Understanding Mass Spectra of Small Molecules

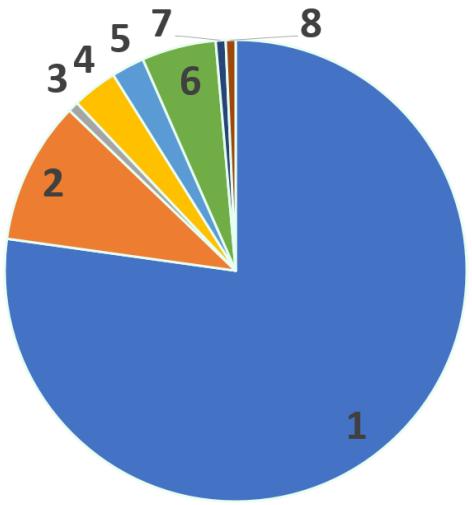
Josef Cvačka

Agenda

9:00 – 9:10	Introduction
9:10 – 10:00	Ionization, ion sources, tandem MS (theory)
10:10 – 12:00	Interpretation of mass spectra (interactive session)

Core facility Mass spectrometry

Services:



Service usage

- Mass spectra of molecules [1]
- Quantitative analysis of small molecules [2]
- Mass spectrometry imaging [3]
- Lipid analysis and lipidomics [4]
- On-demand mass spectrometry [5]
- LC/MS for IOCB Compound Library [6]
- Quality check of solvents [7]
- Open access GC/MS [8]

Core facility Mass spectrometry

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- Data Management
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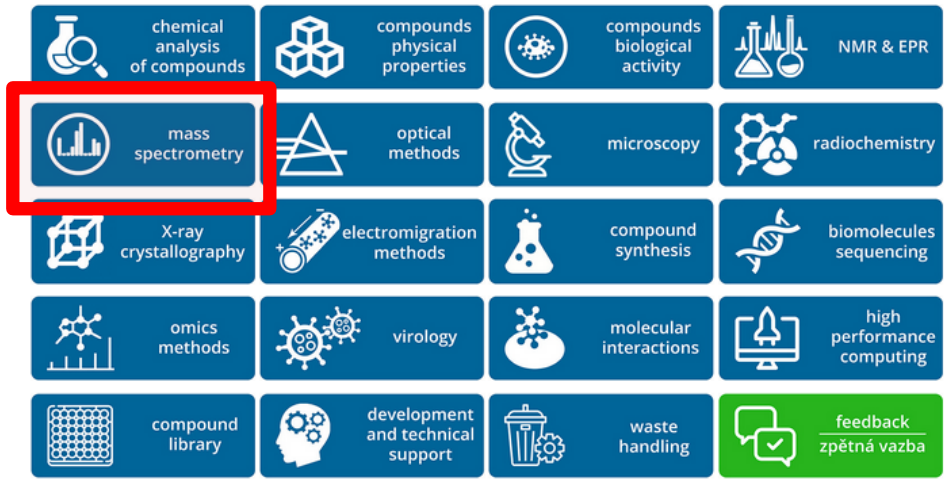
Core Facilities
- Analytical Laboratories
- Biochemical Pharmacology
- Cryo-EM
- Drug Discovery
- Electromigration
- Compoud Library
- Microscopy
- MS
- MS of Biopolymers
- MicroScale Thermophoresis
- NMR
- NGSf
- Radiochemistry
- RTG & bioNMR
- Untargeted Metabolomics

Core Facilities

Core facilities are essential component of IOCB, offering shared resources and expertise to support research activities. They provide reliable services, collaboration, and contribute to the productivity and success of scientific investigations. While core facilities might work on collaborative projects and method development their primary mission is to support researchers at IOCB. This focus ensures that researchers from different areas benefit from the shared resources and expertise in the core facility, helping advance scientific knowledge and discovery.

- Core facilities are either independent units or part of research group as Research core facilities. A list of them is given in the menu on the left and at the bottom of this page.
- Below is a list of provided services and the opportunity for feedback.



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Mass Spectrometry

- Mass spectra of molecules
- Quantitative MS analysis of small molecules
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- Lipid analysis and lipidomics
- On-demand mass spectrometry
- LC/MS for IOCB Compound Library
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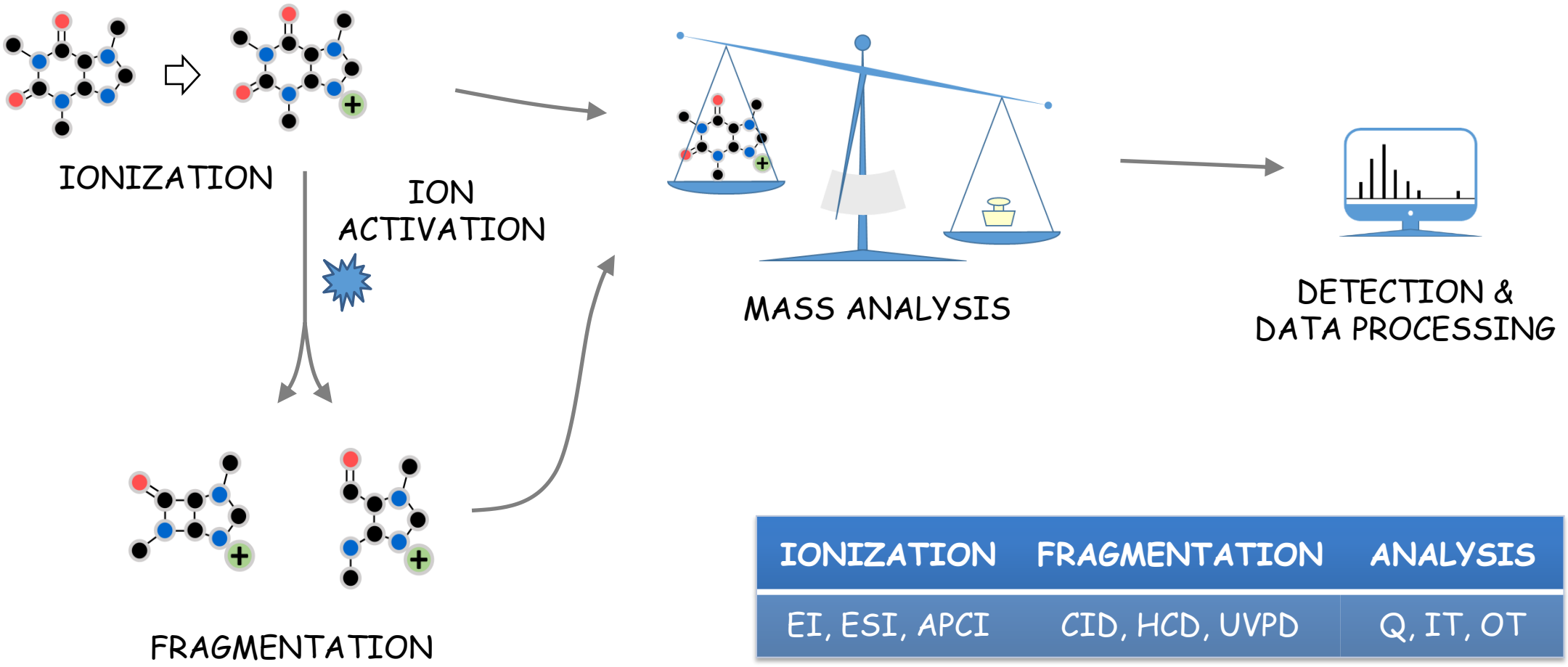
- Open access GC/MS
- Bottom-up proteomics
- Top-down proteomic analysis
- Structural proteomics
- Untargeted metabolomics

«« Core Facilities Signpost

Ionization, ion sources, and tandem MS

Mass spectrometry



IONIZATION	FRAGMENTATION	ANALYSIS
EI, ESI, APCI	CID, HCD, UVPD	Q, IT, OT

Ionization

Ionization: a process during which neutral molecules or ions (in a solution) are converted to ions in the gas phase:

Ionization processes:

$M + e^- \rightarrow M^{+\bullet} + 2e^-$ (formation of radical cation in EI)

$M + e^- \rightarrow M^{2+} + 3e^-$ (formation of multiply charged ions in EI)

$M + e^- \rightarrow M^{-\bullet}$ (resonant electron capture)

$M + hv \rightarrow M^{+\bullet} + e^-$ (formation of radical cation in APPI)

$M + [BH]^+ \rightarrow [M + H]^+ + B$ (proton transfer, CI, ESI, APCI)

$M + B \rightarrow [M - H]^- + [BH]^+$ (formation of negatively charged ions in CI, ESI, APCI)

$M + X^+ \rightarrow [M + X]^+$ (electrophilic addition, cationization, ESI, MALDI)

molecular ion ($M^{+\bullet}$) vs. molecular adduct ($[M + H]^+$, $[M + Na]^+$, $[M + NH_4]^+$)

Ionization techniques

There is no universal ionization technique for all molecules; different ionizations are suitable for different types of compounds.

Classification of ionization techniques:

According to the pressure in the ion source:

ionization
- at reduced pressure (EI, CI, MALDI)
- at atmospheric pressure (ESI, APCI, APPI, MALDI)

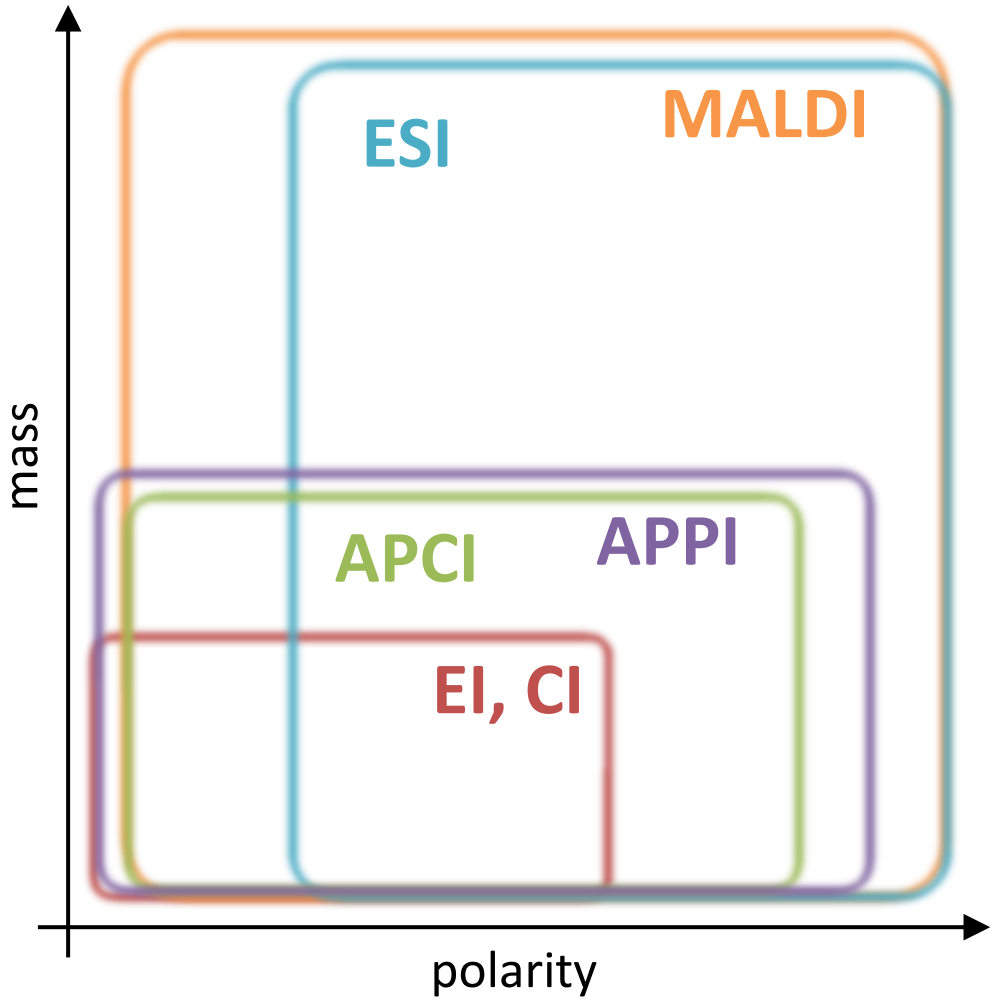
According to the phase in which the sample is in ionization:

ionization
- from gas phase (EI, CI, APCI)
- from liquid phase (ESI, APCI, APPI)
- from solid state (MALDI)

According to the energy transferred to the analyte:

ionization
- Soft (ESI, APCI,APPI, MALDI)
- Hard (EI)

Ionization techniques



EI: electron ionization

CI: chemical ionization

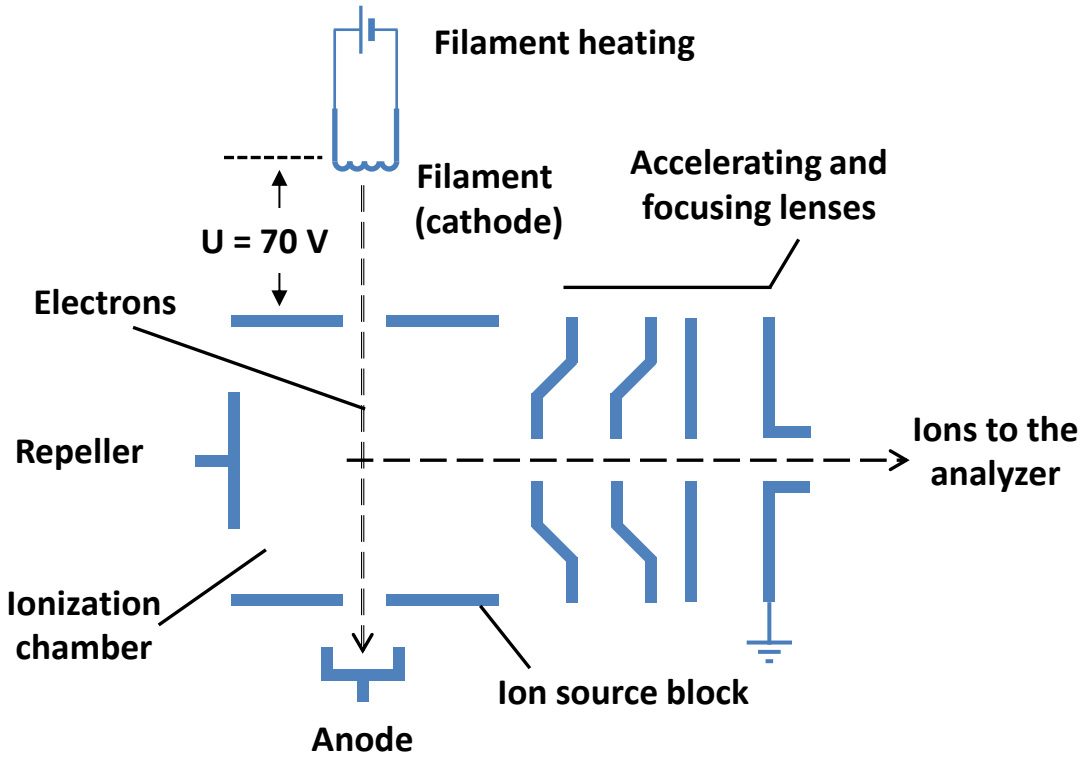
ESI: electrospray ionization

APCI: atmospheric-pressure chemical ionization

APPI: atmospheric-pressure photoionization

MALDI: matrix-assisted laser desorption/ionization

Electron ionization



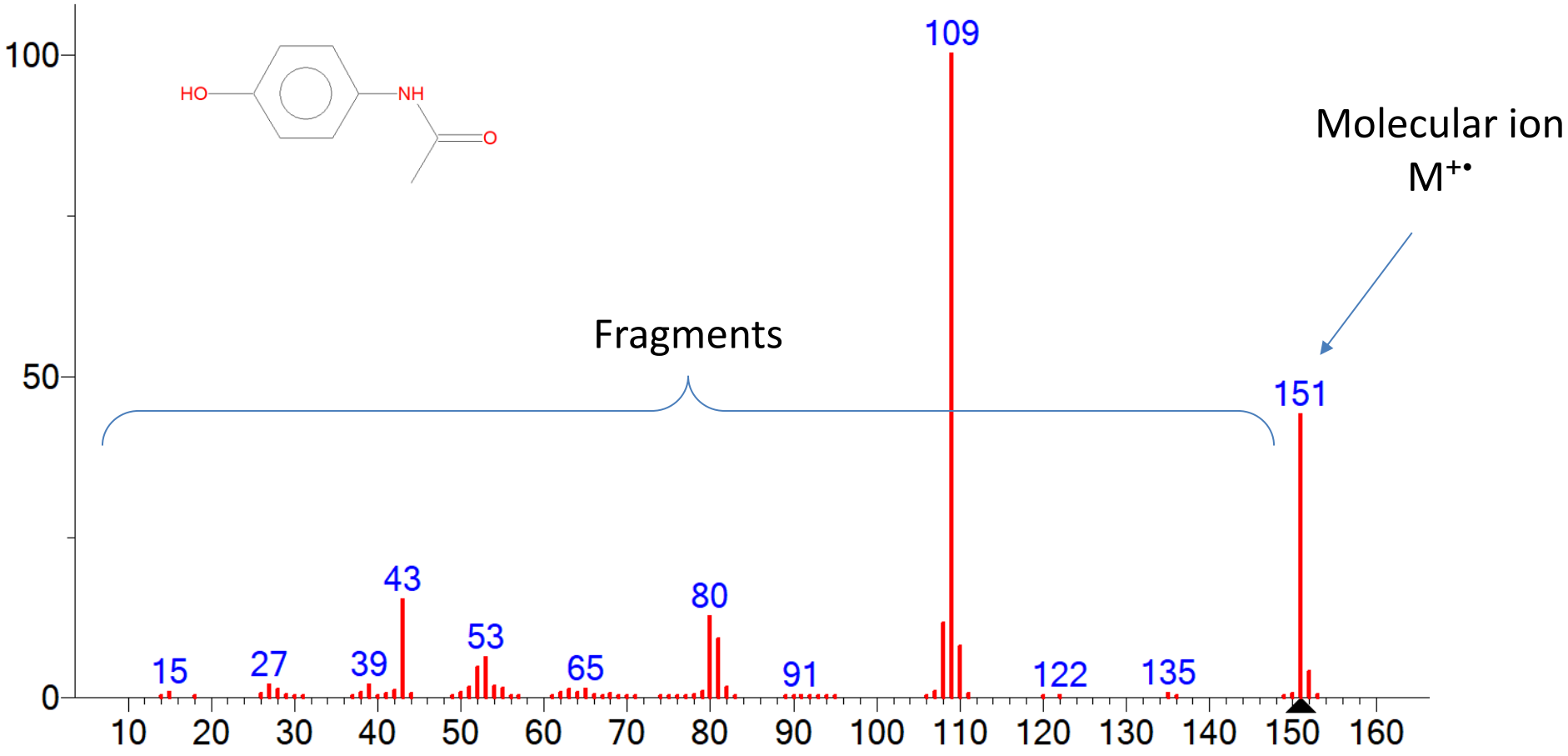
$$M + e^- \rightarrow M^{+\cdot} + 2e^-$$

Ionization energy IE: minimum amount of energy that must be absorbed by a neutral molecule to ionize by removing an electron

→ IE for most molecules 7-15 eV
→ highest ionization efficiency around 70 eV

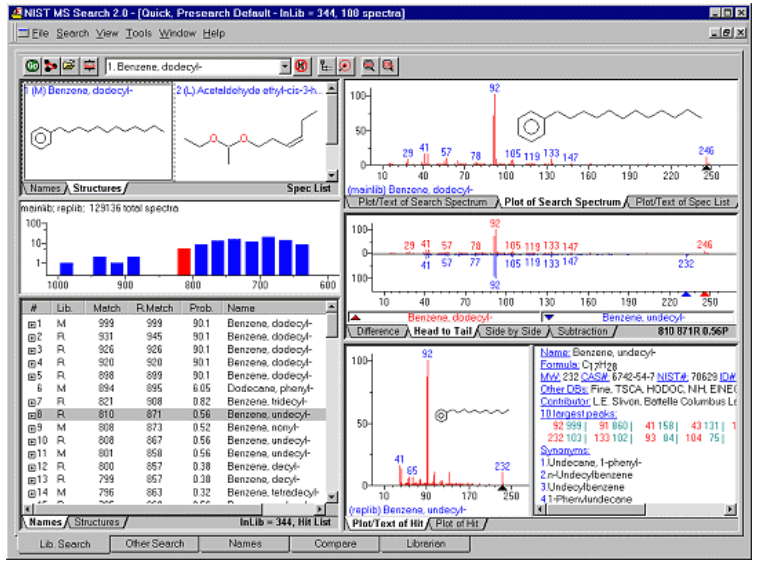
Electron ionization: molecules interact with electrons (70 eV) emitted from a hot tungsten filament. Radical-cations are formed, and excess energy leads to their extensive fragmentation.

EI spectrum


HO
NH
O
109
Molecular ion
M+•
Fragments
151
100
50
0
43
80
53
15
27
39
65
91
122
135
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160

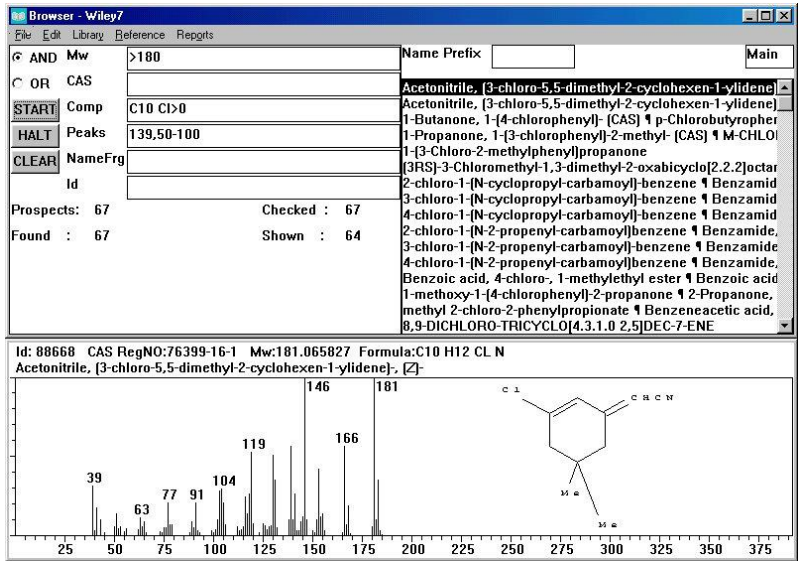
Mass Spectral Libraries

NIST/EPA/NIH Mass Spectral Library



394 000 EI spectra (70 eV)
MS/MS spectra of 51,000 substances,
Retention indices of substances
Structural formulas

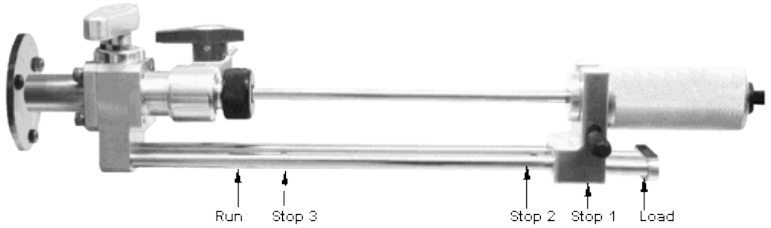
Wiley Registry of Mass Spectral Data

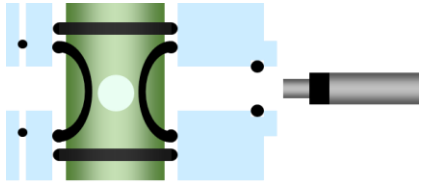


950 200 EI spectra (70 eV)
Structural formulas

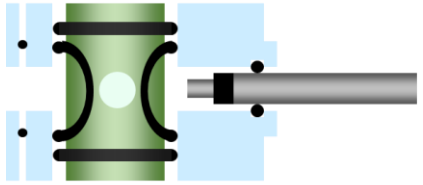
Methods of data recording: DIP, DEP

Samples are introduced using rod probes. After partially inserting the probe, the region around the sample is evacuated. Subsequently, a ball valve is rotated, enabling full insertion of the probe into the EI source.

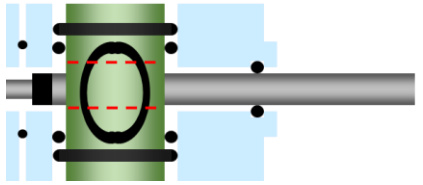


1. 

Inserting the probe into the intermediate position

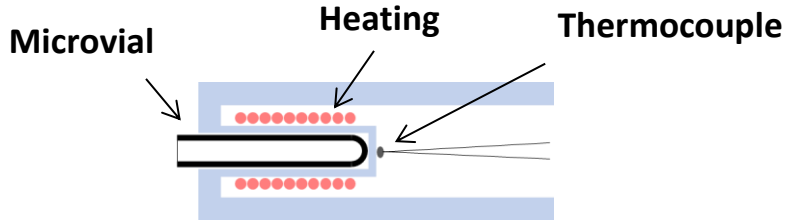
2. 

Vacuum pumping in intermediate position

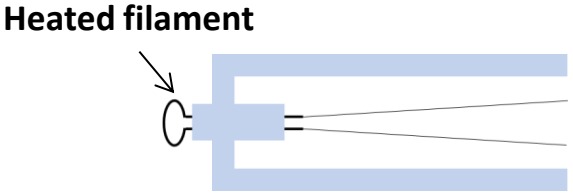
3. 

Slide into final position into the ion source

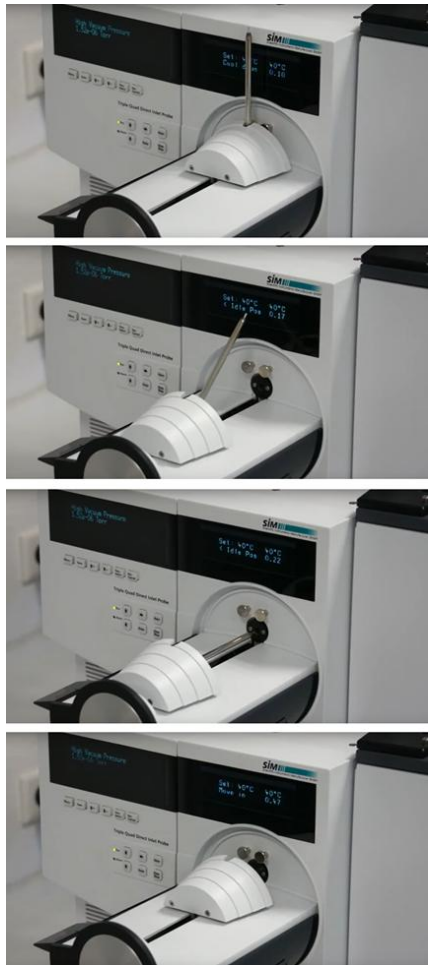
Direct insertion probe (DIP): Sample in microtube, gradual heating, sample vapors flow directly into ion source



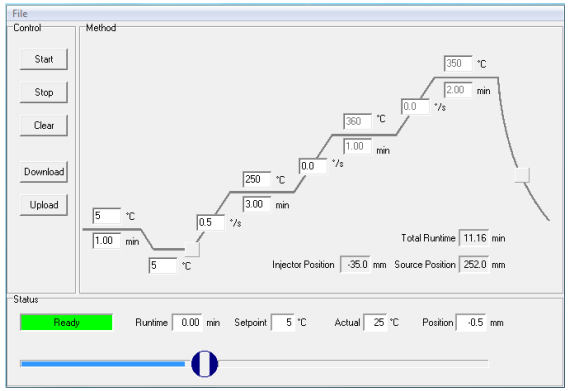
Direct exposure probe (DEP):Very fast evaporation, faster than sample degradation



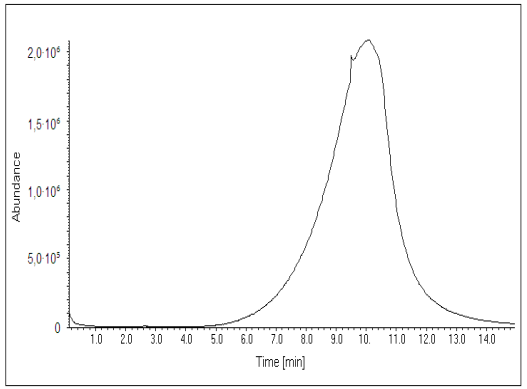
Methods of data recording: Automated DEP



Analyses with the probes can be automated.



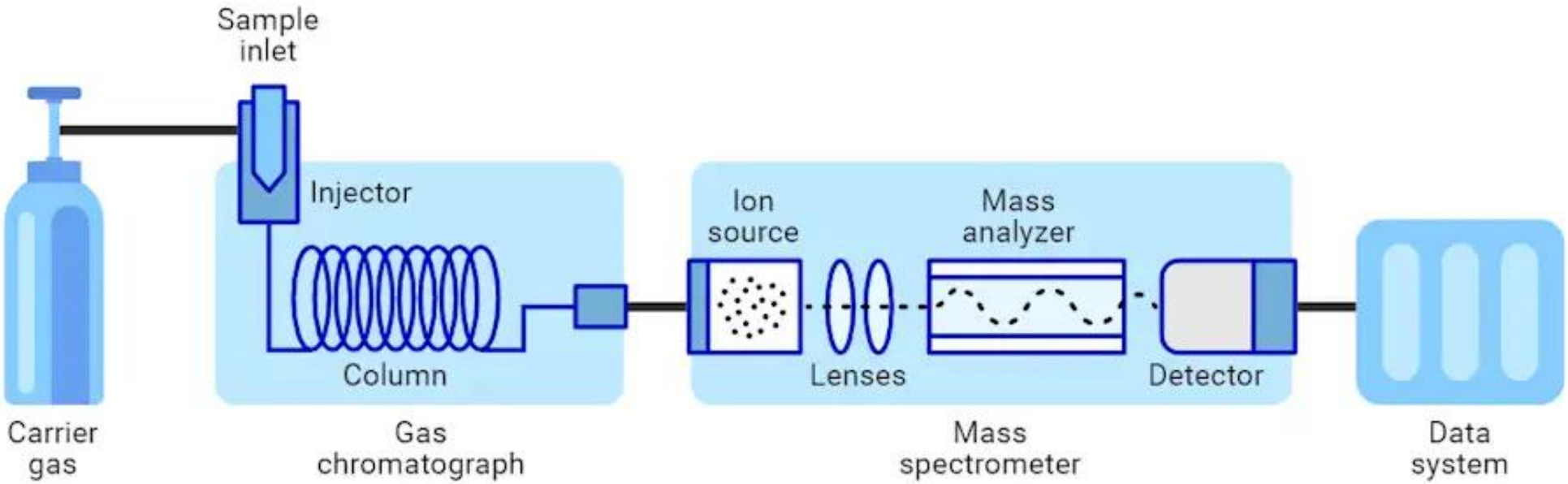
Temperature program



Signal intensity depending on temperature

Pictures from https://sim-gmbh.de/

Methods of data recording: GC/MS



Picture from https://measurlabs.com/

EI: Summary

Major Application Area: small, volatile, and thermally stable organic compounds

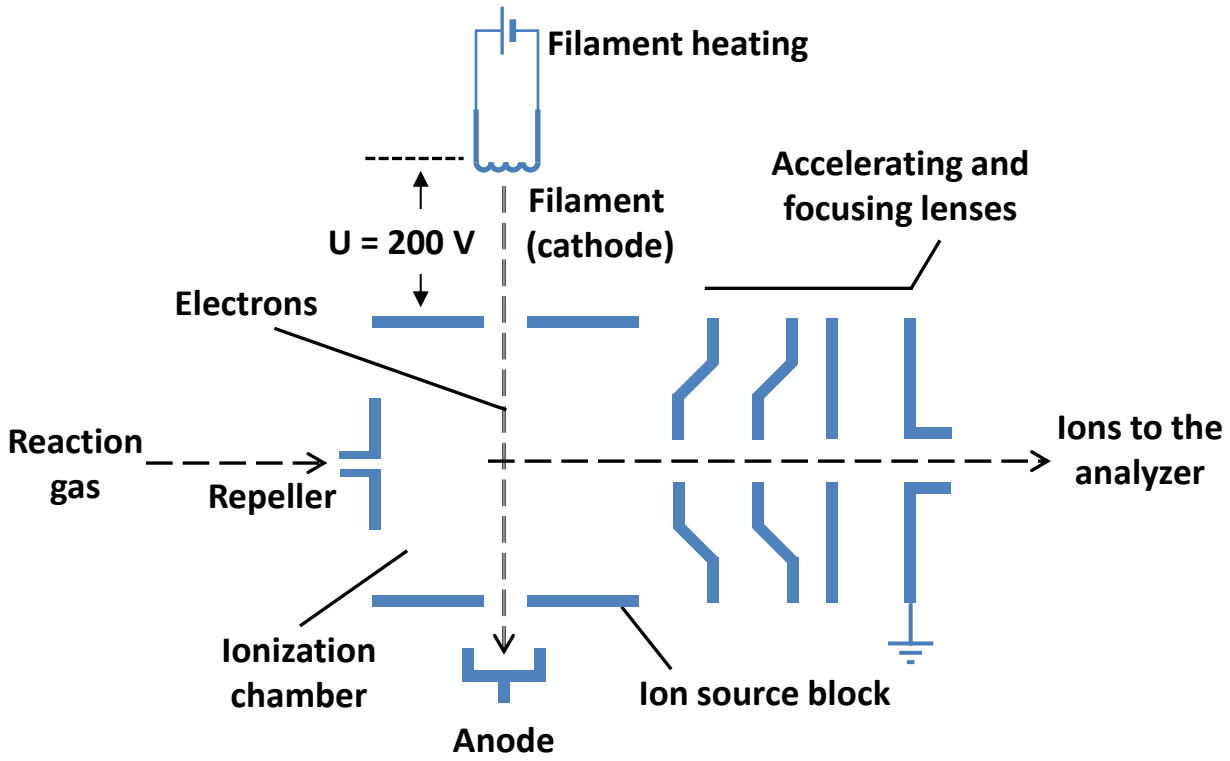
Advantages: 

- Highly reproducible fragmentation patterns.
- Extensive reference libraries facilitate compound identification.
- Excellent sensitivity and quantitative capabilities.

Limitations: 

- Limited to compounds that are volatile and thermally stable.
- Often results in extensive fragmentation, potentially obscuring molecular ions.
- Generally unsuitable for large biomolecules or thermally labile compounds.

Chemical ionization



$$M + BH^+ \rightarrow [M + H]^+ + B$$

Proton affinity PA: enthalpy change associated with protonation.

→ Protonation will only take place if the reaction is exothermic.
→ Reaction gases: methane, isobutane, ammonia.

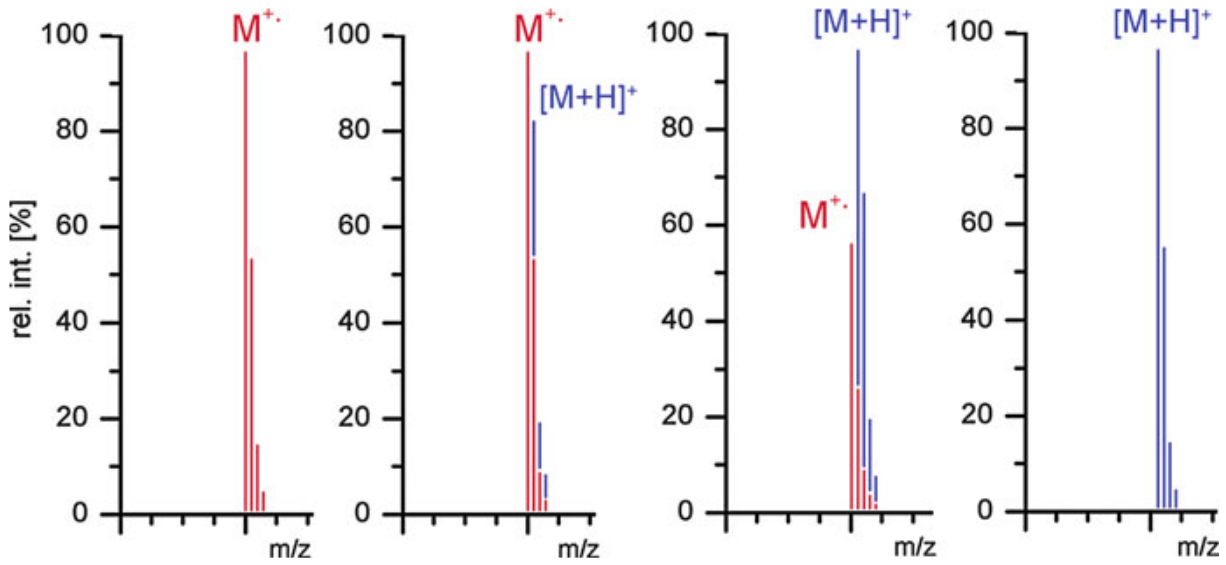
Chemical ionization: a reaction gas is introduced into the ion source, which interacts with electrons emitted from the filament. A reactive plasma is formed, which ionizes the analyte by proton transfer. Fragmentation is suppressed.

Ionization of methane

If we increase the pressure in the EI source,
autoprotonation can occur:

$$M + M^{+\cdot} \rightarrow [M+H]^{+} + [M-H]^{\cdot}$$

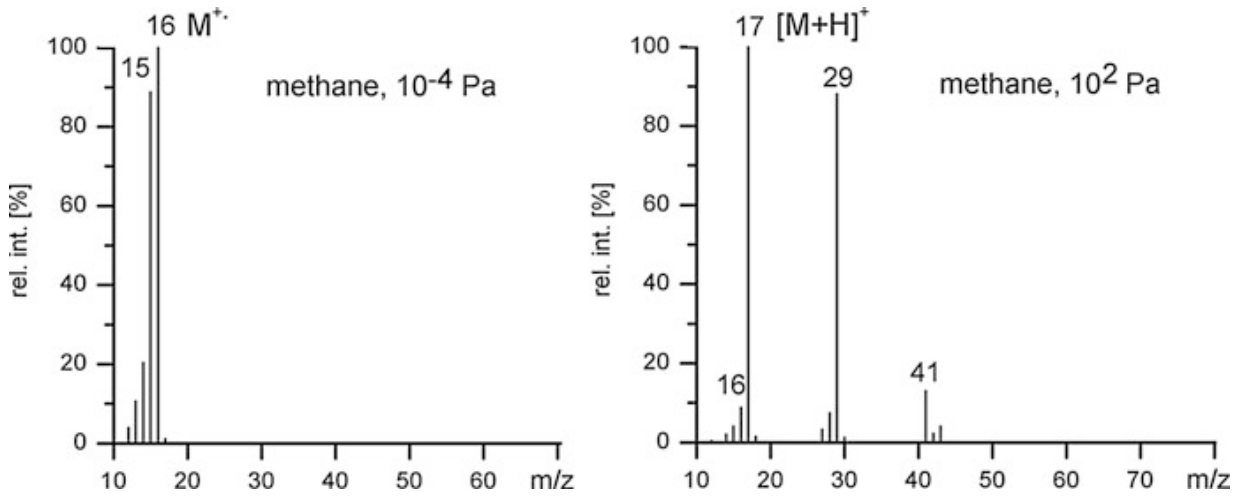
Autoprotonation is undesirable in EI, as it distorts the intensities of isotope peaks.
In EI, it is necessary to maintain a low pressure in the source, while in CI, a higher pressure is required.



