

# 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  $M^+$ , 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**

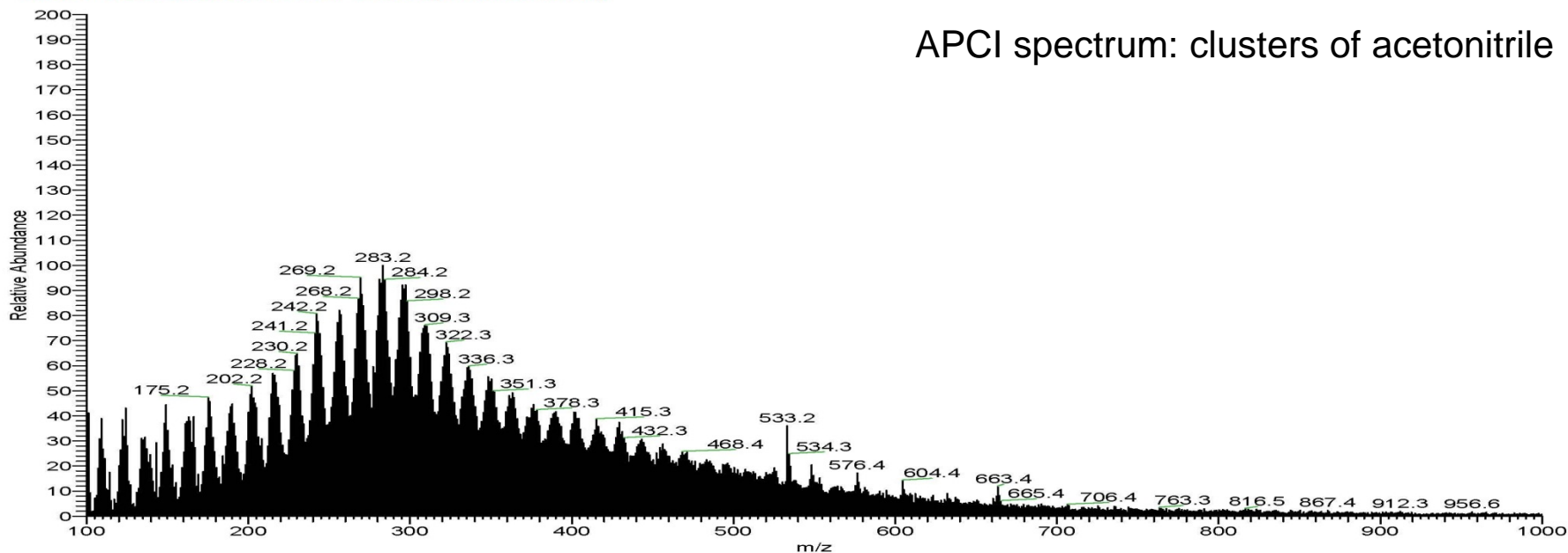
## Background ions, contaminants

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

c:\Documents and Settings\...\0962VV01

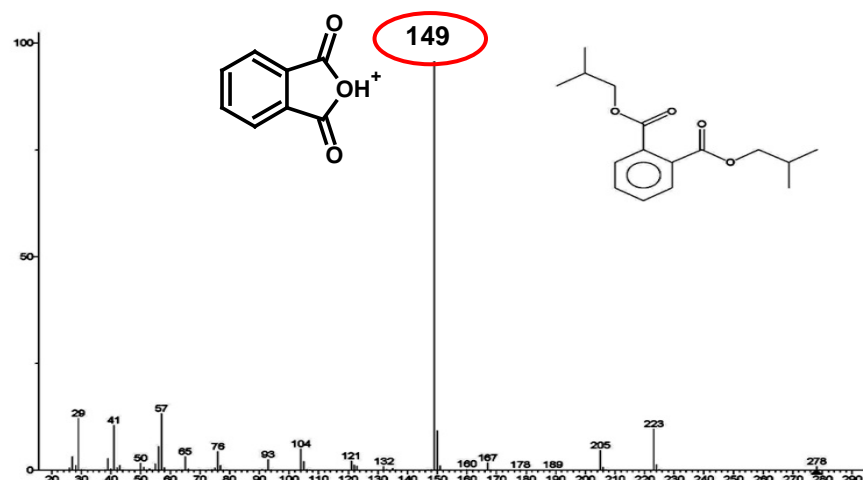
3/10/2009 12:32:40 PM

Bee Wax esters

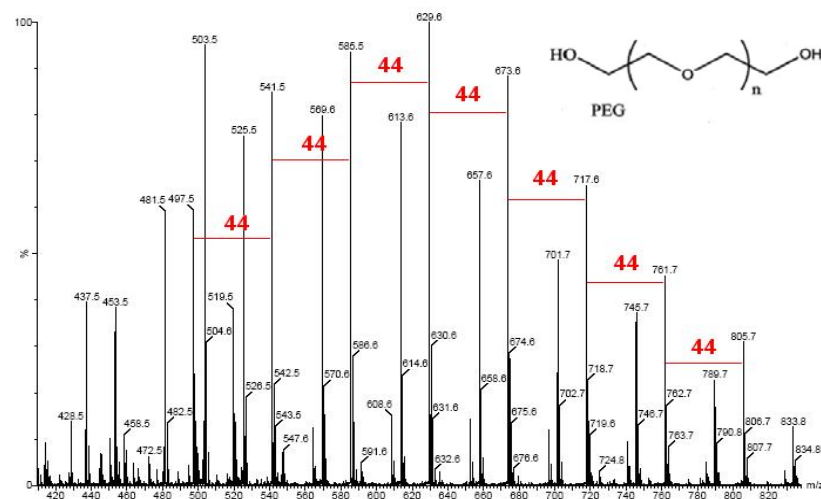
0962VV01 #7-262 RT: 0.08-3.33 AV: 256 NL: 3.63E1  
F: ITMS + c APCI corona sid=8.00 Full ms [100.00-1000.00]

## Background ions, contaminants

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

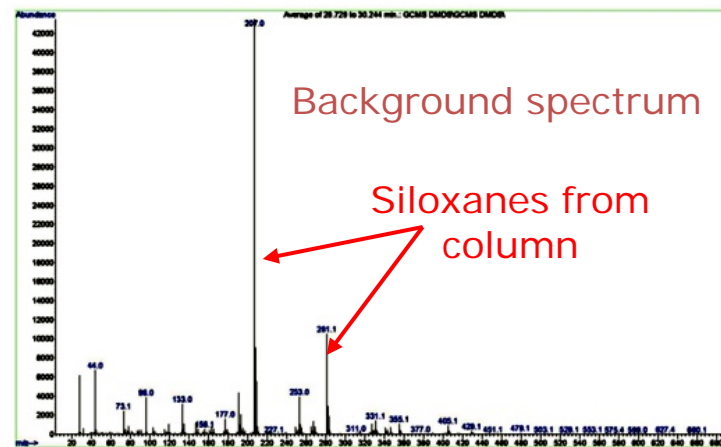
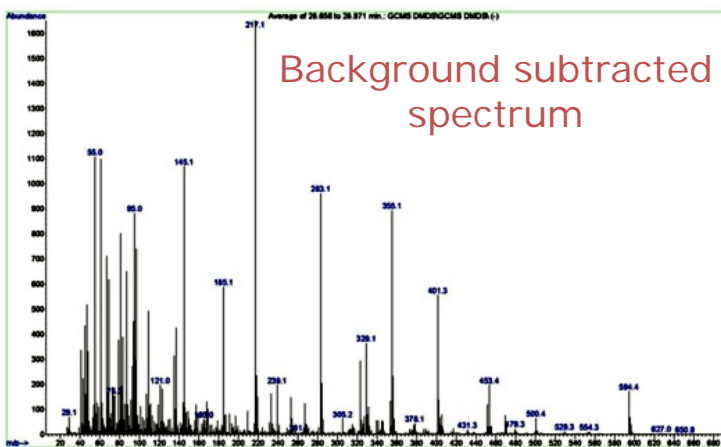
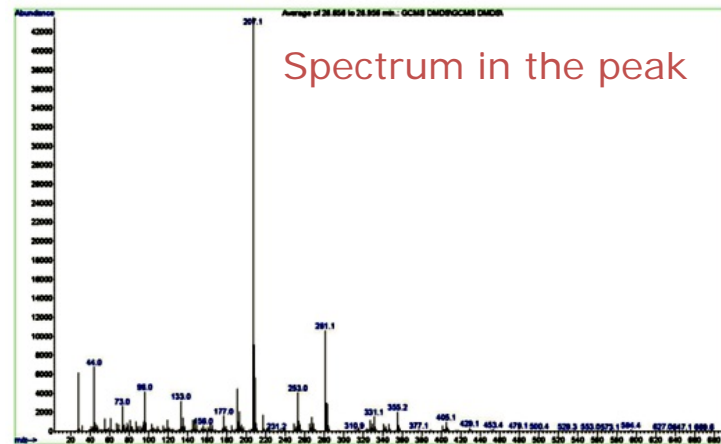
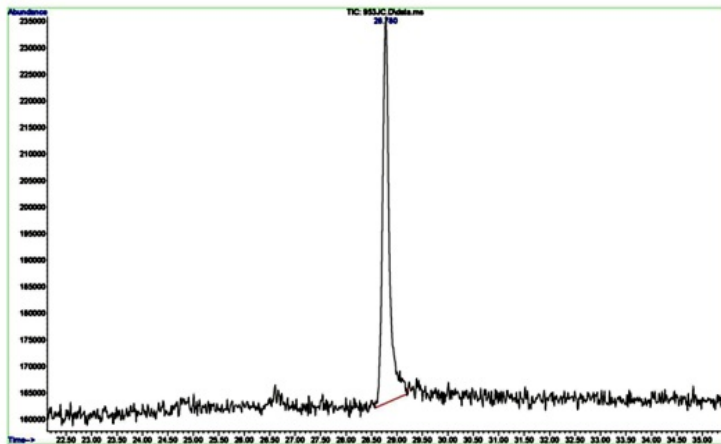


**Polyethyleneglycols:**  
from laboratory plastics, gloves,  
skin lotion  
peak difference 44 u



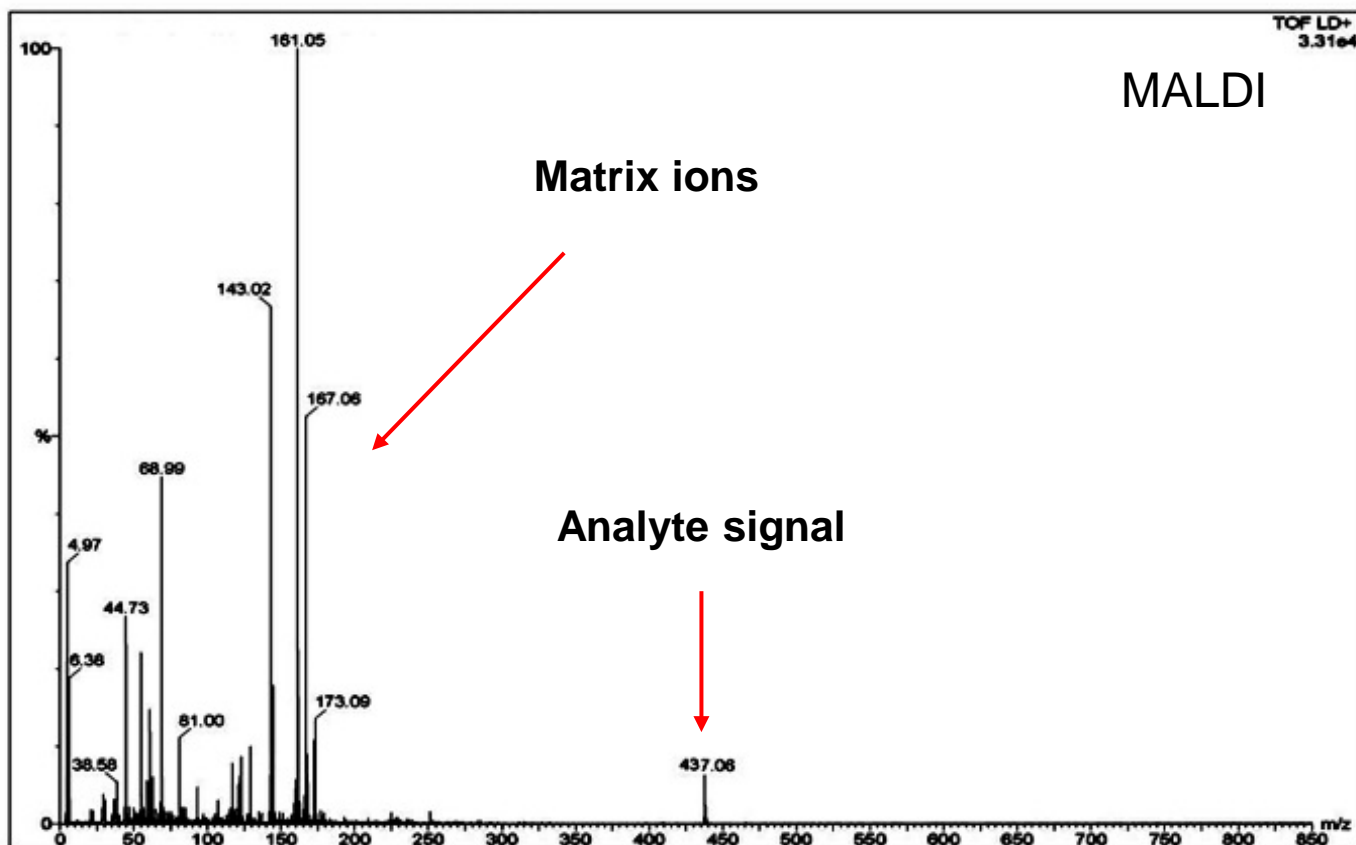
MeOH wash of laboratory gloves

# Background subtraction



## Matrix ions in MALDI

- Clusters, fragments, adducts of the matrix ions in the low mass range; very intense
- MALDI spectra are usually recorded starting from  $\sim m/z$  500



2.

## Determination of the molecular weight



# Determination of molecular weight

## *Identification of molecular ion or molecular adduct*

### I. The electron ionization



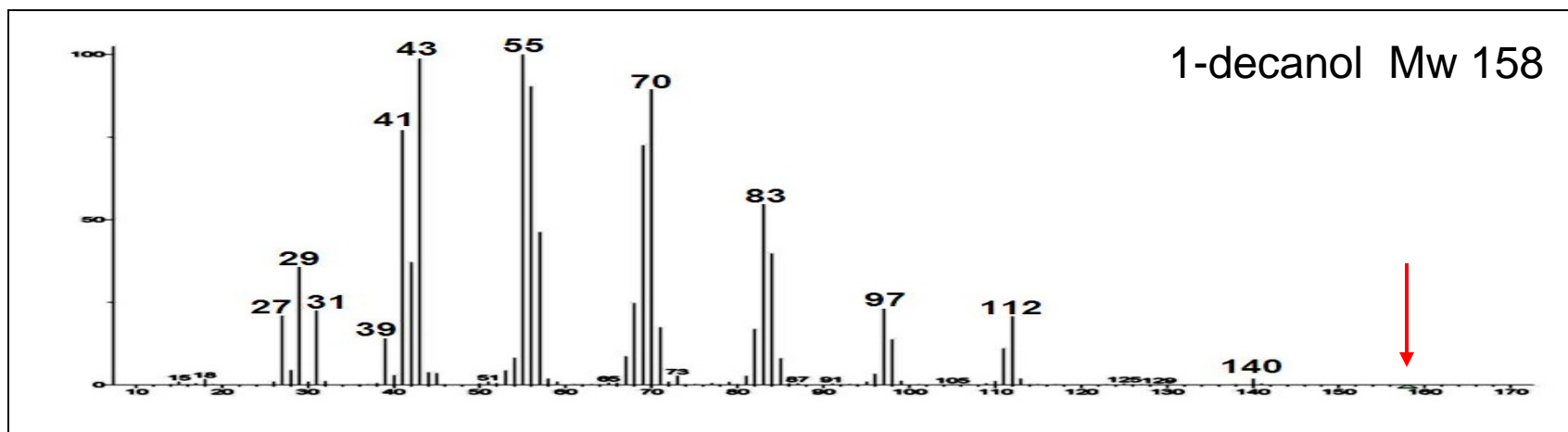
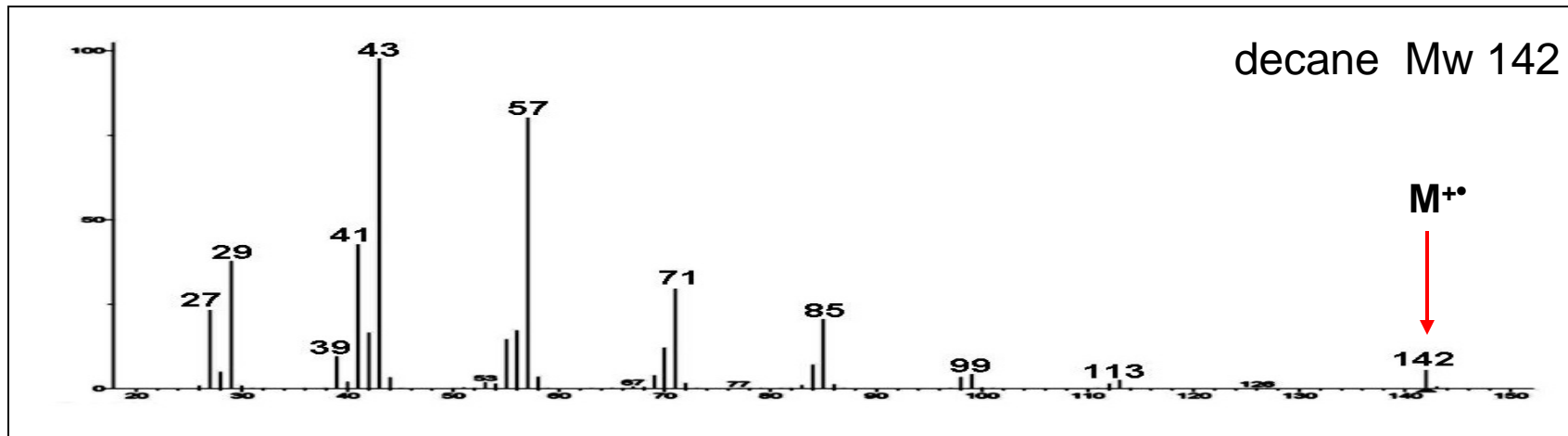
Molecular ion ( $M^{+\bullet}$ ) is a radical cation. The  $m/z$  corresponds to the monoisotopic mass of analyte.

### Identification of the molecular ion in the spectrum

- 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

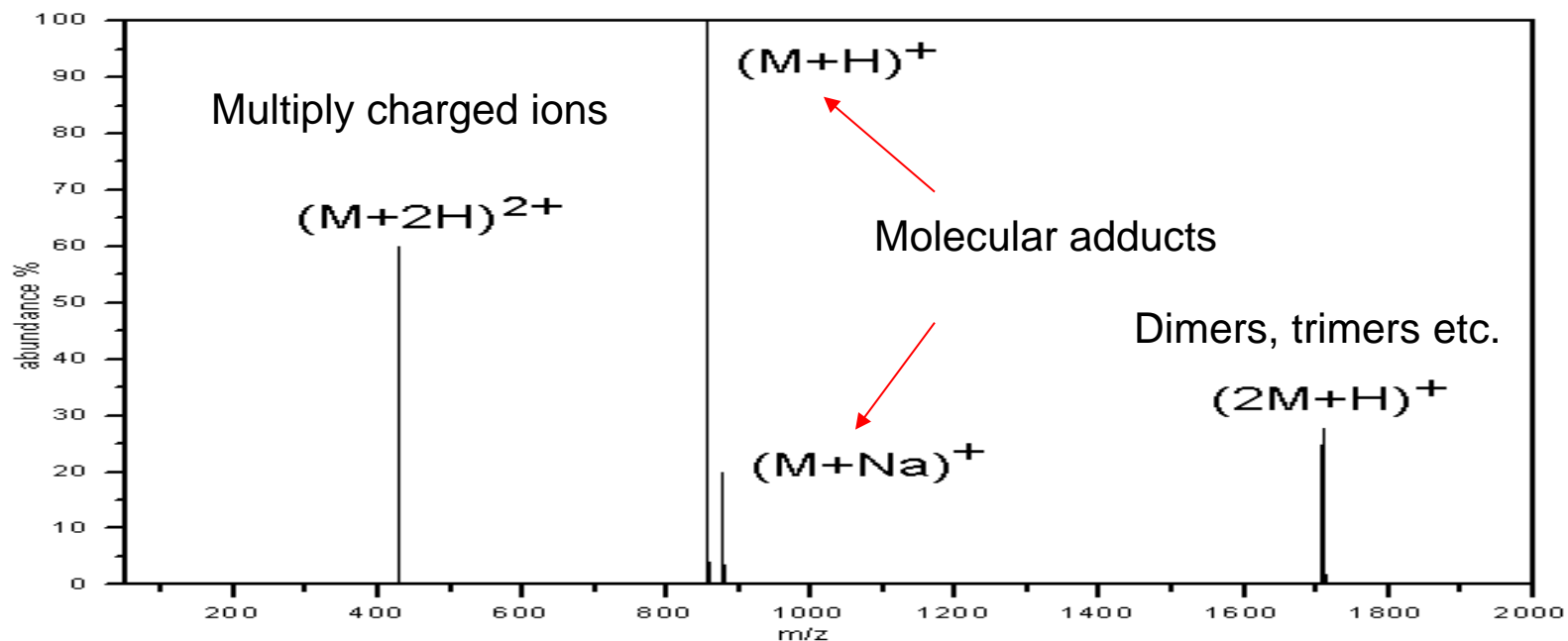


# Determination of molecular weight

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

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

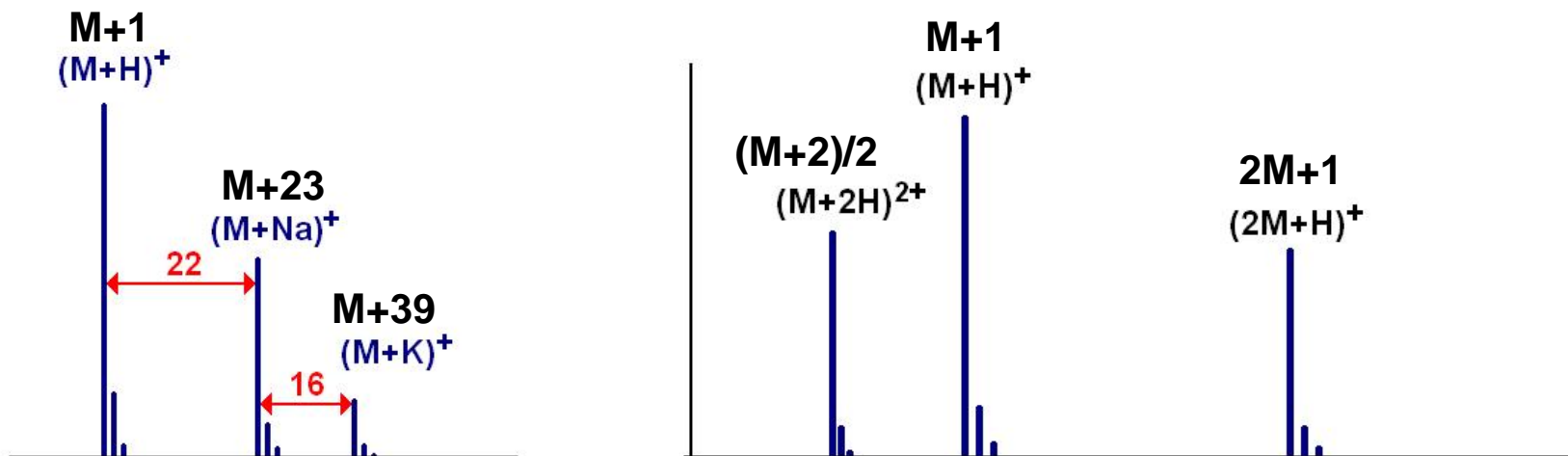
The molecular adduct is an ion with even number of electrons and may not be the highest ion in the spectrum.



