Interpretation of mass spectra

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@Bio_MassSpec

Recommended references on interpretation

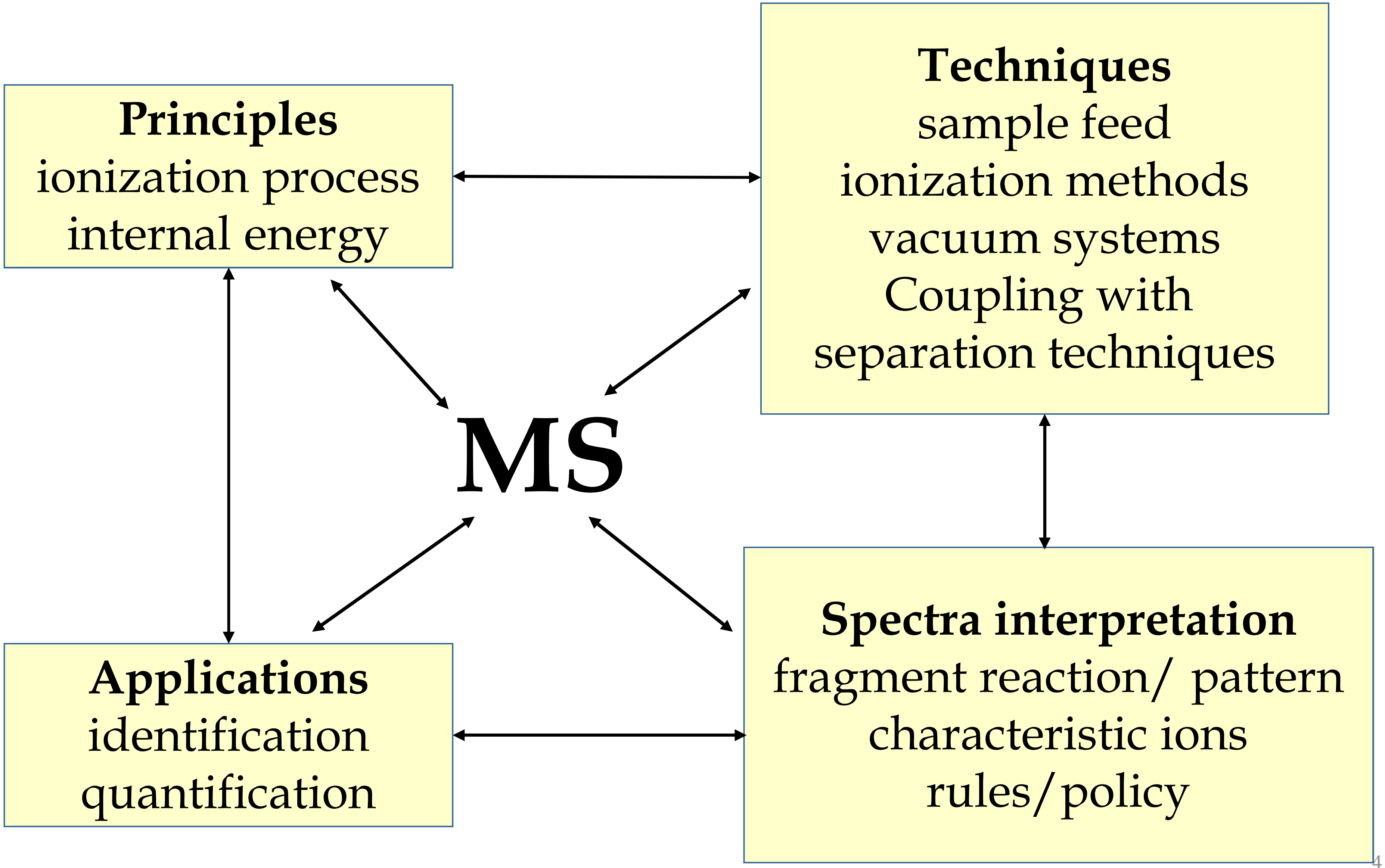
UNIVERSITÄT
DES
SAARLANDES

- McLafferty & Turecek, Interpretation of Mass Spectra, 4th edition, 1993.
- Smith & Busch, Understanding Mass Spectra, 1999.
- Lee, A beginner's Guide to Mass Spectral Interpretation, 1998.
- Ham, Even Electron Mass Spectrometry with Biomolecule Applications, 2008.
- Davis & Frearson, Mass Spectrometry (Analytical Chemistry by Open Learning), 1987

Content of the lecture

- Ion formation via electron ionization
- Unimolecular dissociations
- General strategy for spectral interpretation of EI spectra
- Major fragmentation mechanisms
- Mass spectra of selected compound classes

Aspects of mass spectrometry



Principles
ionization process
internal energy

Techniques
sample feed
ionization methods
vacuum systems
Coupling with
separation techniques

MS

Applications
identification
quantification

Spectra interpretation
fragment reaction/ pattern
characteristic ions
rules/policy