Gradual change of the molecular ion of methane (EI) to the molecular adduct (CI) at increasing pressure in the ion source

Picture from Gross: Mass Spectrometry, A Textbook, 2017

CI with methane



$CH_4 + e^- \rightarrow CH_4^{+\cdot}, CH_3^+, CH_2^{+\cdot}, CH^+, C^{+\cdot}, H_2^{+\cdot}, H^+$ EI

$CH_4^{+\cdot} + CH_4 \rightarrow CH_5^+ + CH_3^{\cdot}$ Autoprotonation of methane, *m/z* 17

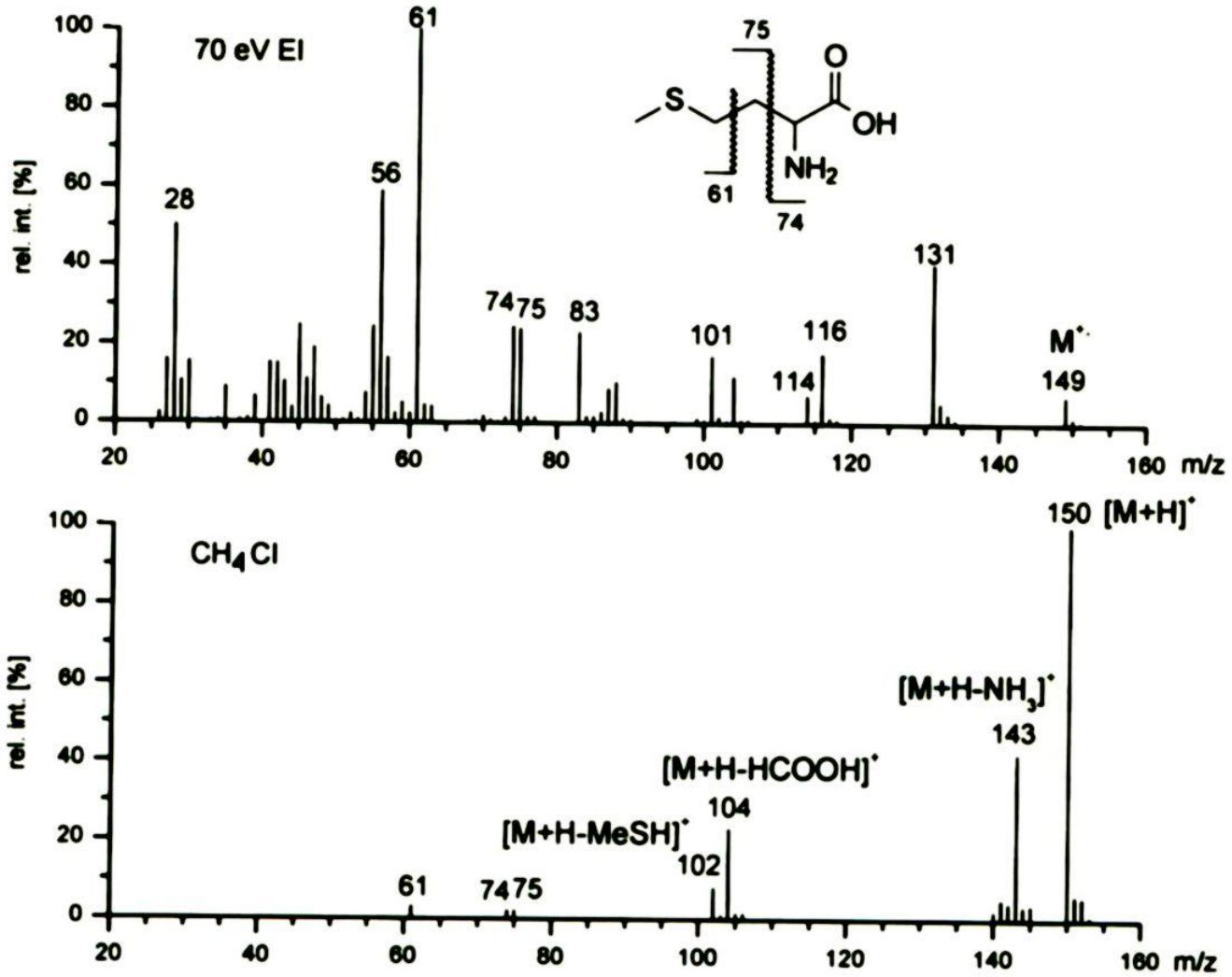
$CH_3^+ + CH_4 \rightarrow C_2H_7^+ \rightarrow C_2H_5^+ + H_2$ C_2H_5, *m/z* 29

$CH_2^{+\cdot} + CH_4 \rightarrow C_2H_3^+ + H_2 + H^{\cdot}$

$C_2H_3^+ + CH_4 \rightarrow C_3H_5^+ + H_2$ C_3H_5, *m/z* 41

Picture from Gross: Mass Spectrometry, A Textbook, 2017

CI with methane



Ions observed in methane-CI spectra: $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$, occasionally $[M - H]^+$ (hydride loss, beware of misinterpretation! ... look at other adducts and losses)

Picture from Gross: Mass Spectrometry, A Textbook, 2017

CI: Summary

Major Application Area: small, volatile, and thermally stable organic compounds

Advantages: 

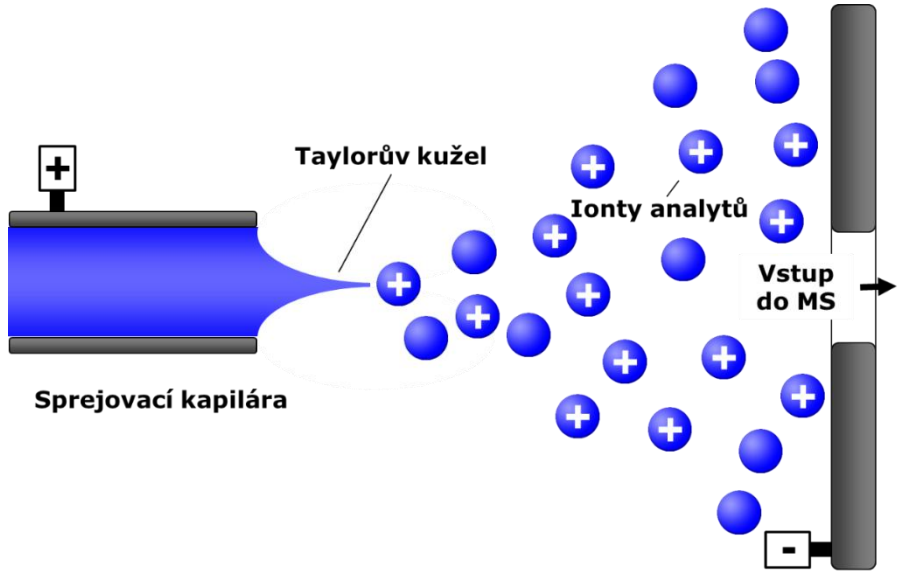
- Produces less fragmentation than EI, providing clearer molecular ions.
- Gentle ionization, useful for confirming molecular weights.
- Allows selective ionization through choice of reagent gas (e.g., methane, ammonia).

Limitations: 

- Lower reproducibility of fragmentation patterns compared to EI.
- Limited to relatively volatile compounds; unsuitable for large biomolecules.

Electrospray Ionization

An **electrospray** is a phenomenon in which a strong electric field applied to the liquid at the tip of a capillary generates a fine mist of electrically charged droplets.

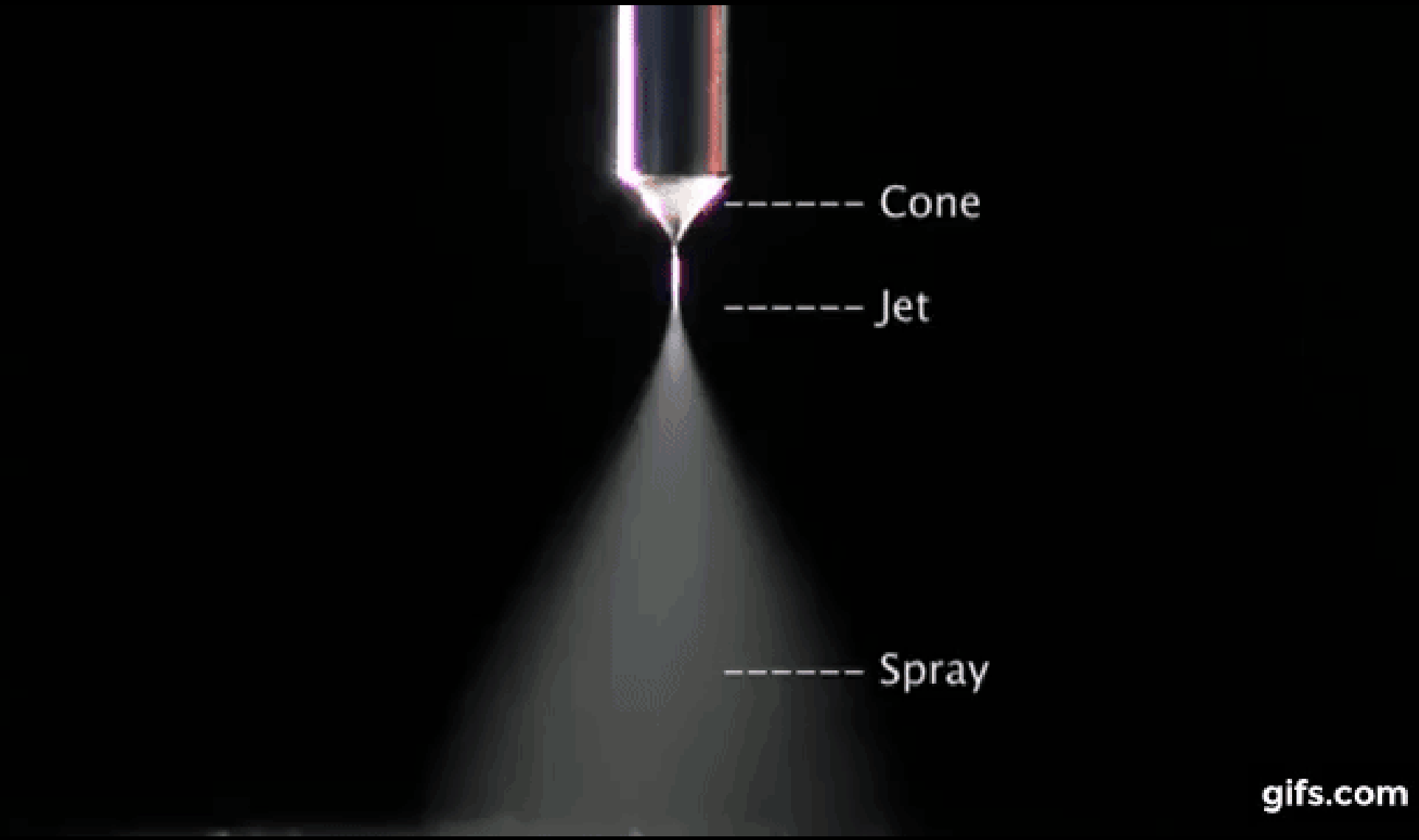


Electrospray Ionization (ESI) source: a liquid sample is delivered through a capillary held at high voltage, generating a strong electric field at its tip. This electric field forms a Taylor cone, emitting a fine spray of charged droplets. Solvent evaporation reduces droplet size, increasing their charge density until ions are released into the gas phase, ready for mass spectrometric analysis.

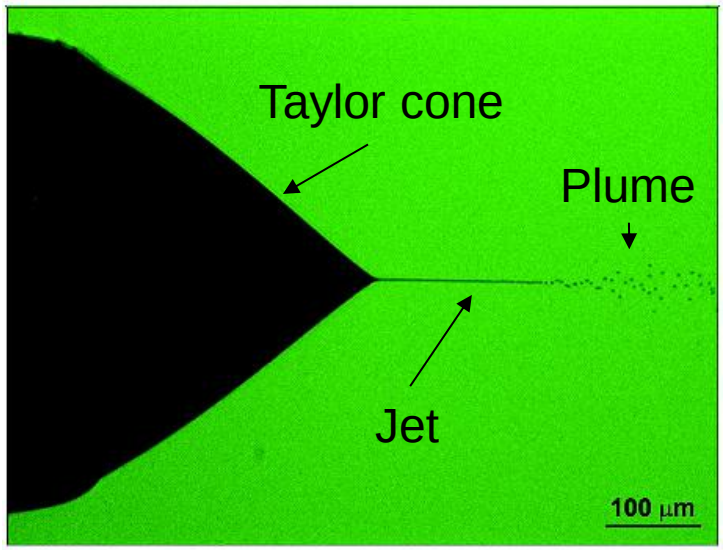
Electrospray Ionization

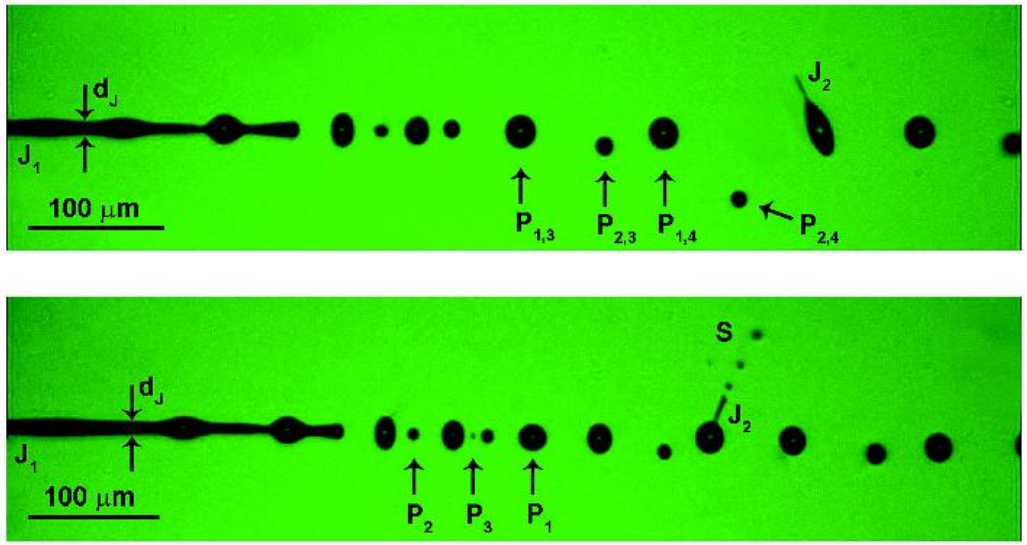
Processes that take place during ESI:

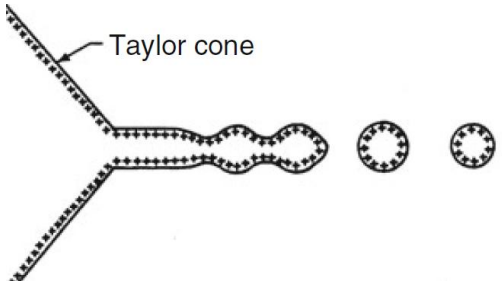
- Taylor cone formation and charged droplet release
- Reduction of charged droplets by gradual evaporation of the solvent
- Release of ions into the gas phase
- Secondary reactions in the gas phase (ion-molecular reactions, fragmentation, etc.)


Cone
Jet
Spray
gifs.com

Droplets formation in ESI







Following the formation of a Taylor cone, a charged liquid jet emerges from its apex, which quickly breaks up into charged droplets.

These droplets repel each other, forming a widening spray plume, and progressively fragment into smaller droplets.

Picture from Nemes et al., 10.1021/ac062382i

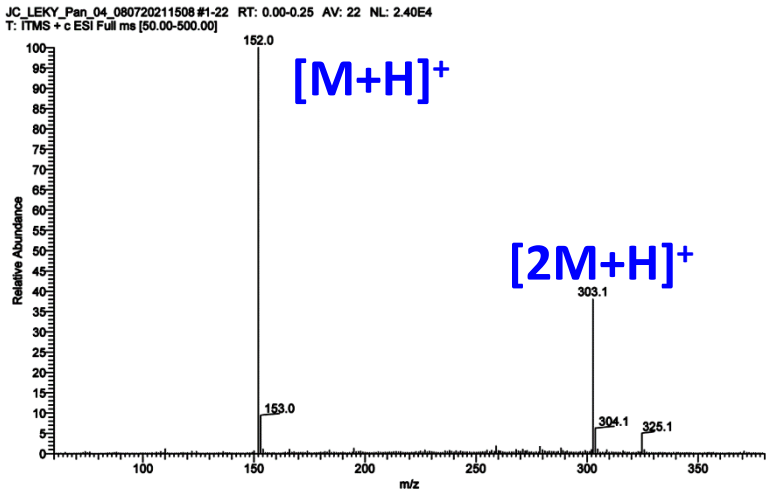
ESI spectra

Soft ionization technique (minimum fragmentation)

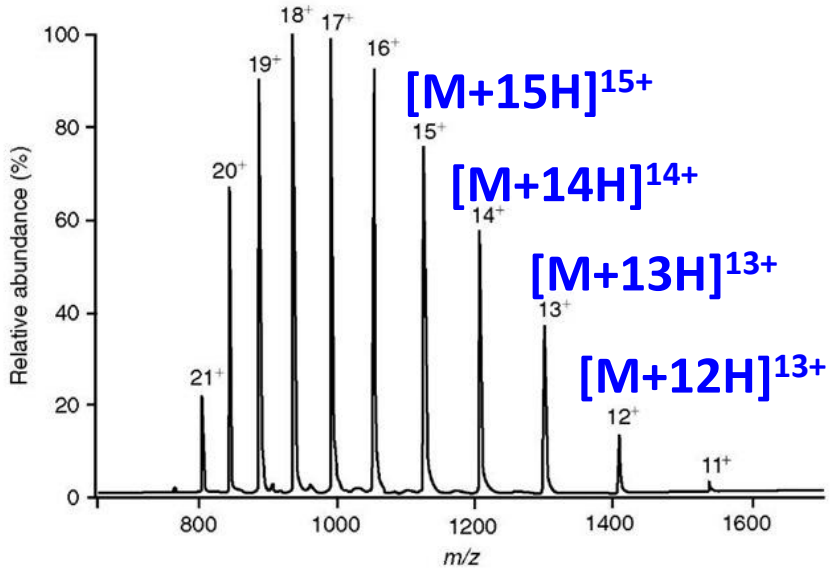
→ Small molecules: single-charged ions $[M + H]^+$, $[M + Na]^+$, $[M + NH_4]^+$ (positive mode) and $[M - H]^-$, $[M + Cl]^-$ (negative mode) depending on the composition of the mobile phase

→ Large biomolecules: multicharged ions $[M + nH]^{n+}$, $[M + nNa]^{n+}$ (positive mode) and $[M - nH]^{n-}$ (negative mode)

Small molecule



Protein



ESI: Summary

Major Application Area: Polar and less polar small organic compounds, including thermally labile or non-volatile compounds and large biomolecules.

Advantages: 

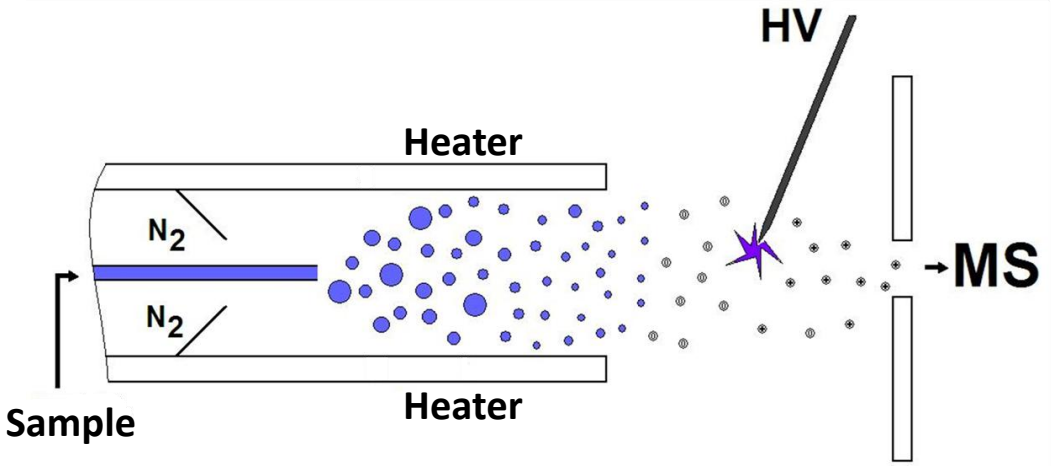
- Ionization of large and thermally labile biomolecules without decomposition.
- Generates multiply charged ions, enabling analysis of very large molecules.
- Excellent compatibility with liquid chromatography (LC/MS).

Limitations: 

- Ionization is influenced by sample matrix and solvent conditions.
- Prone to ion suppression/enhancement effects from complex mixtures.
- Typically provides minimal fragmentation, requiring MS/MS for structural elucidation.

Atmospheric-pressure chemical ionization

Chemical ionization is a process in which charged particles are formed in the gas phase by the reaction of neutral particles with ions.



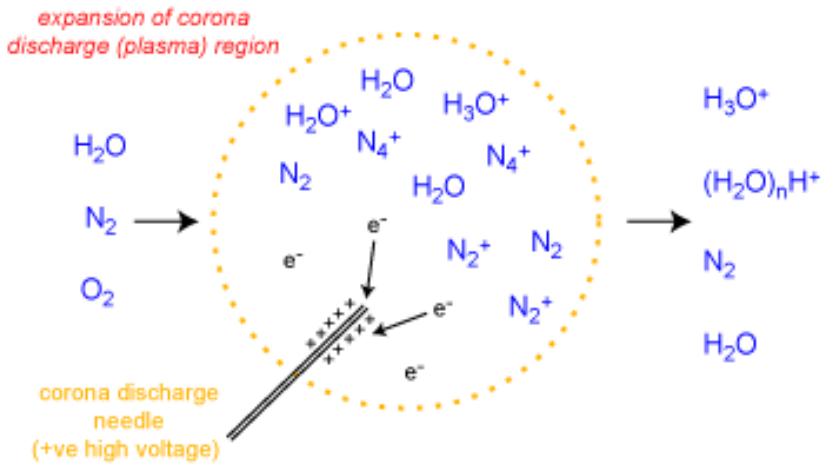
Atmospheric Pressure Chemical Ionization (APCI): the liquid sample is introduced as a fine spray into a heated chamber, rapidly vaporizing the solvent and analyte molecules.

Electrons from a corona discharge needle ionize atmospheric gases and solvent molecules, producing primary reagent ions (e.g., protonated water clusters).

Analyte molecules become ionized via secondary chemical reactions with these reagent ions.

Atmospheric-pressure chemical ionization

Processes that take place during APCI:



Formation of primary ions:

$N_2 + e^- \rightarrow N_2^{+\bullet} + 2e^-$

$N_2^{+\bullet} + 2N_2 \rightarrow N_4^{+\bullet} + N_2$

Formation of solvent reaction ions:

$H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + HO^\bullet$

$H_3O^+ + H_2O + N_2 \rightarrow H^+(H_2O)_2 + N_2$:

$H^+(H_2O)_{n-1} + H_2O + N_2 \rightarrow H^+(H_2O)_n + N_2$

Analyte ionization:

$A + B^{+\bullet} \rightarrow A^{+\bullet} + B$

$A + BH^+ \rightarrow AH^+ + B$

Picture from https://www.microsaic.com/

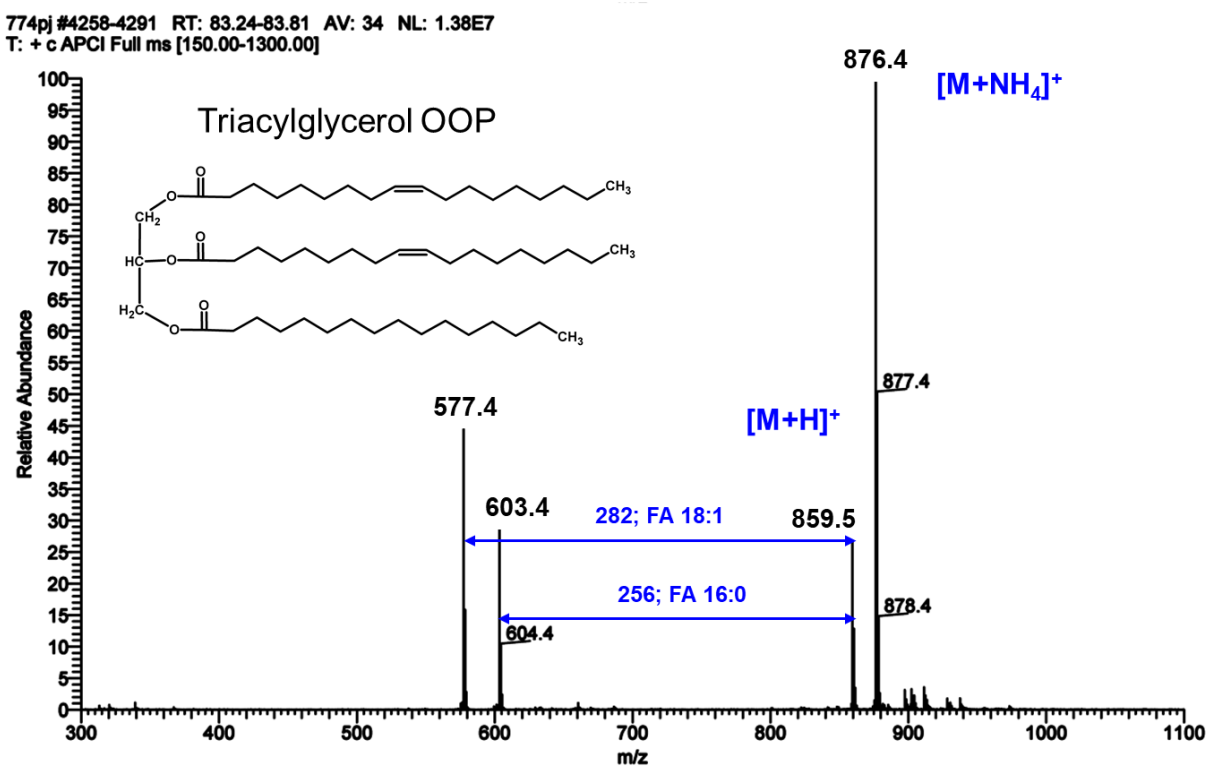
APCI spectra

Soft ionization technique (usually some fragmentation)

Even-electron ions: $[M + H]^+$, $[M + NH_4]^+$ products of reactions with solvents (positive mode), $[M - H]^-$ (negative mode)

Odd-electron ions: $M^{+\bullet}$, products of reactions with solvents

Fragments



APCI: Summary

Major Application Area: moderately polar to nonpolar, small to medium-sized molecules.

Advantages: 

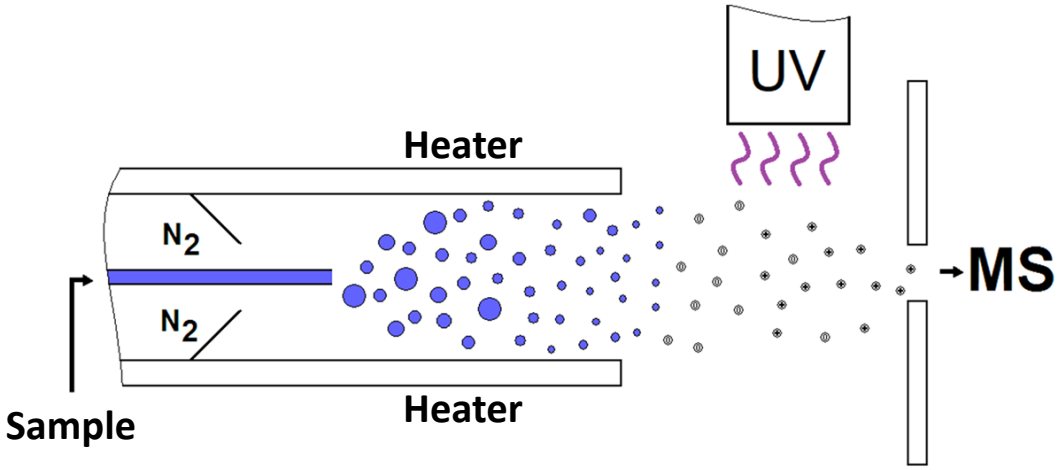
- Effective for relatively nonpolar, thermally stable compounds.
- Good compatibility with liquid chromatography.
- Less susceptible to ion suppression effects compared to ESI.

Limitations: 

- Limited to molecules that can be vaporized without thermal decomposition.
- Usually ineffective for large biomolecules or highly polar and thermally labile compounds.

Atmospheric-pressure photoionization

Photoionization is a process by which neutral species in the gas phase become charged through interactions with photons.



Atmospheric Pressure Photonization (APPI):
The liquid sample is sprayed into a heated chamber.

VUV lamp produces photons that interact with the analyte molecules in the gas phase to form $M^{\bullet+}$; at the same time, proton-solvent transfer reactions can take place.

A low-IE dopant (toluene, acetone) can be used to increase the detection sensitivity.

Atmospheric-pressure photoionization

Processes that take place during APPI:

Direct photoionization

$$M + h\nu \rightarrow M^{+\bullet} + e^{-}$$

Dopant-mediated photoionization

$$D + h\nu \rightarrow D^{+\bullet} + e^{-}$$

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

Formation of protonated molecules:

$$M^{+\bullet} + S \rightarrow MH^{+} + [S-H]^{\bullet}$$

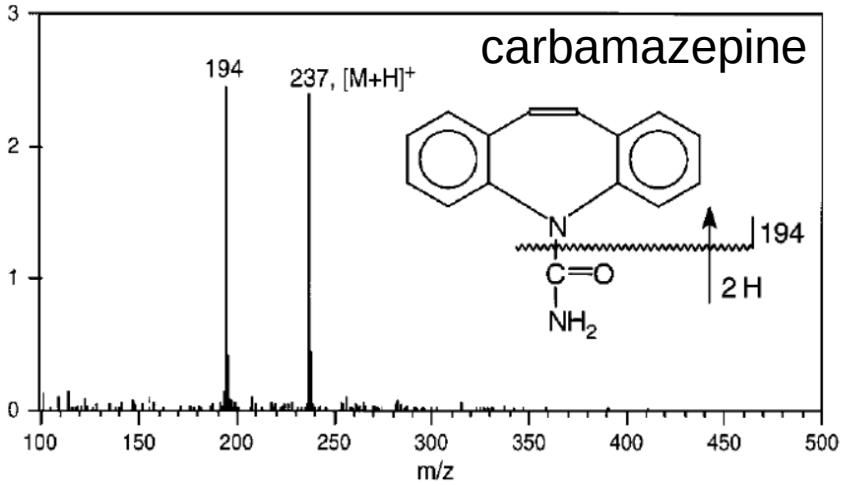
$$D^{+\bullet} + S\ (solvent) \rightarrow [D-H]^{\bullet} + SH^{+}$$

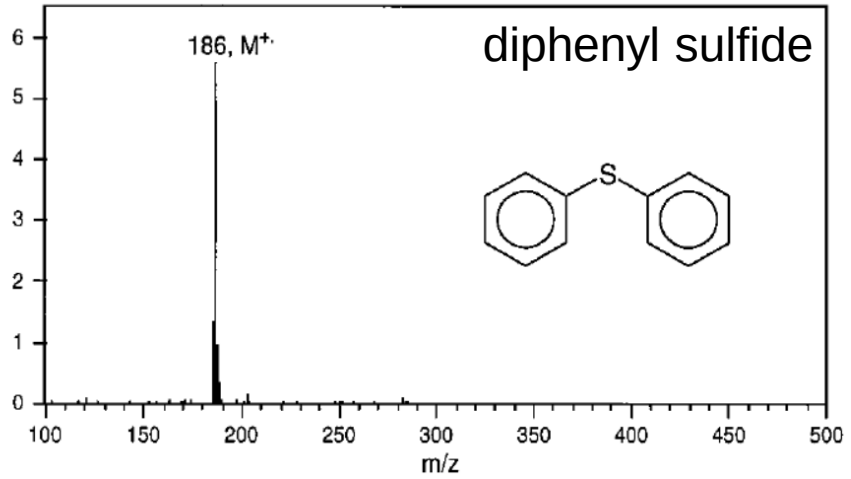
$$SH^{+} + M \rightarrow MH^{+} + S$$

APPI spectra

Soft ionization technique (usually some fragmentation)

Odd electron ions: : $M^{+\bullet}$, products of reactions with solvents
Even electron ions: $[M + H]^+$, products of reactions with solvents (positive mode), $[M - H]^-$ (negative mode)
Fragments





Picture from Robb et al., Anal. Chem. 2000, 72, 3653-3659

APPI: Summary

Major Application Area: moderately polar to nonpolar, small to medium-sized molecules.

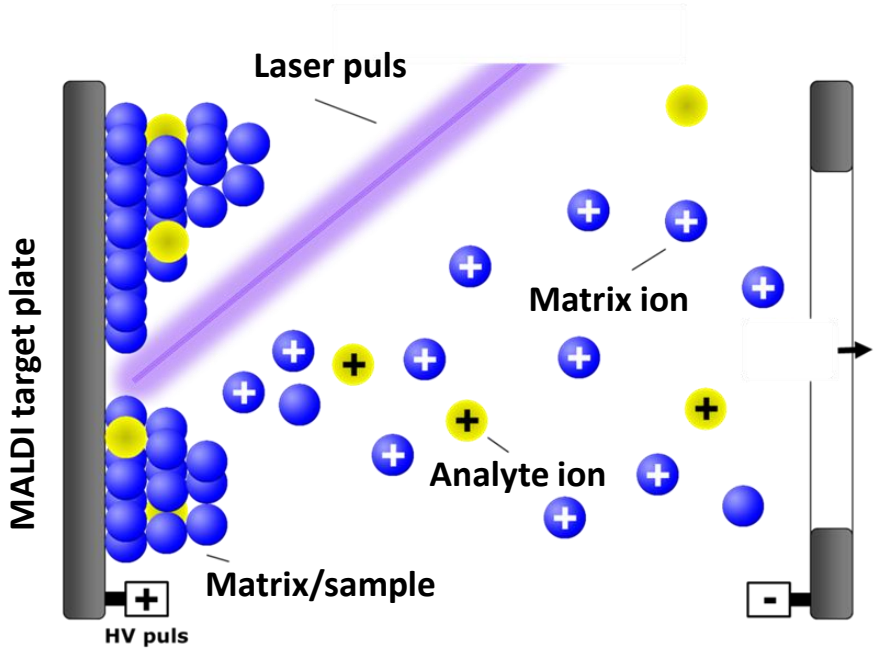
Advantages: 

- Effective for low-polarity or nonpolar molecules that are challenging for ESI or APCI.
- Less affected by ion suppression, enhancing robustness in complex matrices.

Limitations: 

- Requires molecules to have adequate photoionization efficiency (appropriate ionization potentials).
- Limited applicability for highly polar, large, or thermally labile biomolecules.

Matrix-assited laser desorption/ionization

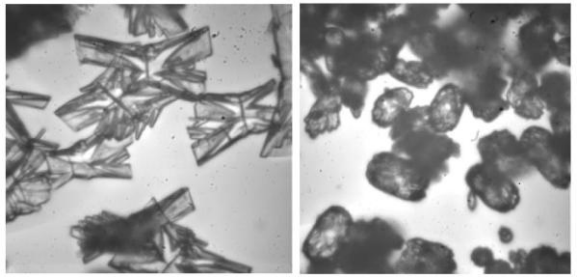


Matrix-Assisted Laser Desorption/Ionization (MALDI) involves mixing the sample with a matrix, applying it to a target plate, and using a laser pulse to cause desorption. The matrix is ionized first, enabling ionization of the analyte—typically through proton transfer.

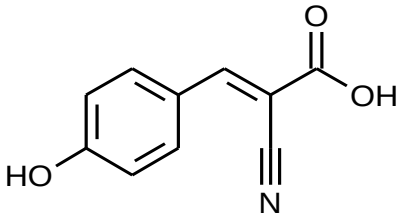
Ions in spectra:

$[M + H]^+$, $[M - H]^-$, alkali metal adducts

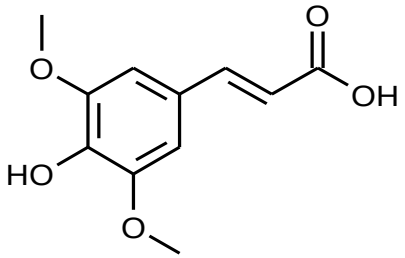
MALDI matrices



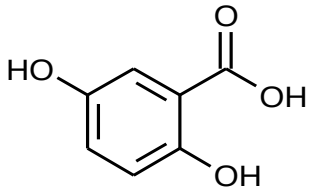
Matrices for UV MALDI:



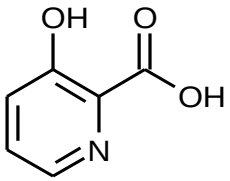
α-Cyano-4-hydroxycinnamic acid
(CHCA or HCCA), peptides



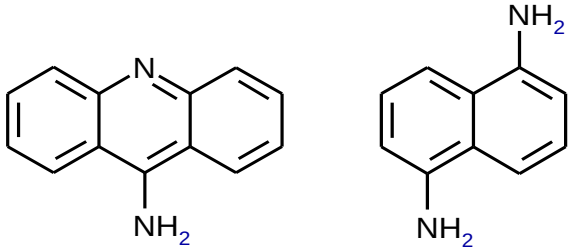
Sinapic acid (SA)
larger peptides, proteins



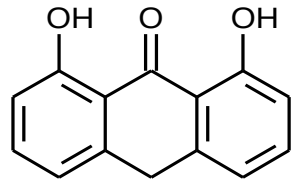
2,5-Dihydroxybenzoic acid (DHB)
general use, lipids, proteins, peptides



3-Hydroxypicolinic acid
(3-HPA), oligonucleotides



9-Aminoacridine (9-AA)
1,5-Diaminonaphthalene (DAN)
for neg. mode, e.g. oligonucleotides, lipids, glycans

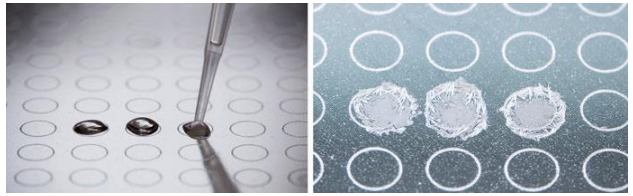


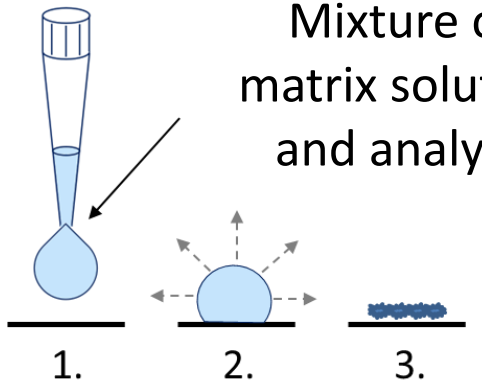
Dithranol (DIT)
Syntetické polymery

Sample preparation for MALDI

Sample preparation for MALDI is a key step in the analysis. It determines the quality of the sample, i.e. its homogeneity and ability to provide quality spectra with good sensitivity and reproducibility.

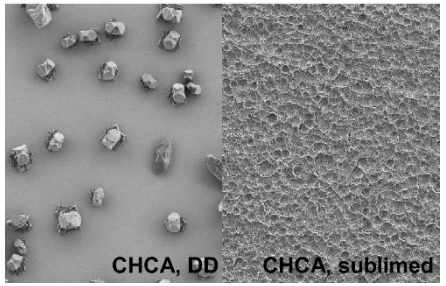
Dried droplet method: matrix-sample solution is mixed (analyte:matrix ratio 1:1000 to 1:10,000), applied to the plate and the solvent is allowed to evaporate





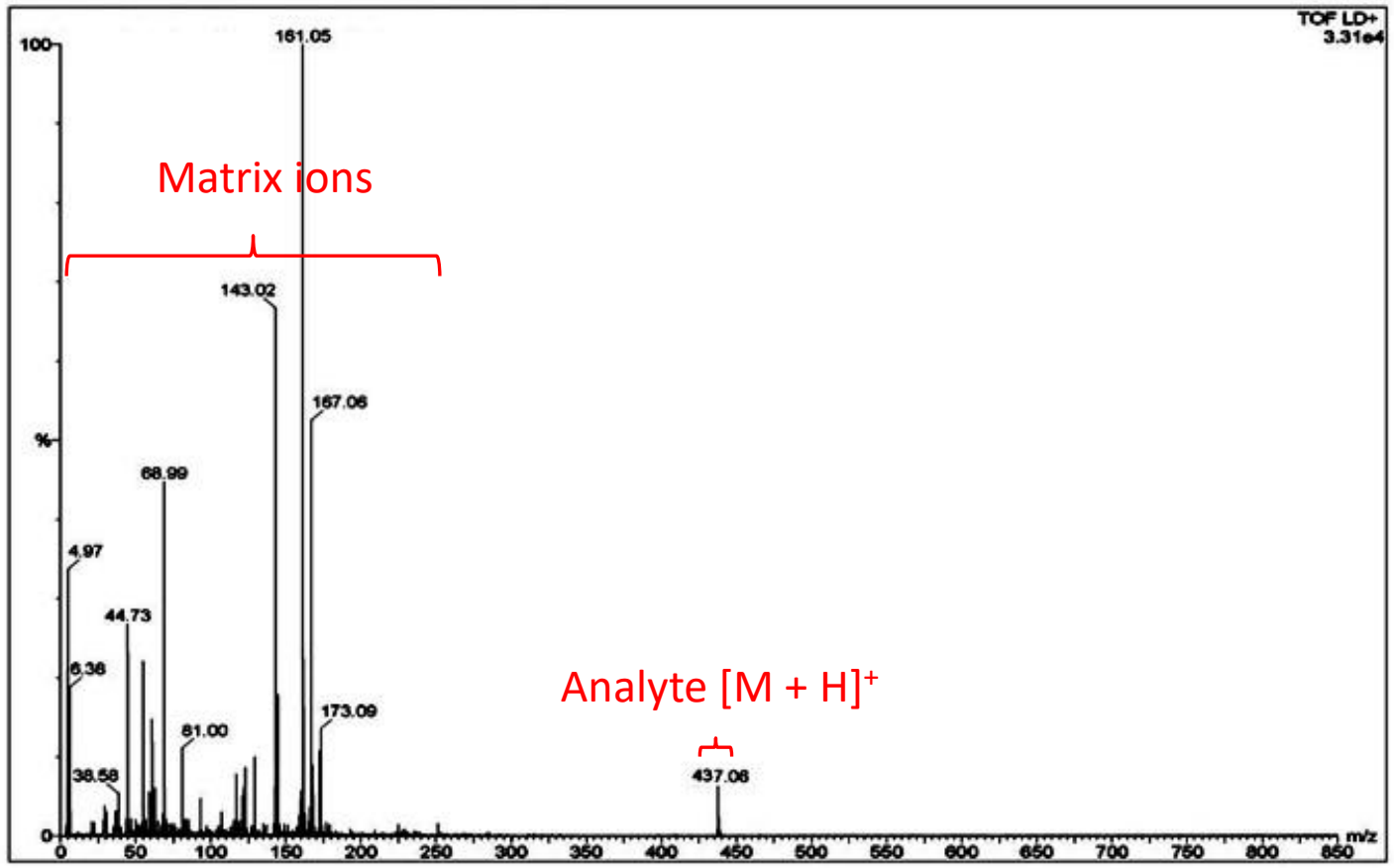
Other application methods:

- Gradual application in the order Sa/Ma, Ma/Sa, Ma/Sa/Ma
- sublimation of the matrix to the sample surface
- Mechanical spraying of the matrix
- Electrospraying of the matrix



Pictures from Jürgen H Gross, Mass Spectrometry: A Textbook; https://doi.org/10.1016/j.jasms.2009.02.010

MALDI spectra

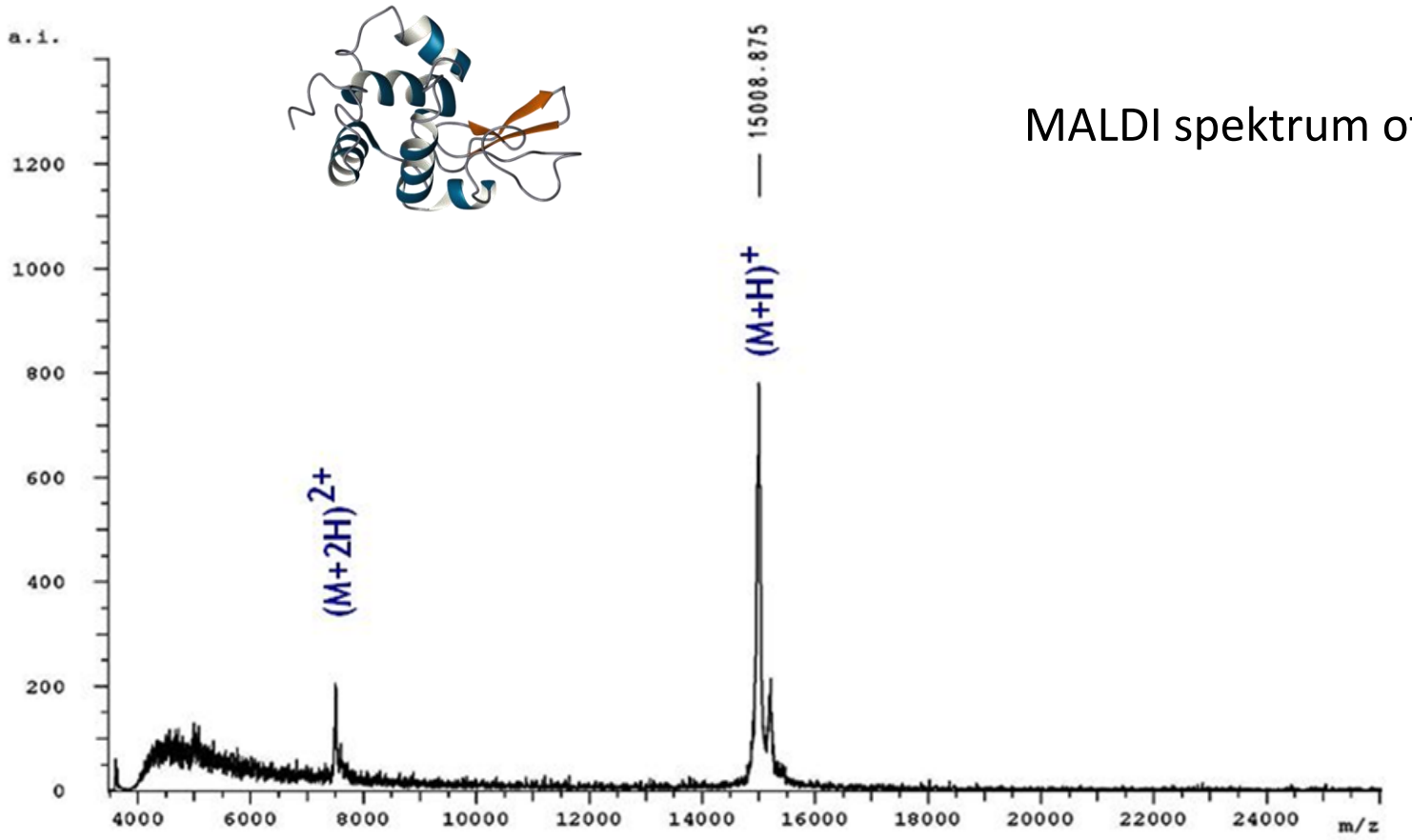


Singly charged ions.

