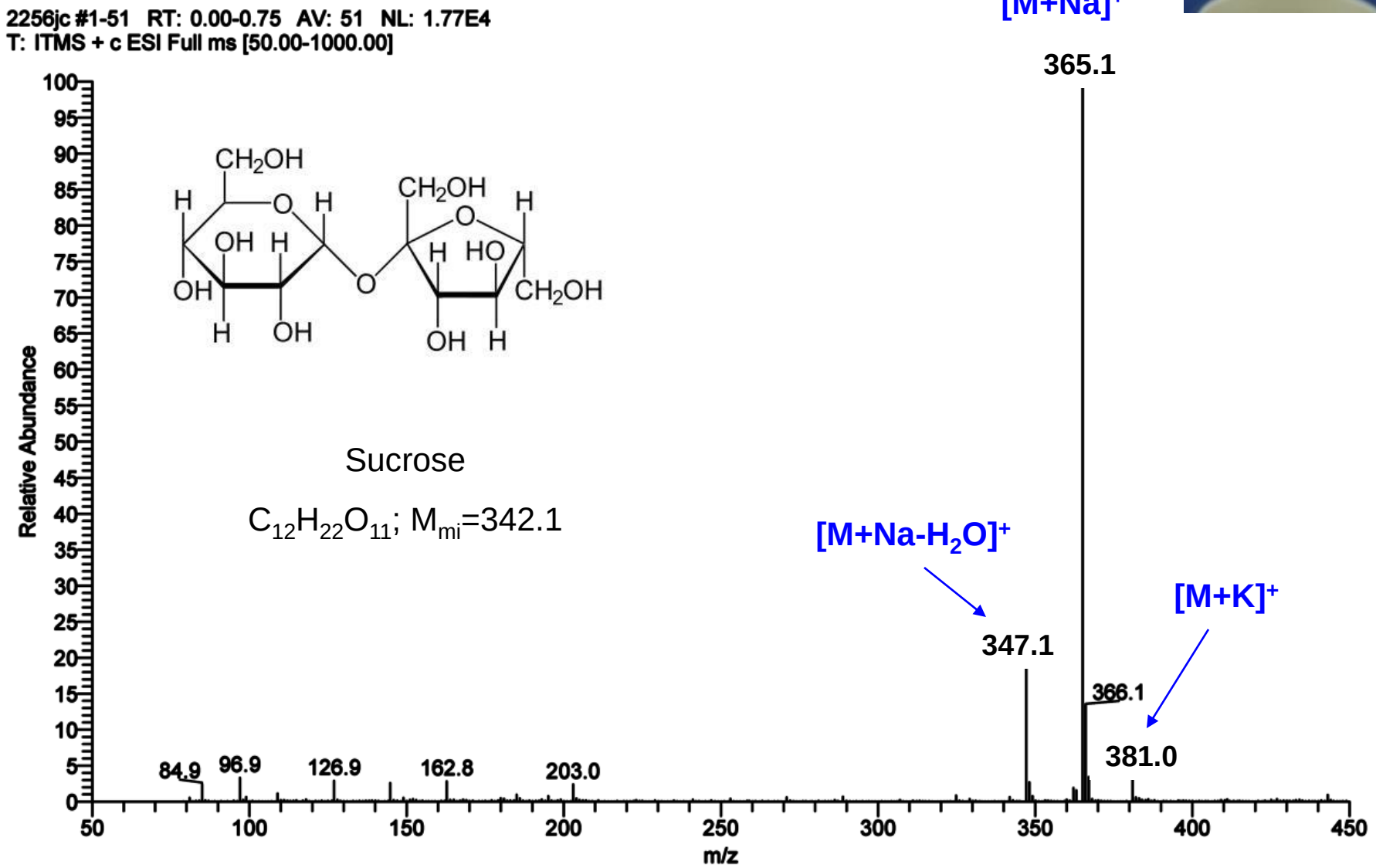
The molecular weight is determined based on the presence of adducts, dimers or multiply charged ions.





Calculation of adducts, dimers or multiply charged ions: software EIC
I:\MISC\MS\DOWNLOAD\

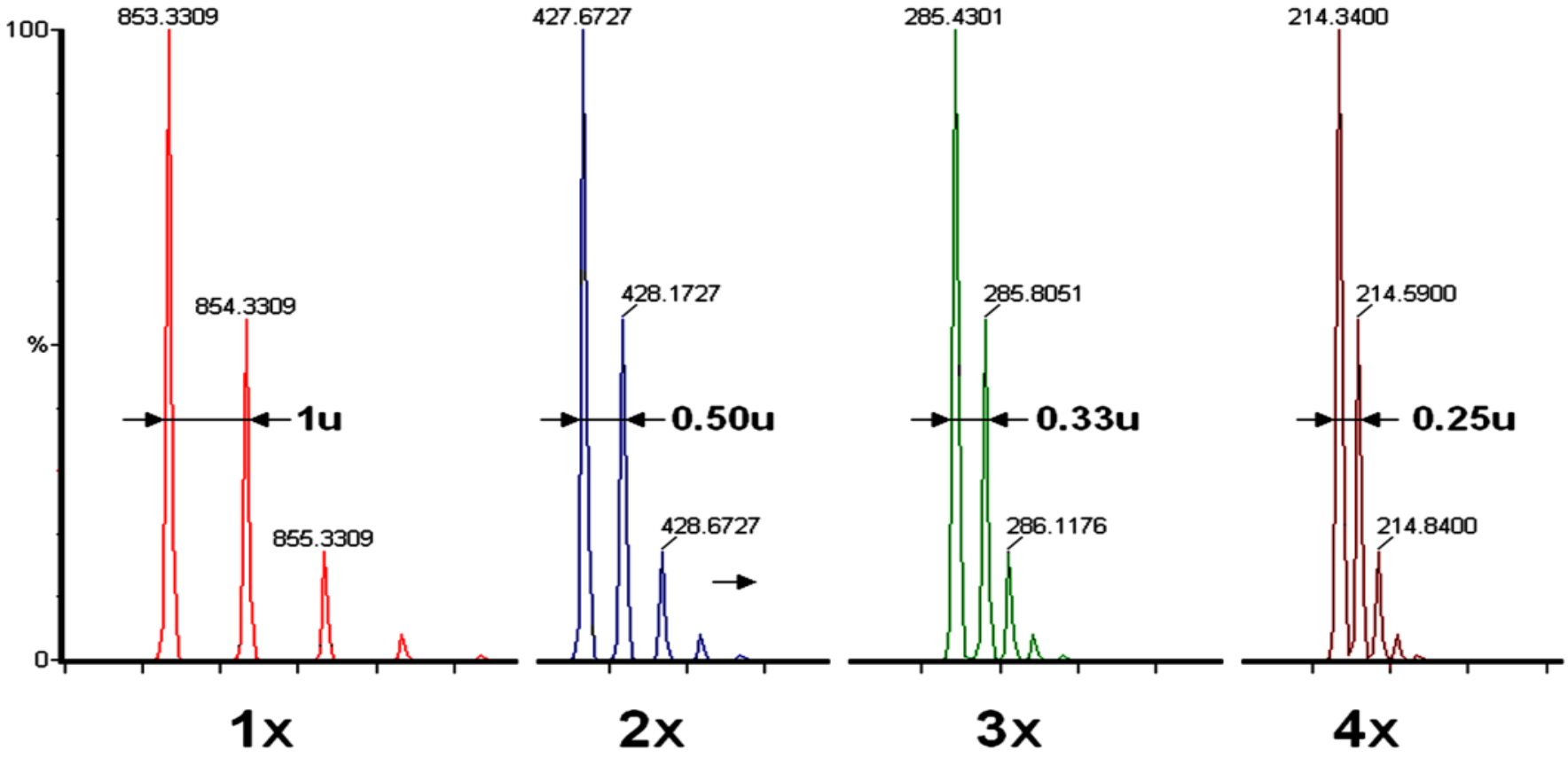
Determination of molecular weight



Determination of molecular weight – charge state

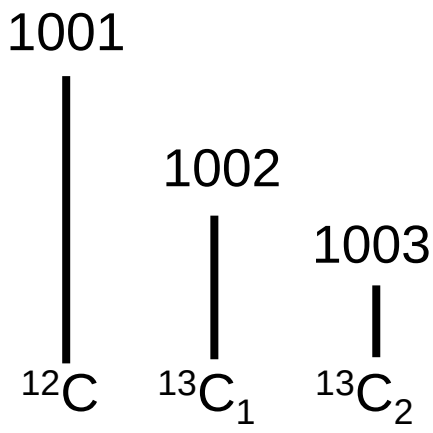
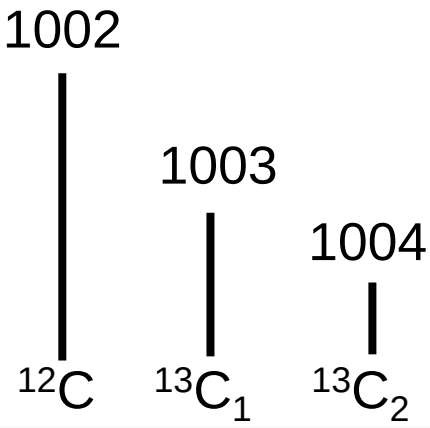
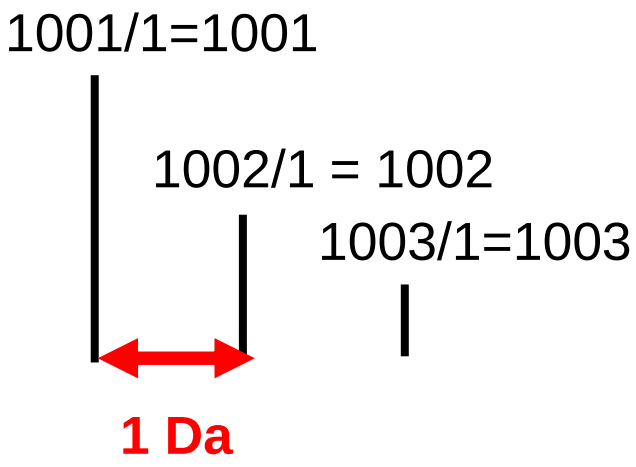
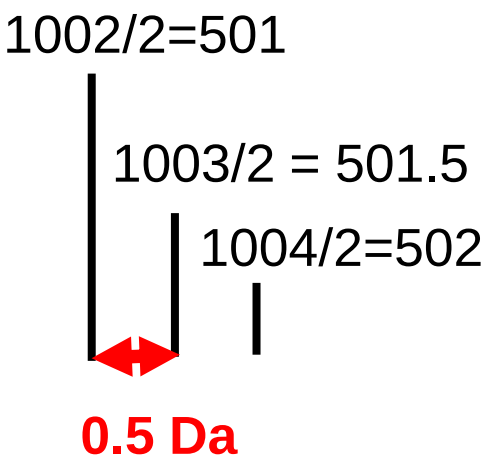
Determining number of charges

Number of charges is determined from the distance between the peaks in the isotopic clusters.



Determination of molecular weight – charge state

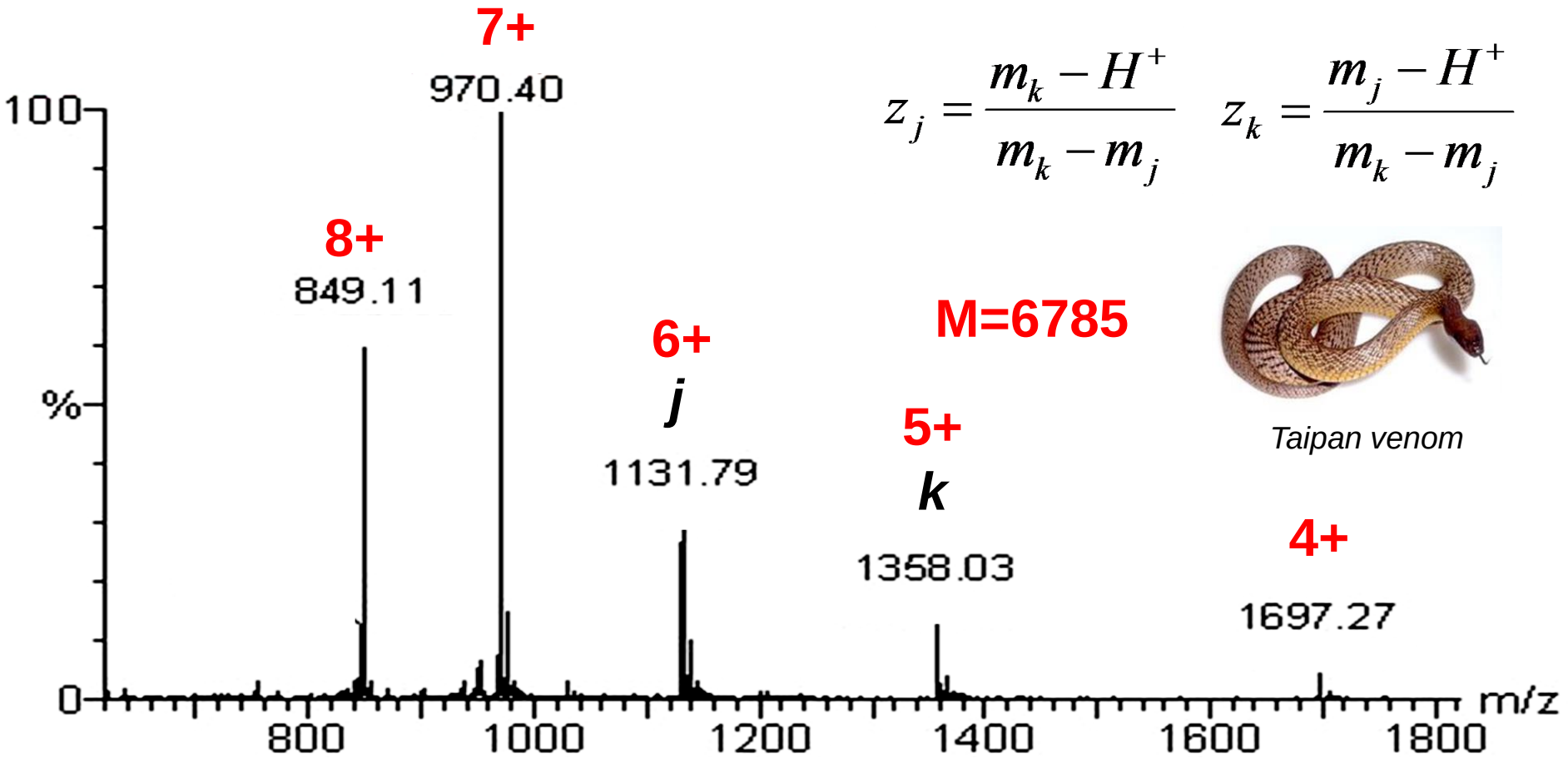
Example: relative mass 1000

	$[M + H]^+$	$[M + 2H]^{2+}$
Isotopes		
Spectrum (*m/z*!)		

Determination of molecular weight – charge state

Determining number of charges

Number of charges can be determined from the distance between the neighboring peaks representing different charge states.