Terminology

Memory effect = contamination of previous measurement

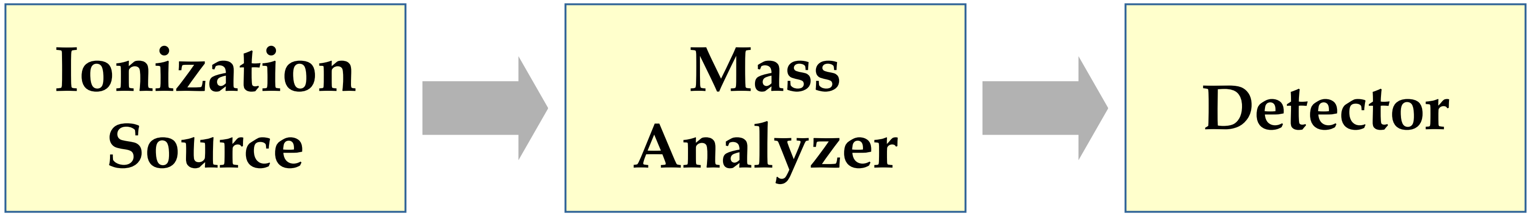
Sensitivity = specifies the total response of analytical system of a specific target compound under def. conditions

Limit of detection (LOD) = lowest measurable signal of target compound

Signal-to-noise ratio (S/N) = uncertainty of an intensity measurement ; quantitative statement of signal quality

Principle operation of a mass spectrometer

- Introduce sample to the instrument
- Generate ions in the gas phase
- Separate ions on the basis of differences in *m/z* with a mass analyzer
- Detect ions