Low m/z regions of MALDI spectra often show intense matrix-derived signals (e.g., fragments, degradation products, adducts)

→ Spectra are typically recorded from higher *m/z*.

MALDI spectra



MALDI spektrum of lysozyme

In the MALDI spectra of peptides and proteins, only single-charged ions are usually observed. The multiple-charged ions known from ESI are (almost) missing.

MALDI: Summary

Major Application Area: Large (bio)molecule and synthetic polymers, insoluble samples

Advantages: 

- Enables soft ionization of large, labile molecules with minimal fragmentation.
- Produces mostly singly charged ions, simplifying spectral interpretation.
- Highly effective for high-mass analytes.

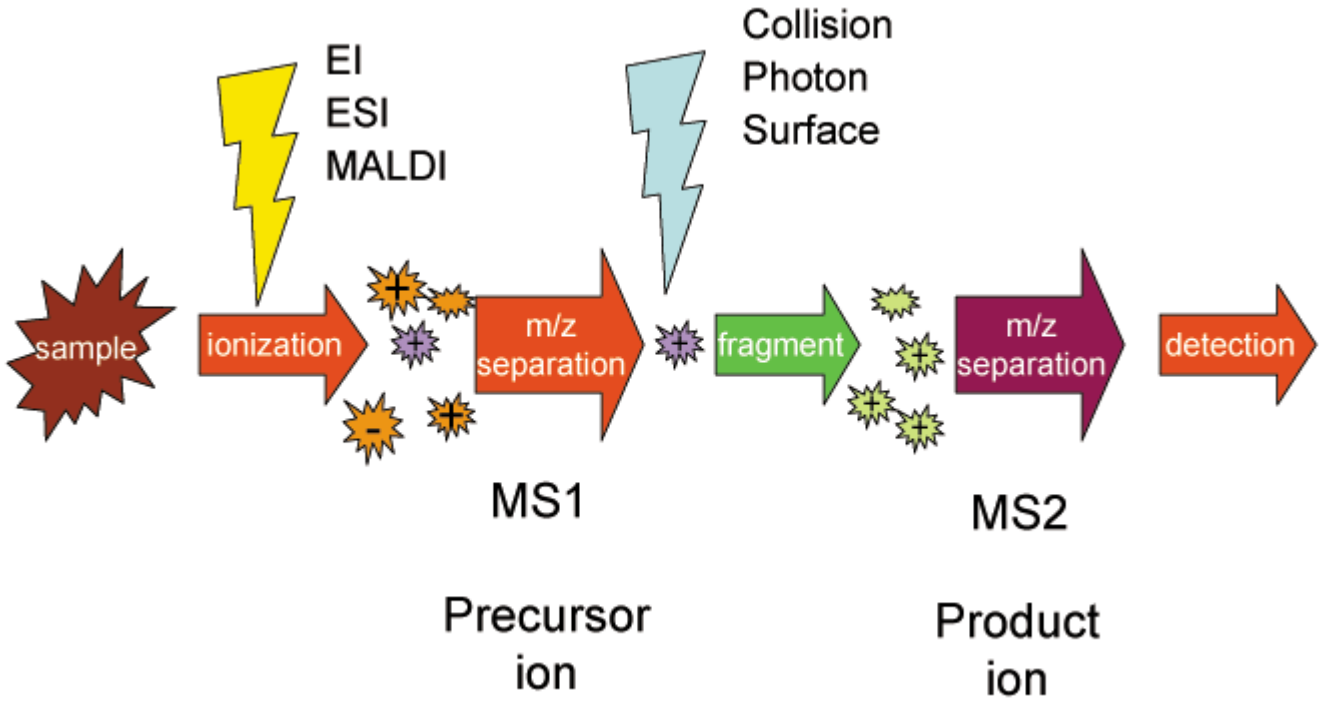
Limitations: 

- Less effective for small molecules (<500 Da) due to matrix interference.
- Requires co-crystallization with a suitable matrix; matrix needs to be optimized

Tandem mass spectrometry

Tandem mass spectrometry: methods that involve two or more consecutive mass spectrometric analyses separated from each other by ion fragmentations.

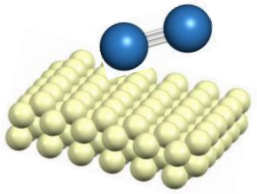
Used for the identification, quantification and structural characterization of substances.



Picture K. Murray, https://commons.wikimedia.org/w/index.php?curid=1943319

Tandem mass spectrometry

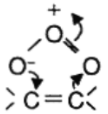
Activation is the supply of energy to ions in the gas phase in order to induce their fragmentation.



Activation by collision with atoms, molecules and surfaces
CID, SID

Activation by interactions with photons
(UV)PD, IRMPD, BIRD

Activation by interactions with electrons
EID, ECD, ETD

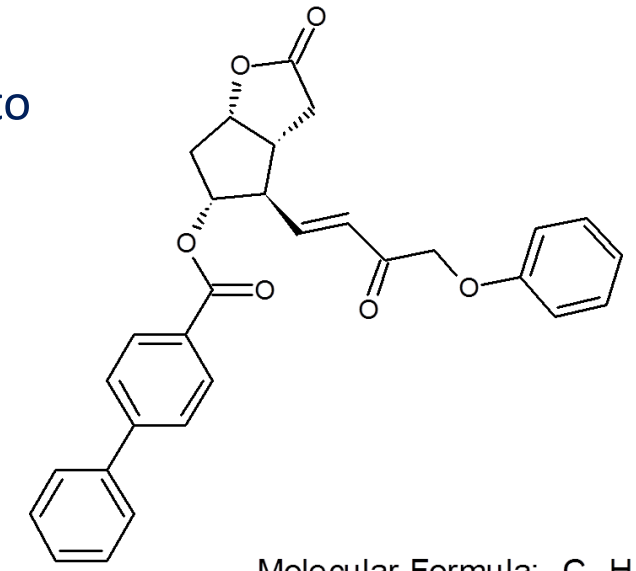


Activation by chemical reaction
OzID

Basic principles of mass spectra interpretation

C:\Xcalibur\data\Pepa\4_ENONCX1_01 3/17/2016 12:43:34 PM ENON-CX1 RO-2-37-2
ESI, ACN/H2O
4_ENONCX1_01 #1-41 RT: 0.00-0.49 AV: 41 NL: 2.37E3
T: ITMS + c ESI Full ms [150.00-600.00]

Can the spectrum correspond to this molecule?



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

General interpretation procedure for mass spectra

1/ Identification of signals unrelated to the analyte

2/ Spectra Library Search

Good Match: GoTo 6 *No or Poor Match: GoTo 3*

3/ Search for ions carrying information about the entire molecule

Determination of molecular weight, determination of elemental composition

4/ Interpretation of fragment ions in MS or MS^n spectra

Spectra interpretation based on knowledge of fragmentation mechanisms, analogies, empirical rules

5/ Structure suggestion

6/ Structure confirmation (comparison of MS and chromatographic data with a standard compound)

I.
Ions, which are not related to the analyte

Background ions, contaminants, co-eluting components

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

- Synthetic byproducts, co-isolated components
- Contaminants from sample handling, solvents, etc.
- Persistent contamination of ion sources, solvent clustering
- Column bleeding peaks (GC/MS)
- Matrix ions (MALDI)

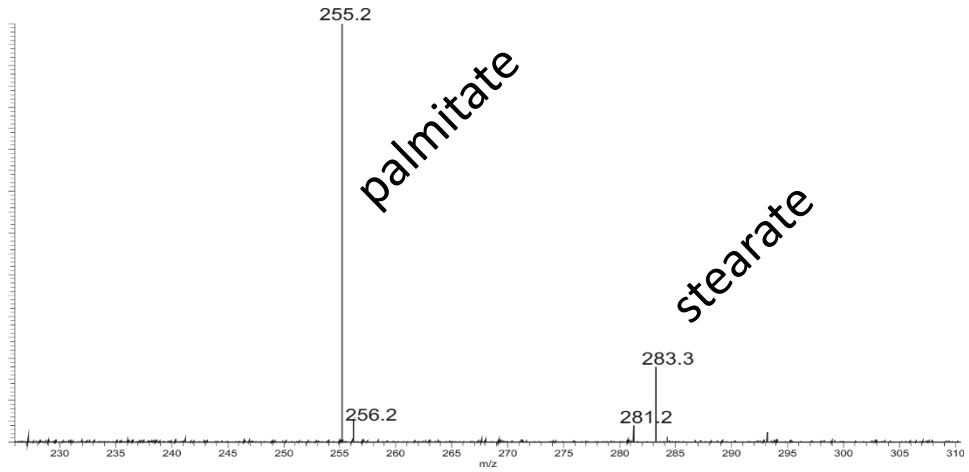
Mass spectrometry Contaminant Database:

MaConDa
Mass spectrometry Contaminants Database

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

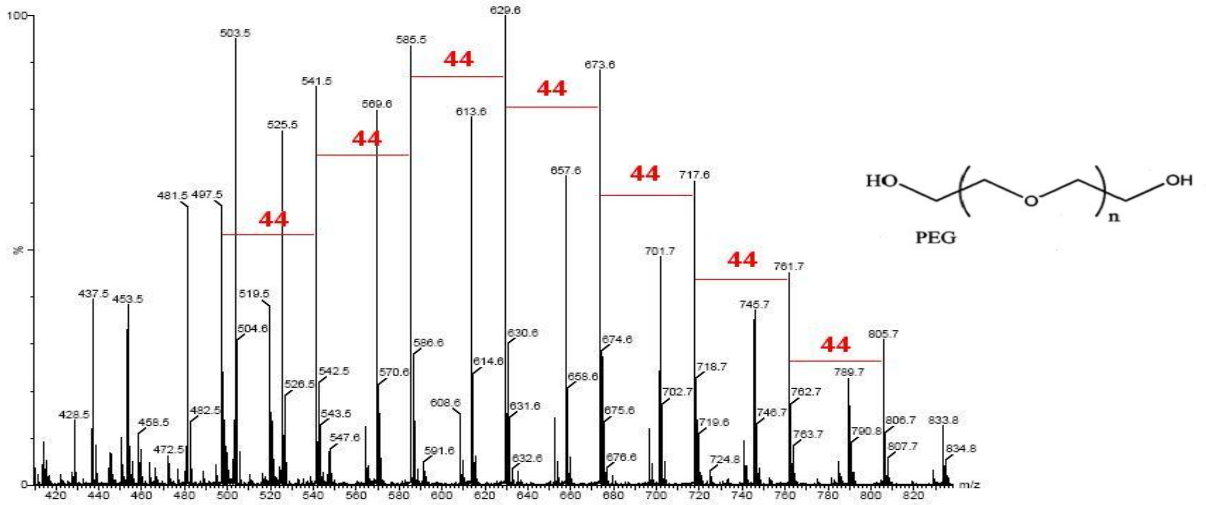
Background ions, contaminants, co-eluting components

ESI-MS: easily ionizable impurities



Negative mode: fatty acids

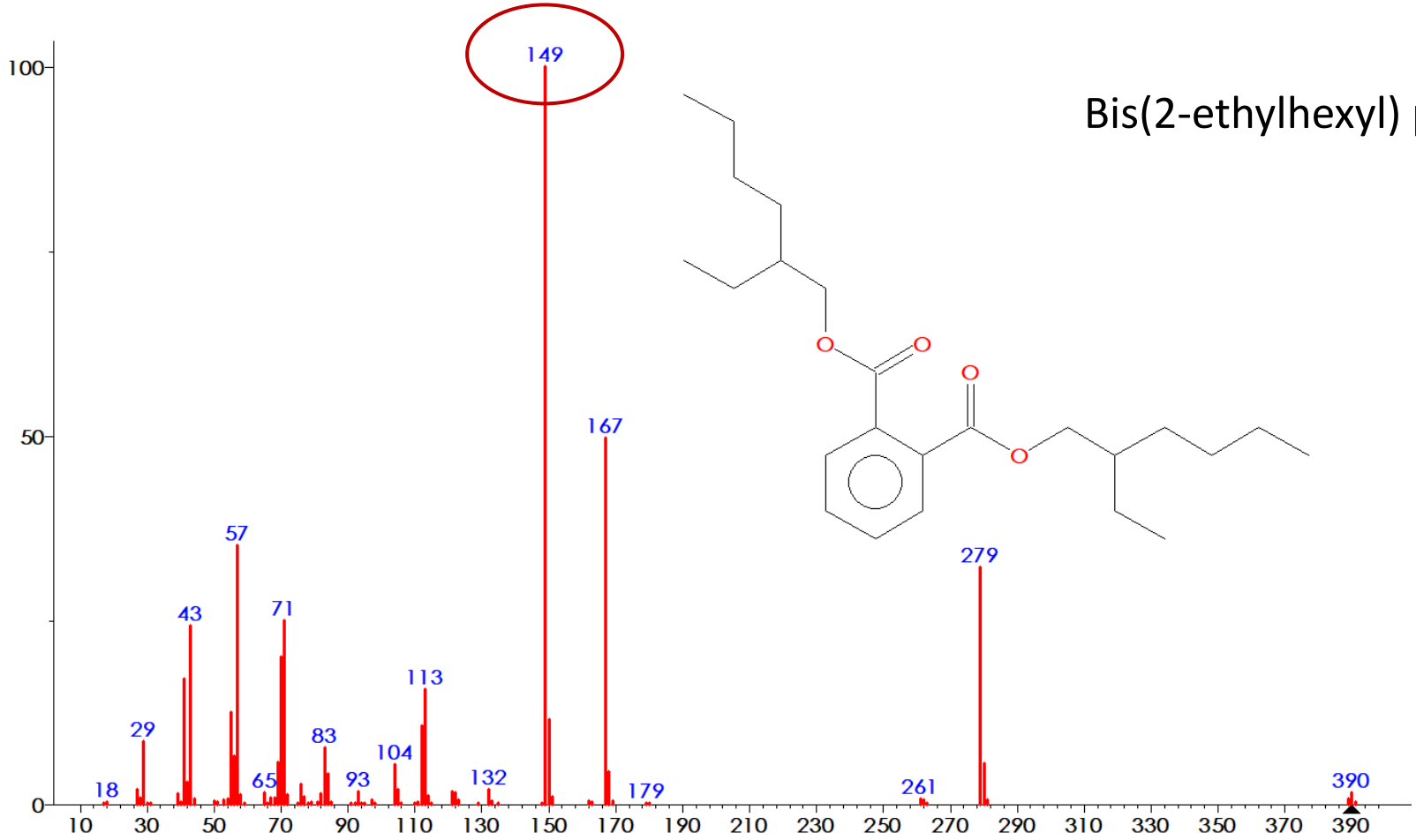
Positive mode: PEGs, phthalates (*m/z* 149, 279, 301, 391, 413 ...)



<u>Polyethylene glycols</u>:
from laboratory plastics, gloves, skin lotion; peak difference 44 Da

Background ions, contaminants, co-eluting components

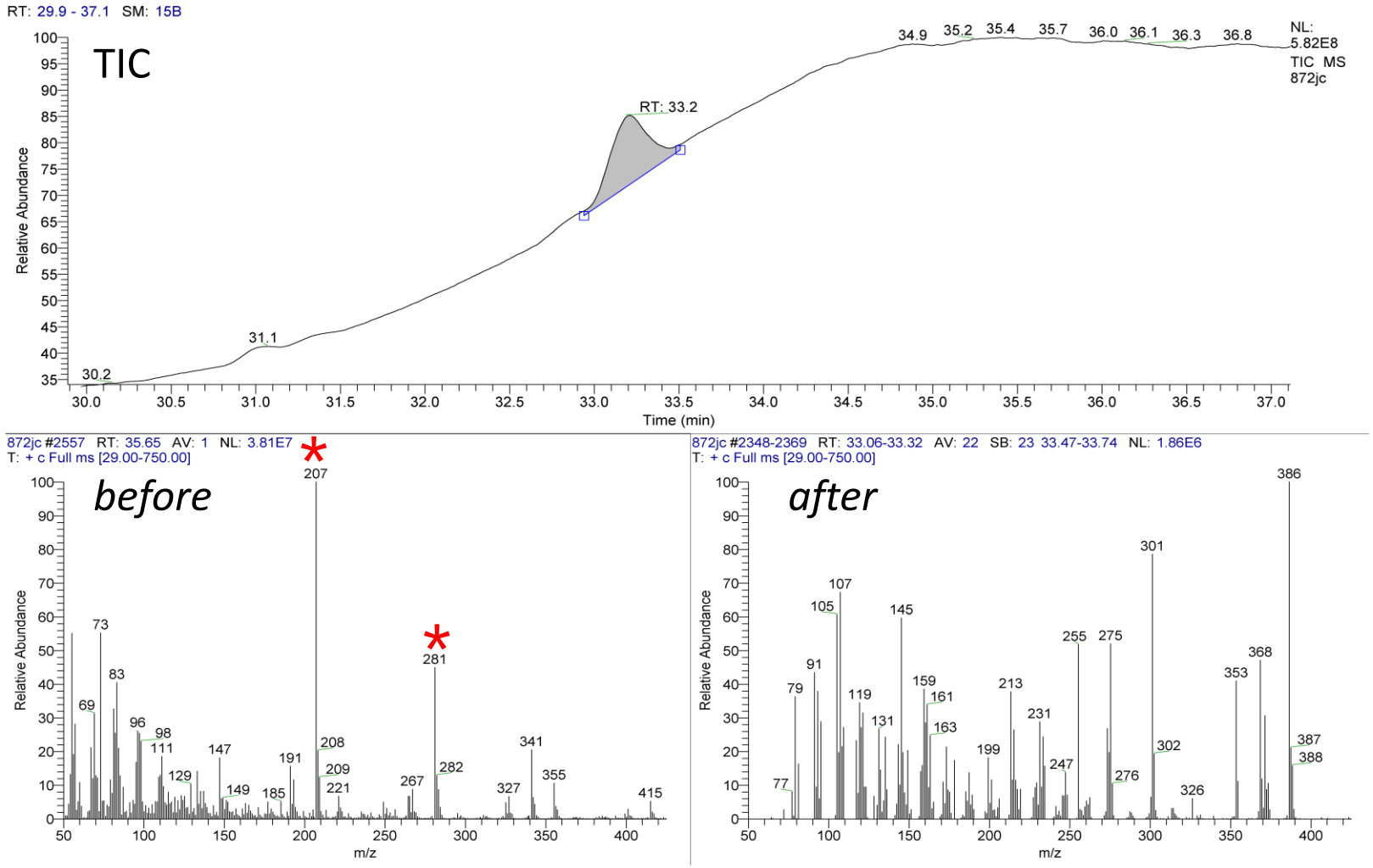
GC/MS: phthalates, *m/z* 149



Bis(2-ethylhexyl) phthalate

Background ions, contaminants, co-eluting components

Background subtraction in chromatographic data.

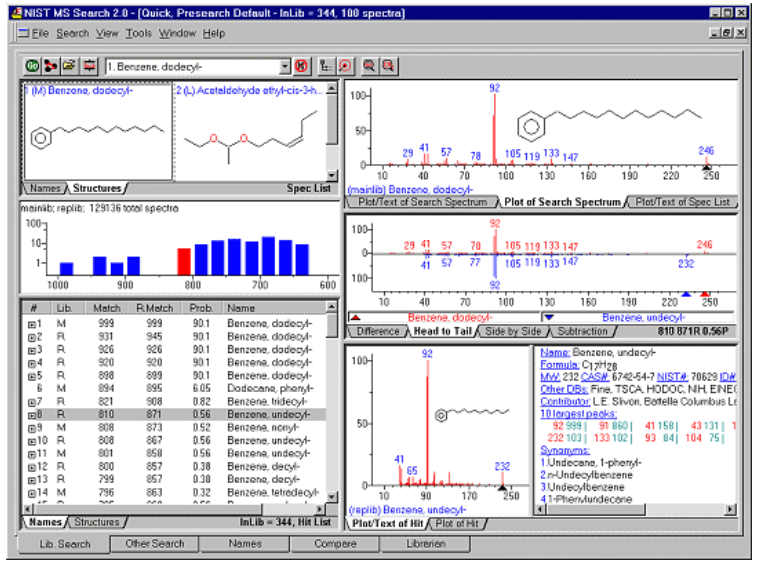


* Siloxanes from GC column bleeding.

II.
Mass spectra library search

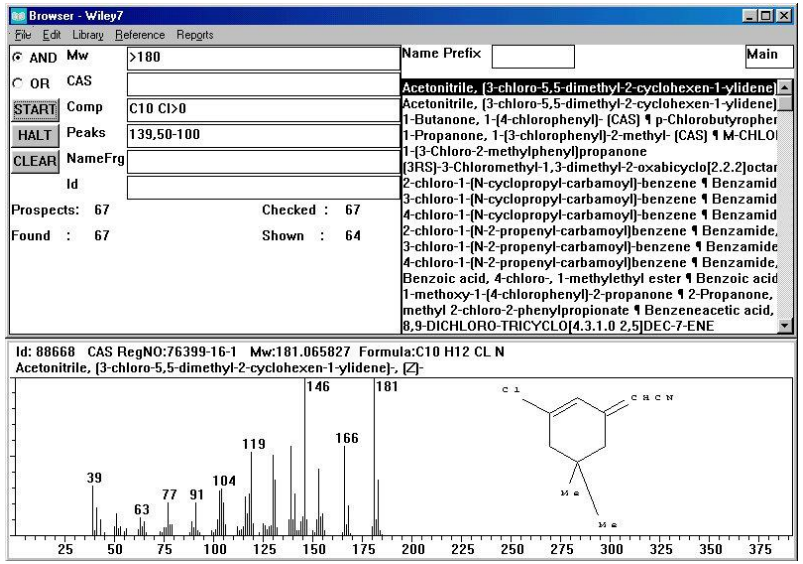
Libraries of EI spectra

NIST/EPA/NIH Mass Spectral Library



394 000 EI spectra (70 eV)
MS/MS spectra of 51,000 substances,
Retention indices of substances
Structural formulas

Wiley Registry of Mass Spectral Data



950 200 EI spectra (70 eV)
Structural formulas

Libraries of soft ionization spectra

Problems with the creation of libraries:

- Spectra strongly depend on the experimental conditions (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

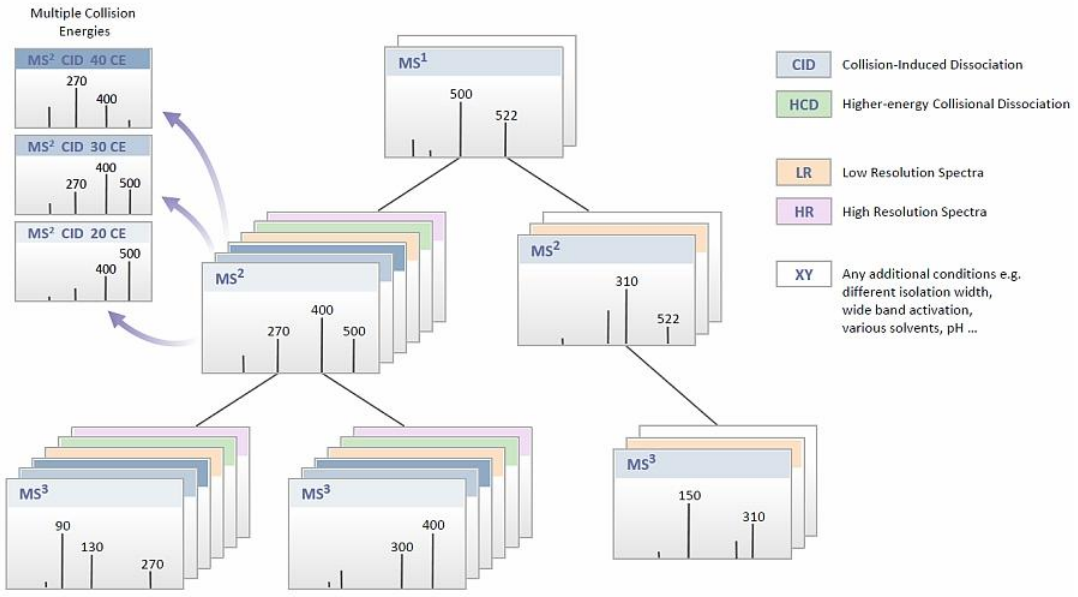
https://www.mzcloud.org/

Libraries of soft ionization spectra: mzCloud

Freely accessible database of spectra, spectral trees, structures, fragments of chromatographic data, links, etc. (2 862 932 spectra, 8 321 compounds)

Spectral tree: database structure of tandem mass spectra

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



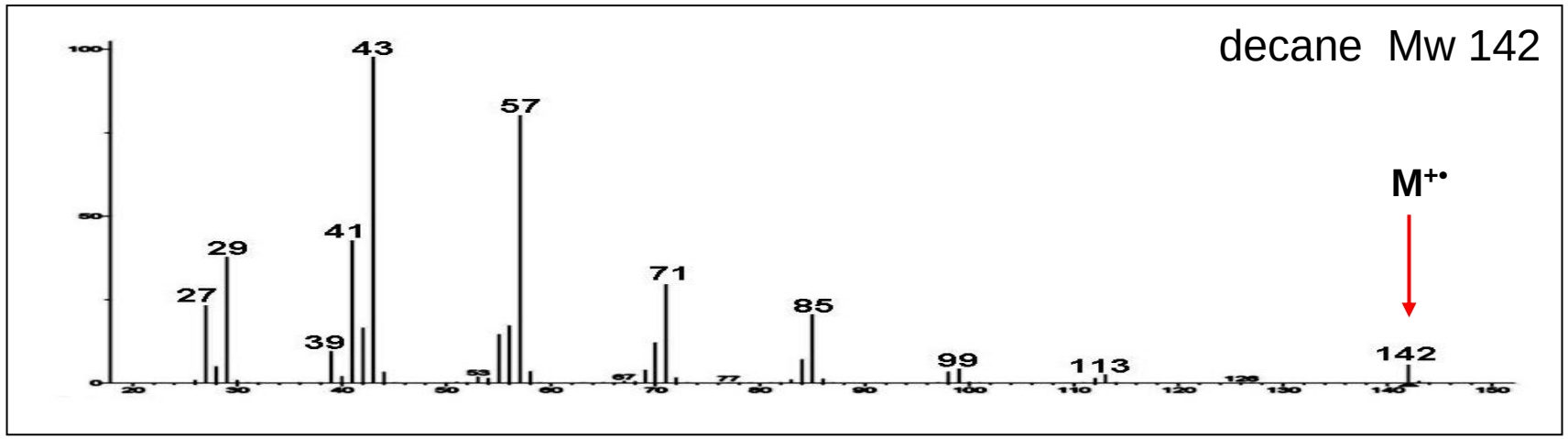
- Free to use for a single compound search
- Works only with Internet Explorer

III.
Molecular ions, molecular adducts and deprotonated molecules

Molecular ion in EI spectra

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

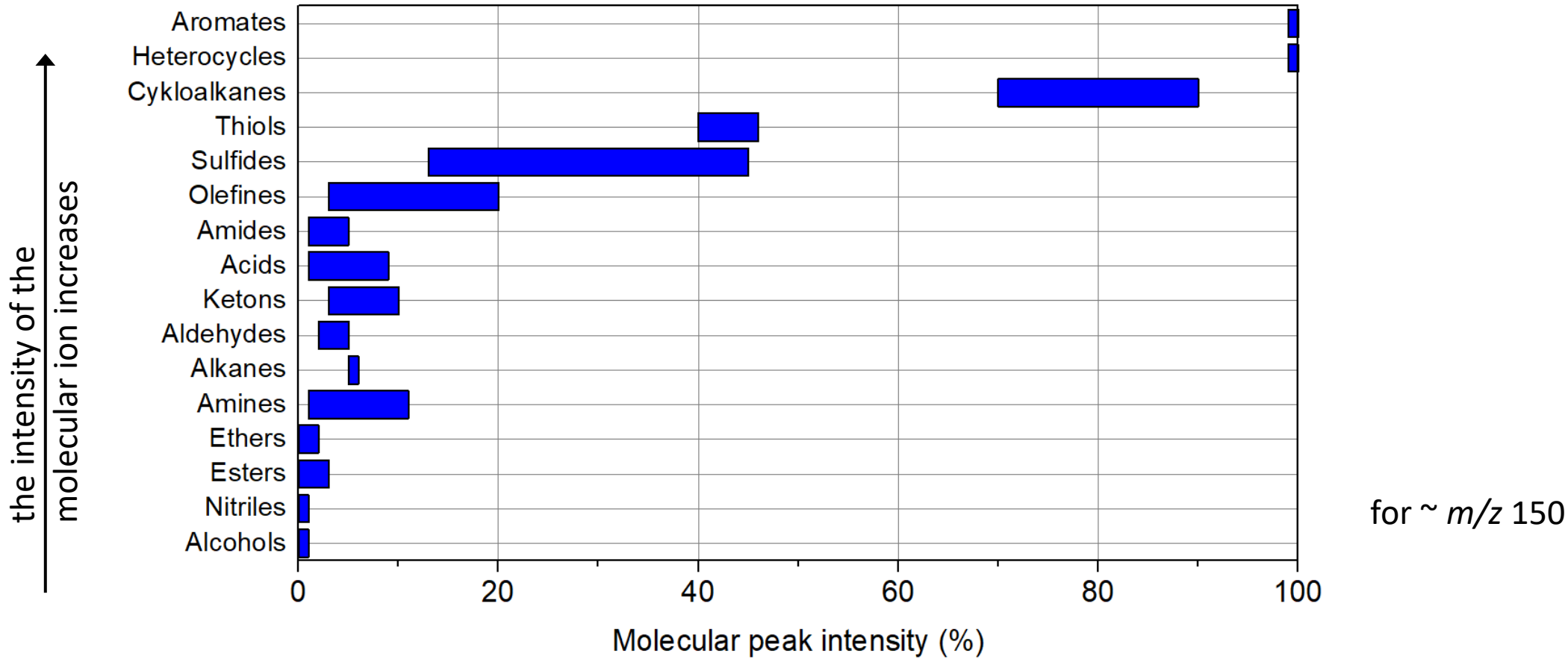
Molecular ion (**$M^{+\bullet}$**) is a radical cation (odd number of electrons, OE $^{+\bullet}$). The *m/z* corresponds to the mass of the analyte.



Identification of the molecular ion in EI spectra:

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

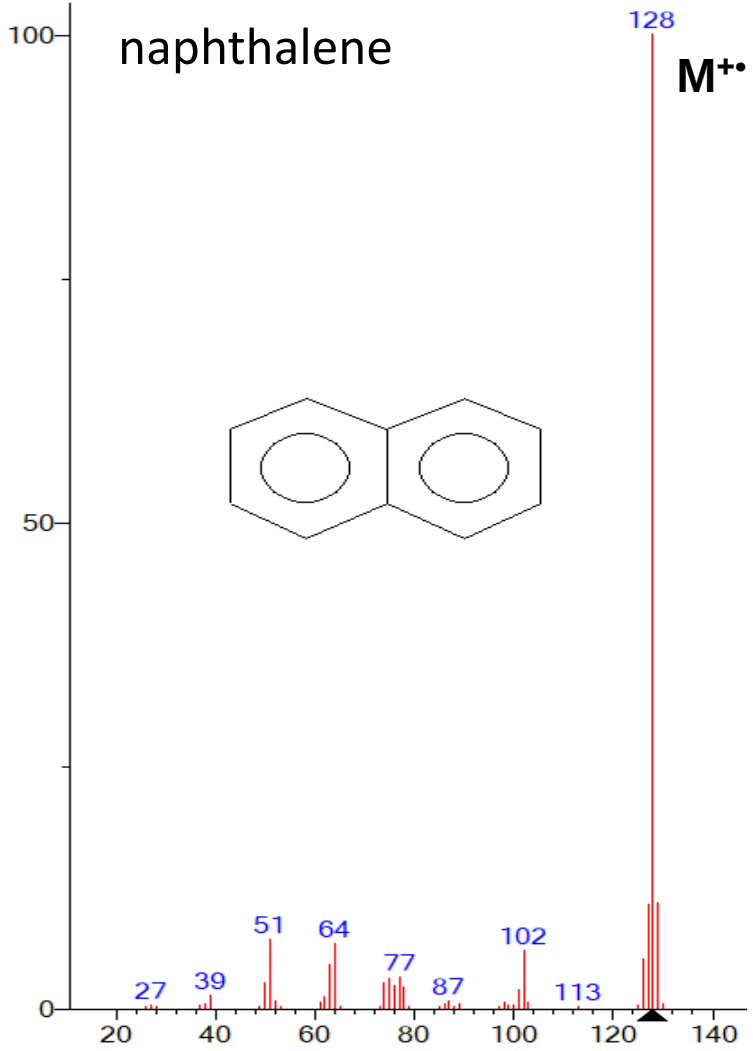
Molecular ion in EI spectra

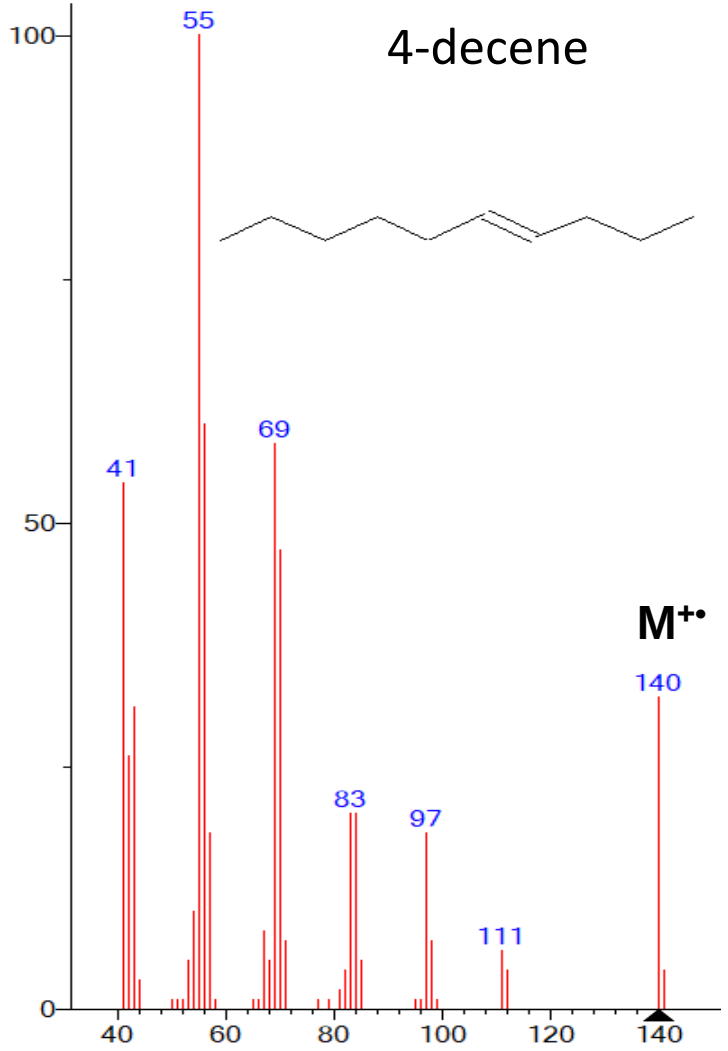


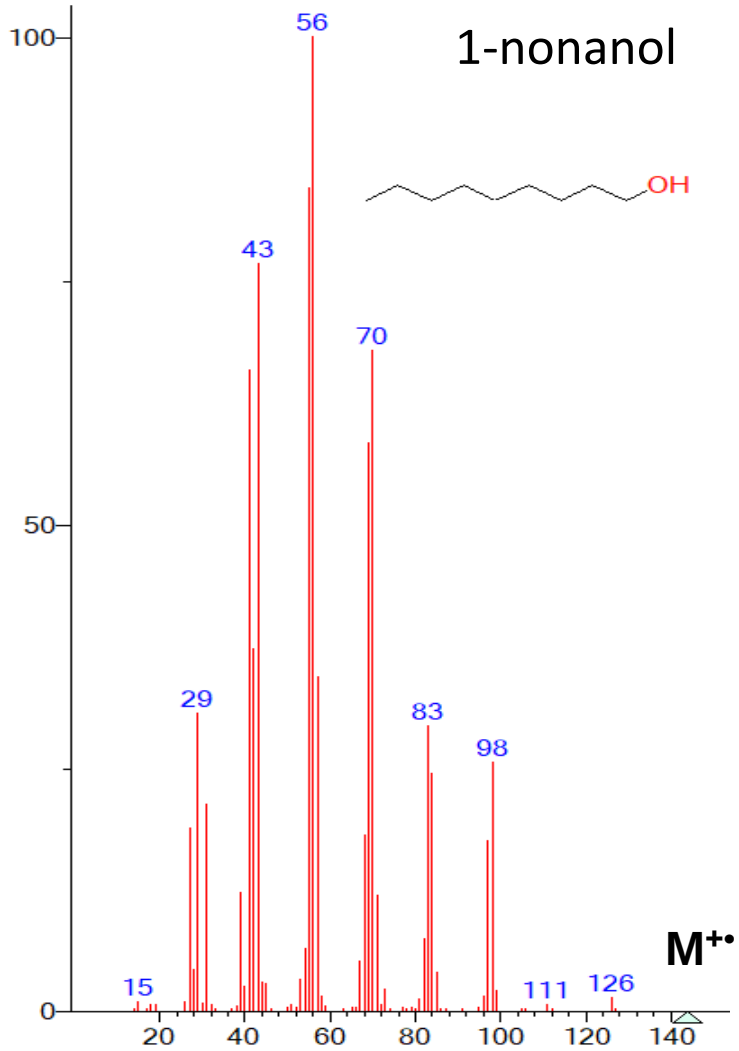
for ~ *m/z* 150

- The intensity reflects stability of the molecular ion
- Type of the compound can be estimated based on molecular peak intensity