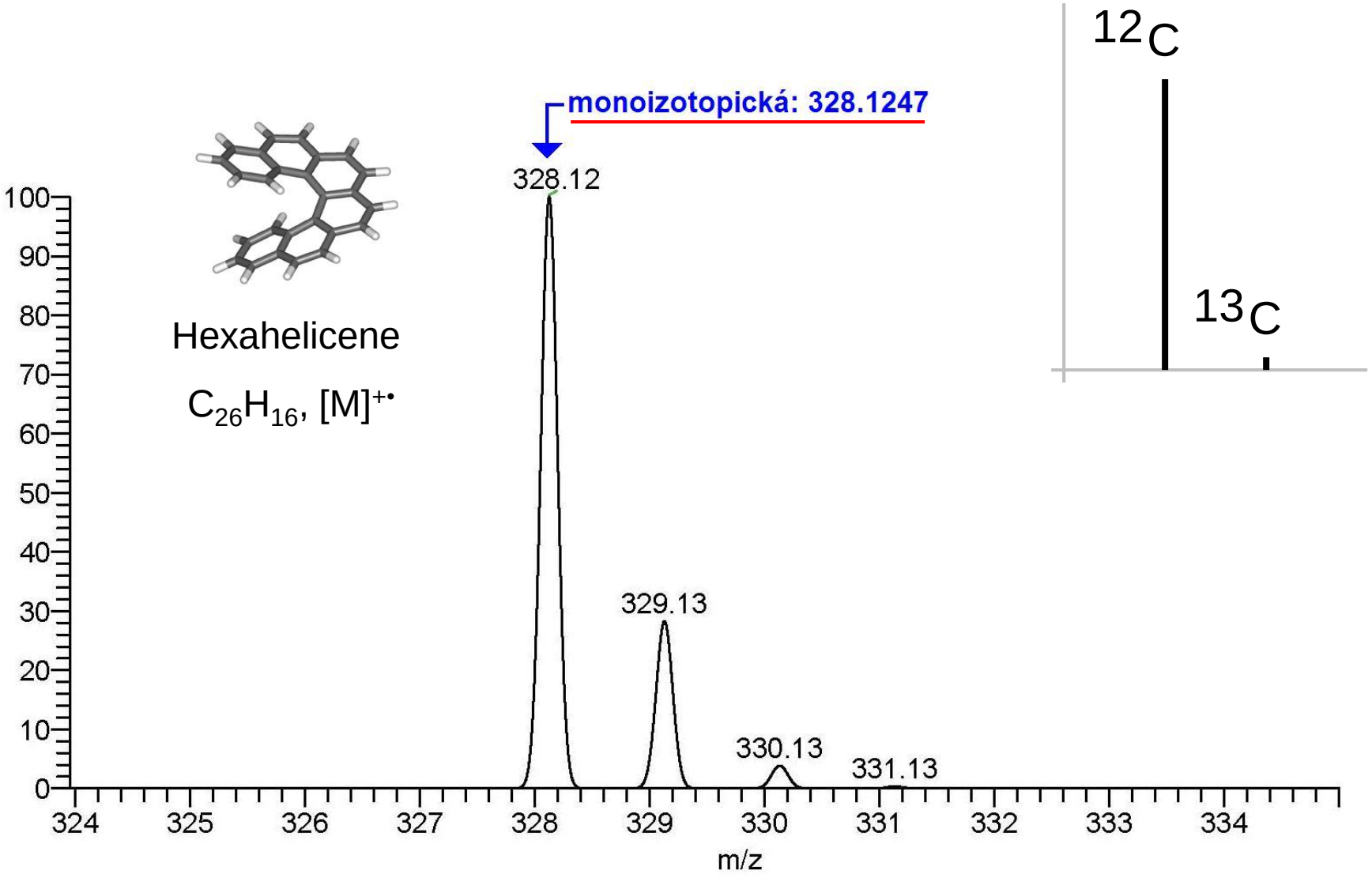


$$z_j = \frac{m_k - H^+}{m_k - m_j} \qquad z_k = \frac{m_j - H^+}{m_k - m_j}$$

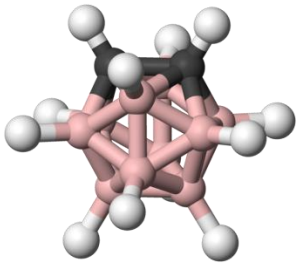
M=6785

Taipan venom

Monoisotopic mass

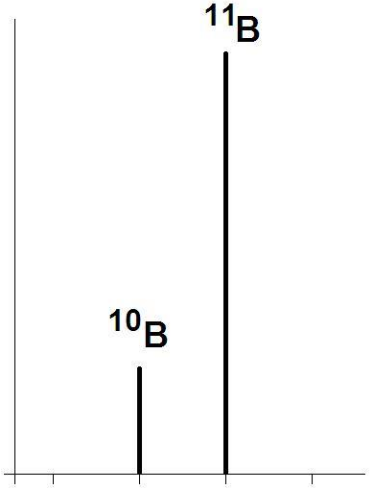


Monoisotopic mass

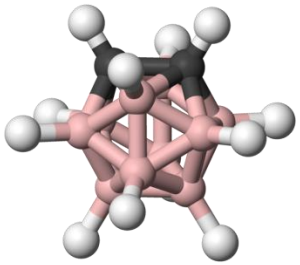


Carborane

$C_2B_{10}H_{12}$, $[M-H]^-$

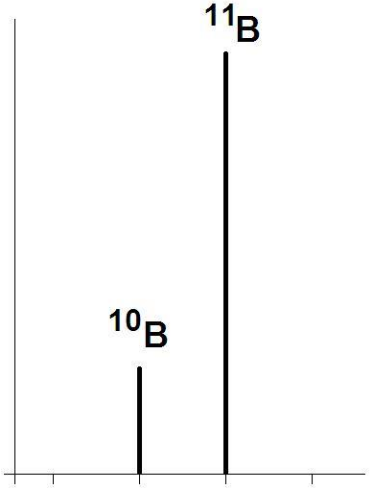


Monoisotopic mass



Carborane

$C_2B_{10}H_{12}$, $[M-H]^-$

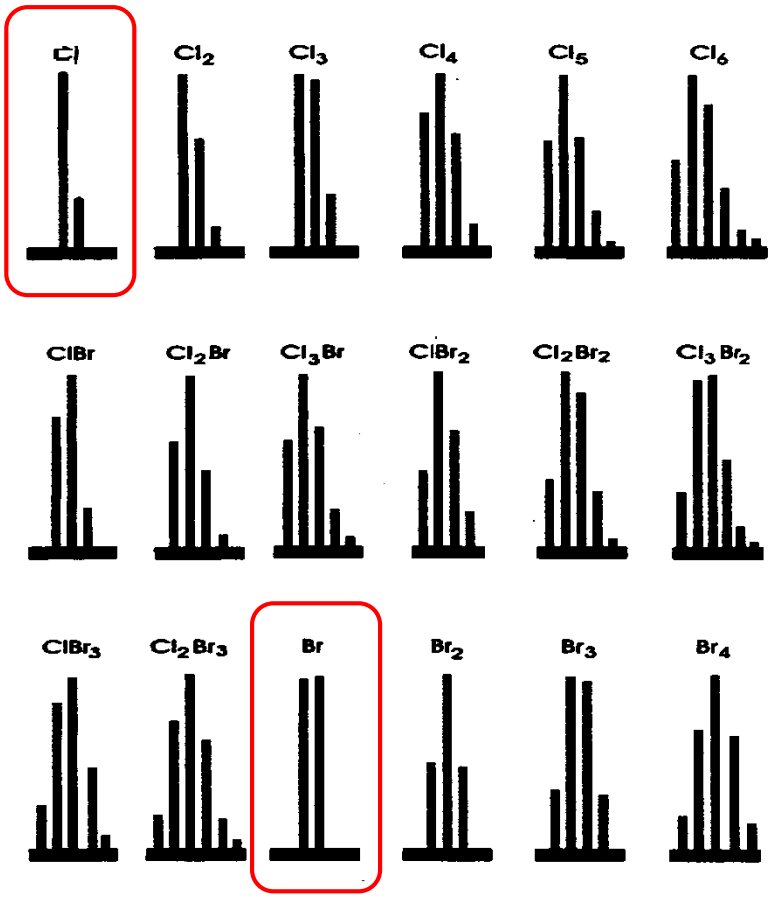


3.

Elemental composition from isotope cluster (and mass)

Isotope clusters

Isotopic clusters indicate the presence of some elements (e.g., Cl, Br, metals etc.).

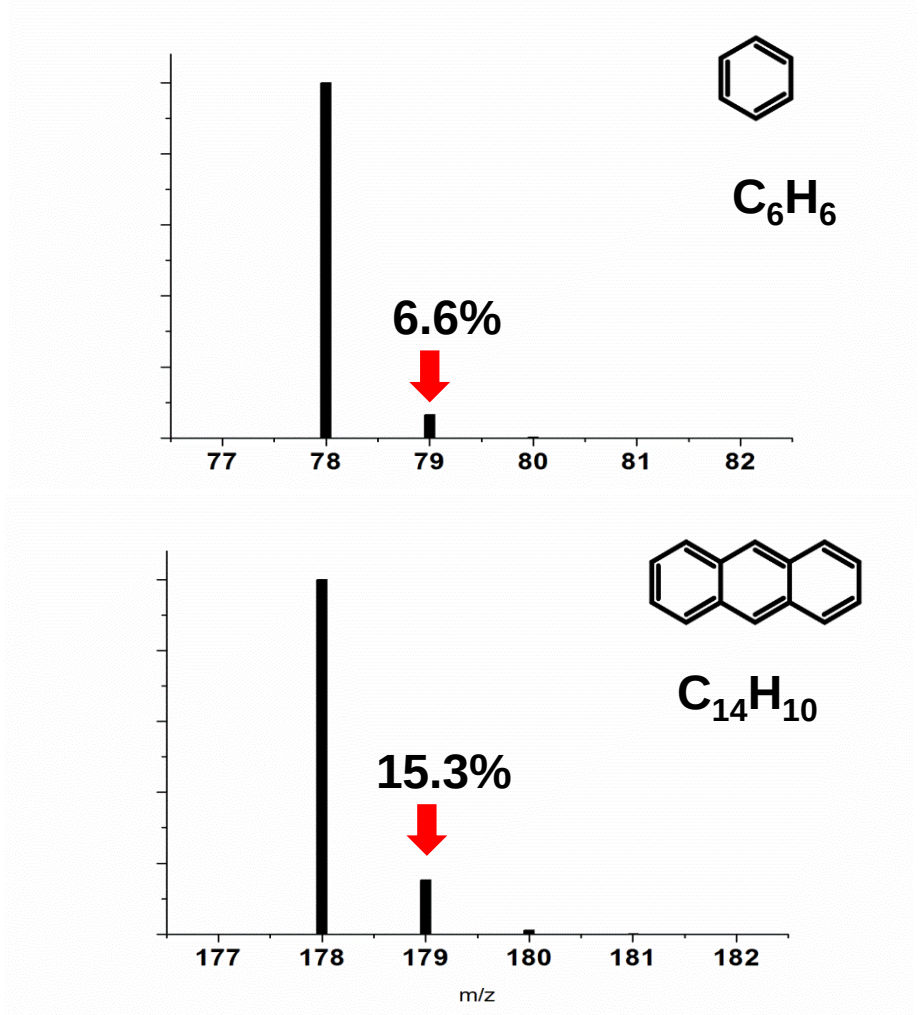


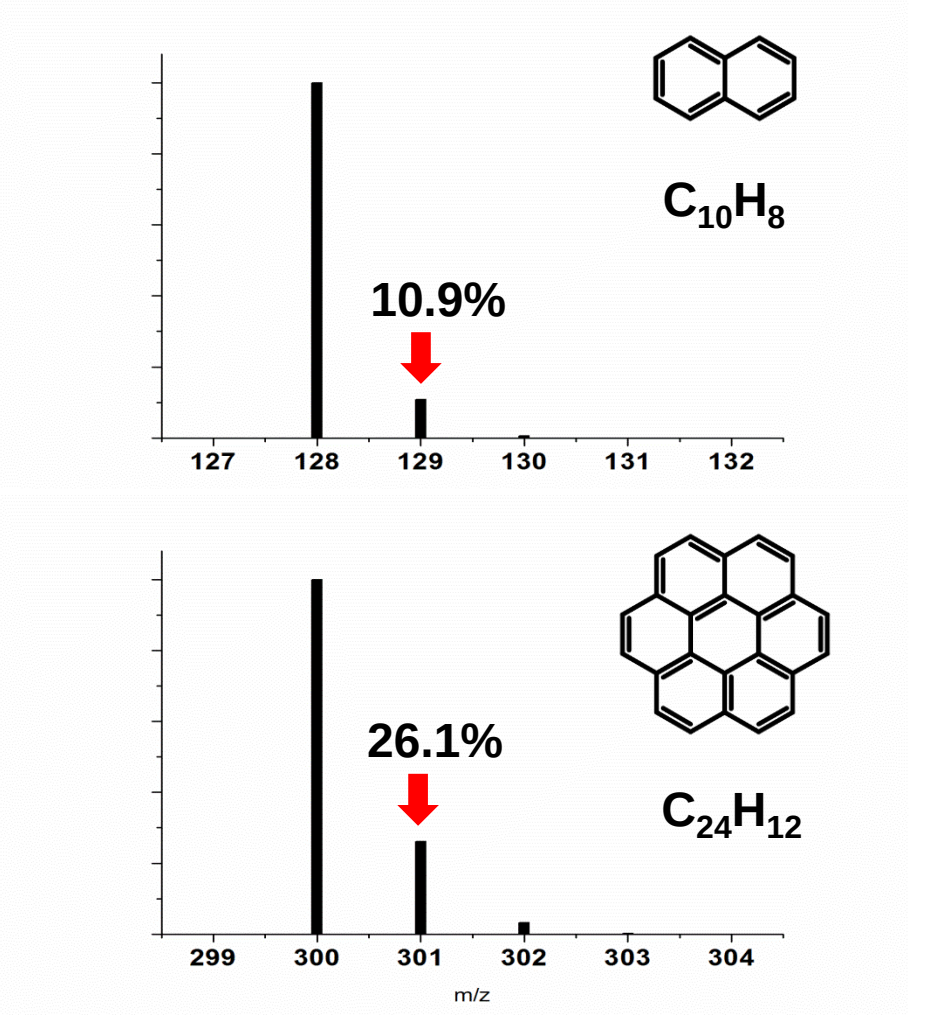
Computer programs allow you to calculate the composition of the cluster from the specified summary formula –you can compare it with your experiment.

http://www.colby.edu/chemistry/NMR/IsoClus.html

Number of carbon atoms

The number of carbons in an ion can be estimated based on the intensity of ^{13}C isotope (relative ratio $^{13}C/^{12}C$ is ~1.1%)





Nitrogen rule

- Elements with odd nominal masses form odd numbers of covalent bonds.
- Elements with even masses form even numbers of covalent bonds, with the exception of nitrogen (nominal mass of 14, valency of 3).

Nitrogen rule applies to organic compounds containing C, H, N, O, S, P, F, Cl, Br, I

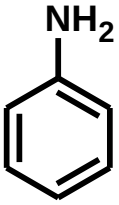
Odd value of molecular weight = odd number of nitrogens

Even value of molecular weight = even (zero) number of nitrogens

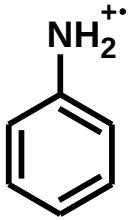
Applying the rule for ions

EI – valid for $M^{+\bullet}$ as stated above

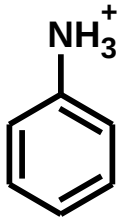
ESI, APCI, MALDI – the rule must be reversed for molecular adducts!



M=93



m/z 93



m/z 94

4.

Determination of elemental composition from accurate mass

Elemental composition from accurate mass

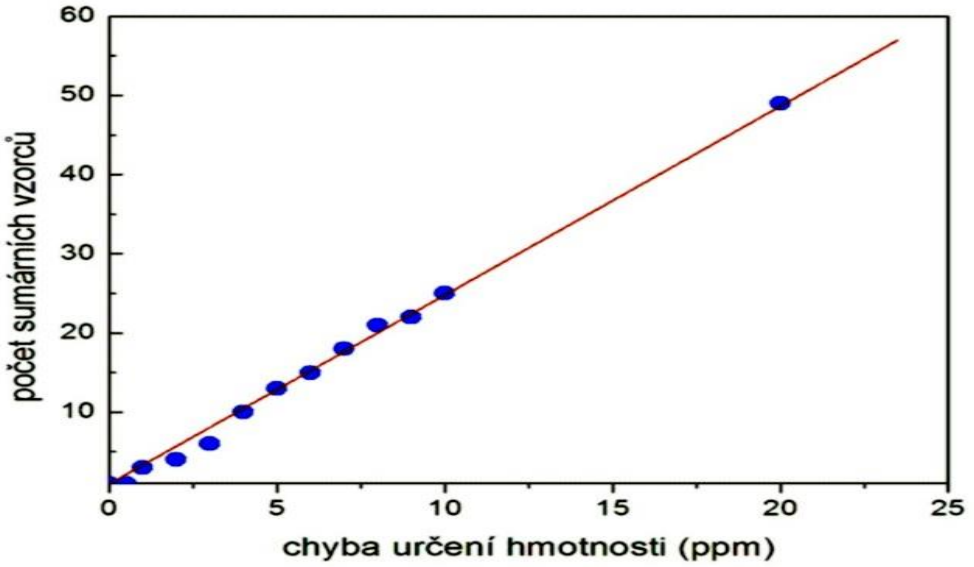
Each combination of elements has a unique exact mass => we can use accurately measured masses for calculating elemental formula

Absolutely correct measurement of an ion mass would give us a single elemental composition. In real word, we have to consider an error of the measurement.

The more accurately we determine the mass of an ion, the less number of possible structures we get

Example. paclitaxel, $C_{47}H_{51}NO_{14,}$ mon. mass 854.3388

Constrains:
C: 0-100
H: 0-100
N: 0-10
O: 0-30

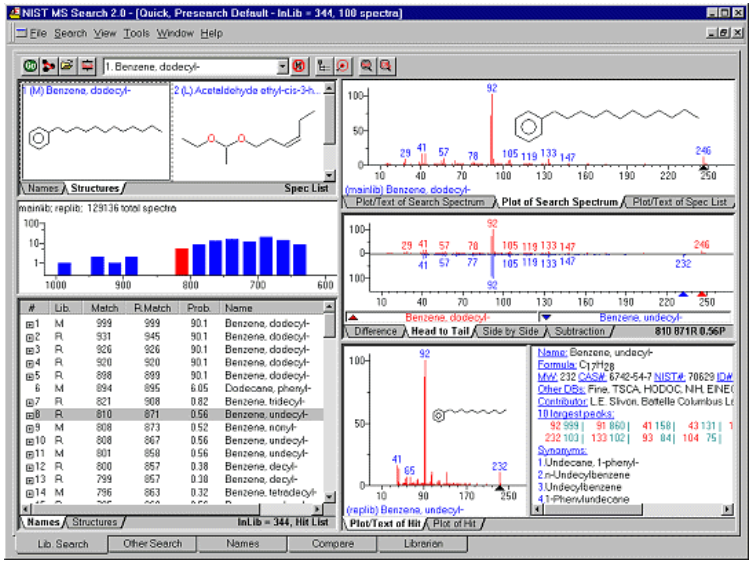


5.

Searching mass spectra libraries

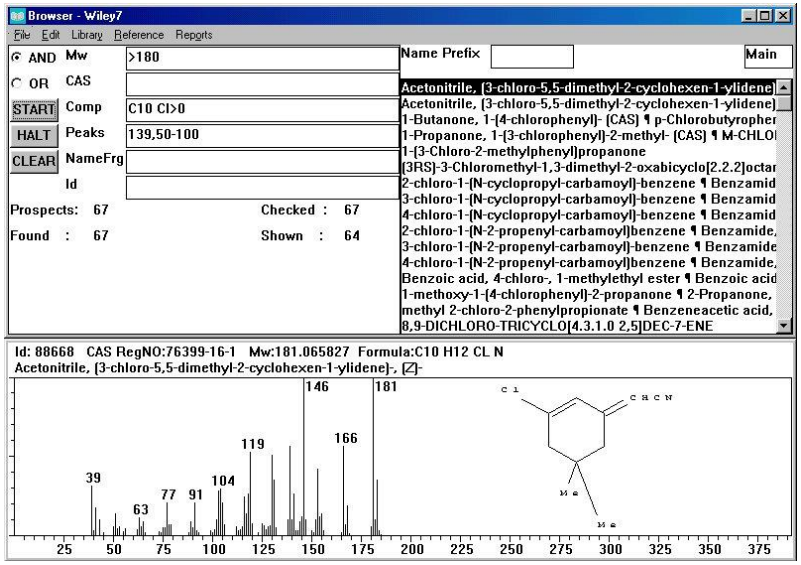
Libraries of EI mass spectra

NIST/EPA/NIH Mass Spectral Library



276 248 EI spectra (70 eV)
234 284 MS/MS spectra,
retention indices

Wiley Registry of Mass Spectral Data



719 000 EI spectra (70 eV)

NIST 05 installed on the open access GC/MS

Libraries of soft ionization techniques spectra

Problems with the creation of libraries:

- Appearance of the spectra is strongly dependent on the experimental conditions (formation of various adducts depends on the composition of the mobile phase and ion source settings)

- MS spectra are usually without fragment ions -> library spectra at the MS^n level

- MS^n spectra depends on the experimental conditions (ionization energy, type of the analyzer, etc.).

→ spectra libraries are measured at several experimental conditions

MassBank

http://www.massbank.jp/

mzCLOUD™

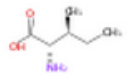
https://www.mzcloud.org/

Libraries of soft ionization techniques spectra

MassBank

Public repository MS data for sharing within the scientific community (~ 40 thousands spectra).

Merged spectra from data measured under different conditions.

