Spectra Interpretation: Small Molecules

Josef Cvačka

General interpretation procedure for mass spectra

1/ Disclosing ions that are related to the analyte and that are not

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

3/ Estimating elements which are present: inspecting isotope clusters, application of nitrogen rule

4/ Determination of the elemental formula from exact mass measurement

5/ Comparison the spectrum with a library, searching 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
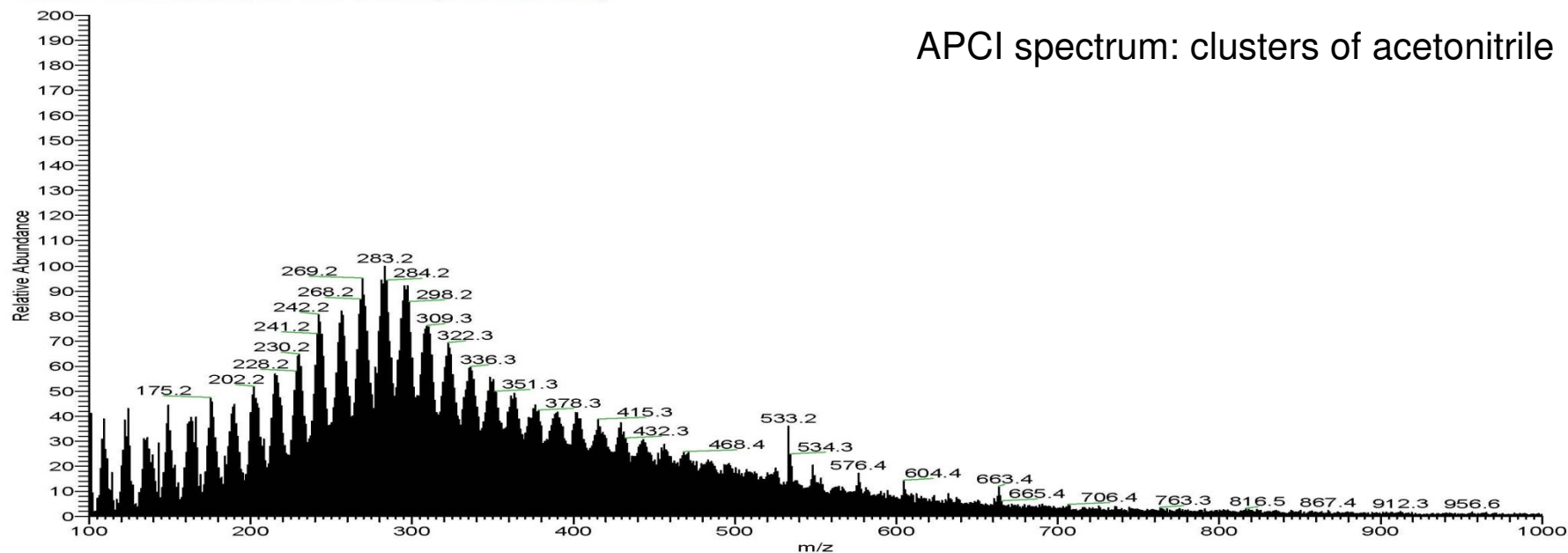
Background ions, contaminants

- solvent cluster ions
- impurities form solvents
- impurities from plastic ware and glassware
- impurities in the mass spectrometer from previous injections
- column bleeding
- impurities from sample
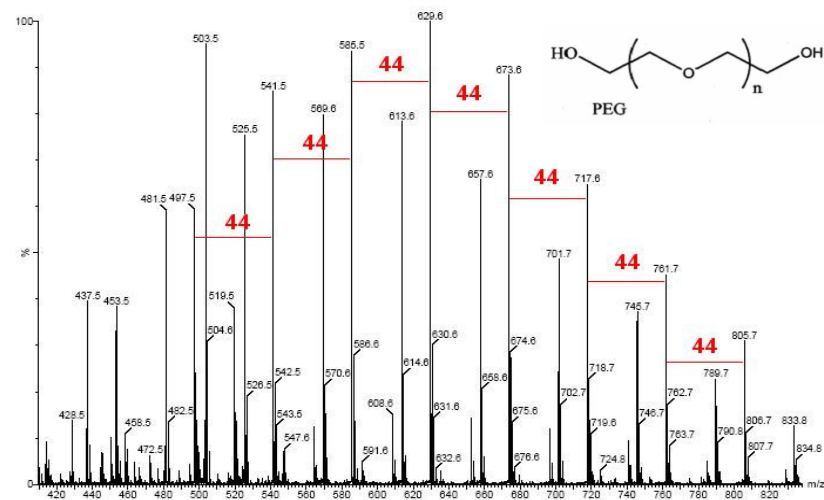

APCI spectrum: clusters of acetonitrile

Background ions, contaminants

Phthalates: common plasticizers, from laboratory plastic
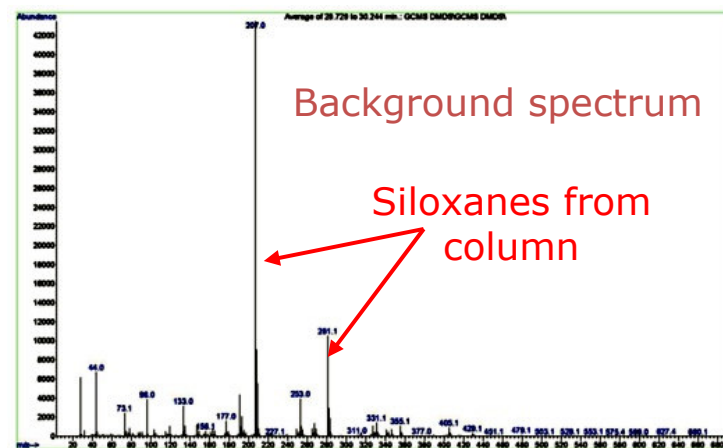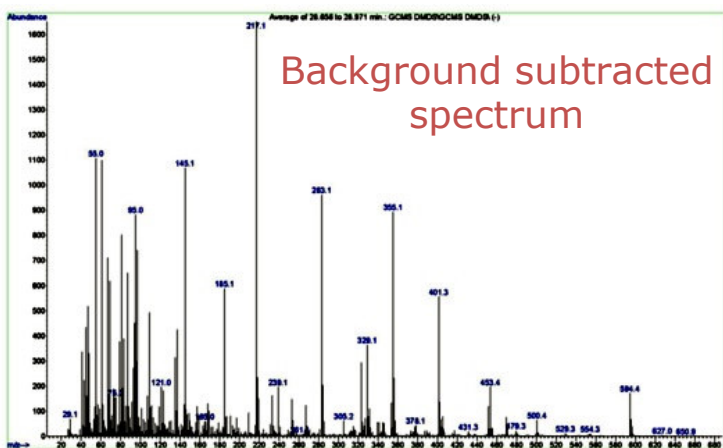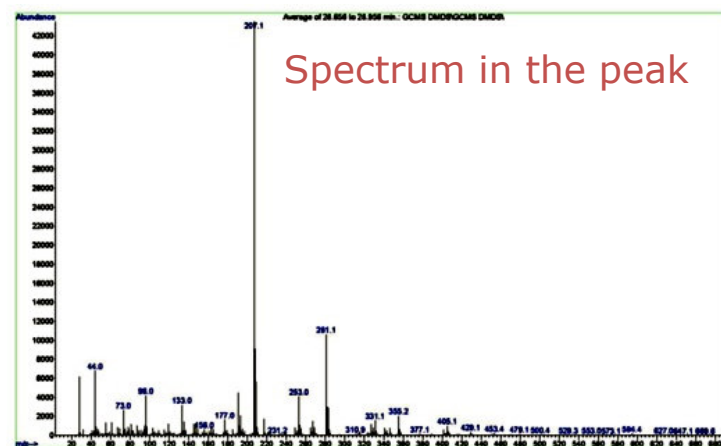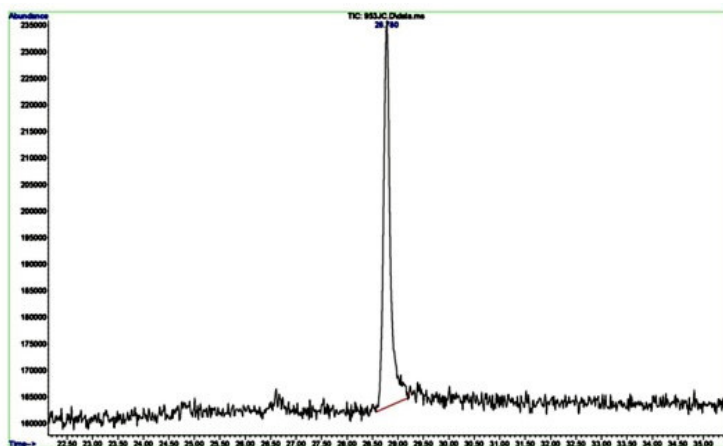*m/z* 149, 279, 301, 391, 413 ...
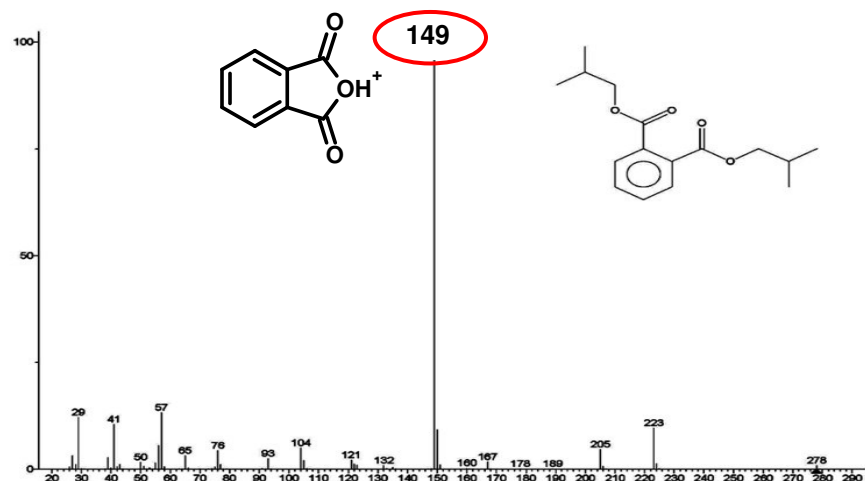
Polyethylenglycols:
from laboratory plastic, gloves, skin lotion
peak difference 44 u

MeOH wash of laboratory gloves

Background subtraction

Spectrum in the peak

Background subtracted spectrum

Background spectrum

Siloxanes from column

Matrix ions in MALDI

> ➤ 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.
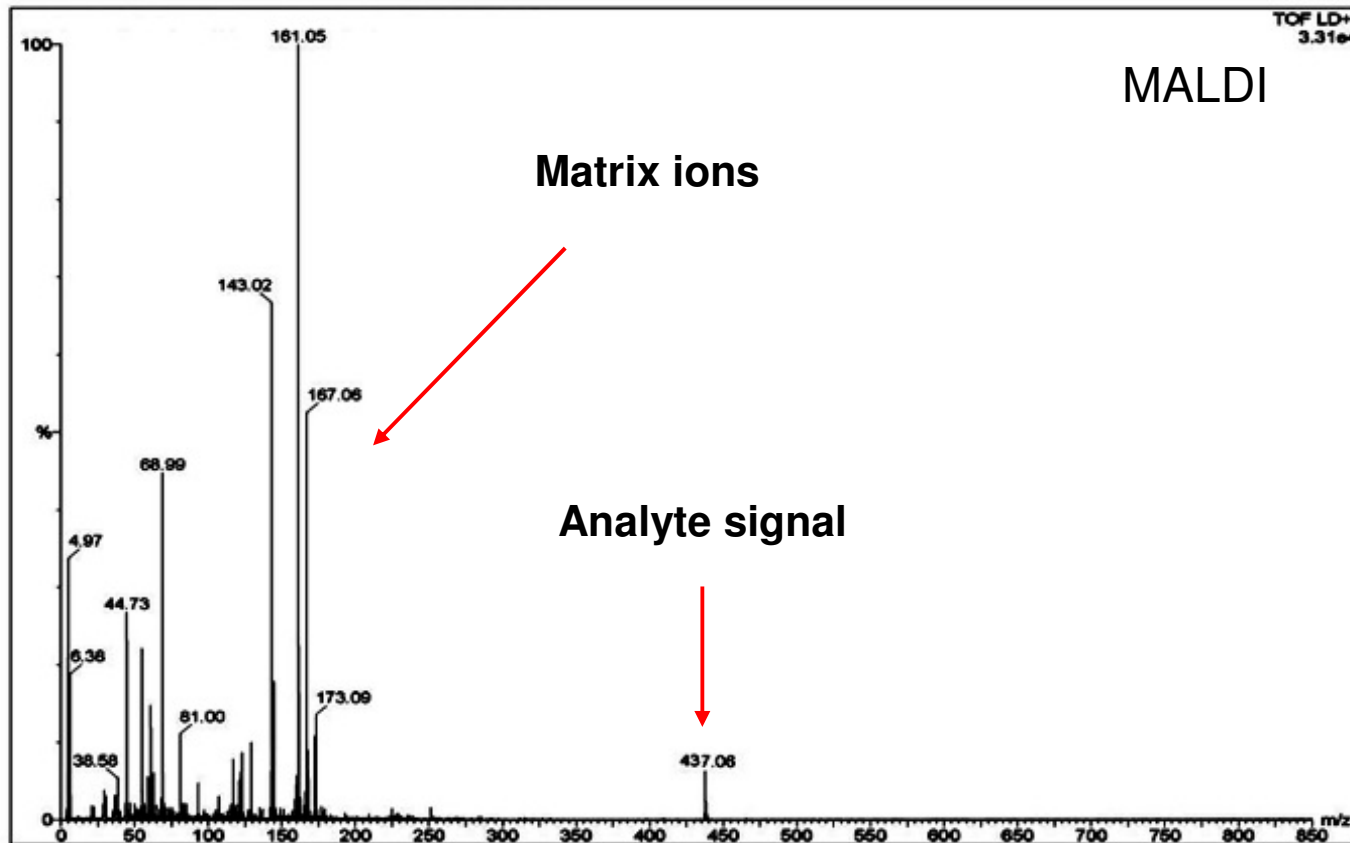
Determination of the molecular weight

<u>Determination of molecular weight</u>

Identification of molecular ion or molecular adduct
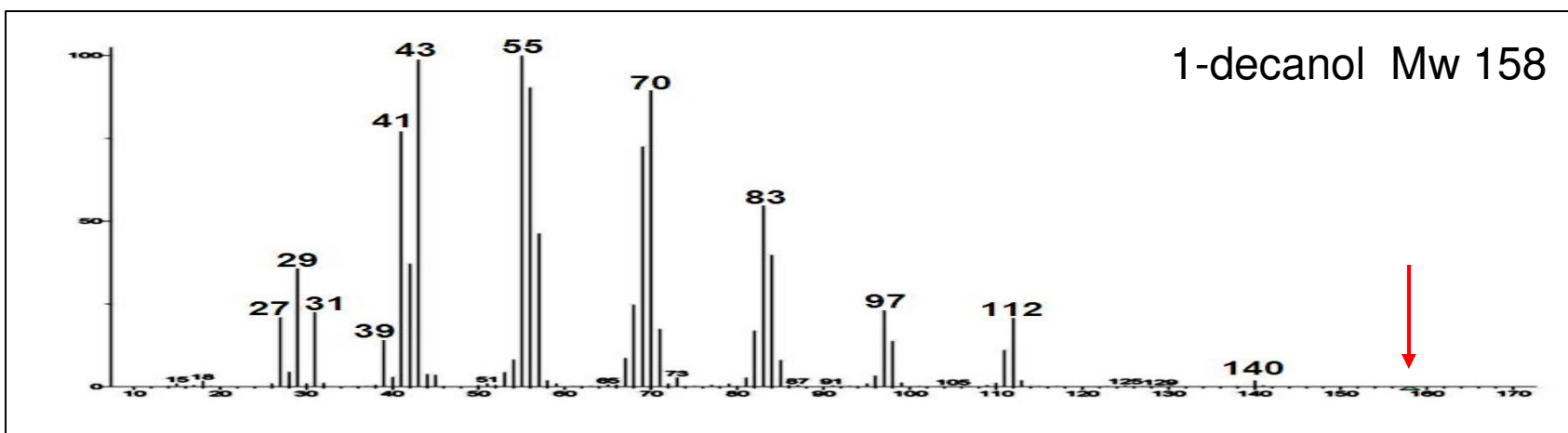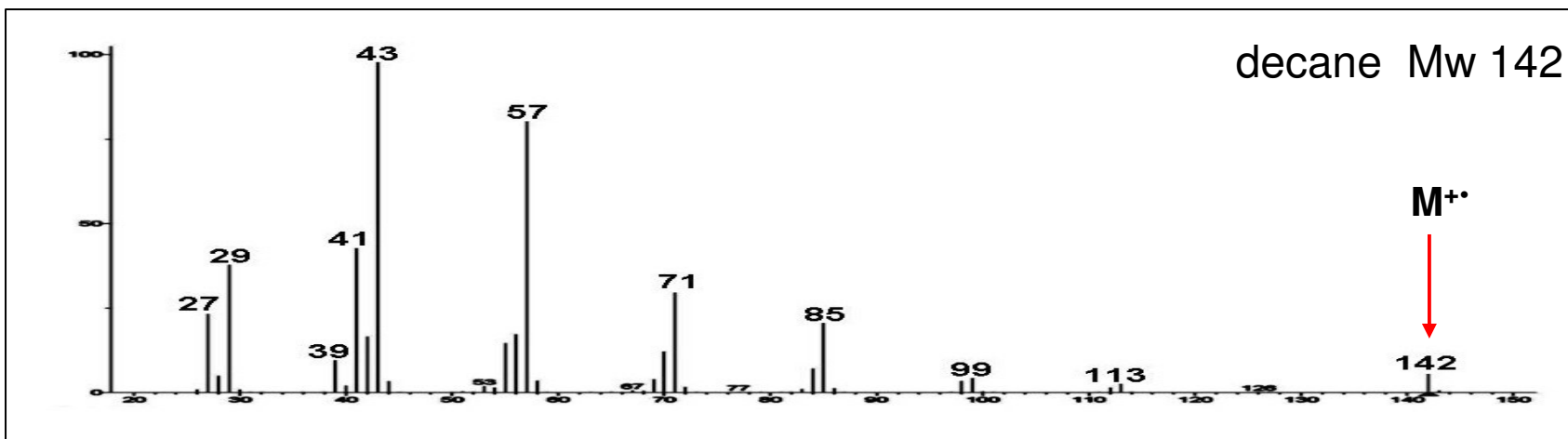
I. The electron ionization

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

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

<u>Identification of the molecular ion in the spectrum</u>

1/ the 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
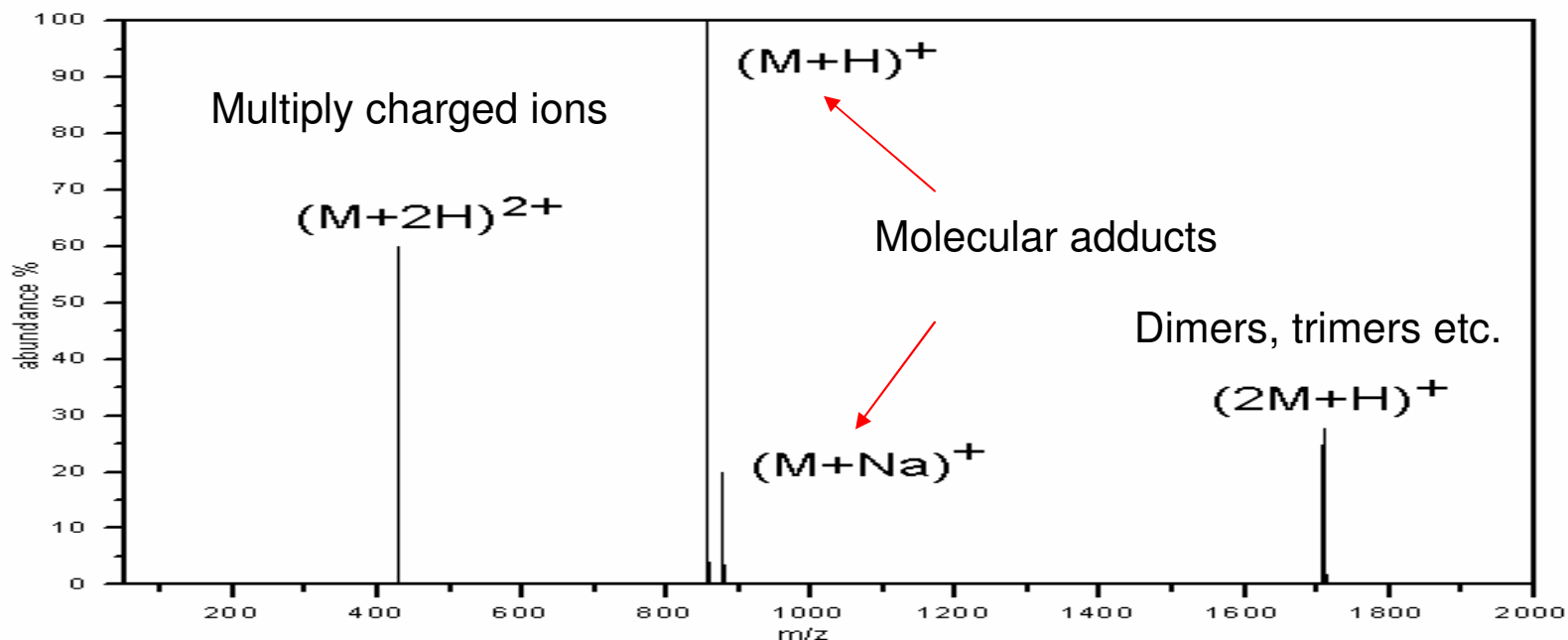
I. The electron ionization

Determination of molecular weight

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

Molecular adducts (**[M+H]$^+$, [M+Na]$^+$**) or deprotonated molecules (**[M-H]$^-$**)
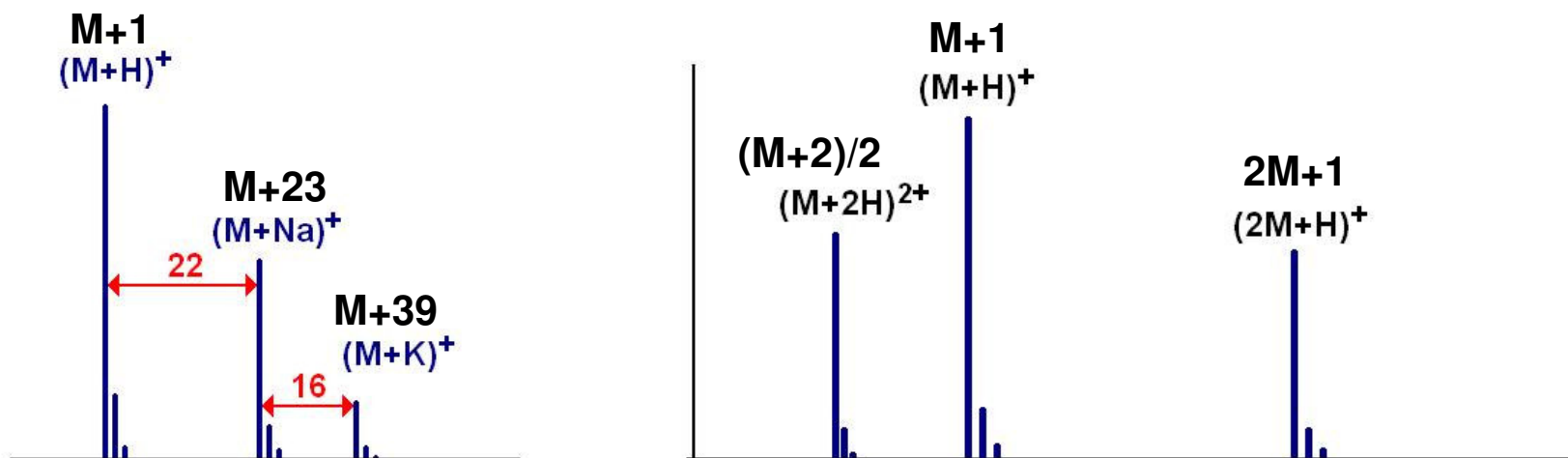
The molecular adduct is an ion with even number of electrons and may not be the highest ion in the spectrum.