Molecular ion in EI spectra

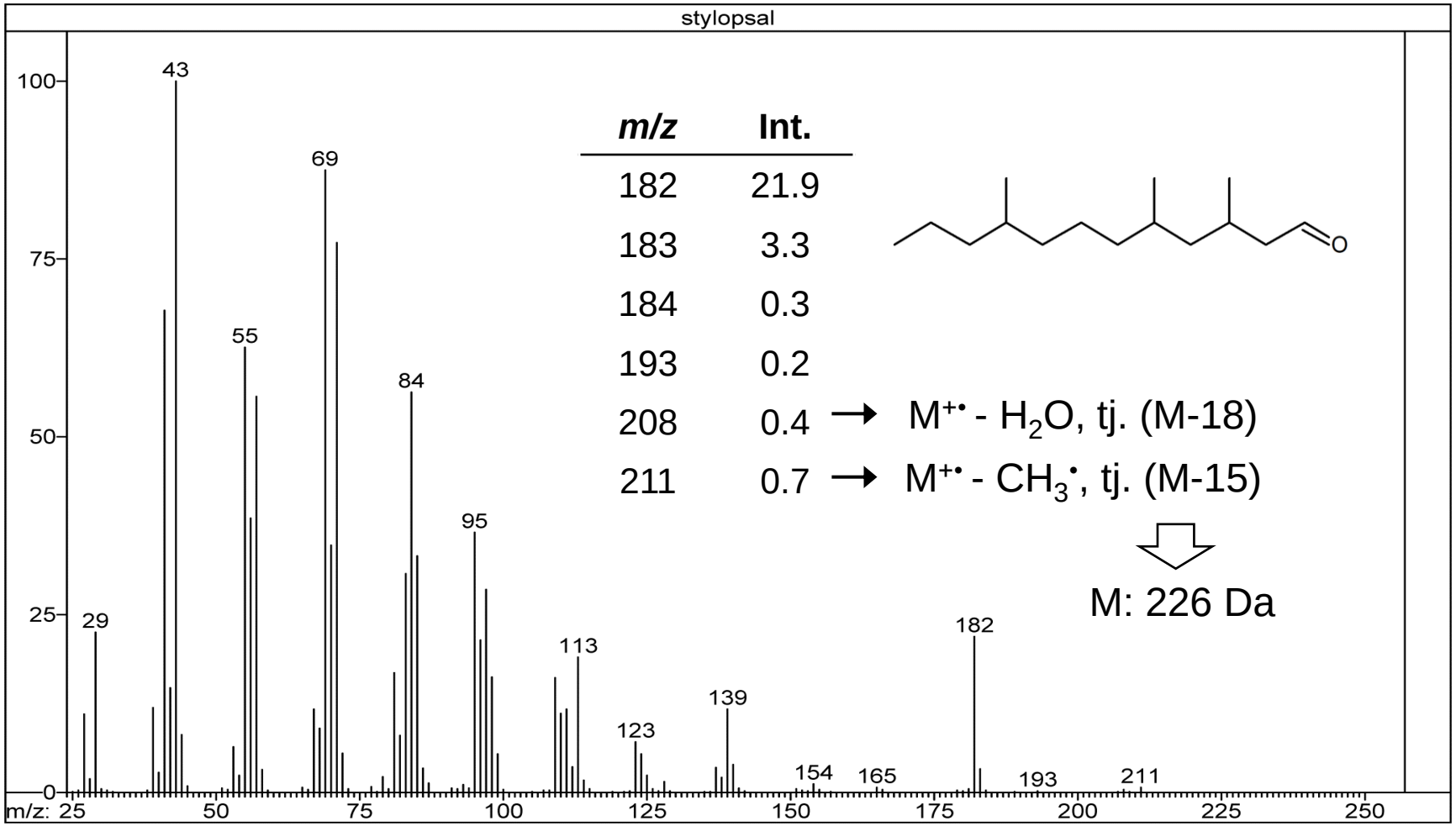






Molecular ion in EI spectra

- if the molecular ion is missing, its m/z can be derived from logical neutral losses

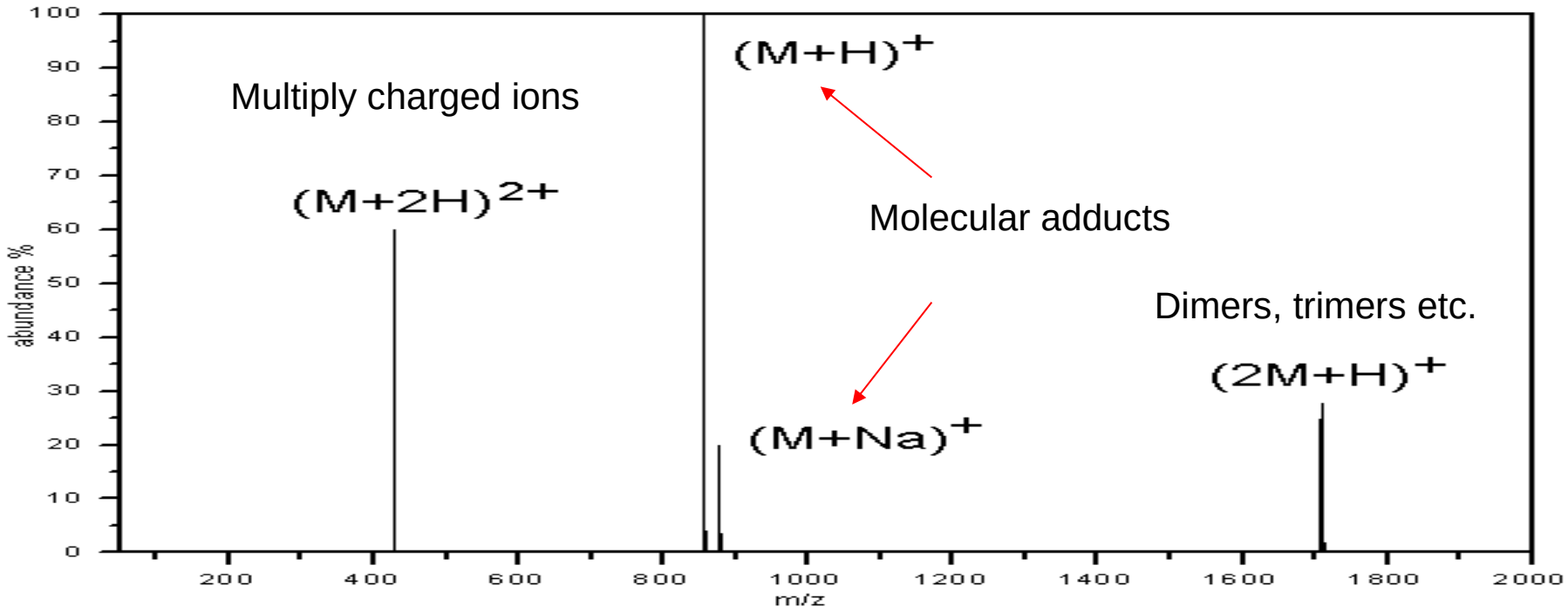


DOI 10.1007/s10886-012-0214-7

Molecular adducts in ESI (and other soft ionizations)

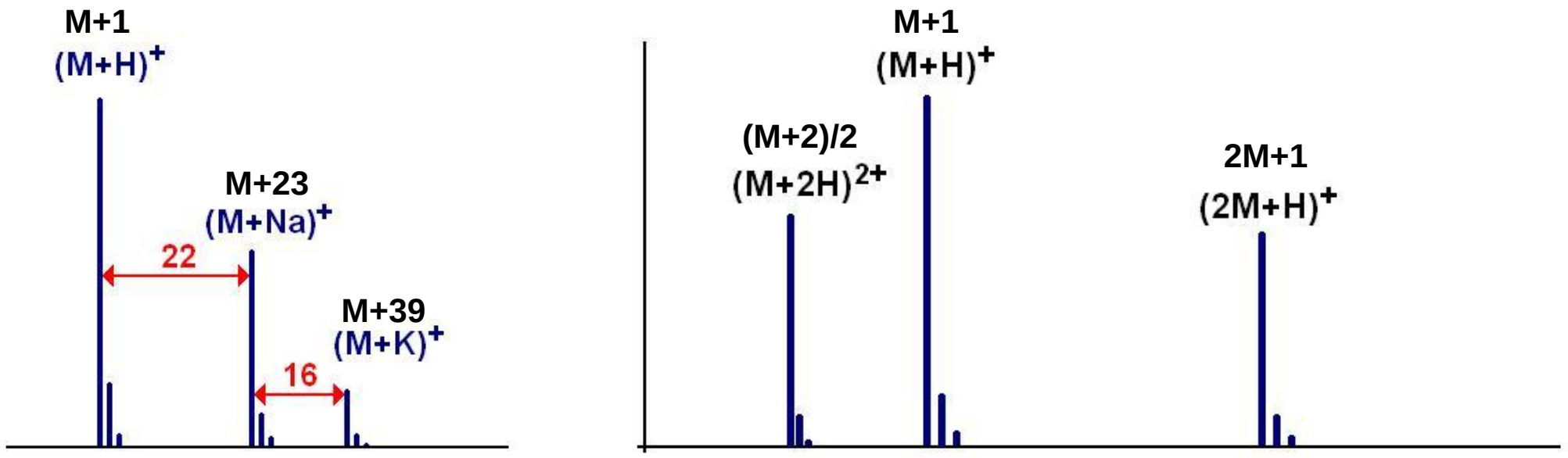
Molecular adducts (**$[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, $[M+Cl]^-$**) or deprotonated molecules (**$[M-H]^-$**)

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



Molecular adducts in ESI (and other soft ionizations)

The molecular weight can be determined based on the presence of adducts, dimers and/or multiply charged ions

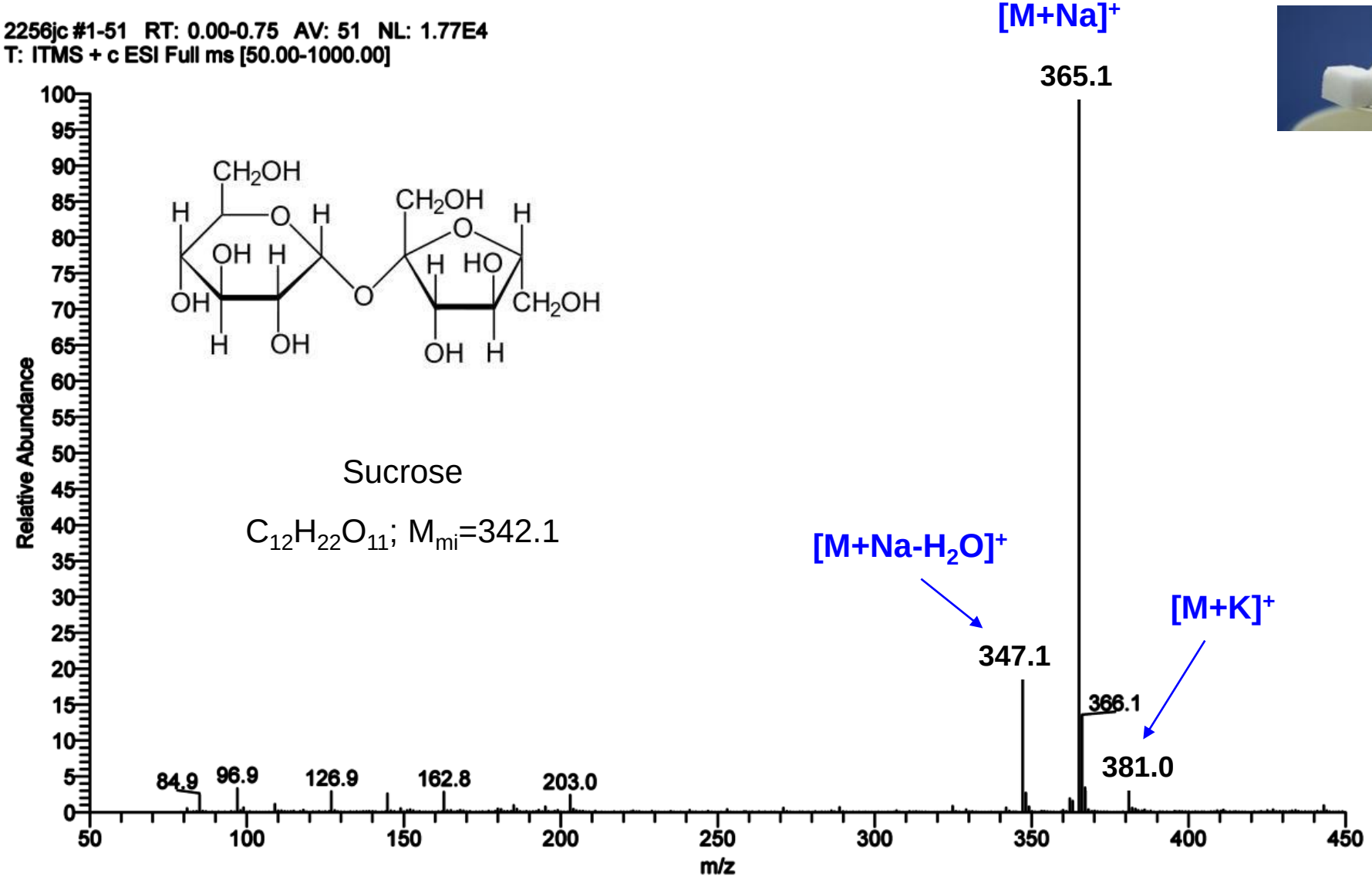


***EIC**: Calculation of adducts, dimers or multiply charged ions available from reQuest/Services*

What is the molecular weight?

MS, ESI+

1

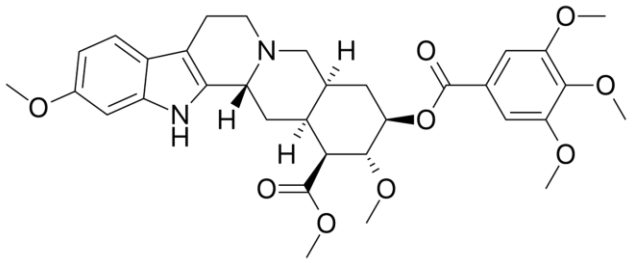


Sucrose

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

MS, ESI+

What is the molecular weight of reserpine?



Reserpine

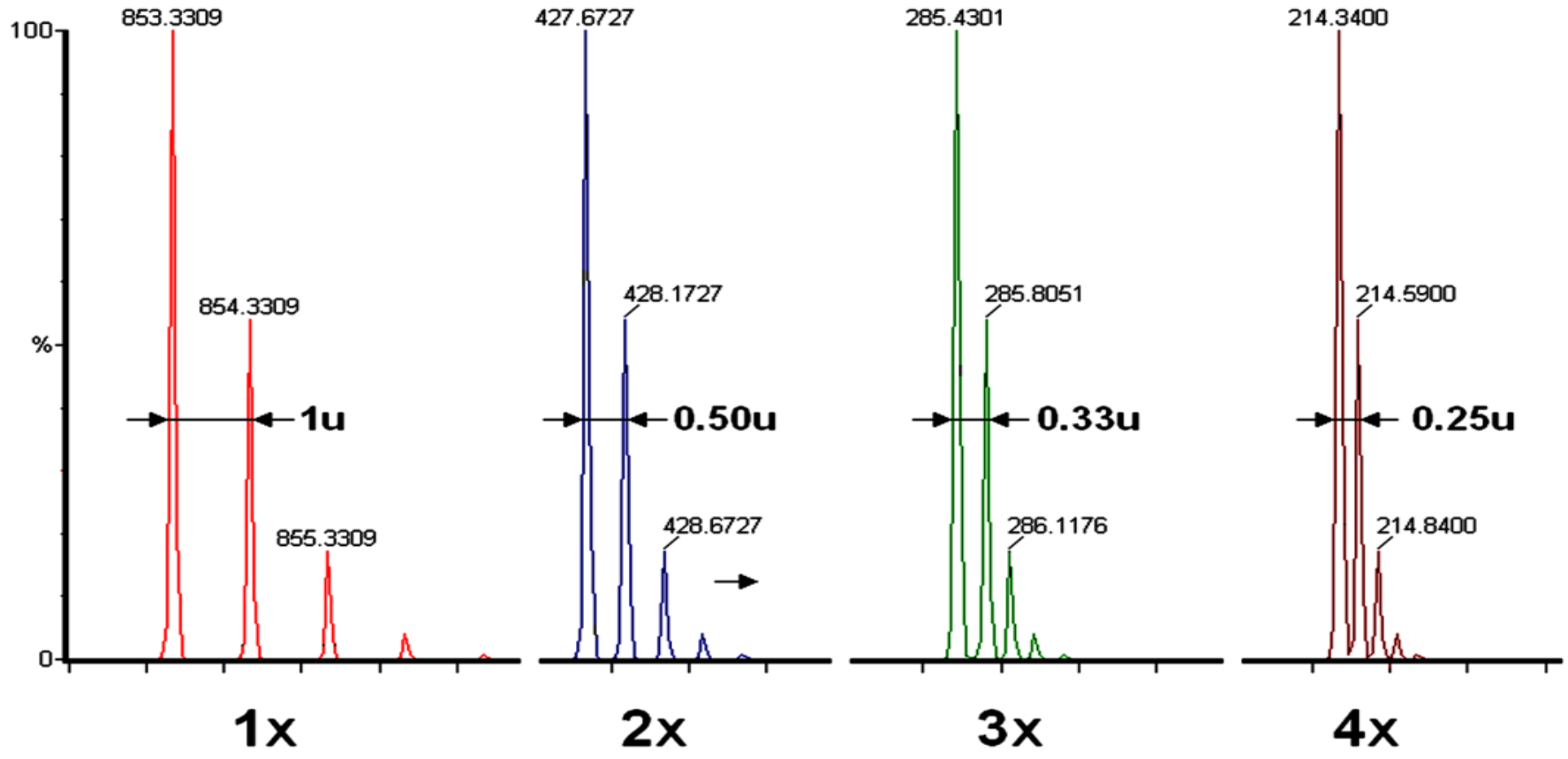
$C_{33}H_{40}N_2O_9$; M_{mi}=608.3

2

Charge state

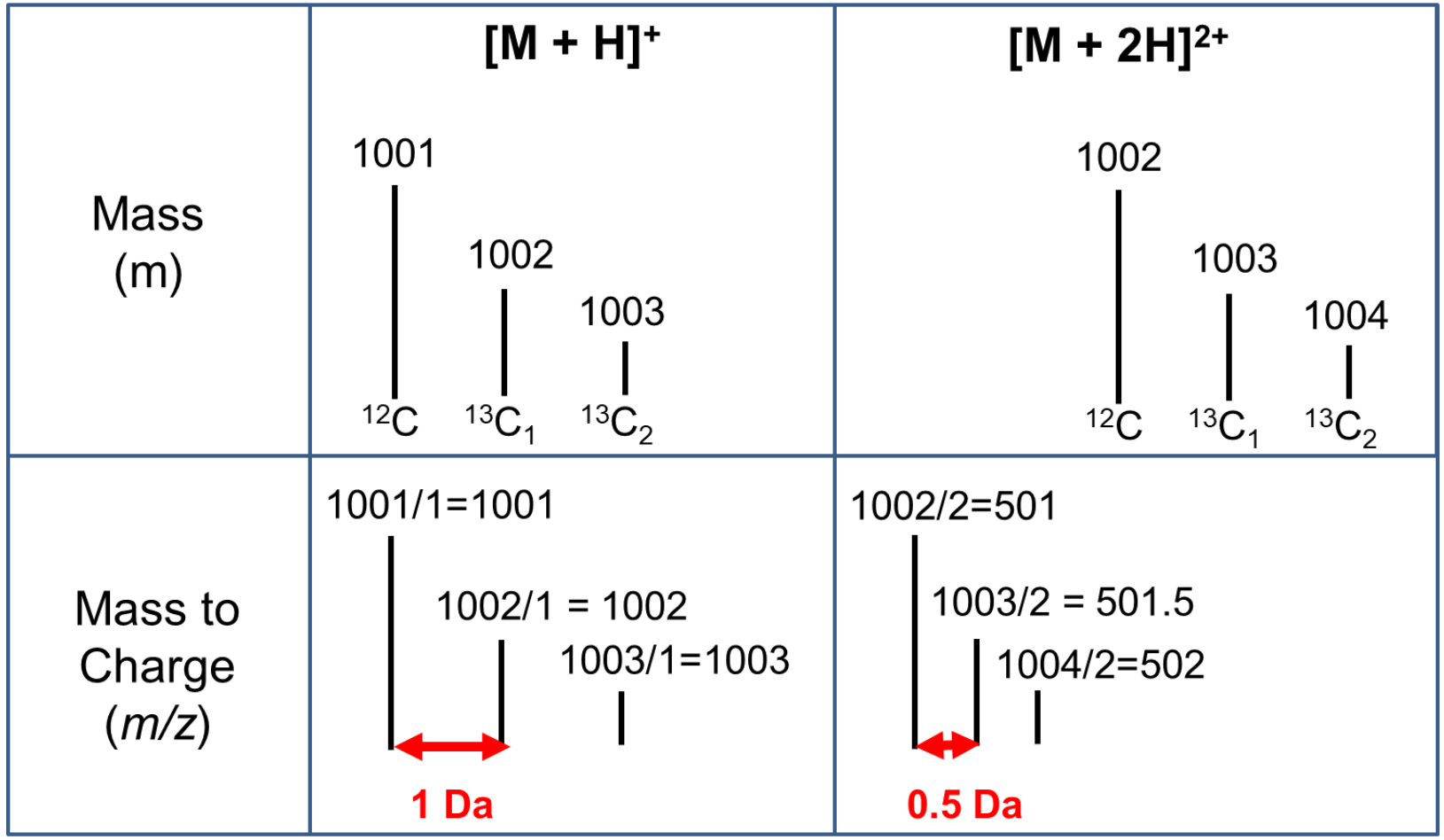
Determining the number of charges

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



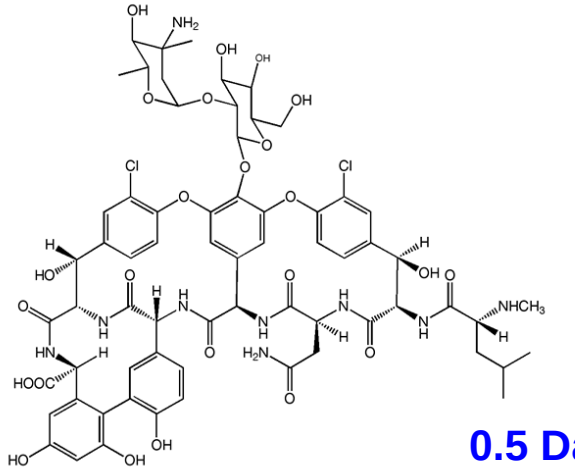
Charge state

Example: a compound with relative mass of 1000



MS, ESI-

What is the molecular weight of vancomycine?



Vancomycine

$C_{66}H_{75}Cl_2N_9O_{24}$; M_{mi}=1447.4

M = (722.7 x 2) + 2 = 1447.4

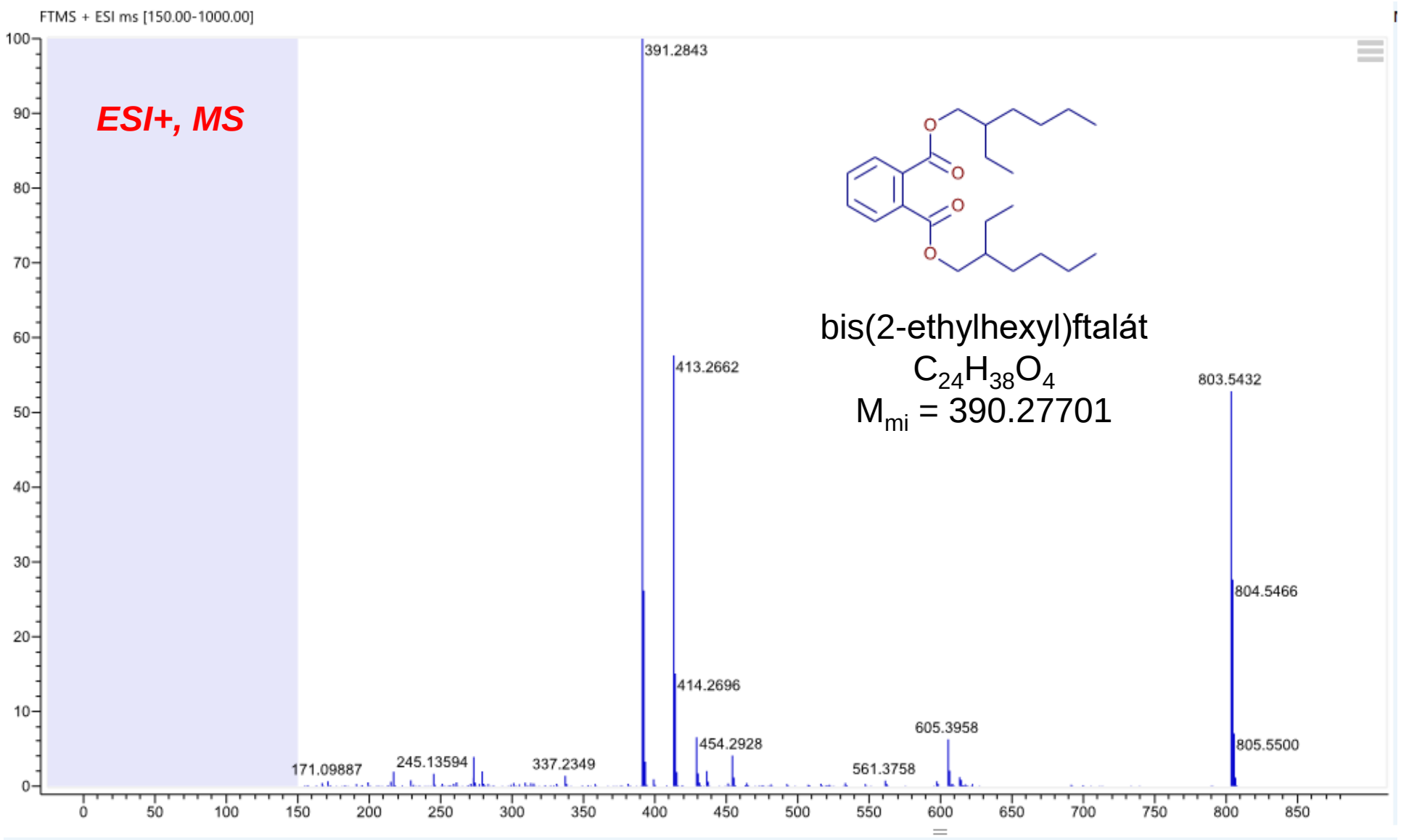
$[M-2H]^{2-}$

722.7

3

MS, ESI+

What is the molecular weight ?

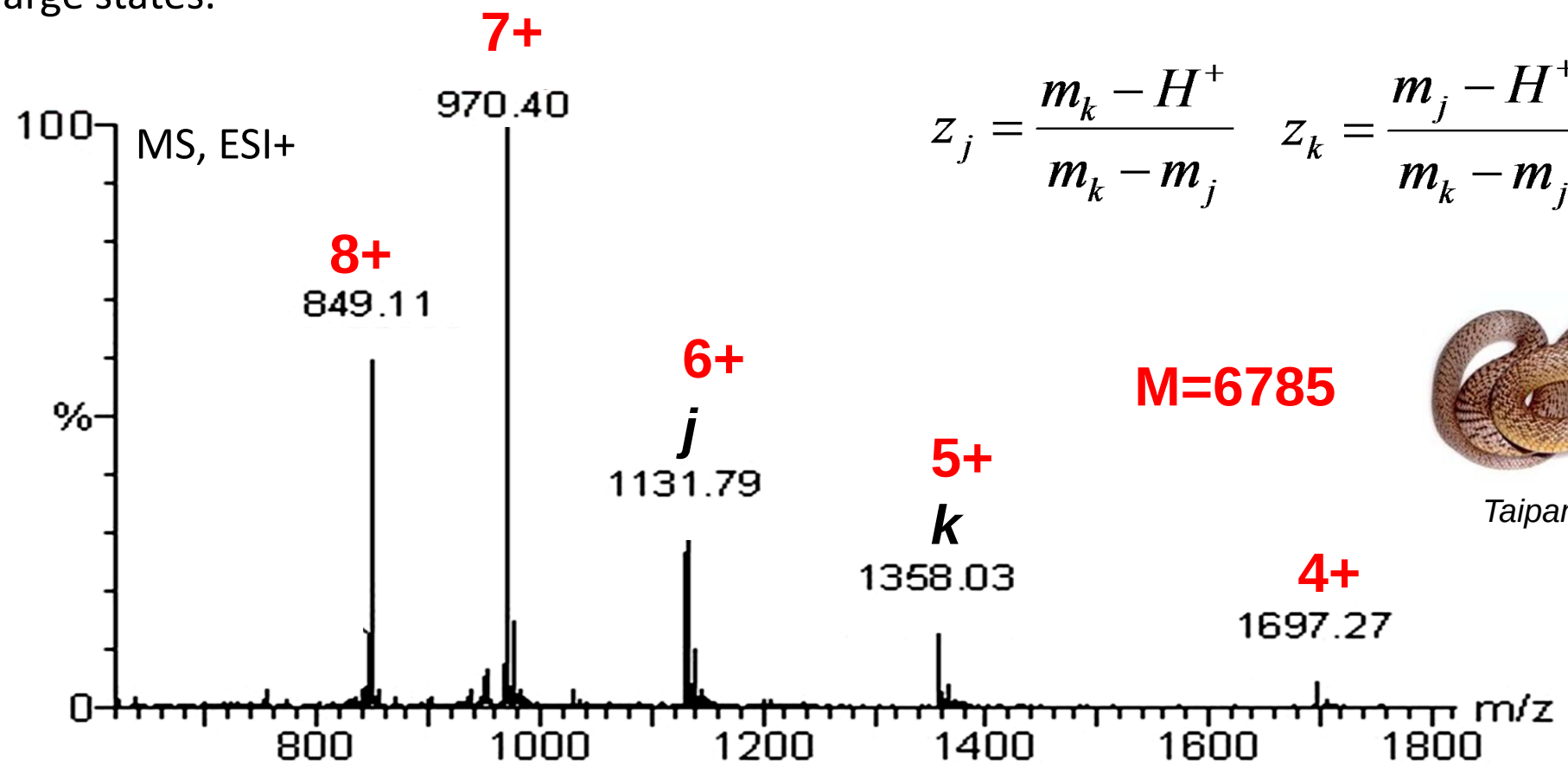


4

Charge state: multiply charged peptides

Determining the number of charges

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



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

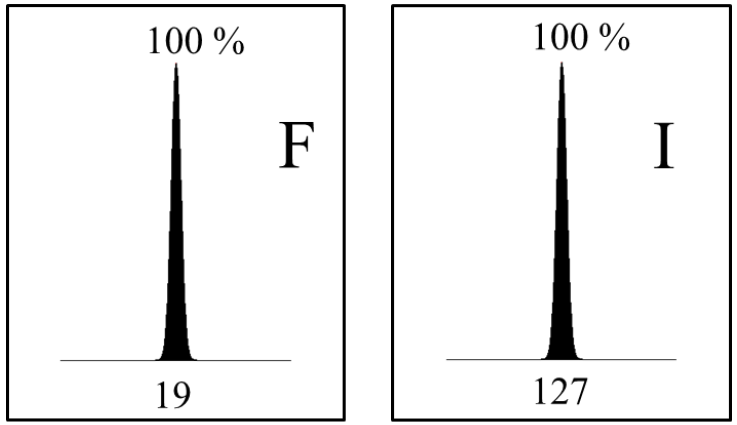
M=6785

Taipan venom

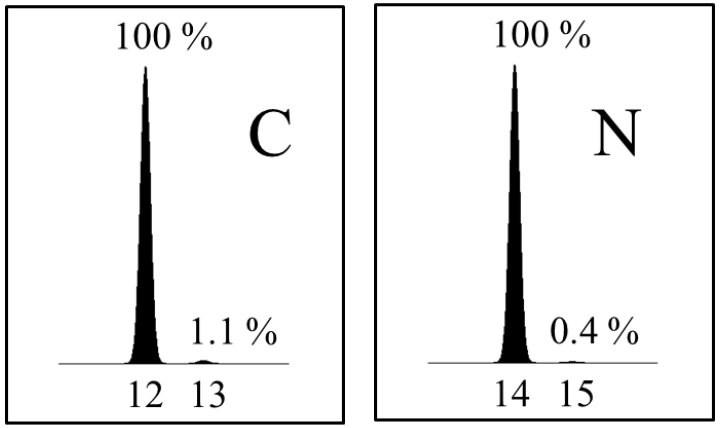
Elemental composition: information from isotope clusters

Isotope clusters of polyatomic ions are linear superposition of clusters of all elements. Ratios of stable isotopes of elements are fixed.

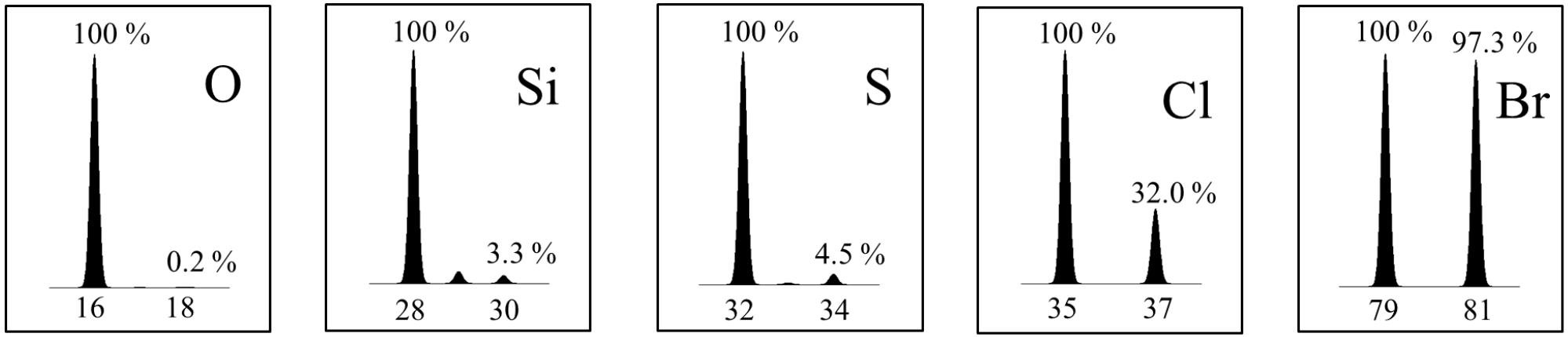
„X“ elements



„X+1“ elements

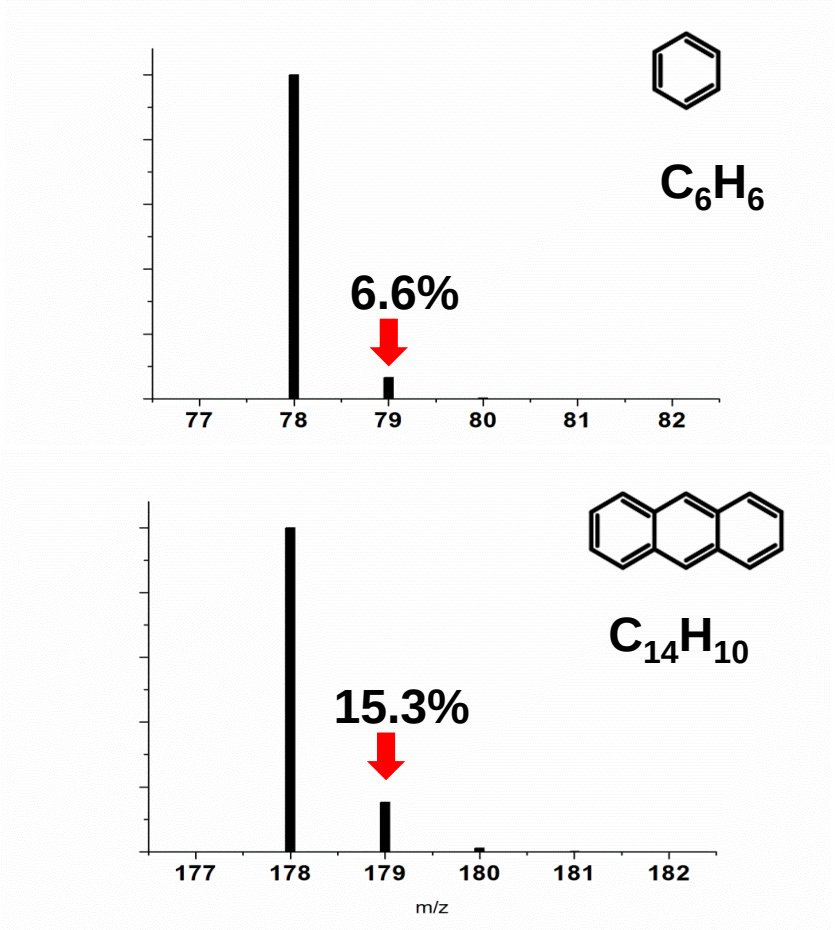


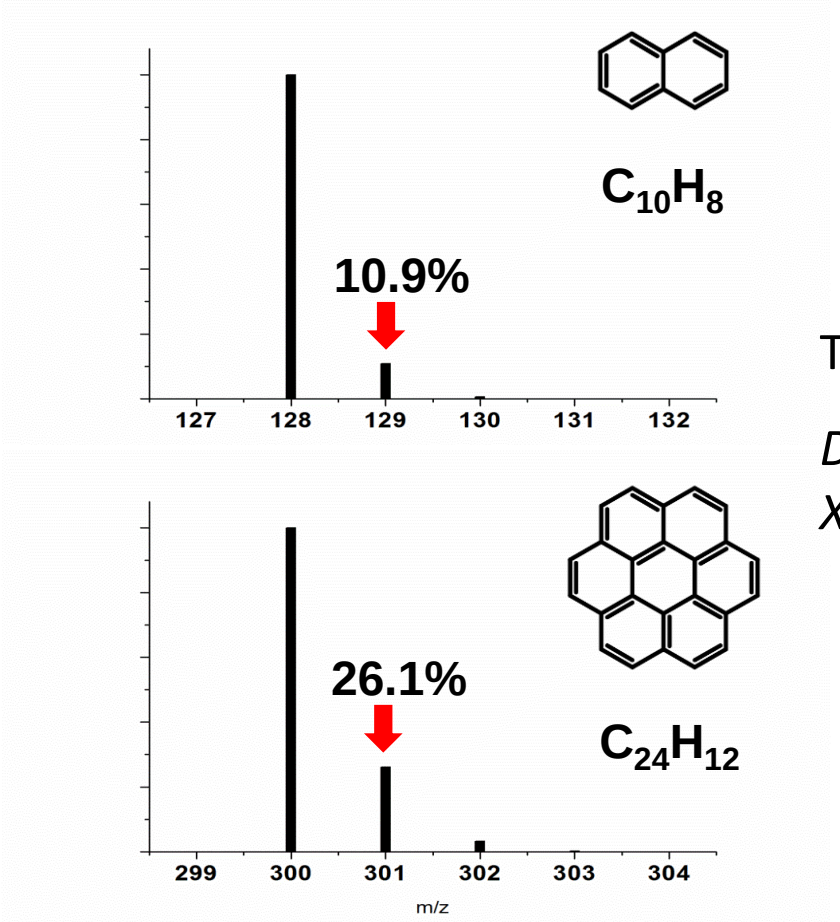
„X+2“ elements



Elemental composition: information from isotope clusters

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%)



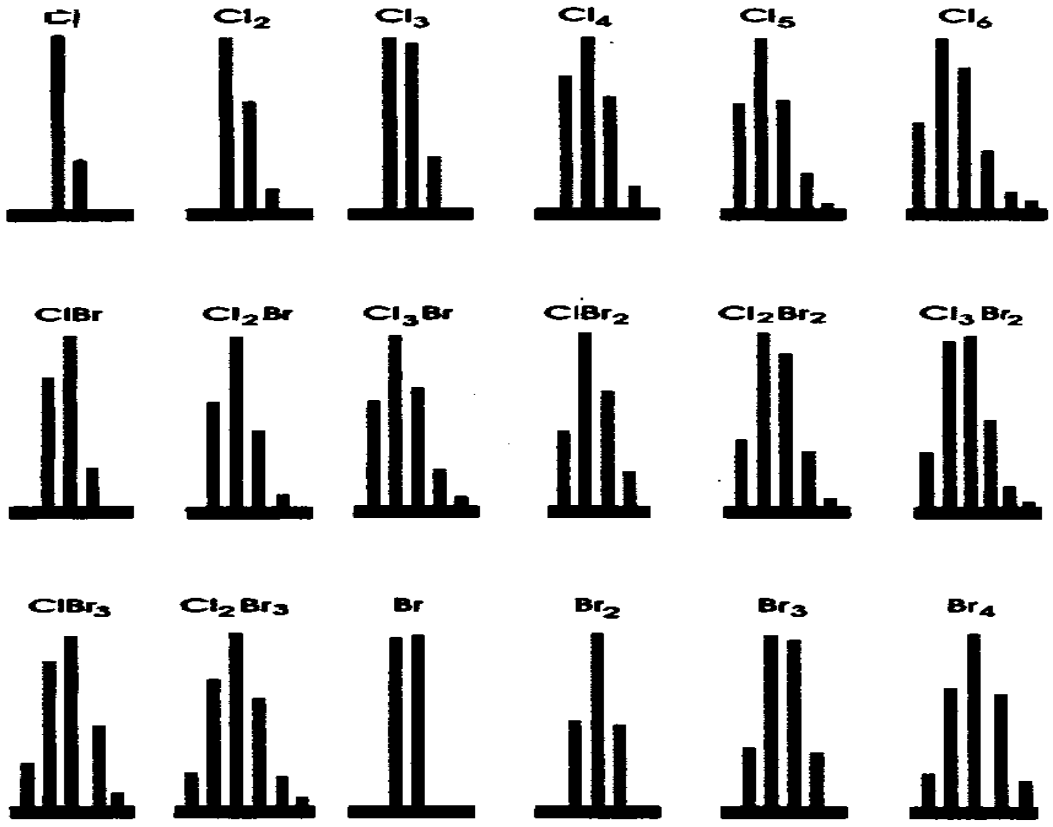


To get the number of carbons:

Divide the relative intensity of X+1 peak by 1.1

Elemental composition: information from isotope clusters

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

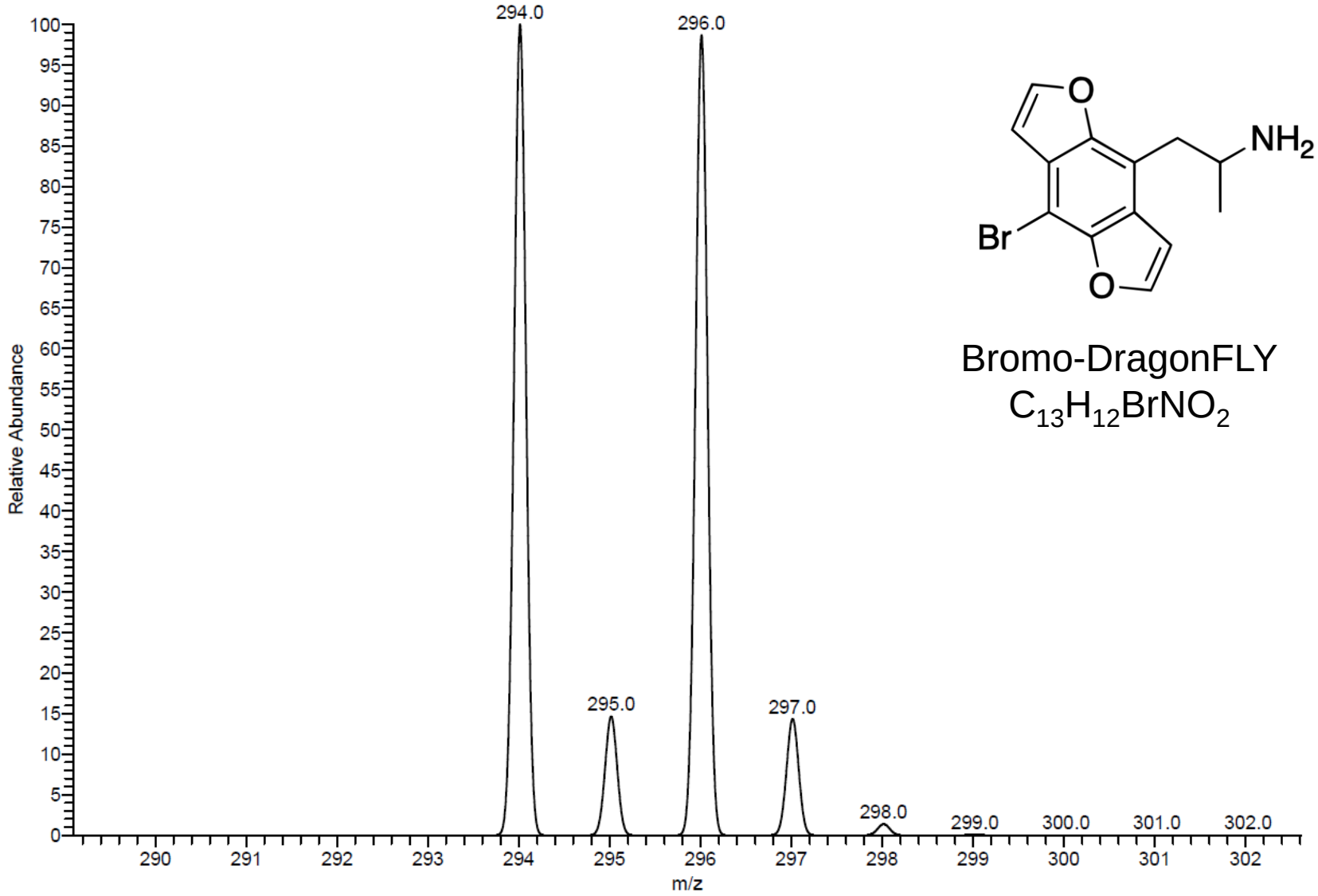


Compare experimental data with simulations of isotope clusters.