# 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.

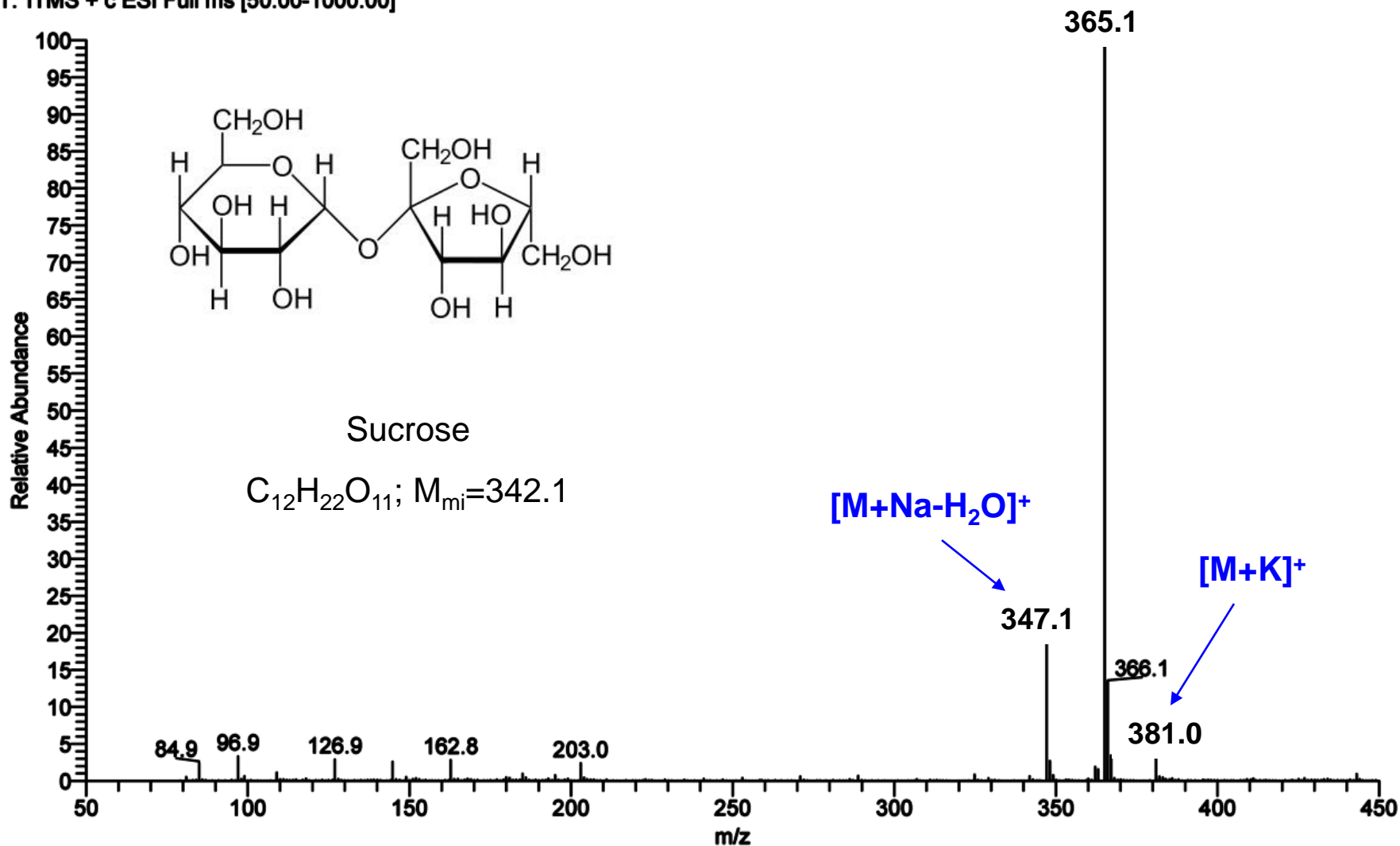


Calculation of adducts, dimers or multiply charged ions: software EIC  
I:\MISC\MS\DOWNLOAD\

# Determination of molecular weight



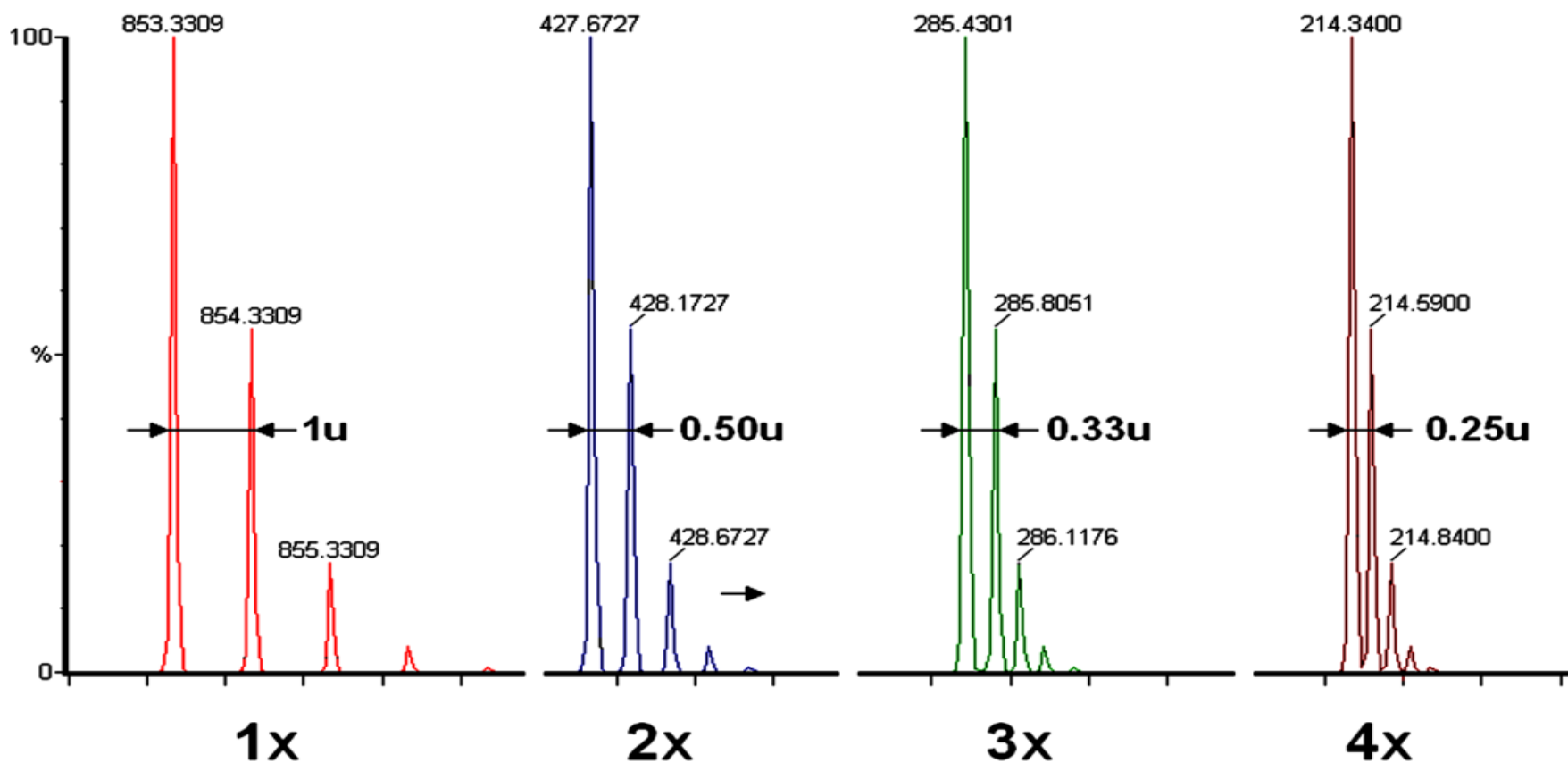
2256jc #1-51 RT: 0.00-0.75 AV: 51 NL: 1.77E4  
T: ITMS + c ESI Full ms [50.00-1000.00]



# Determination of molecular weight – charge state

## Determining number of charges

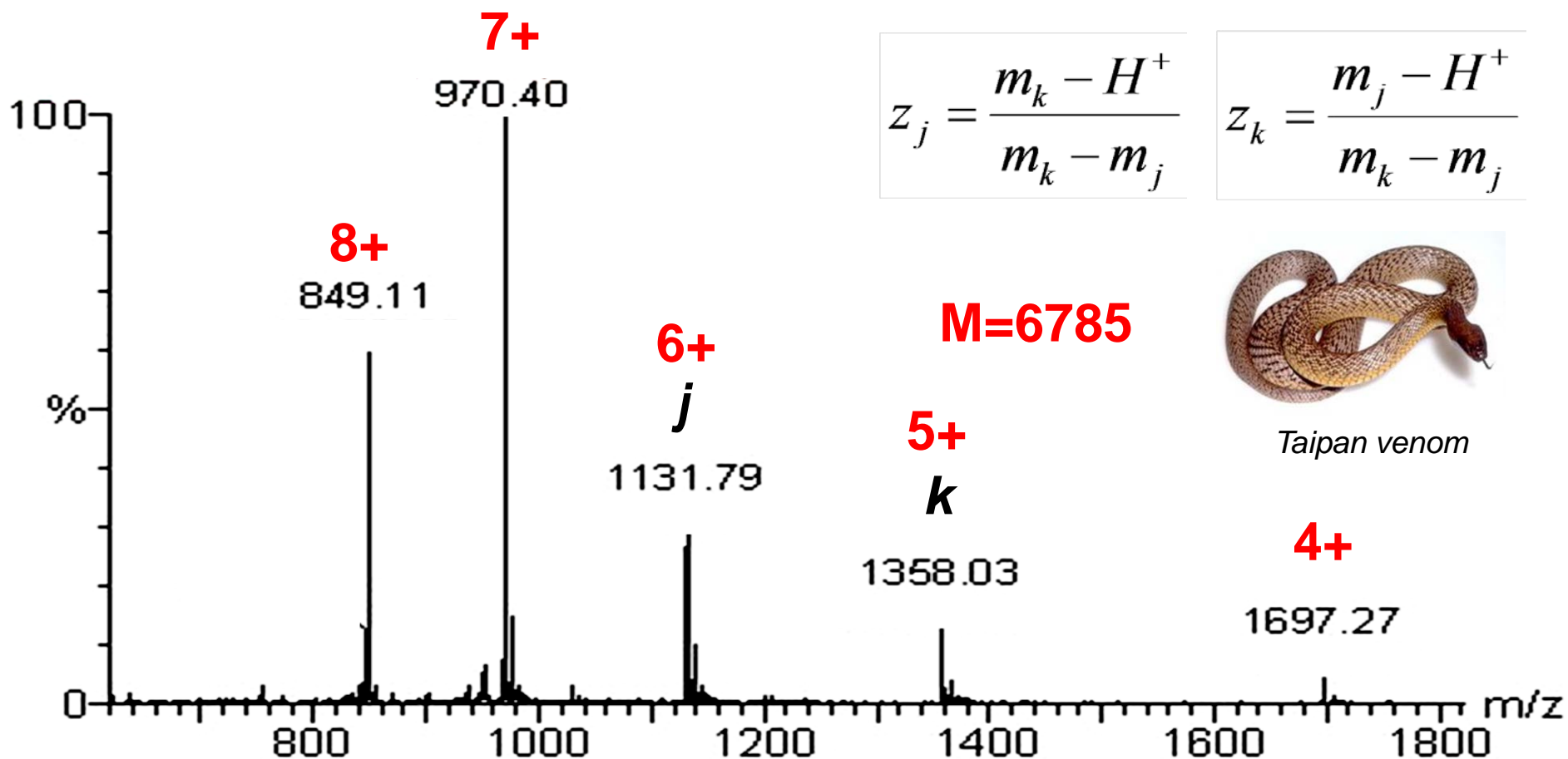
Number of charges can be determined from the distance between the peaks in the isotopic clusters.



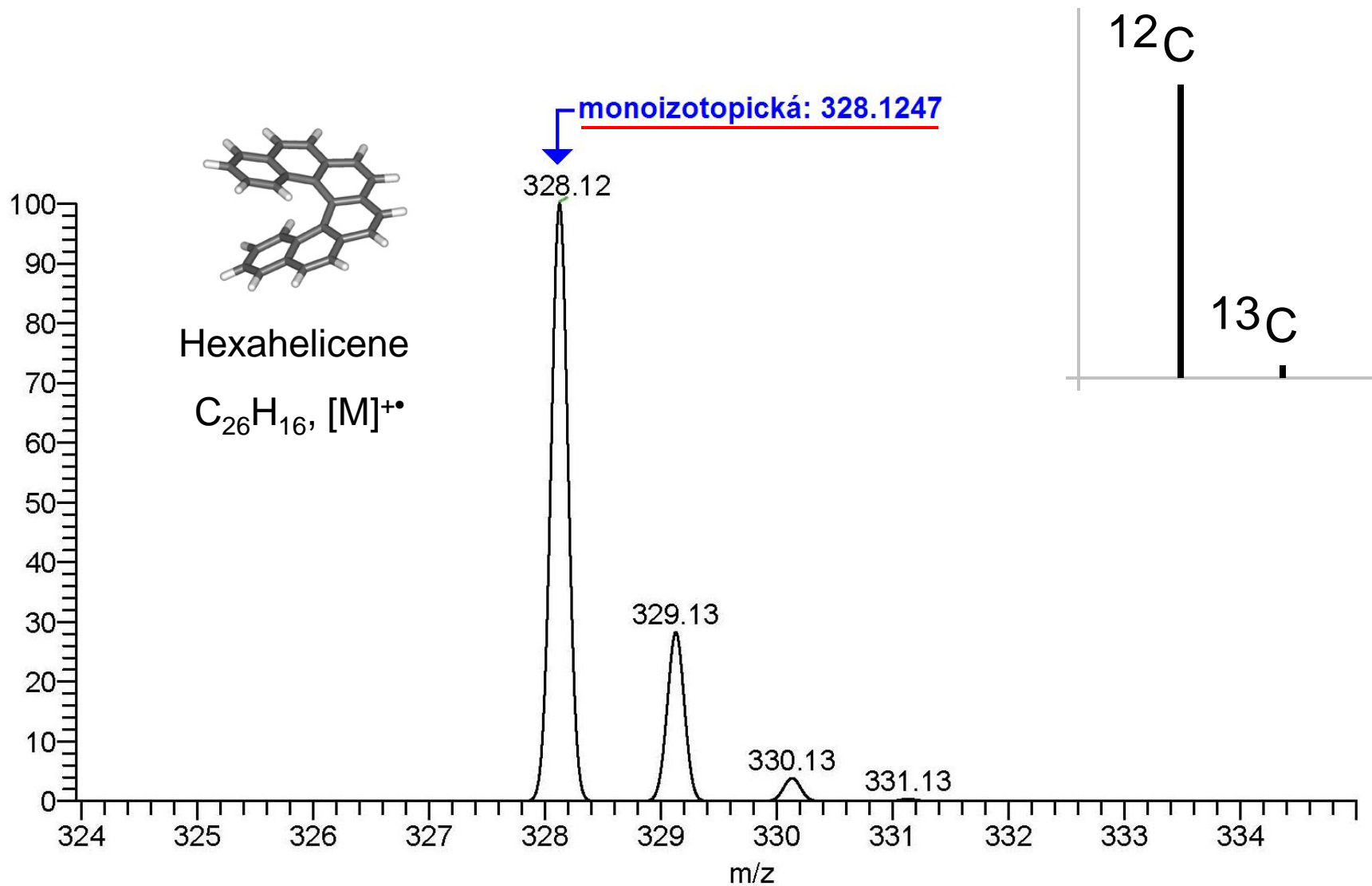
## Determination of molecular weight – charge state