Determination of molecular weight

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

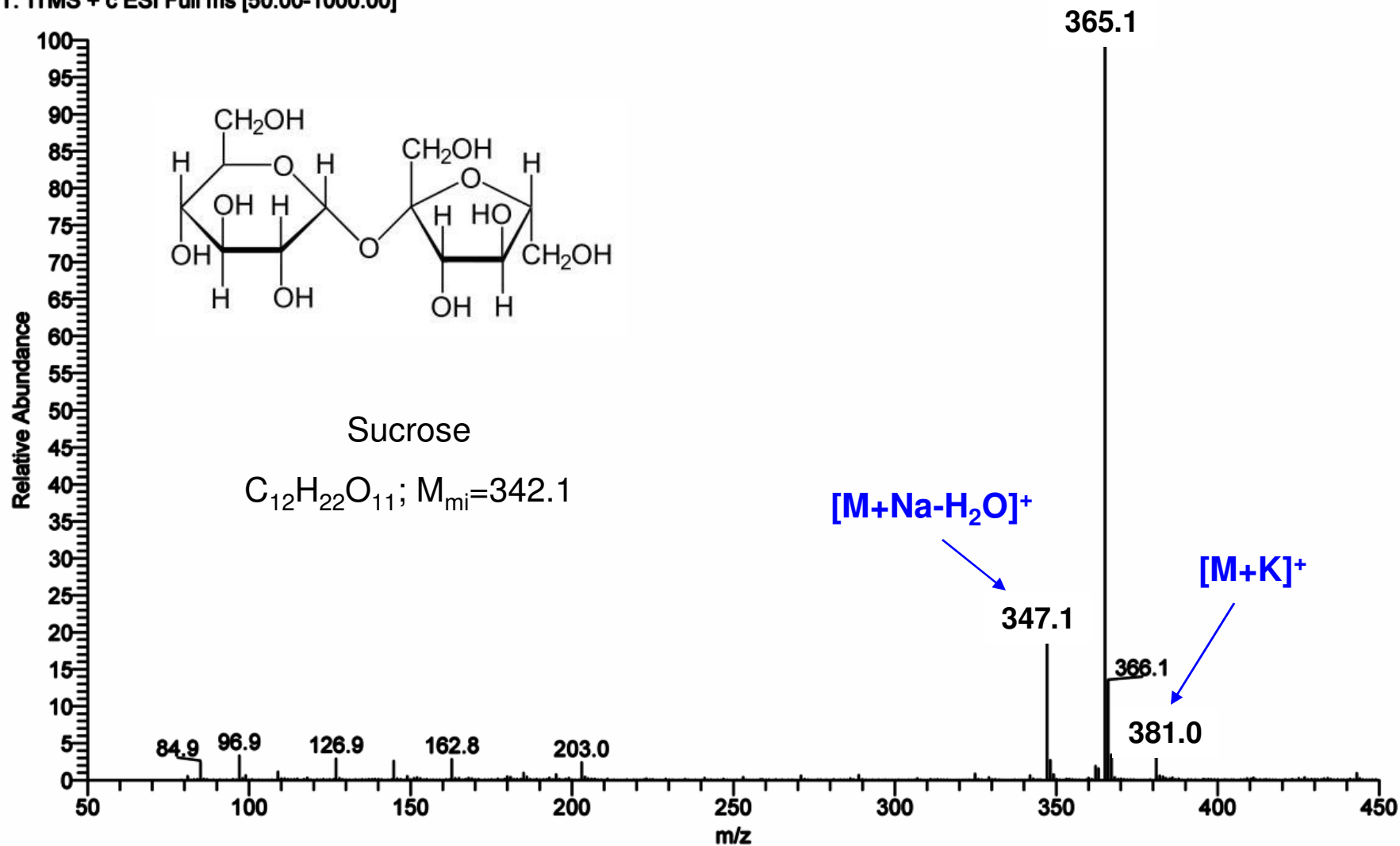The molecular weight is determined based on the presence of adducts, dimers or multiply charged ions.

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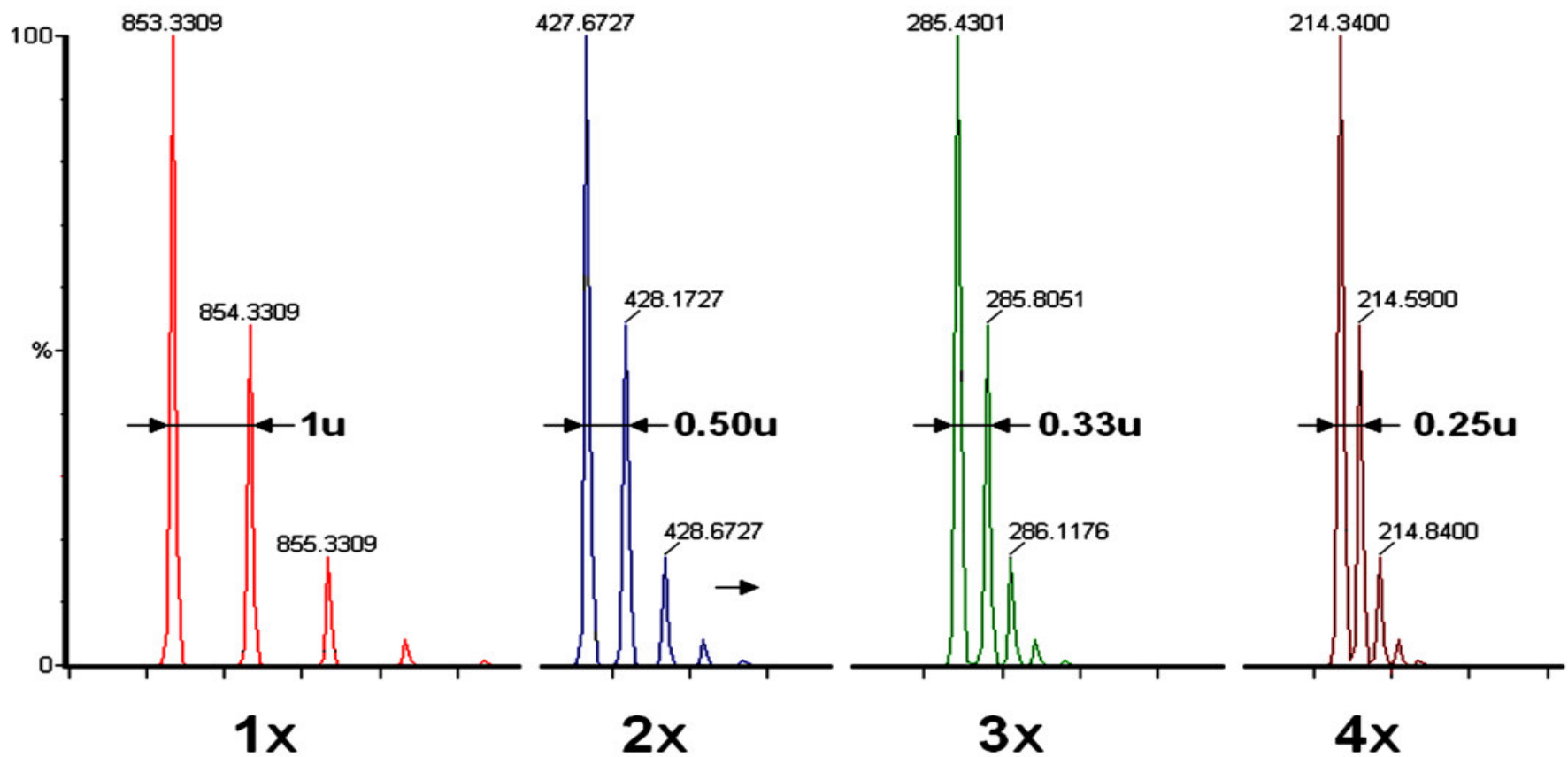
Determination of molecular weight

2256jc #1-51 RT: 0.00-0.75 AV: 51 NL: 1.77E4
T: ITMS + c ESI Full ms [50.00-1000.00]

Sucrose

$C_{12}H_{22}O_{11}$; M_{mi}=342.1

$[M+Na]^+$

365.1

$[M+Na-H_2O]^+$

347.1

$[M+K]^+$

366.1

381.0

84.9 96.9 126.9 162.8 203.0

Determination of molecular weight – charge state
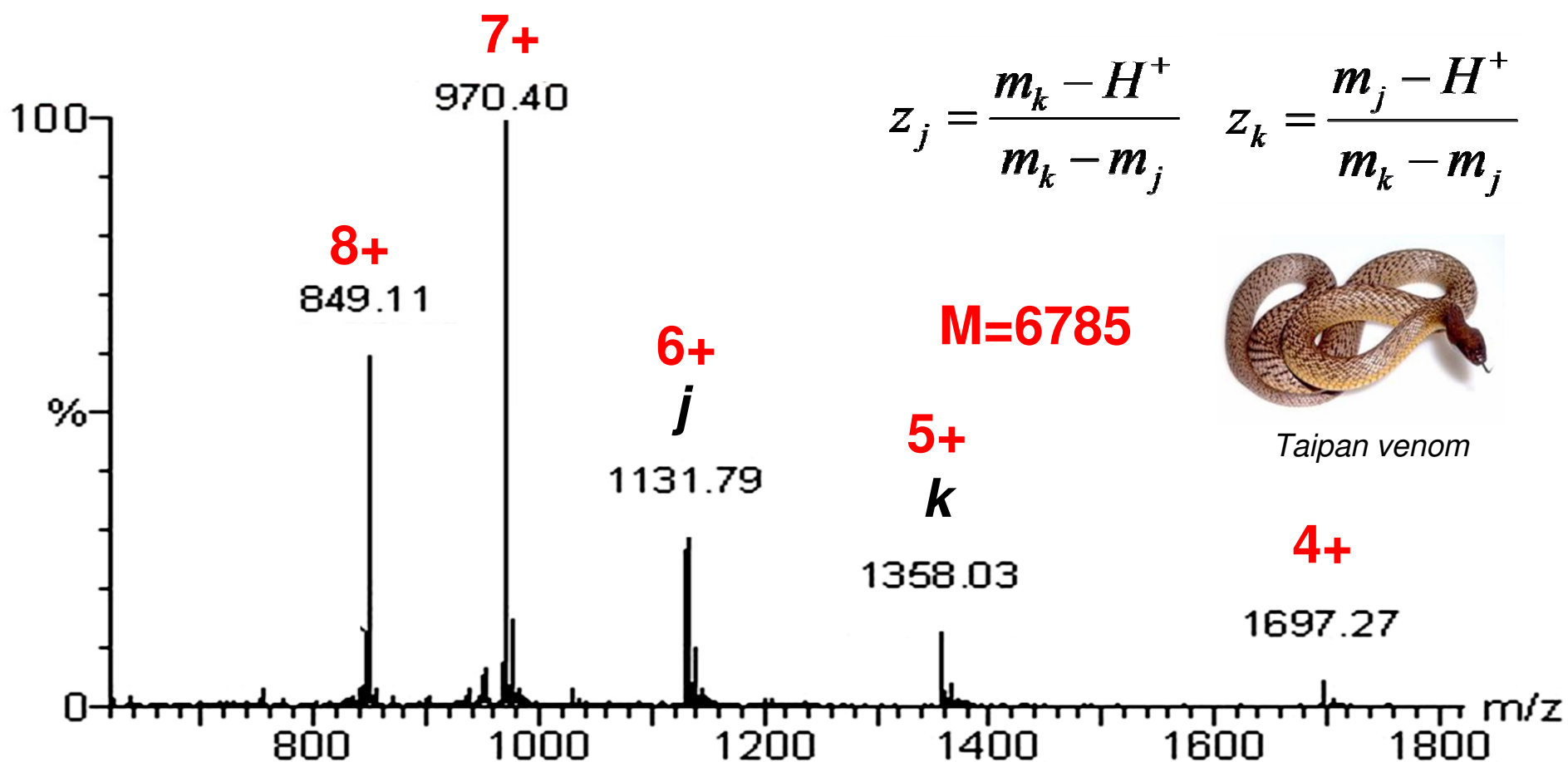
Determining number of charges

Number of charges can be determined from the distance between the peaks in the isotopic clusters.
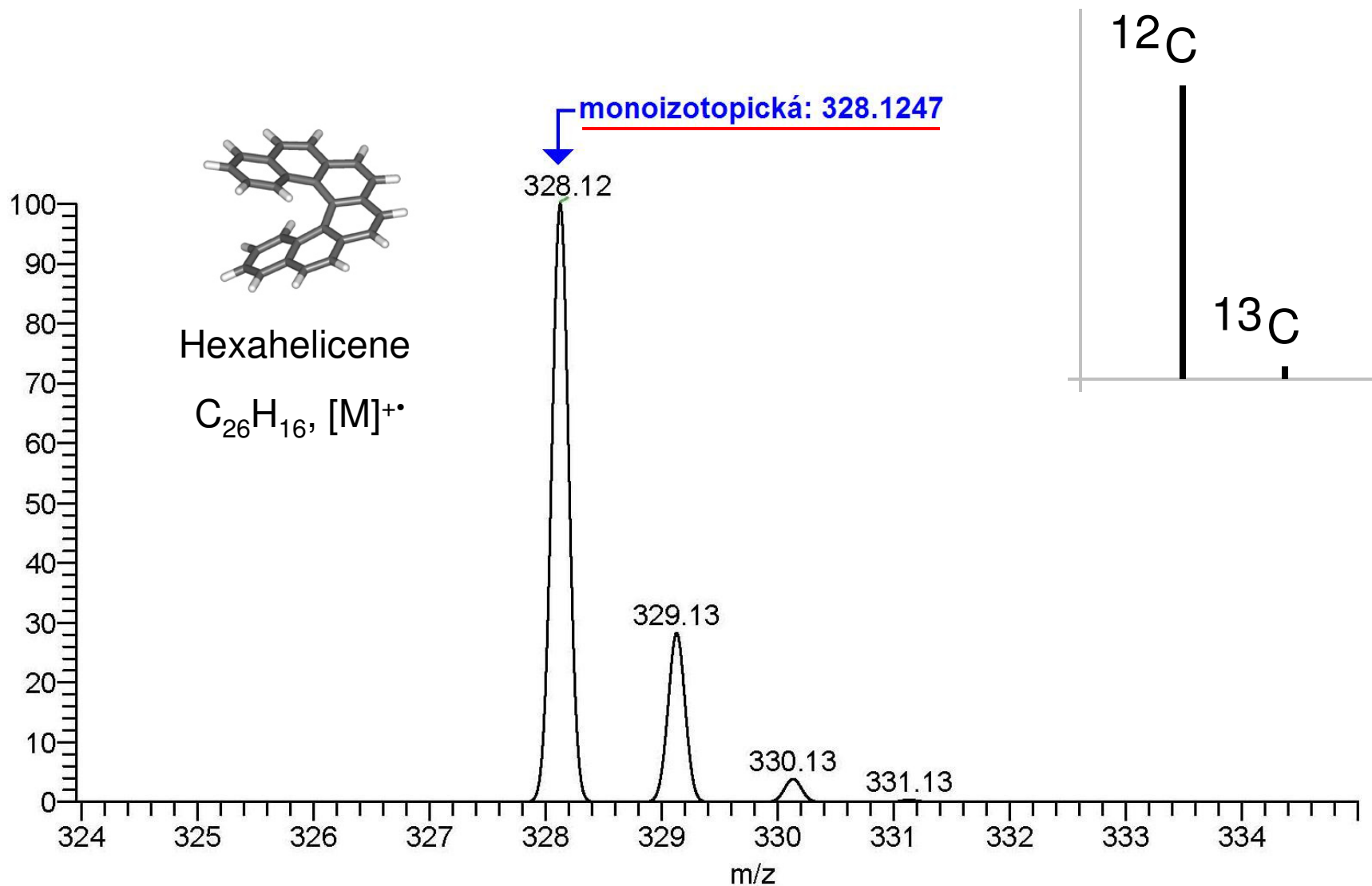
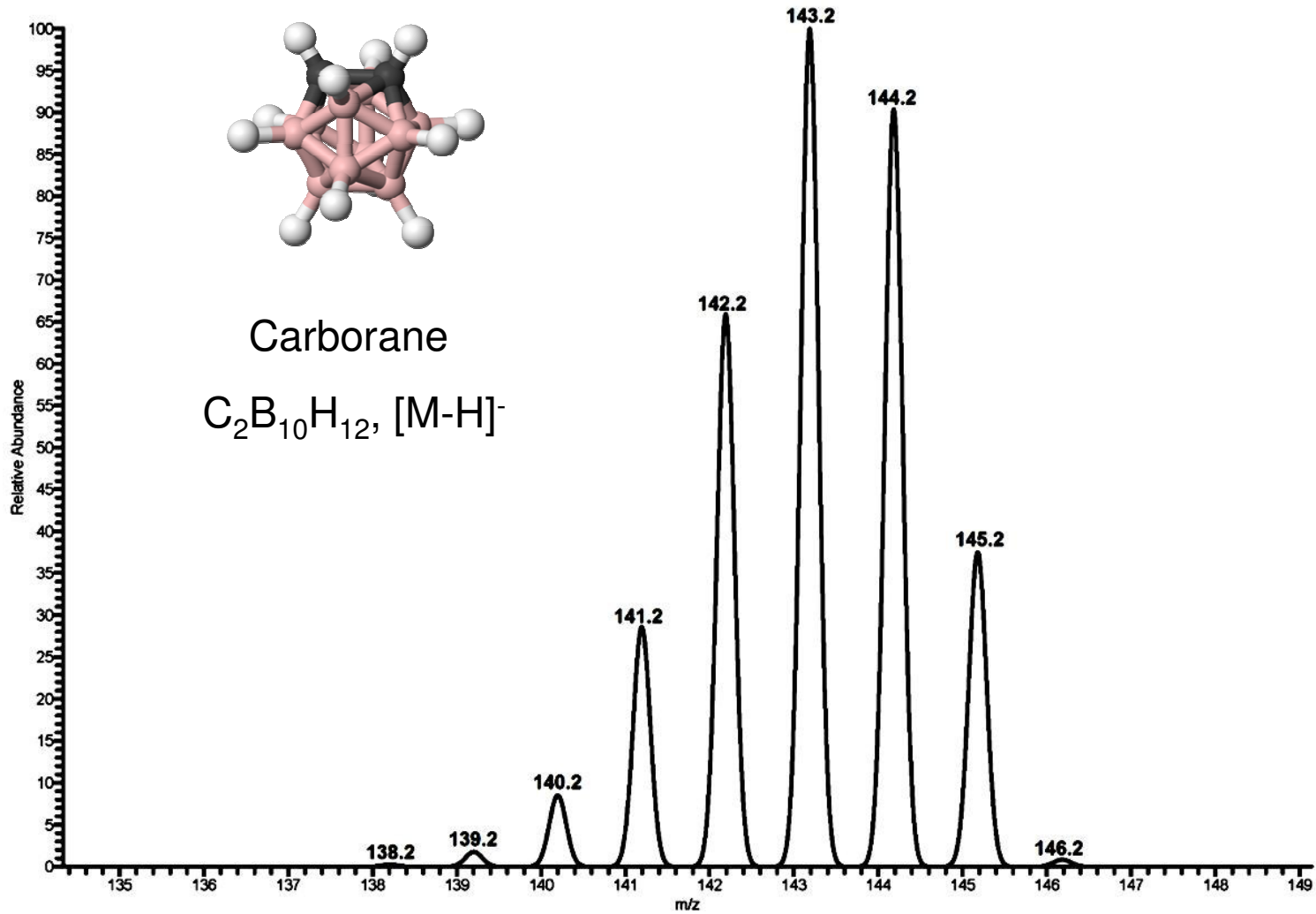# **Determination of molecular weight – charge state**