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

MS, ESI+

What element would you expect in the structure of this ion ?



Bromo-DragonFLY

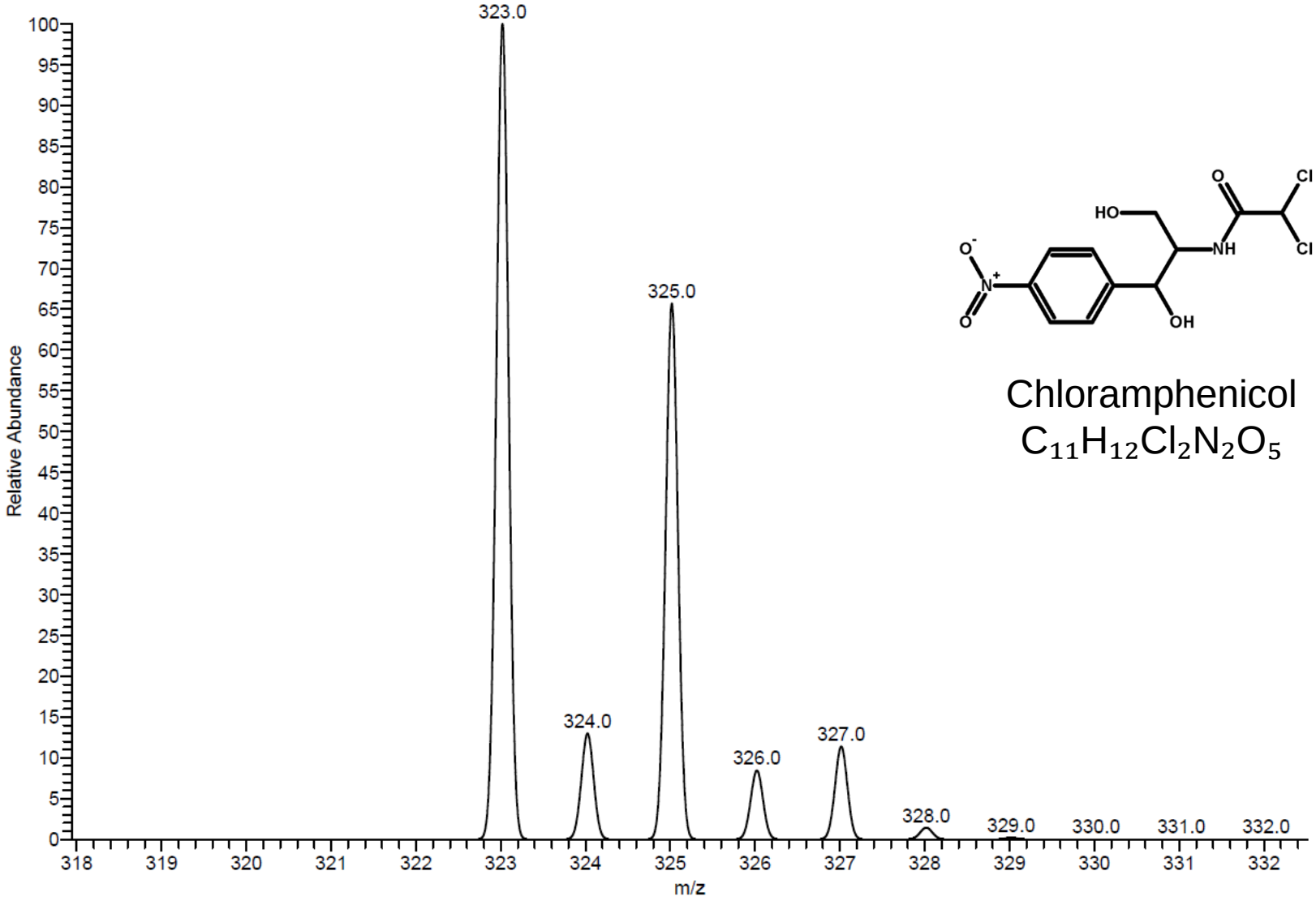
$C_{13}H_{12}BrNO_2$

5

MS, ESI+

What element would you expect in the structure of this ion ?

6

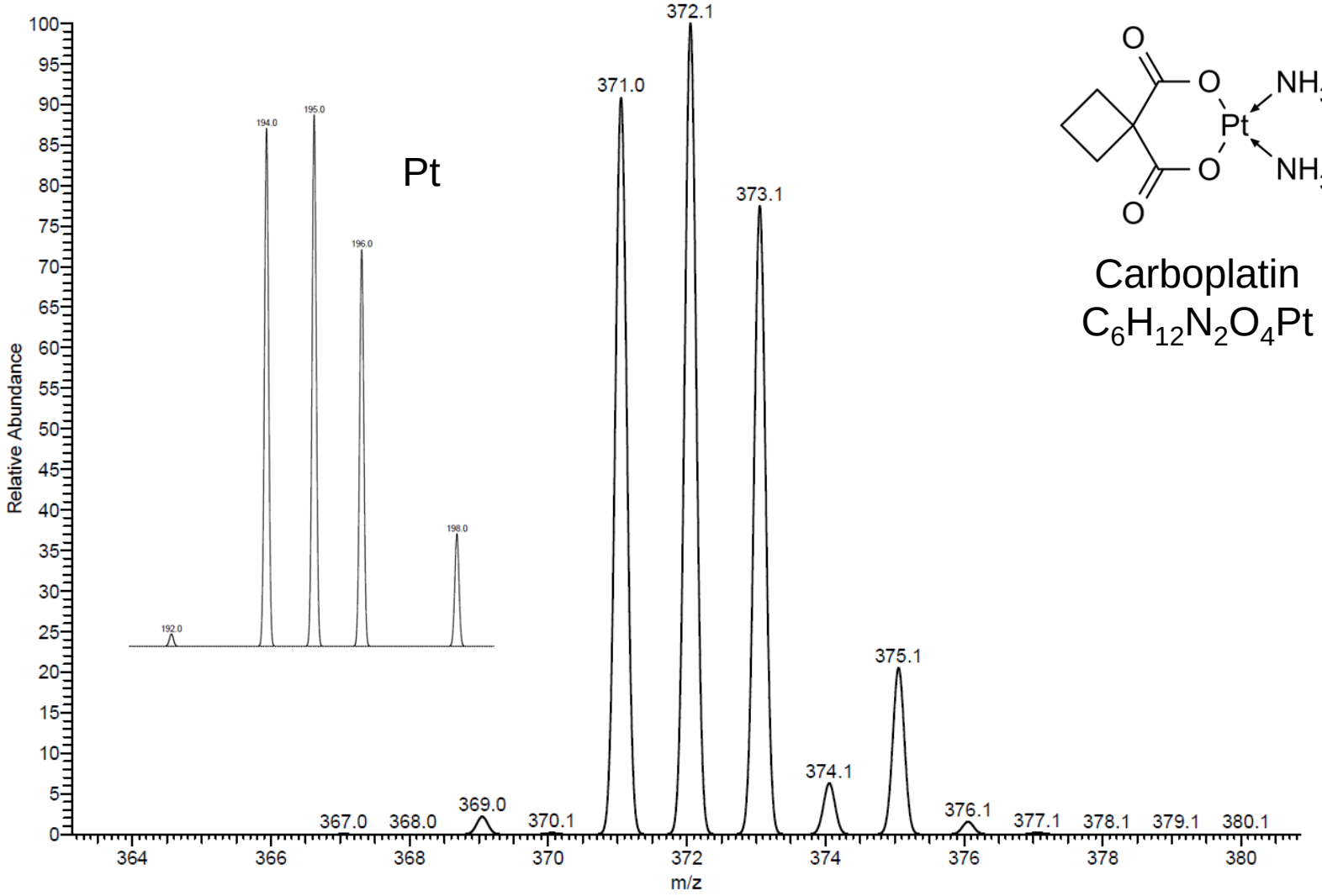


Chloramphenicol

$C_{11}H_{12}Cl_2N_2O_5$

MS, ESI+

What element would you expect in the structure of this ion ?



Carboplatin

$C_6H_{12}N_2O_4Pt$

7

Elemental composition: information from nominal mass

- 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

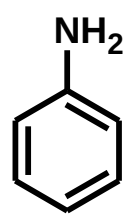
Odd value of molecular weight = odd number of nitrogens

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

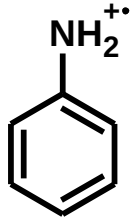
Applying the rule for ions:

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

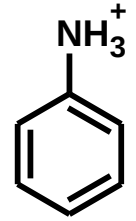
ESI, APCI, MALDI: the rule must be reversed for molecular adducts!



M=93



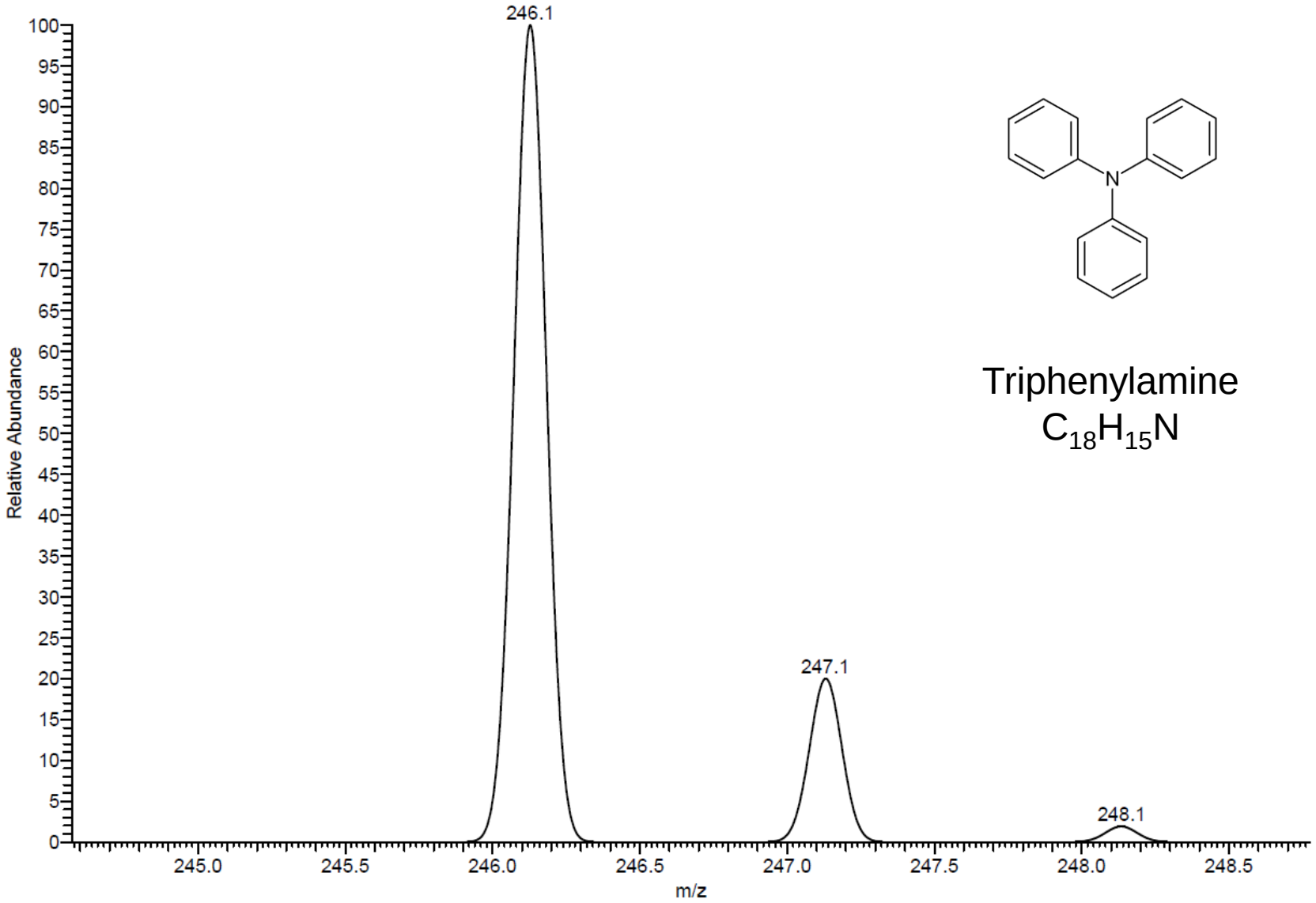
m/z 93



m/z 94

MS, ESI+

Does this ion contain nitrogen?



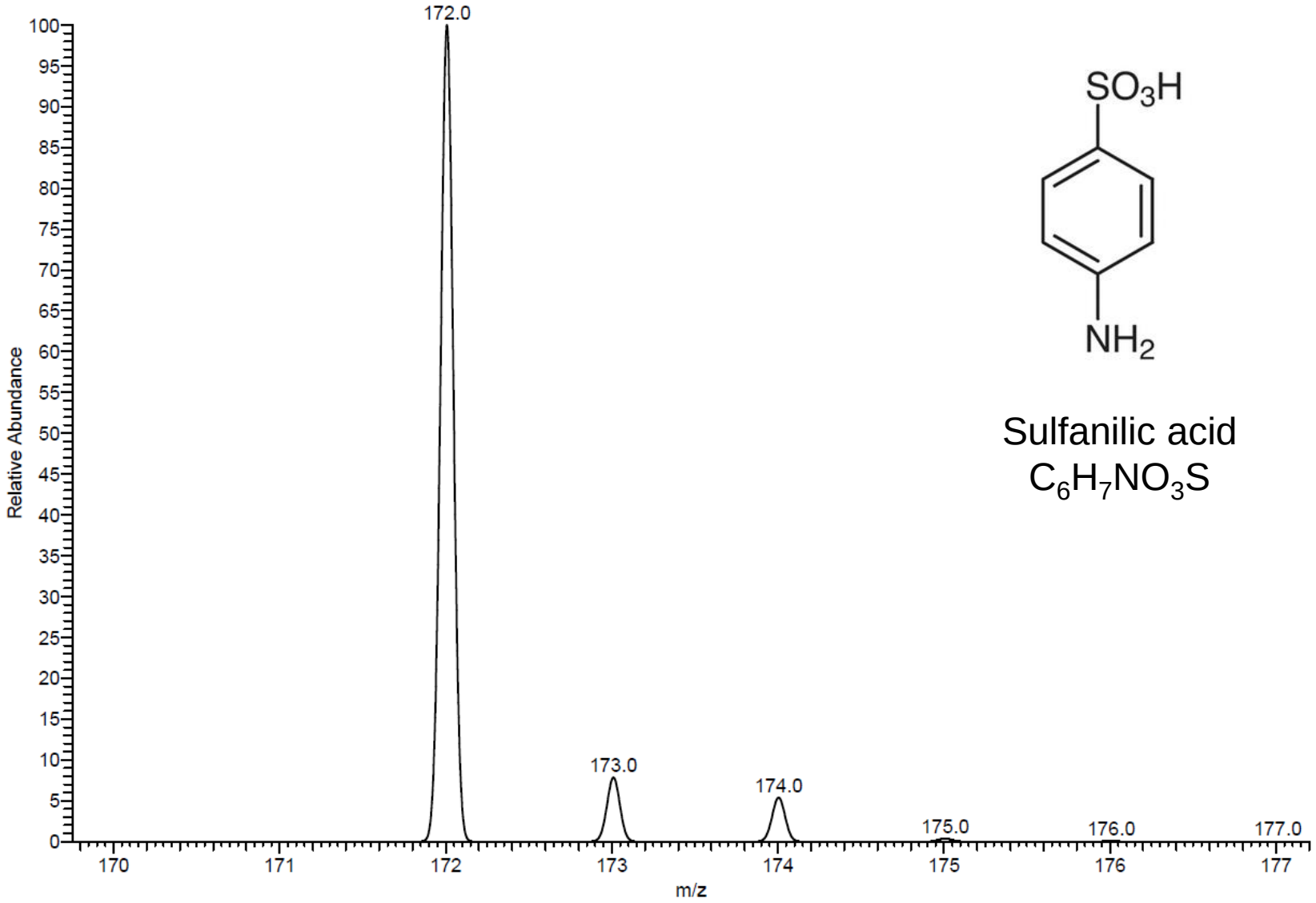
Triphenylamine

$C_{18}H_{15}N$

8

MS, ESI-

Does this ion contain nitrogen?



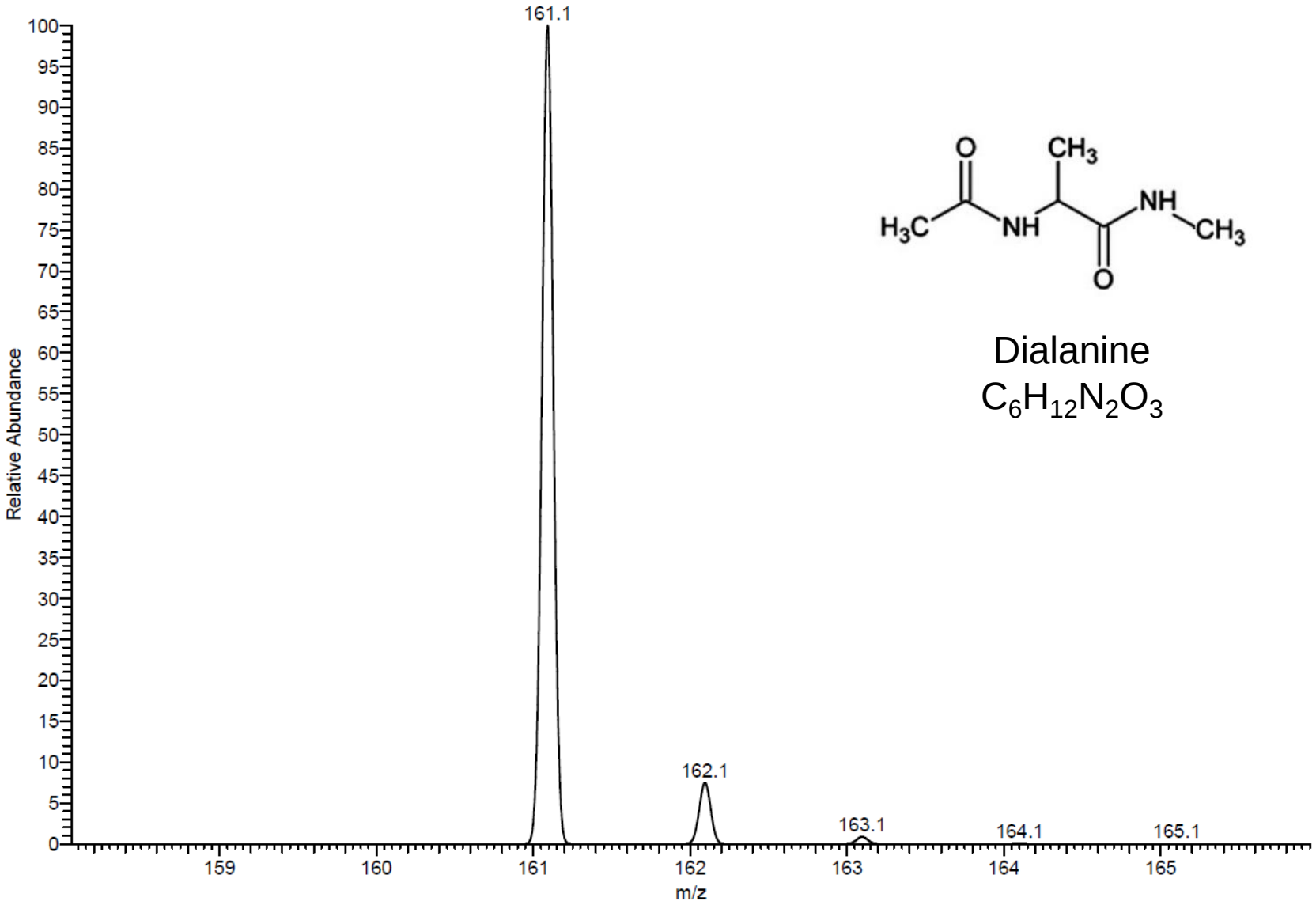
Sulfanilic acid

$C_6H_7NO_3S$

9

MS, ESI+

Does this ion contain nitrogen?



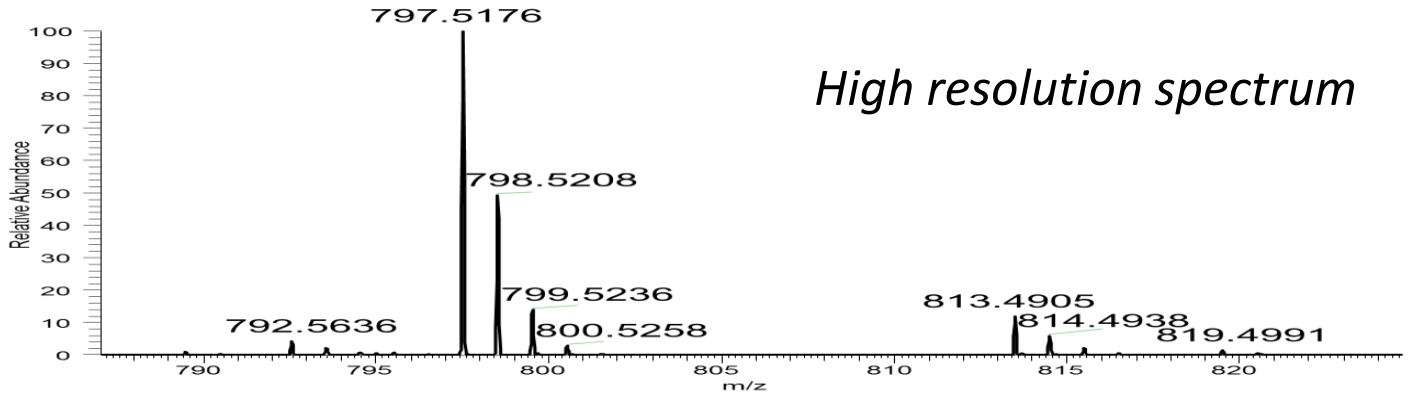
Dialanine

$C_6H_{12}N_2O_3$

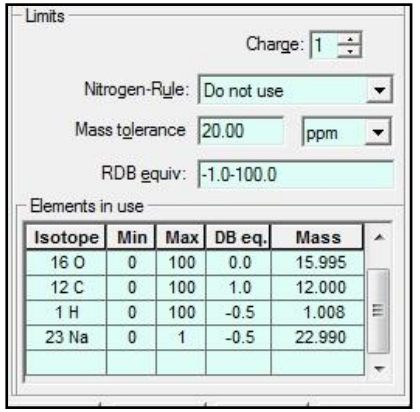
10

Elemental composition: HRAM measurement

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



High resolution spectrum



Calculation limits

Results – usually several possibilities

Elemental composition search on mass 797.52

m/z= 792.52-802.52

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
797.5176	797.5174	0.20	8.5	$C_{45}H_{74}O_{10}Na$
	797.5198	-2.81	11.5	$C_{47}H_{73}O_{10}$
	797.5140	4.55	20.5	$C_{54}H_{69}O_5$

Odd or even electron ion? RDBE

RDBE (Ring and Double Bond Equivalent): the number of unsaturations present in a organic molecule

Number of rings and double bonds in $C_xH_yN_zO_n$:

$$RDBE = x - \frac{1}{2}y + \frac{1}{2}z + 1$$

Other elements are added to CHNO based on their valence, e.g. Si (+C), P (+N), halogens (+H)…

RDBE of ions:

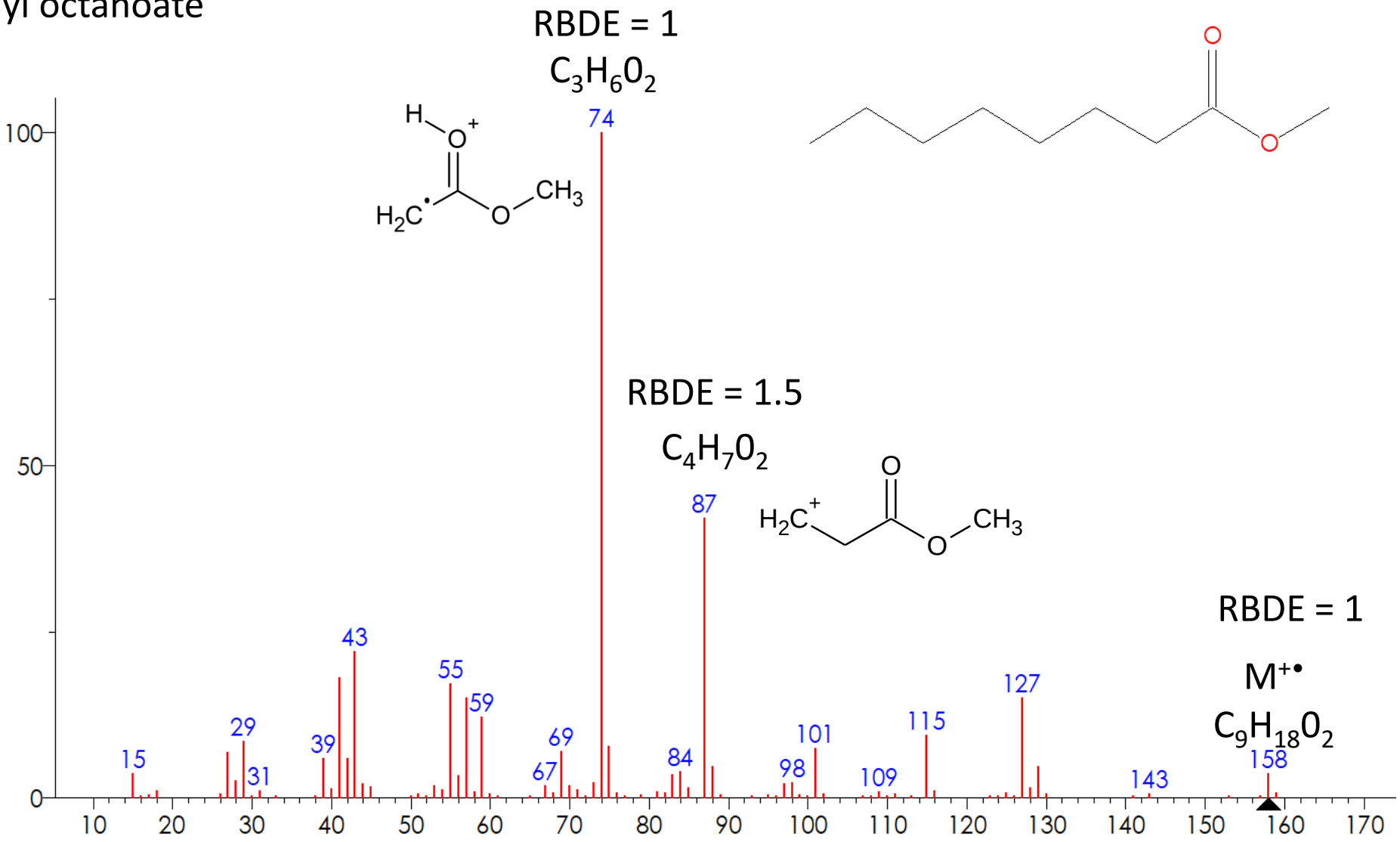
integer value of RDBE → $OE^{+\bullet}$

fractional value of RDBE → EE^{+}

Odd or even electron ion? RDBE

Example: methyl octanoate

MS, EI



IV.
Fragment ions: EE^+

Even-electron ions from soft ionizations

Fragmentation of even electron ions (EE^+)

Ions generated by MS/MS of EE^+ (e.g. $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, or $[M-H]^-$), fragments formed during APCI

FRAGMENTATION of EE^+:

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

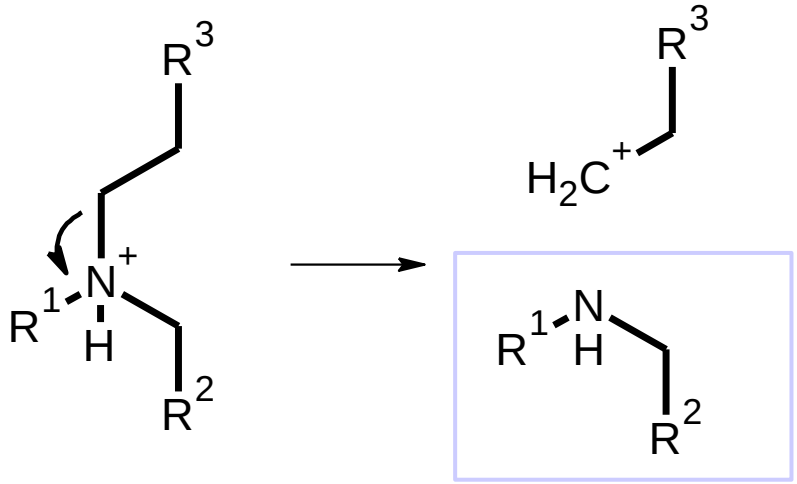
$$EE^+ \rightarrow EE^+ + M$$

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

- Cleavage of neighboring bond to the charge site
- EE^+ ions are more stable than $OE^{+\bullet}$
- The spectra are simpler than EI spectra (less information). They are sensitive to small changes in the structure

Fragmentation of even electron ions (EE^+)

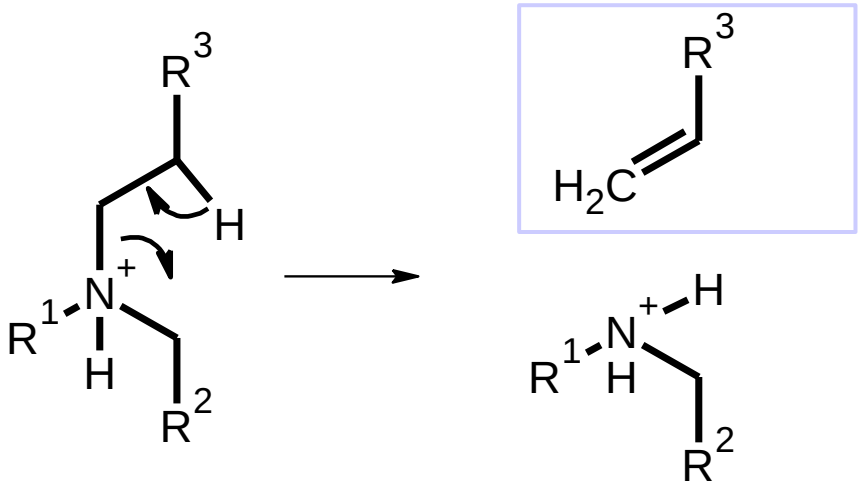
Example. Fragmentation of tertiary amines - two competing processes



A. Inductive cleavage

- positive charge attracts electron pair of the C-N bond

- charge migration, neutral loss of secondary amine



B. Rearrangement of β-hydrogen

- migration of hydrogen to nitrogen accompanied by cleavage of the C-N bond

- charge retention, neutral loss of alkene

Fragmentation of even electron ions (EE^+)

Fragmentation of EE^+ can often be described as a series of losses of neutral molecules.

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 (+/-)

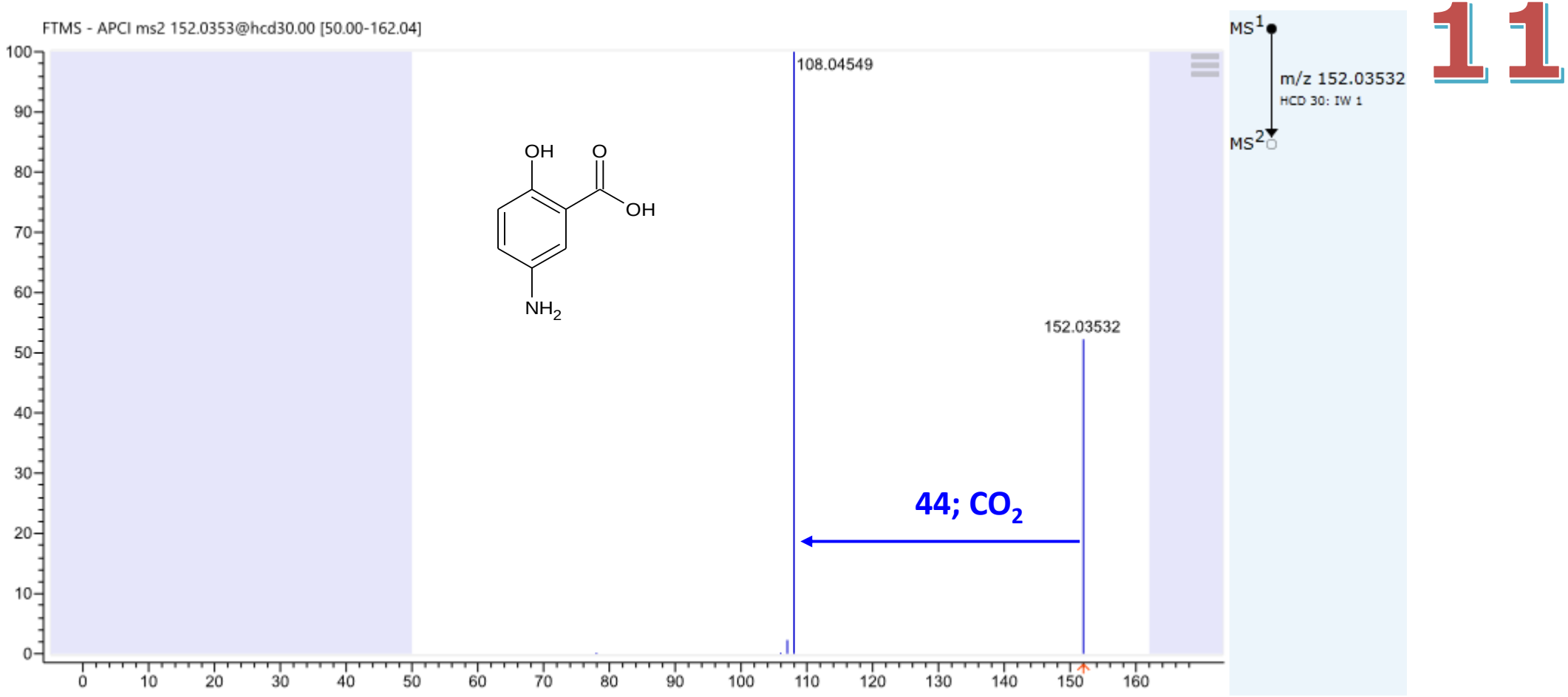
80: SO_3 – sulfonic acids(+/-)

162: anhydroglucose – glucosides (+/-)

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

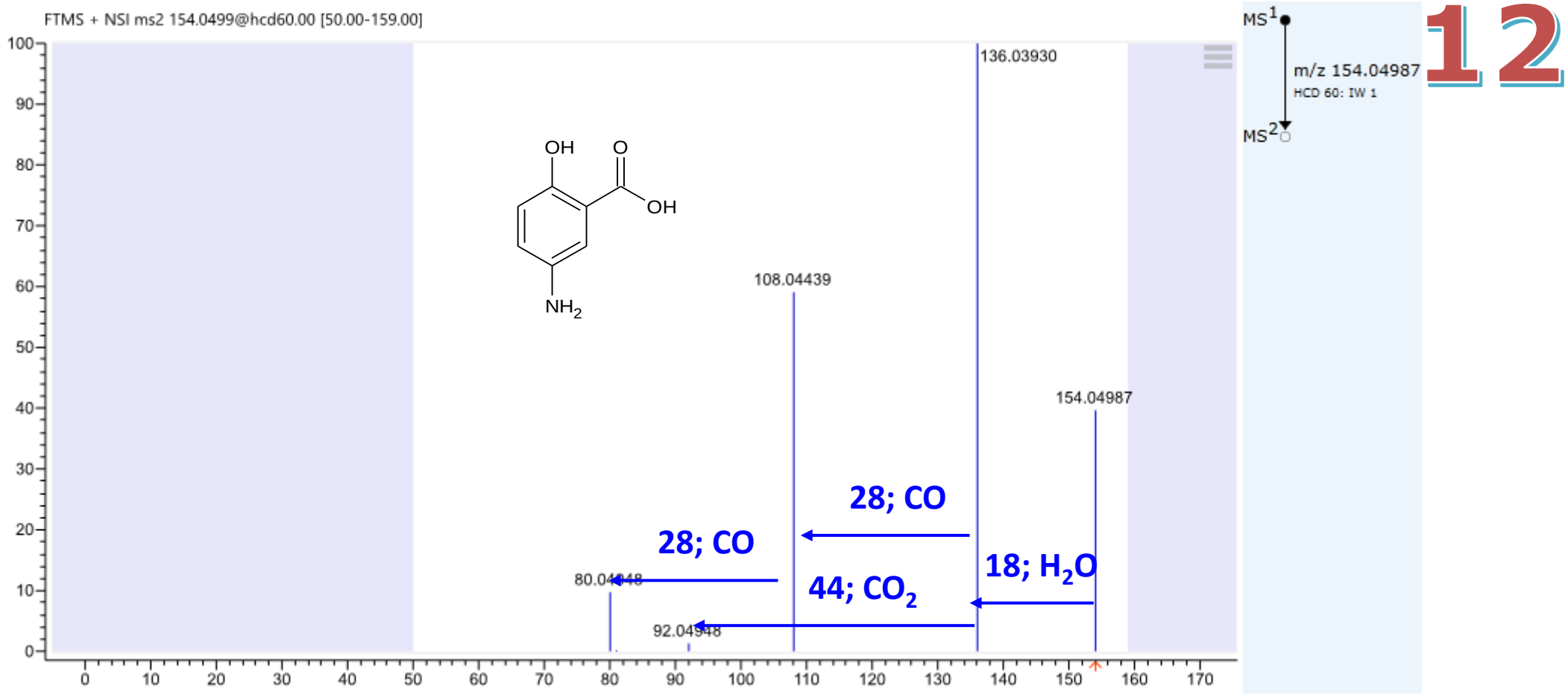
What neutral molecule is lost during fragmentation?