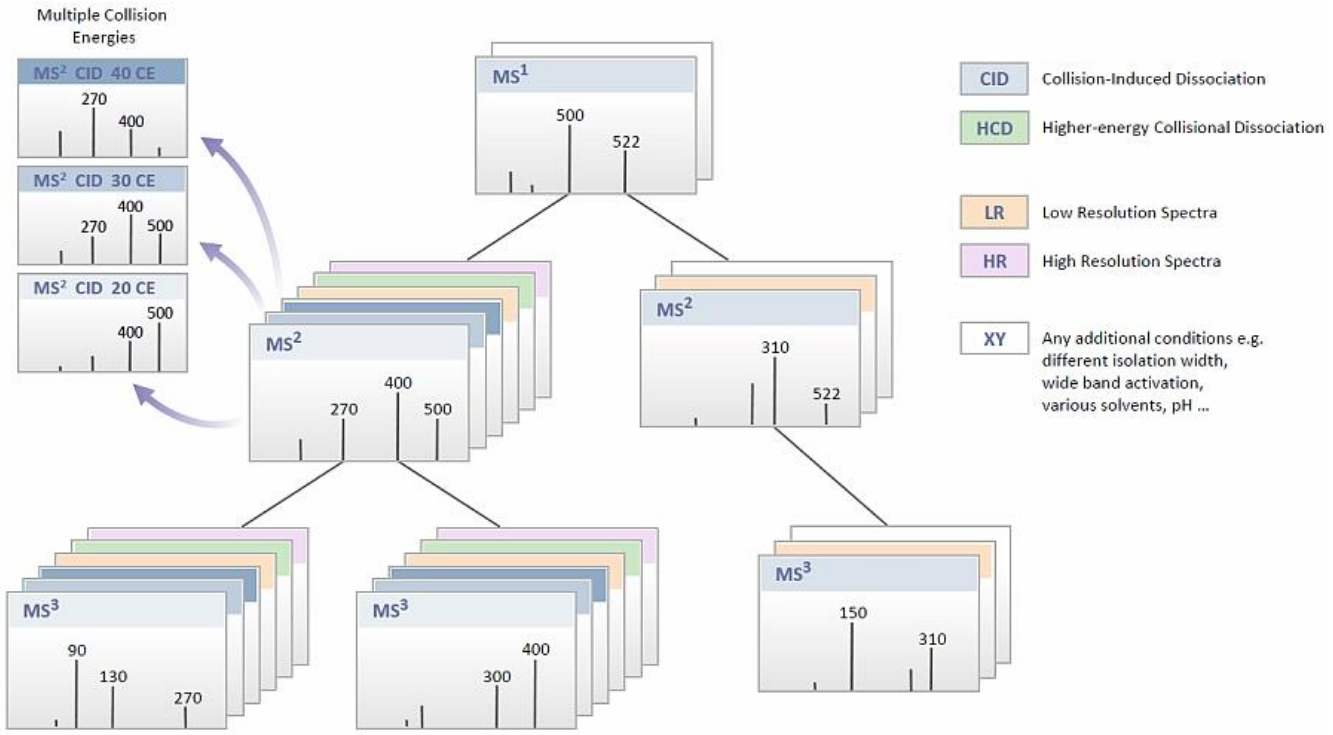
L-Isoleucine (13 spectra)	C6H13NO2 	131.09463	
CE-ESI-TOF; MS; [M+H]+			PR030010
LC-ESI-ITFT; MS2; m/z:132.10; POS			KNA00321
LC-ESI-ITFT; MS2; m/z:132.10; POS			KNA00042
LC-ESI-ITFT; MS2; m/z:133.10; POS			KNA00322
LC-ESI-ITFT; MS2; m/z:133.10; POS			KNA00043
LC-ESI-ITFT; MS; POS			KNA00320
LC-ESI-ITFT; MS; POS			KNA00041
LC-ESI-QQ; MS2; CE:10 V; [M+H]+			KO003172
LC-ESI-QQ; MS2; CE:20 V; [M+H]+			KO003173
LC-ESI-QQ; MS2; CE:30 V; [M+H]+			KO003174
LC-ESI-QQ; MS2; CE:40 V; [M+H]+			KO003175
LC-ESI-QQ; MS2; CE:50 V; [M+H]+			KO003176
LC-ESI-QTOF; MS2; MERGED; [M+H]+			KOX00364

Libraries of soft ionization techniques spectra

Freely accessible database of spectra, spectral trees, structures, fragments of chromatographic data, links, etc. (~ 170 thousand spectra of 2,600 substances).

Spectral tree: database structure of tandem mass spectra

Identification substructures - the possibility of identifying substances which are not in the database



6.

Solving fragmentation spectra

Fragmentation of ions with even number of electrons (EE^+)

ESI, APCI (APPI, MALDI, DESI ...)

Fragmentation of EE^+

1/ CID (MS/MS) of EE^+ (**$[M+H]^+$**, **$[M+Na]^+$**, **$[M-H]^-$**) formed by ESI

2/ fragmentation of (**$[M+H]^+$**, **$[M+Na]^+$**, **$[M-H]^-$**) during APCI, APPI

Cleavage of neighboring bond to the charge site, charge migration

$$R\text{-}OH + H \rightarrow R\text{-}OH_2^+$$

$$R\text{-}OH_2^+ \rightarrow R^+ + H_2O$$

FRAGMENTATION of EE^+:

The fragments are EE^+ and a neutral fragment (not seen in the spectra)

$$EE^+ \rightarrow EE^+ + M$$

EE^+ ions are more stable than $OE^{+\bullet}$

The spectra are simpler than EI spectra, thus provide less information.
They are sensitive to small changes in the structure.

Fragmentation of EE^+

Elimination of a neutral molecule depends on basicity and stability of the forming ion

Typical logical neutral losses:

17: NH_3 – amines aliphatic, aromatic (+)

18: H_2O – oxygen-containing compounds (+/-)

27: HCN – amines aliphatic, aromatic, nitriles aromatic (+/-)

28: CO – aldehydes, ketones, nitroaromates (+/-)

32: CH_3OH – methyl esters (+)

42: $CH_2C{=}O$ – N-acetyl derivatives (+/-)

44: CO_2 – carboxylic acids, carbamates (+/-)

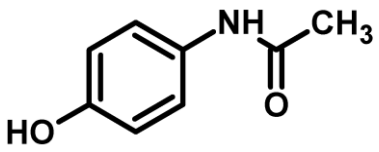
80: SO_3 – sulfonic acids(+/-)

162: anhydroglucose – glucosides (+/-)

Impossible "forbidden" neutral losses: 3-14, 21-25, 37-40

Paracetamol N-(4-hydroxyphenyl)acetamide

ESI+, MS



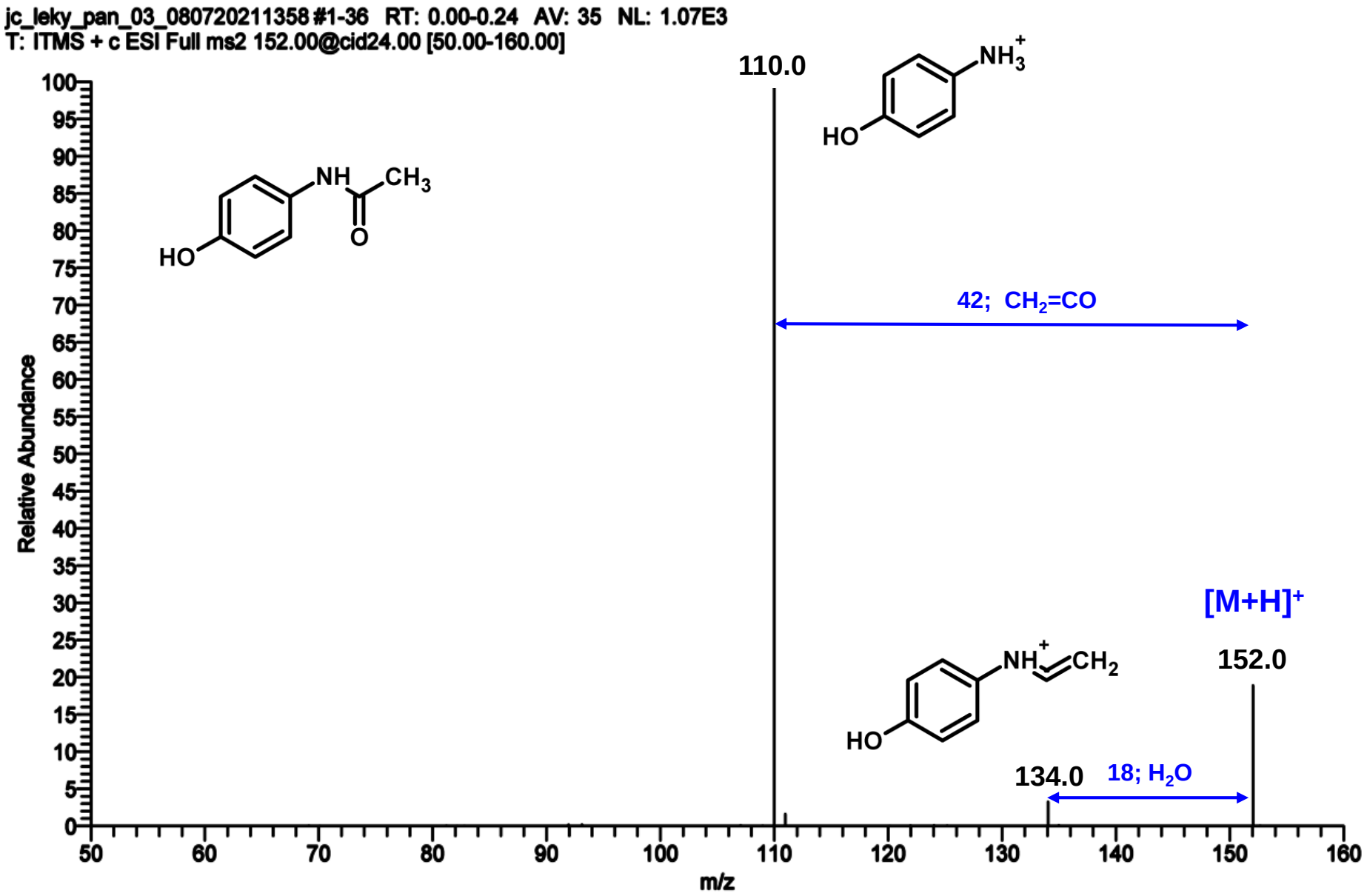
$C_8H_9NO_2$; M=151.1

JC_LEKY_Pan_04_080720211508 #1-22 RT: 0.00-0.25 AV: 22 NL: 2.40E4
T: ITMS + c ESI Full ms [50.00-500.00]

Mass	Intensity
152	100.0
153	8.7
154	0.7

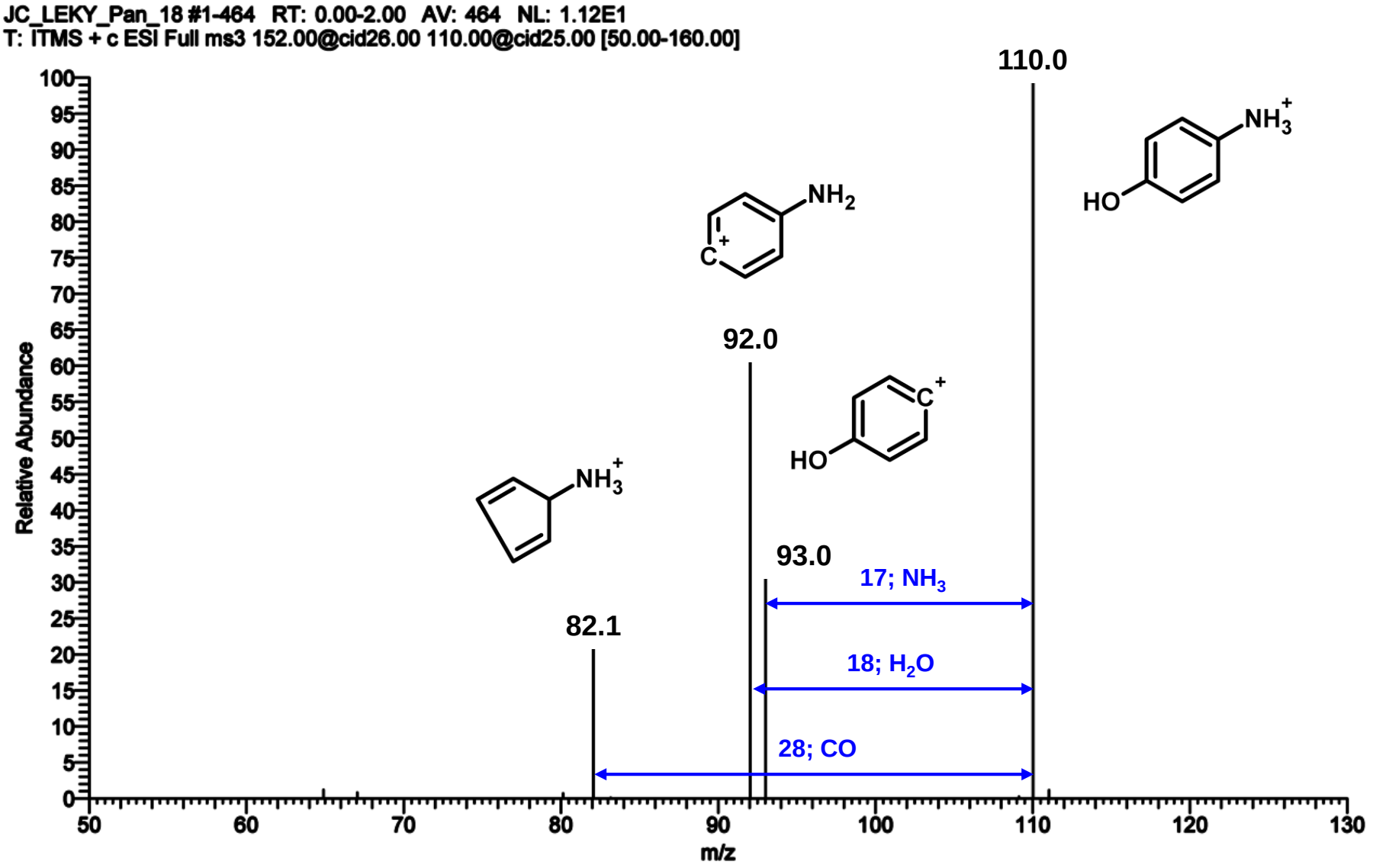
Paracetamol N-(4-hydroxyphenyl)acetamide

ESI+, MS/MS



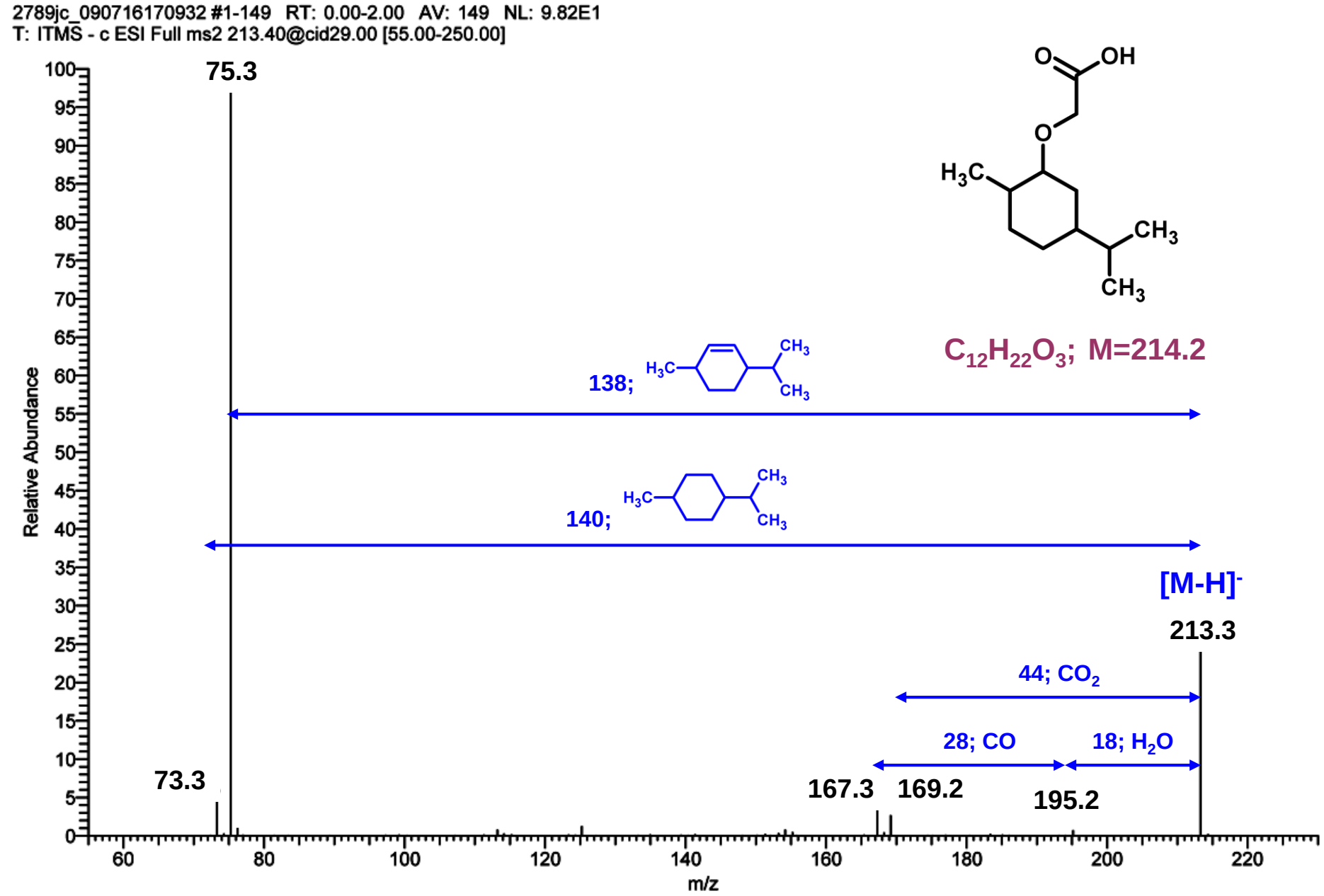
Paracetamol N-(4-hydroxyphenyl)acetamide

ESI+, MS³



Menthyloxyacetic acid

ESI-, MS/MS

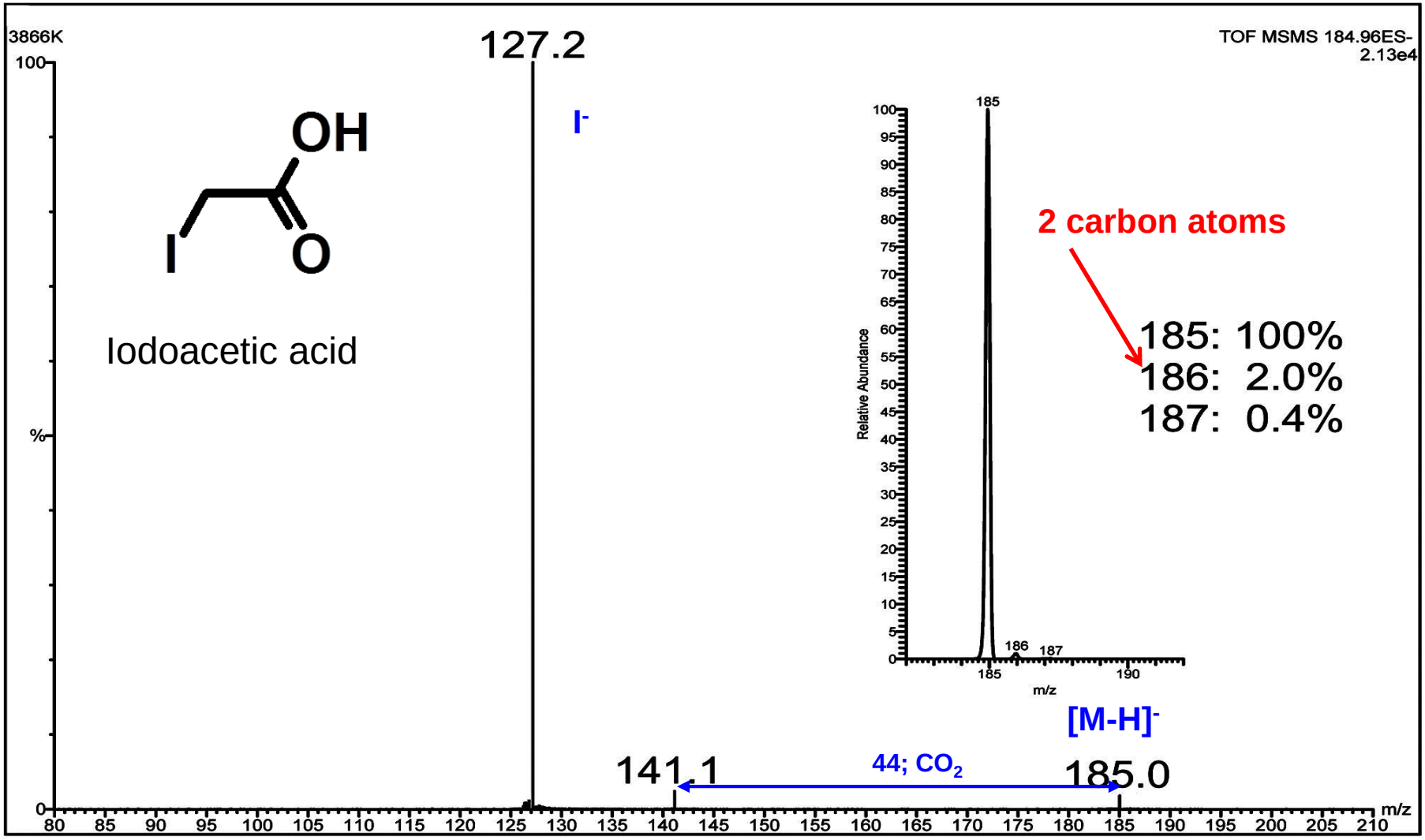


Unknown

What is the structure?