Determining number of charges

Number of charges can be determined from the distance between the neighboring peaks representing different charge states.
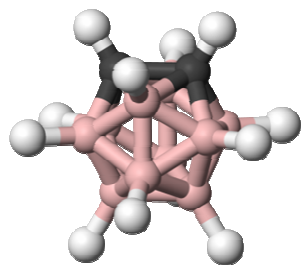
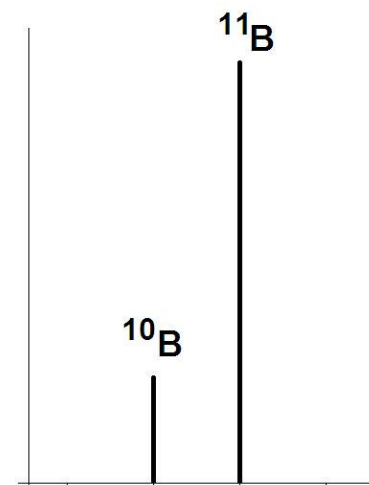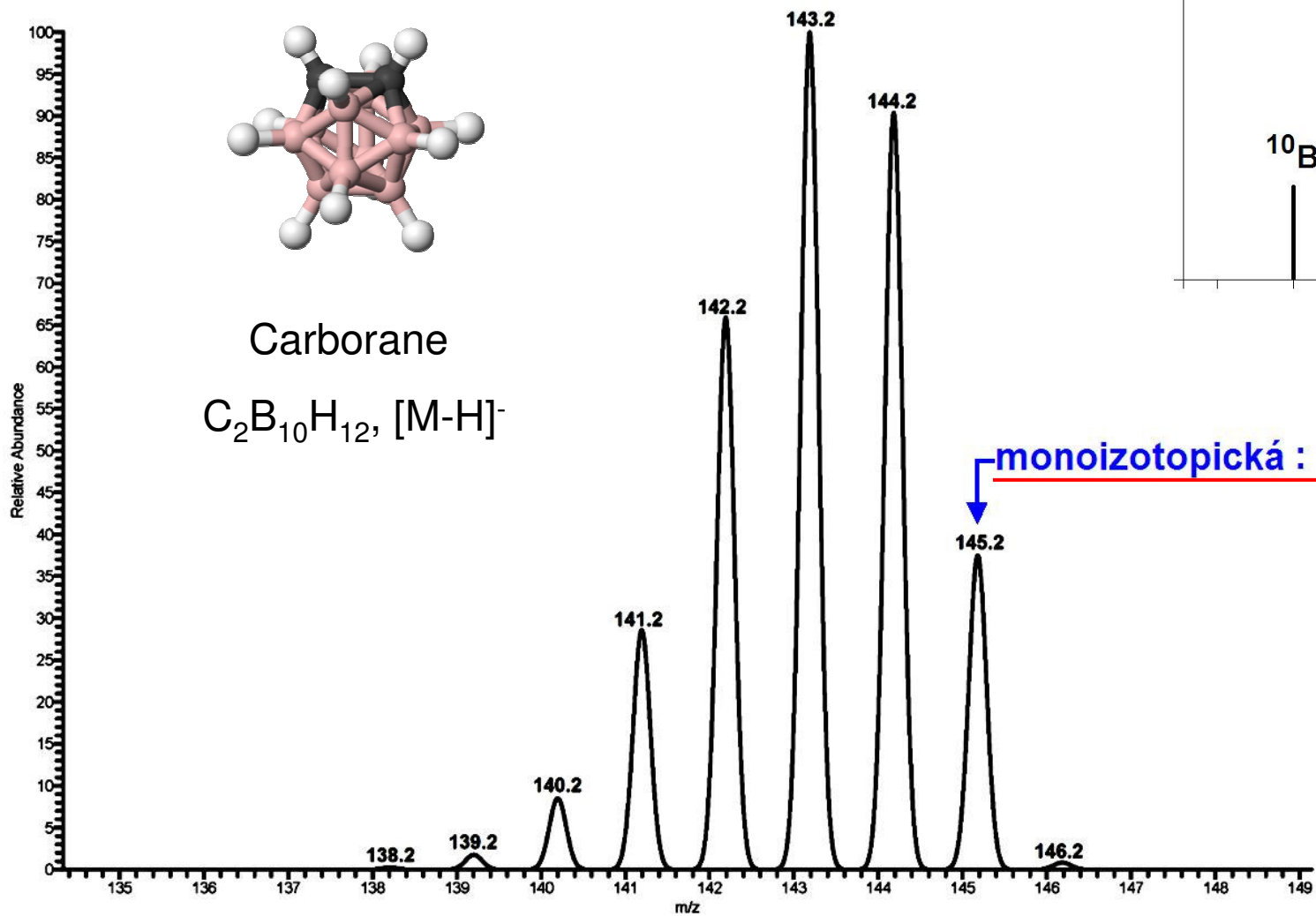$$z_j = \frac{m_k - H^+}{m_k - m_j} \qquad z_k = \frac{m_j - H^+}{m_k - m_j}$$

7+
970.40

8+
849.11

6+
j
1131.79

5+
k
1358.03

4+
1697.27

M=6785

Taipan venom

100

%

0

800   1000   1200   1400   1600   1800   m/z

Monoisotopic mass

monoizotopická: 328.1247

328.12

Hexahelicene

$C_{26}H_{16}$, $[M]^{+\bullet}$

^{12}C

^{13}C

Monoisotopic mass

Carborane

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

Monoisotopic mass

Carborane

$C_2B_{10}H_{12}$, [M-H]$^-$
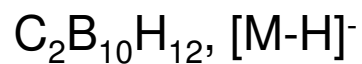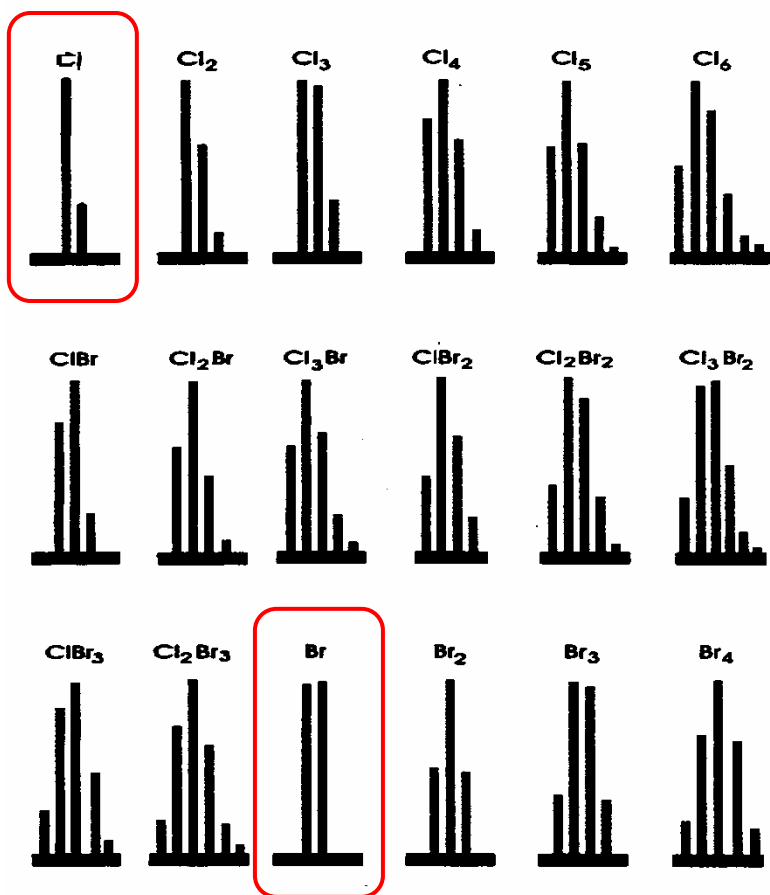
monoizotopická : 145.1791

3.

Elements present in a ion

Isotope clusters

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

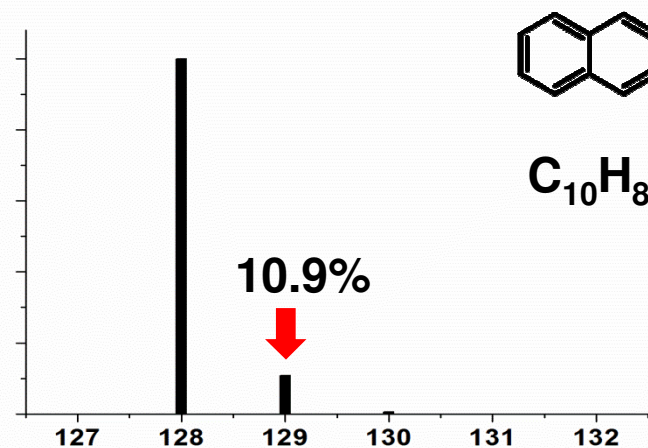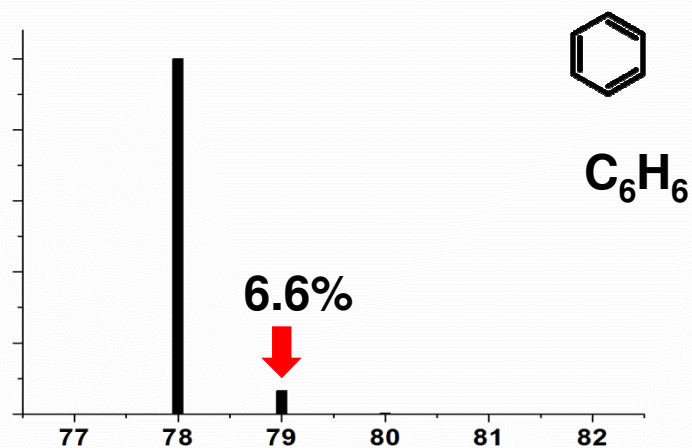Computer programs allow you to calculate the composition of the cluster from the specified summary formula and compare it with experiment.

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%)
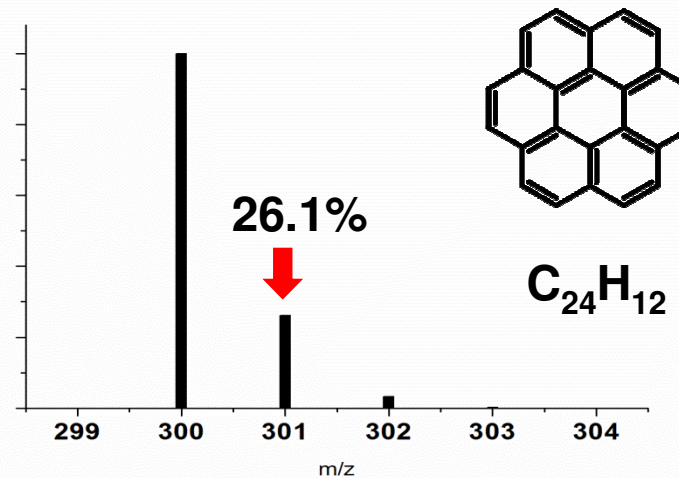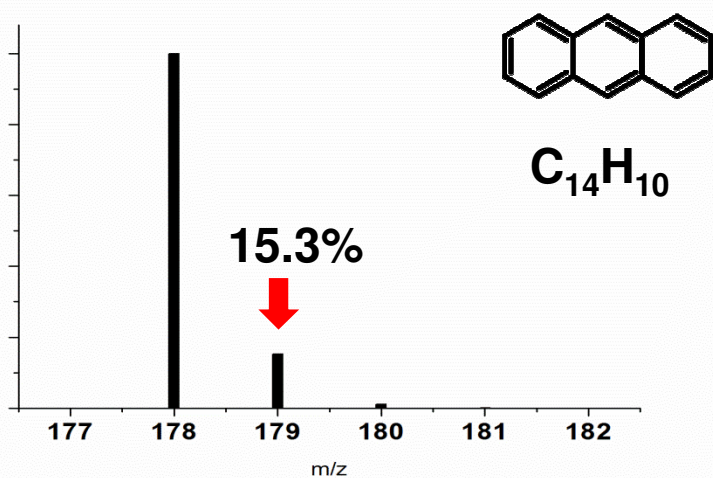
Nitrogen rule

➢ 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).

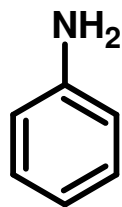Nitrogen rule applies to organic compounds containing C, H, N, O, S, P, F, Cl, Br, I

Odd value of molecular weight = odd number of nitrogens

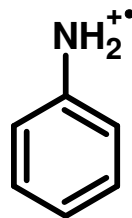**Even value of molecular weight = even (zero) number of nitrogens**

Applying the rule for ions
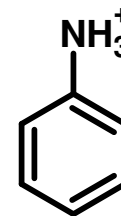
EI – valid for $M^{+\bullet}$ as stated above

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

NH₂

M=93

NH₂⁺•

m/z 93

NH₃⁺

m/z 94

4.

Determination of elemental composition from accurate mass

Elemental composition from accurate mass

The more accurately we determine mass of the ion, the more we restrict the number of possible structures.

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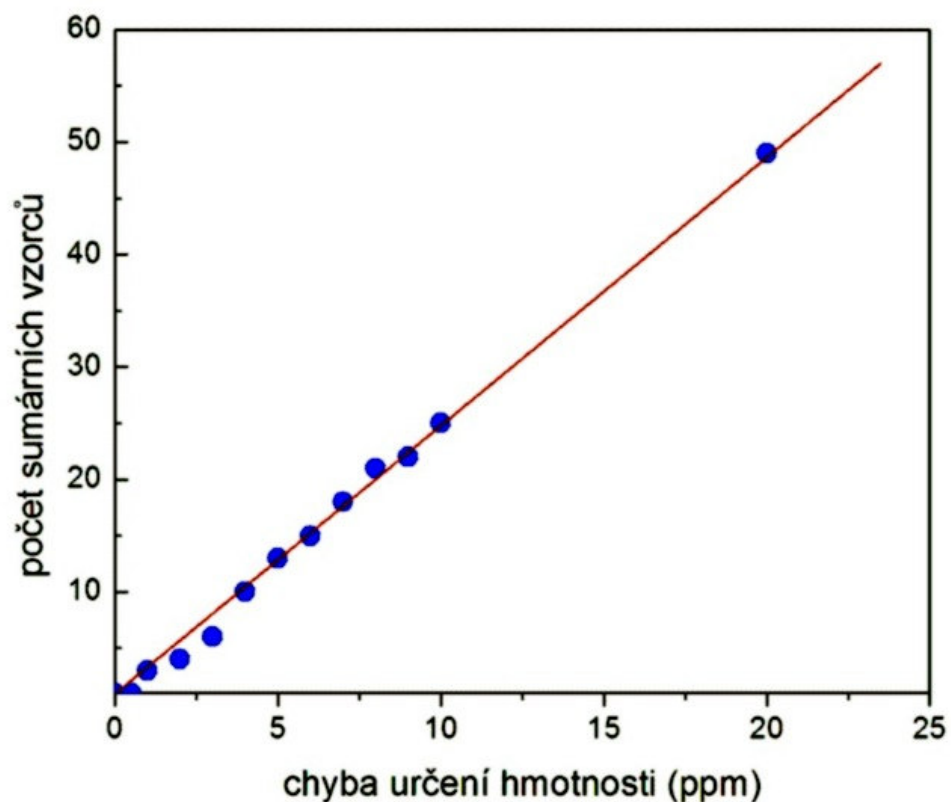$C_{47}H_{51}NO_{14,}$ mon. mass
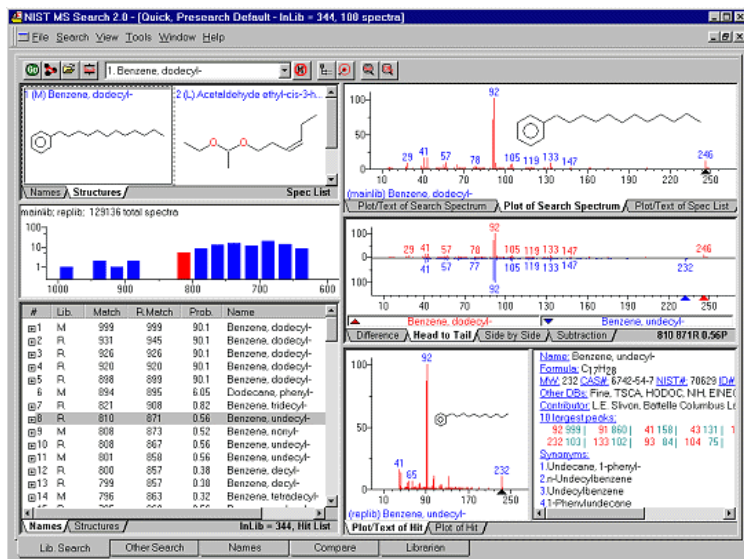854.3388

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

5.

Searching mass spectra libraries

Libraries of EI mass spectra

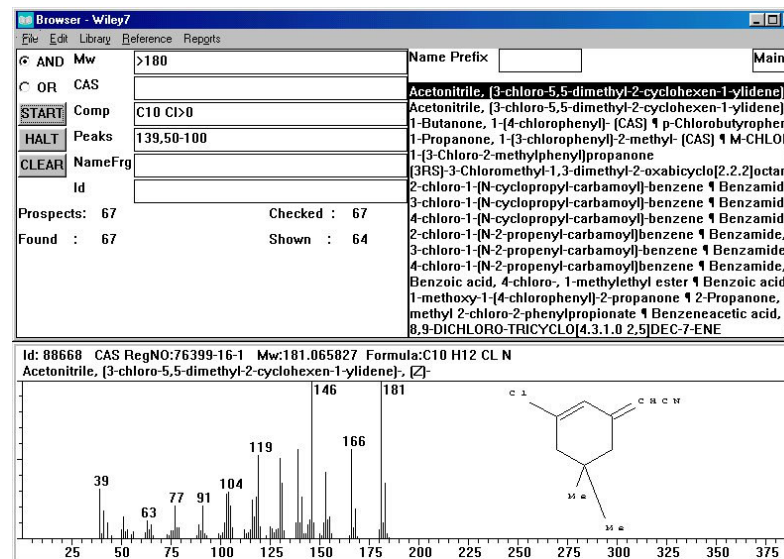## NIST/EPA/NIH Mass Spectral Library

Wiley Registry of Mass Spectral Data

NIST 11:

EI spectra of 212,961 compounds
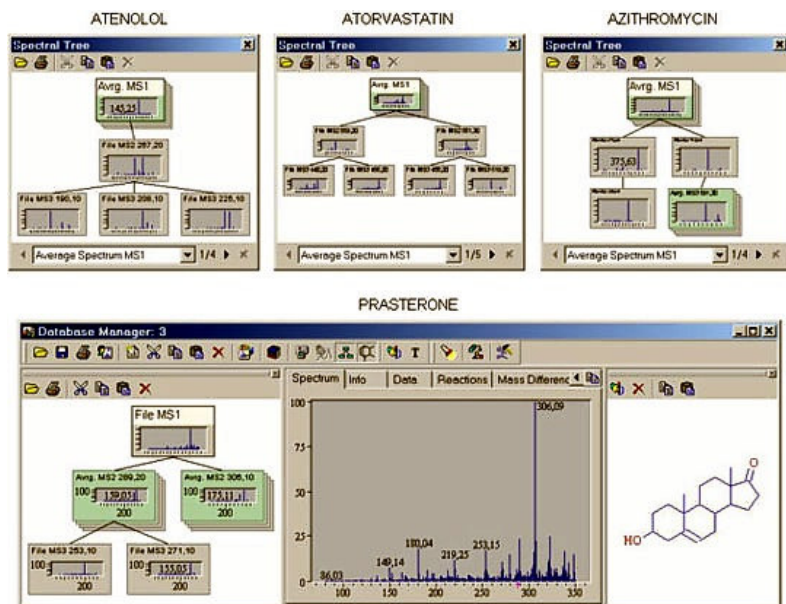MS/MS spectra
Kovats retention index values

9th Edition:

663,000 EI spectra(70 eV)

NIST 05 installed on open access GC/MS

Libraries of ESI MS/MS mass spectra

HighChem's spectral library collection

MS Libraries for LC-MS/MS and ESI in-source CID-MS, University of Freiburg

MS/MS spectra > 1000 compounds
Drugs, natural products
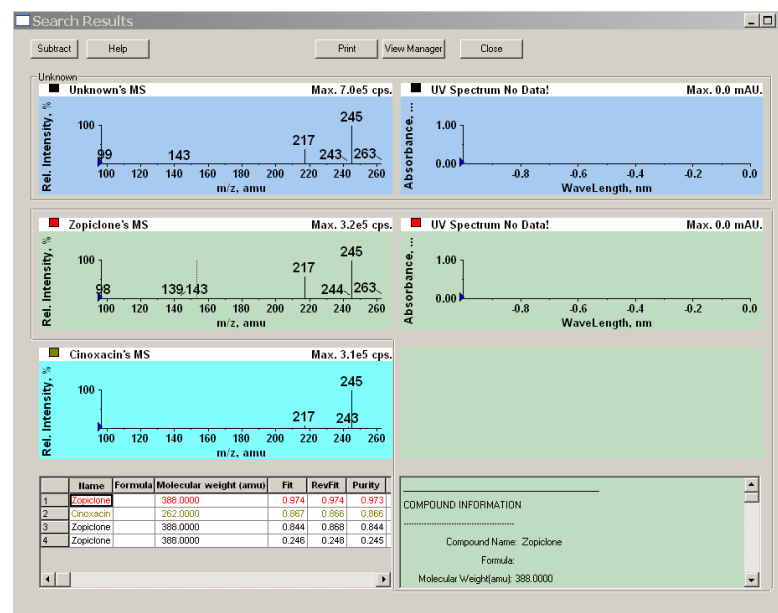
MS/MS spectra ~ 800
compounds, drugs

http://www.massbank.jp

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

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

$$R\text{-}OH_2^+ \rightarrow R^+ + H_2O$$
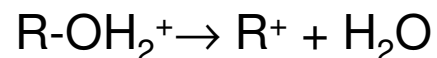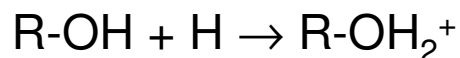
FRAGMENTATION of EE[+]:

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

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

EE+ ions are more stable than OE[+]•

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
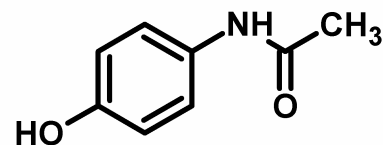
Typical logical neutral losses:

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 (+/-)