Carboxylic acid: neutral loss of CO_2



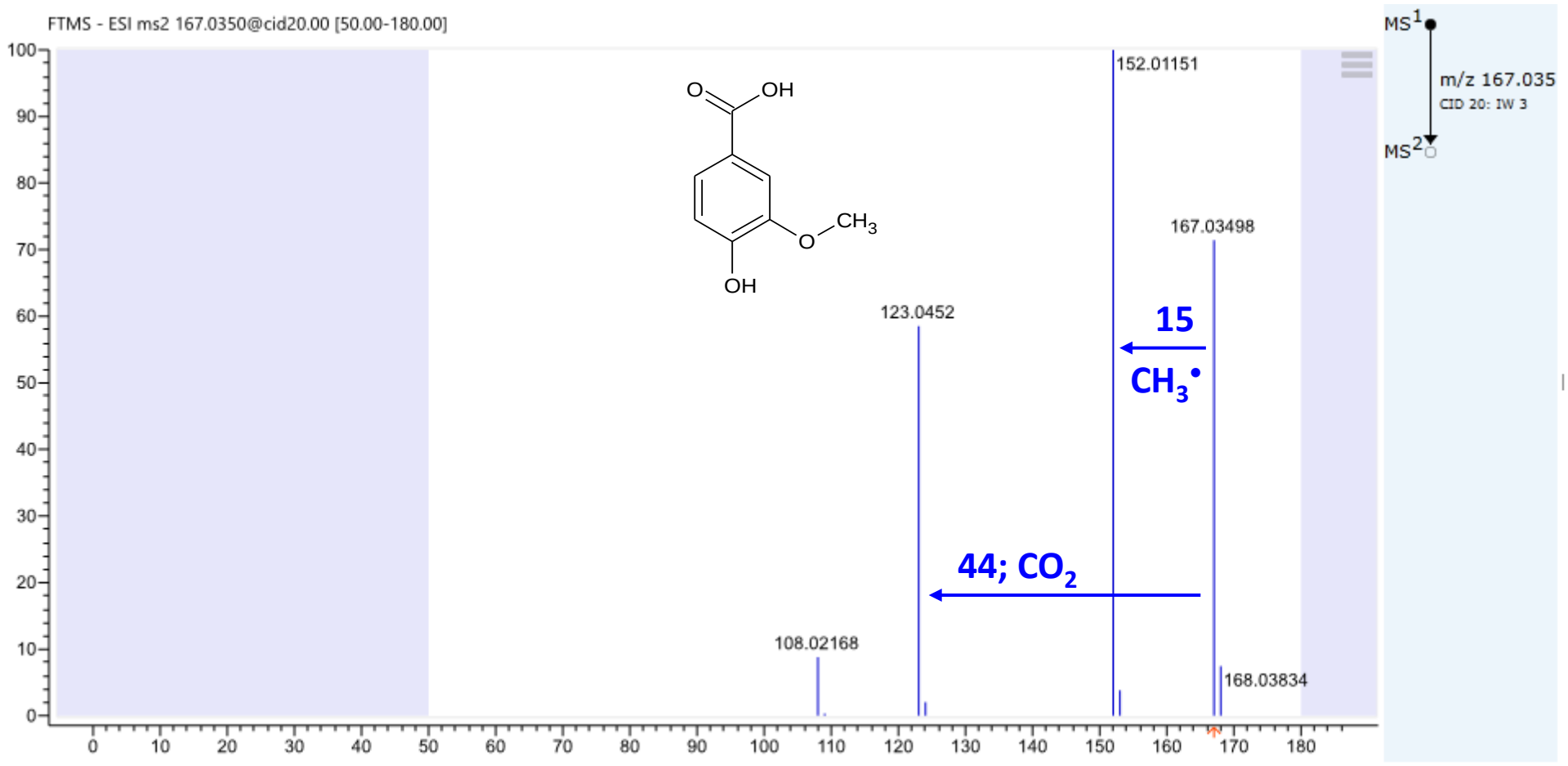
What neutral molecule is lost during fragmentation?

Carboxylic acid: neutral loss of H_2O, (CO_2)



What neutral molecule is lost during fragmentation?

Carboxylic acid: neutral loss of CO_2
Methoxy group: neutral loss of $CH_3^{\bullet}$

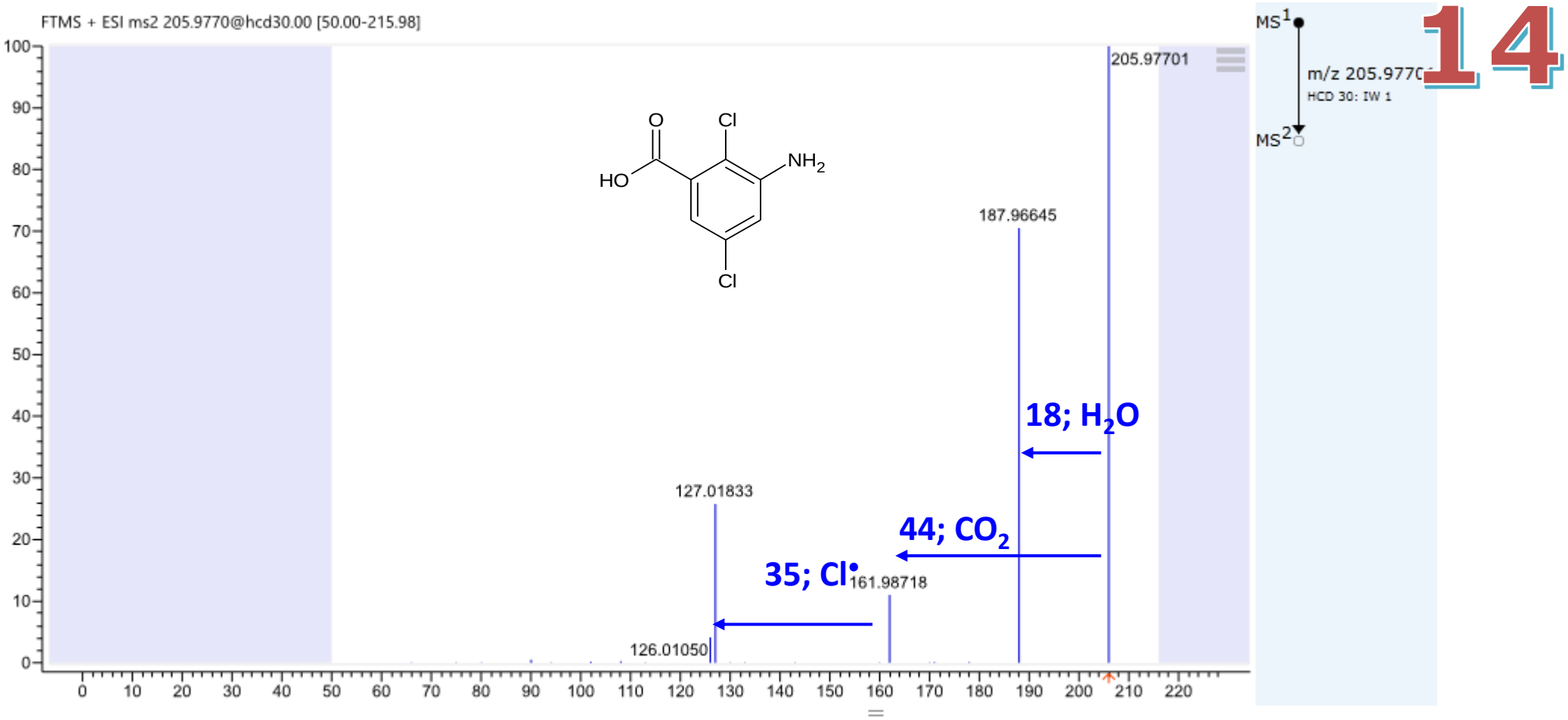


13

What neutral molecule is lost during fragmentation?

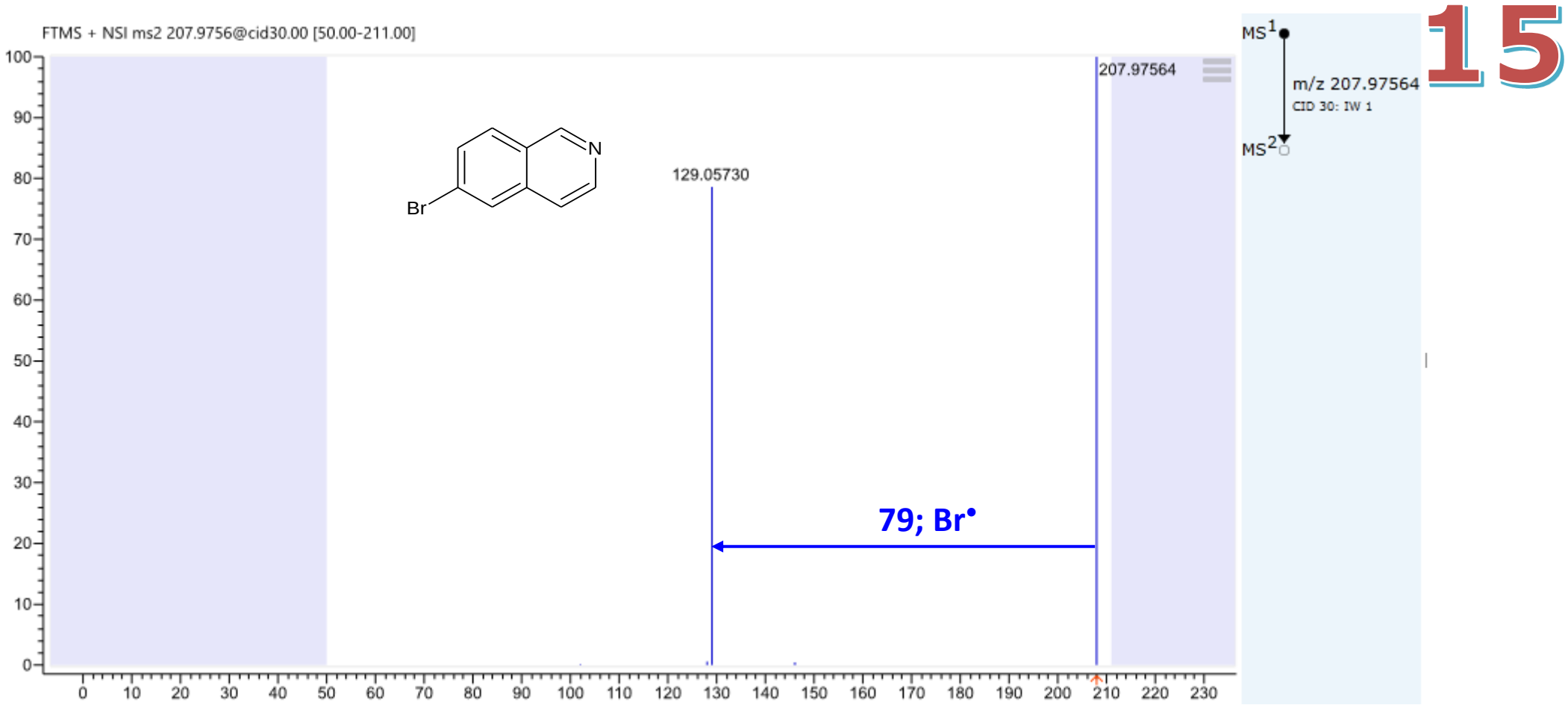
Carboxylic acid: neutral loss of H_2O, CO_2

Arylhalogenderivatives: loss of halogen radical



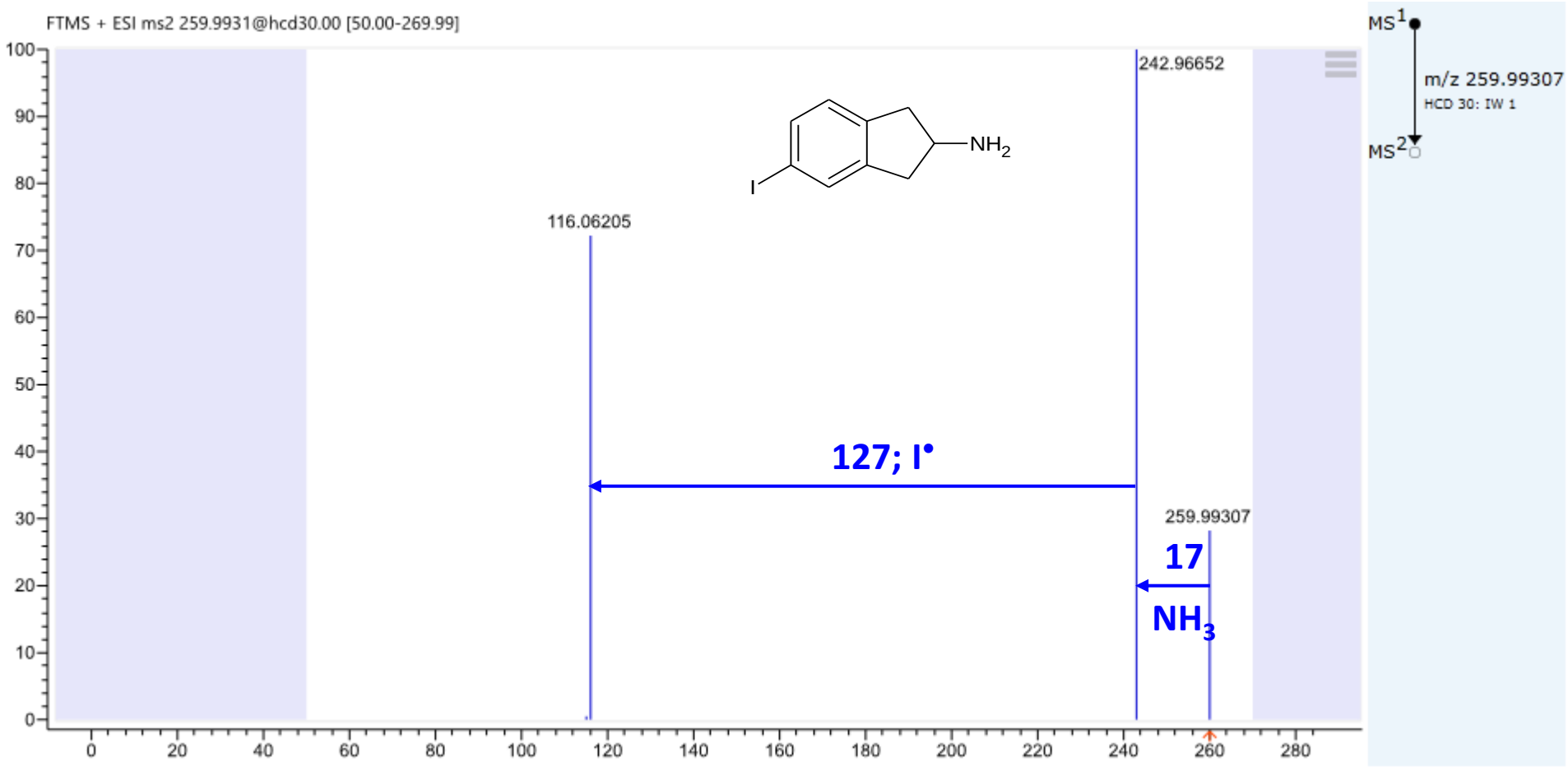
What neutral molecule is lost during fragmentation?

Arylhalogenderivatives: loss of halogen radical



What neutral molecule is lost during fragmentation?

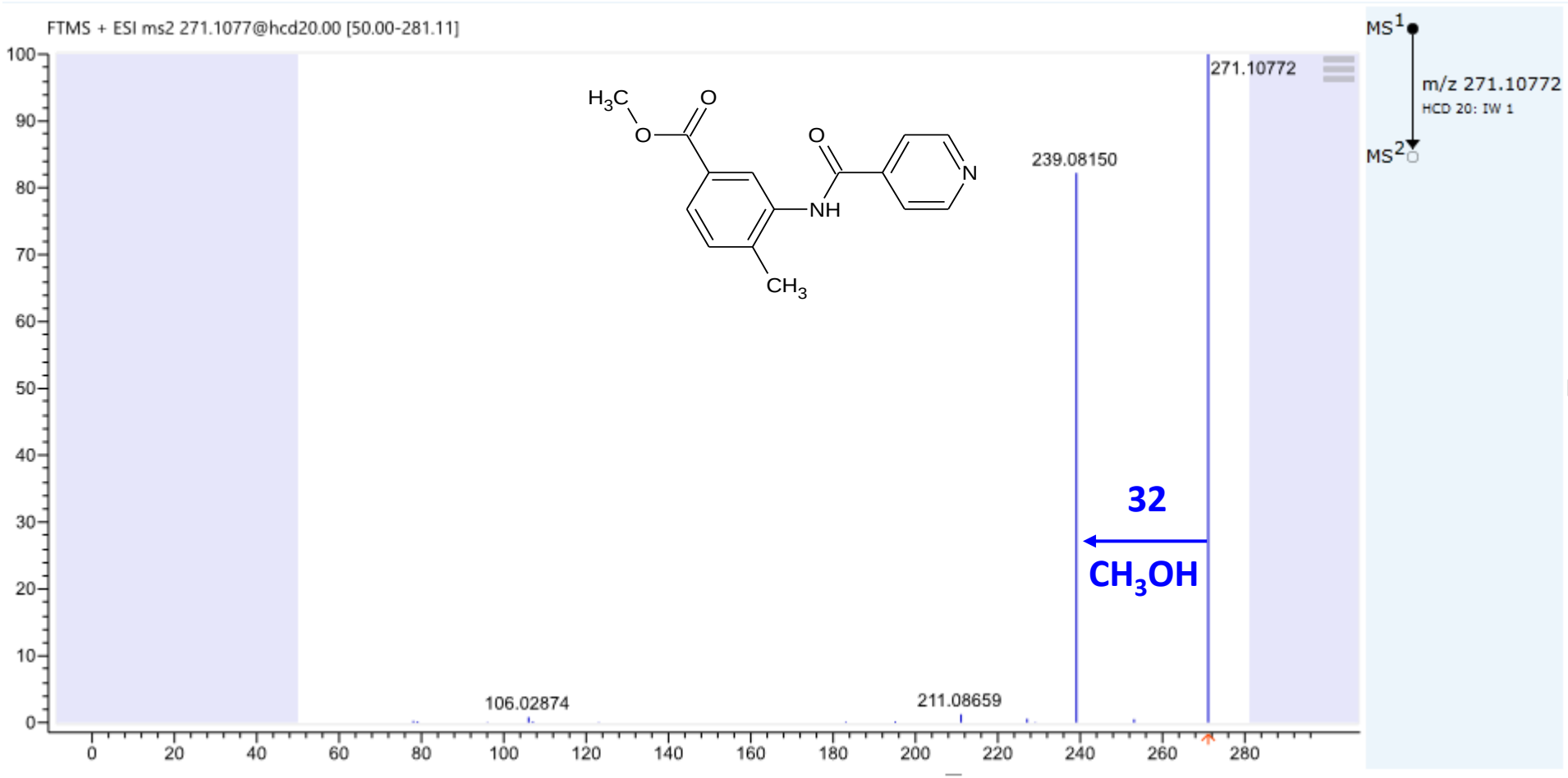
Arylhalogenderivatives: loss of halogen radical



16

What neutral molecule is lost during fragmentation?

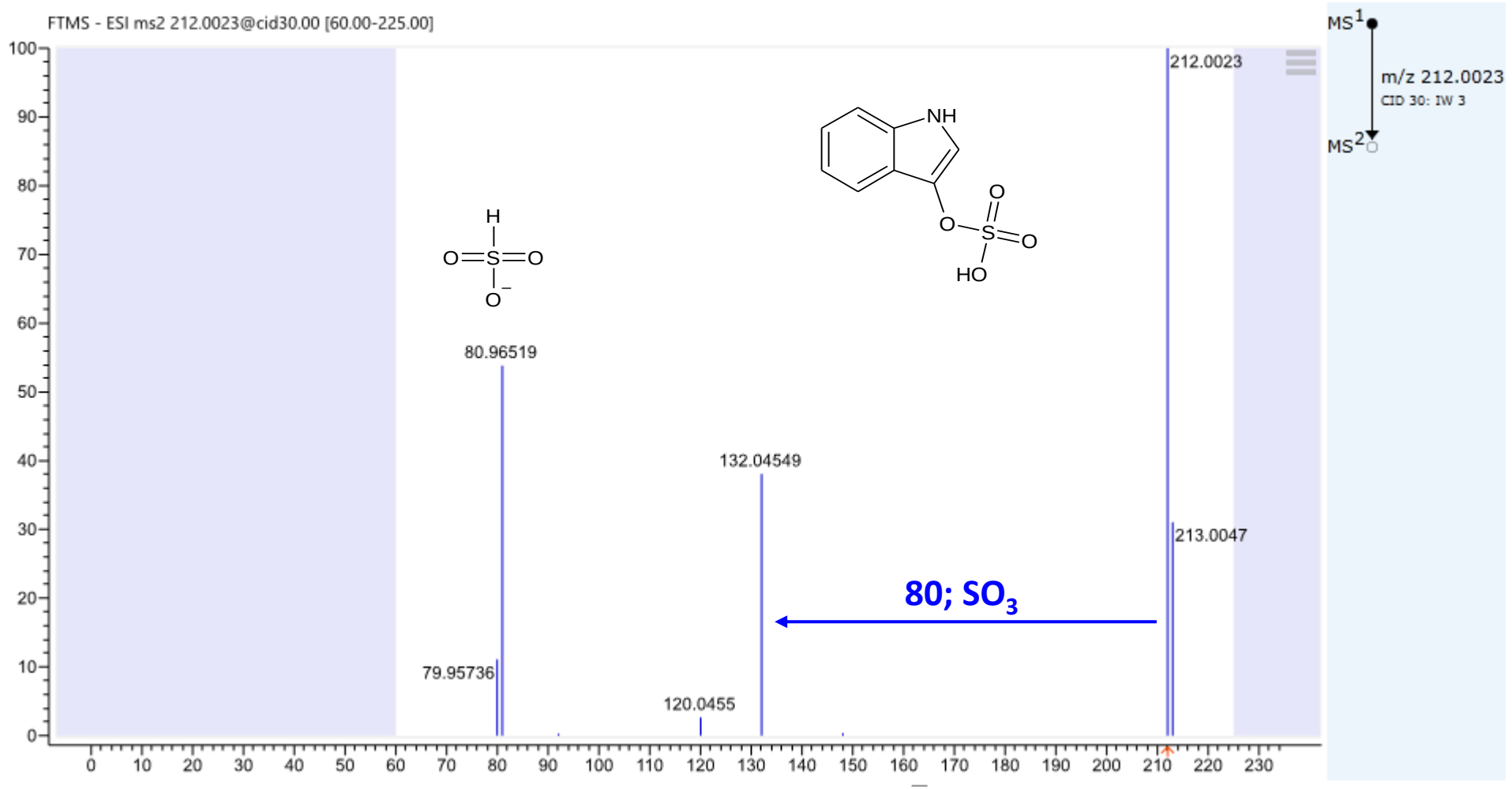
Esters of carboxylic acids: neutral loss of alcohol



17

What neutral molecule is lost during fragmentation?

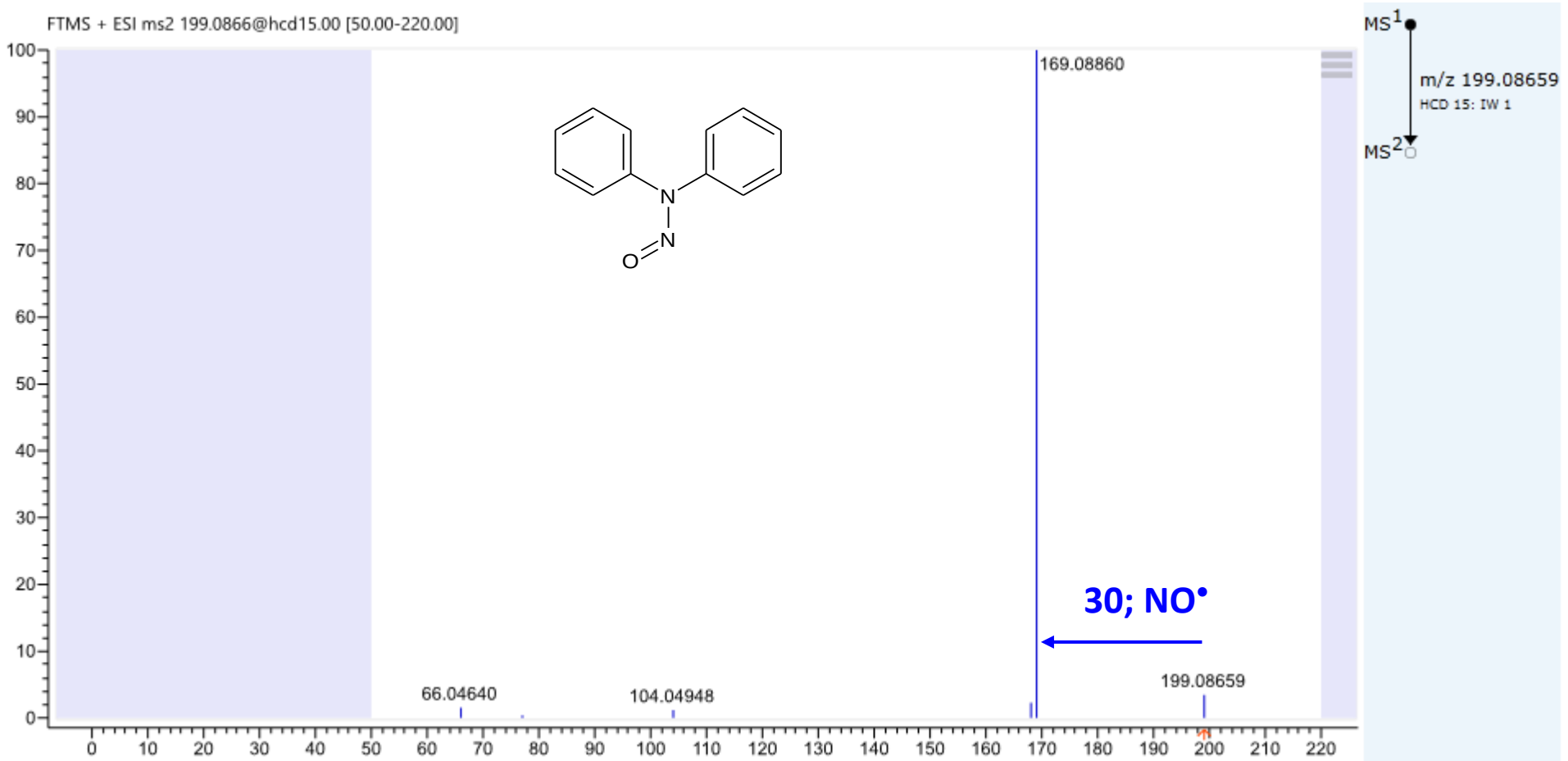
Sulphates: neutral loss of sulfur trioxide



18

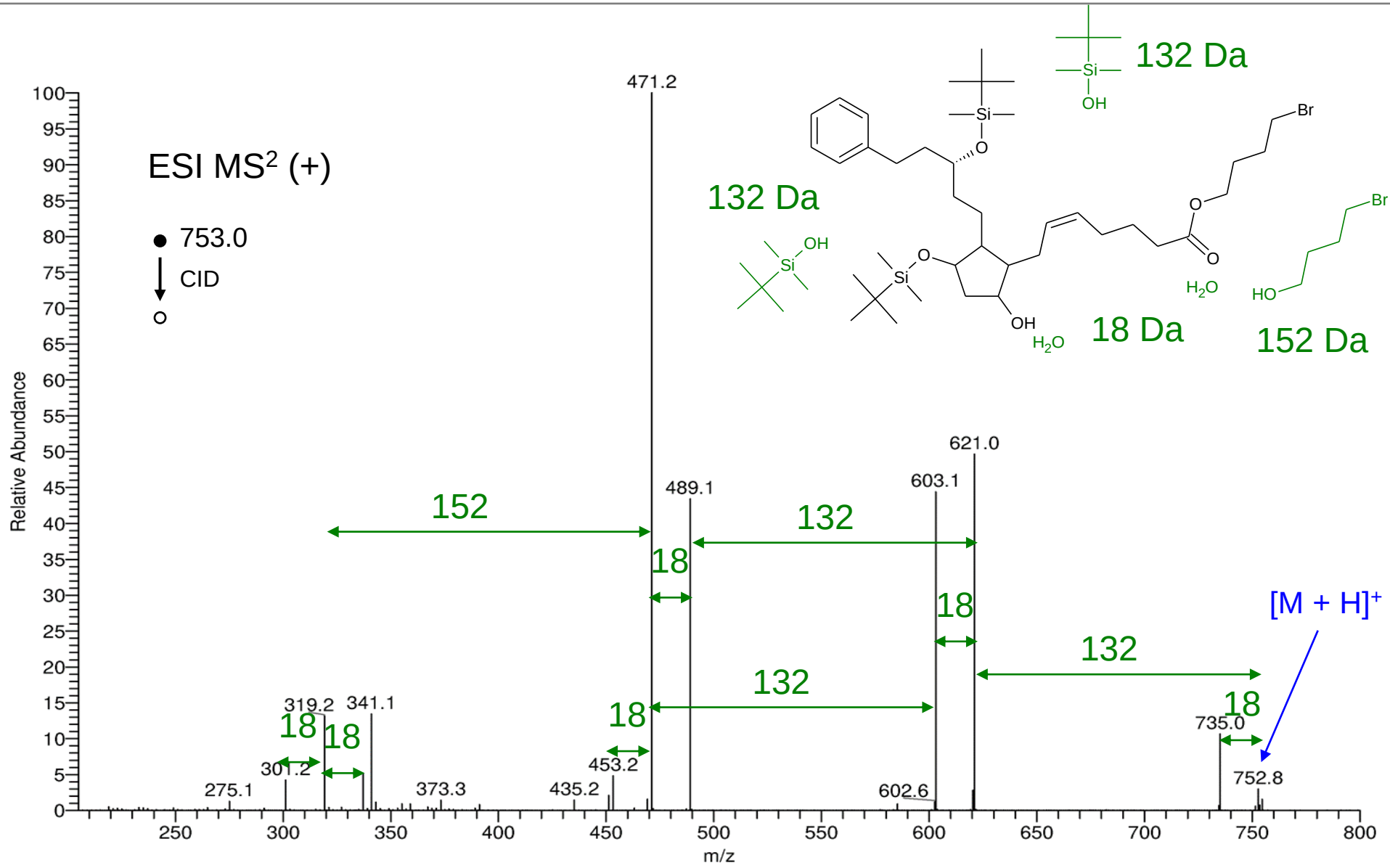
What neutral molecule is lost during fragmentation?

N-nitroso: homolytic cleavage, loss of nitric oxide NO•

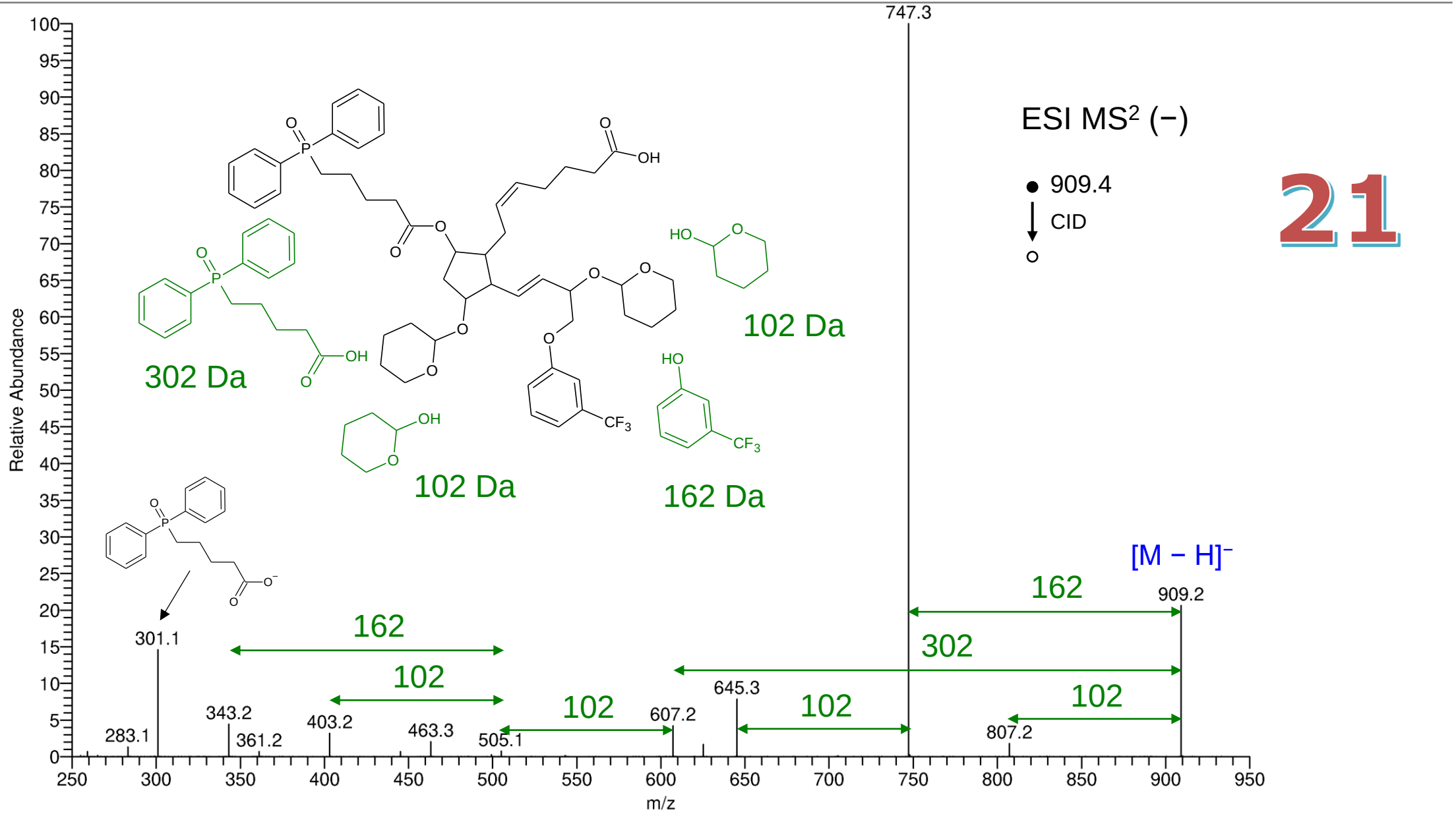


19

Interpretion by a series of successive neutral losses

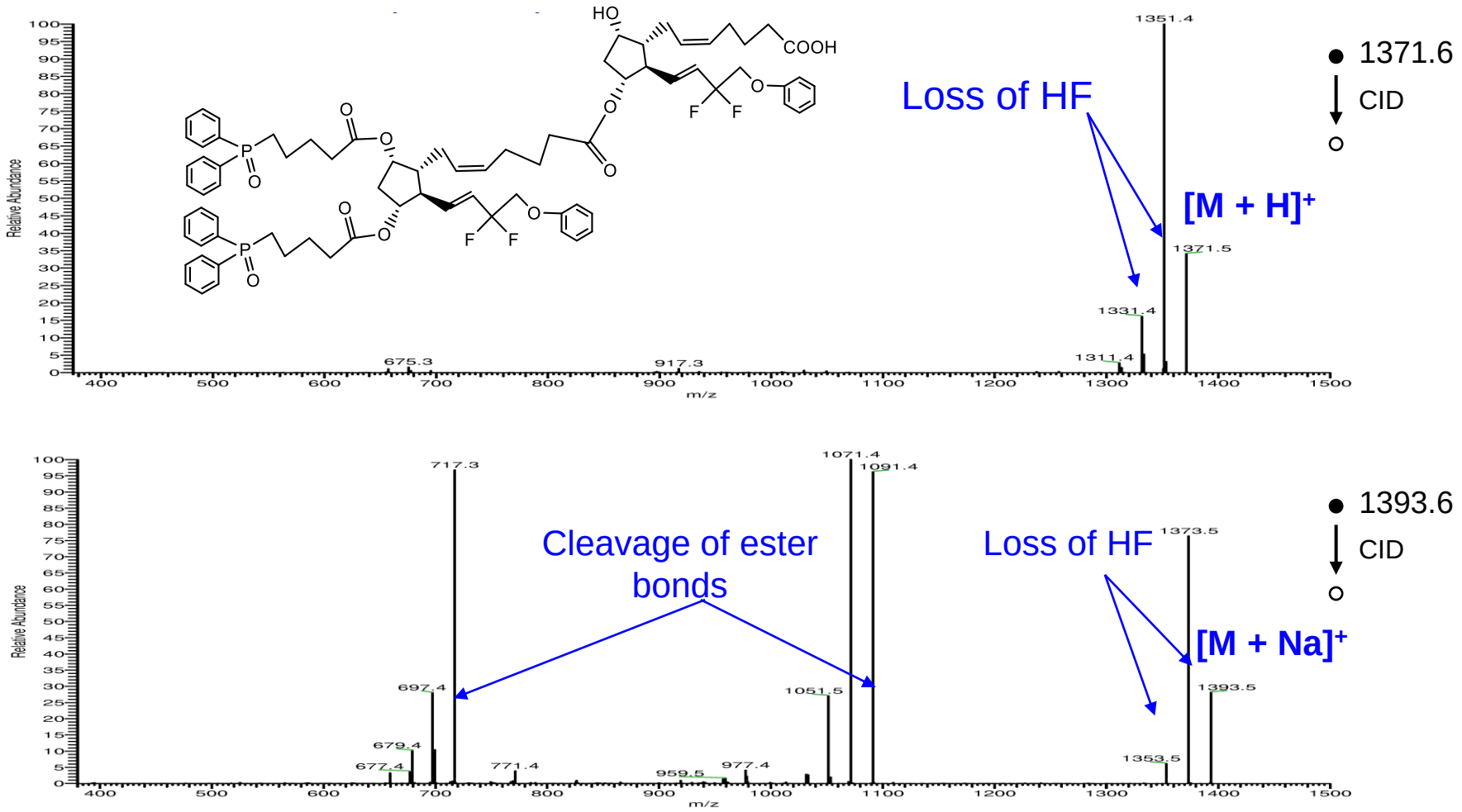


Interpretion by a series of successive neutral losses



Fragmentation of even electron ions (EE^+)

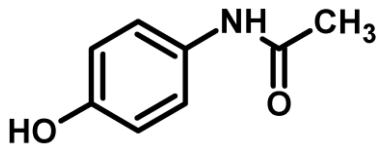
Fragmentation depends on the ion type: the CID spectra of different adducts of the same molecule may differ



What are the ions in the spectrum of paracetamol?

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]

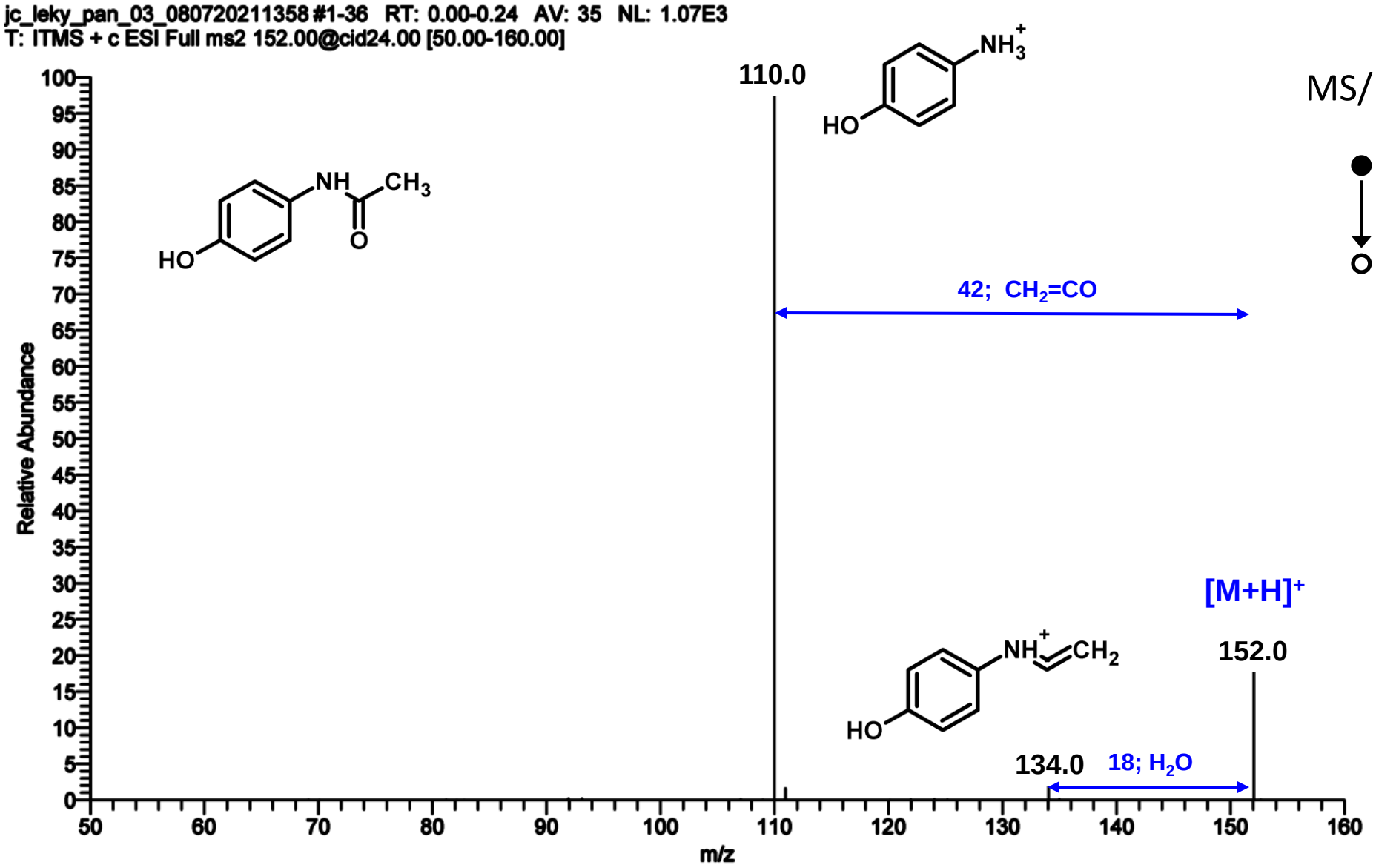
MS, ESI+



$C_8H_9NO_2$; M=151.1

22

What are the ions in the spectrum of paracetamol?