### Determining number of charges

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

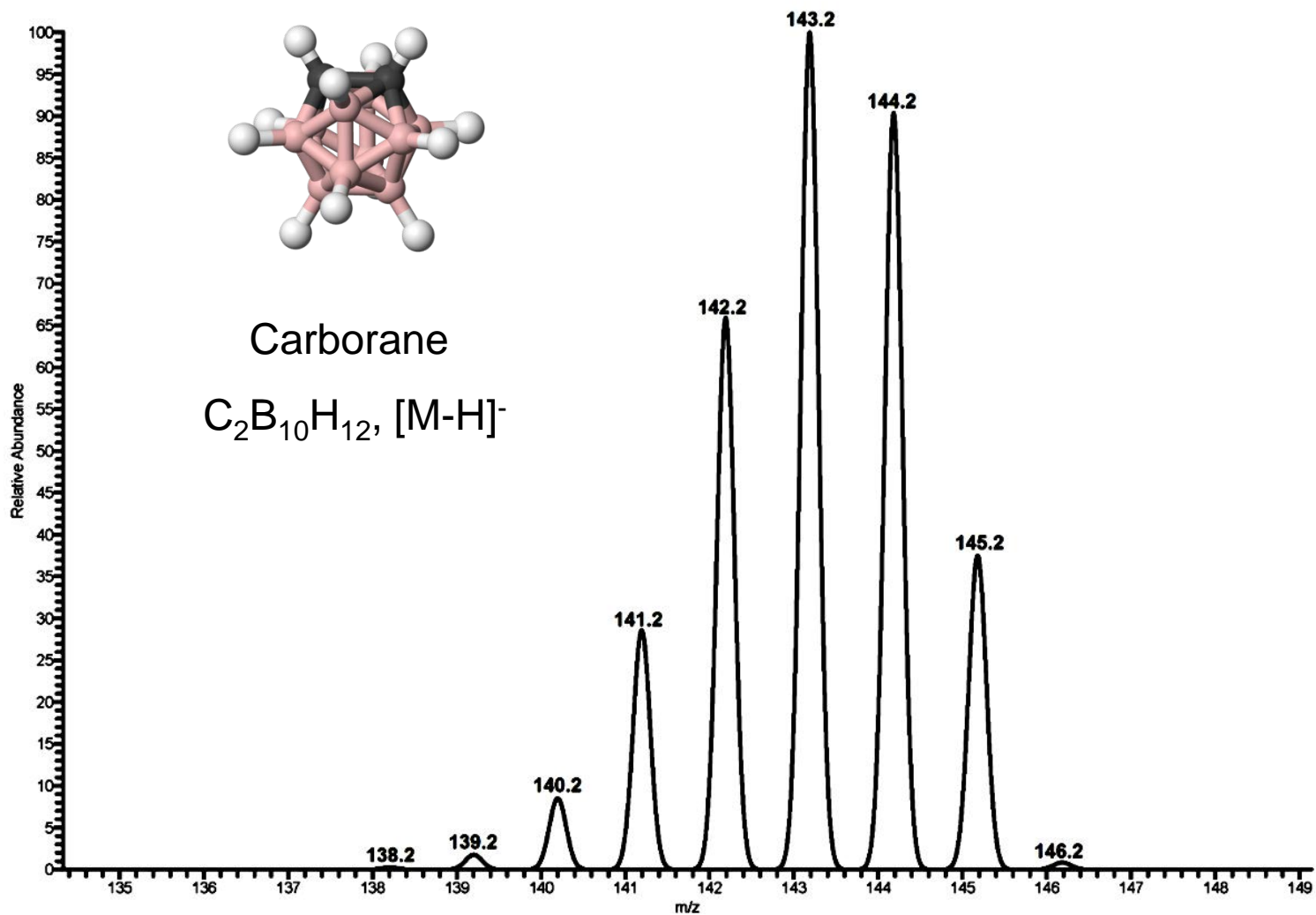


# Monoisotopic mass

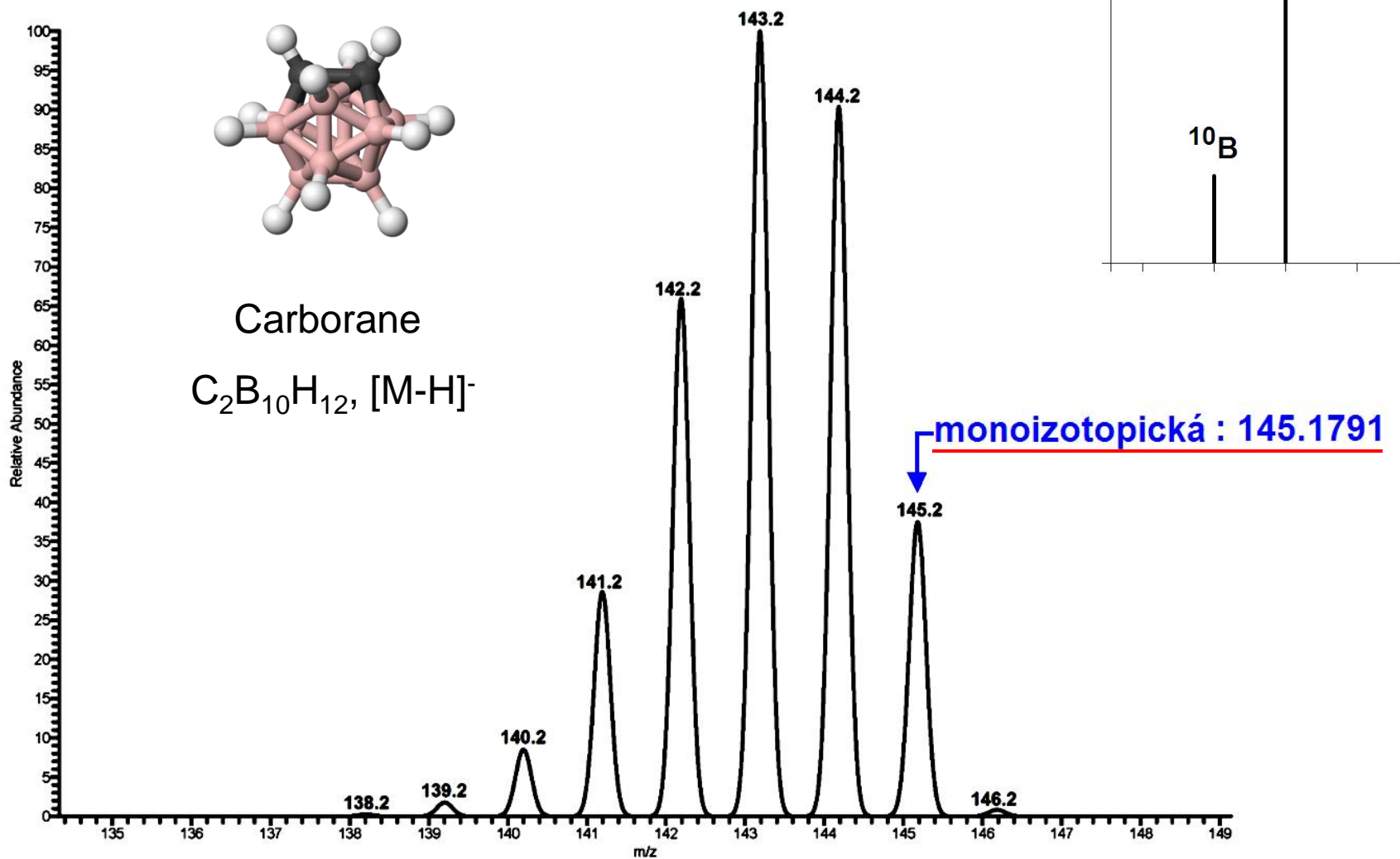




# Monoisotopic mass



# Monoisotopic mass

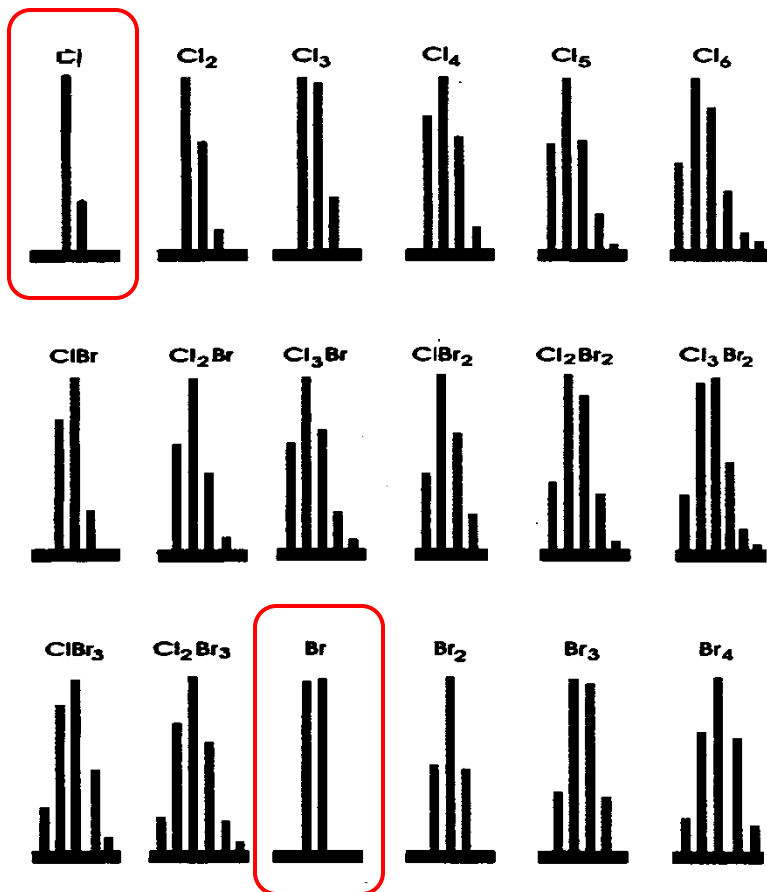


3.

**Elements present in a ion**

## Isotope clusters

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



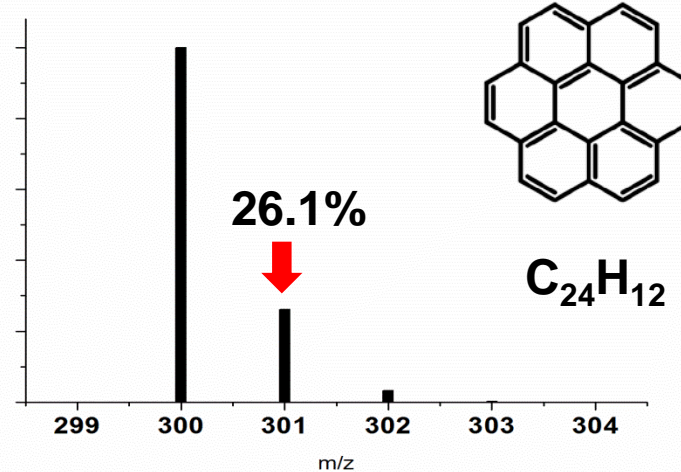
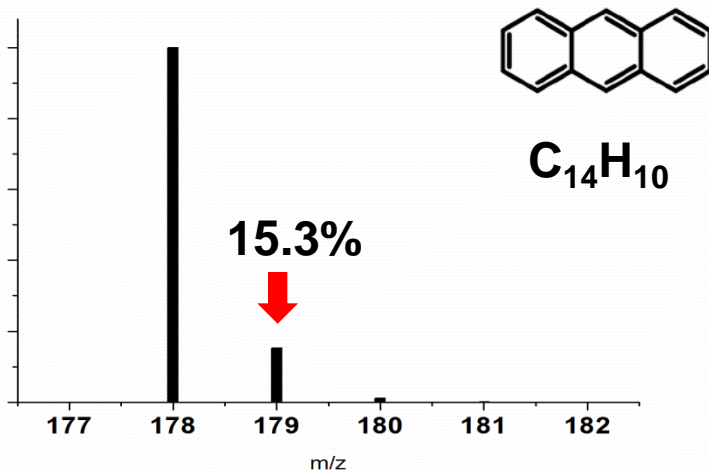
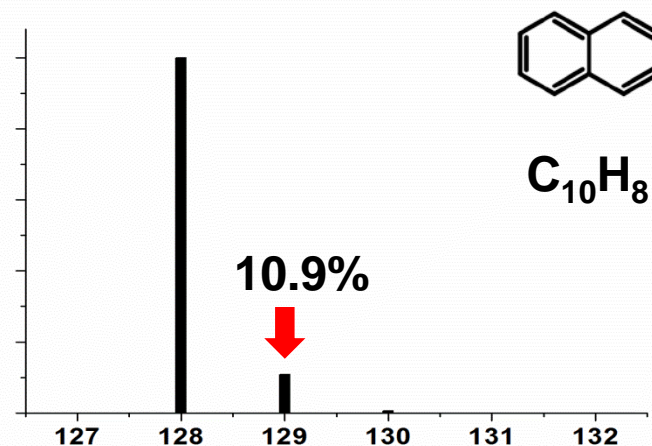
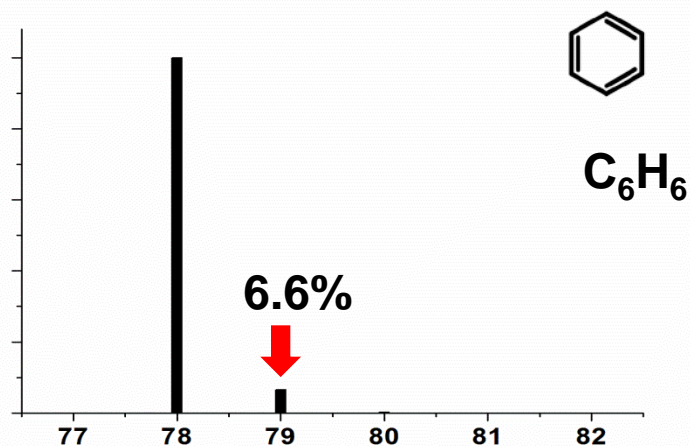
Computer programs allow you to calculate the composition of the cluster from the specified summary formula and compare it with experiment.



<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  $^{13}\text{C}$  isotope (relative ratio  $^{13}\text{C}/^{12}\text{C}$  is  $\sim 1.1\%$ )



## 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

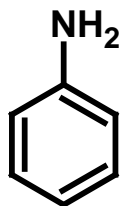
**Odd value of molecular weight = odd number of nitrogens**

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

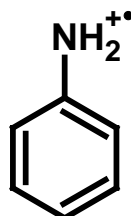
### *Applying the rule for ions*

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

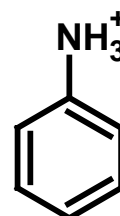
**ESI, APCI, MALDI** – the rule must be reversed for molecular adducts!



M=93



m/z 93



m/z 94

4.

**Determination of elemental composition from  
accurate mass**

## Elemental composition from accurate mass

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

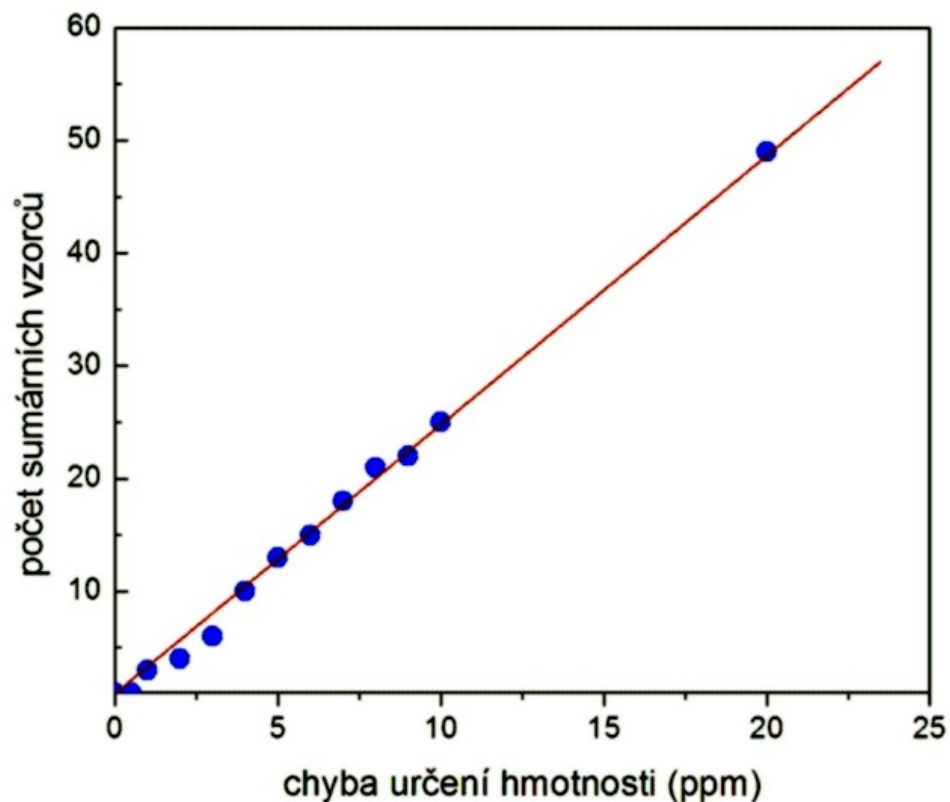
Constrains:

C: 0-100

H: 0-100

N: 0-10

O: 0-30



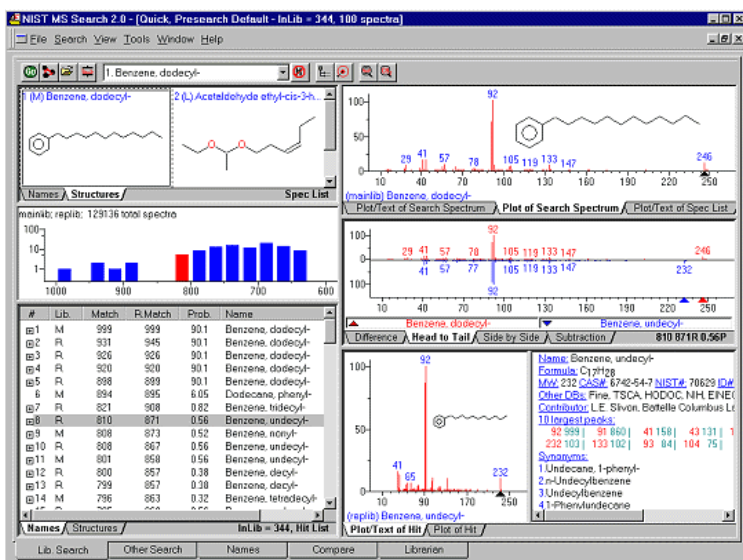


5.

## Searching mass spectra libraries

# Libraries of EI mass spectra

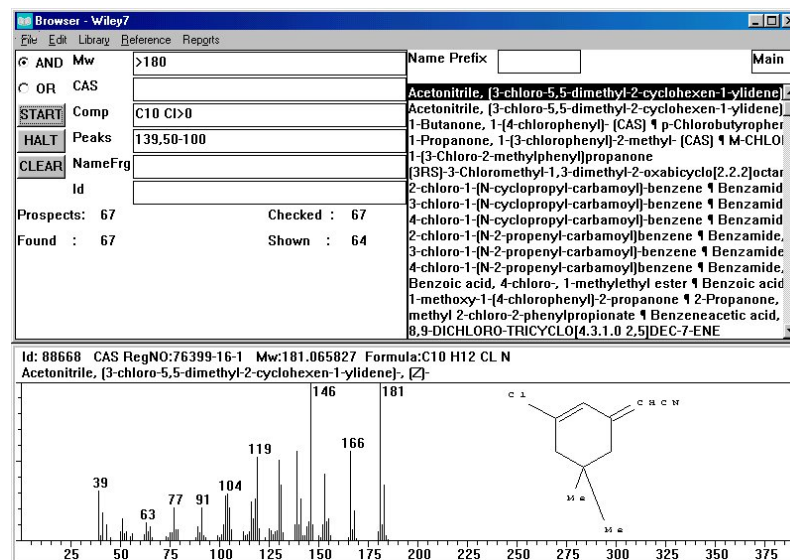
## NIST/EPA/NIH Mass Spectral Library



**NIST 11:**

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

## Wiley Registry of Mass Spectral Data



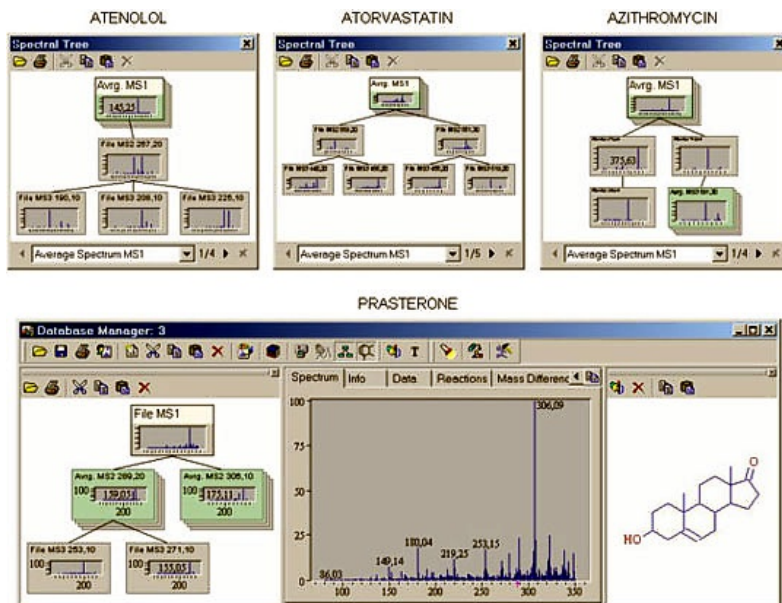
**9<sup>th</sup> Edition:**

663,000 EI spectra(70 eV)

*NIST 05 installed on open access GC/MS*

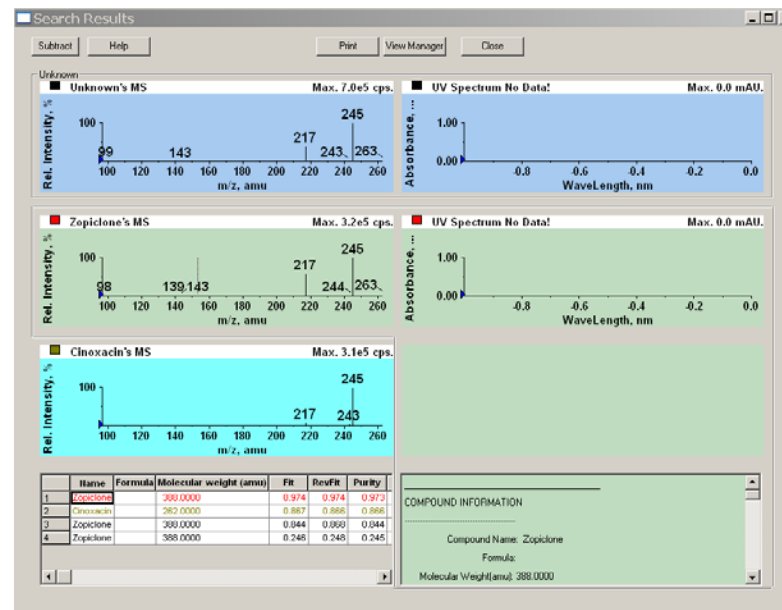
# Libraries of ESI MS/MS mass spectra

## HighChem's spectral library collection



MS/MS spectra > 1000 compounds  
Drugs, natural products

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



MS/MS spectra ~ 800  
compounds, drugs



<http://www.massbank.jp>

6.