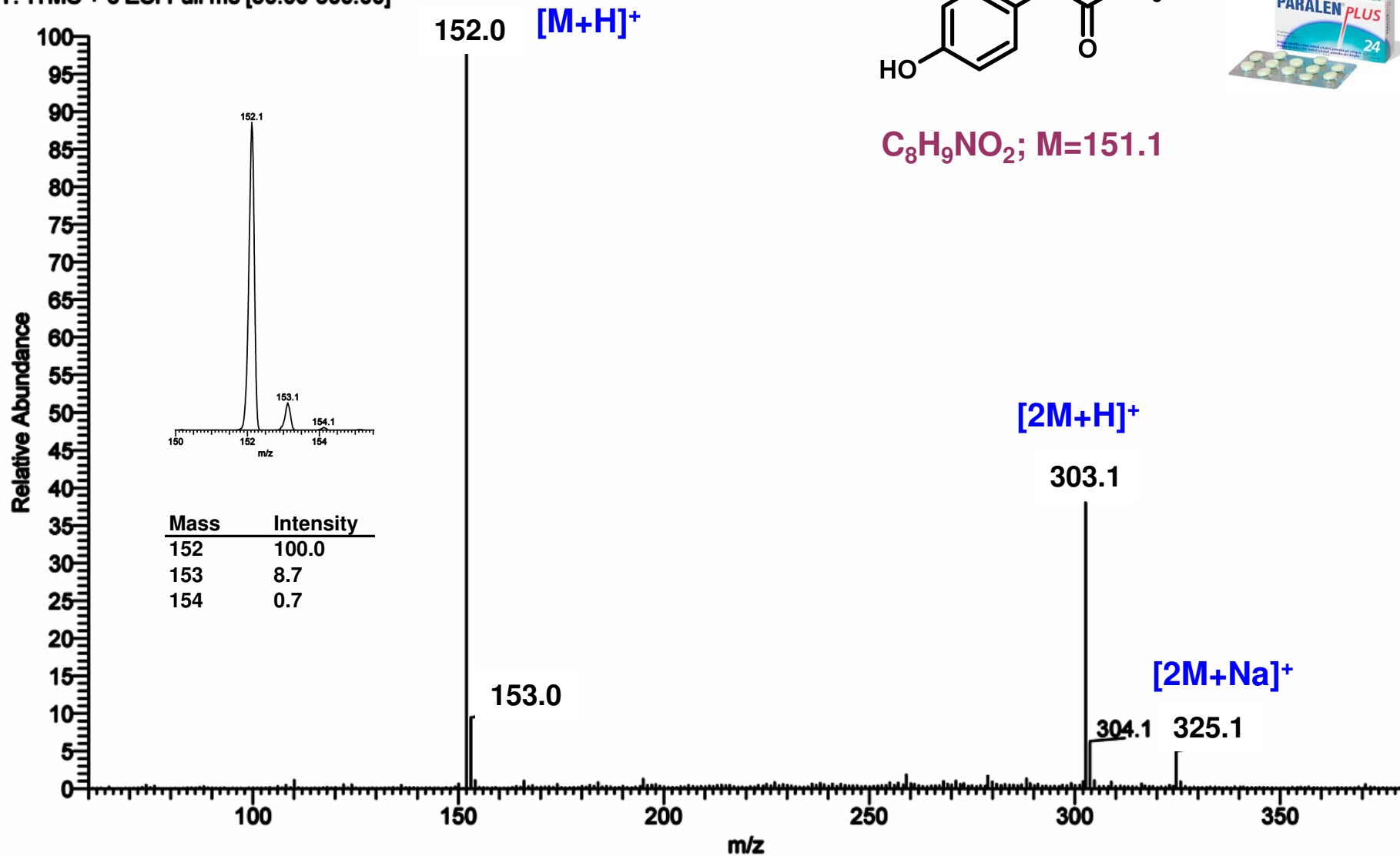**Impossible "forbidden" neutral losses**: 3-14, 21-25, 37-40

Paracetamol N-(4-hydroxyphenyl)acetamide

ESI+, MS

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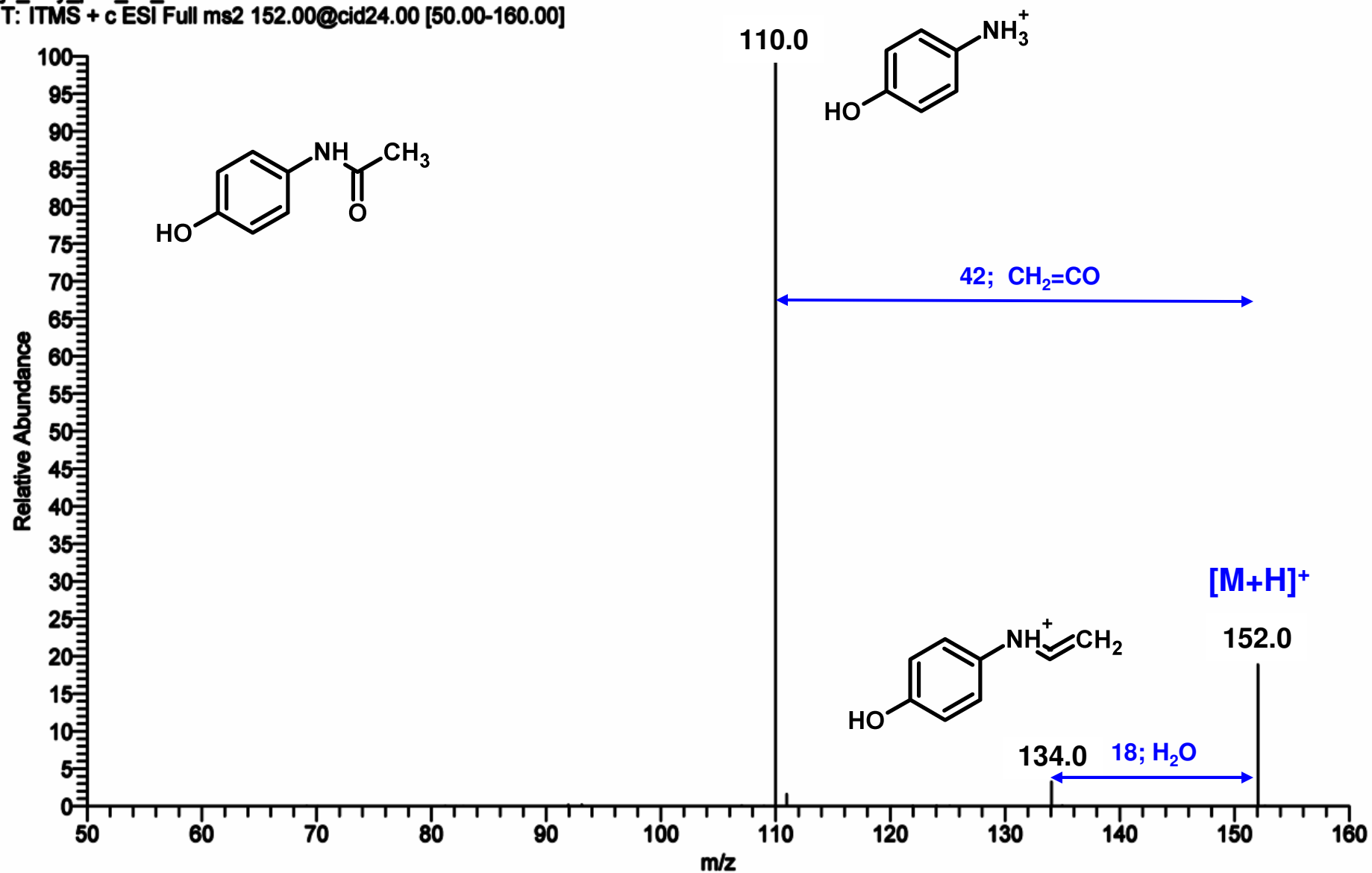T: ITMS + c ESI Full ms [50.00-500.00]

152.0 [M+H]⁺

303.1 [2M+H]⁺

325.1 [2M+Na]⁺

153.0

304.1

$C_8H_9NO_2$; M=151.1

Mass	Intensity
152	100.0
153	8.7
154	0.7

Paracetamol N-(4-hydroxyphenyl)acetamide

ESI+, MS/MS

Paracetamol N-(4-hydroxyphenyl)acetamide
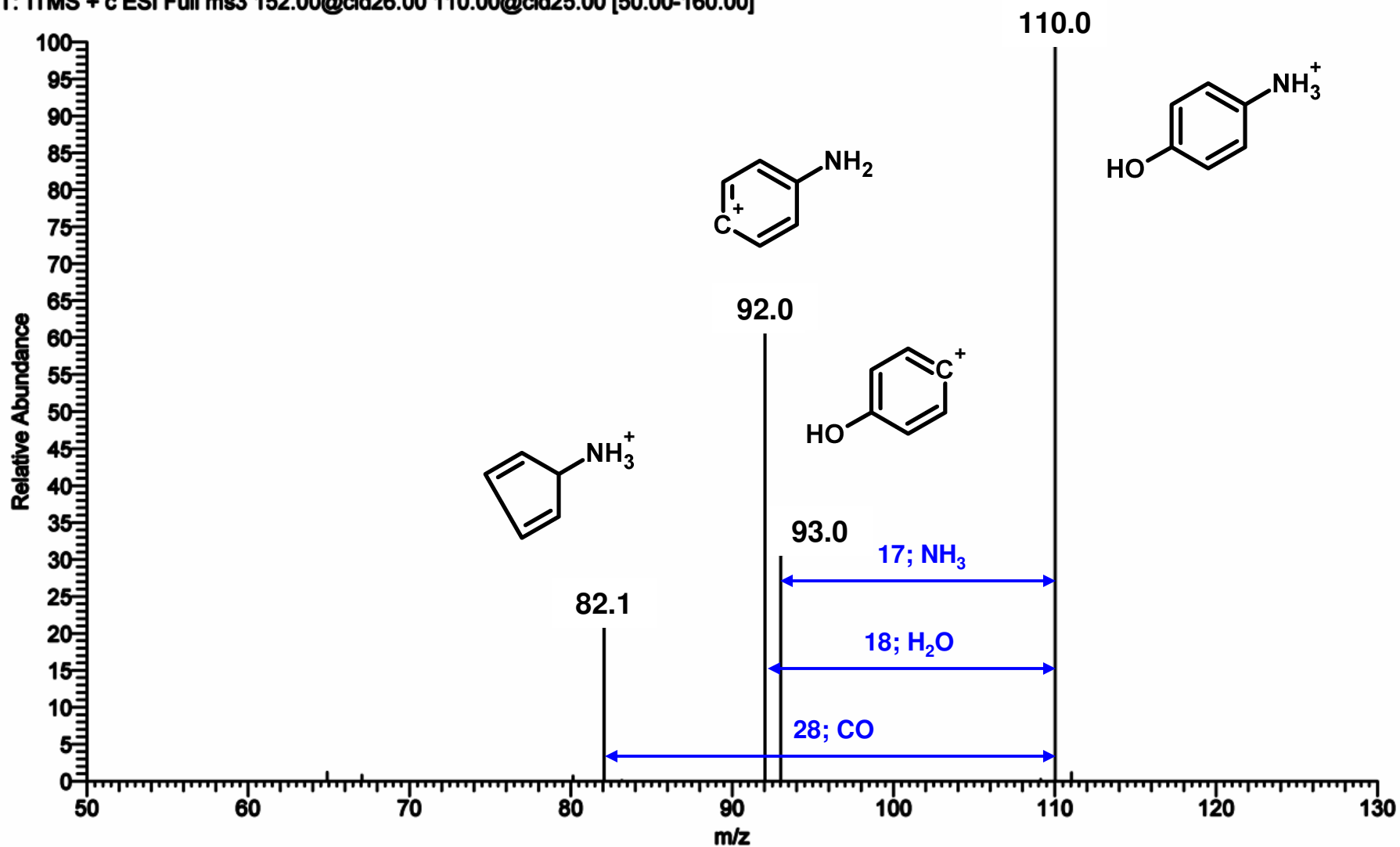
ESI+, MS^3

Menthyloxyacetic acid

ESI-, MS/MS
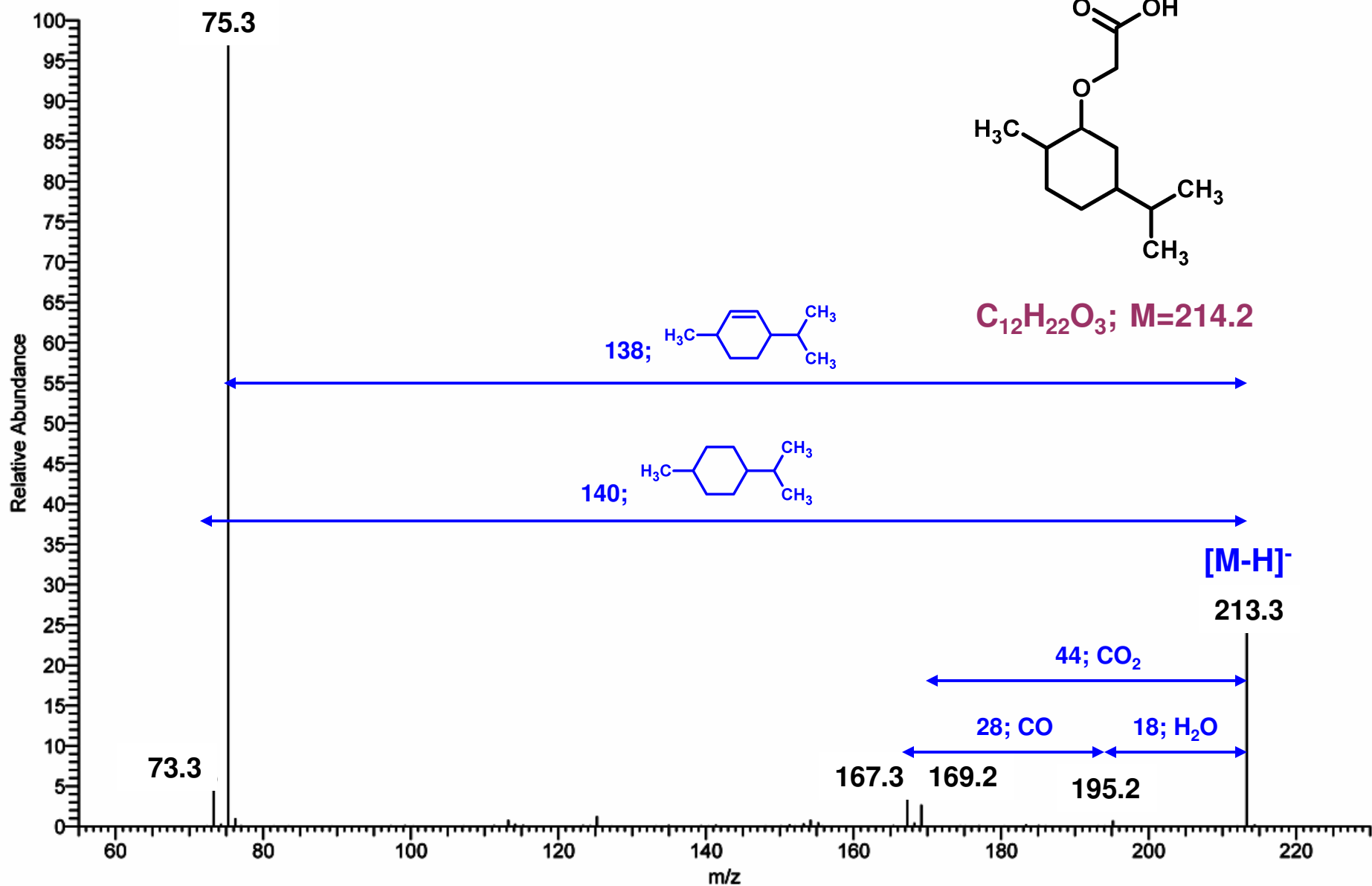
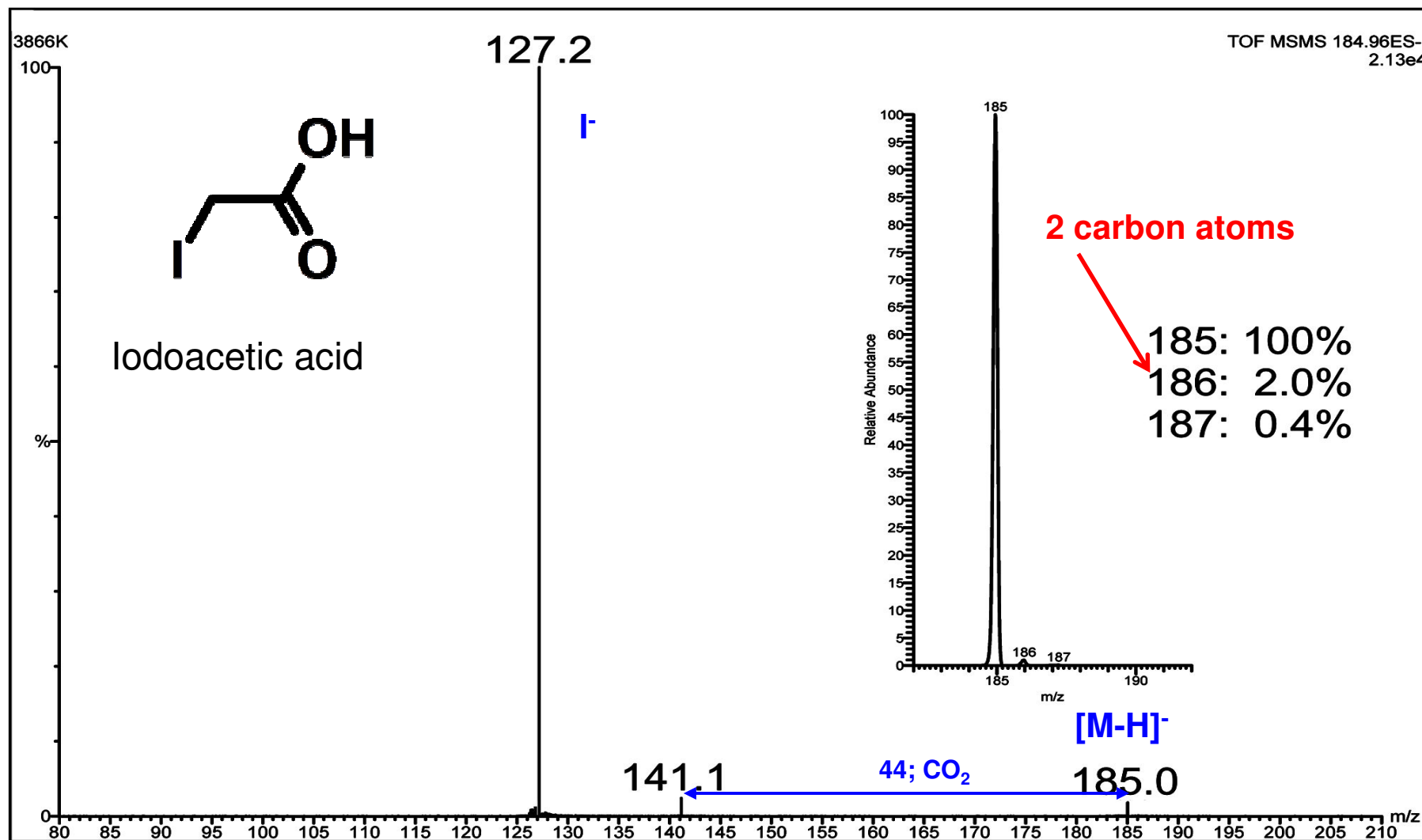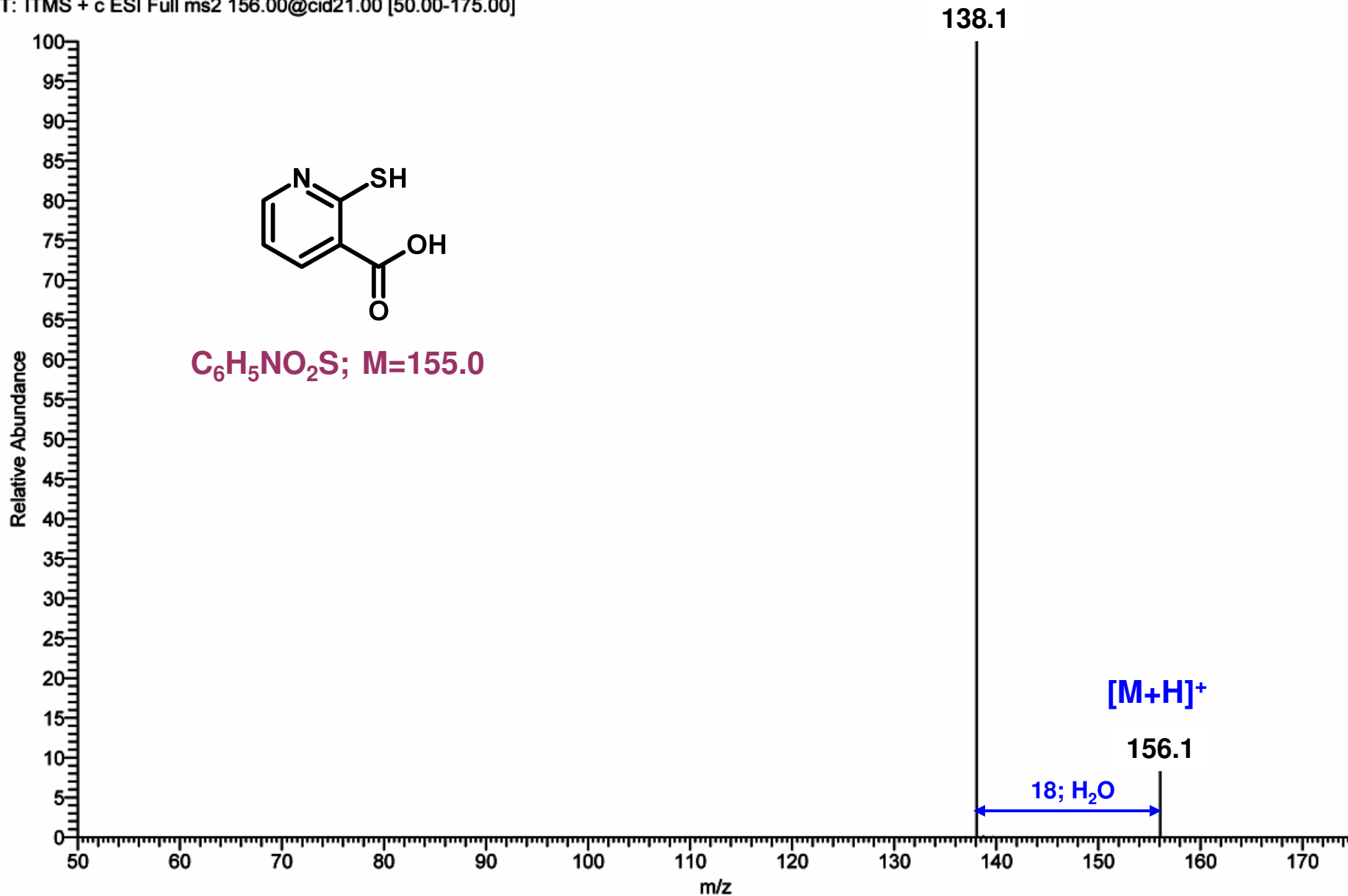# Unknown

ESI-, MS/MS

What is the structure?