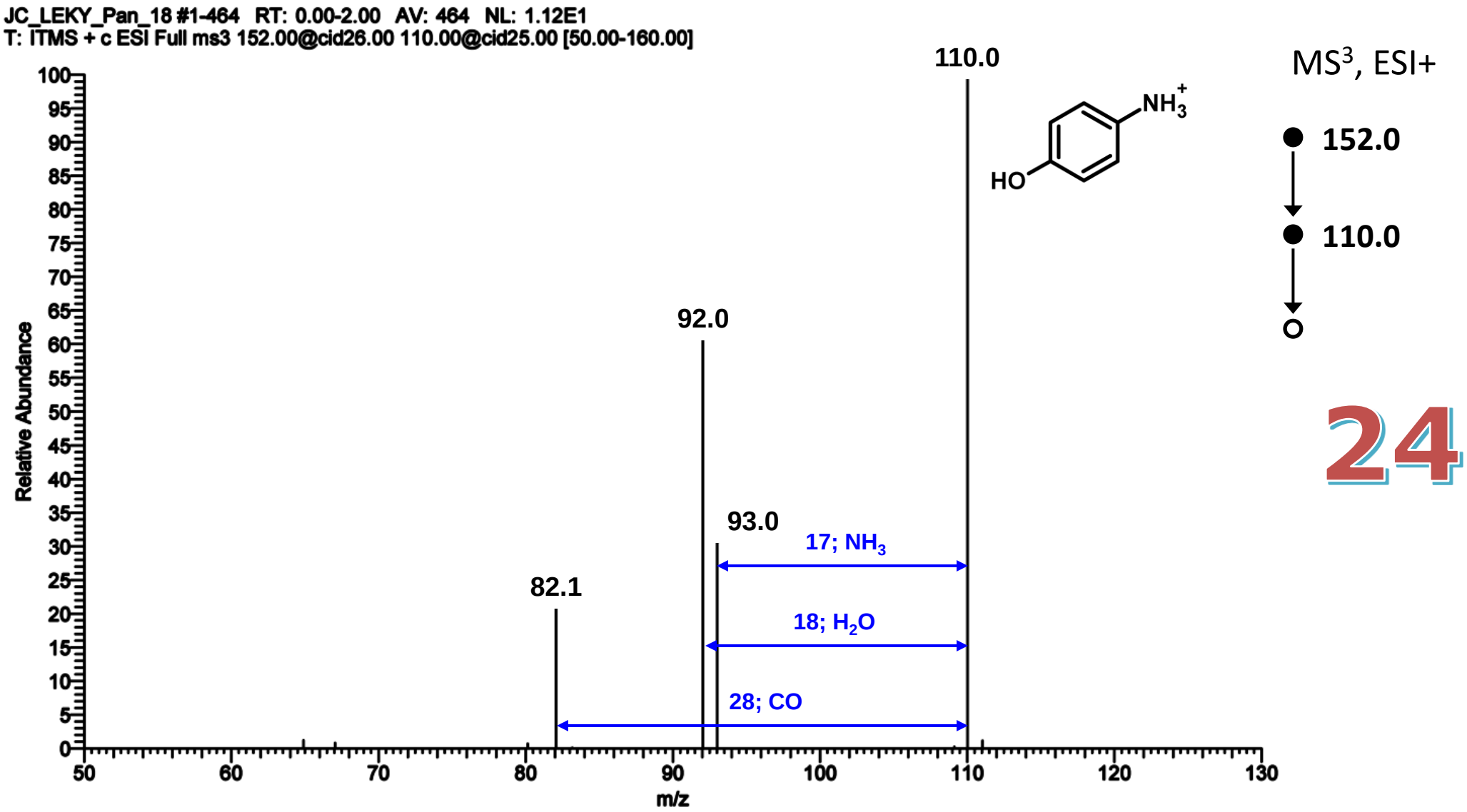


MS/MS, ESI+

152.0

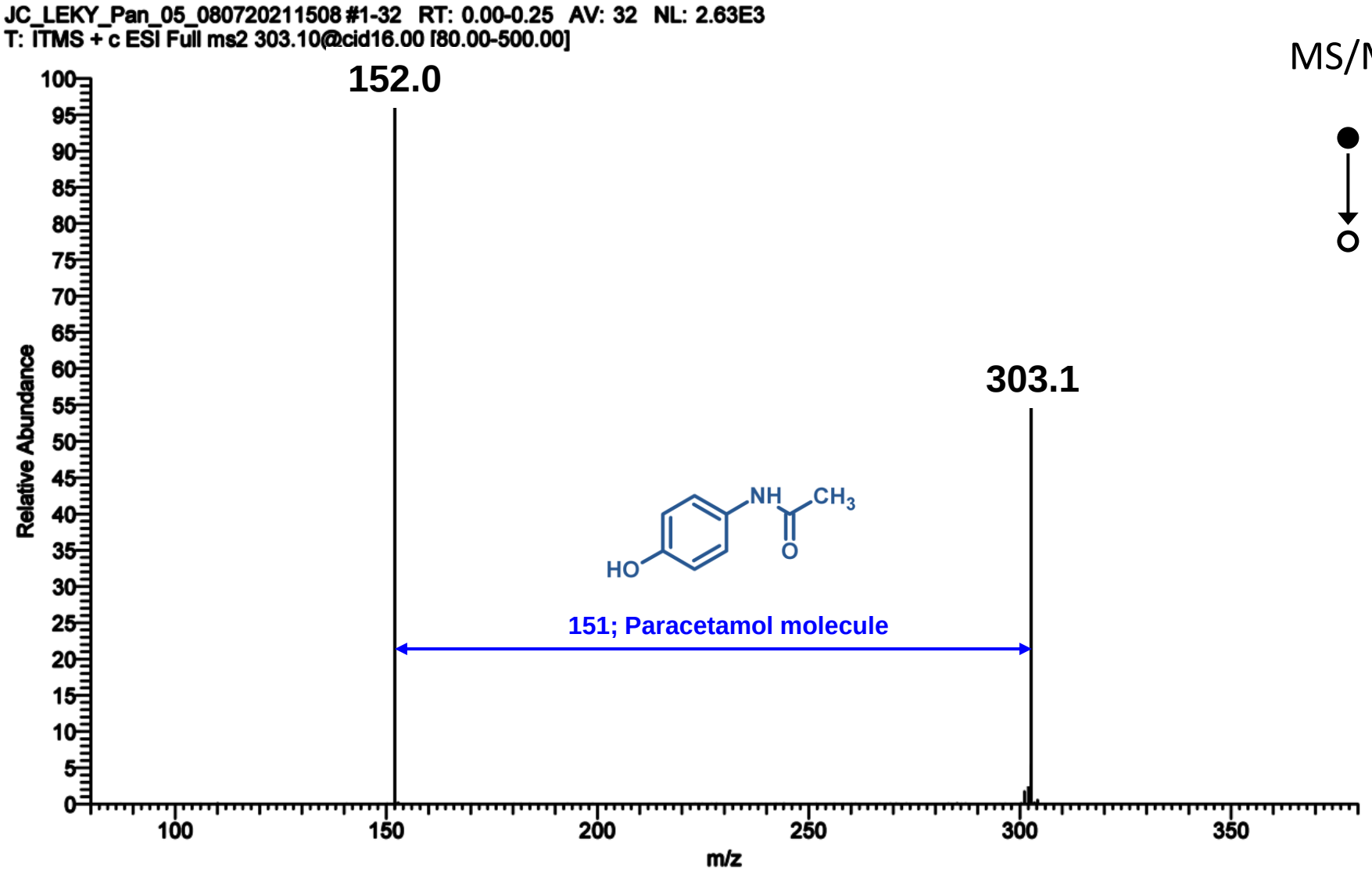
23

What are the ions in the spectrum of paracetamol?



24

What fragments are expected from paracetamol dimer?



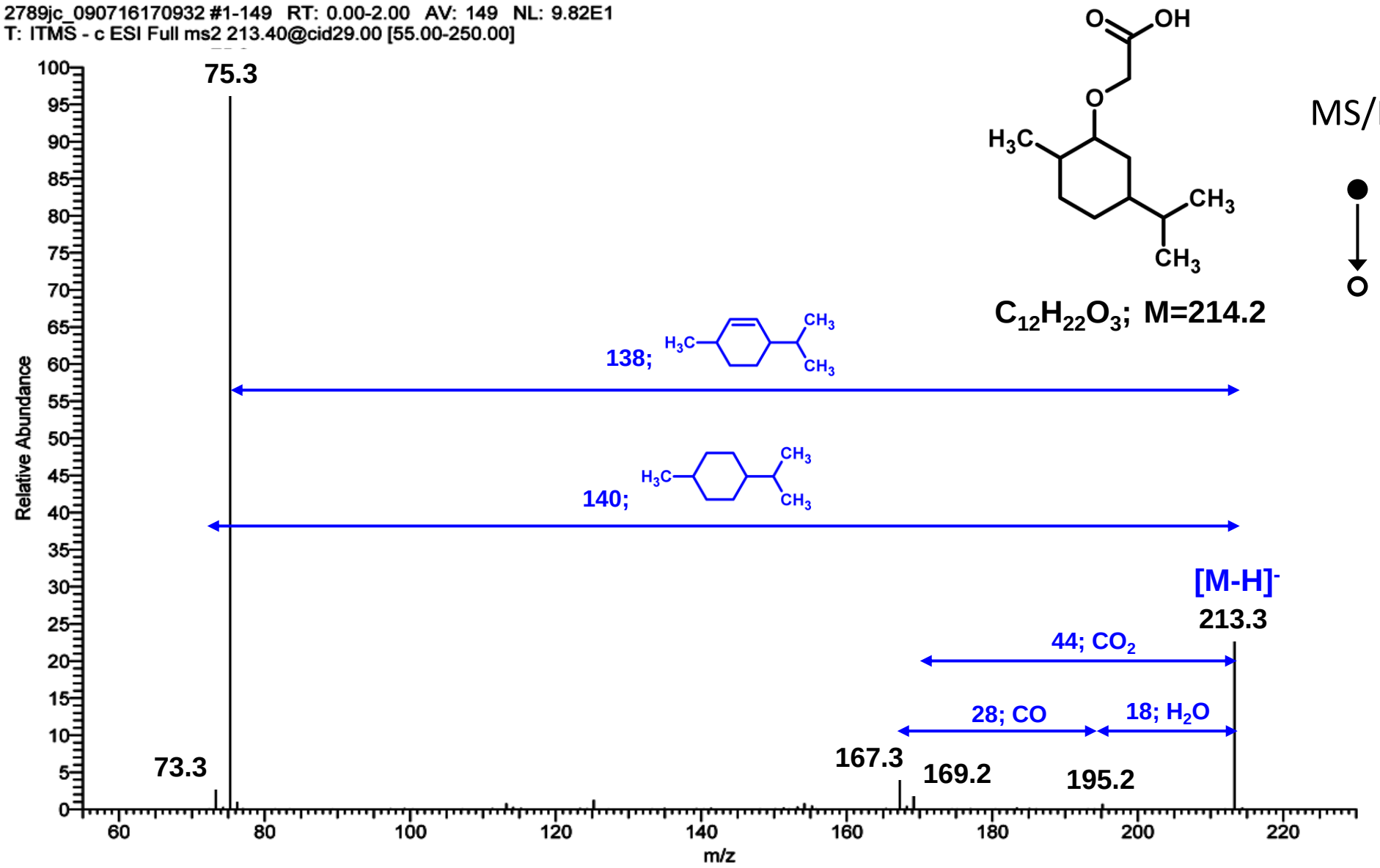
MS/MS, ESI+

303.1

O

25

What are the ions in the spectrum of menthyloxyacetic acid?



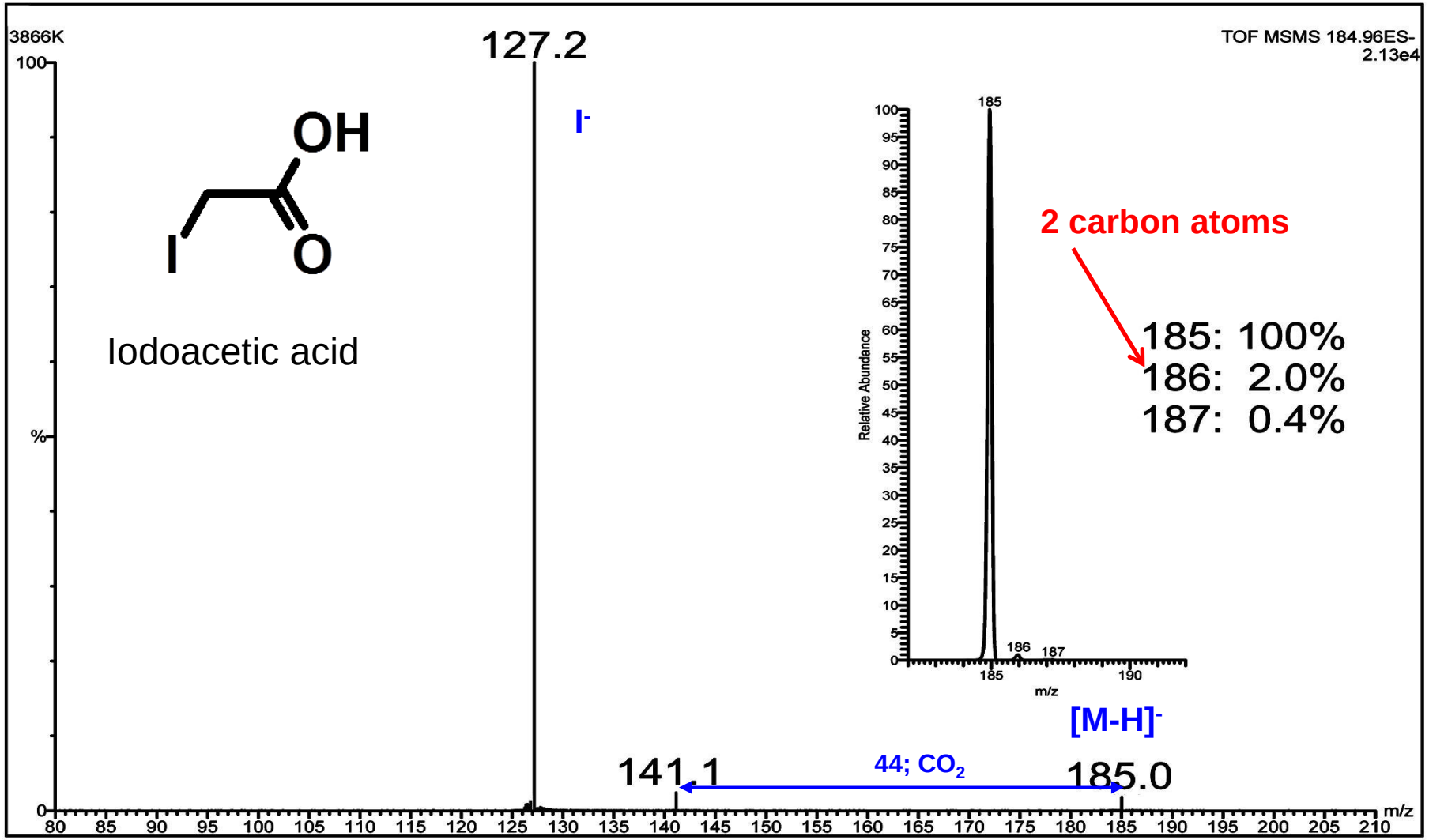
$C_{12}H_{22}O_3$; M=214.2

MS/MS, ESI-

213.3

26

What compound is it?

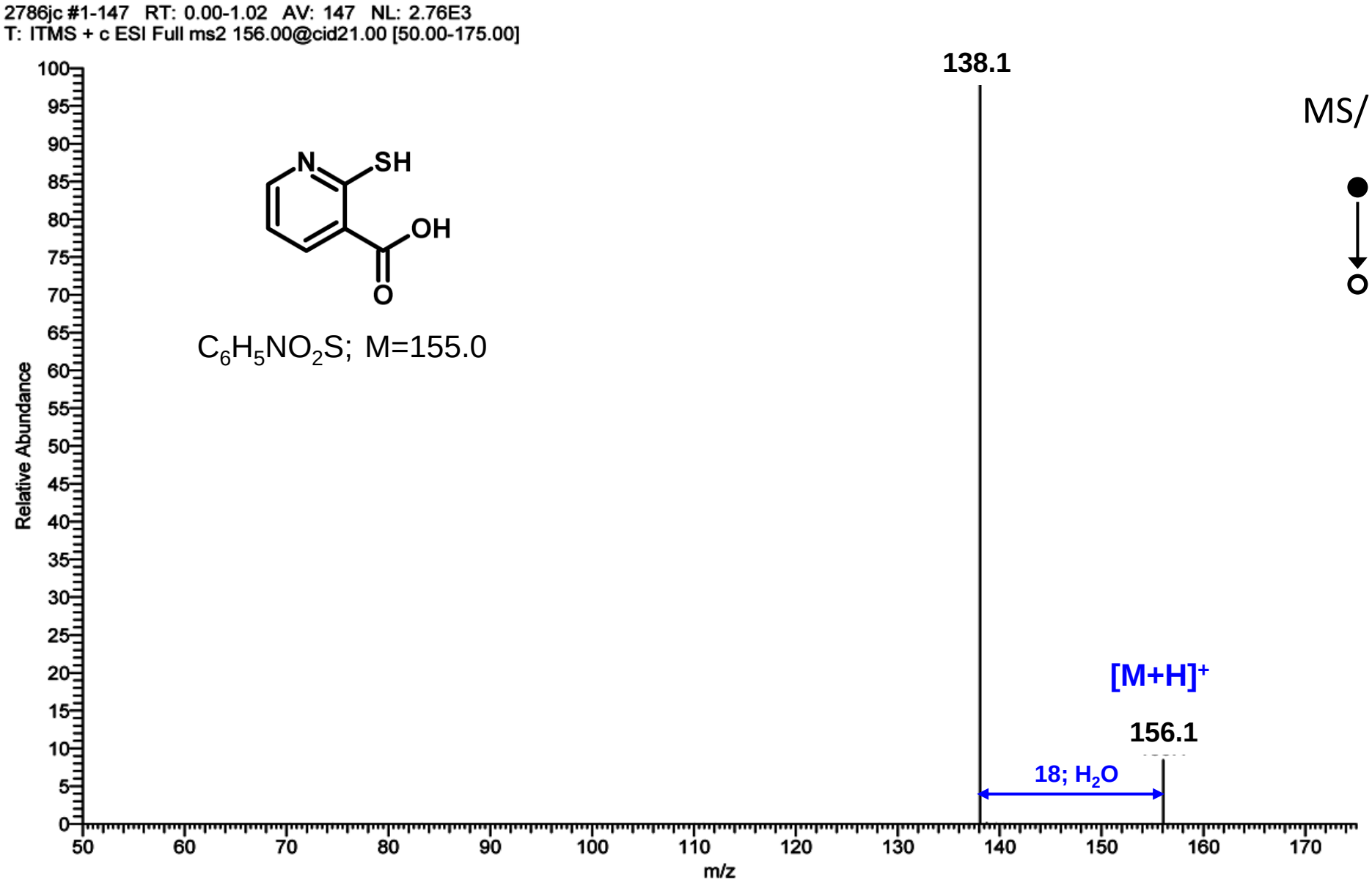


MS/MS, ESI-

185.0

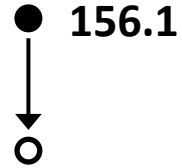
27

What are the ions in the spectrum of 2-mercaptonicotinic acid?



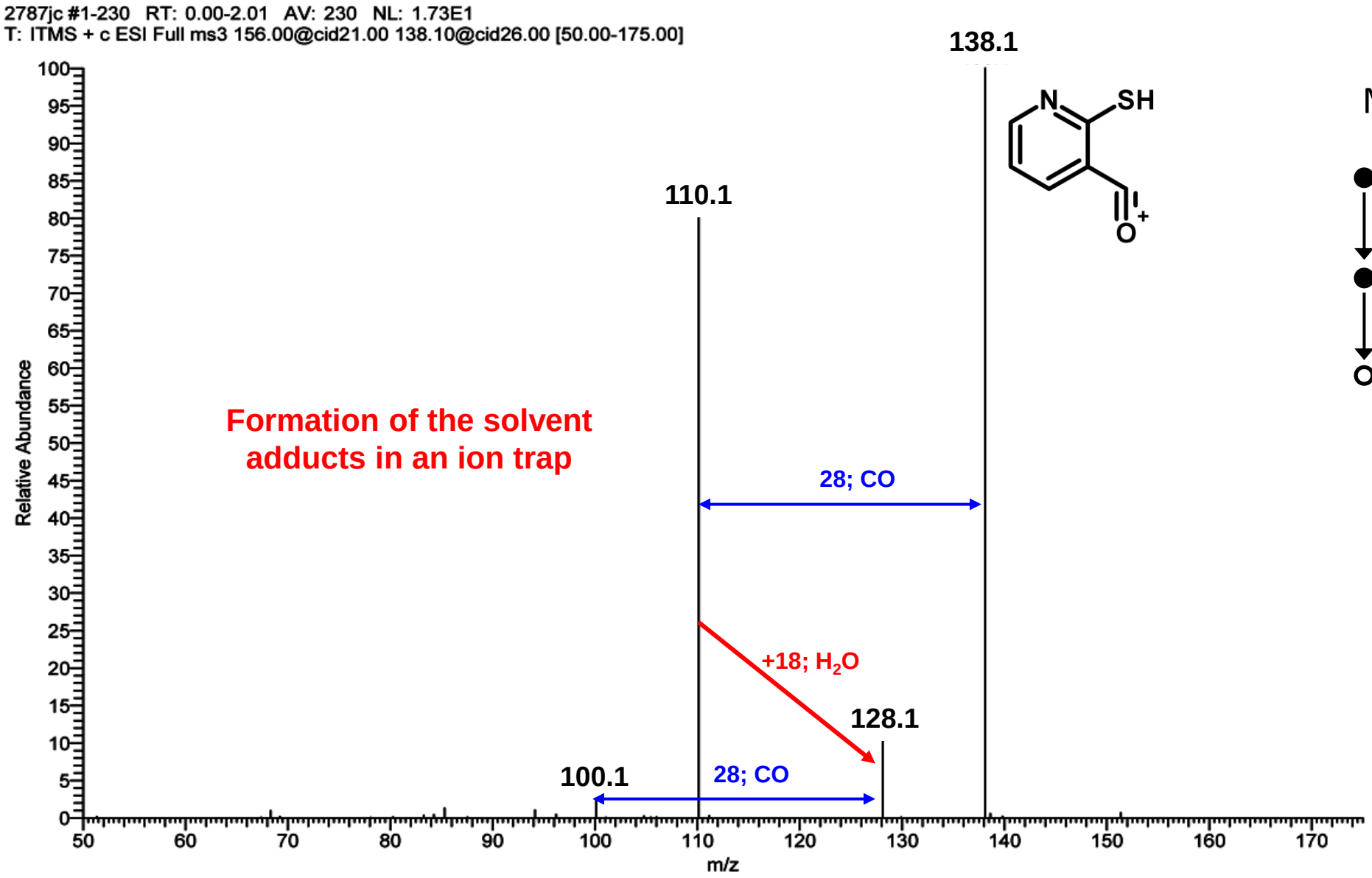
$C_6H_5NO_2S$; M=155.0

MS/MS, ESI+



28

What are the ions in the spectrum of 2-mercaptonicotinic acid?



Formation of the solvent adducts in an ion trap

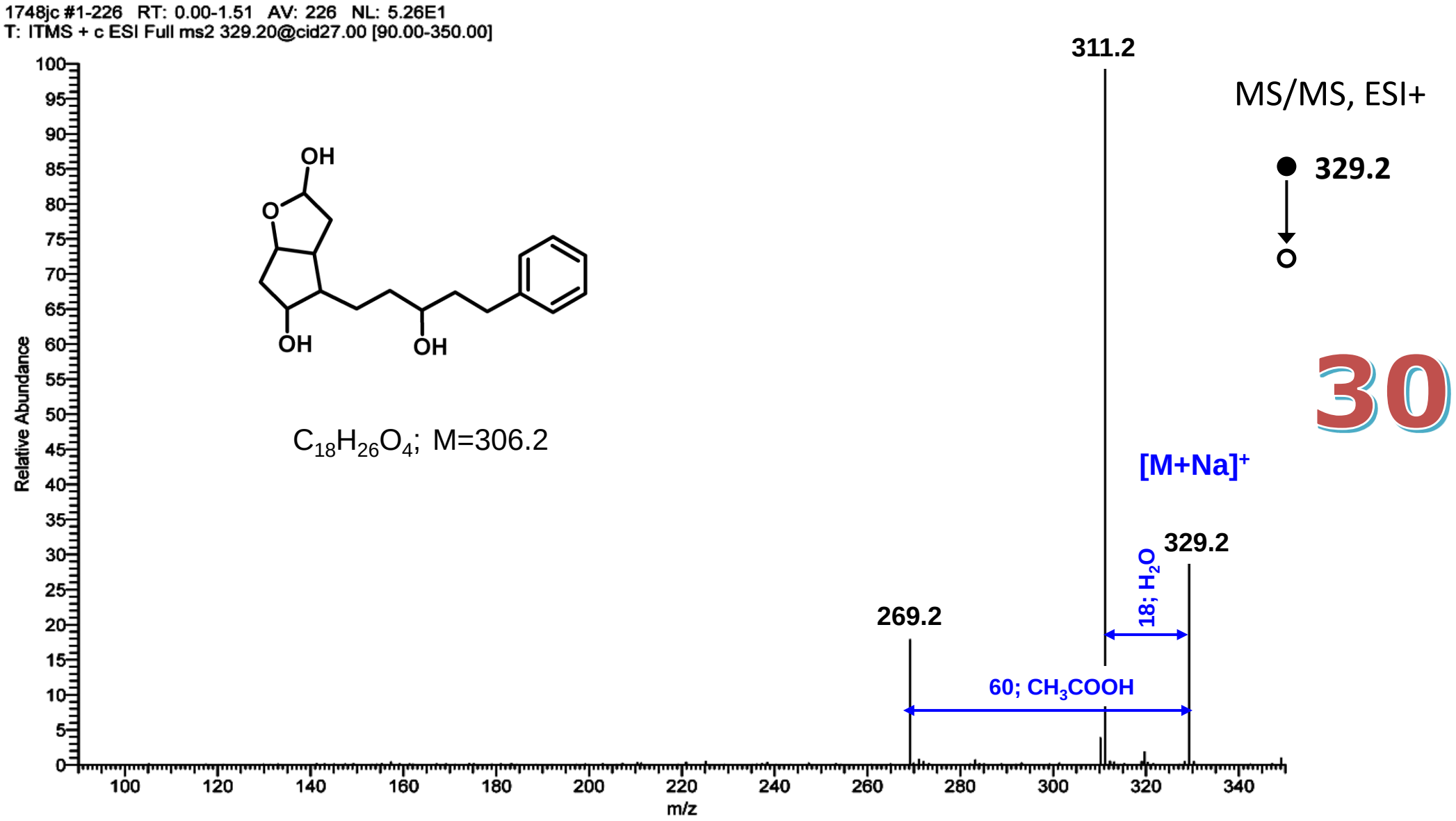
MS^3, ESI+

156.1

138.1

O

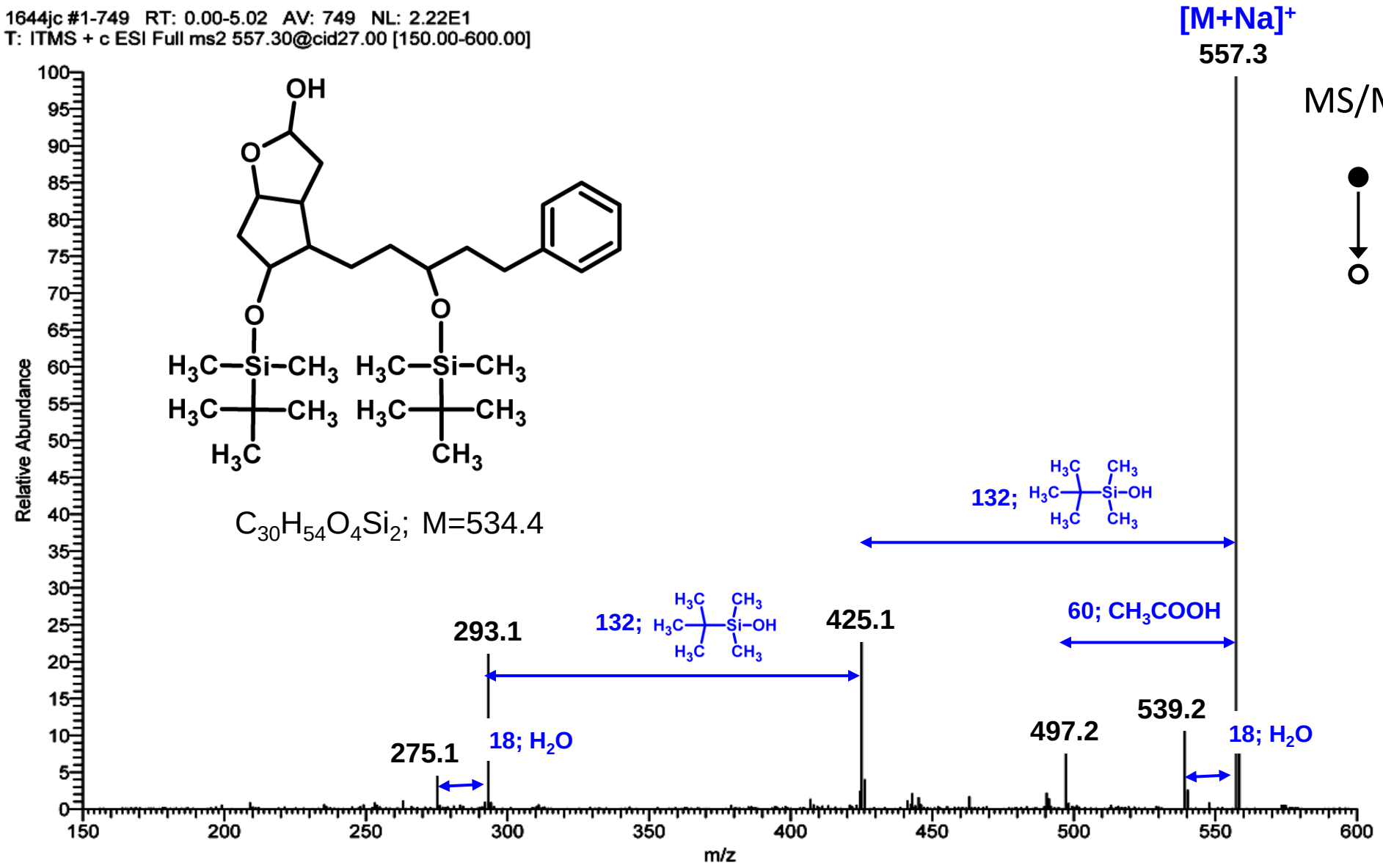
What are the ions in this spectrum?



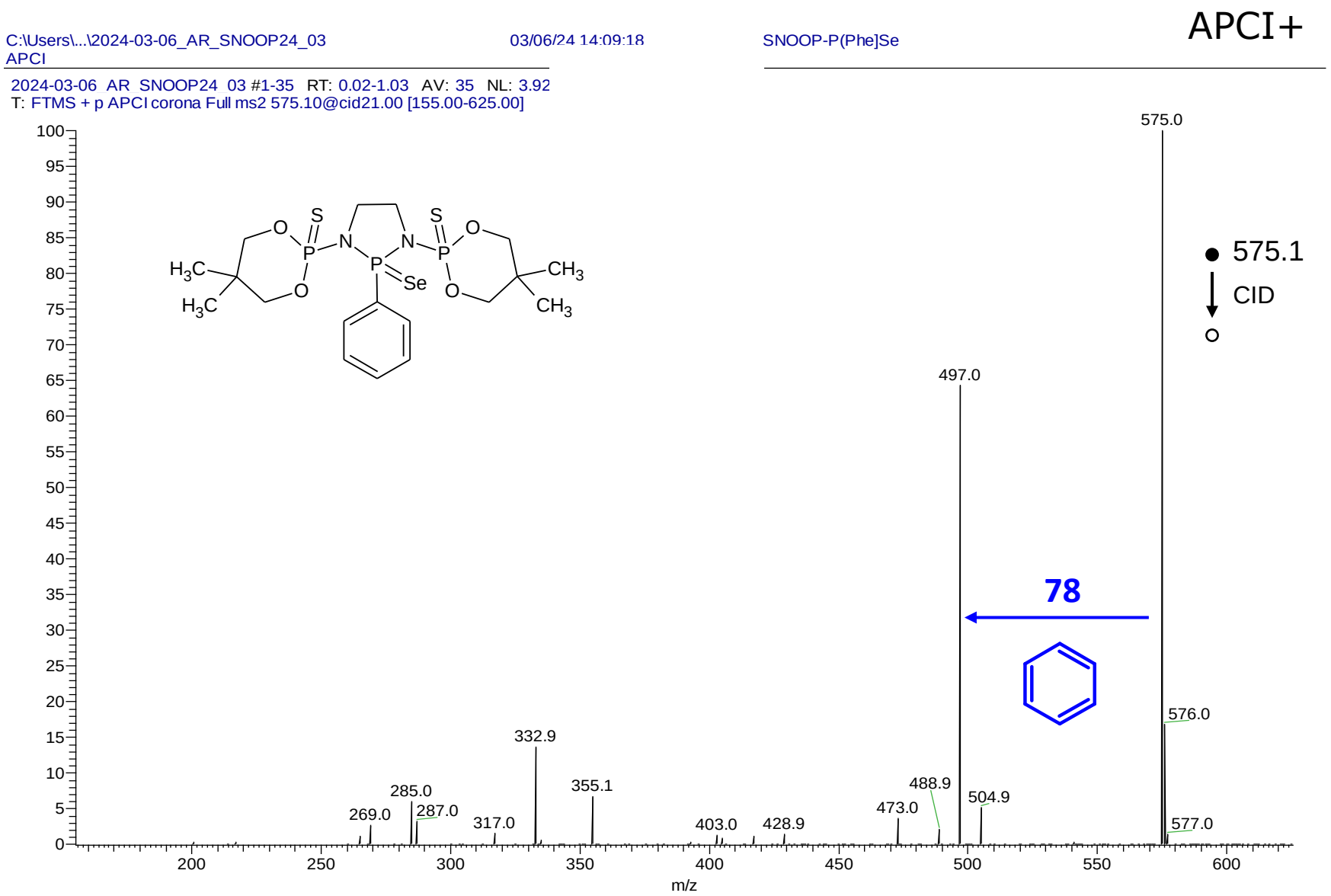
$C_{18}H_{26}O_4$; M=306.2

30

What are the ions in this spectrum?

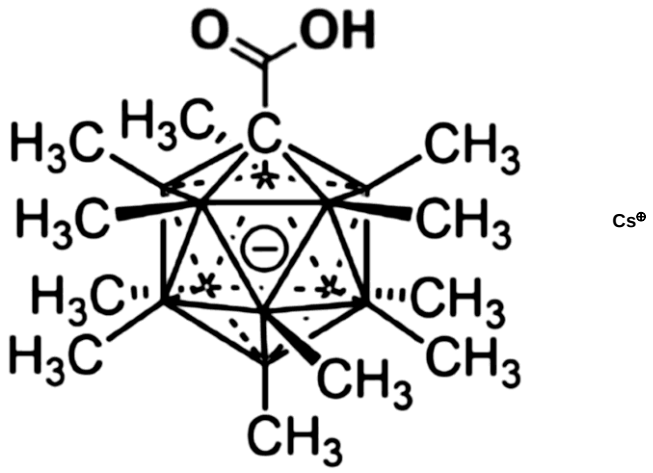


CURIOSITY: What neutral molecule is lost during fragmentation?



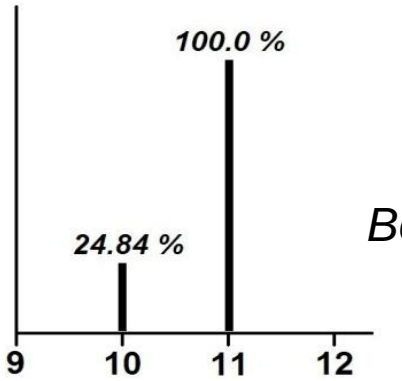
32

CURIOSITY: Loss of methane, eliminations of ethylene

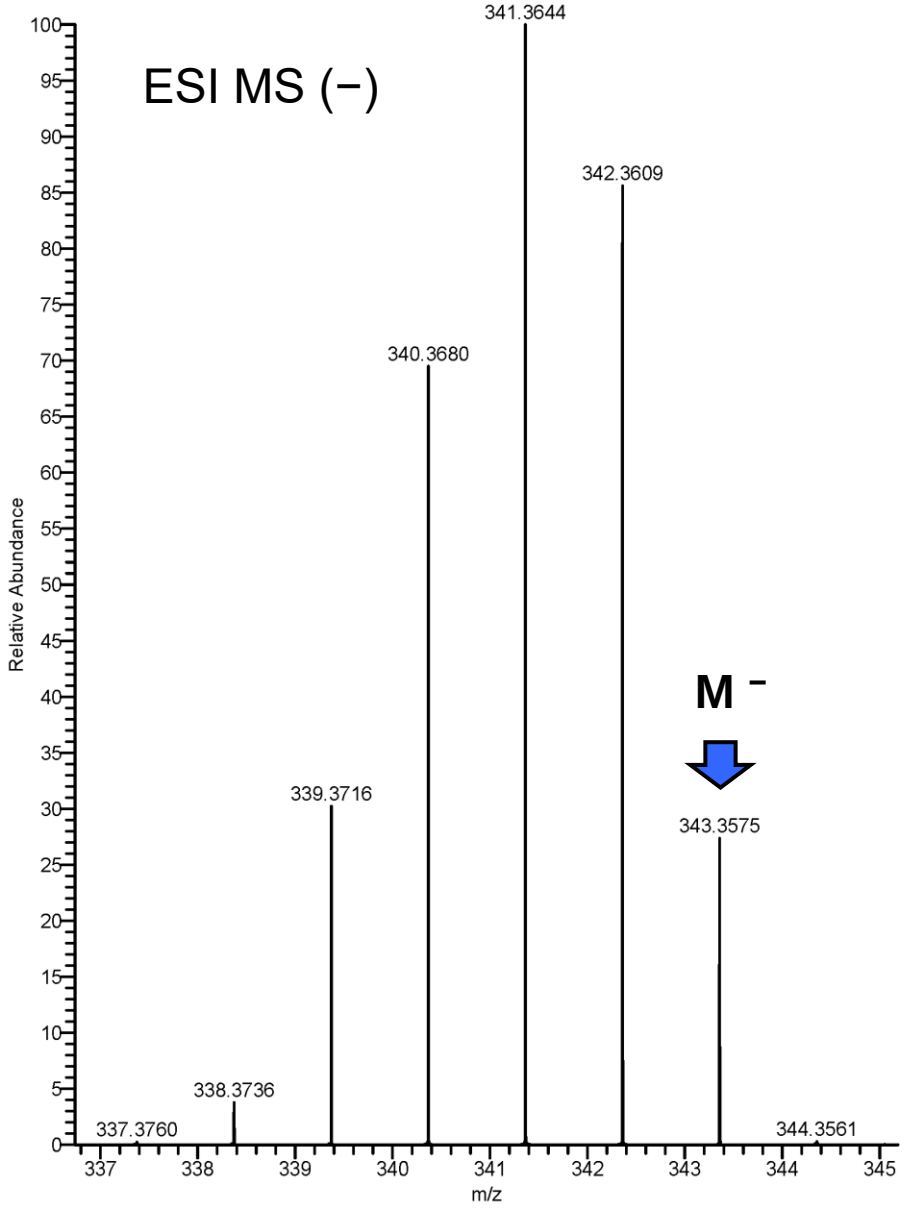


Carborane HOOC-$CB_{11}Me_{11}$

$C_{13}H_{34}B_{11}O_2^-$, 343.358246 Da

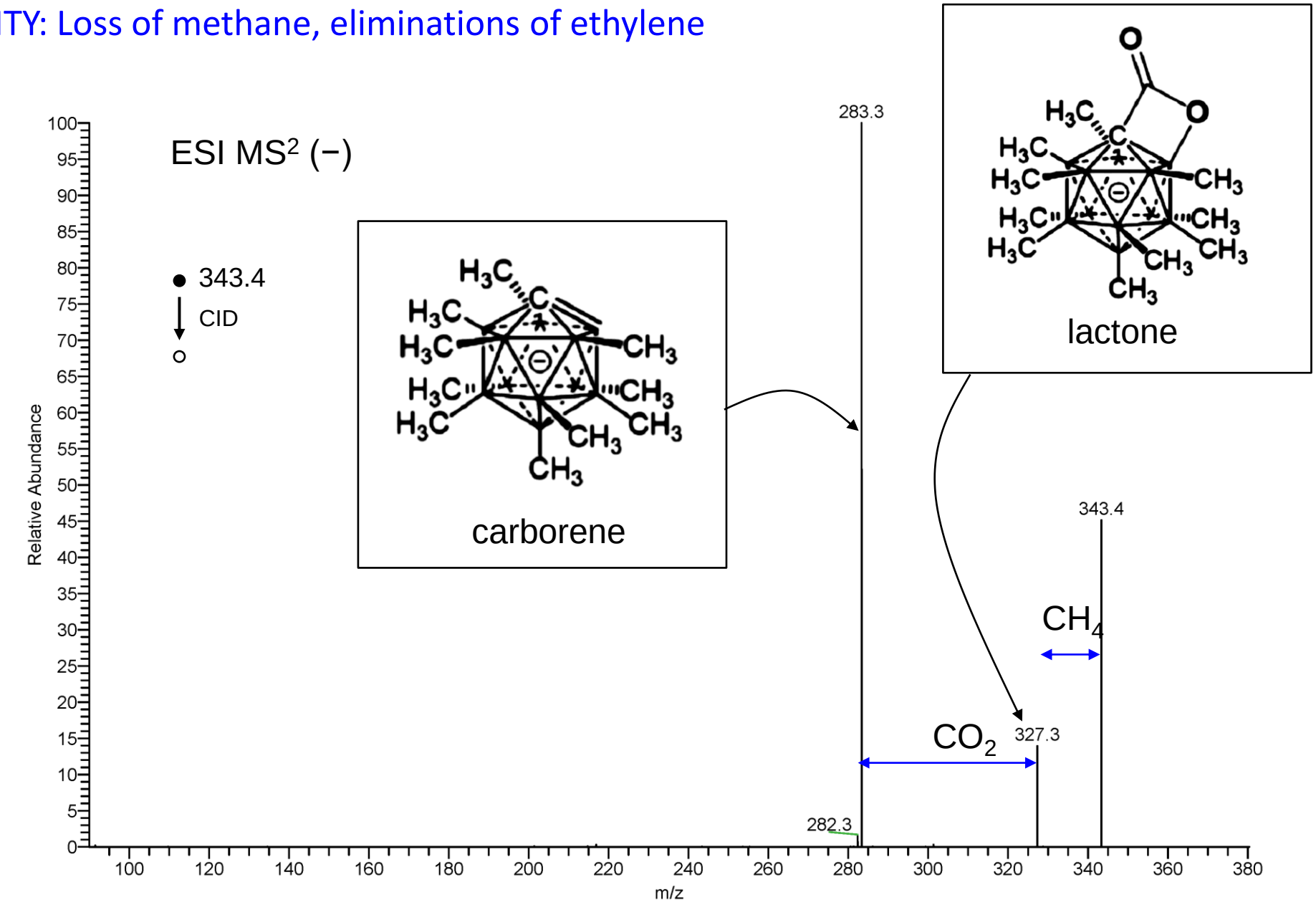


Boron isotopes



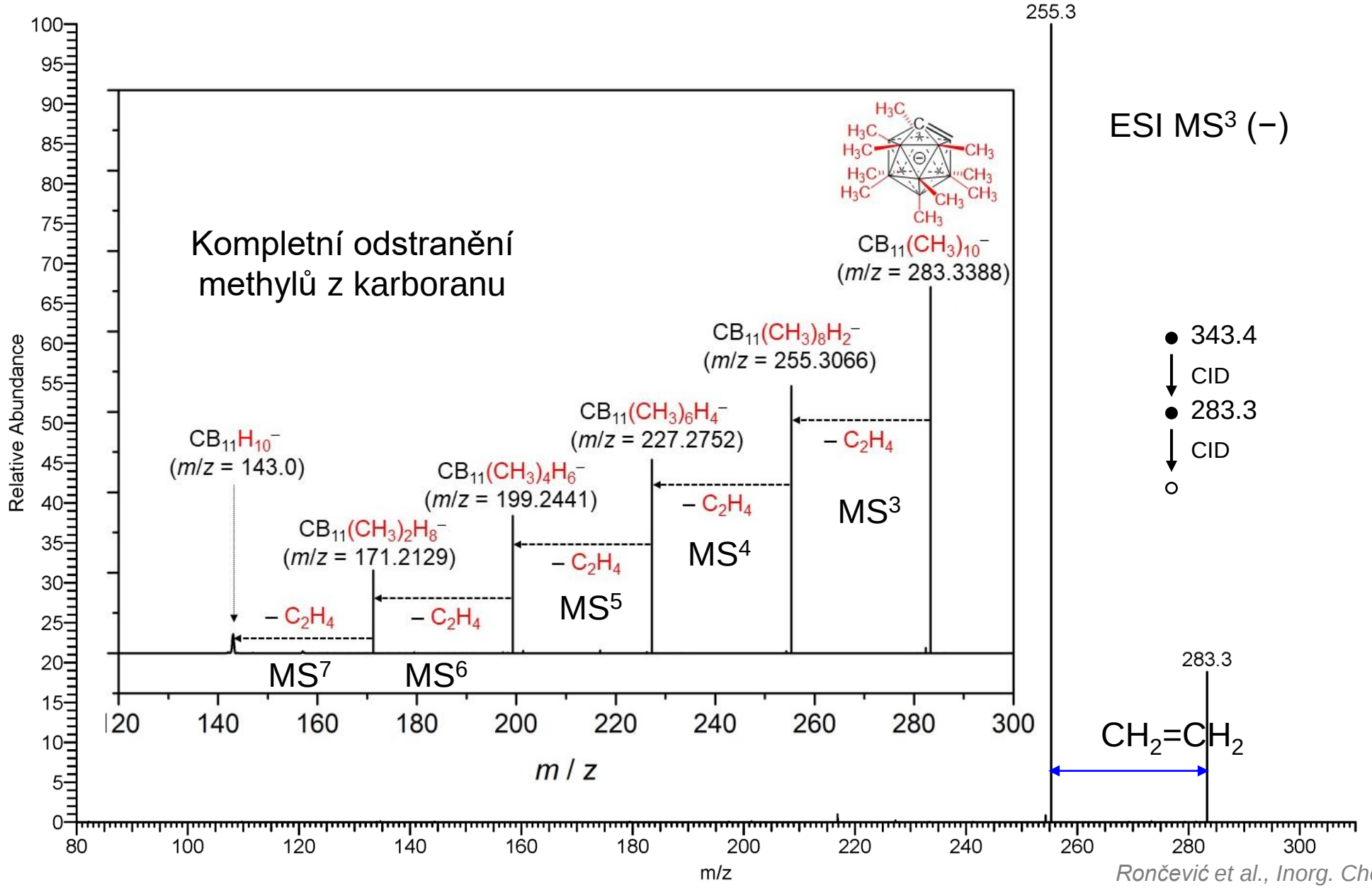
Rončević et al., Inorg. Chem. 59, 12453, 2020