## Solving fragmentation spectra

## Fragmentation of ions with even number of electrons (EE<sup>+</sup>)

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

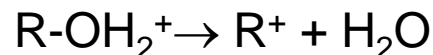
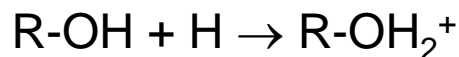
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## Fragmentation of EE<sup>+</sup>

1/ CID (MS/MS) of EE<sup>+</sup> ([M+H]<sup>+</sup>, [M+Na]<sup>+</sup>, [M-H]<sup>-</sup>) formed by ESI

2/ fragmentation of ([M+H]<sup>+</sup>, [M+Na]<sup>+</sup>, [M-H]<sup>-</sup>) during APCI, APPI

Cleavage of neighboring bond to the charge site, charge migration



FRAGMENTATION of EE<sup>+</sup>:

The fragments are EE<sup>+</sup> and a neutral fragment (not seen in the spectra)



EE<sup>+</sup> ions are more stable than OE<sup>+</sup>

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

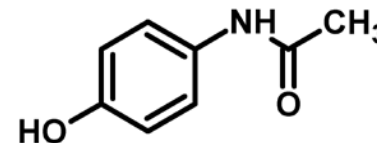
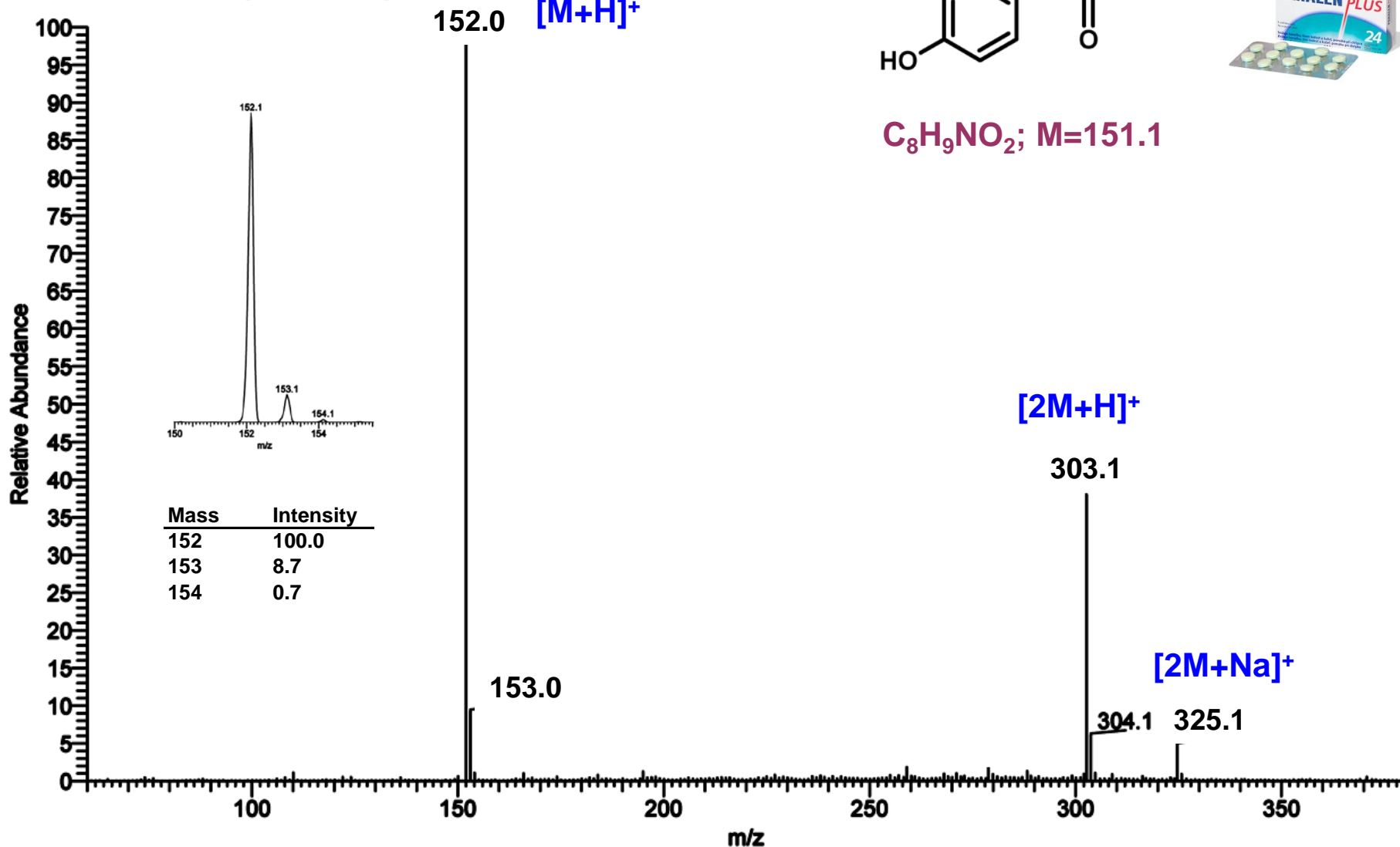
### **Typical logical neutral losses:**

- 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

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# Paracetamol N-(4-hydroxyphenyl)acetamide

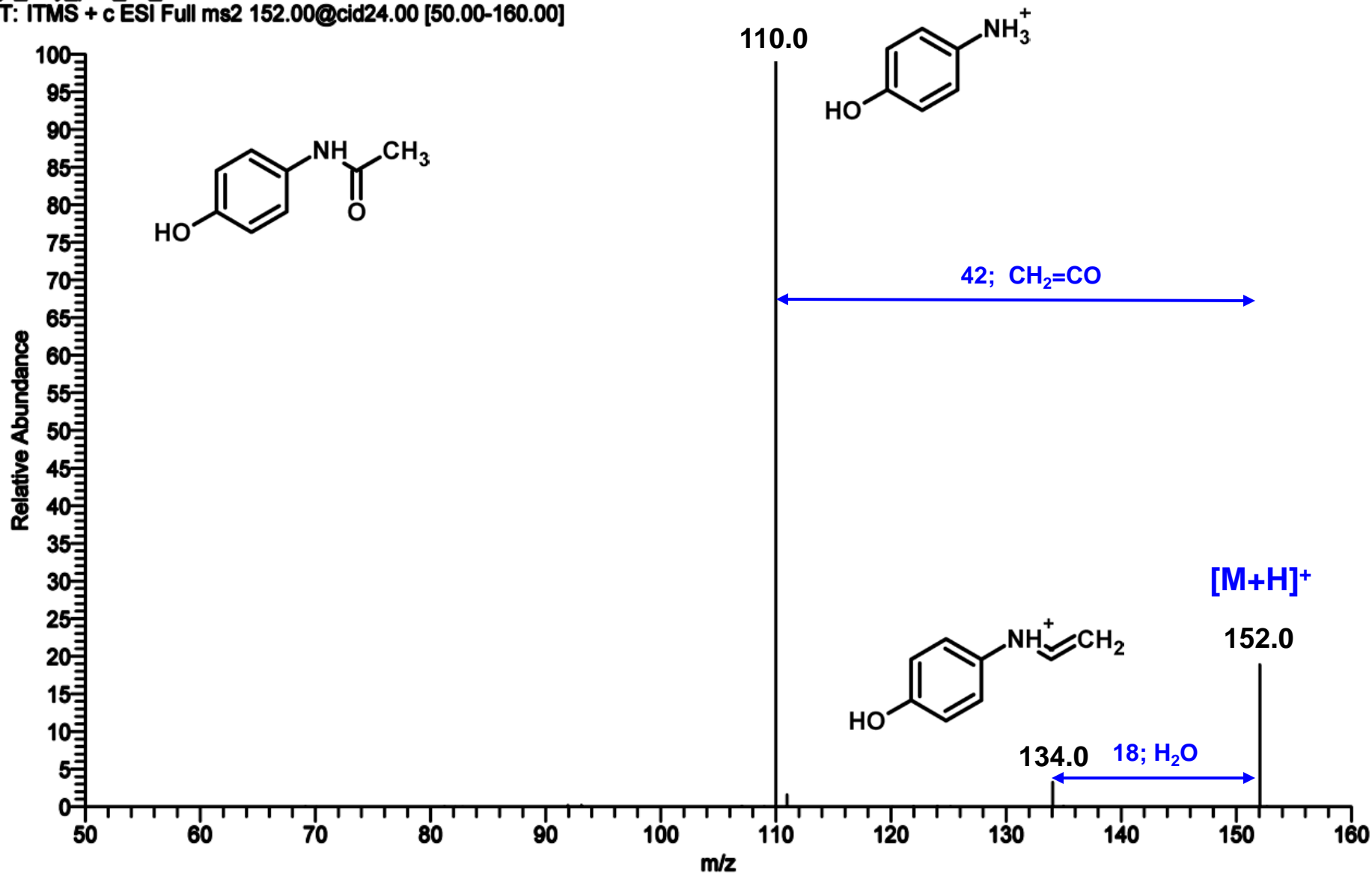
**ESI+, MS**JC\_LEKY\_Pan\_04\_080720211508 #1-22 RT: 0.00-0.25 AV: 22 NL: 2.40E4  
T: ITMS + c ESI Full ms [50.00-500.00] $C_8H_9NO_2$ ;  $M=151.1$ 



# Paracetamol N-(4-hydroxyphenyl)acetamide

**ESI+, MS/MS**

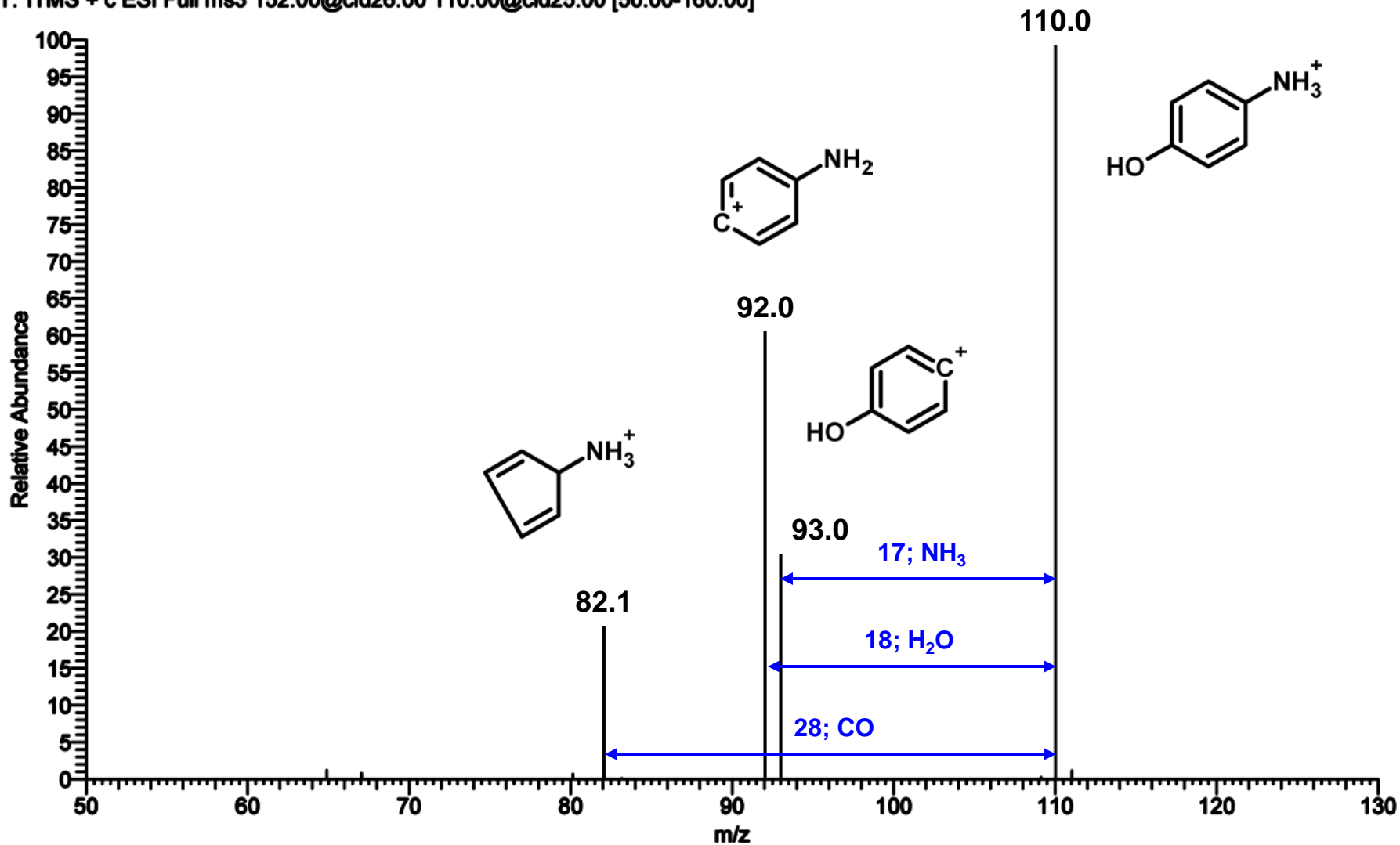
jc\_leky\_pan\_03\_080720211358 #1-36 RT: 0.00-0.24 AV: 35 NL: 1.07E3  
T: ITMS + c ESI Full ms2 152.00@cid24.00 [50.00-160.00]



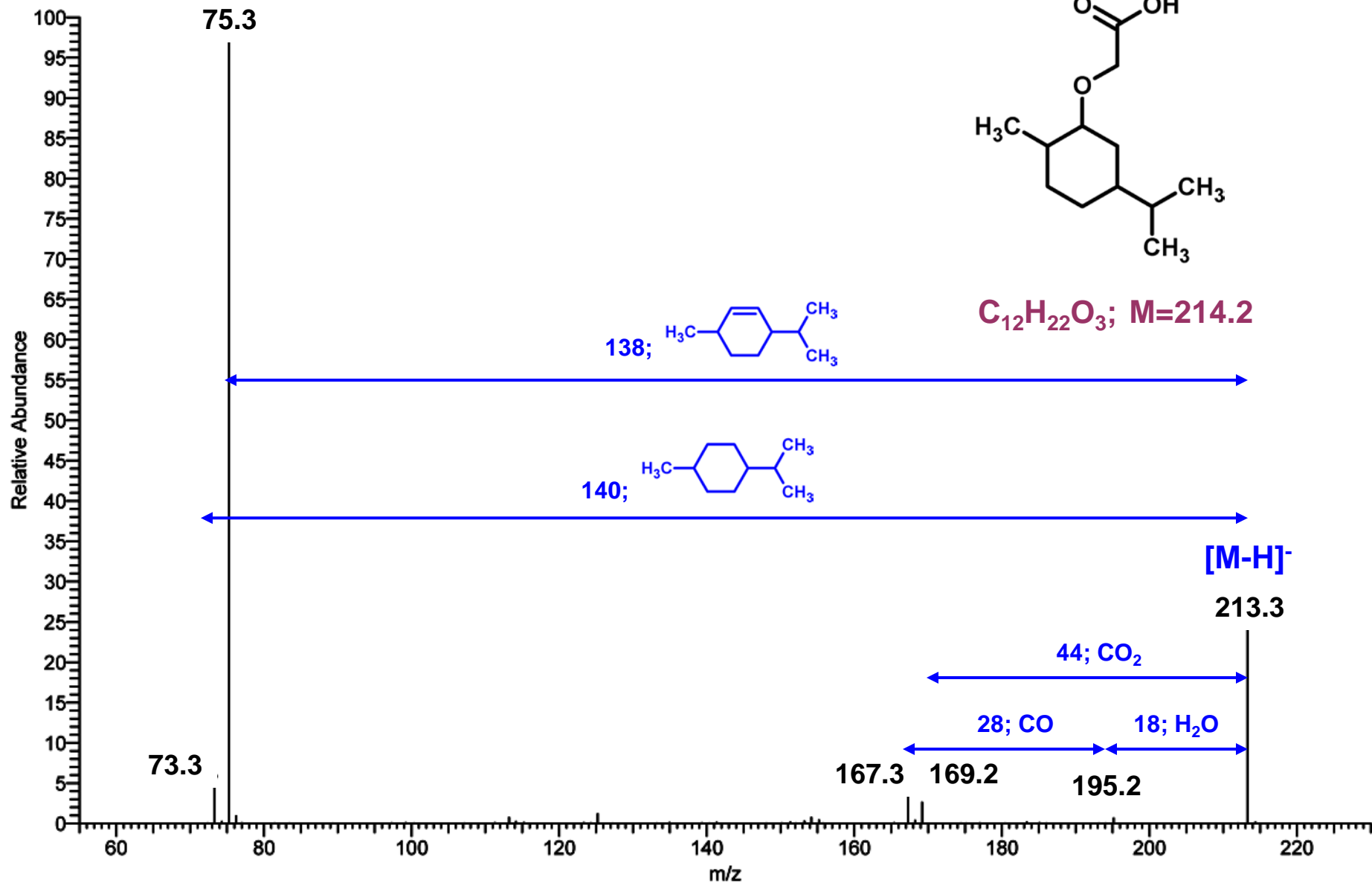
# Paracetamol N-(4-hydroxyphenyl)acetamide

**ESI+, MS<sup>3</sup>**

JC\_LEKY\_Pan\_18 #1-464 RT: 0.00-2.00 AV: 464 NL: 1.12E1  
T: ITMS + c ESI Full ms3 152.00@cid26.00 110.00@cid25.00 [50.00-160.00]



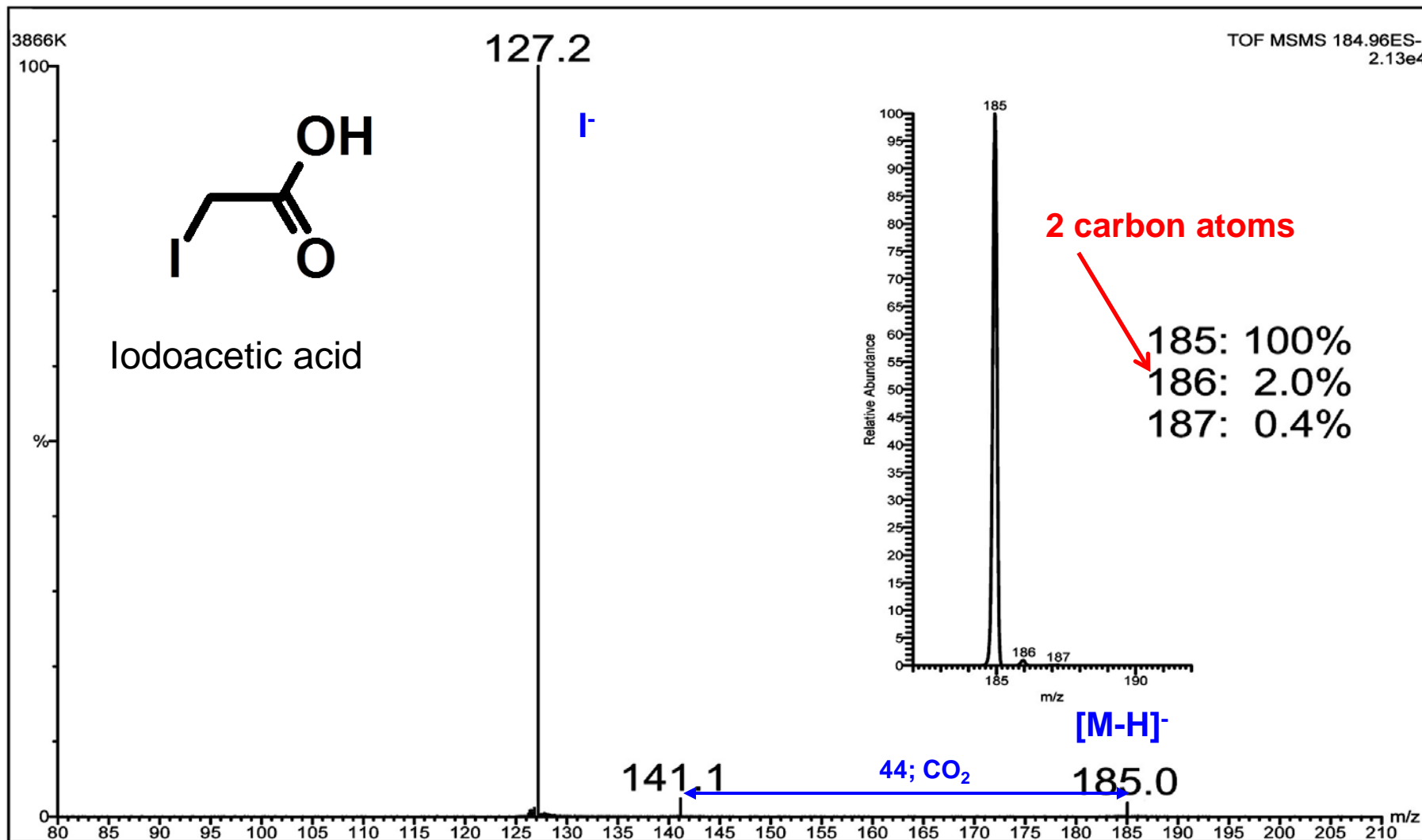
# Menthylacetic acid

**ESI-, MS/MS**2789jc\_090716170932 #1-149 RT: 0.00-2.00 AV: 149 NL: 9.82E1  
T: ITMS - c ESI Full ms2 213.40@cid29.00 [55.00-250.00]

# Unknown

**ESI-, MS/MS**

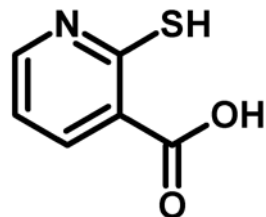
What is the structure?