MS/MS *m/z* 185

2-Mercaptonicotinic acid

ESI+, MS/MS

2786jc #1-147   RT: 0.00-1.02   AV: 147   NL: 2.76E3
T: ITMS + c ESI Full ms2 156.00@cid21.00 [50.00-175.00]

$C_6H_5NO_2S$; M=155.0

2-Mercaptonicotinic acid
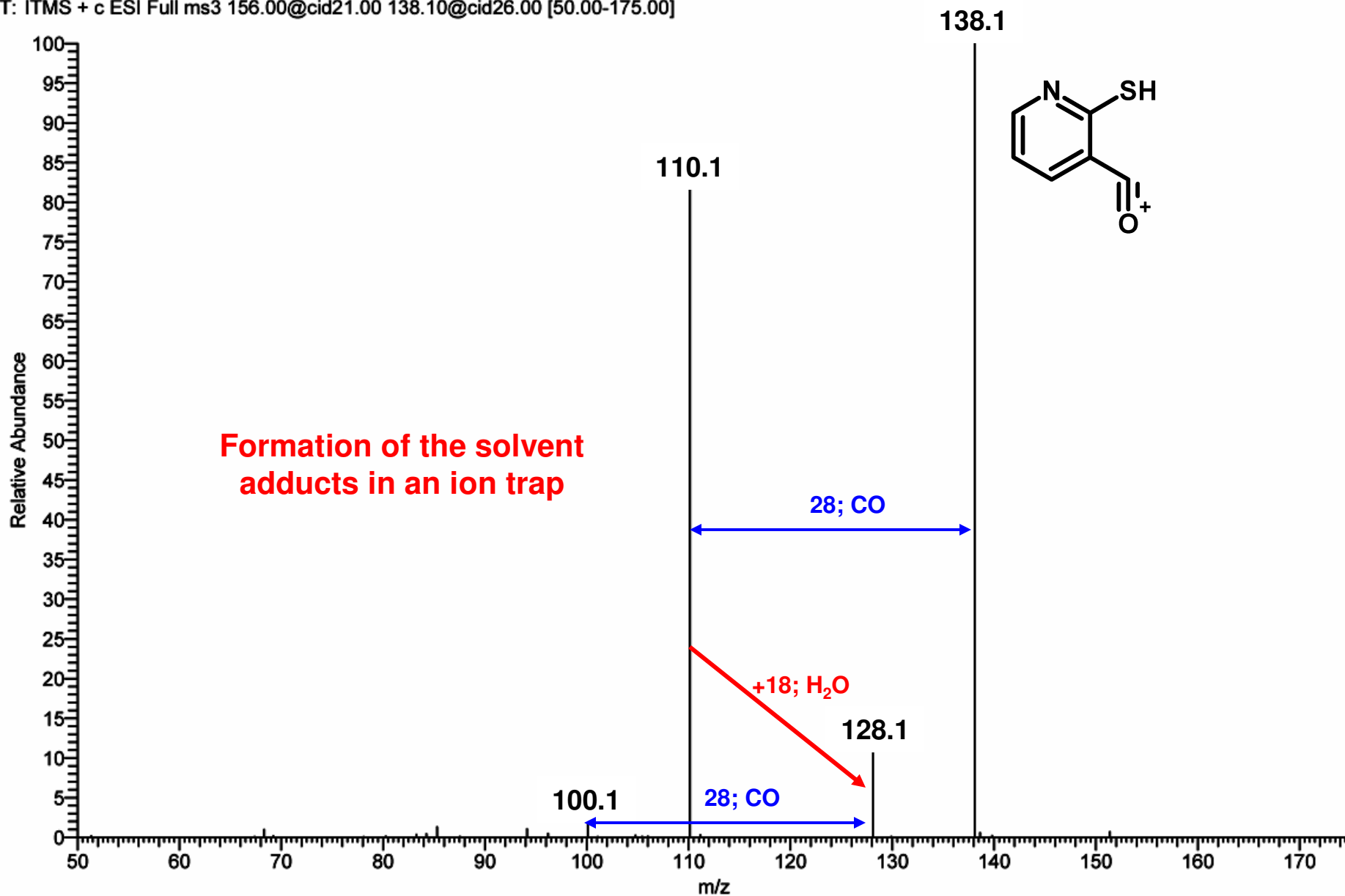
ESI+, MS³

Cyclopentafuranol-derivative

ESI+, MS/MS

1748jc #1-226   RT: 0.00-1.51   AV: 226   NL: 5.26E1
T: ITMS + c ESI Full ms2 329.20@cid27.00 [90.00-350.00]

$C_{18}H_{26}O_4$; M=306.2

311.2

$[M+Na]^+$

329.2

18; H_2O

269.2

60; CH_3COOH
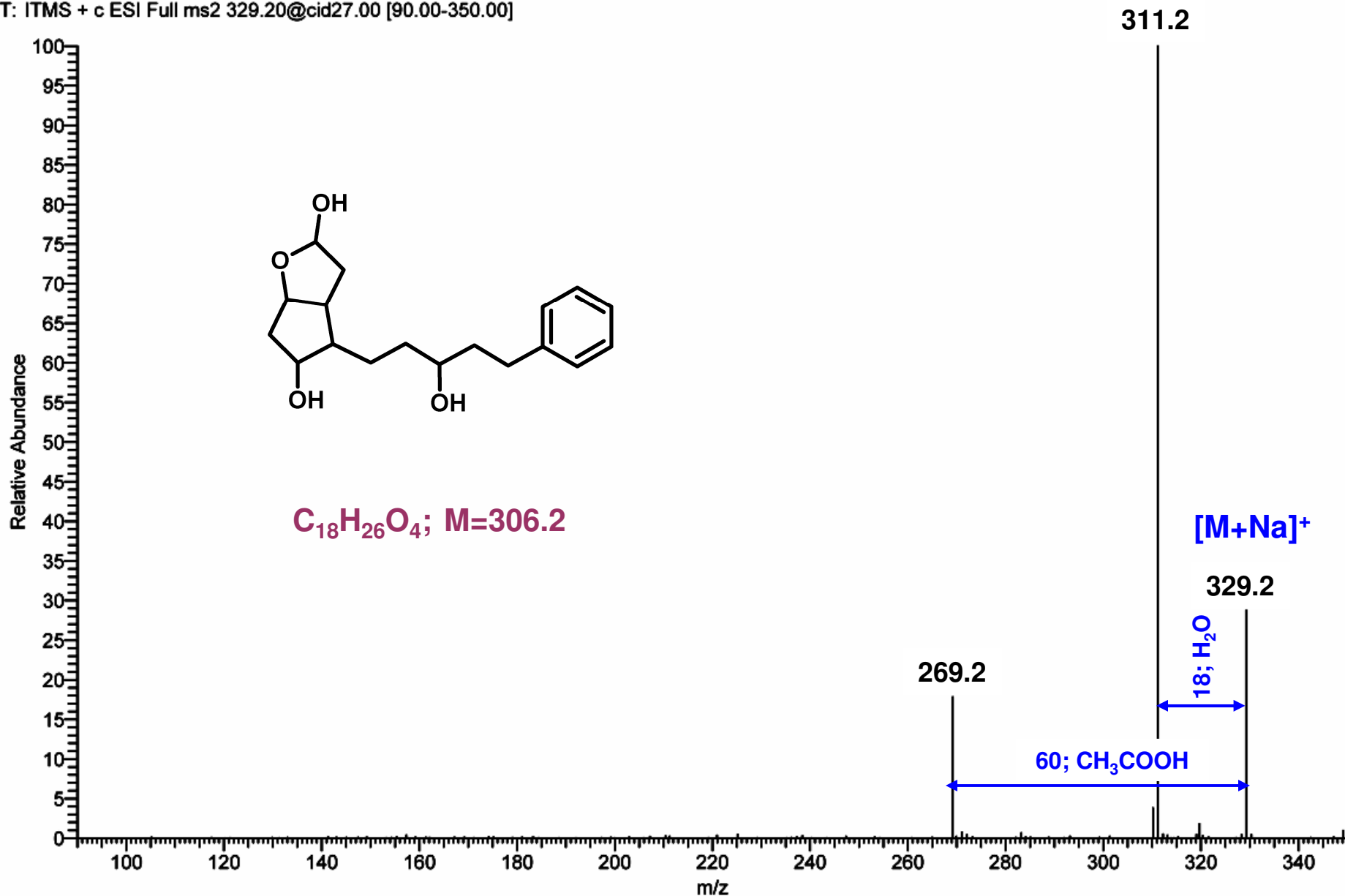
Cyclopentafuranol-derivative, disil

ESI+, MS/MS

[M+Na]+

557.3

1644jc #1-749   RT: 0.00-5.02   AV: 749   NL: 2.22E1
T: ITMS + c ESI Full ms2 557.30@cid27.00 [150.00-600.00]

$C_{30}H_{54}O_4Si_2$; M=534.4

132; H$_3$C—Si—OH (with H$_3$C, CH$_3$, CH$_3$ groups)

60; CH$_3$COOH

132; H$_3$C—Si—OH (with H$_3$C, CH$_3$, CH$_3$ groups)

293.1

425.1

497.2

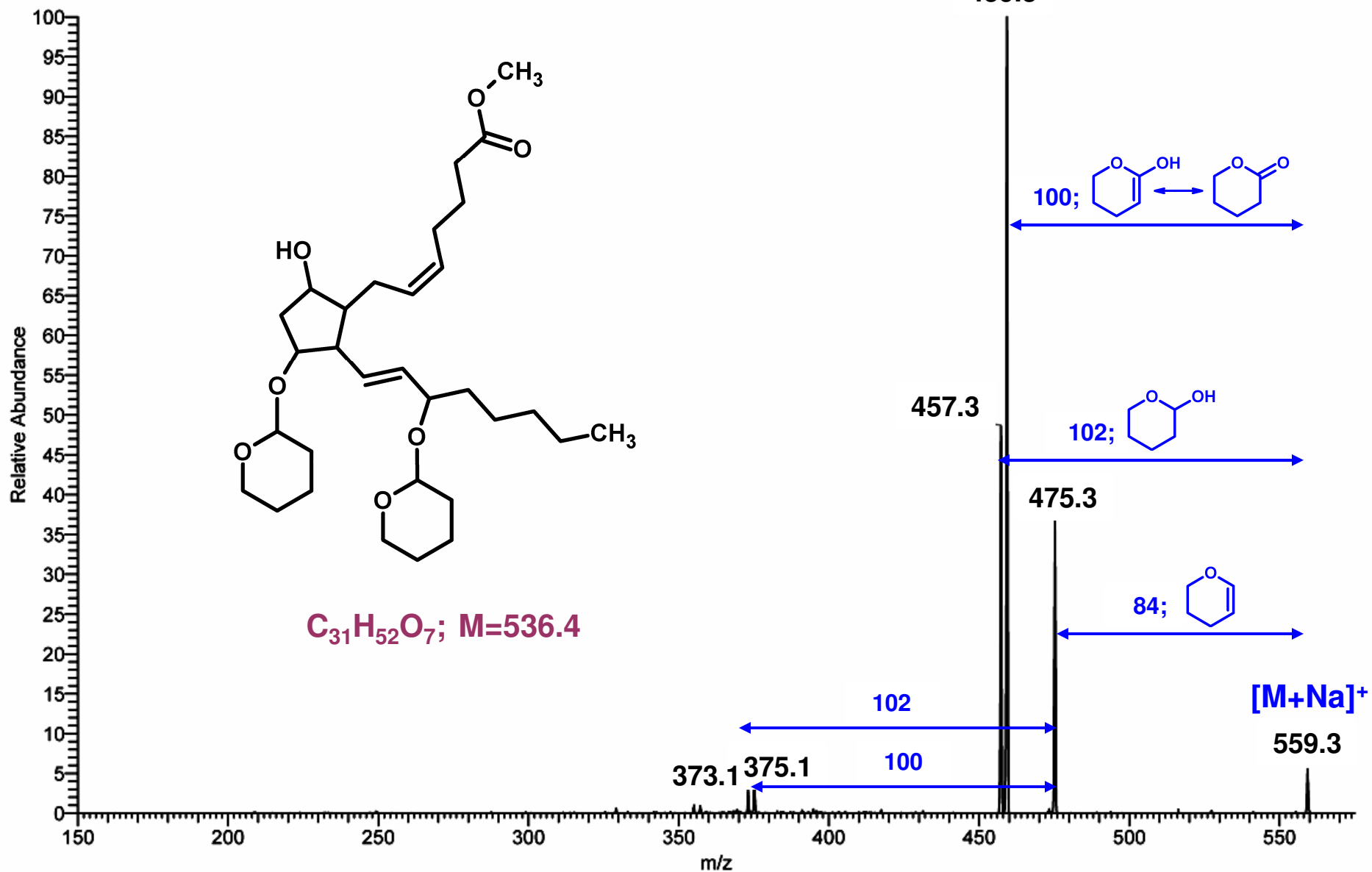539.2

275.1

18; H$_2$O

18; H$_2$O

Relative Abundance

m/z
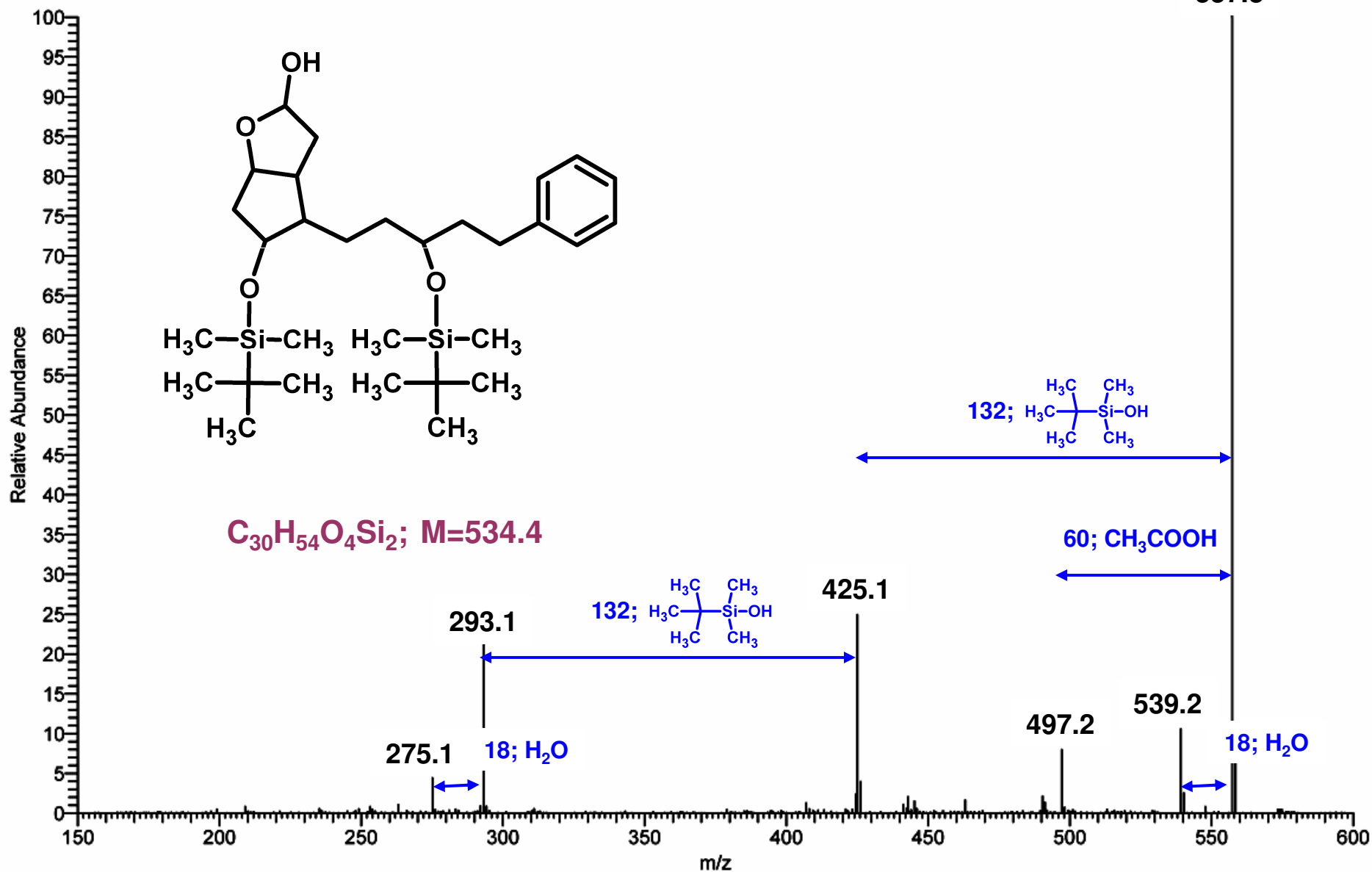
PGF$_{2a}$-methylester, diTHP

ESI+, MS/MS

3378jc #1-64 RT: 0.01-0.99 AV: 64 NL: 8.64E5
T: + p Full ms2 559.40@cid37.00 [150.00-600.00]

C$_{31}$H$_{52}$O$_{7}$; M=536.4
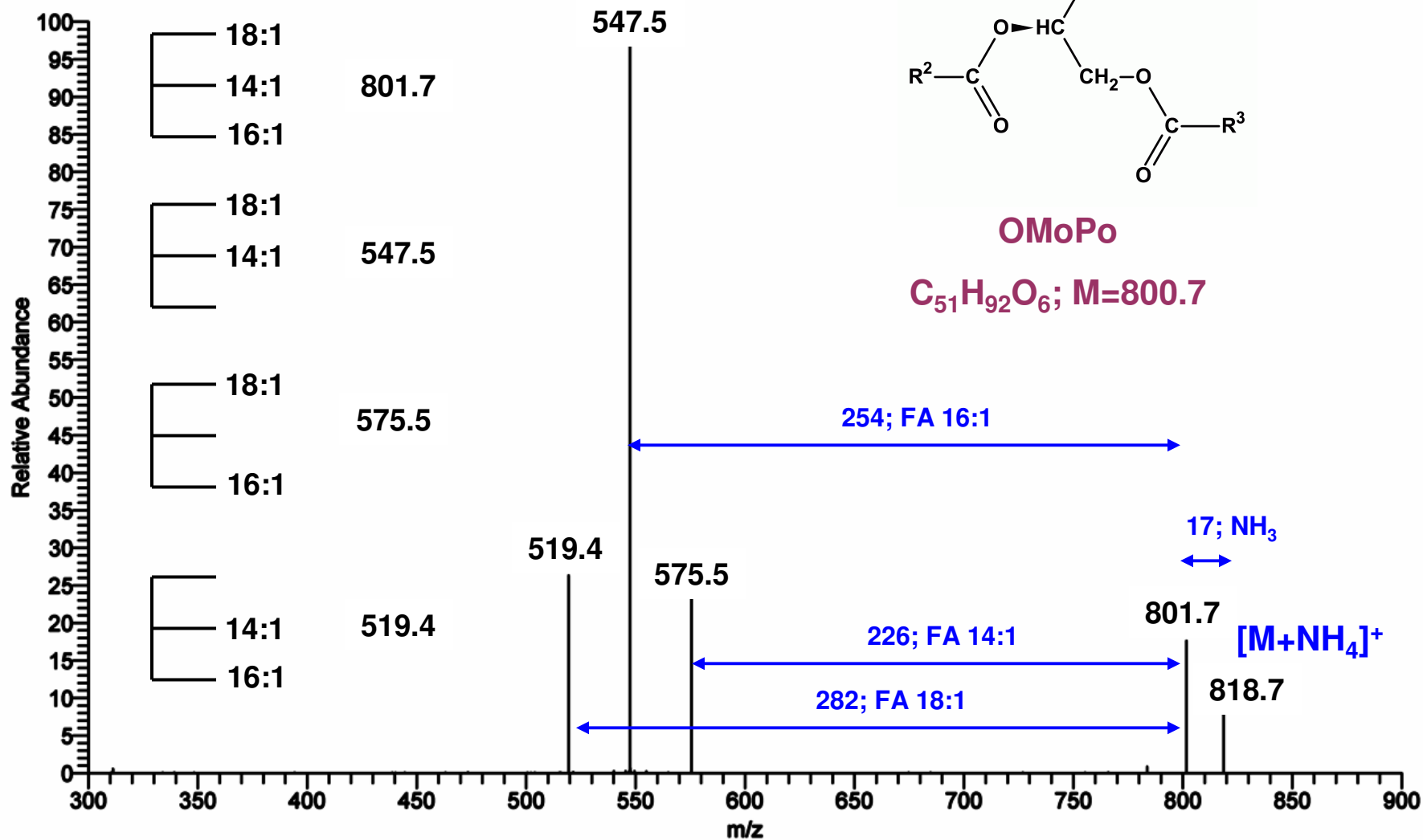
Triacylglycerols

APCI+, MS/MS

JC_TAG_Bter_MSMS01 #6162   RT: 64.99   AV: 1   NL: 3.82E6
T: FTMS + c APCI corona d Full ms2 818.72@cid30.00 [215.00-830.00]
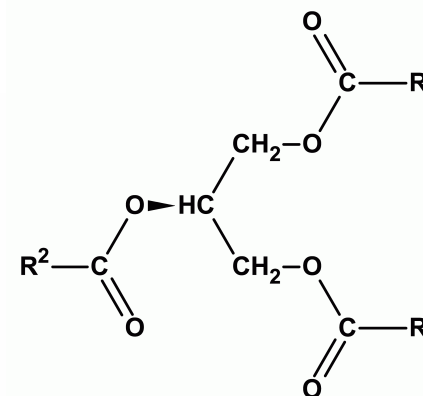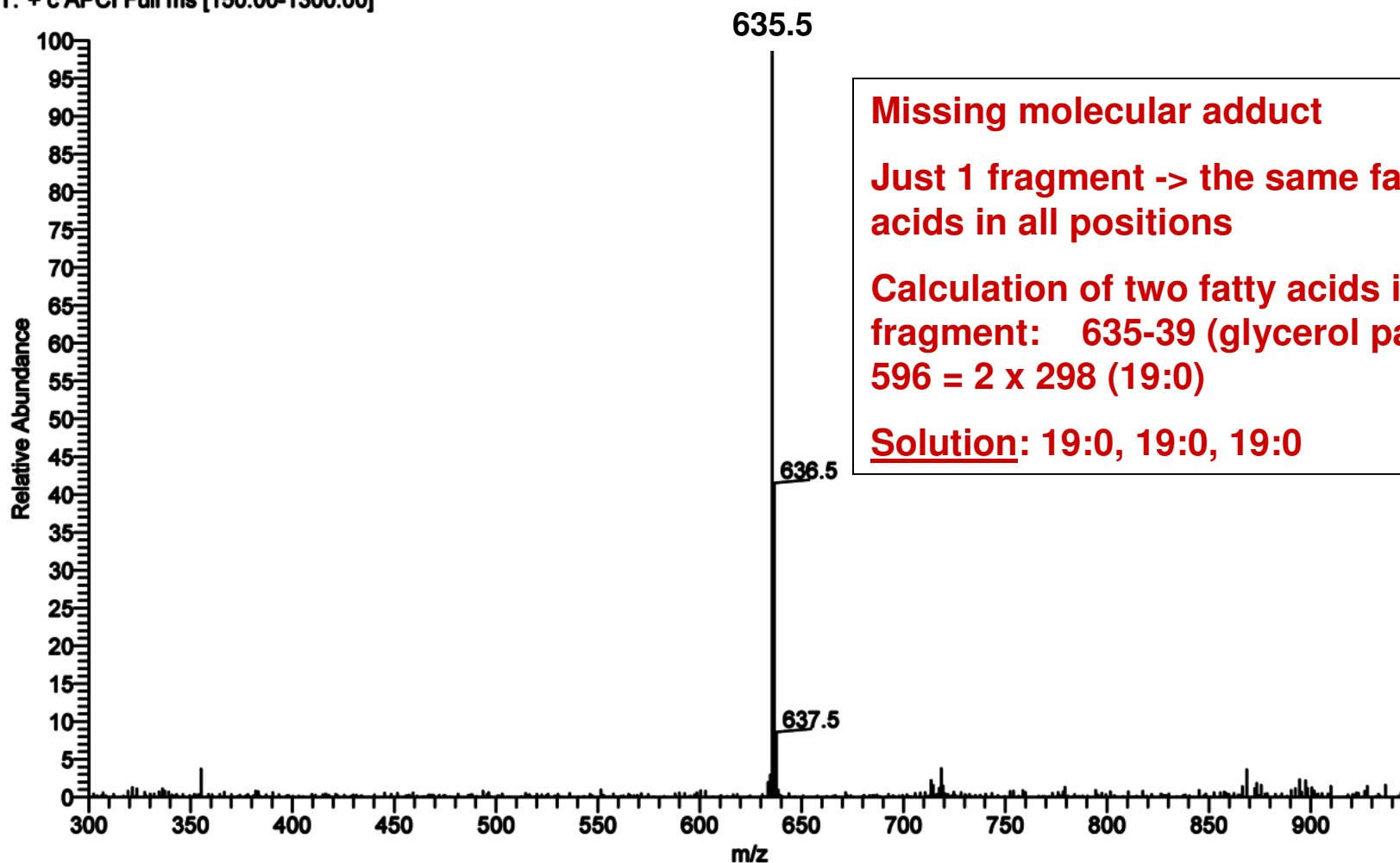
OMoPo

$C_{51}H_{92}O_6$; M=800.7

Triacylglycerols

What is the structure?