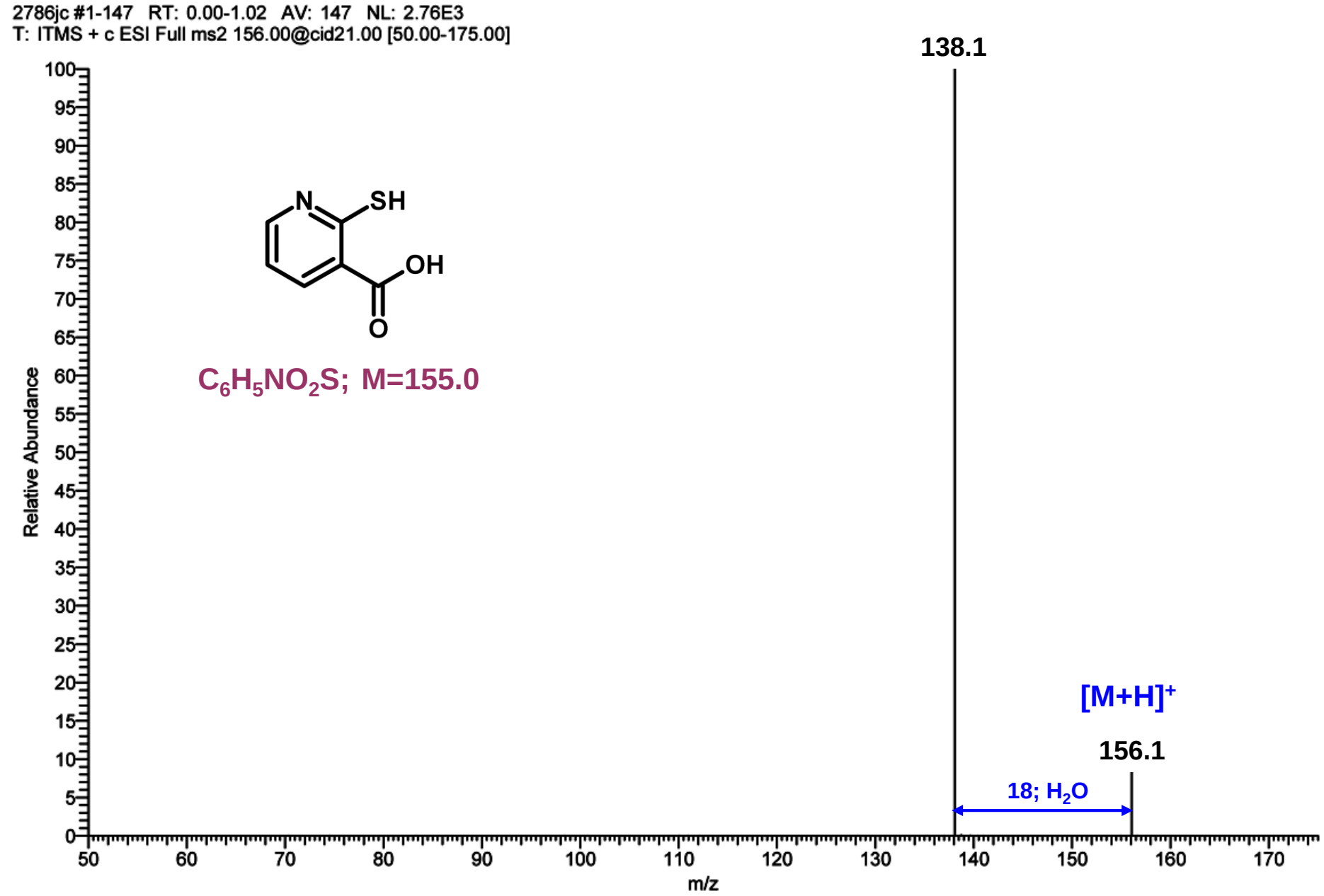
ESI-, MS/MS

MS/MS *m/z* 185



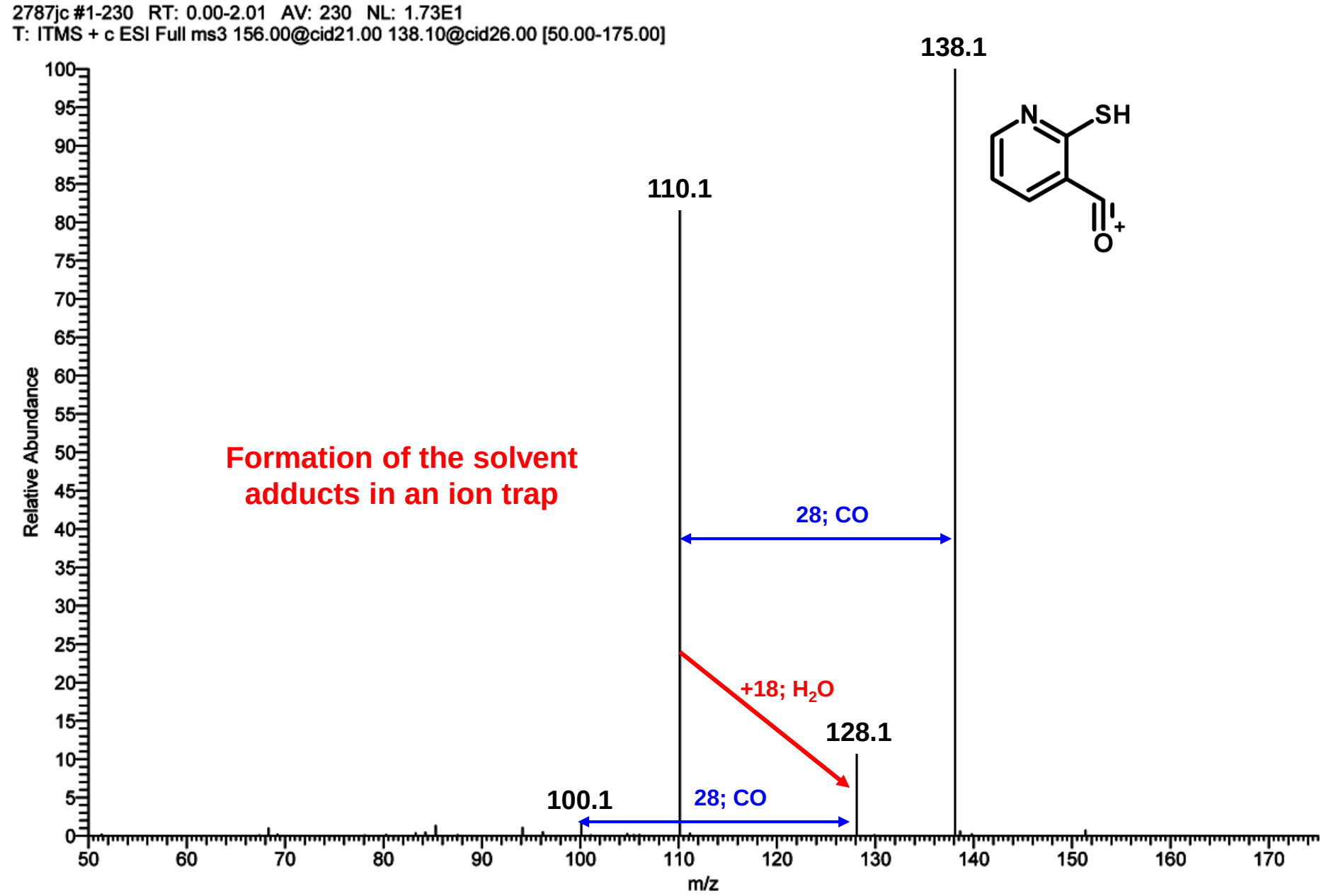
2-Mercaptonicotinic acid

ESI+, MS/MS



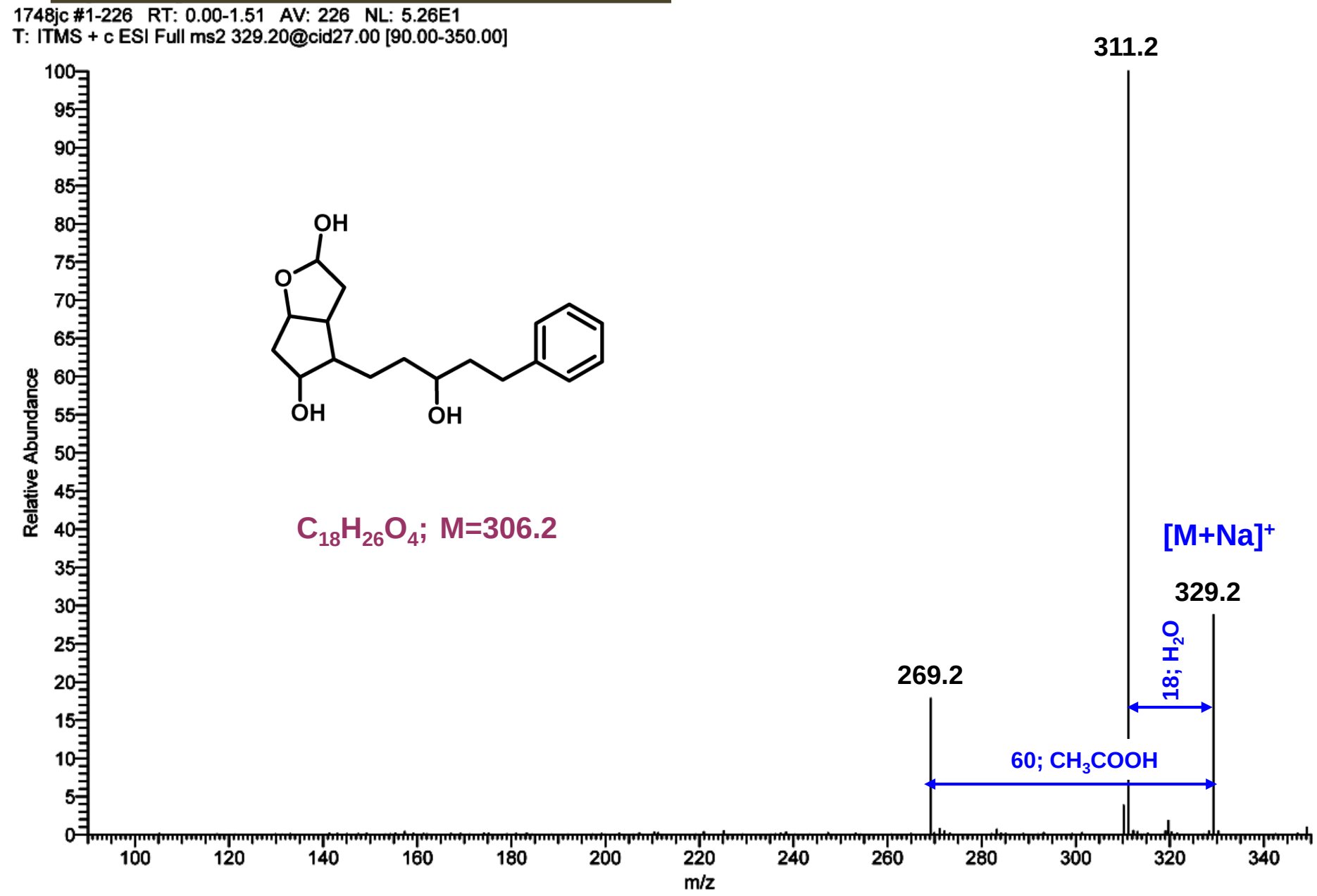
2-Mercaptonicotinic acid

ESI+, MS³



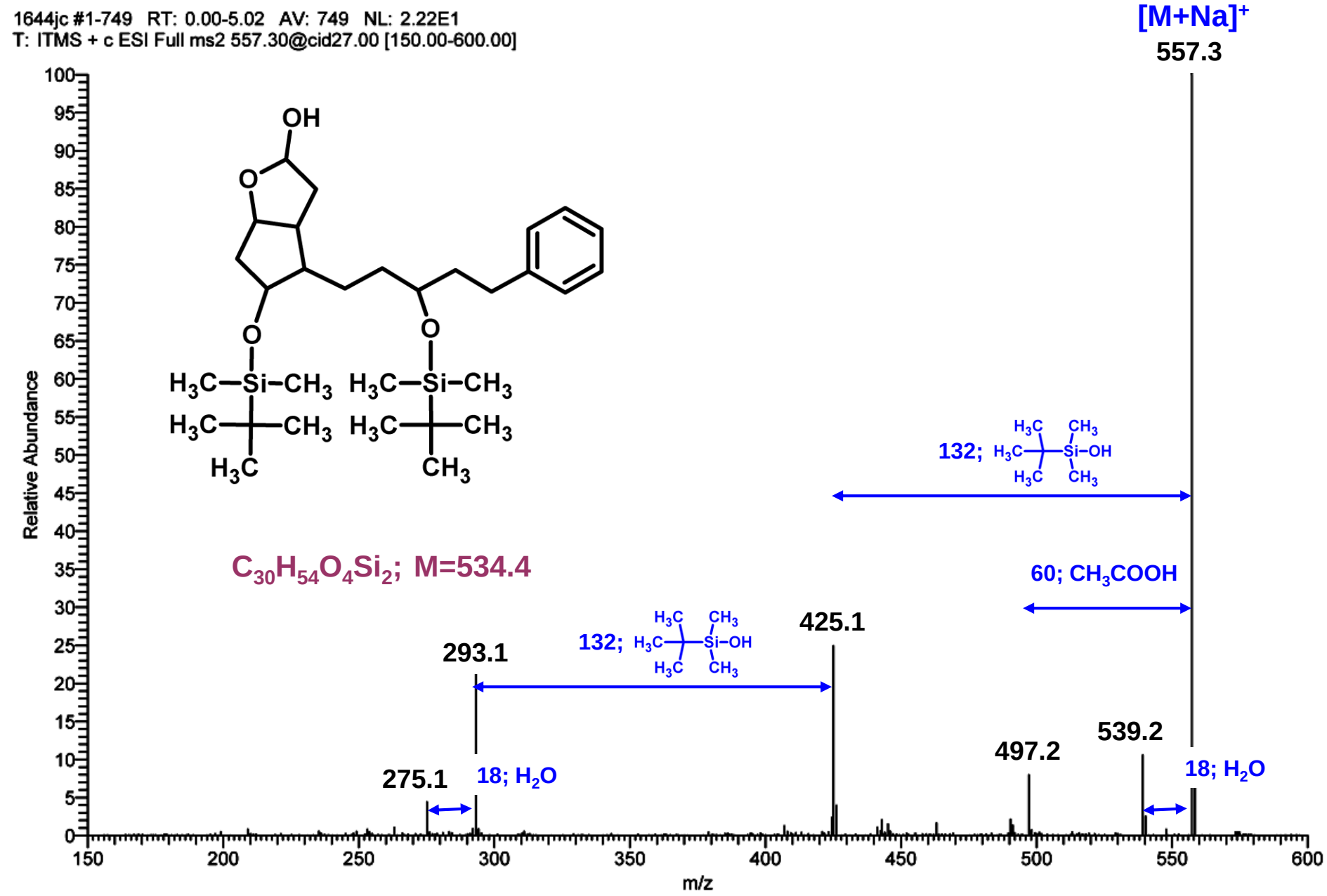
Cyclopentafuranol-derivative

ESI+, MS/MS



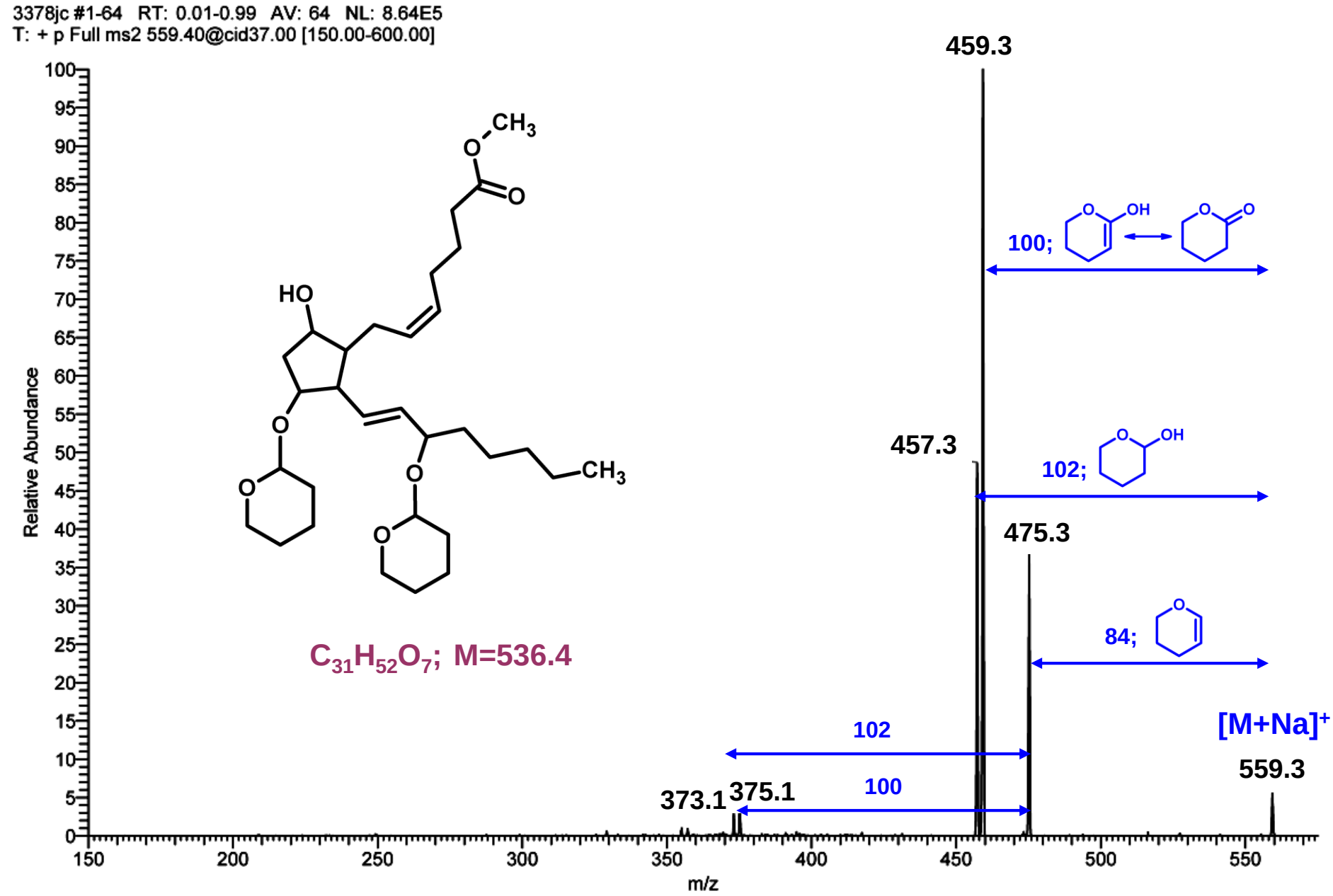
Cyclopentafuranol-derivative, disil

ESI+, MS/MS



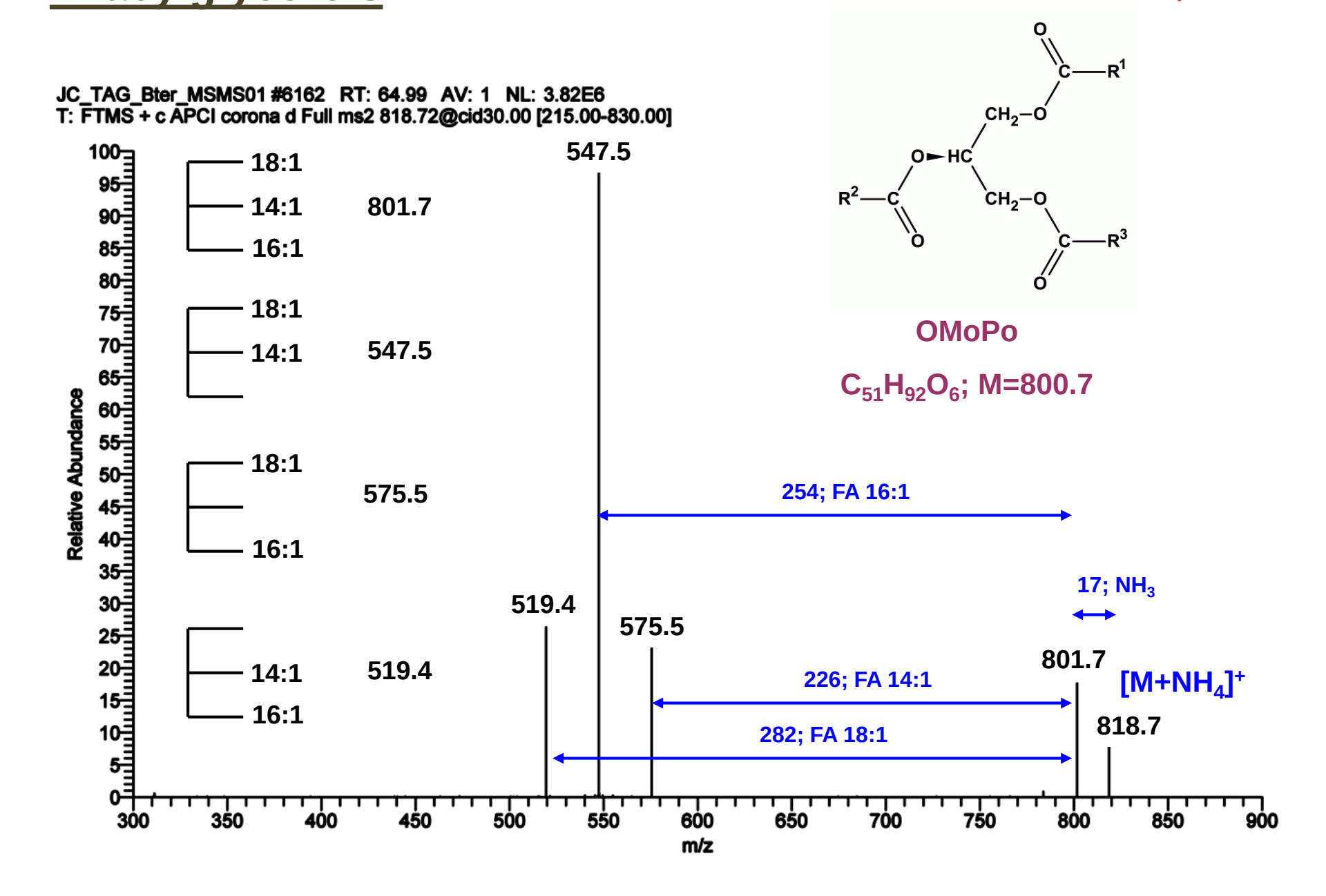
PGF_{2a}-methylester, diTHP

ESI+, MS/MS



Triacylglycerols

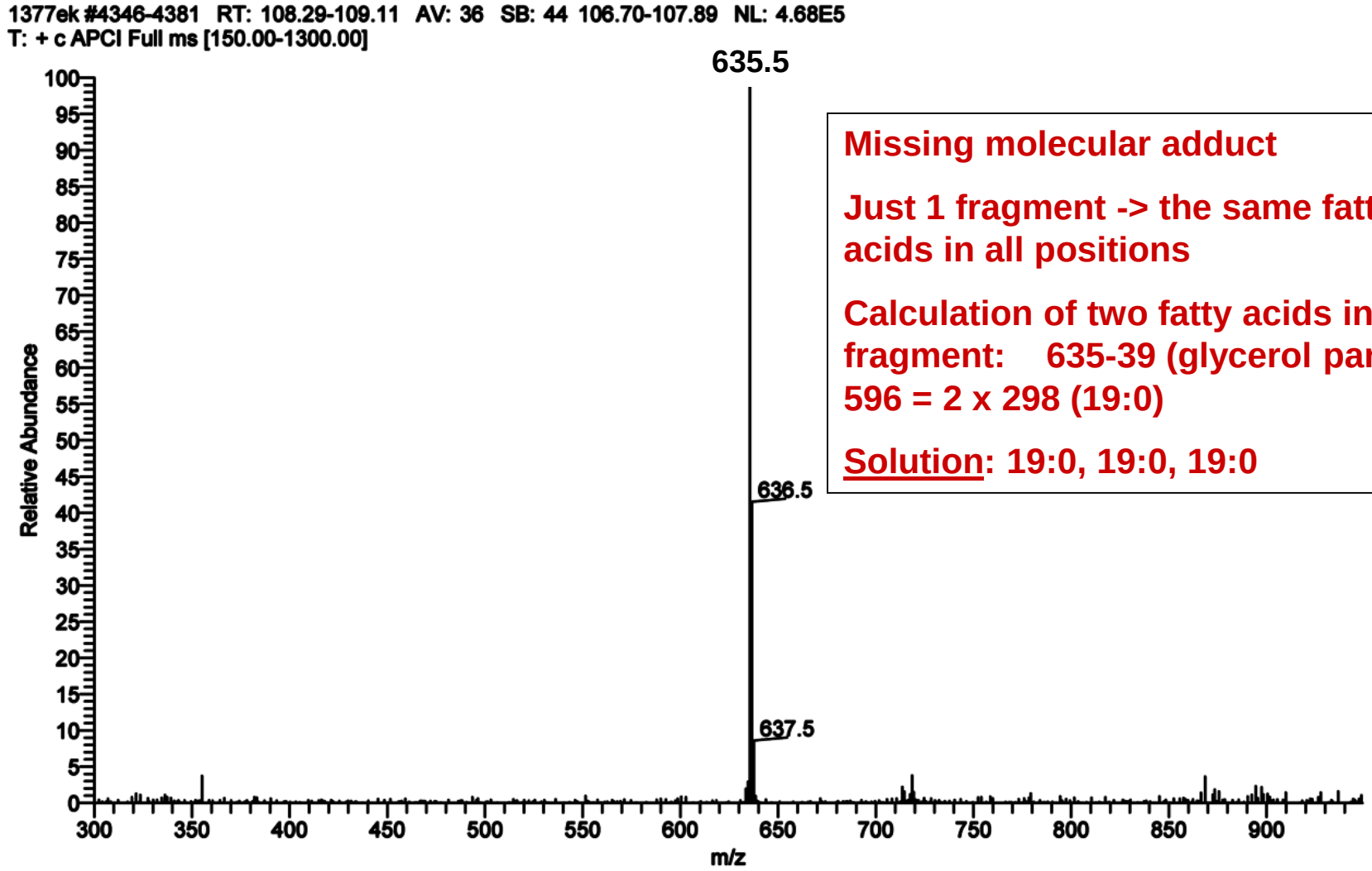