CURIOSITY: Loss of methane, eliminations of ethylene



Rončević et al., Inorg. Chem. 59, 12453, 2020

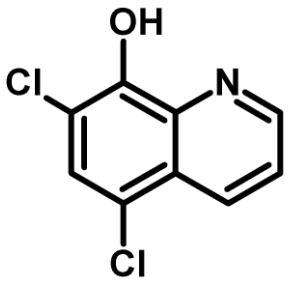
CURIOSITY: Loss of methane, eliminations of ethylene



Rončević et al., Inorg. Chem. 59, 12453, 2020

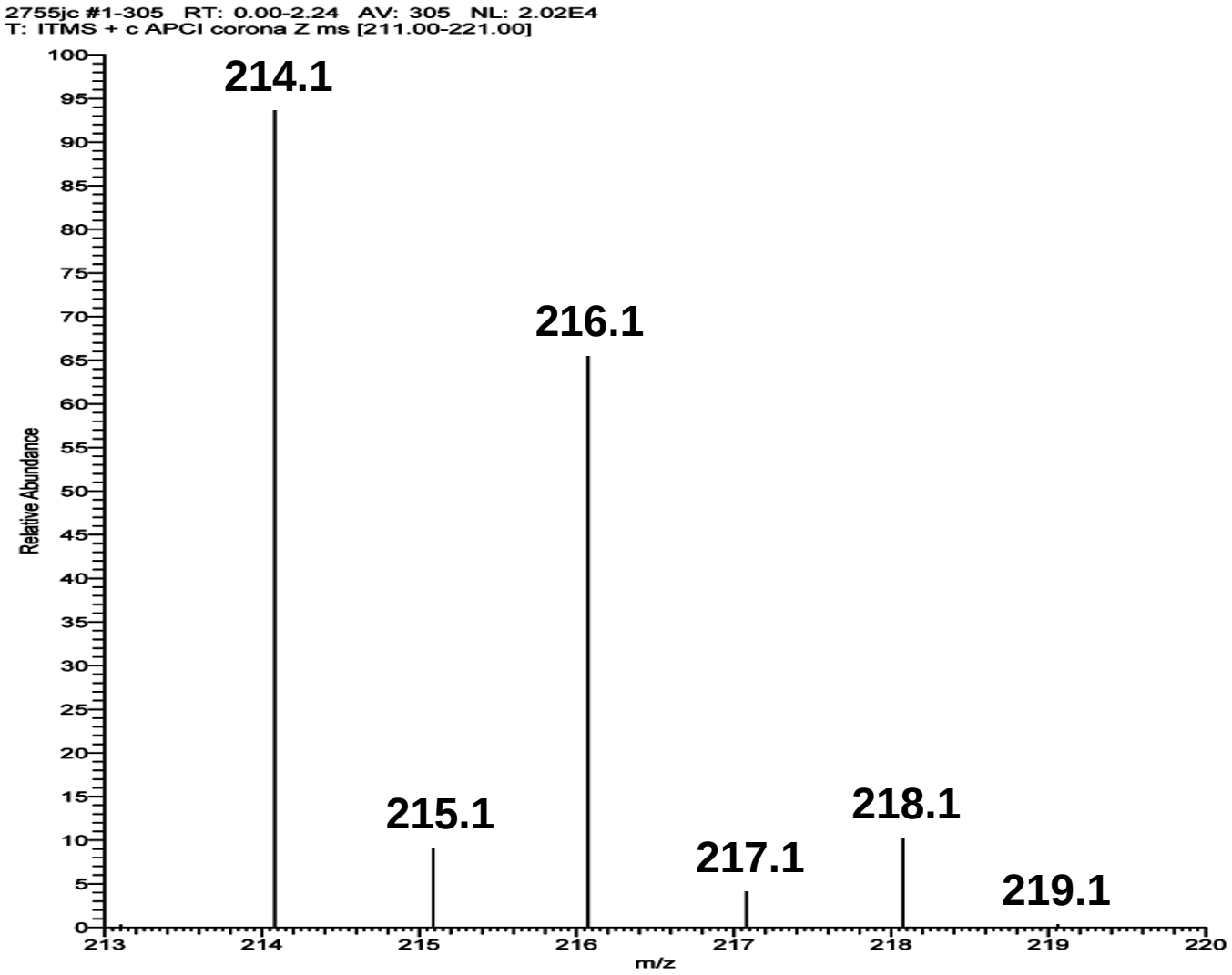
Fragmentation of isotopic peaks (EE^+)

MS, APCI+

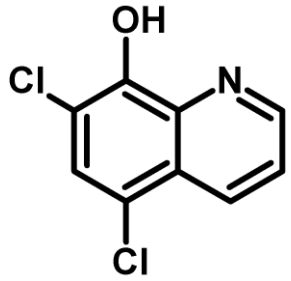


Chloroxine

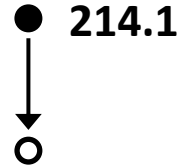
$C_9H_5Cl_2NO$; M=213.0



What are the ions in the spectrum of chloroxine?



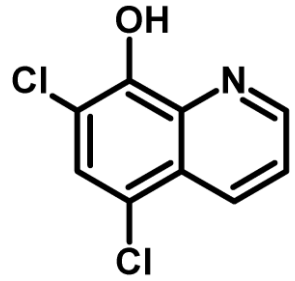
MS/MS, APCI+



FORMATION OF A RADICAL SPECIES FROM EE^+

33

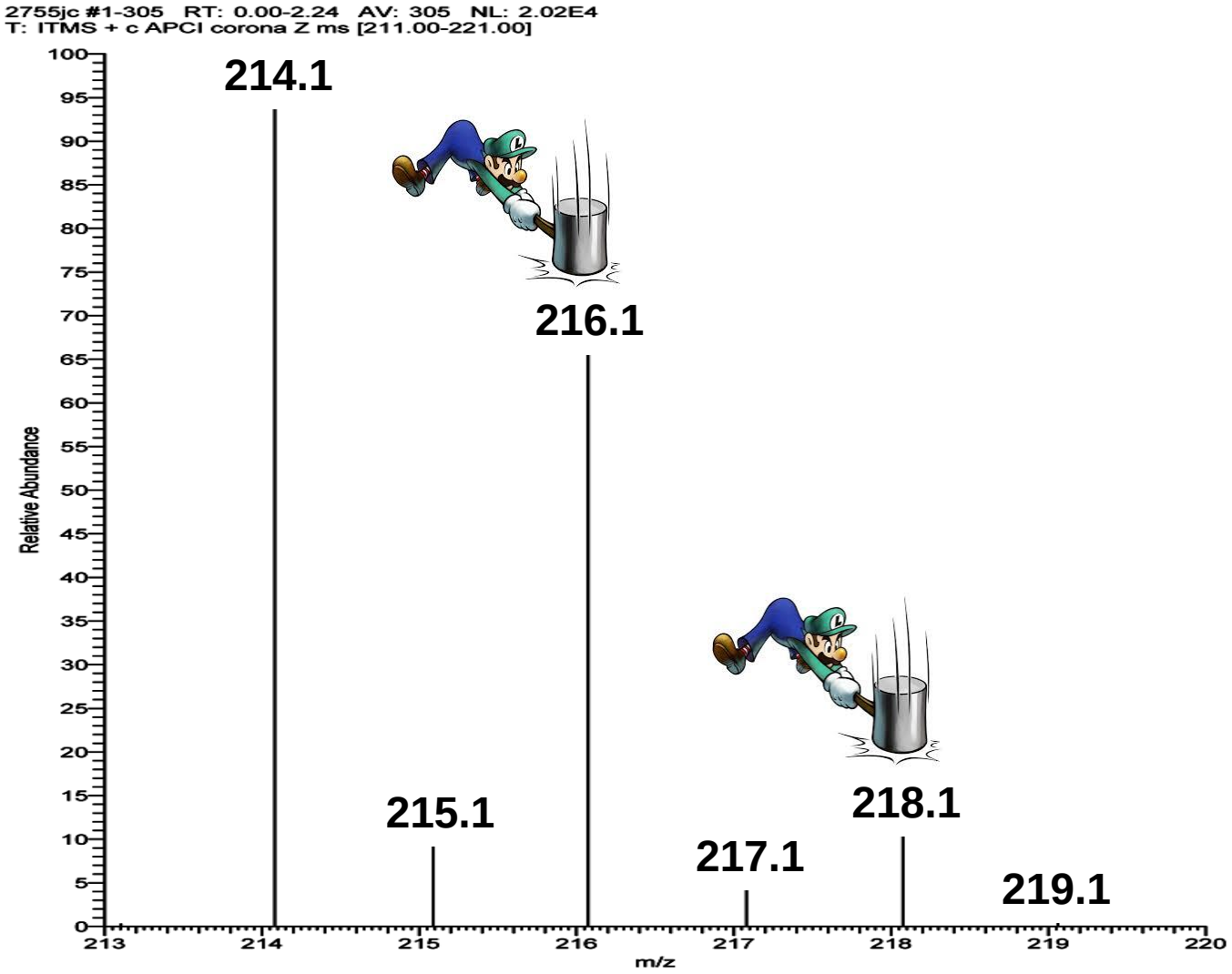
What ions are expected in the fragmentation spectra of isotopologues *m/z* 216.1 and *m/z* 218.1?



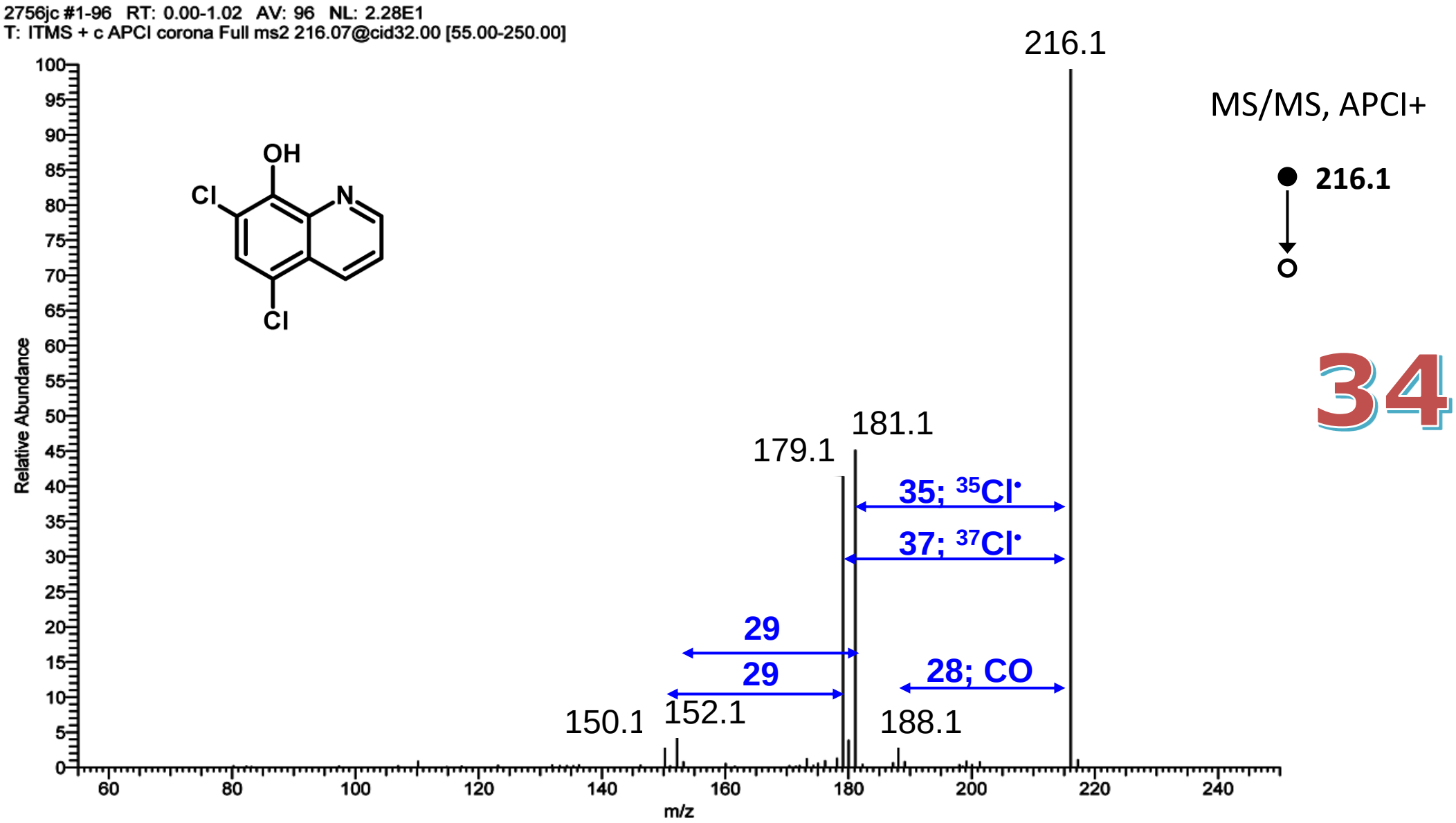
Chloroxine

$C_9H_5Cl_2NO$; M=213.0

MS, APCI+

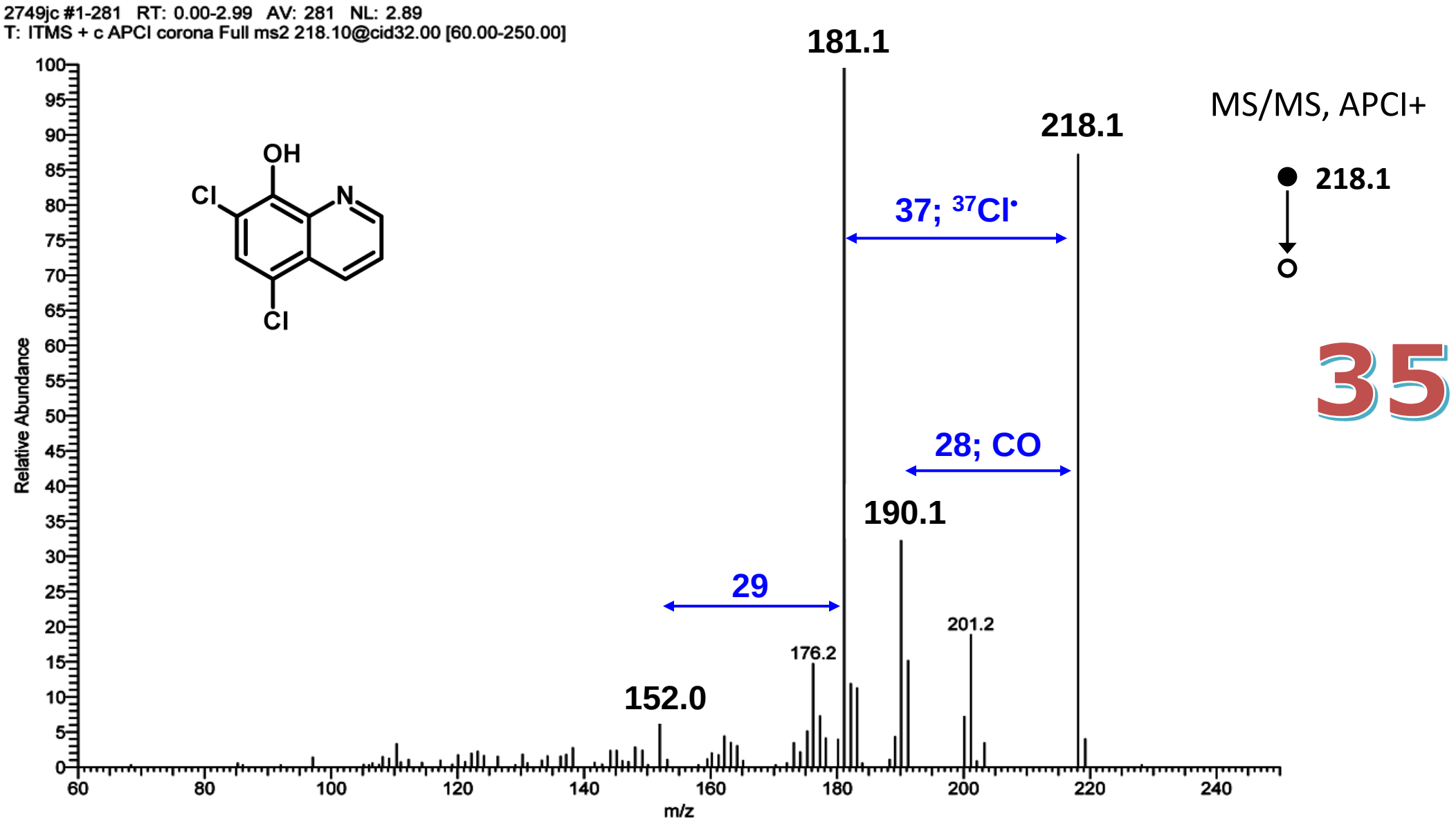


What ions are expected in the fragmentation spectra of isotopologues *m/z* 216.1 and *m/z* 218.1?



34

What ions are expected in the fragmentation spectra of isotopologues *m/z* 216.1 and *m/z* 218.1?



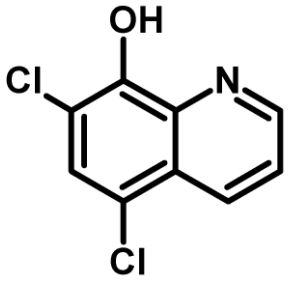
MS/MS, APCI+

218.1

O

35

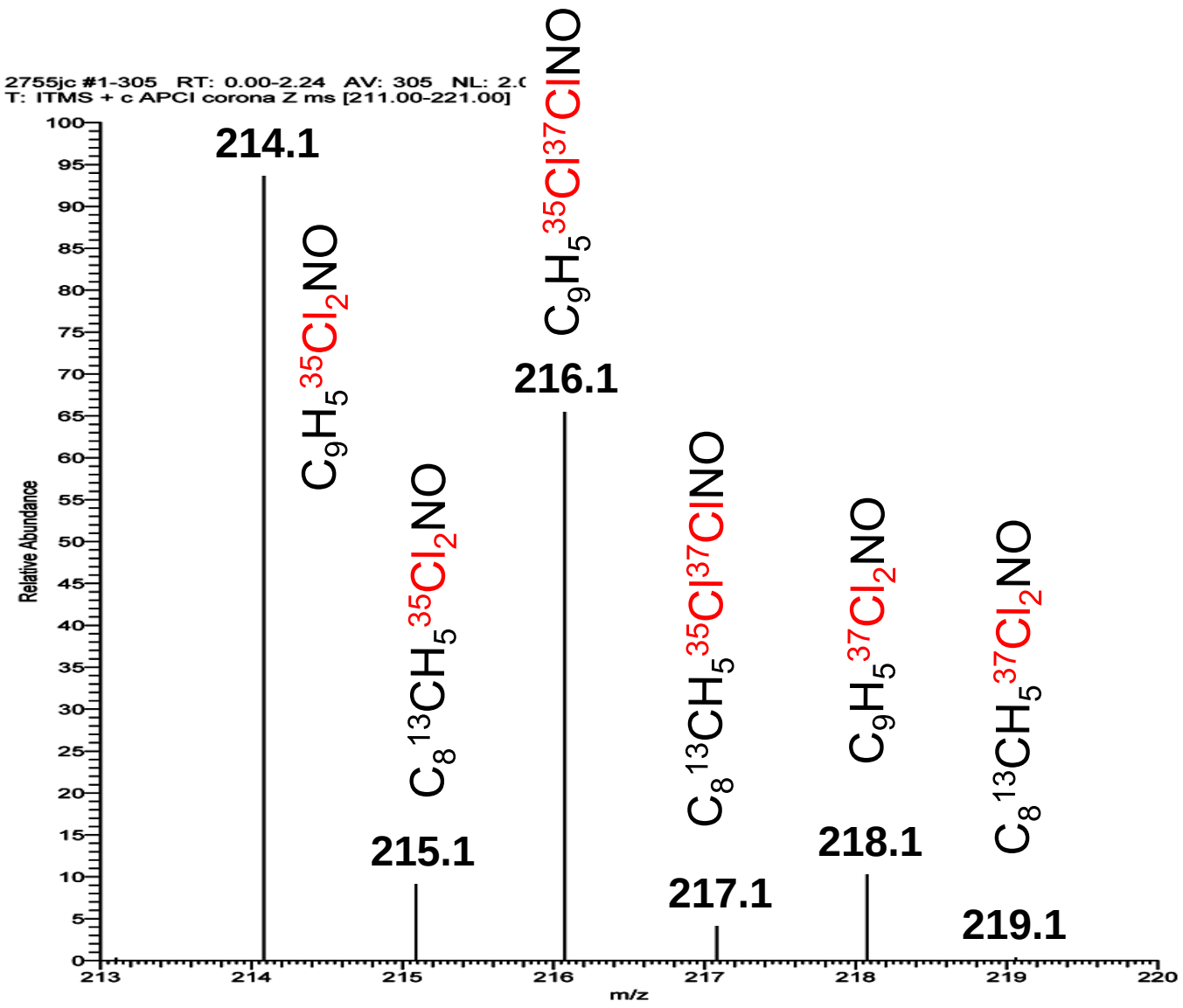
What ions are expected in the fragmentation spectra of isotopologues *m/z* 216.1 and *m/z* 218.1?



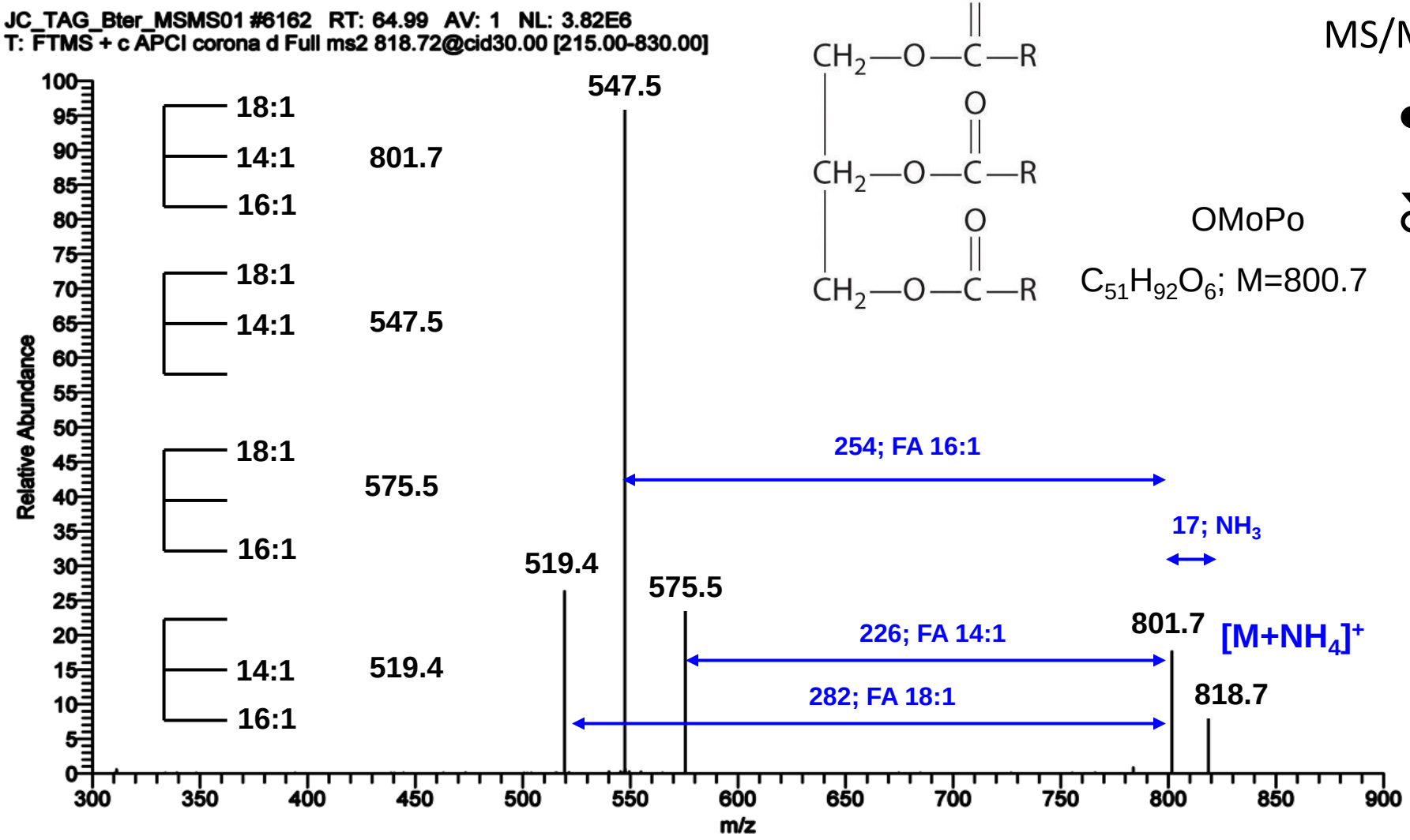
Chloroxine

$C_9H_5Cl_2NO$; M=213.0

MS, APCI+



What are the ions in the spectrum of a triacylglycerol?



MS/MS, APCI+

818.7

OMoPo

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

36

What is the structure of this triacylglycerol?

MS, APCI+

37

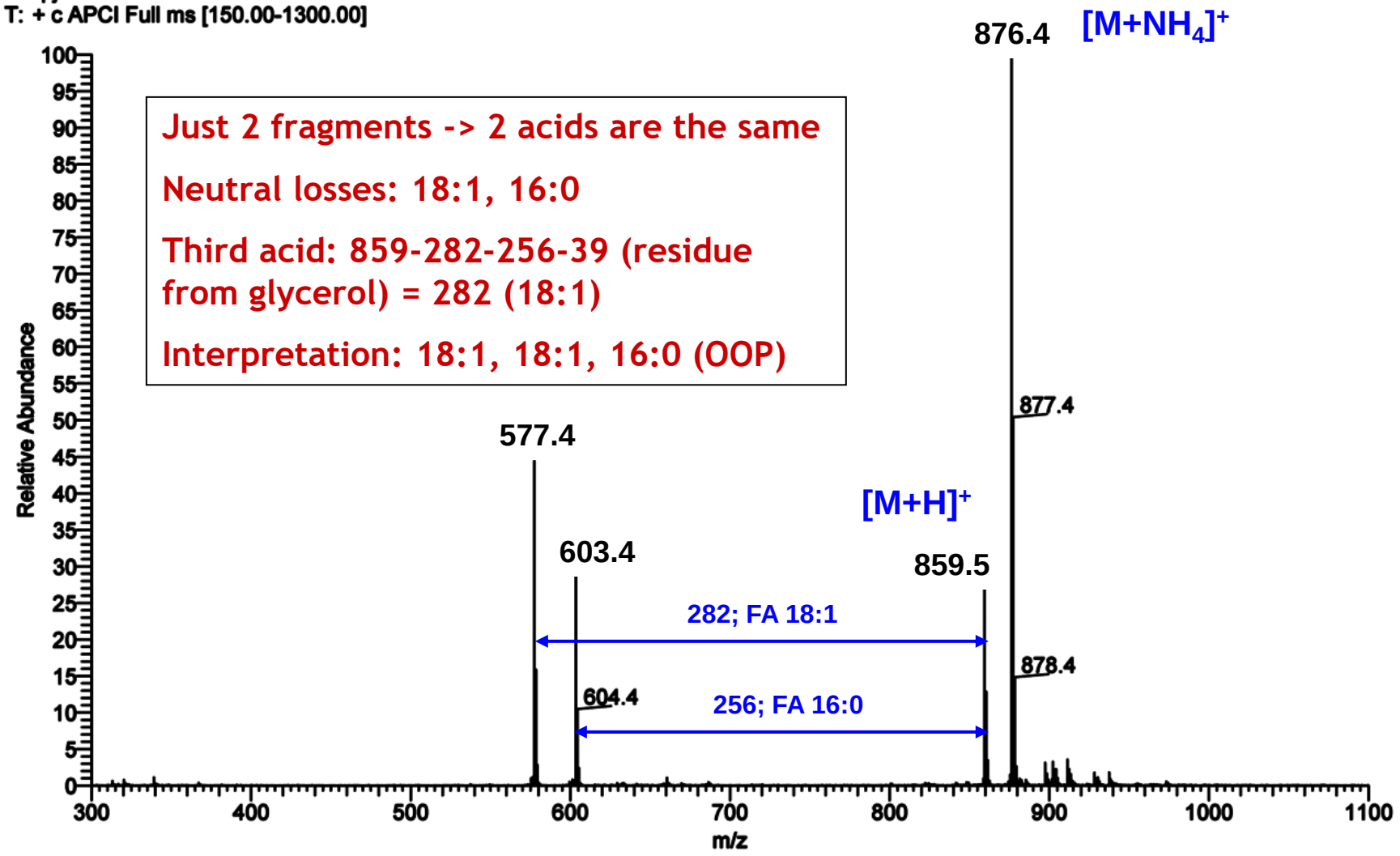
774pj #4258-4291 RT: 83.24-83.81 AV: 34 NL: 1.38E7
T: + c APCI Full ms [150.00-1300.00]

Just 2 fragments -> 2 acids are the same

Neutral losses: 18:1, 16:0

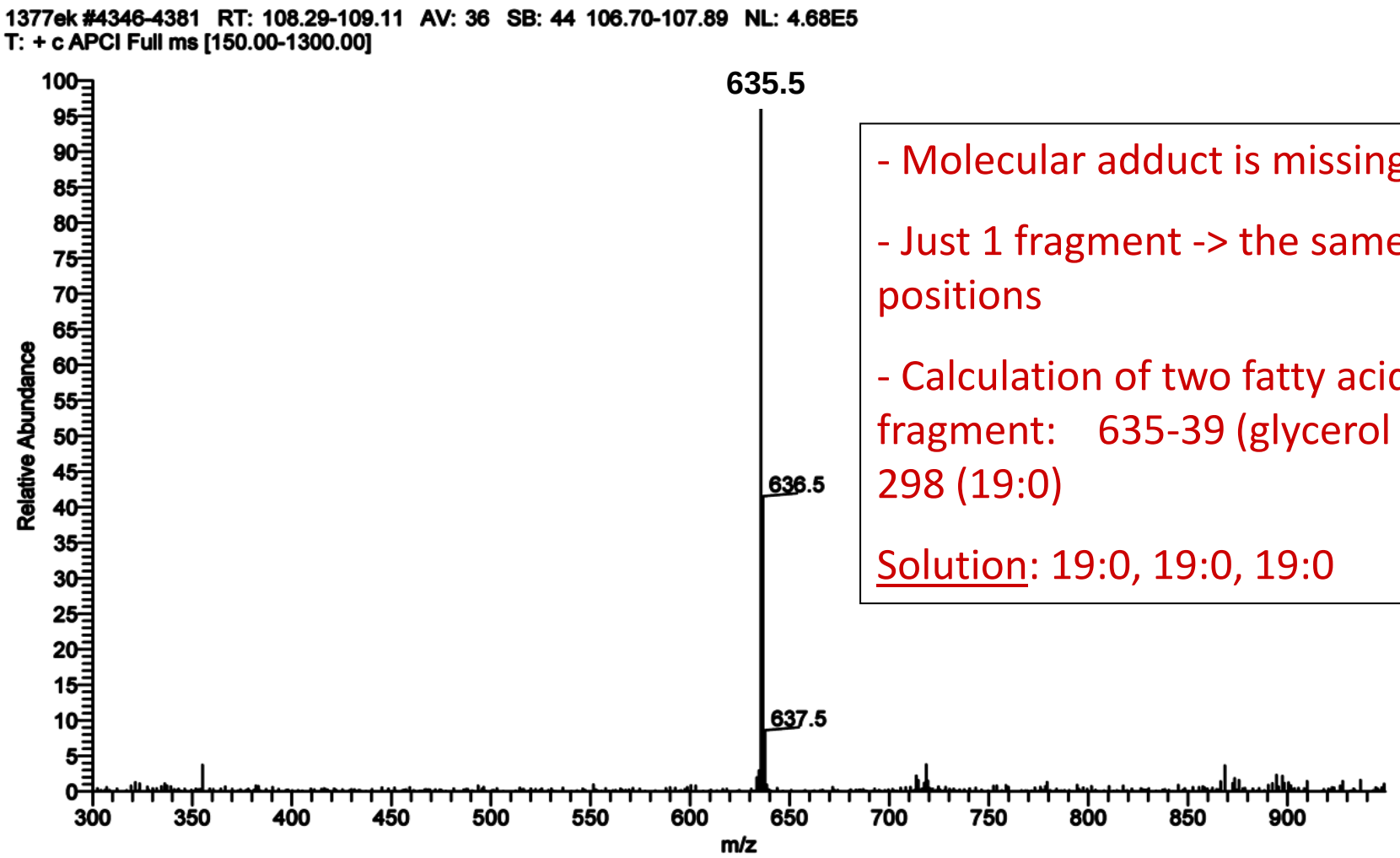
Third acid: 859-282-256-39 (residue from glycerol) = 282 (18:1)

Interpretation: 18:1, 18:1, 16:0 (OOP)



What is the structure of this triacylglycerol?

MS, APCI+



- Molecular adduct is missing

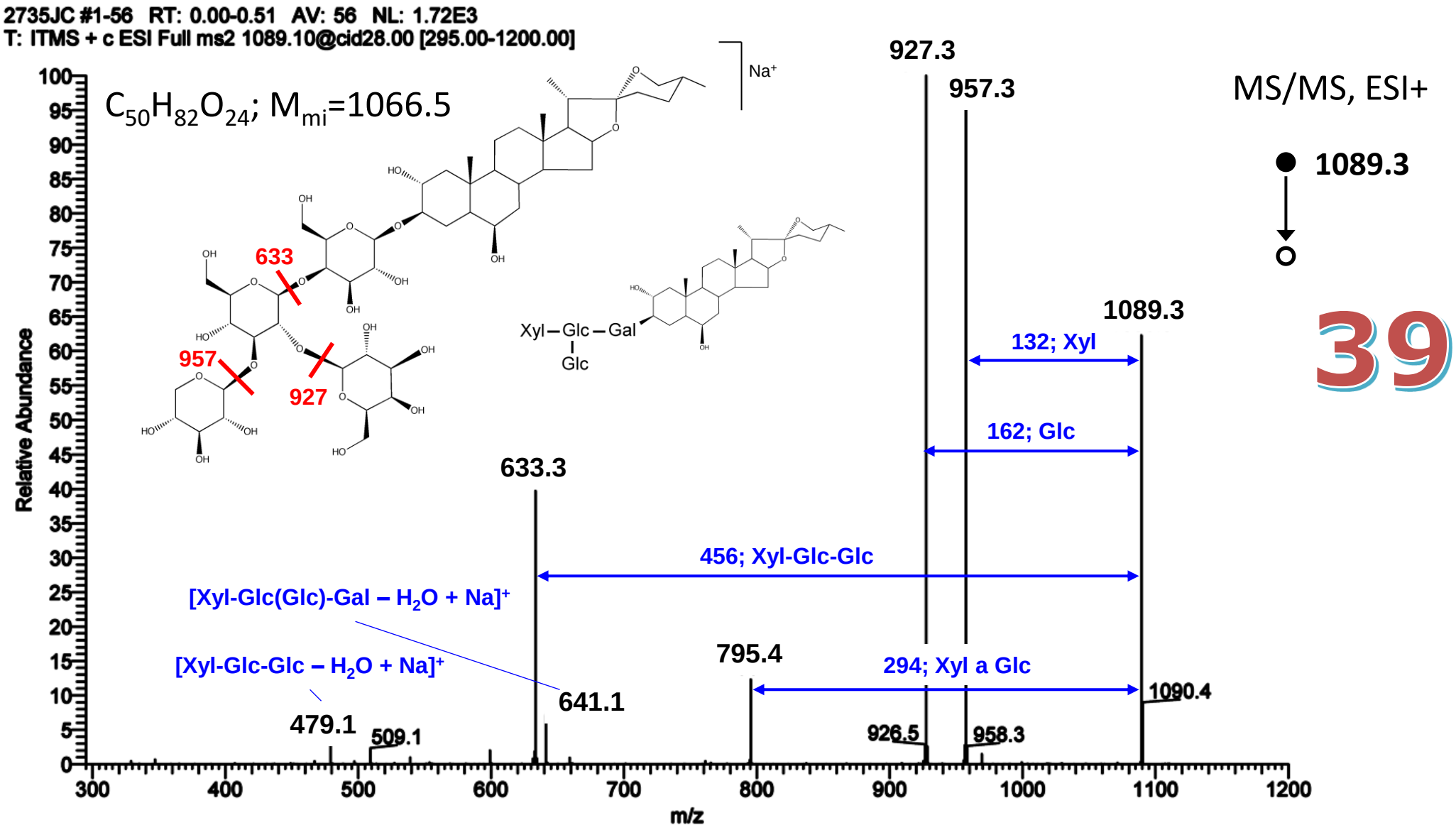
- Just 1 fragment -> the same fatty acids in all positions

- 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

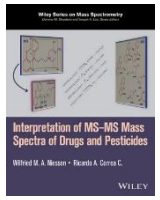
What are the ions in the spectrum of aginoside?

Leek saponins



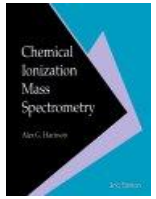
Recommended reading

ESI
APCI



Wilfried M. A. Niessen, Ricardo A. Correa C.: Interpretation of MS-MS Mass Spectra of Drugs and Pesticides, John Wiley & Sons (2017), ISBN: 978-1-118-50018-7

CI



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

EI

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