MS/MS  $m/z$  185

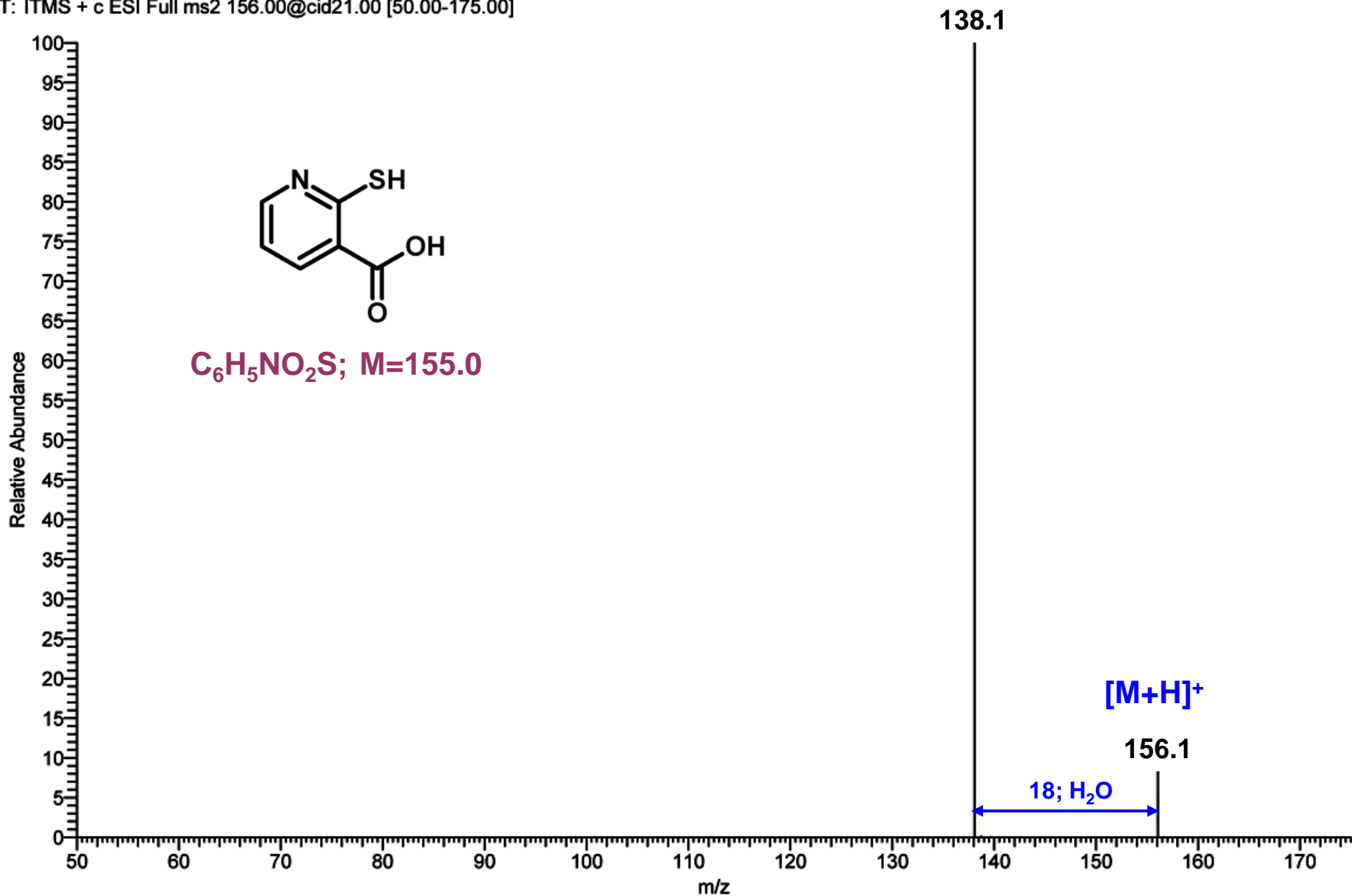
# 2-Mercaptopyridine-3-carboxylic acid

**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]



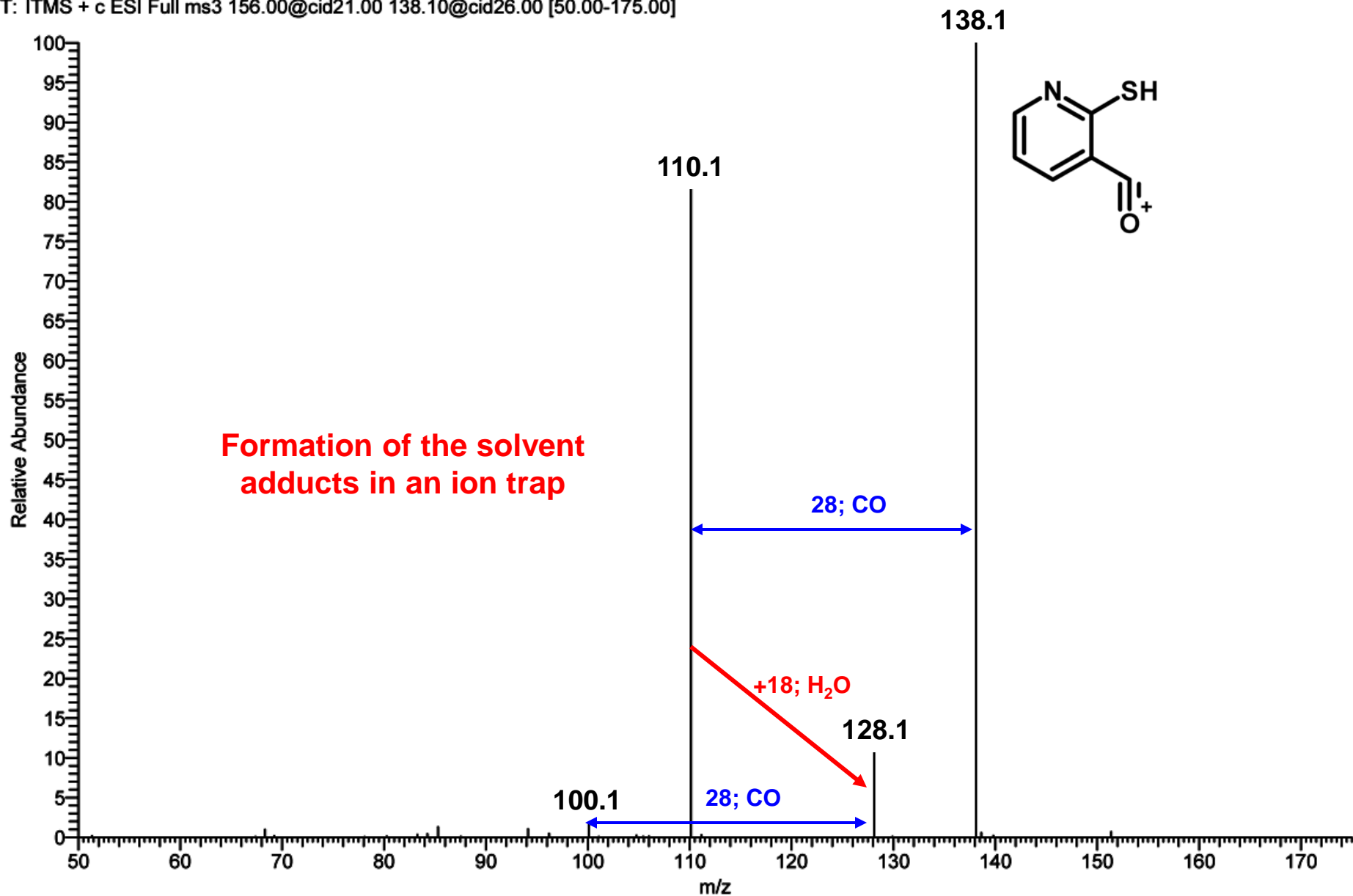
$C_6H_5NO_2S$ ;  $M=155.0$



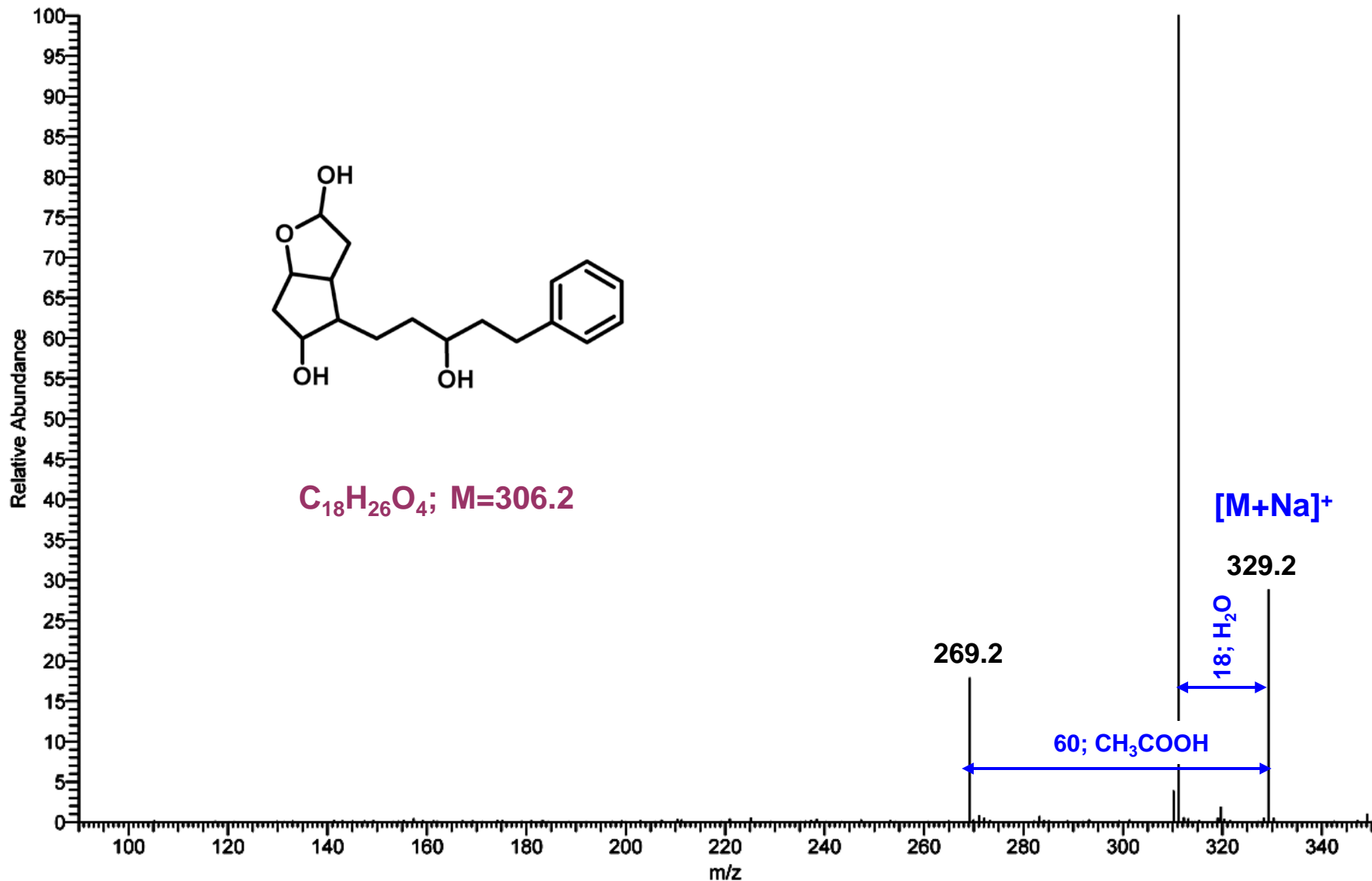
# 2-Mercaptonicotinic acid

**ESI+, MS<sup>3</sup>**

2787jc #1-230 RT: 0.00-2.01 AV: 230 NL: 1.73E1  
T: ITMS + c ESI Full ms3 156.00@cid21.00 138.10@cid26.00 [50.00-175.00]



# 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]

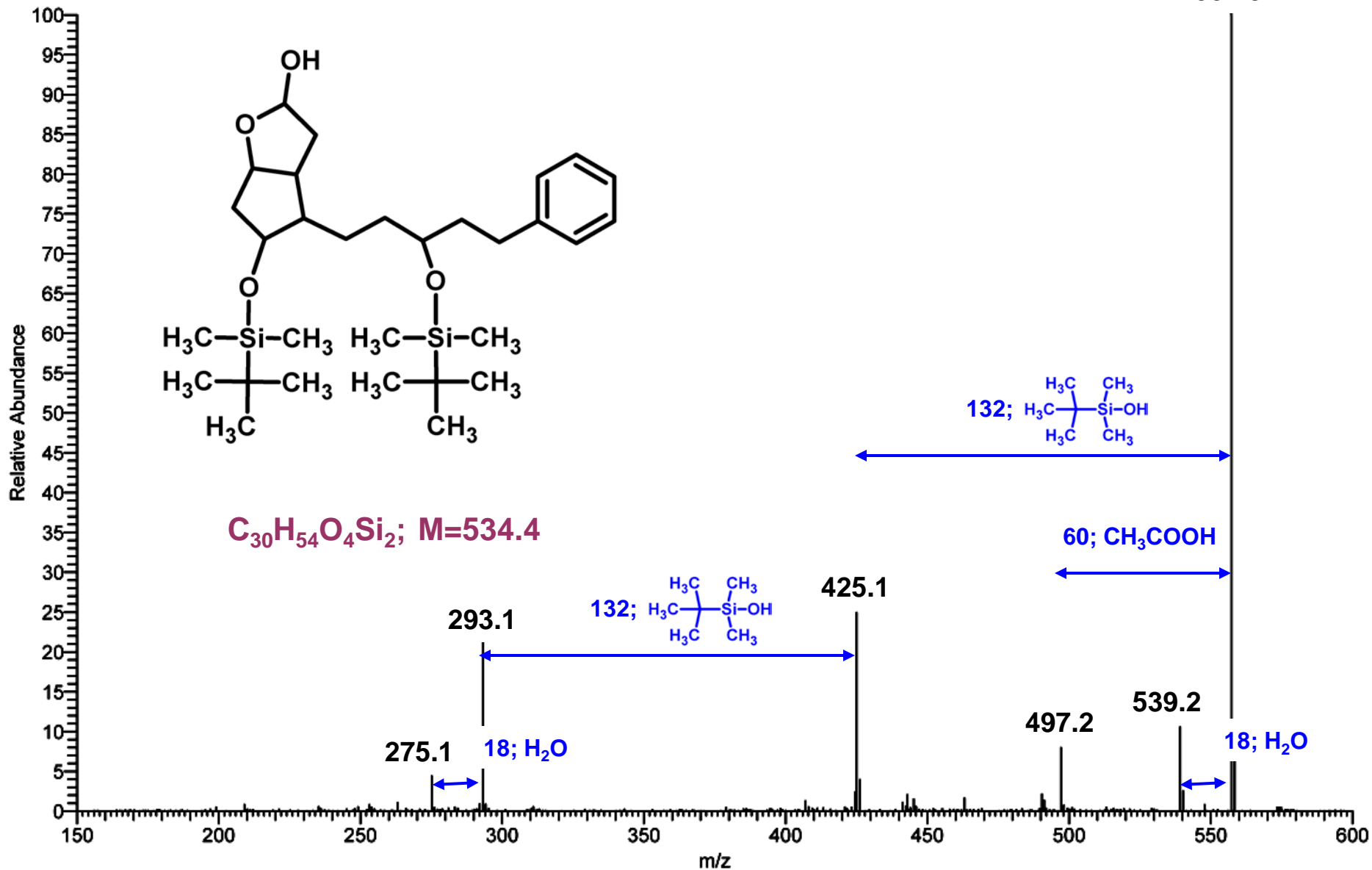
# Cyclopentafuranol-derivative, disil

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]

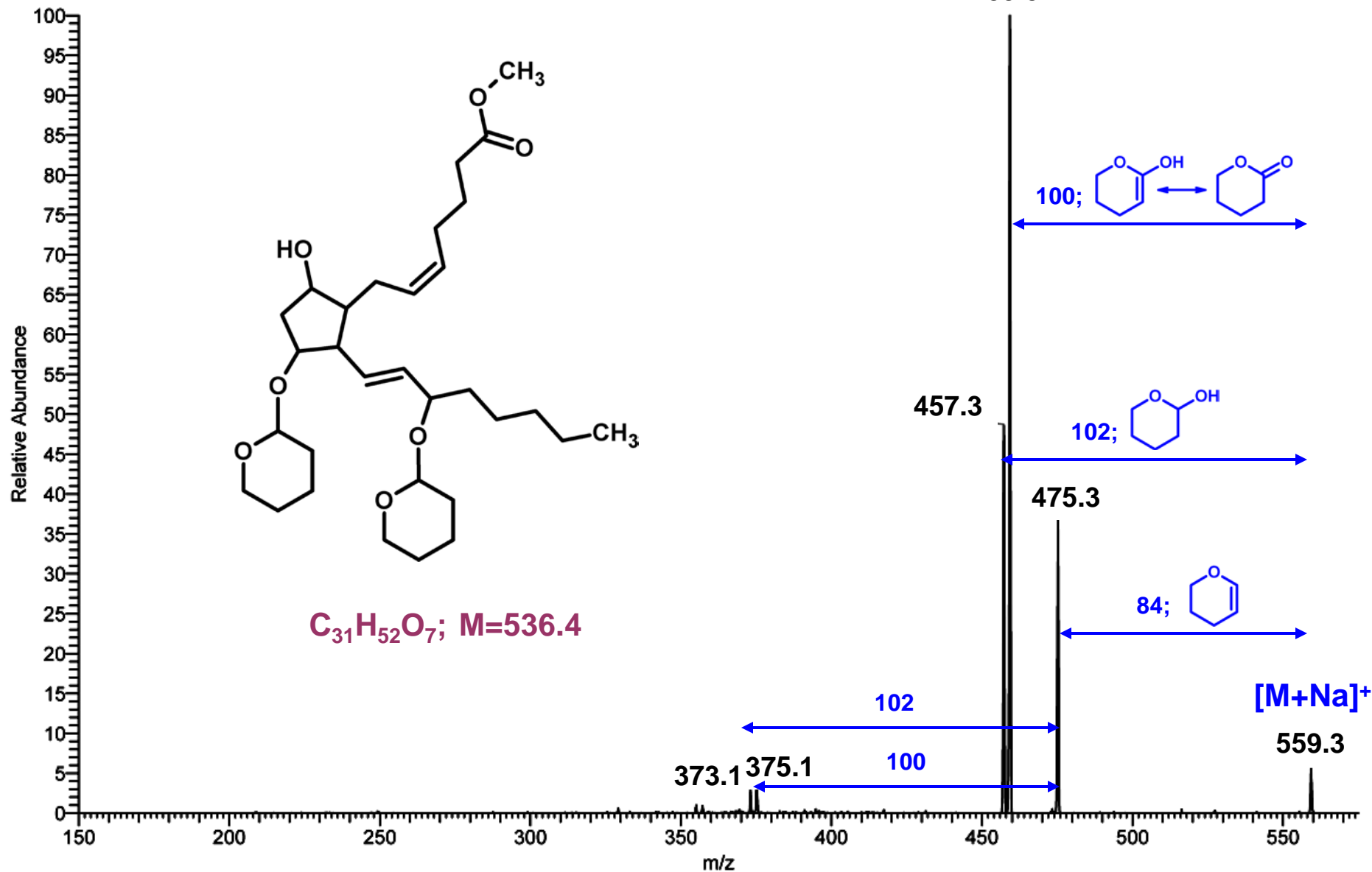




**PGF<sub>2a</sub>-methylester, diTHP****ESI+, MS/MS**

3378jc #1-64 RT: 0.01-0.99 AV: 64 NL: 8.64E5

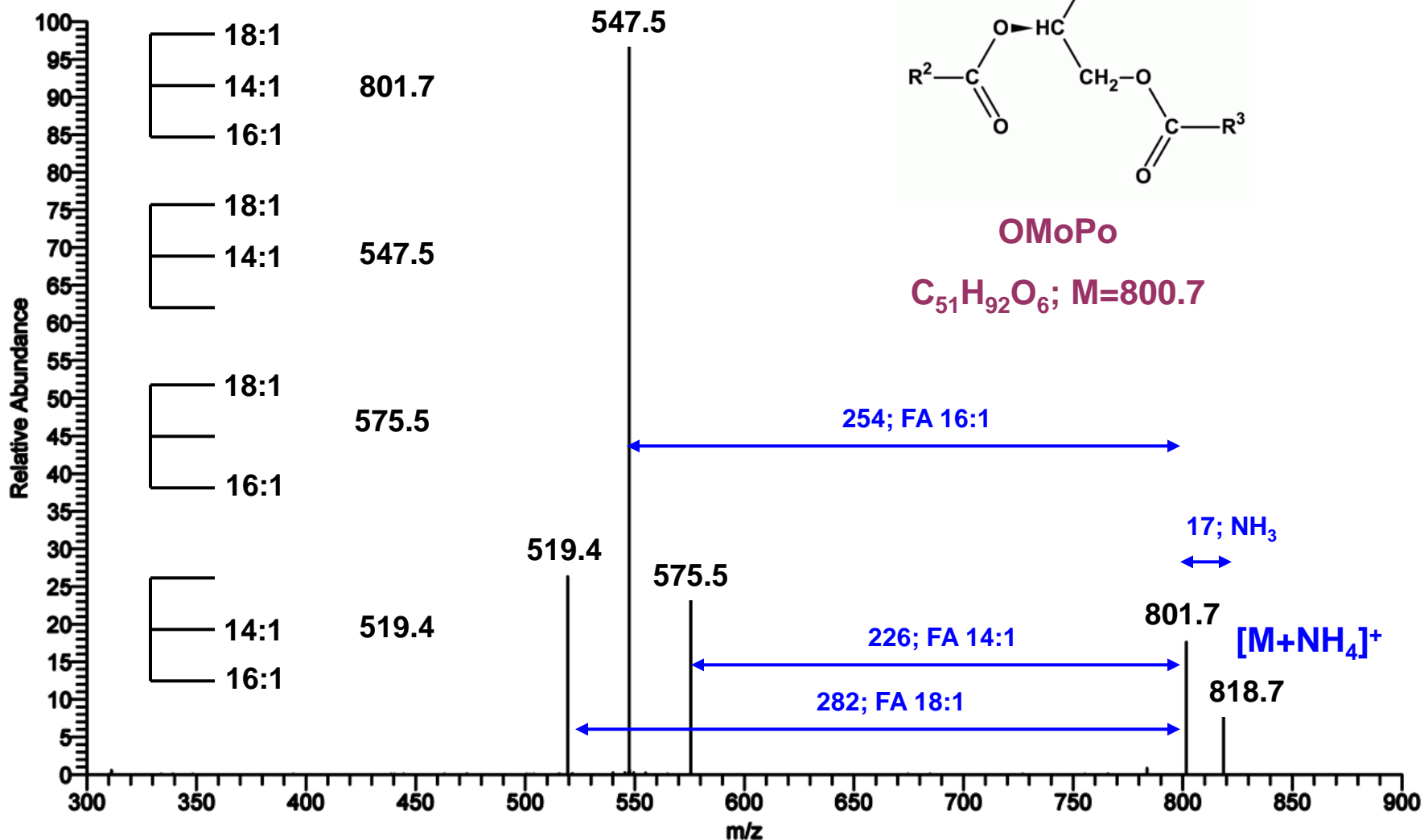
T: + p Full ms2 559.40@cid37.00 [150.00-600.00]