APCI+, MS/MS



Triacylglycerols

What is the structure?

APCI+, MS



Missing molecular adduct

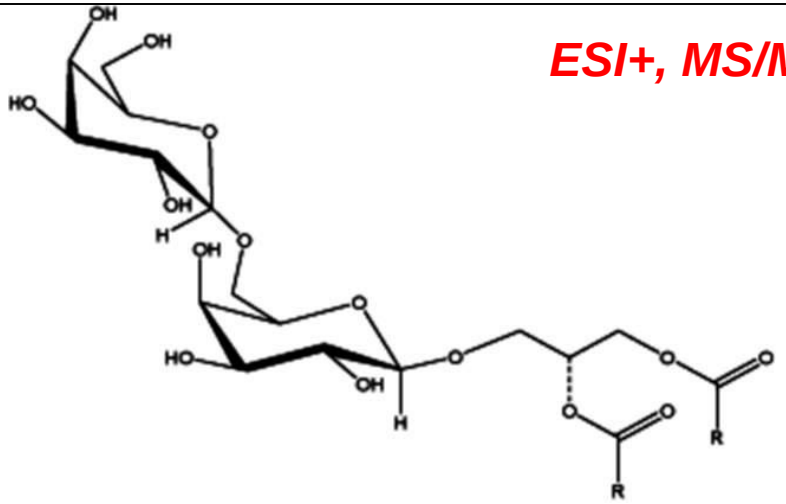
Just 1 fragment -> the same fatty acids in all positions

Calculation of two fatty acids in the fragment: 635-39 (glycerol part) = 596 = 2 x 298 (19:0)

Solution: 19:0, 19:0, 19:0

Digalaktosyldiacylglycerols

ESI+, MS/MS

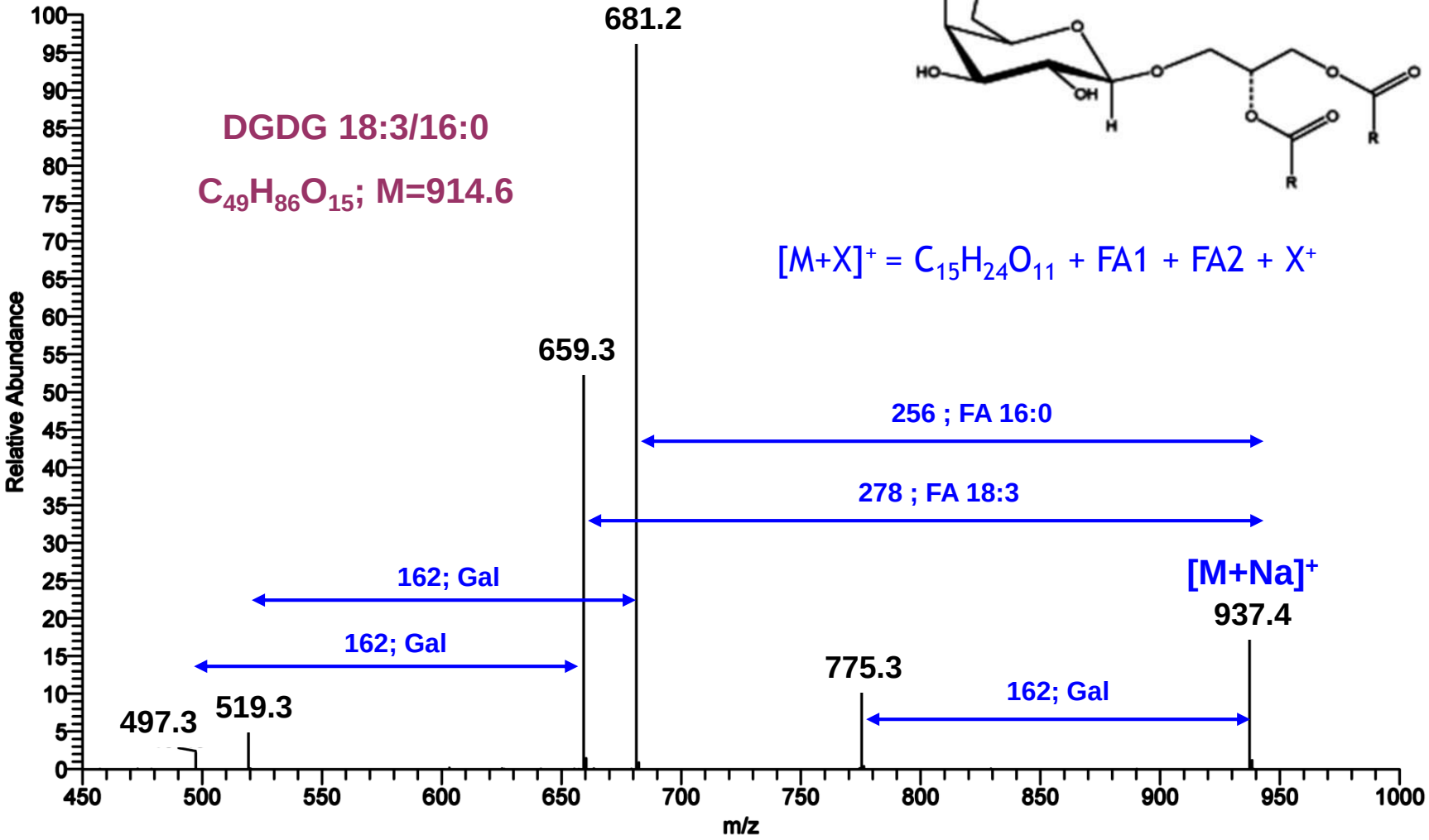


1656MK01 #1342-1375 RT: 21.08-21.58 AV: 17 NL: 1.26E3
T: ITMS + c ESI d Full ms2 937.47@cid28.00 [245.00-950.00]