APCI+, MS

1377ek #4346-4381 RT: 108.29-109.11 AV: 36 SB: 44 106.70-107.89 NL: 4.68E5
T: + c APCI Full ms [150.00-1300.00]

Missing molecular adduct

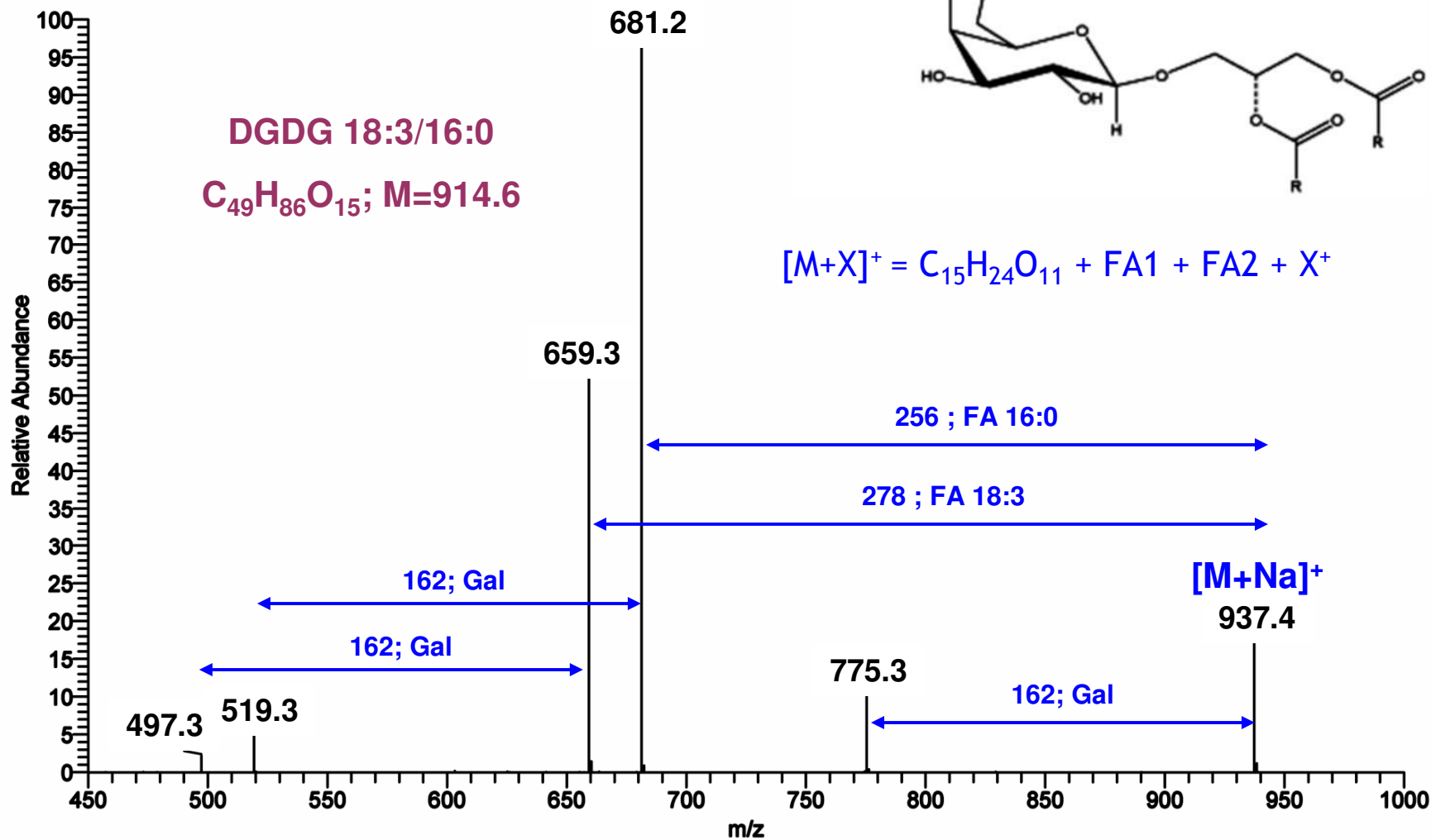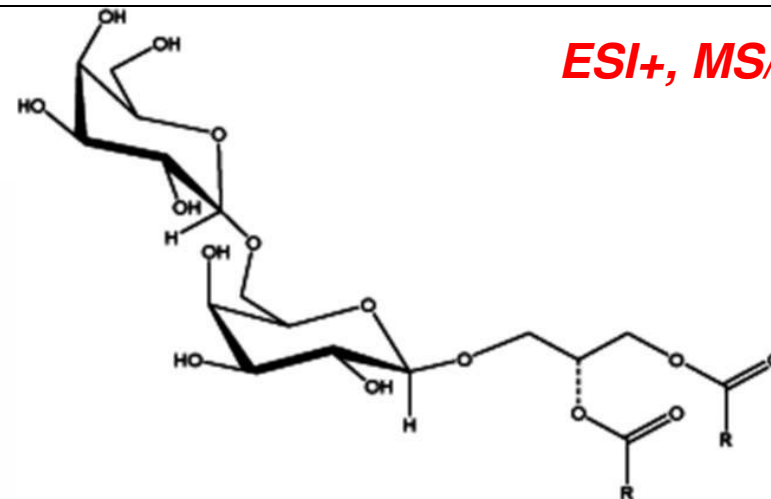**Just 1 fragment -> the same fatty acids in all positions**

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

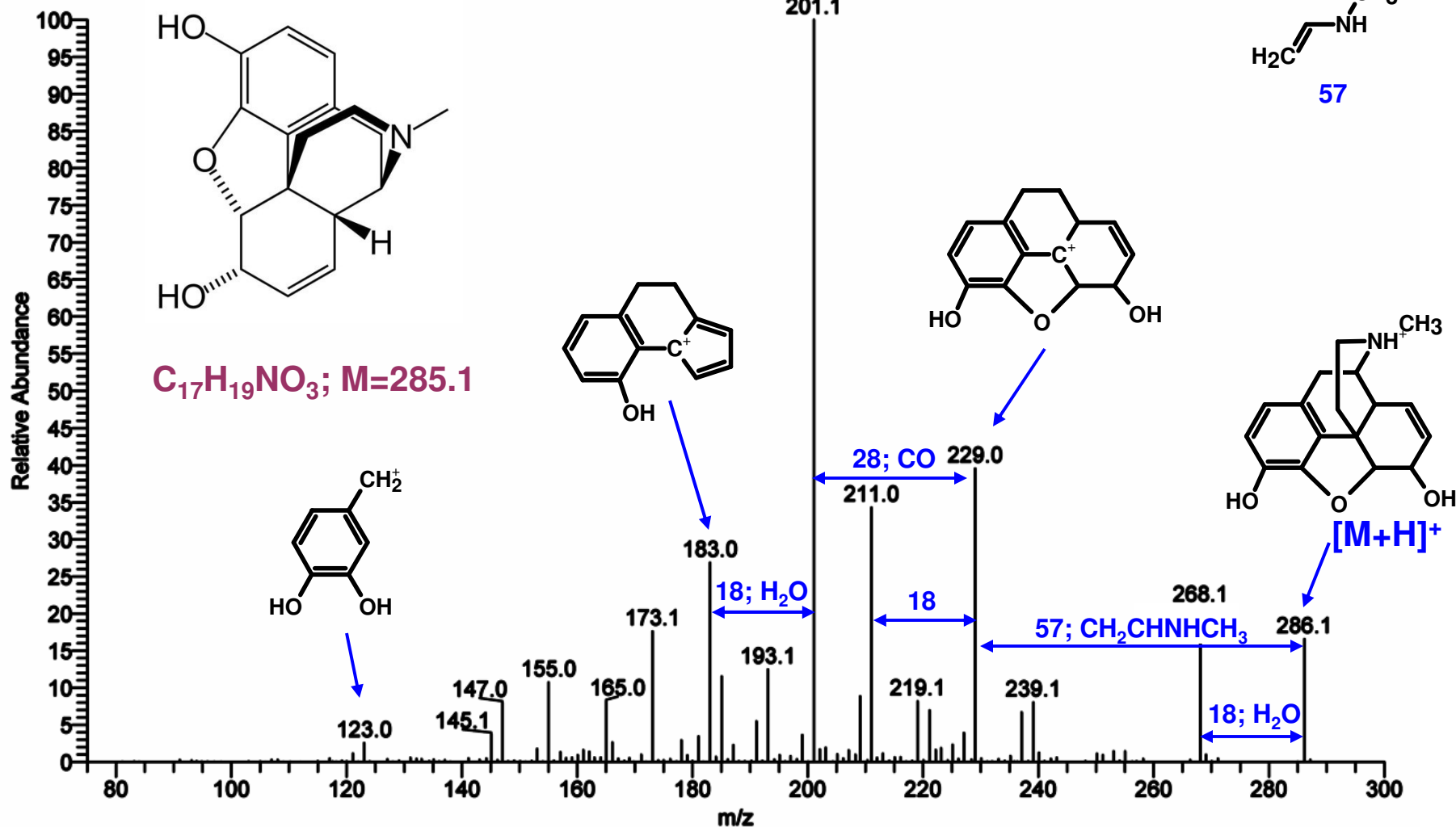

1656MK01 #1342-1375   RT: 21.08-21.58   AV: 17   NL: 1.26E3
T: ITMS + c ESI d Full ms2 937.47@cid28.00 [245.00-950.00]

DGDG 18:3/16:0

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

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

681.2

659.3

256 ; FA 16:0

278 ; FA 18:3

162; Gal

162; Gal

$[M+Na]^+$

937.4

775.3

162; Gal

497.3   519.3

m/z

Morphine

ESI+, MS/MS

2229jc_080801195950 #1-11   RT: 0.00-0.47   AV: 11   NL: 1.81E2
T: ITMS + c ESI Full ms2 286.10@cid27.00 [75.00-300.00]

$C_{17}H_{19}NO_3$; M=285.1

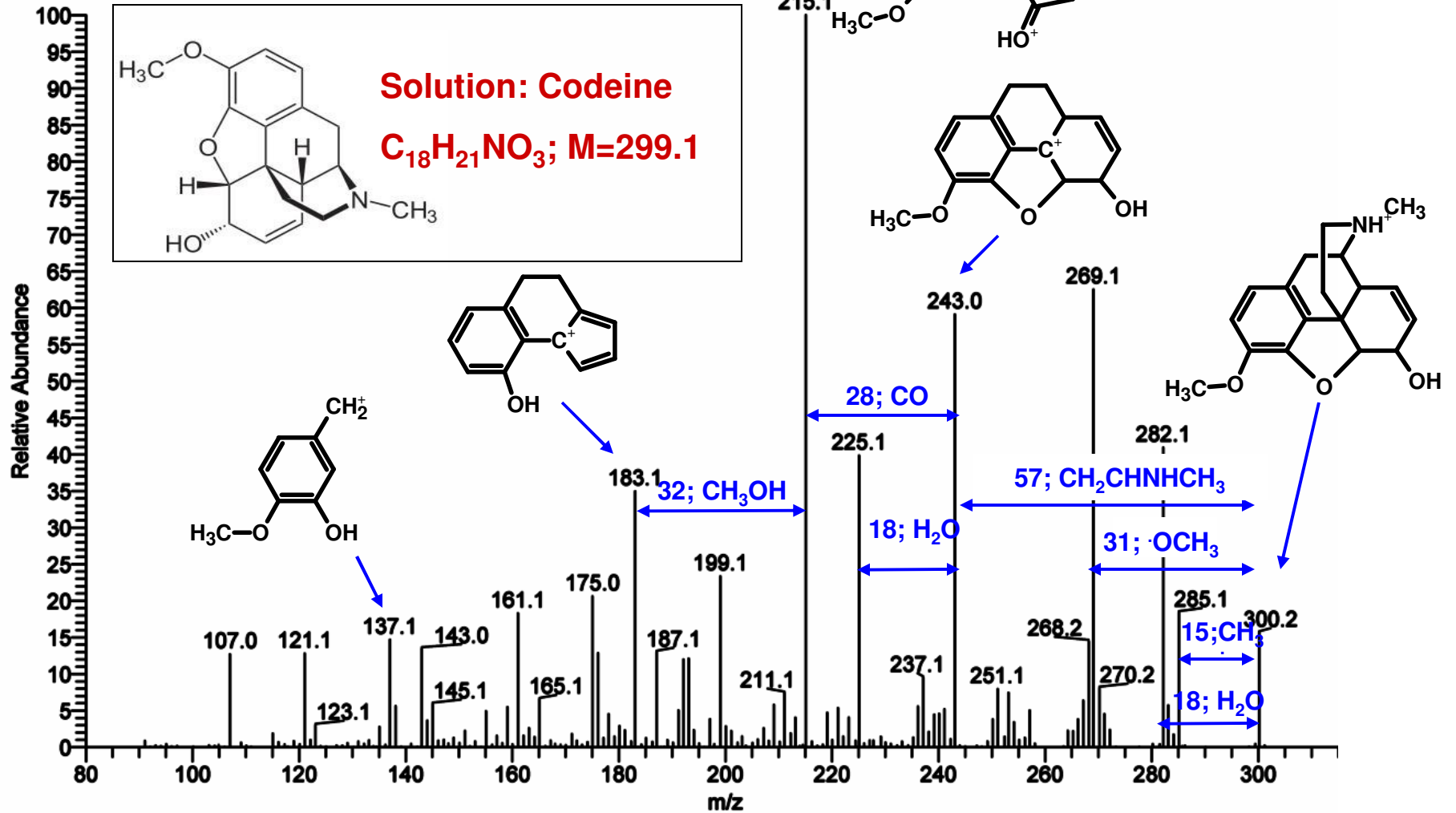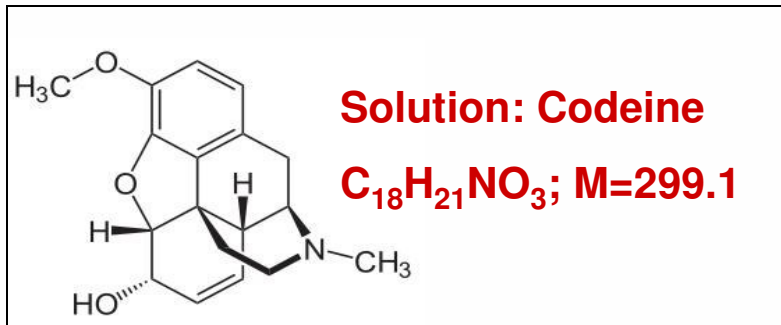Struktury iontů:Peoaknapo et al., Phytochemistry 65 (2004) 1413.
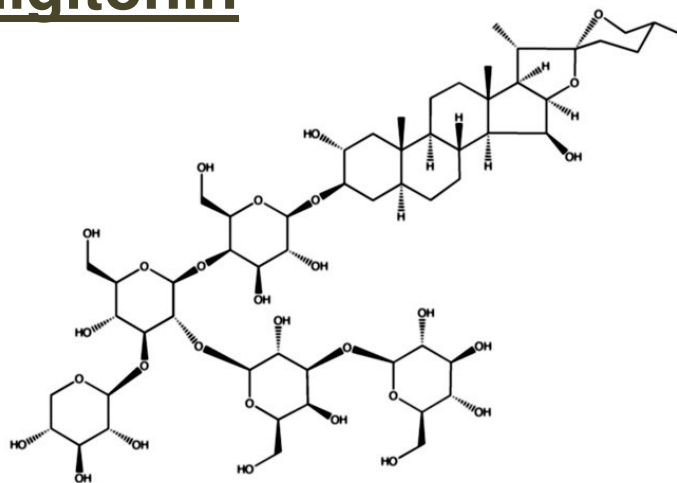
Unknown

What is the structure?

ESI+, MS/MS

2233jc #1-22 RT: 0.00-2.00 AV: 22 NL: 1.51E1
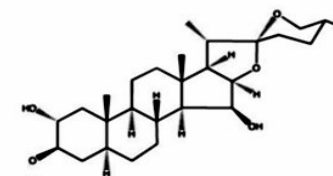F: ITMS + c ESI Full ms2 300.10@cid27.00 [80.00-320.00]

Solution: Codeine

$C_{18}H_{21}NO_3$; M=299.1

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

Glycosides - digitonin

Digitonin
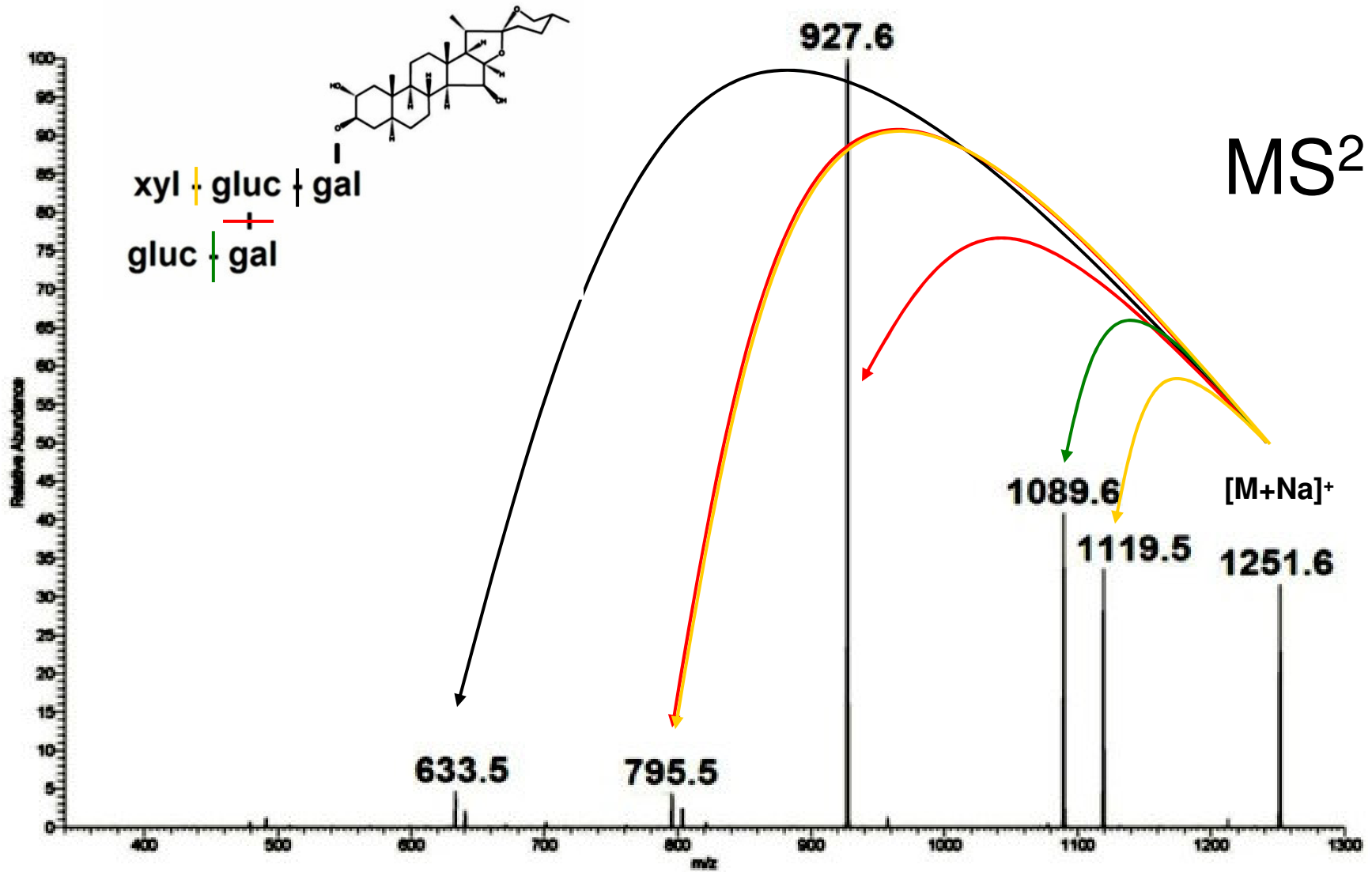$C_{56}H_{92}O_{29}$

xyl - gluc - gal
|
gluc - gal

ESI ionization – formation of the molecular adduct **[M+Na]⁺**

MS/MS fragmentation – clevage by elimination of neutral sugars
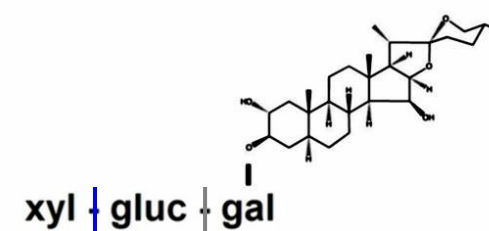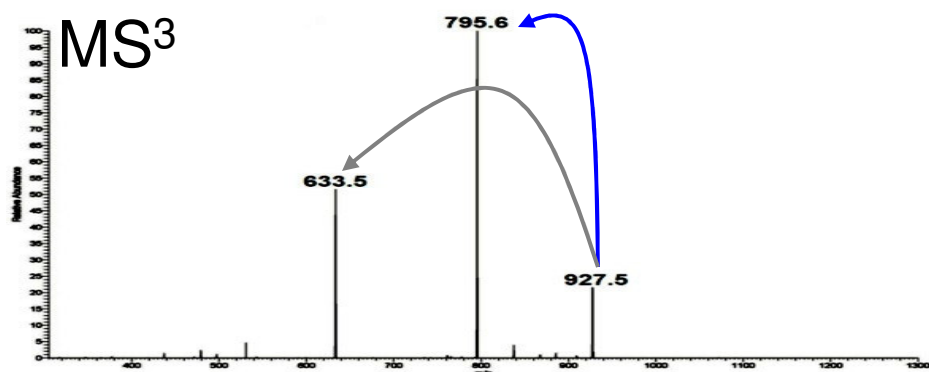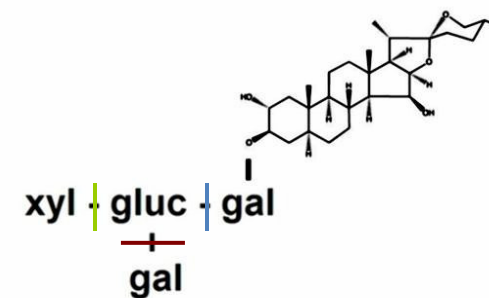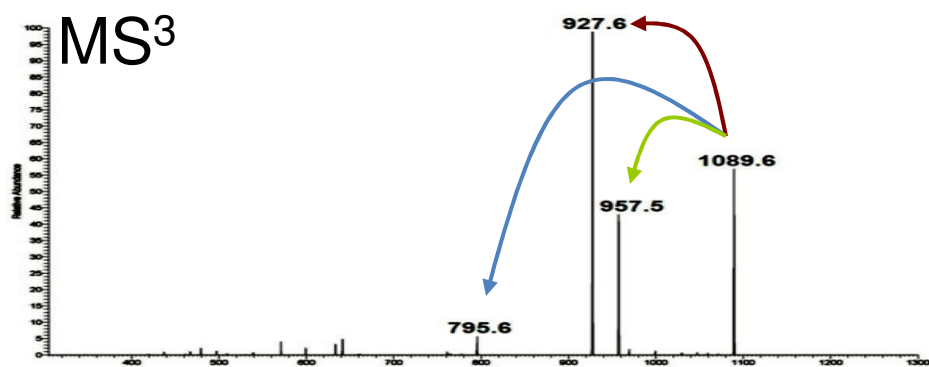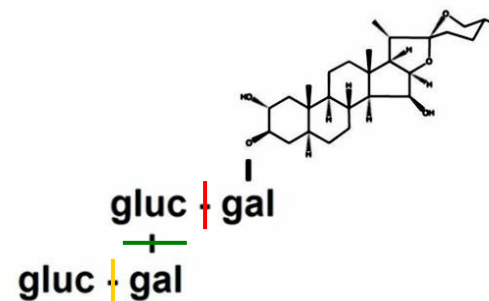
Hexose: loss of **162 u**   (the molecule – H_2O)