# 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]



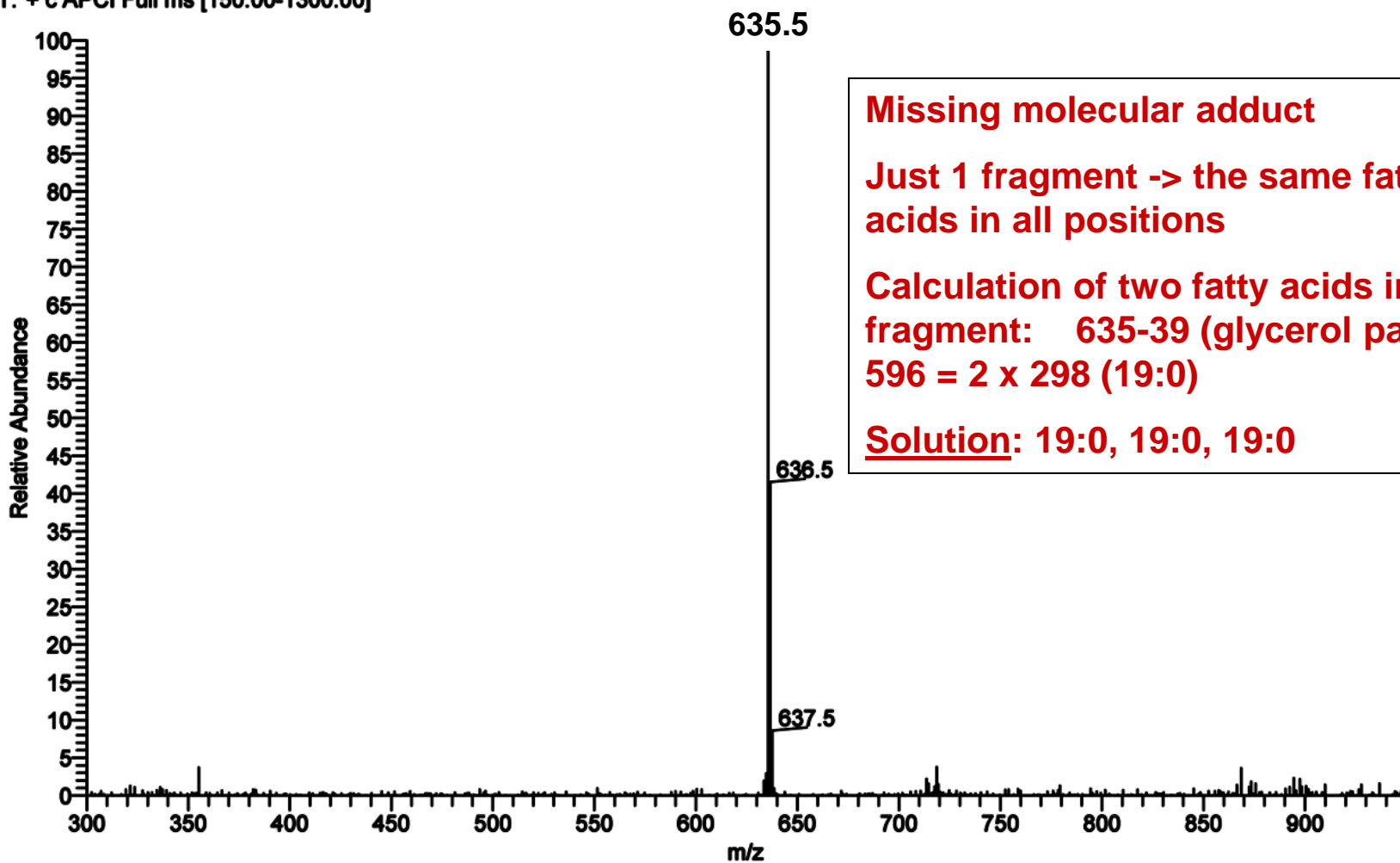
# Triacylglycerols



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**

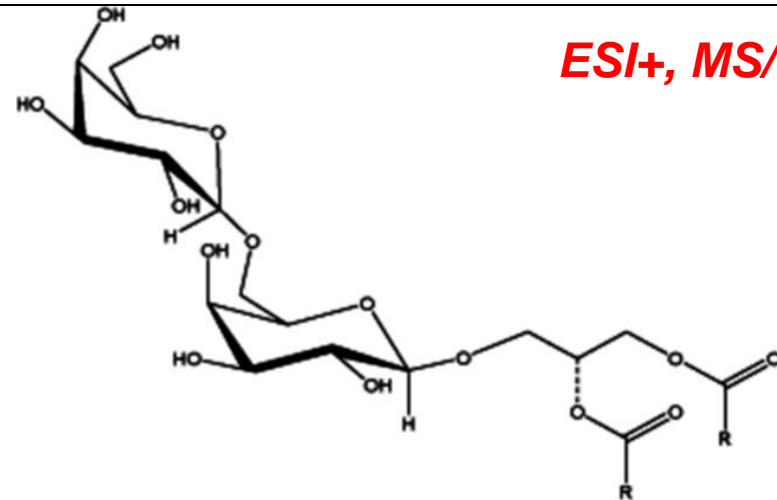
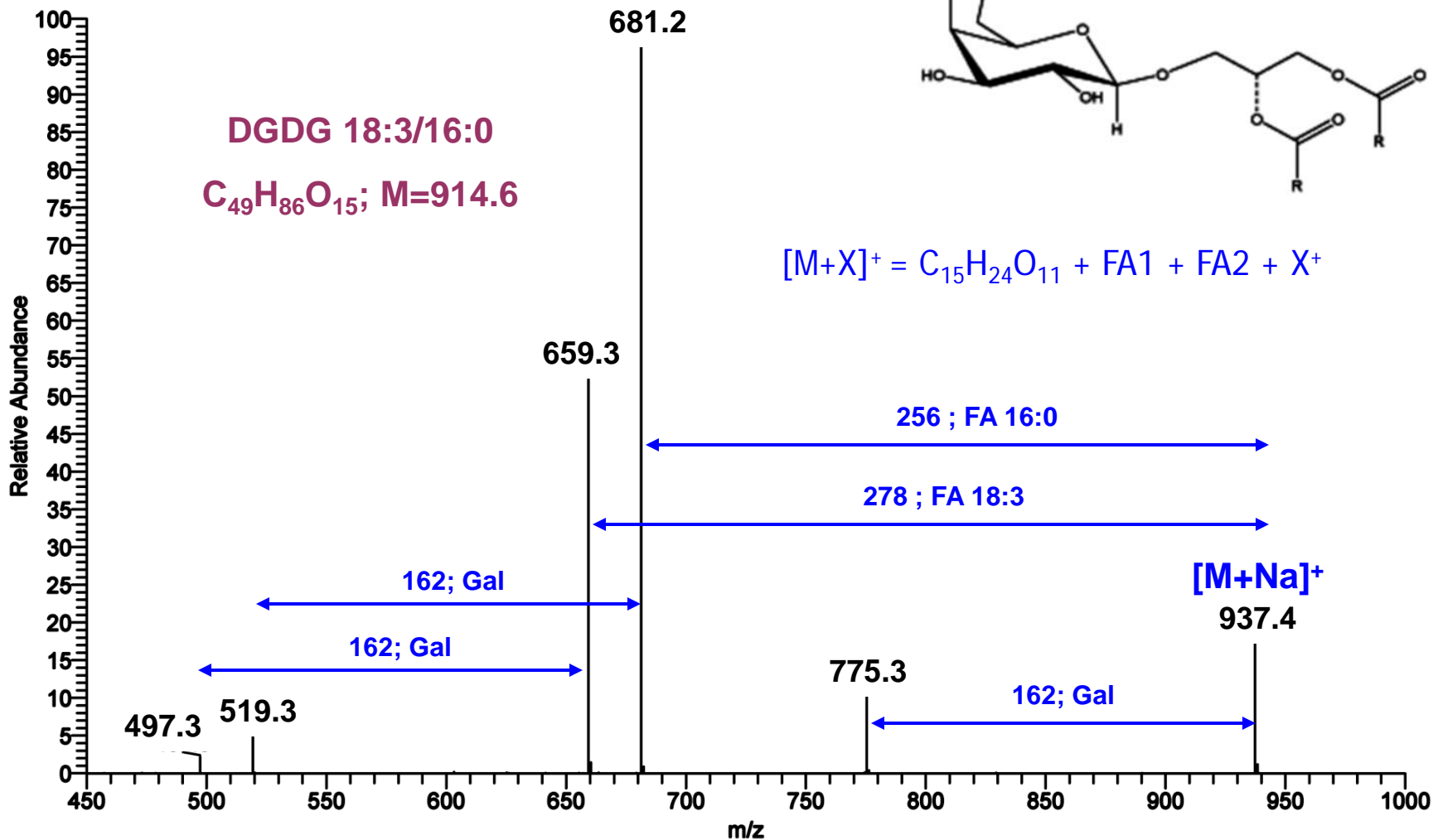
**Calculation of two fatty acids in the fragment:  $635 - 39$  (glycerol part) =  $596 = 2 \times 298$  (19:0)**

**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]





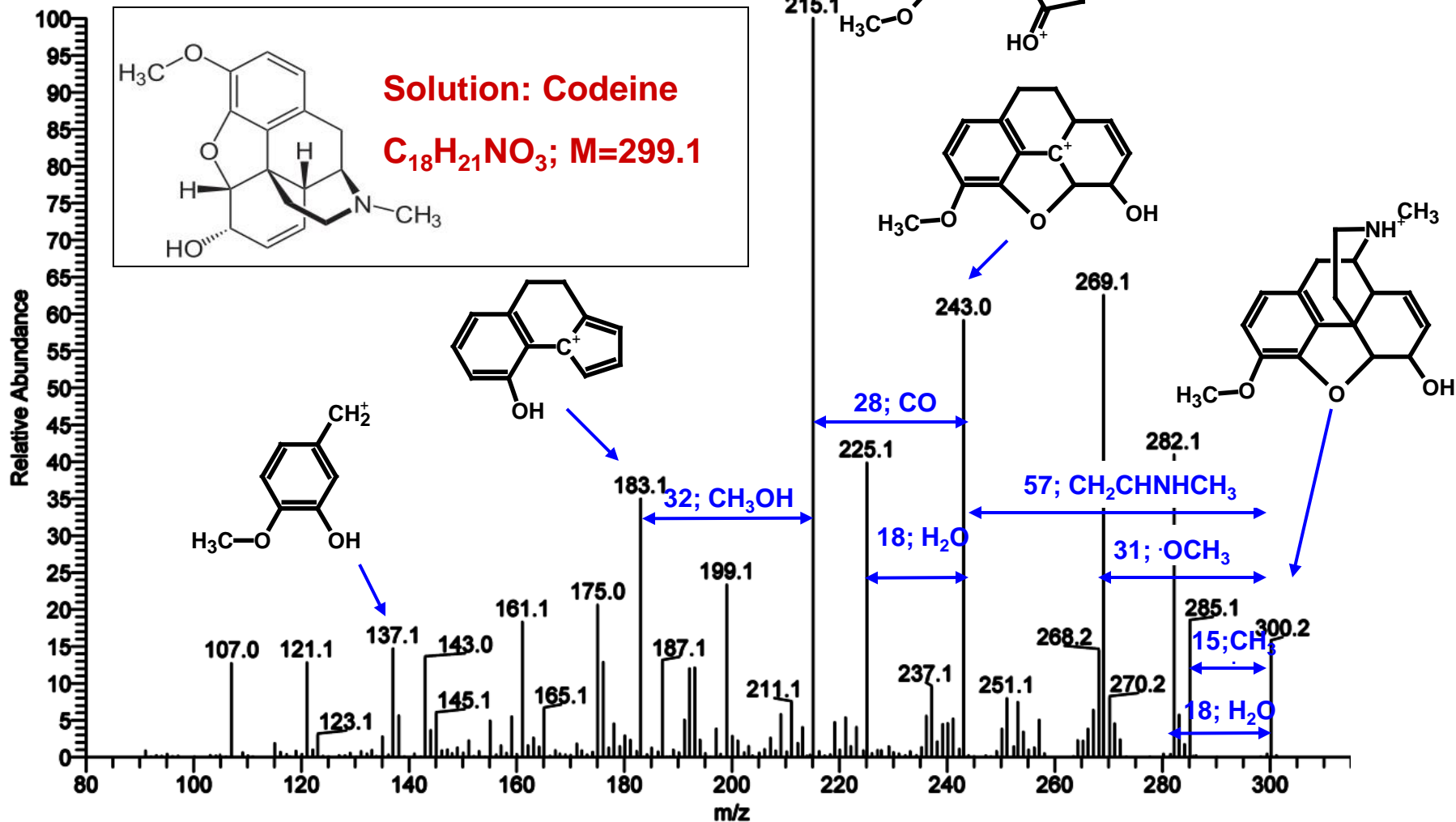
# Unknown



What is the structure?

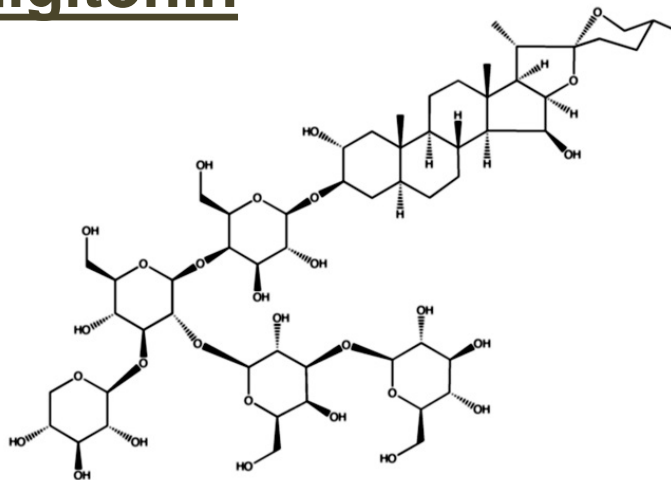
**ESI+, MS/MS**

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]

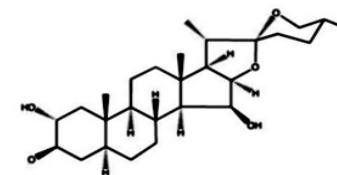


# Glycosides - digitonin

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



xyl - gluc - gal  
 |  
 gluc - gal



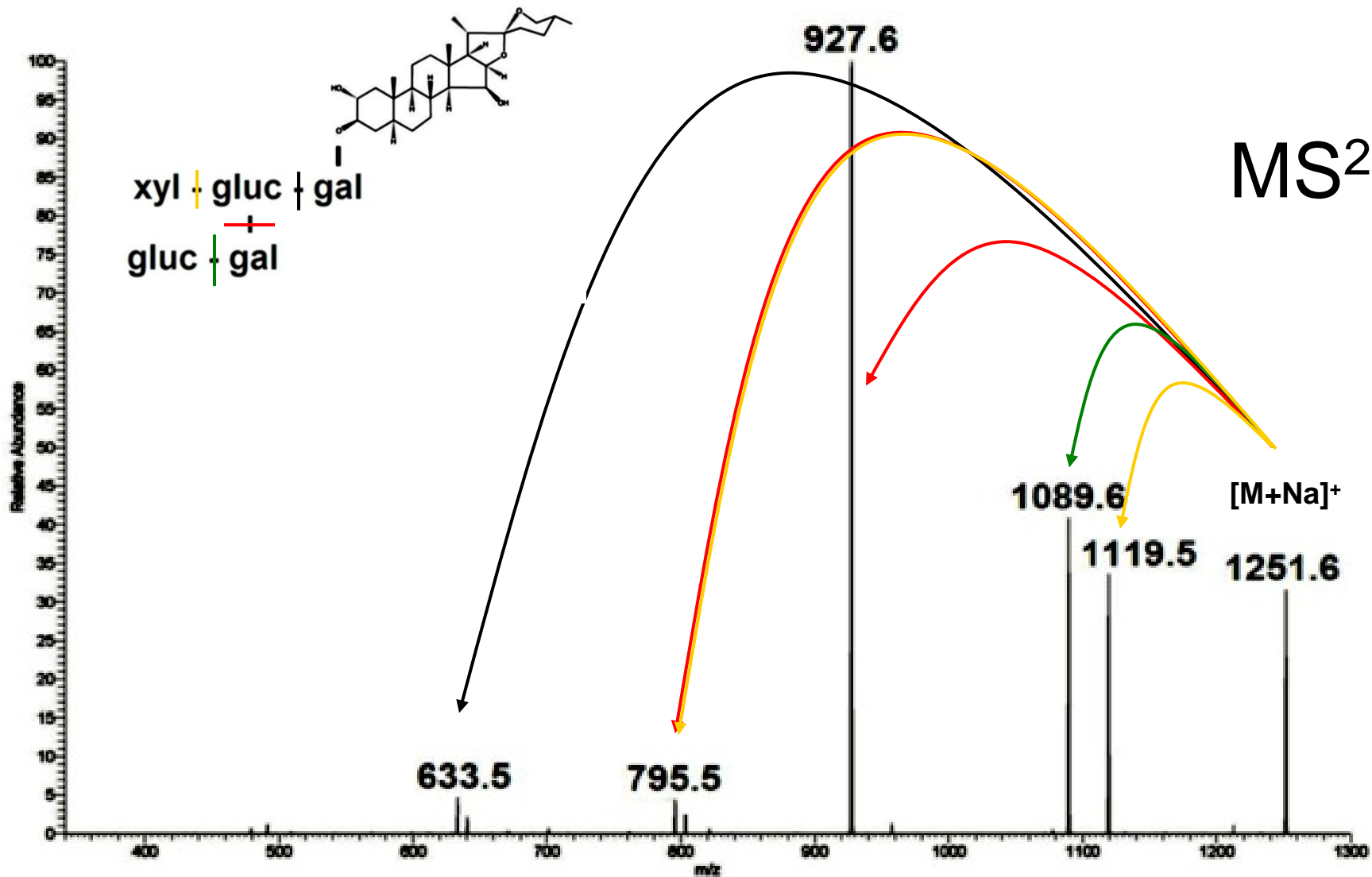
ESI ionization – formation of the molecular adduct  $[M+Na]^+$

MS/MS fragmentation – cleavage by elimination of neutral sugars

Hexose: loss of **162 u** (the molecule –  $H_2O$ )