DGDG 18:3/16:0

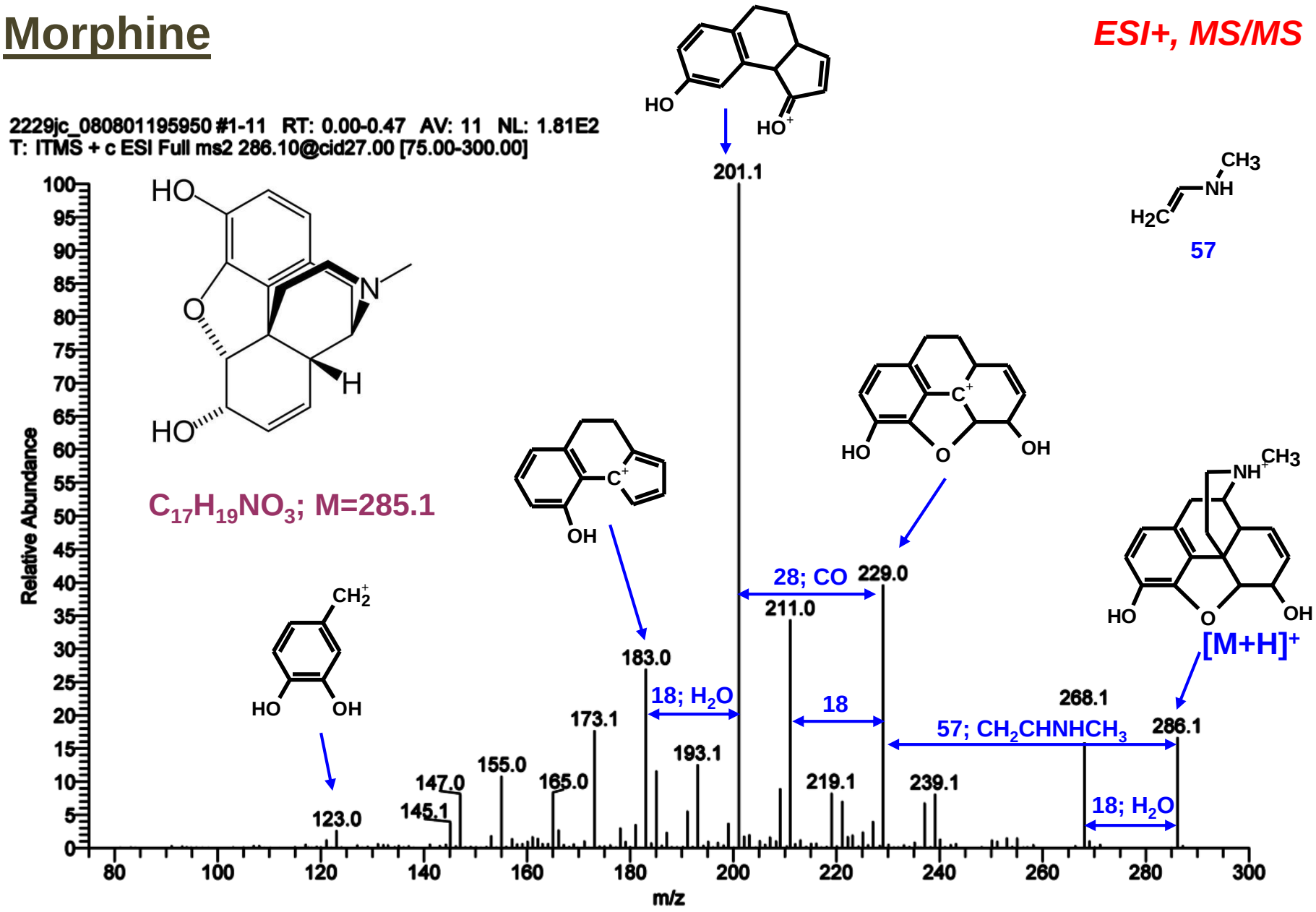
$C_{49}H_{86}O_{15}$; M=914.6

$[M+X]^+ = C_{15}H_{24}O_{11} + FA1 + FA2 + X^+$



Morphine

ESI+, MS/MS

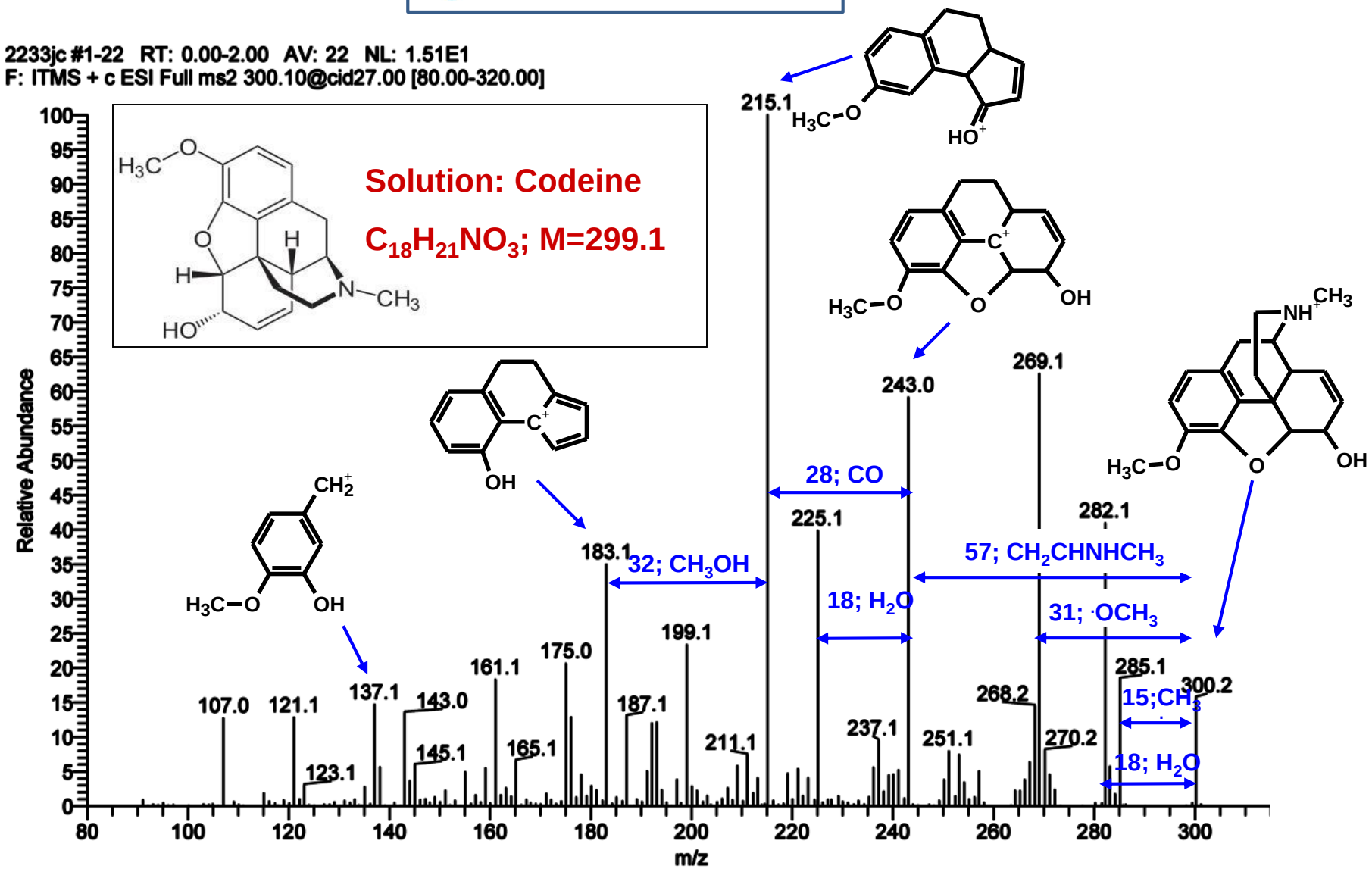


Struktury iontů:Peoaknapo et al., Phytochemistry 65 (2004) 1413.

Unknown

What is the structure?

ESI+, MS/MS



Ion structures: Peoaknapo et al., Phytochemistry 65 (2004) 1413.

Fragmentation of ions with odd number of electrons ($OE^{+\bullet}$)

EI

Fragmentation of $OE^{\bullet+}$

EI fragments are formed already in MS step (it is not necessary to use fragmentation techniques such as CID, etc.)

FRAGMENTATION of $OE^{+\bullet}$

I. formation of an ion with even number of electrons and a radical

$$OE^{+\bullet} \rightarrow EE^{+} + R^{\bullet}$$

II. formation of an ion with odd number of electrons and a neutral specie

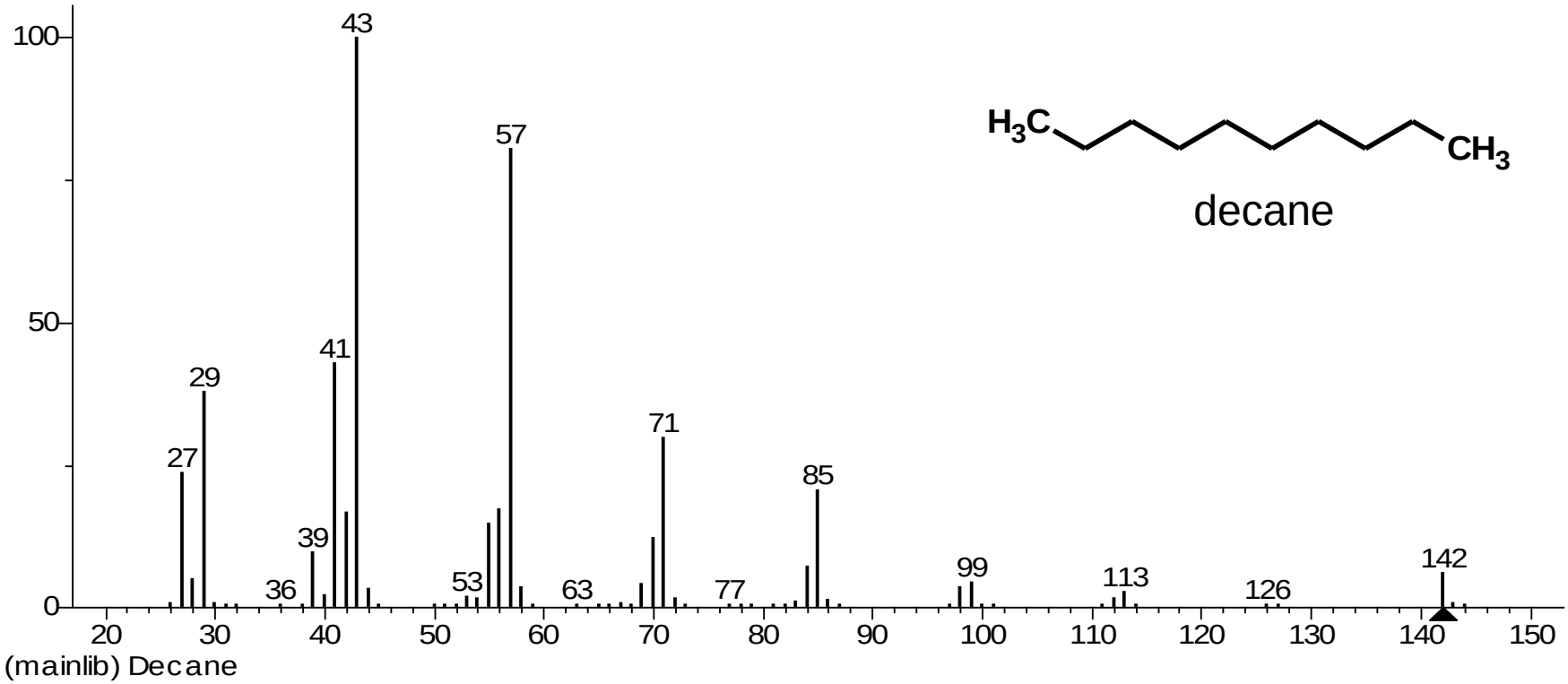
$$OE^{+\bullet} \rightarrow OE^{+\bullet} + M$$

Information-rich spectra are obtained, can be used as a "fingerprint" for the creation of libraries of spectra

Only monomolecular reactions take place

σ-bond cleavage

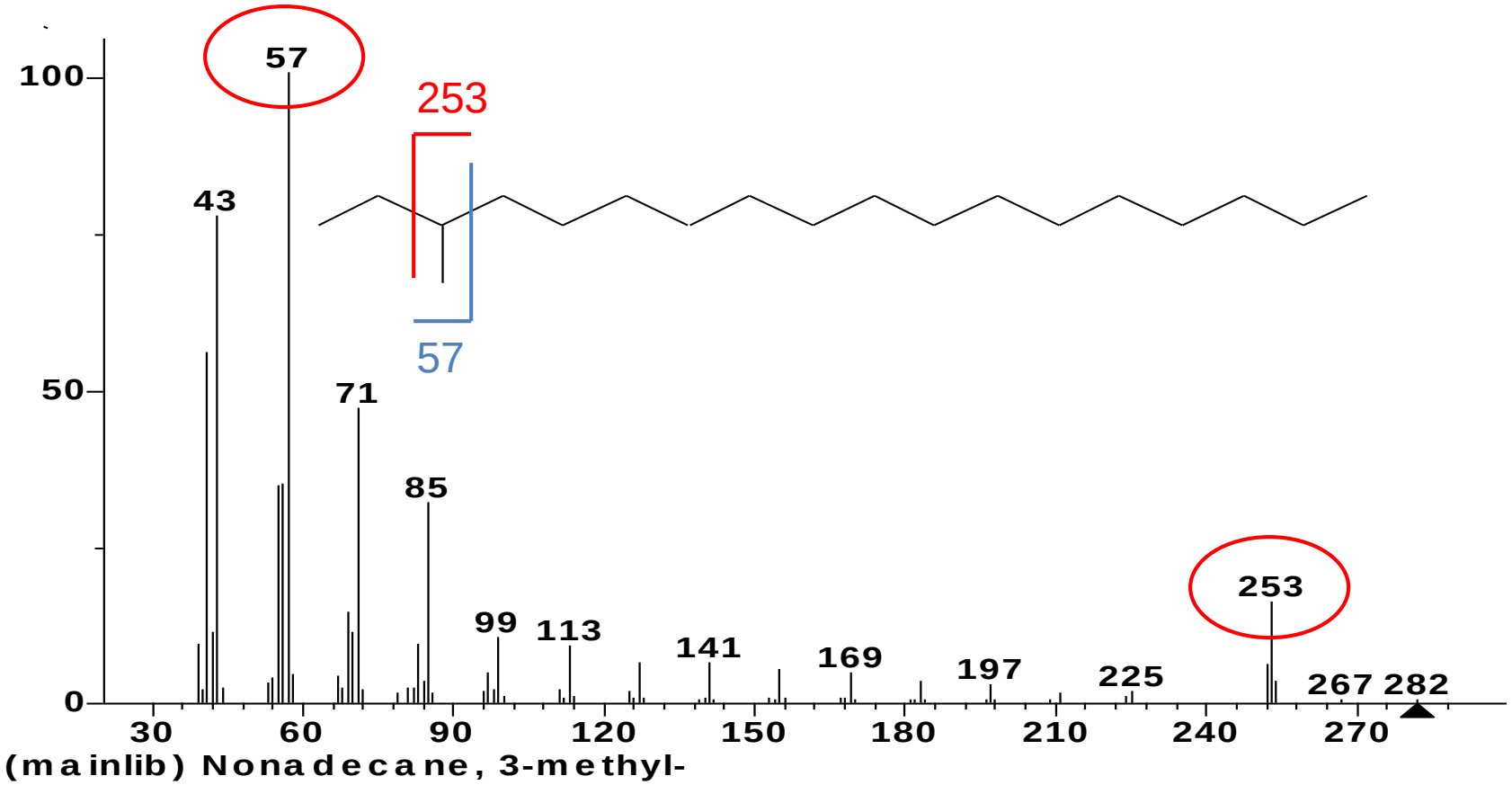
- The electron is expelled from a sigma bond
- Typical fragmentation for **alkanes**, or F-, Cl-, CN- substituted alkanes



decane

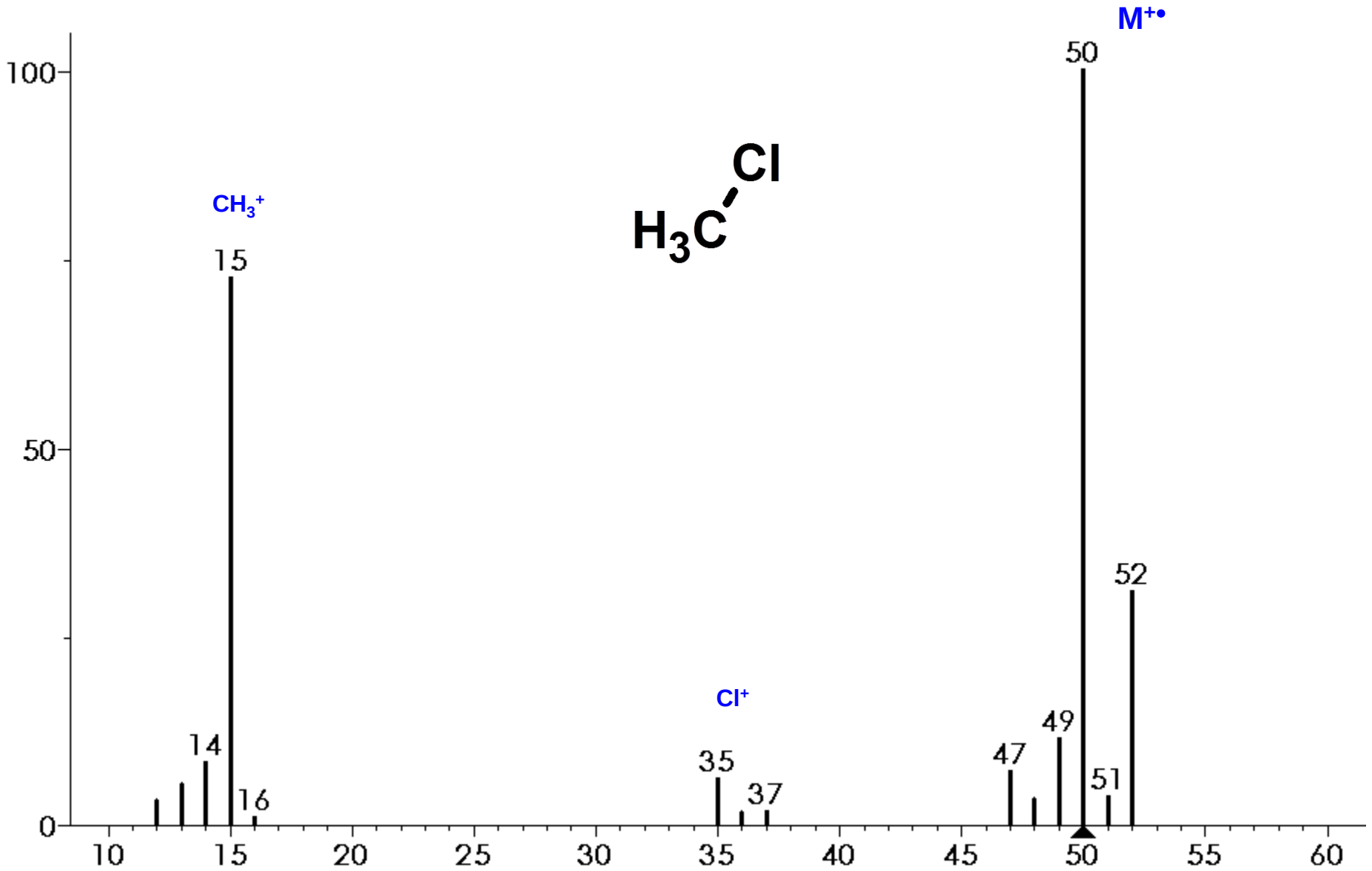
Branched hydrocarbons

- the ion intensity depends on the ability of fragments to stabilize the charge



Chloromethane

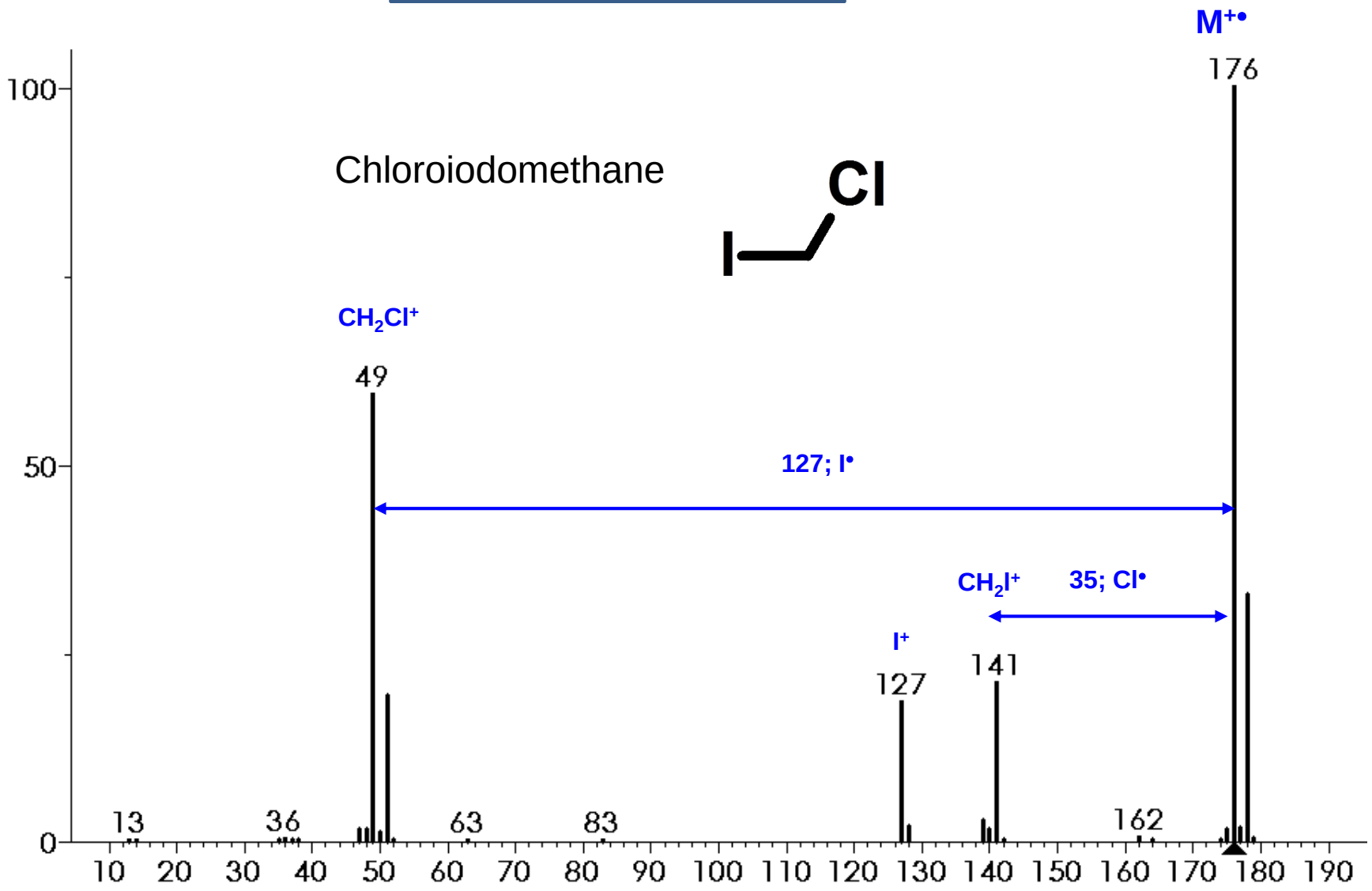
EI



Unknown

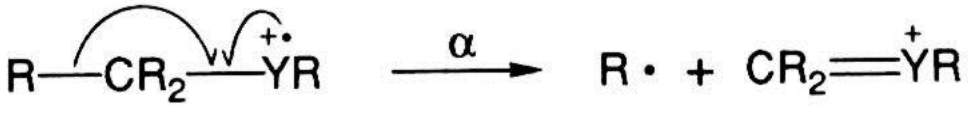
What is the structure?