Pentose: loss of **132 u**   (the molecule – H_2O)

Glycosides - digitonin

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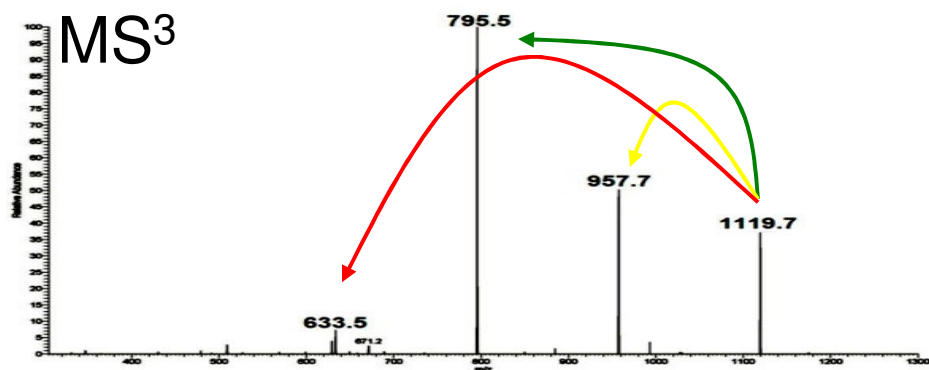
Fragmentation of ions with odd number of electrons (OE$^{+\bullet}$)

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

$$OE^{+\bullet} \rightarrow EE^+ + R^{\bullet}$$
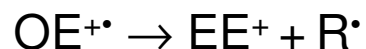
II. formation of an ion with odd number of electrons and a neutral specie

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

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

$$R^{+\bullet}CR_3 \xrightarrow{\sigma} R\bullet + \overset{+}{C}R_3$$

decane

(mainlib) Decane

Branched hydrocarbons

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

(mainlib) Nonadecane, 3-methyl-
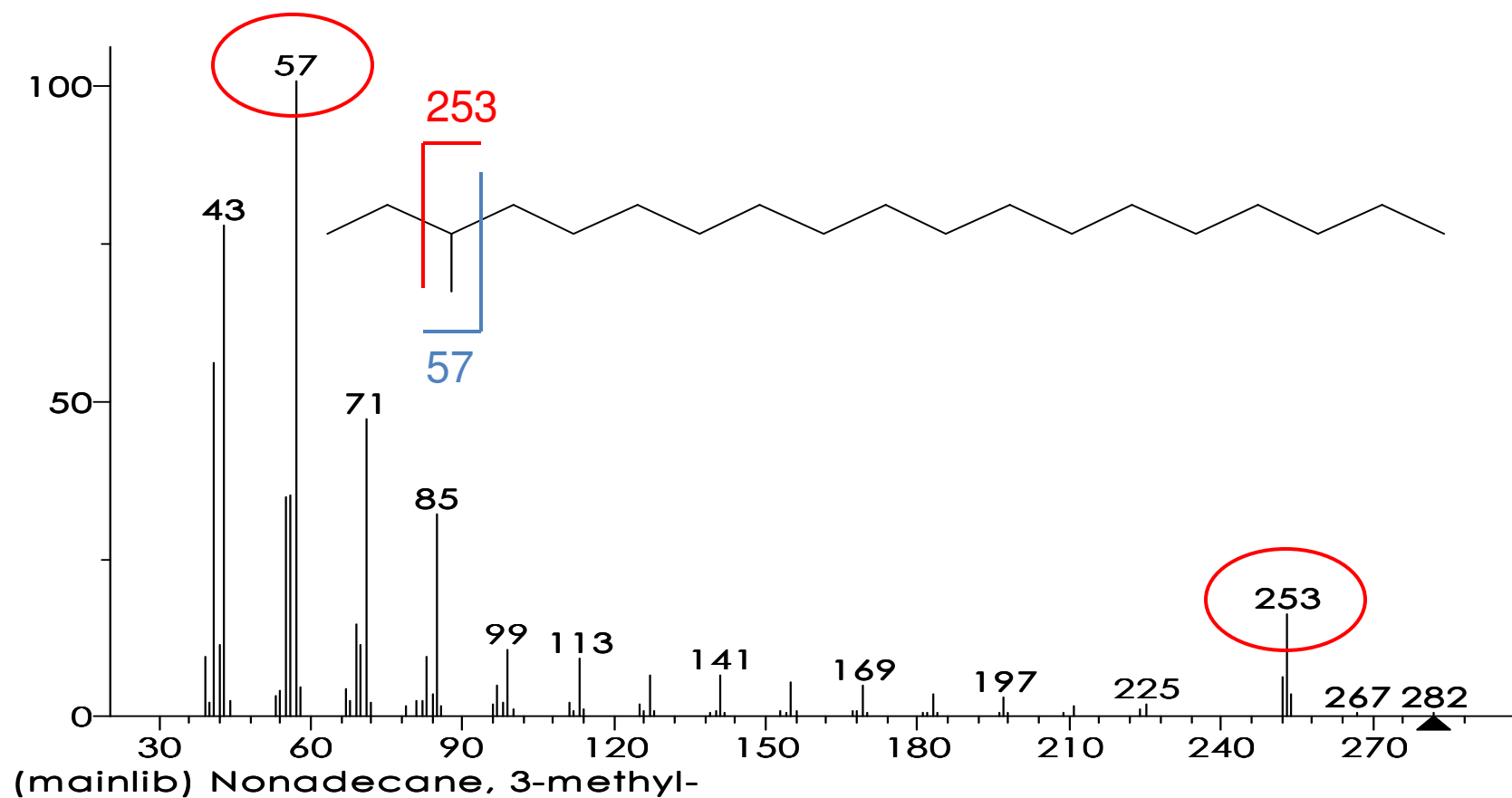
Chloromethane

EI

Unknown

What is the structure?

EI

$M^{+\bullet}$
176

100 —

Chloroiodomethane

I—Cl

CH_2Cl^+

49

127; I$^\bullet$

50 —

CH_2I^+ 35; Cl$^\bullet$

I$^+$
141

127

13   36   63   83   162

0

10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190
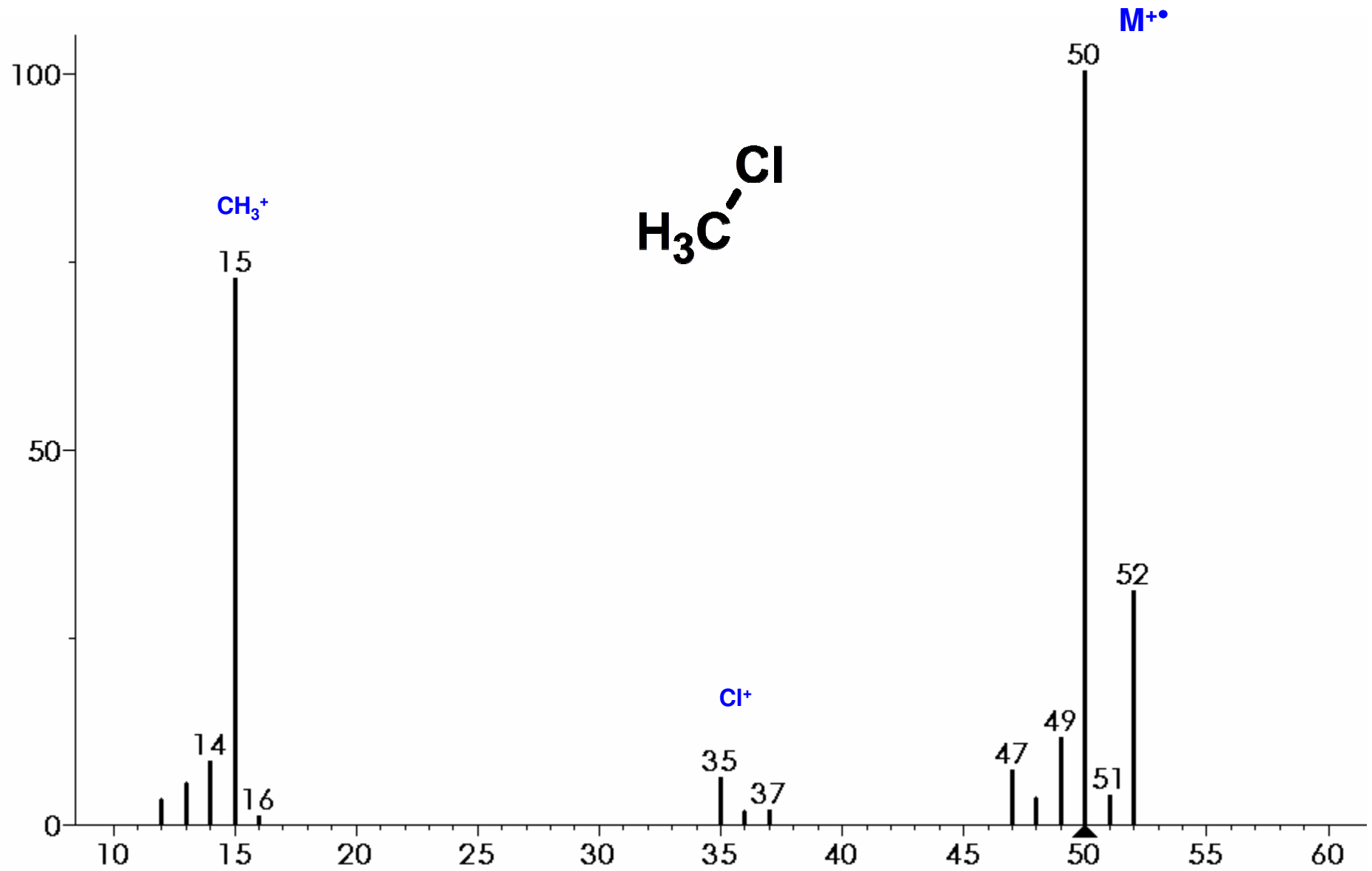
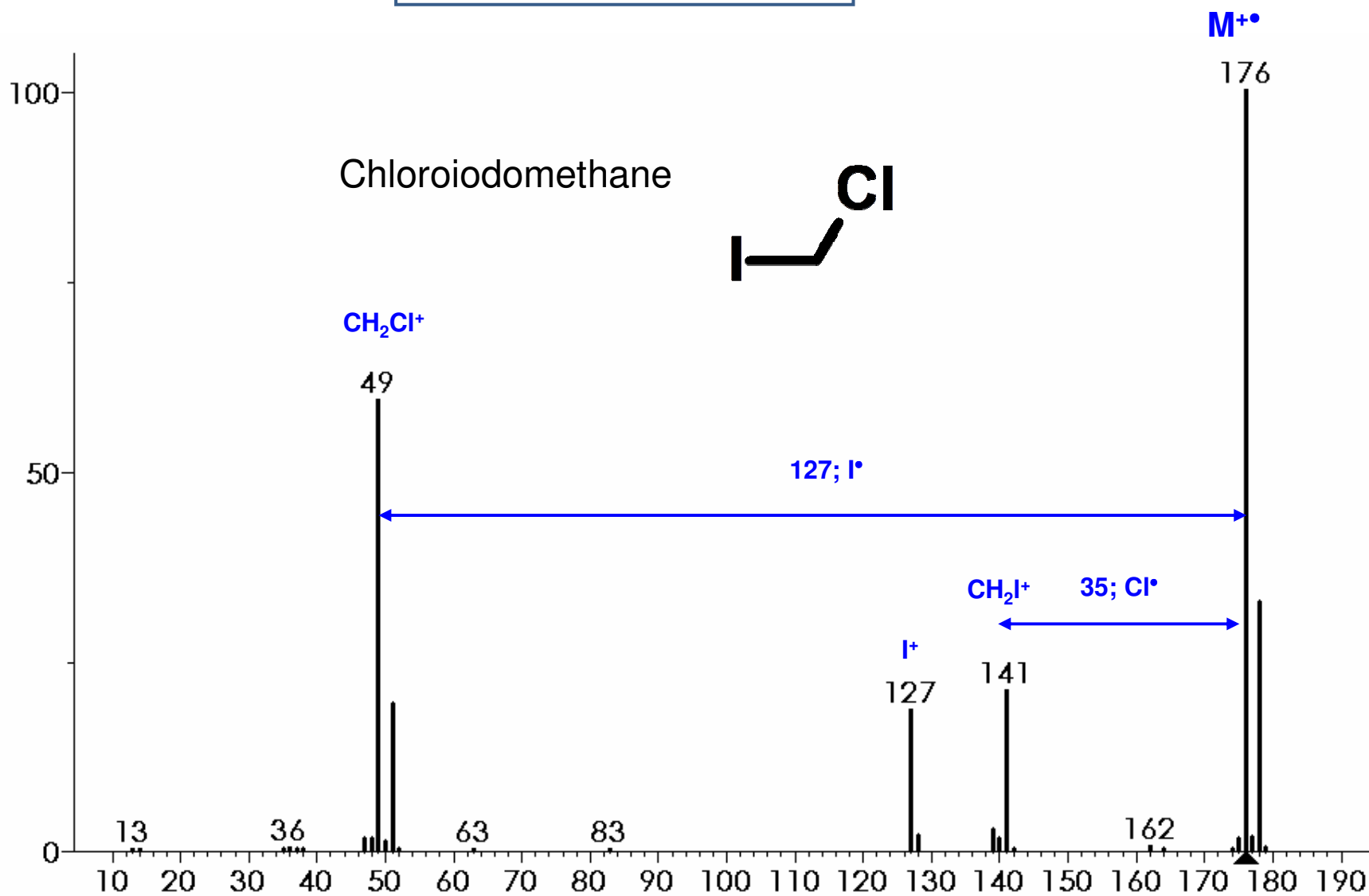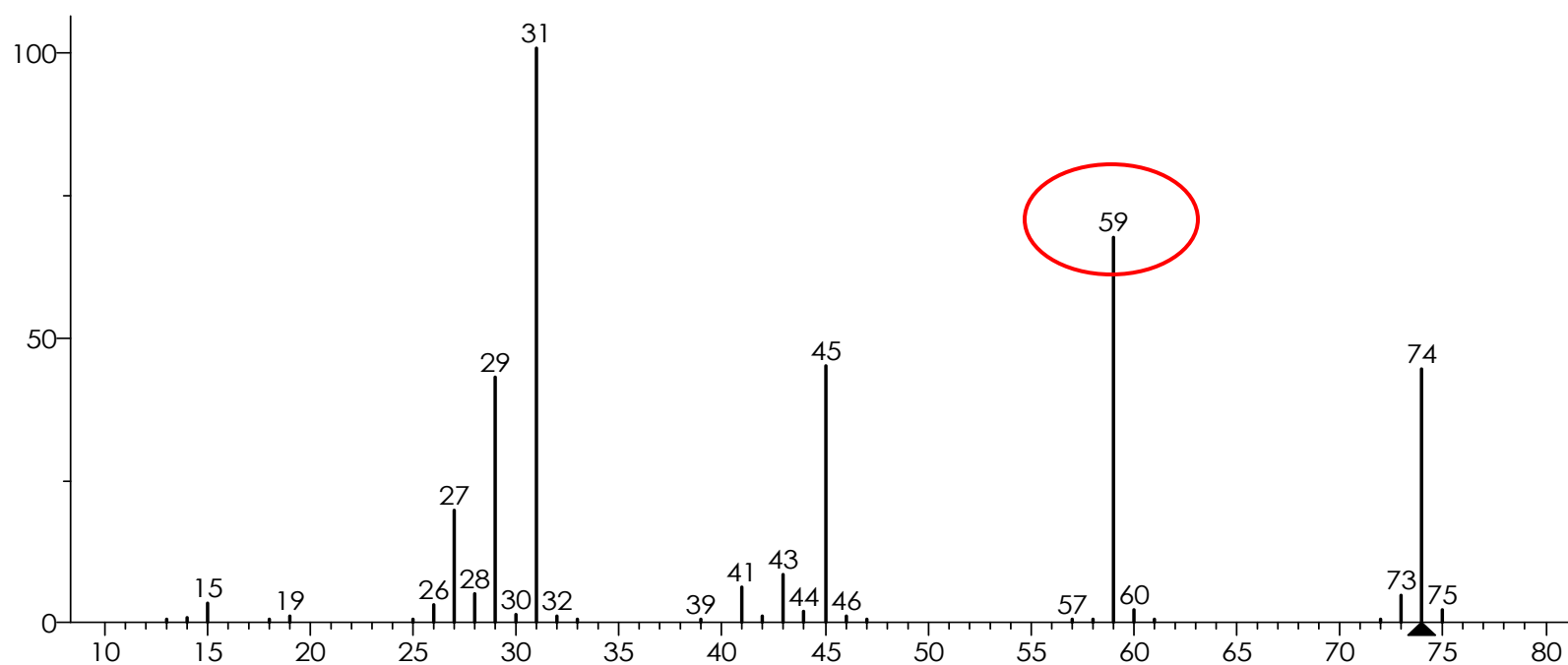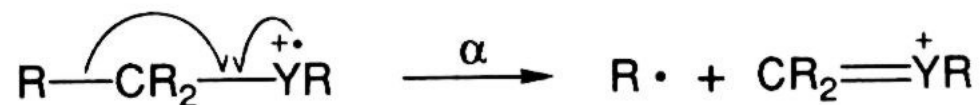# α-cleavage: fragmentation initiated by radical site

• 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

$$R\text{---}CR_2\text{---}\overset{+\bullet}{Y}R \quad \xrightarrow{\ \alpha\ } \quad R\bullet \ + \ CR_2\!\!=\!\!\overset{+}{Y}R$$

$$CH_3\text{---}CH_2\text{---}\overset{+\bullet}{O}C_2H_5 \quad \xrightarrow{\ \alpha\ } \quad \bullet CH_3 \ + \ CH_2\!\!=\!\!\overset{+}{O}C_2H_5 \quad (\leftrightarrow \overset{+}{C}H_2\text{---}OC_2H_5)$$
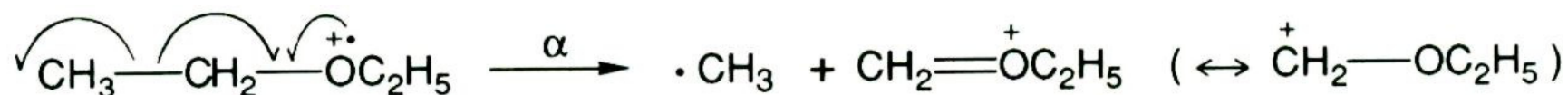
(mainlib) Ethyl ether