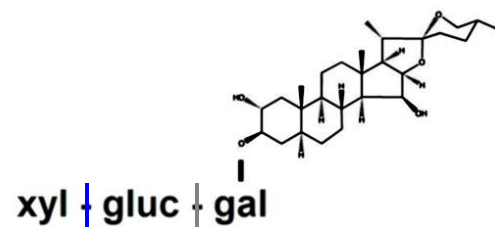
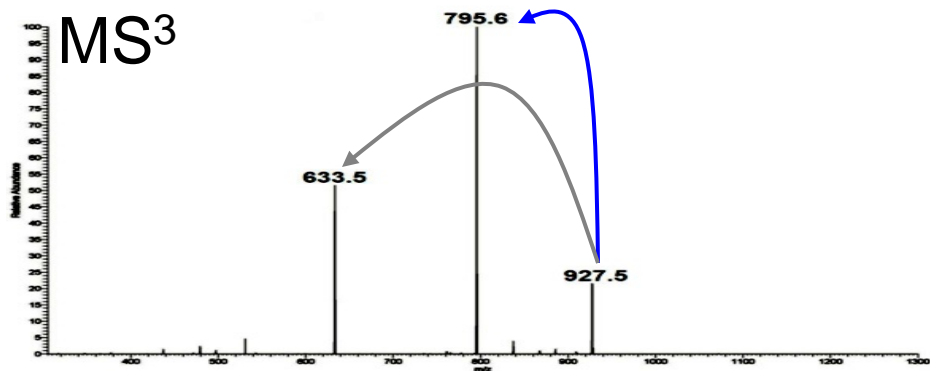
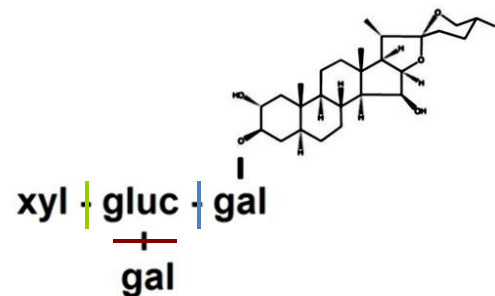
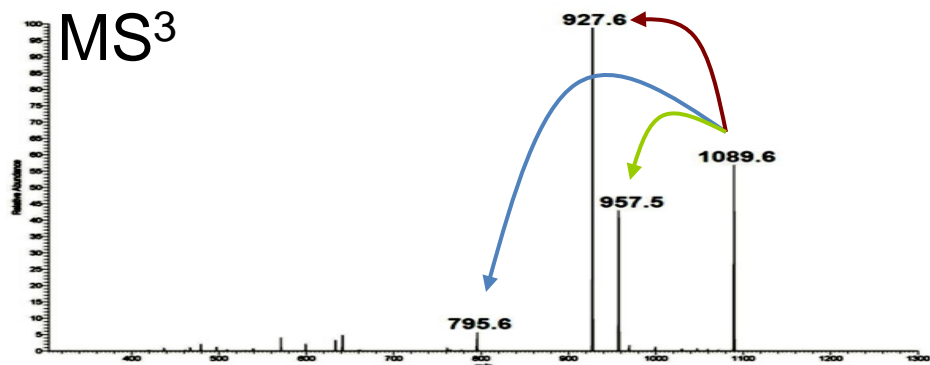
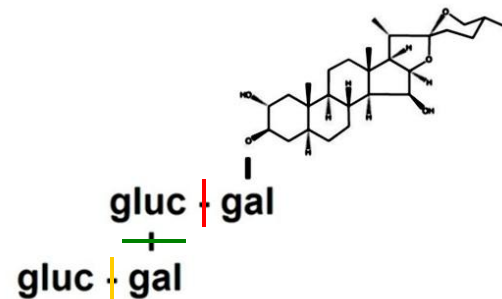
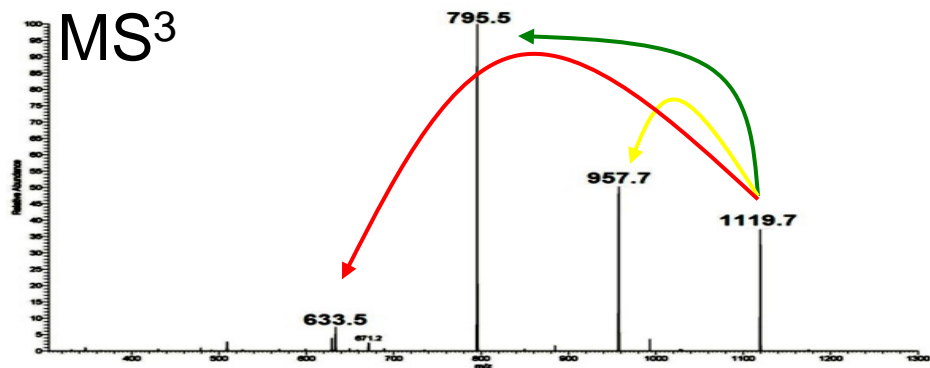
Pentose: loss of **132 u** (the molecule –  $H_2O$ )

# Glycosides - digitonin





# Glycosides - digitonin



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

**EI**

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## Fragmentation of OE<sup>+•</sup>

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

### FRAGMENTATION of OE<sup>+•</sup>

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



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



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

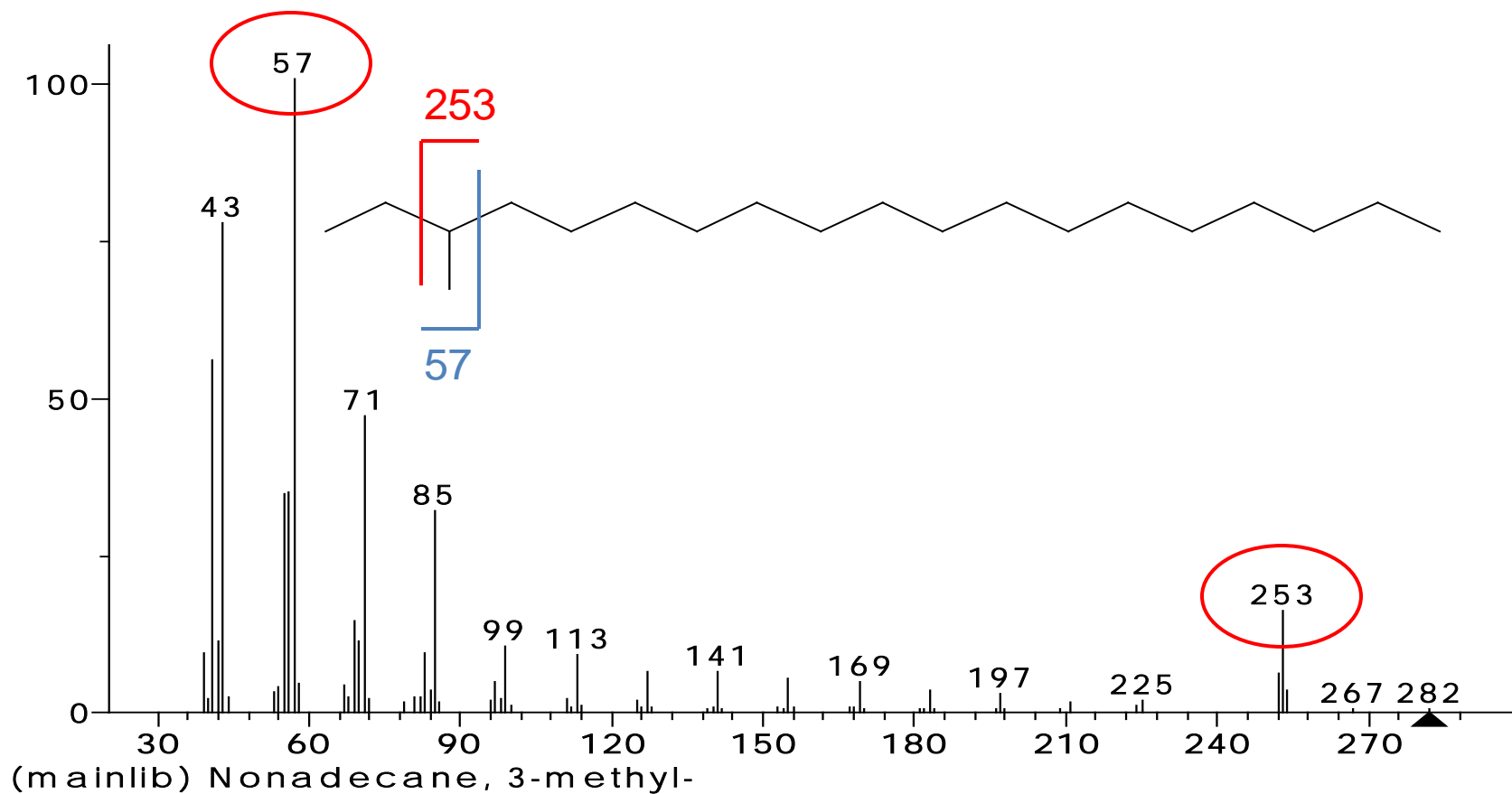
Only monomolecular reactions take place

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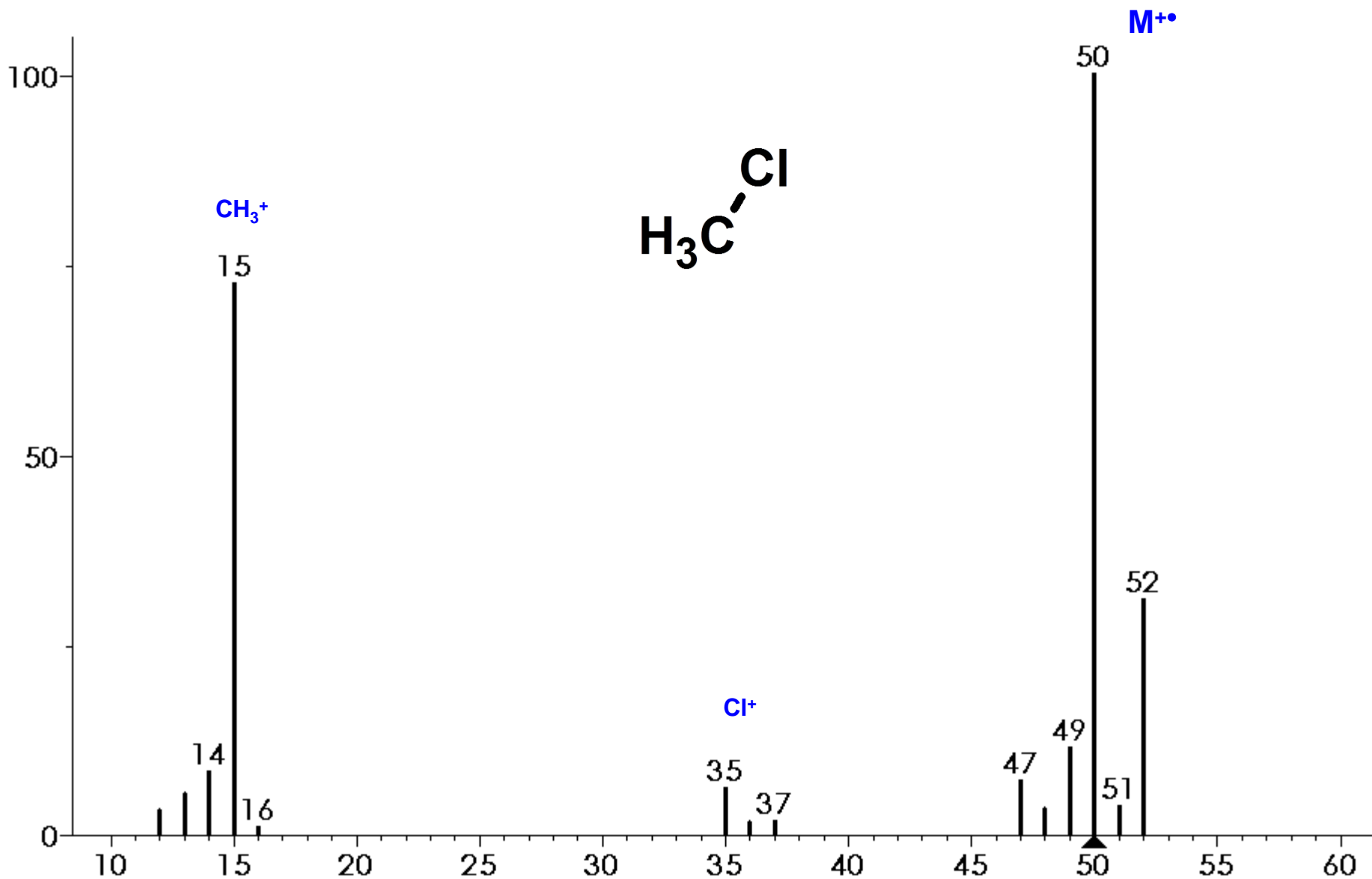


## Branched hydrocarbons

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



# Chloromethane

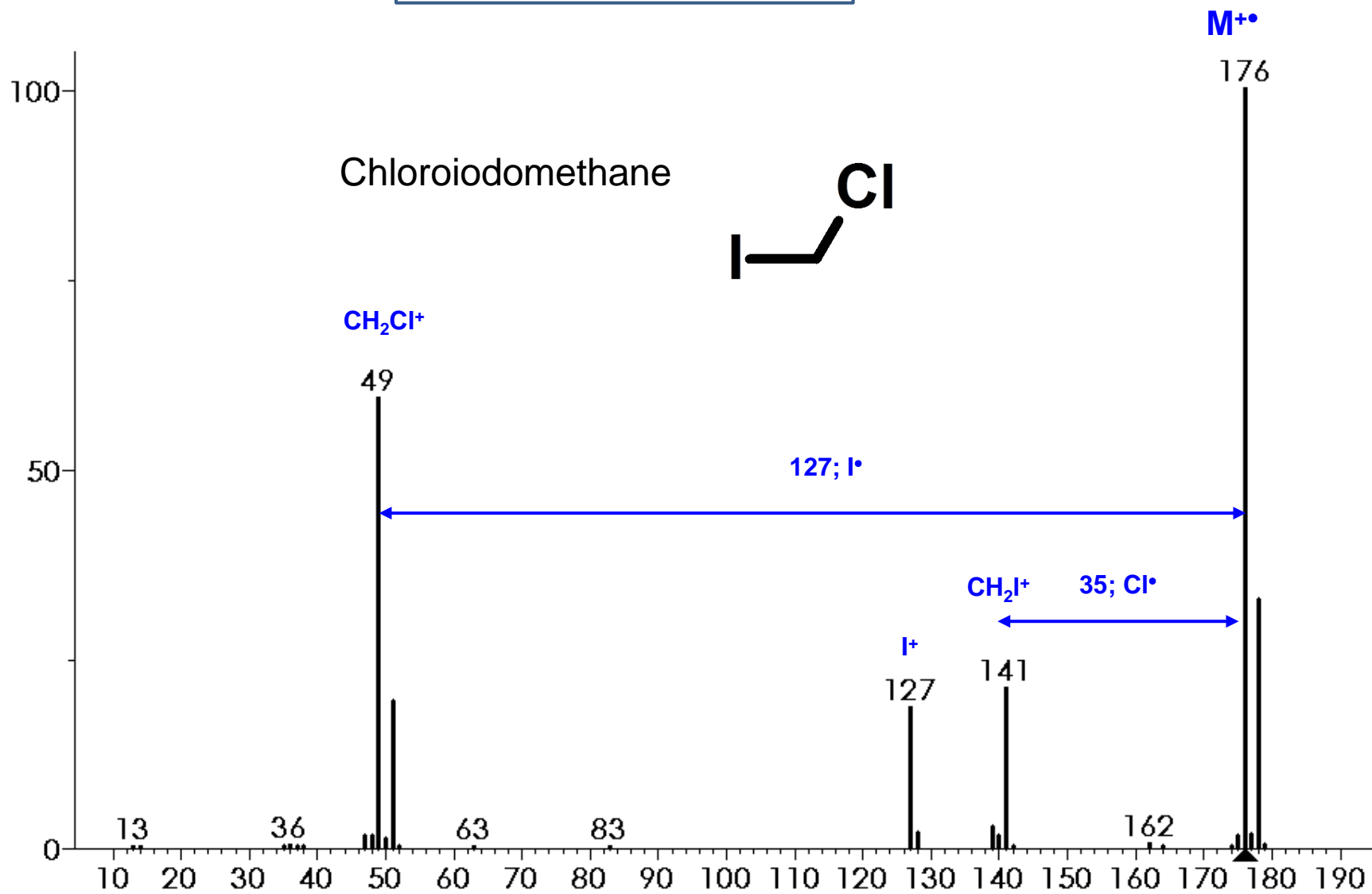
*EI*

# Unknown



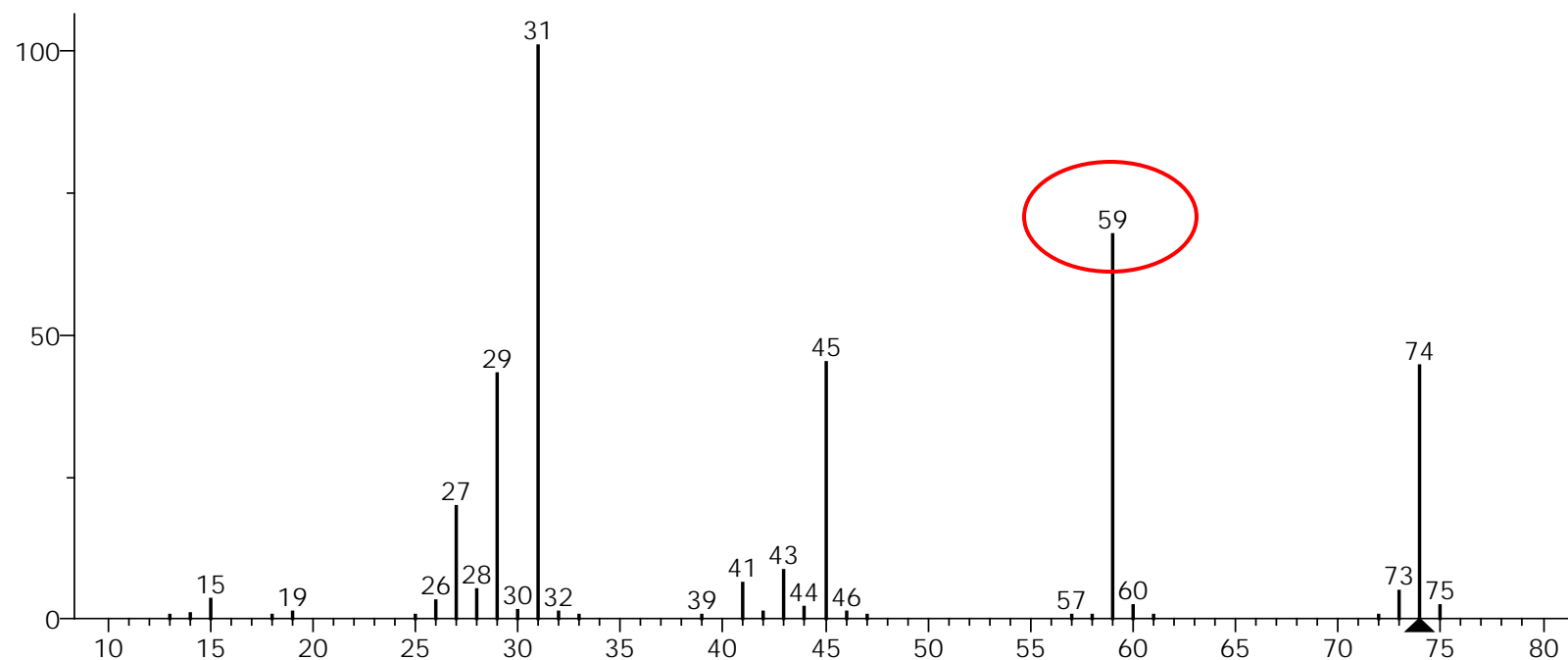
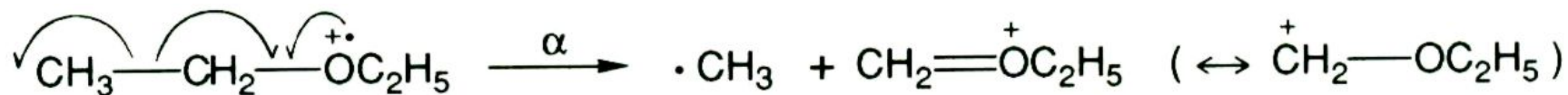
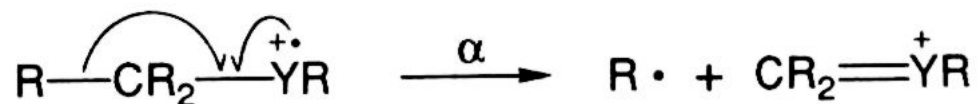
What is the structure?