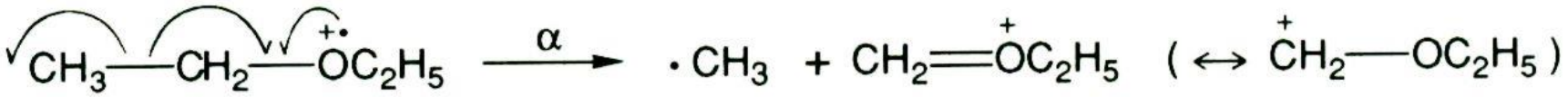
EI

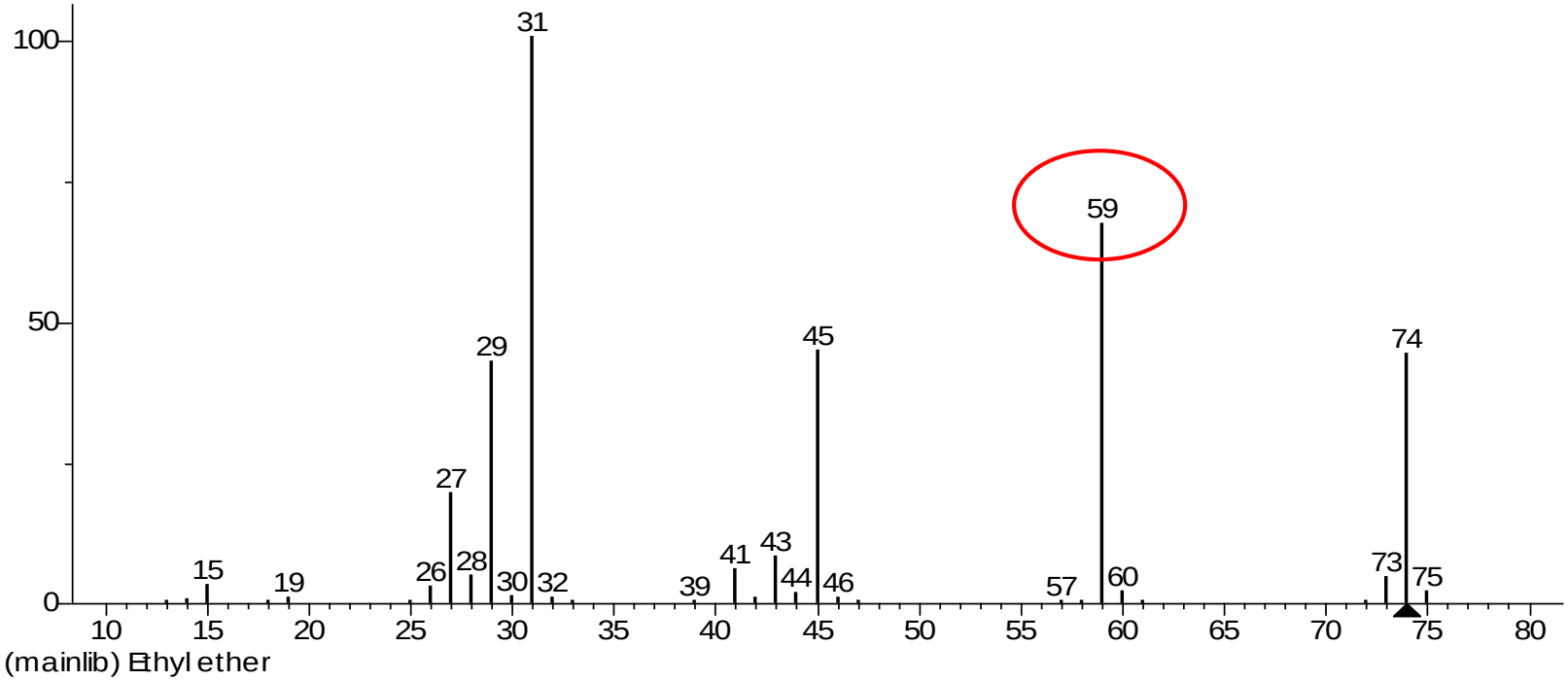


α-cleavage: fragmentation initiated by radical site

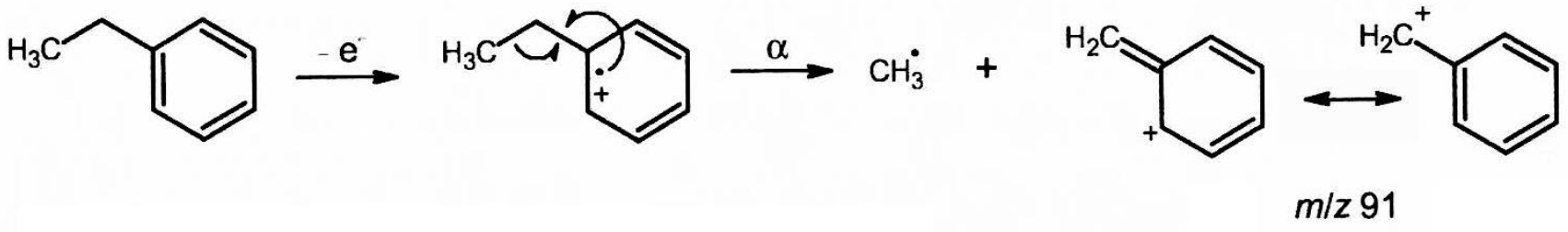
• cleavage induced by a strong tendency of electrons to form pairs – the odd electron is provided for the creation of a new bond; the neighboring bond is cleaved

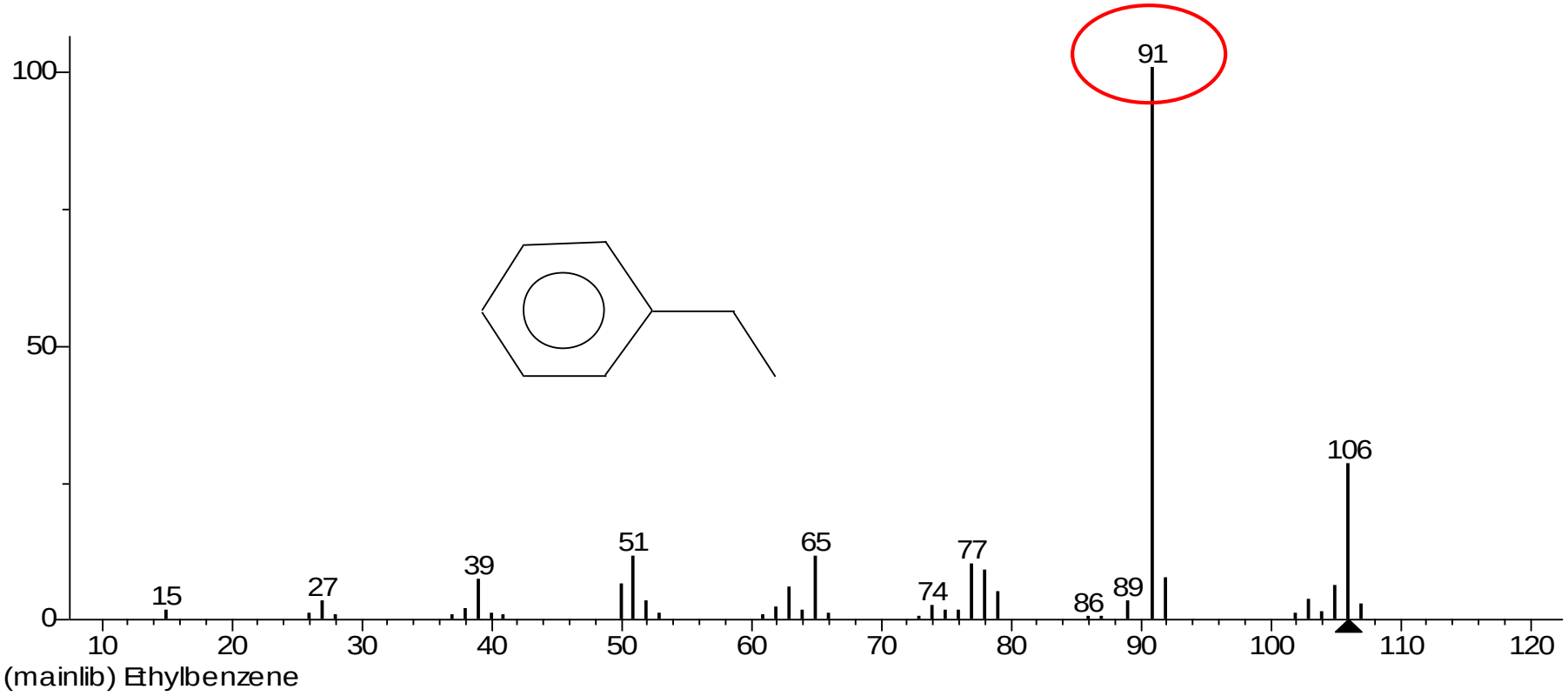






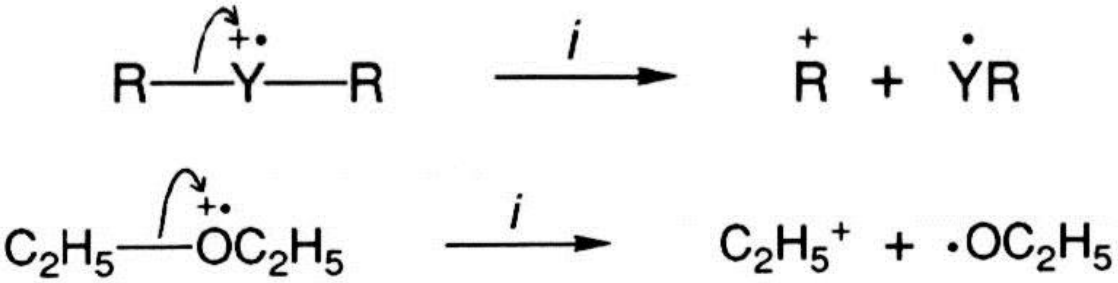
α-cleavage: benzylic clevage

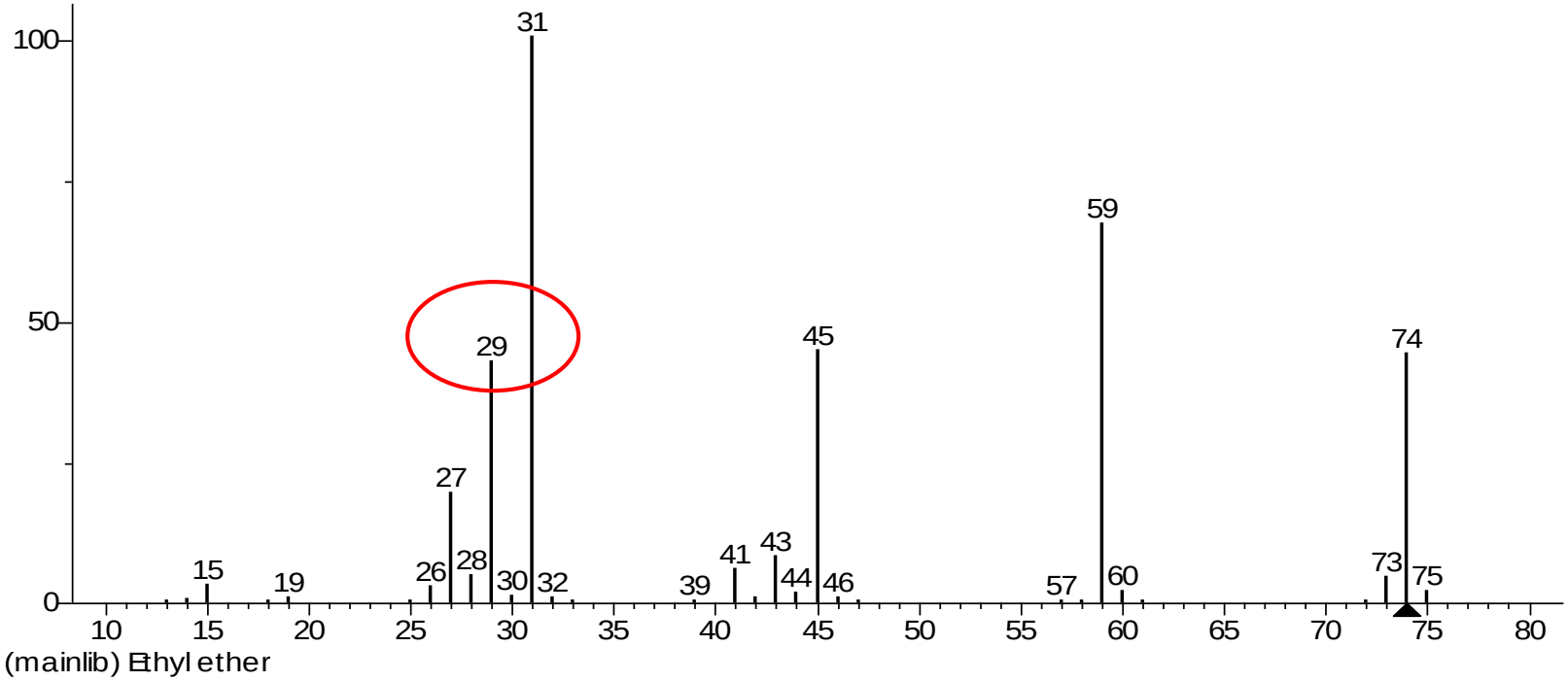




Inductive cleavage: fragmentation caused by a charge

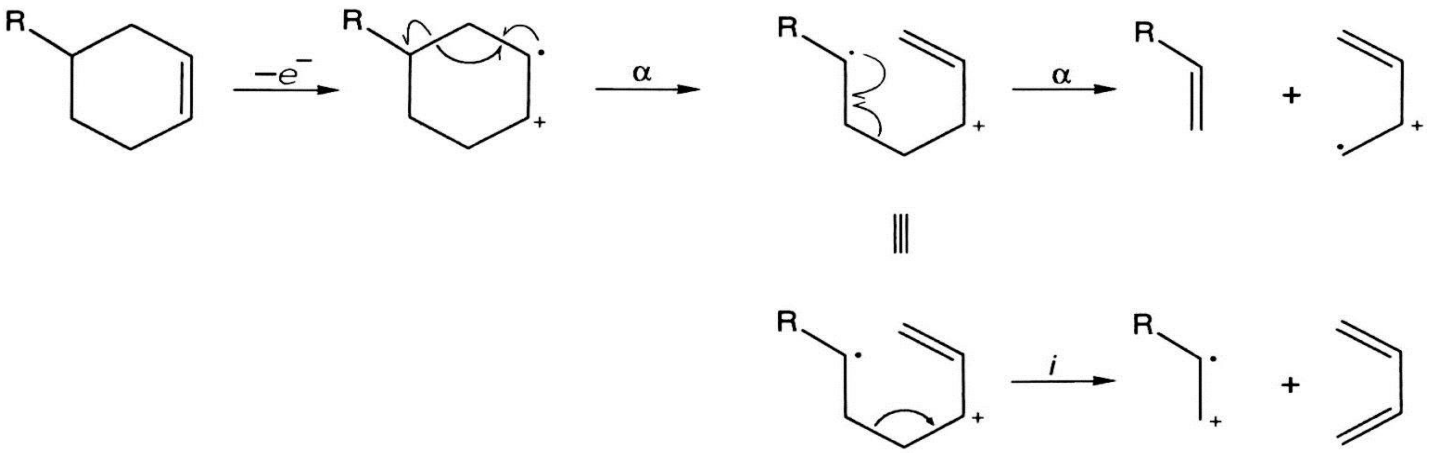
- cleavage initialized by attraction of an electron pair by the charge