α-cleavage: benzylic clevage

(mainlib) Ethylbenzene
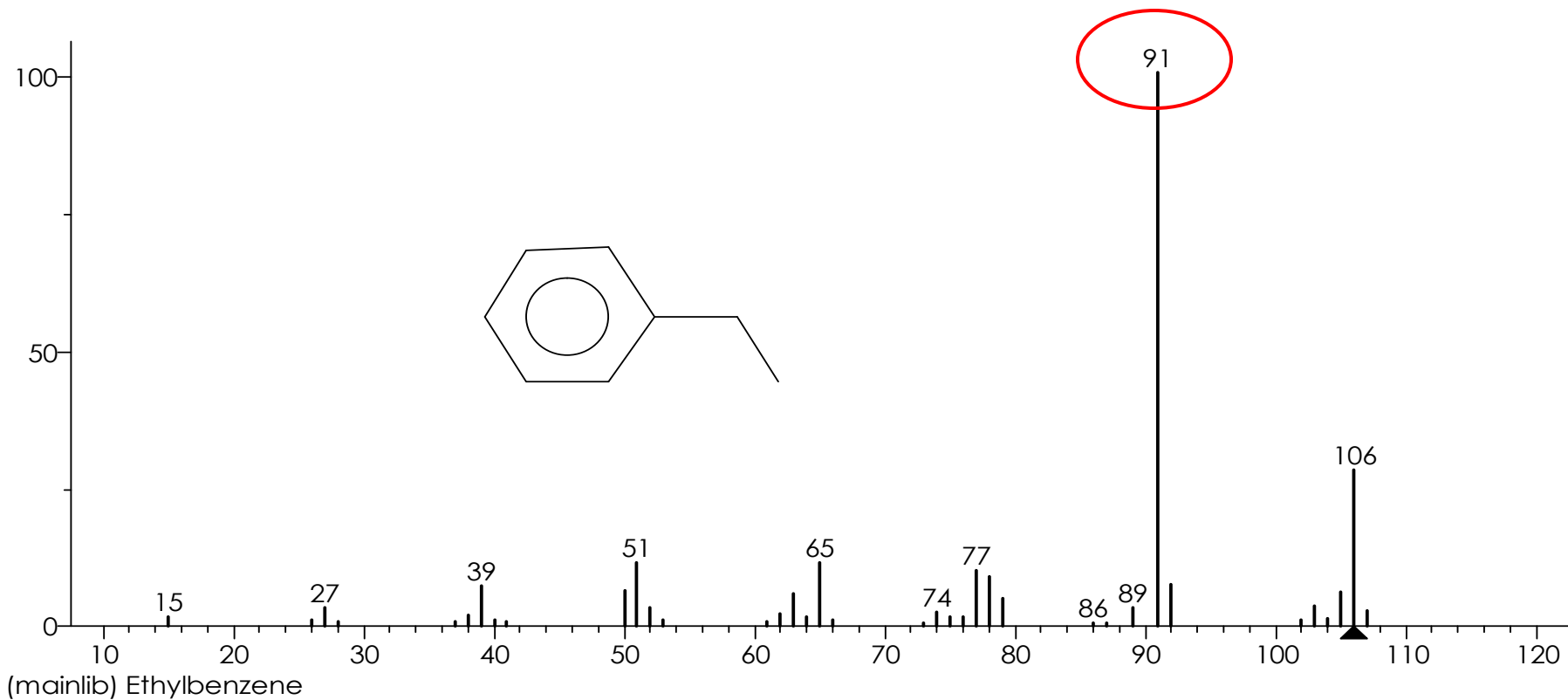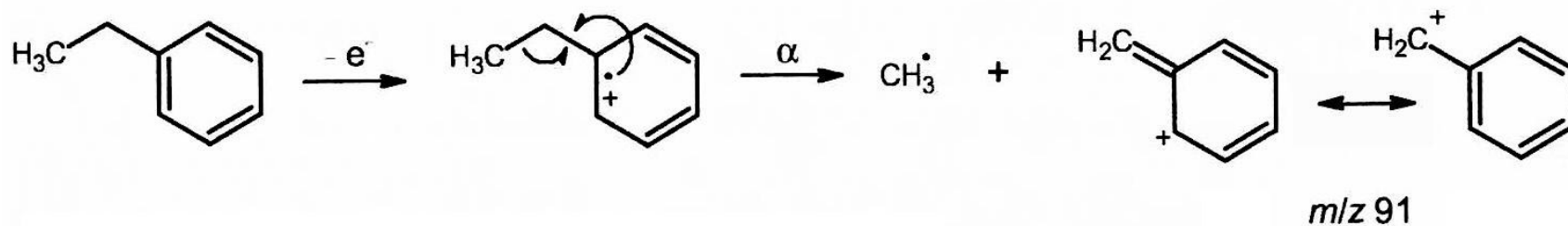
Inductive cleavage: fragmentation caused by a charge

• cleavage initialized by attraction of an electron pair by the charge
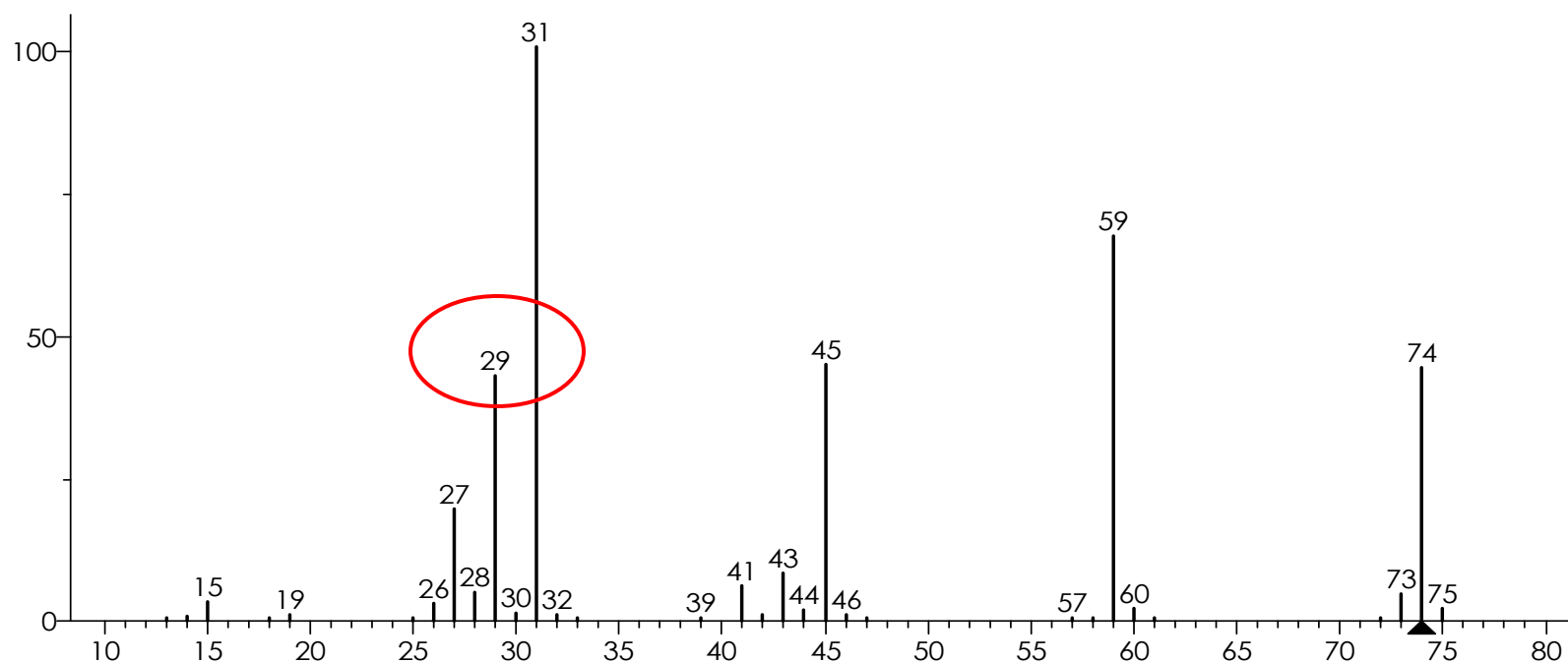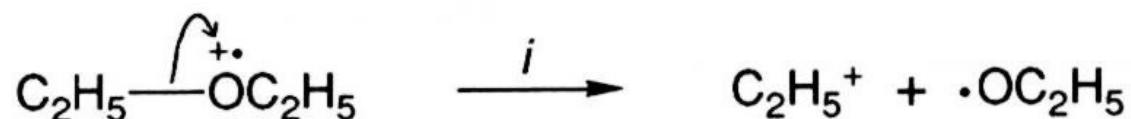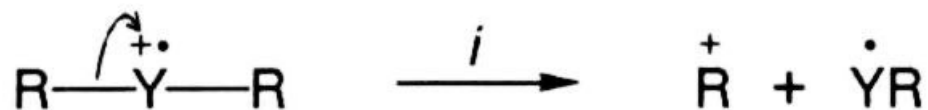
(mainlib) Ethyl ether

Fragmentation of cyclic structures – retro Diels-Alder

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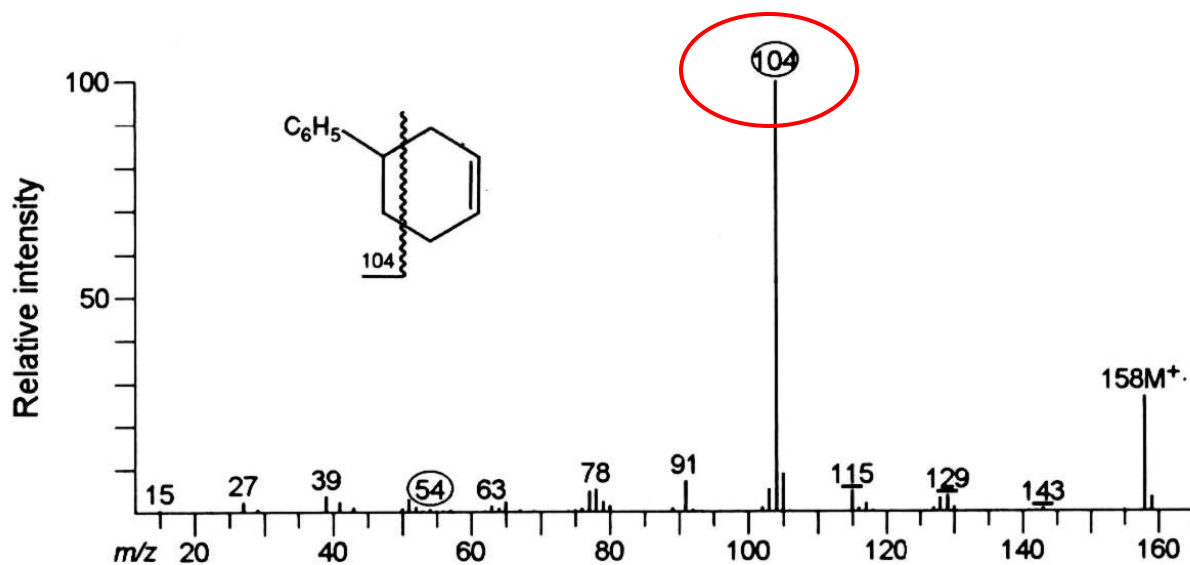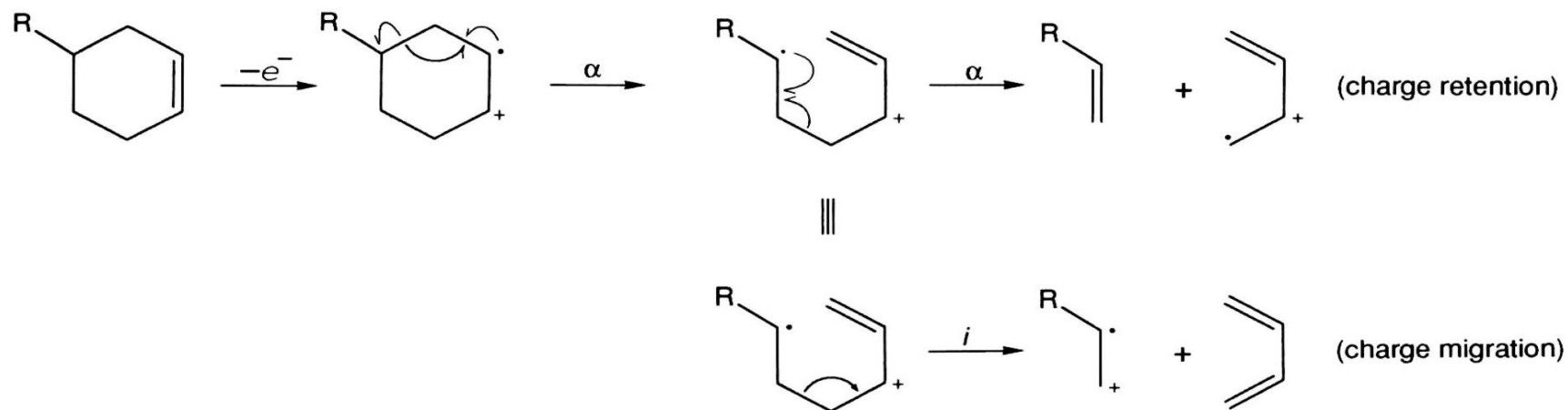
4-fenylcyklohexen

Hydrogen rearrangement – McLafferty rearrangement

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

(charge retention)

(charge migration)
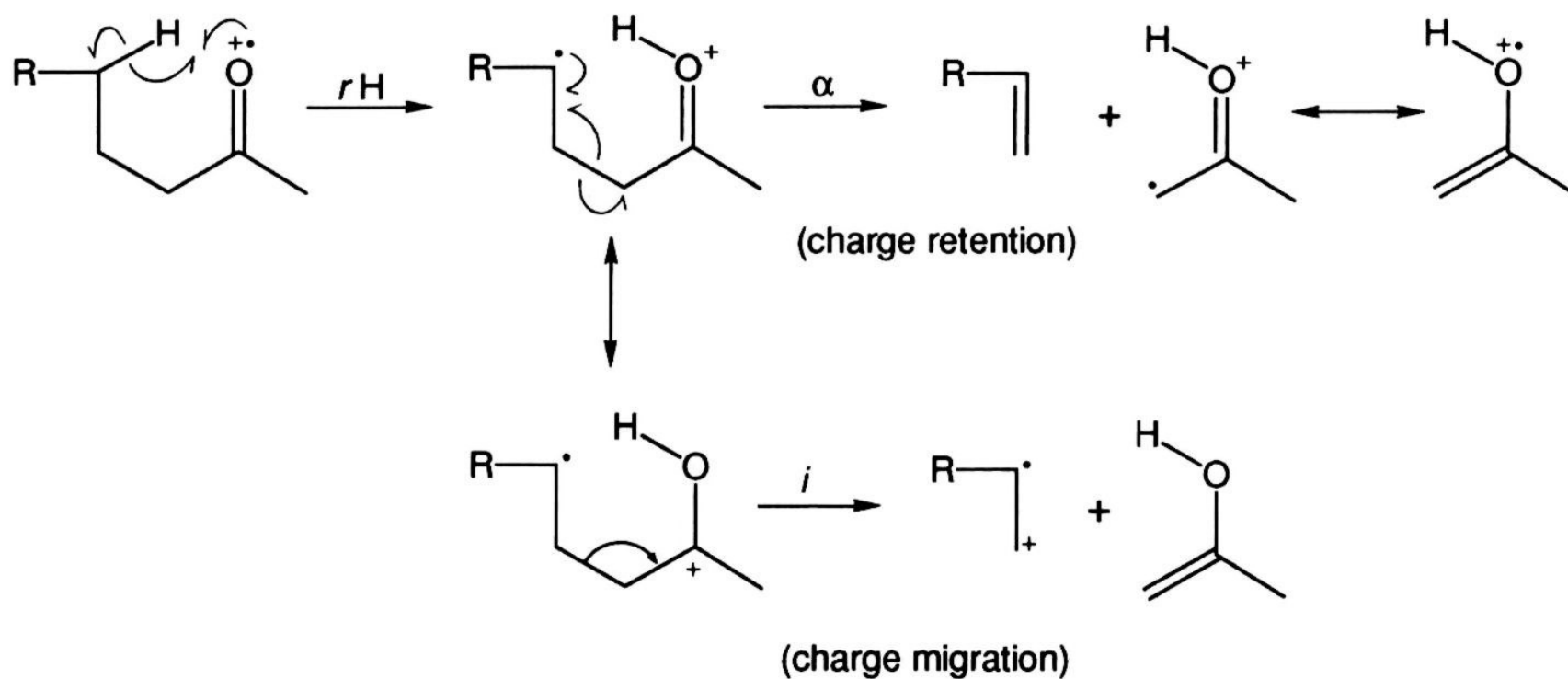
Hydrogen rearrangement – McLafferty rearrangement

• The OE$^{+\bullet}$ fragments are typical for many functional groups - aldehydes, ketones, esters, acids, amides, carbonates, phosphonates, etc.
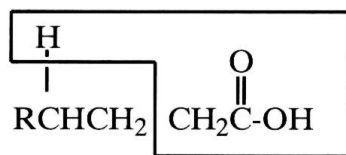
Acids — m/z 60

Aldehydes — m/z 44

Amides — m/z 59

Esters — m/z 74

Ketones — m/z 58

(mainlib) Dodecanoic acid, methyl ester
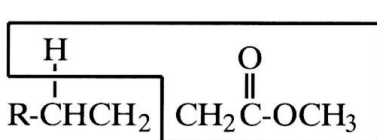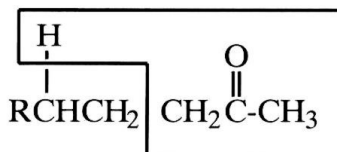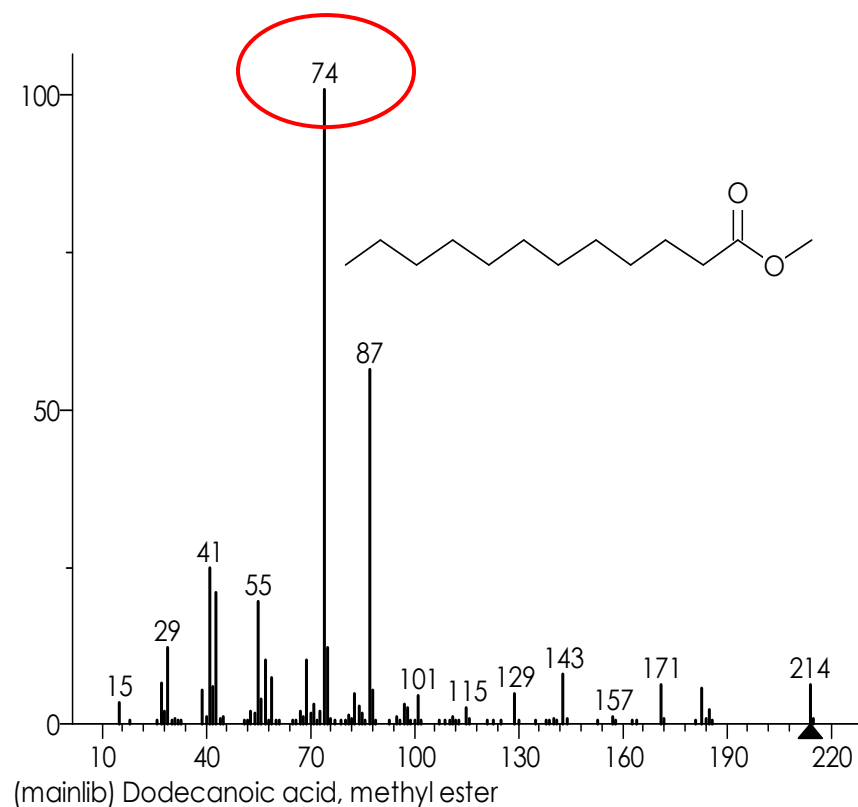
Characteristic ion series

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

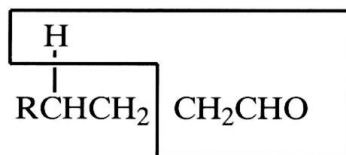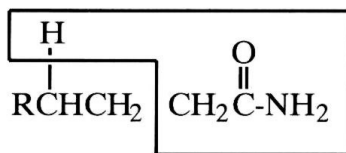| Ionty	Série	Funkční skupiny
m/z 29, 43, 57, 71, 85, 99, ...	$C_nH_{2n+1}^+$	alkyl („alifatika")
m/z 31, 45, 59, 73, 87, ...	$C_nH_{2n+1}O^+$	alkoholy, ethery
m/z 33, 47, 61, 75, 89, ...	$C_nH_{2n+1}S^+$	thioly, sulfidy
m/z 30, 44, 58, 72, 86, ...	$C_nH_{2n+2}N^+$	aminy
m/z 29, 43, 57, 71, 85, 99, ...	$C_nH_{2n-1}O^+$	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.
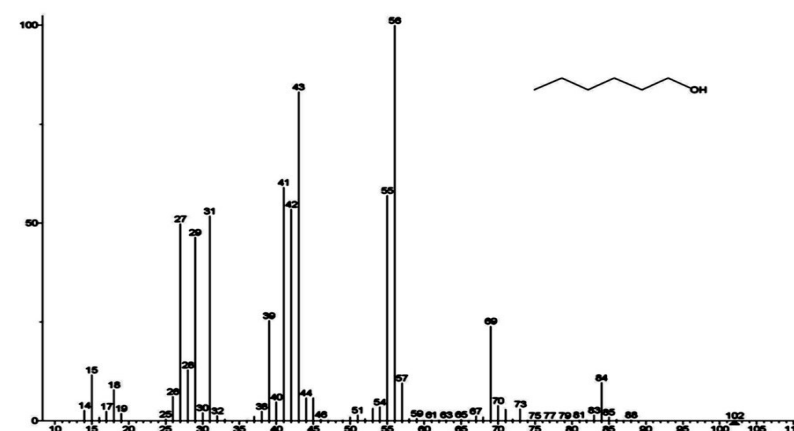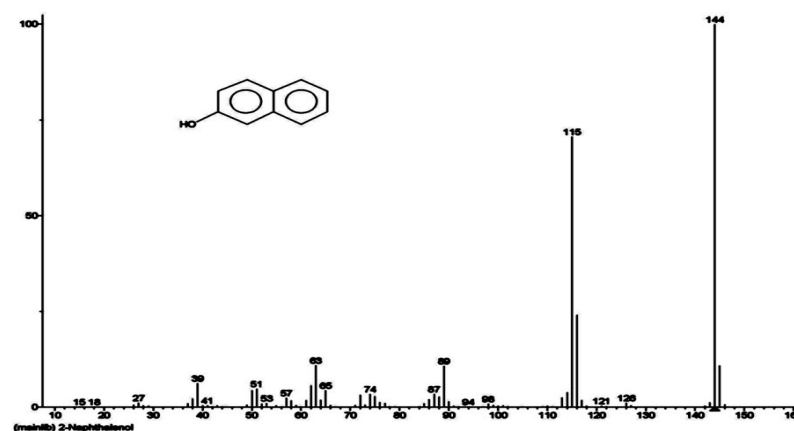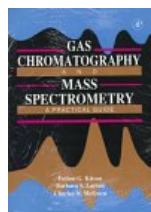
Literature

EI

Fred W. McLafferty and Frantisek Turecek: Interpretation of Mass Spectra. University Science Books (1993). ISBN-10: 0935702253, ISBN-13: 978-0935702255
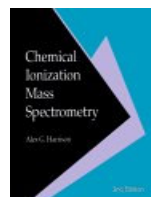
Fulton G. Kitson, Barbara S. Larsen, and Charles N. McEwen: Gas Chromatography and Mass Spectrometry. Academic Press (1996). ISBN-10: 0124833853, ISBN-13: 978-0124833852

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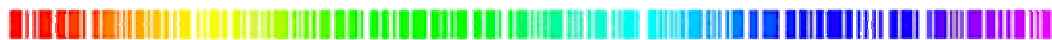
Alex. G. Harrison: Chemical Ionization Mass Spectrometry, CRC(1992). ISBN-10: 0849342546, ISBN-13: 978-0849342547

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13th Škola MS: Srní, **3.-7.9.2012**

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Thank you for your attention !