EI



## $\alpha$ -cleavage: fragmentation initiated by radical site

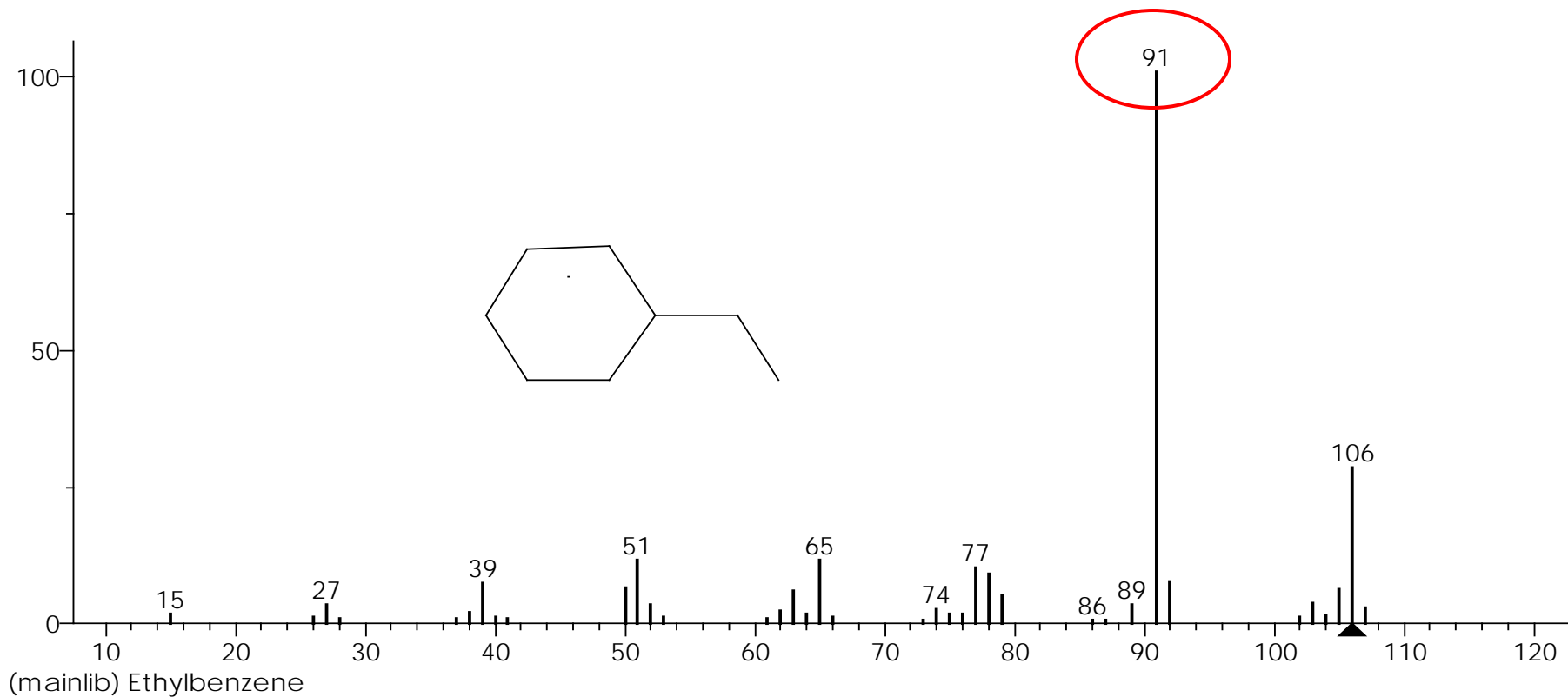
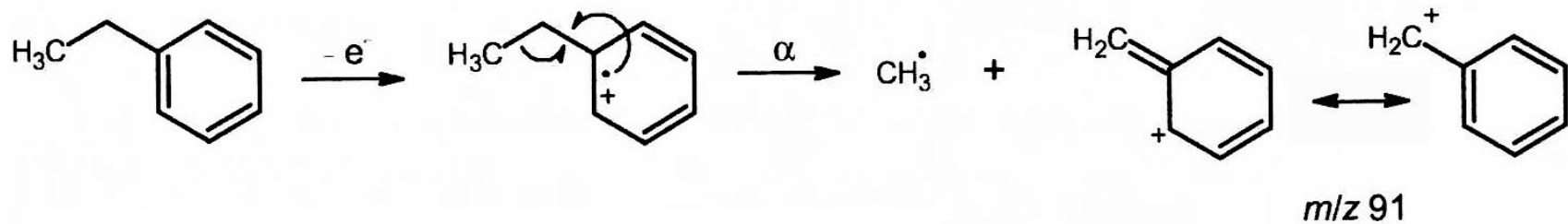
- cleavage induced by a strong tendency of electrons to form pairs – the odd electron is provided for the creation of a new bond; the neighboring bond is cleaved



(mainlib) Ethyl ether

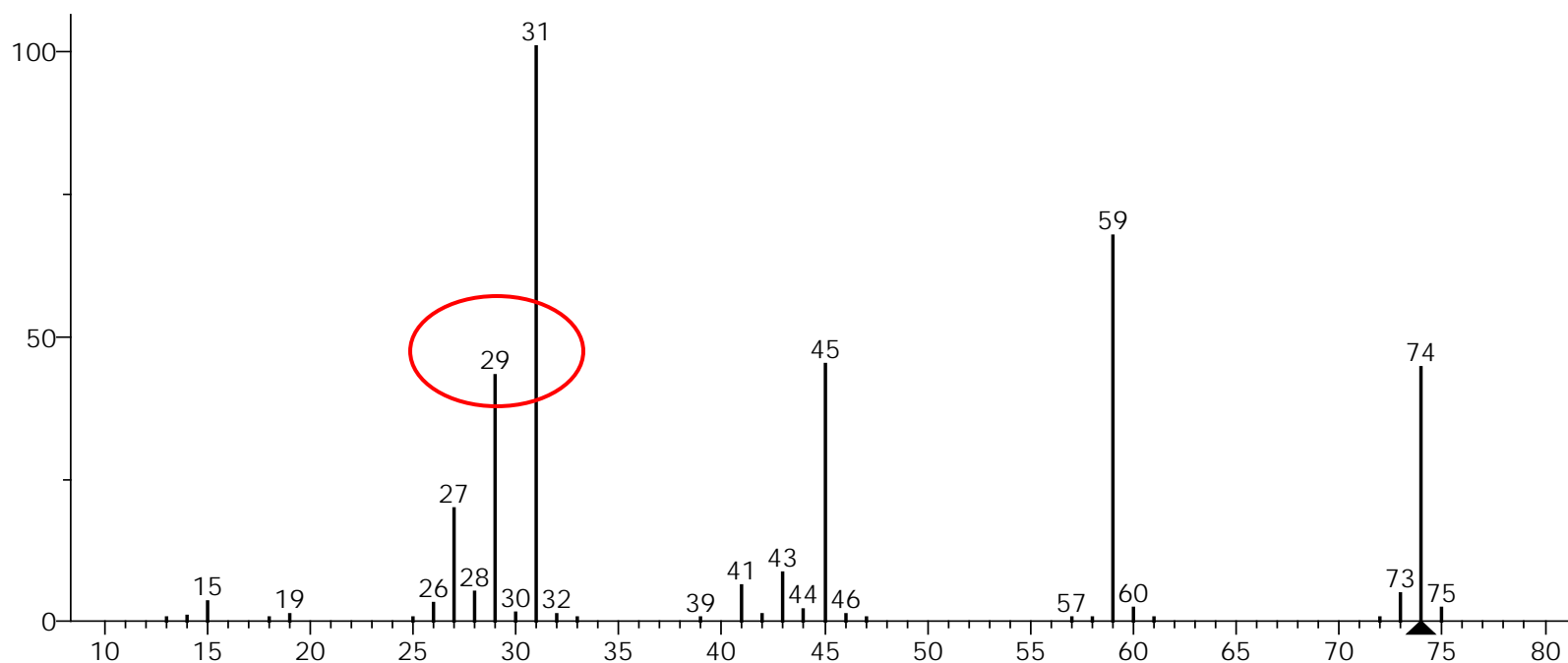
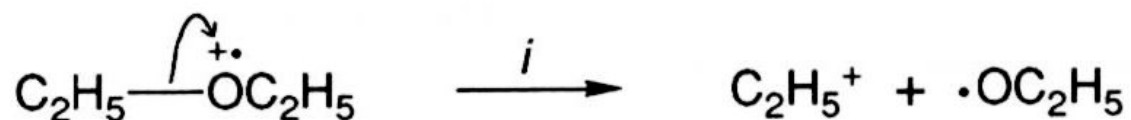
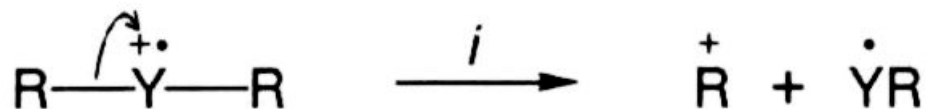


## $\alpha$ -cleavage: benzylic cleavage



## Inductive cleavage: fragmentation caused by a charge

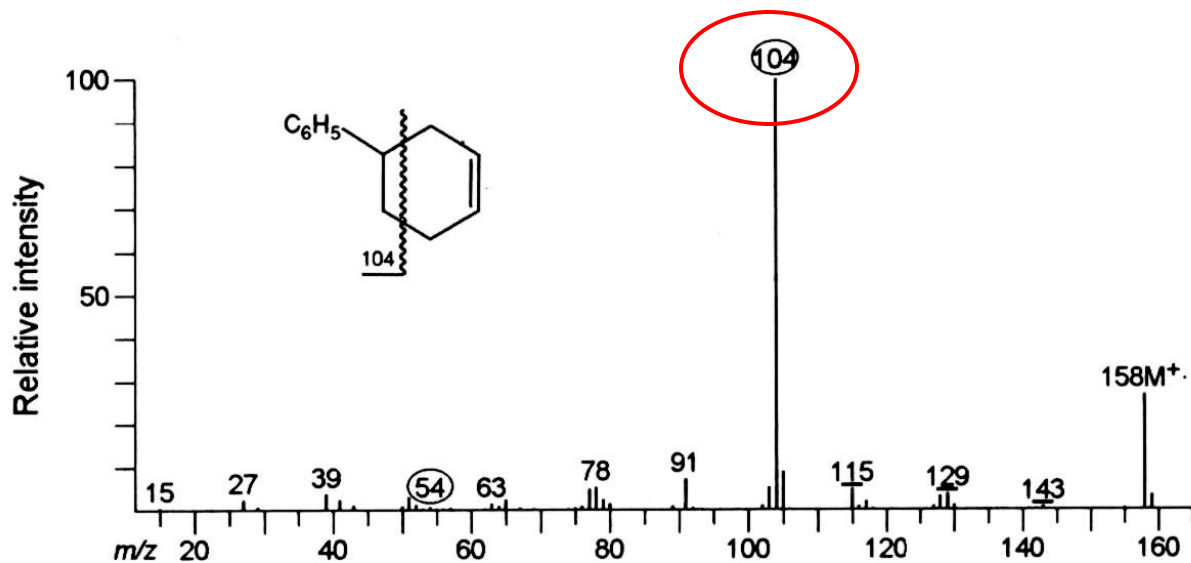
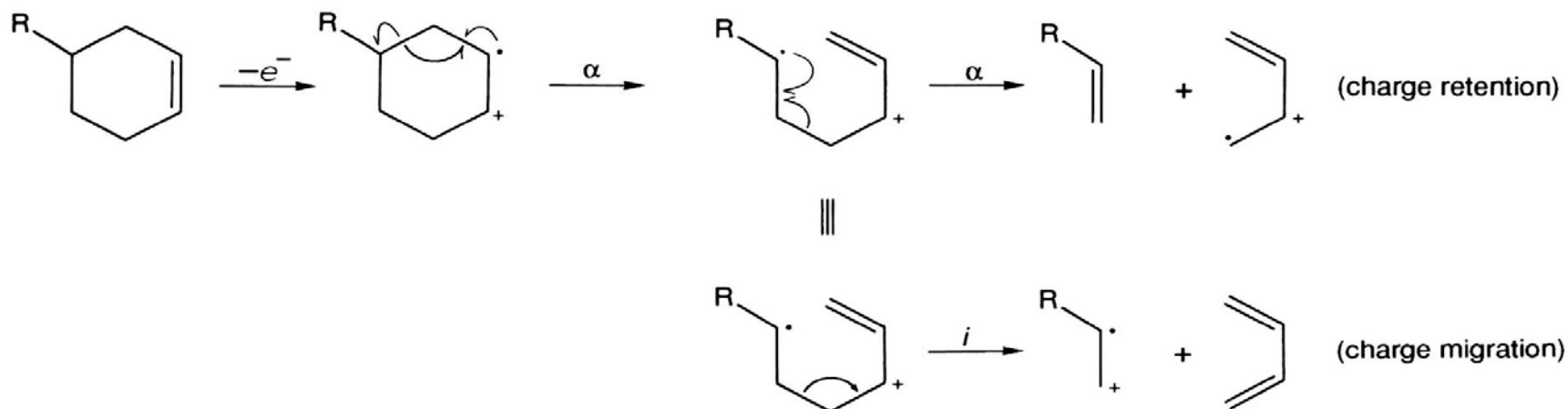
- cleavage initialized by attraction of an electron pair by the charge



(mainlib) Ethyl ether

# Fragmentation of cyclic structures – retro Diels-Alder

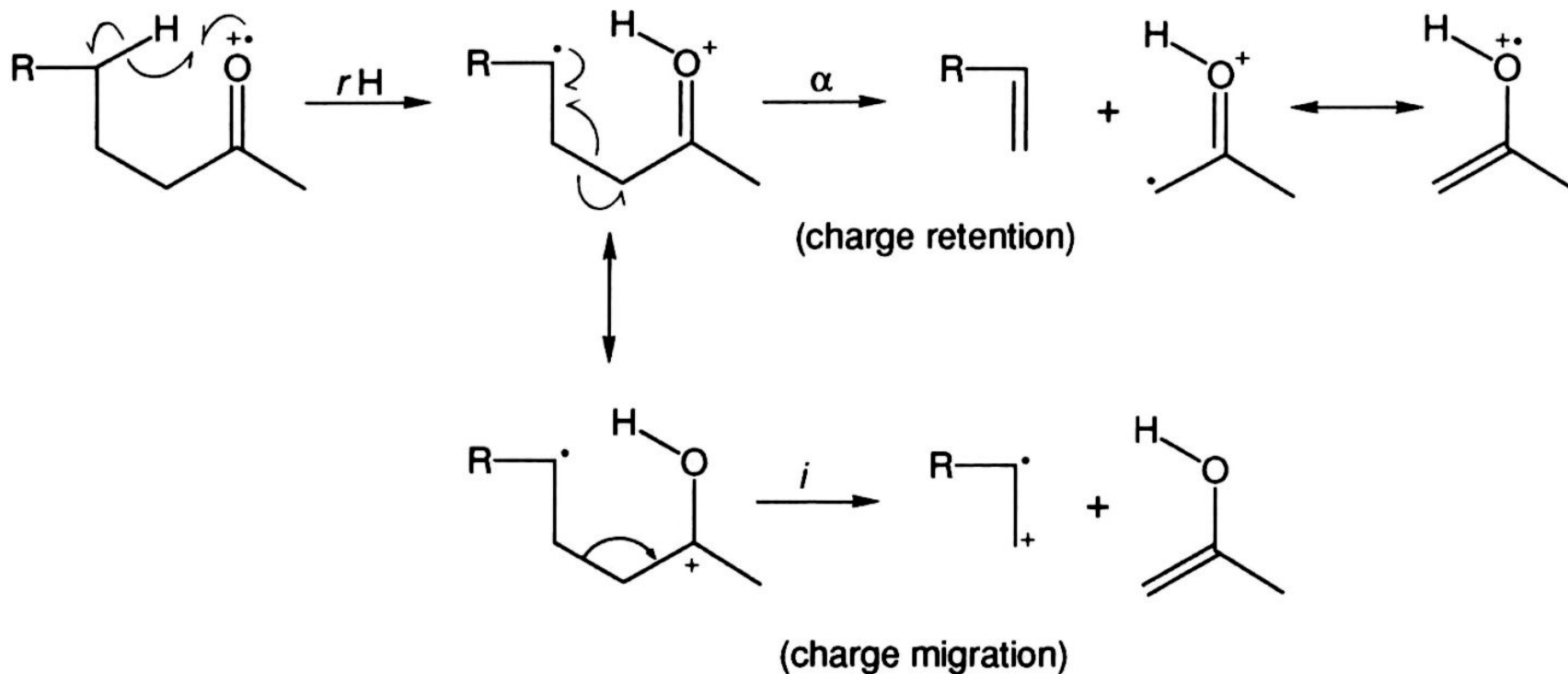
- $\pi$ -electrons of double bonds in the cyclic structures are the primary site of ionization



4-fenylokyklohexen

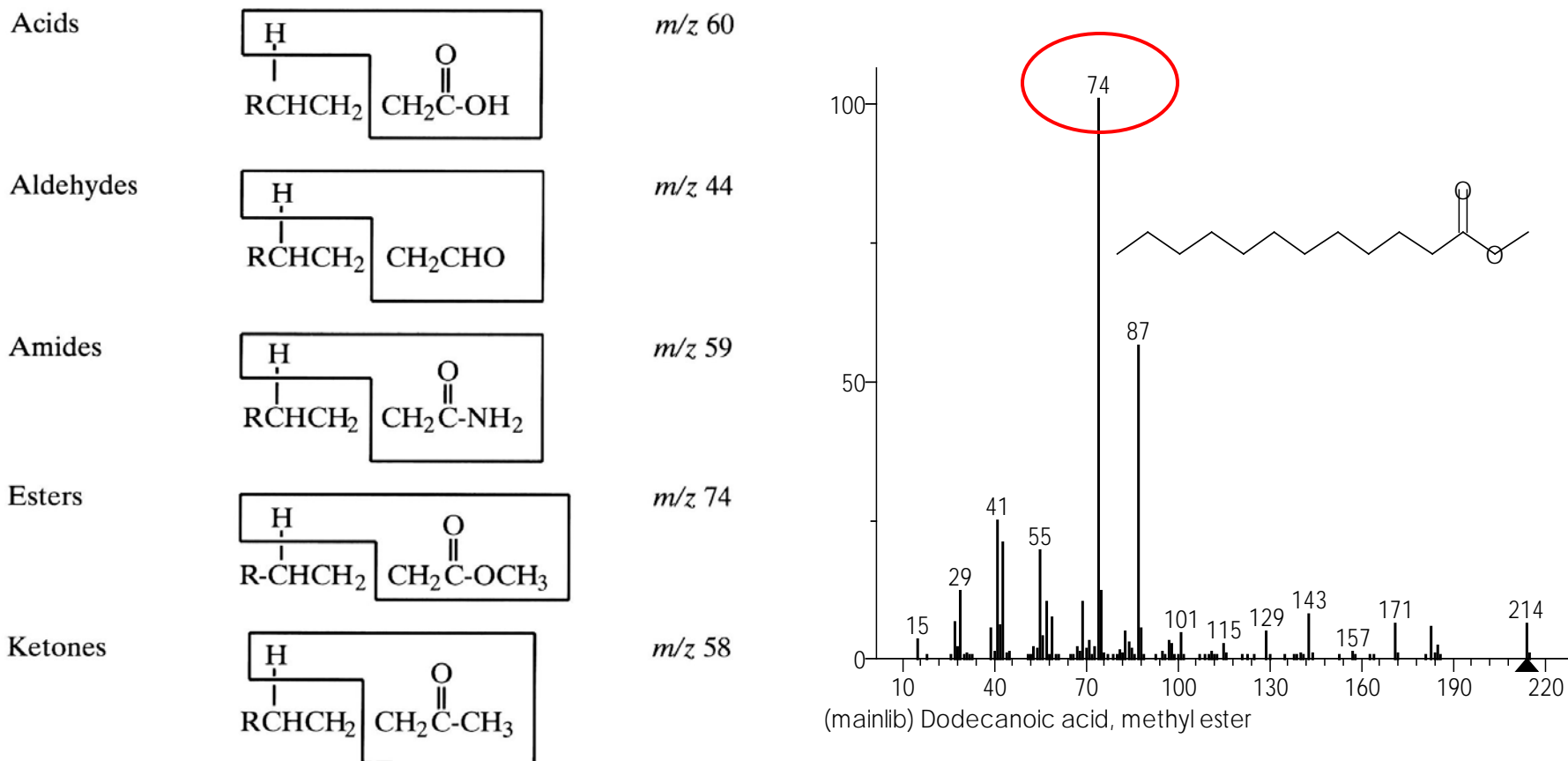
## Hydrogen rearrangement – McLafferty rearrangement

- rearrangement of  $\gamma$ -hydrogen on an unsaturated group over a 6-membered ring. The new radical site initializes  $\alpha$ -cleavage.



## Hydrogen rearrangement – McLafferty rearrangement

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



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

## Logical neutral losses

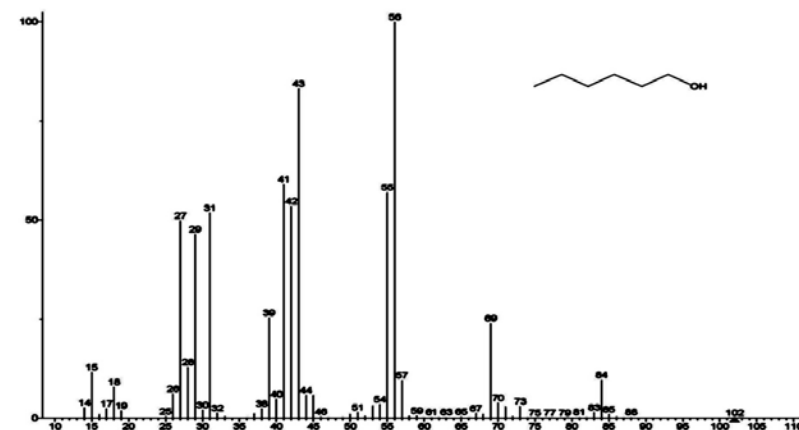
Neutral losses of radicals and neutral molecules must make chemical sense.

Ztráta (u)	Logická ztráta ?	Složení
1	ano	H
2	ano	H <sub>2</sub>
3 - 14	NE	-
15	ano	CH <sub>3</sub>
16	ano	NH <sub>2</sub> , O
17	ano	OH, NH <sub>3</sub>
18	ano	H <sub>2</sub> O
19	ano	F
20	ano	HF
21 - 25	NE	-
26	ano	C <sub>2</sub> H <sub>2</sub> , CN
27	ano	C <sub>2</sub> H <sub>3</sub> , HCN
28	ano	C <sub>2</sub> H <sub>4</sub> , CO
29	ano	HCO, C <sub>2</sub> H <sub>5</sub> , CH <sub>3</sub> N
30	ano	CH <sub>2</sub> O
31	ano	CH <sub>3</sub> O
32	ano	CH <sub>3</sub> OH, S
33	ano	SH
34	ano	H <sub>2</sub> S
35	ano	Cl
36	ano	HCl
37 - 40	NE	-
41 a výše	ano	homologické ztráty



## The molecular ion intensity

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

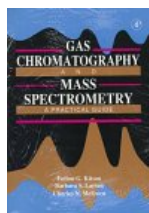


# Literature

EI



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

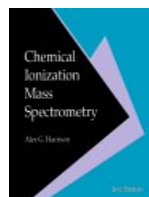


Fulton G. Kitson, Barbara S. Larsen, and Charles N. McEwen: Gas Chromatography and Mass Spectrometry. Academic Press (1996). ISBN-10: 0124833853, ISBN-13: 978-0124833852

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

## Courses on spectra interpretation

Advanced courses on mass spectrometry – **Škola MS**

*organized by*

**SPEKTROSKOPICKÁ SPOLEČNOST JANA MARKA MARCI**



<http://www.spektroskopie.cz/>

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## Acknowledgements



- MS team members
- Support from IOCB



**ÚOCHB AV ČR**

***Thank you for your attention !***

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