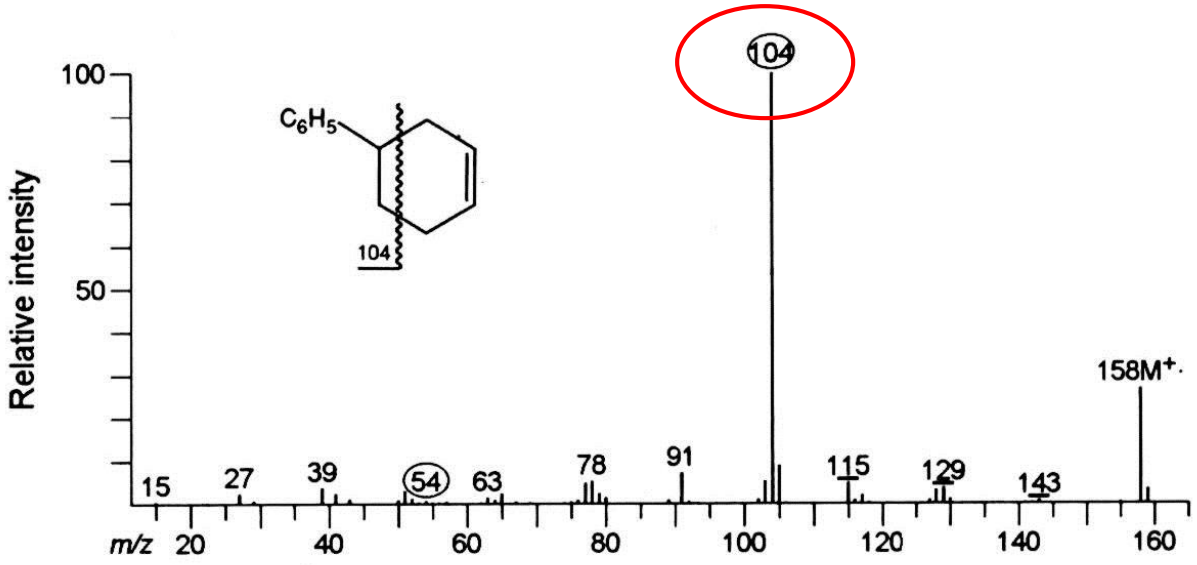




Fragmentation of cyclic structures – retro Diels-Alder

- π-electrons of double bonds in the cyclic structures are the primary site of ionization

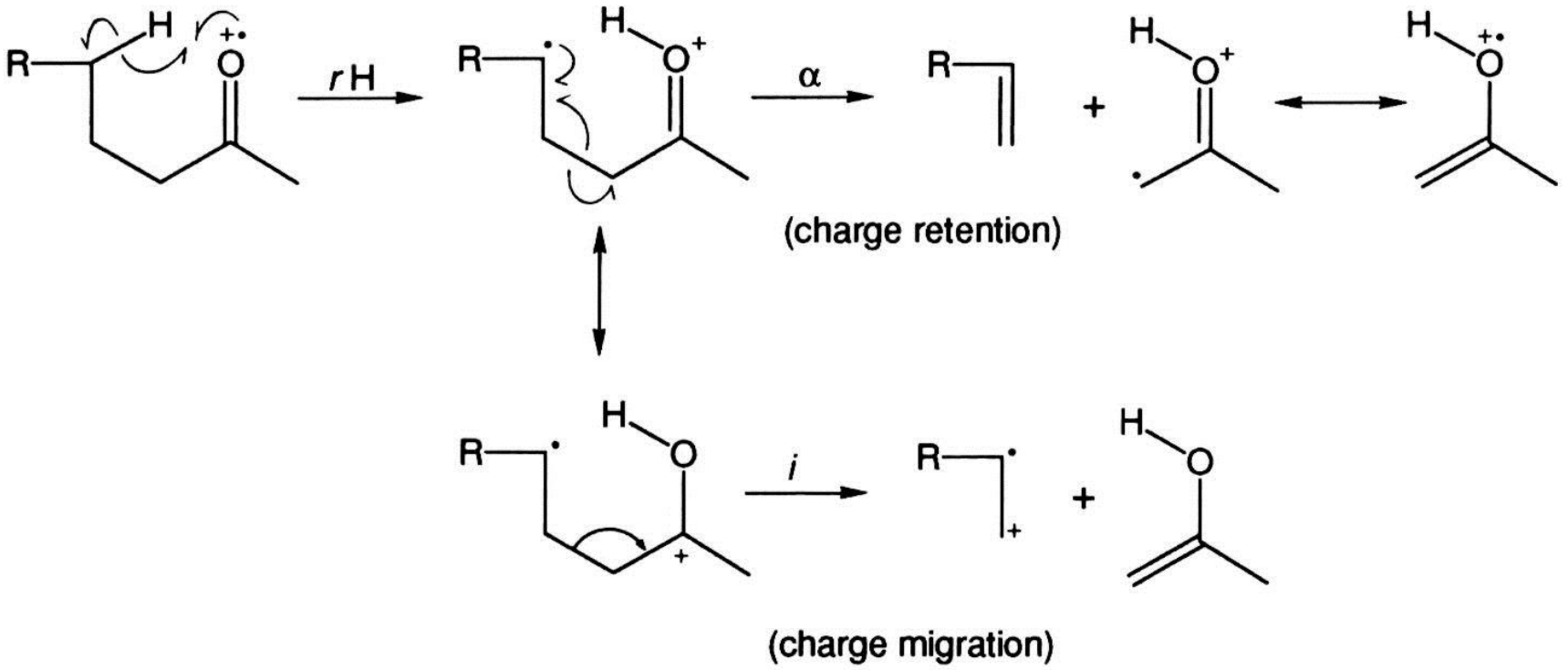




4-phenylcyklohexene

Hydrogen rearrangement – McLafferty rearrangement

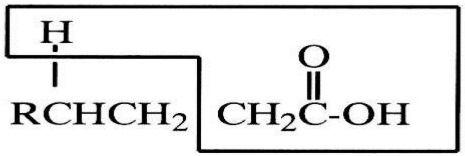
• rearrangement of γ-hydrogen on an unsaturated group over a 6-membered ring. The new radical site initializes α-cleavage.



Hydrogen rearrangement – McLafferty rearrangement

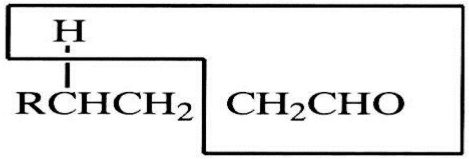
• The $OE^{+\bullet}$ fragments are typical for many functional groups - aldehydes, ketones, esters, acids, amides, carbonates, phosphonates, etc.

Acids



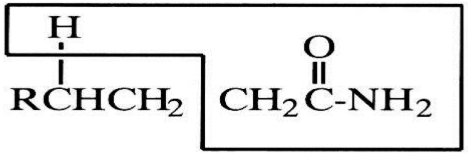
m/z 60

Aldehydes



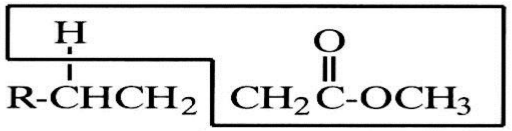
m/z 44

Amides



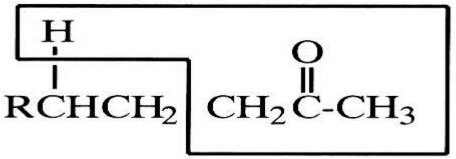
m/z 59

Esters



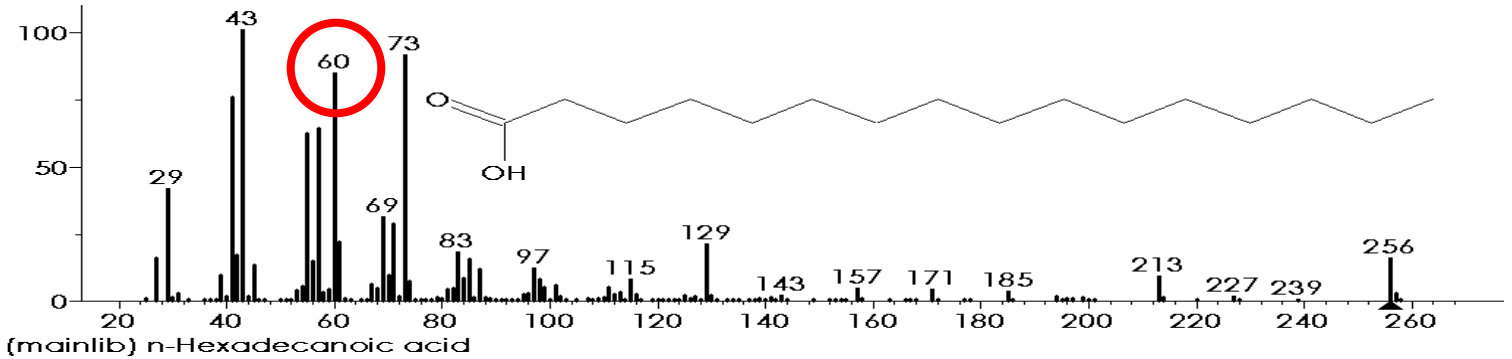
m/z 74

Ketones

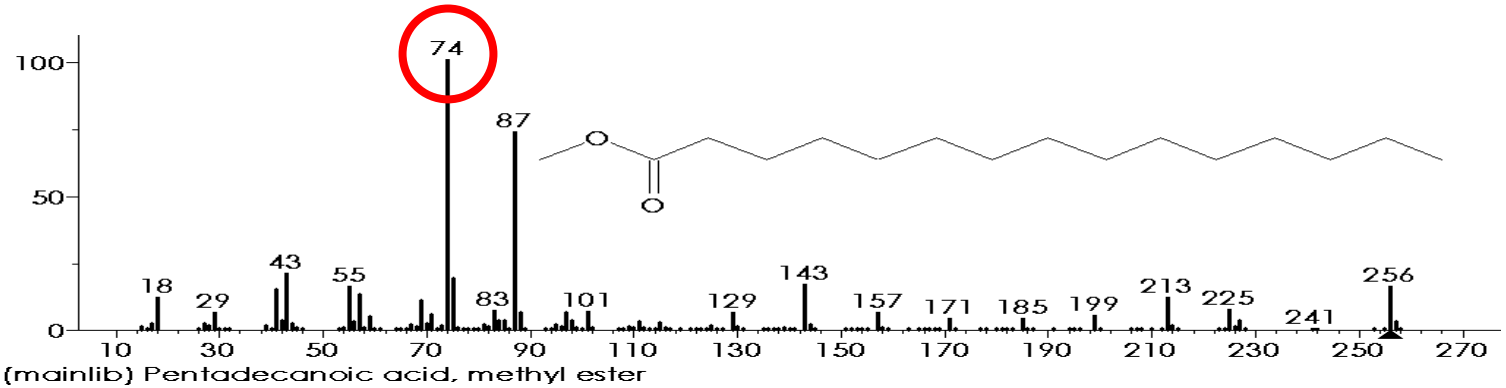


m/z 58

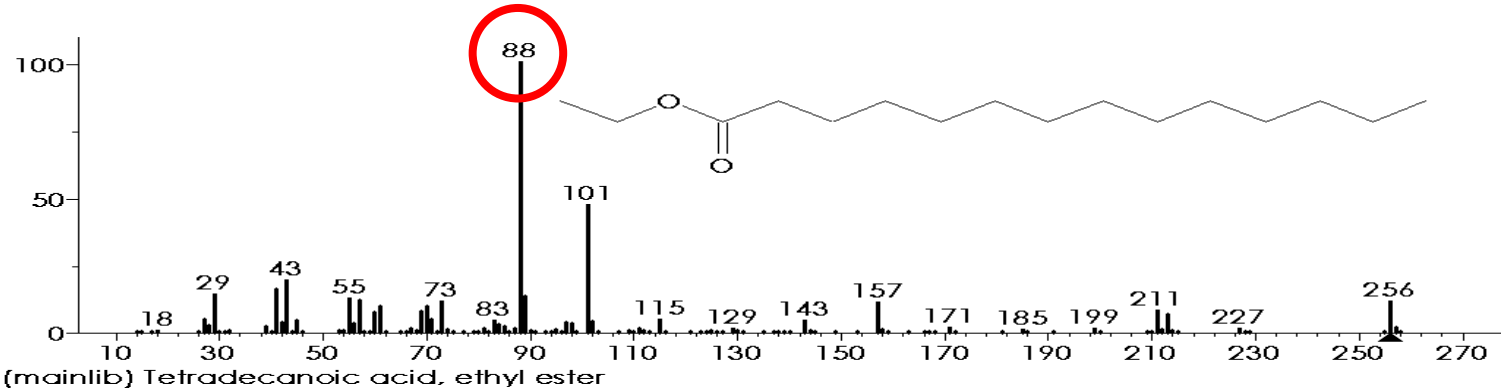
Hydrogen rearrangement – McLafferty rearrangement



(mainlib) n-Hexadecanoic acid



(mainlib) Pentadecanoic acid, methyl ester



(mainlib) Tetradecanoic acid, ethyl ester

Characteristic ion series

Homologous series in the low m/z range provide information on structural elements in the molecule.

Ionty	Série	Funkční skupiny
m/z 29, 43, 57, 71, 85, 99, ...	$C_nH_{2n+1}^+$	alkyl („alifatika“)
m/z 31, 45, 59, 73, 87, ...	$C_nH_{2n+1}O^+$	alkoholy, ethery
m/z 33, 47, 61, 75, 89, ...	$C_nH_{2n+1}S^+$	thioly, sulfidy
m/z 30, 44, 58, 72, 86, ...	$C_nH_{2n+2}N^+$	aminy
m/z 29, 43, 57, 71, 85, 99, ...	$C_nH_{2n-1}O^+$	aldehydy, ketony
m/z 45, 59, 73, 87, ...	$C_nH_{2n-1}O_2^+$	kyseliny, estery
m/z 40, 54, 68, 82, 96, ...	$C_nH_{2n-2}N^+$	nitrily
m/z 38, 39, 50-52, 63-65, 75-78, 89-92	-	aromatika

Characteristic ions

m/z	Ion
19	F^+, H_3O^+
20	$HF^{+\bullet}$
30	$CH_2NH_2^+$, indikuje aminy
31	indikuje CH_3O- nebo -CH_2OH
33,34	HS^+, $H_2S^{+\bullet}$
35,36,37,38	Cl^+, $HCl^{+\bullet}$
46	NO_2^+, indikuje nitrosloučeniny
47	CCl^+, $HC(OH)_2^+$, CH_3S^+
61	$CH_3C(OH)_2^+$ indikuje „dlouhé" estery kyseliny octové
73	$(CH_3)_3Si^+$, $CH_5Si^{+\bullet}$
77	fenyl (doprovázen m/z 51 a 50)
105	benzoyl (pokud doprovázen m/z 77)

podle M.Poláška – Škola MS

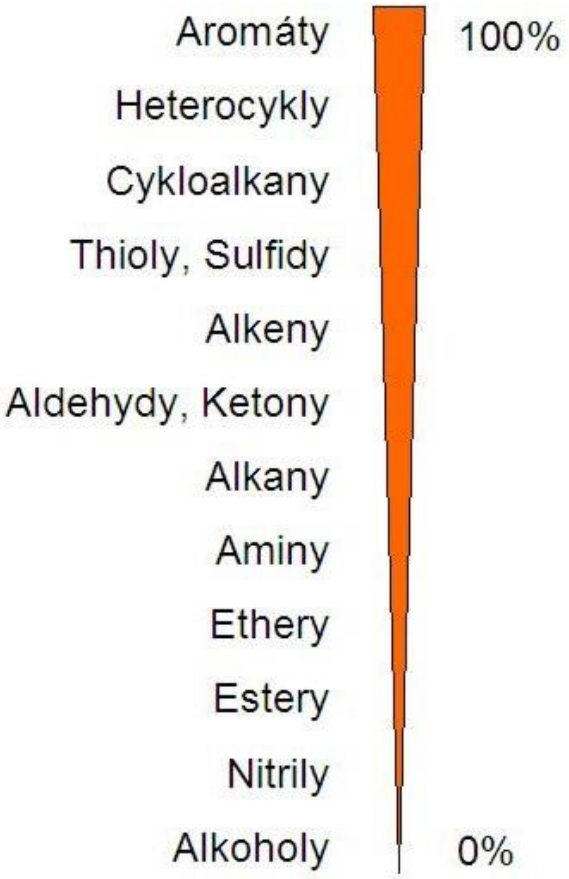
Logical neutral losses

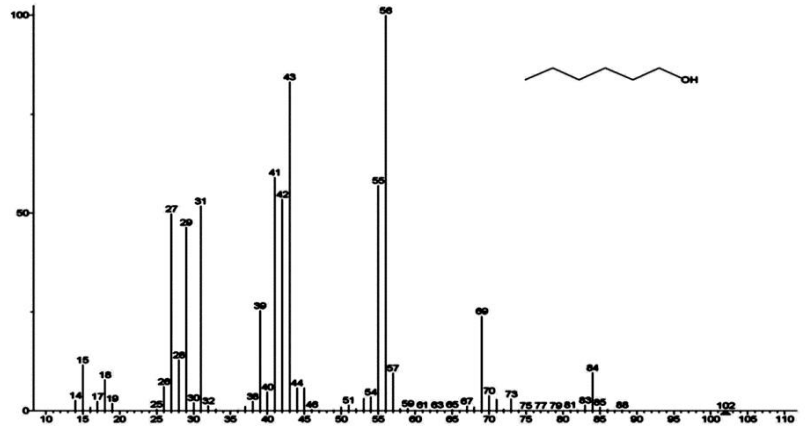
Neutral losses of radicals and neutral molecules must make chemical sense.

Ztráta (u)	Logická ztráta ?	Složení
1	ano	H
2	ano	H_2
3 - 14	NE	-
15	ano	CH_3
16	ano	NH_2, O
17	ano	OH, NH_3
18	ano	H_2O
19	ano	F
20	ano	HF
21 - 25	NE	-
26	ano	C_2H_2, CN
27	ano	C_2H_3, HCN
28	ano	C_2H_4, CO
29	ano	HCO, C_2H_5, CH_3N
30	ano	CH_2O
31	ano	CH_3O
32	ano	CH_3OH, S
33	ano	SH
34	ano	H_2S
35	ano	Cl
36	ano	HCl
37 - 40	NE	-
41 a výše	ano	homologické ztráty

The molecular ion intensity

The olecular ion intensity is related to its stability. The intensity suggests the presence of certain structural elements in the molecule.

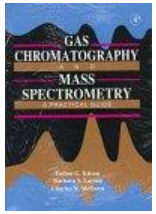




Literature

EI

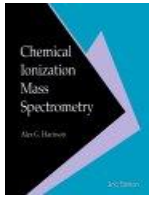
Fred W. McLafferty and Frantisek Turecek: Interpretation of Mass Spectra. University Science Books (1993). ISBN-10: 0935702253, ISBN-13: 978-0935702255



Fulton G. Kitson, Barbara S. Larsen, and Charles N. McEwen: Gas Chromatography and Mass Spectrometry. Academic Press (1996). ISBN-10: 0124833853, ISBN-13: 978-0124833852

ESI
APCI
CI

Levsen et al.: Even-electron ions: a systematic study of the neutral species lost in the dissociation of quasi-molecular ions. J. Mass Spectrom. 42, 1024 – 1044, 2007



Alex. G. Harrison: Chemical Ionization Mass Spectrometry, CRC(1992). ISBN-10: 0849342546, ISBN-13: 978-0849342547

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Thank you for your attention !