Ionization Source

Mass Analyzer

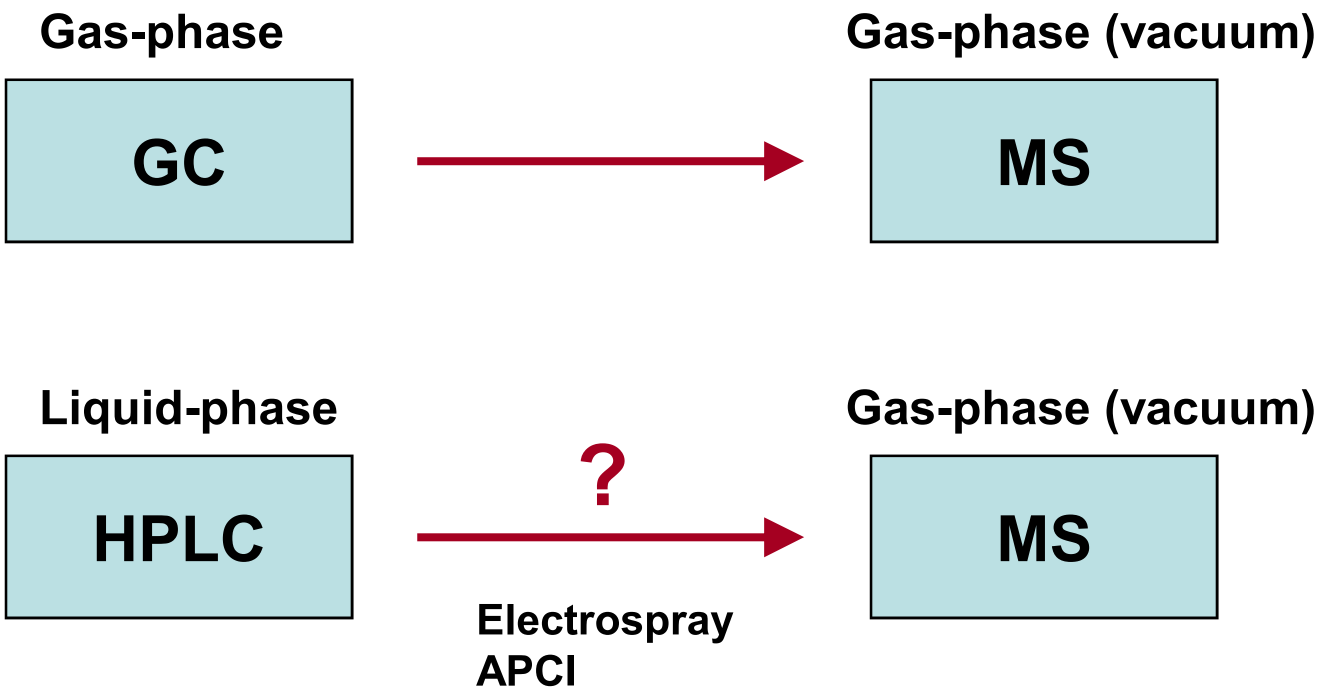
Detector

high vacuum

10^{-5} - 10^{-6} mbar

10^{-6} - 10^{-9} mbar

GC-MS / LC-MS



Gas-phase → **Gas-phase (vacuum)**

GC → **MS**

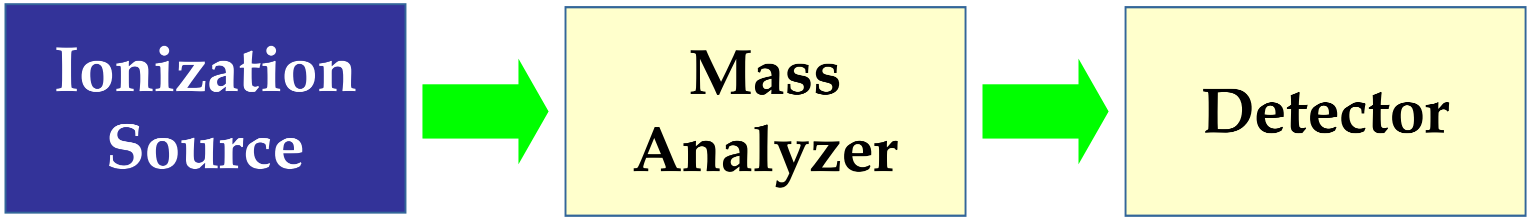
Liquid-phase → **Gas-phase (vacuum)**

HPLC → ? → **MS**

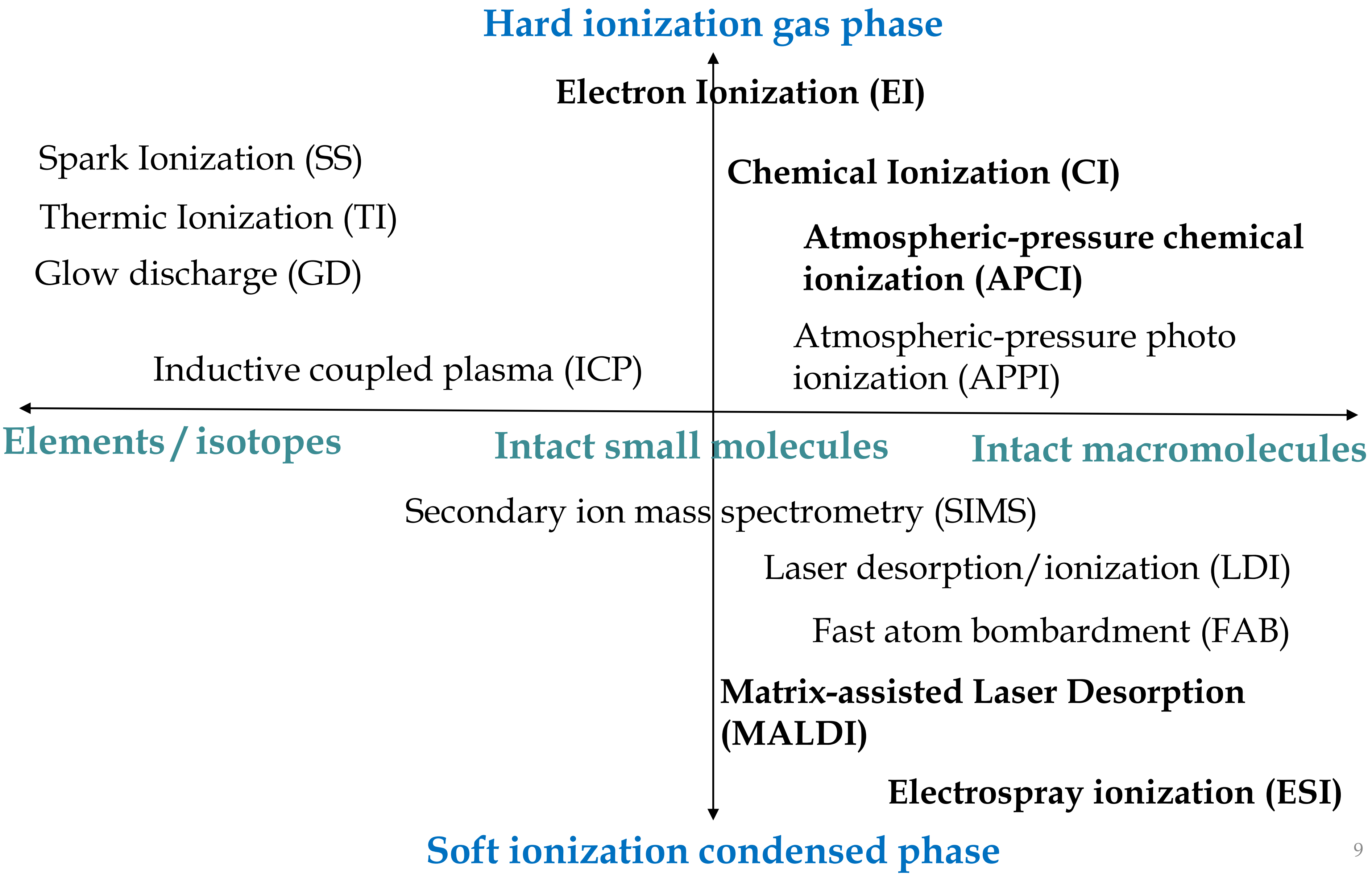
Electrospray
APCI

Ionization techniques

- **Electron Ionization (EI)**
- Chemical Ionization (CI)
- Electrospray ionization (ESI)
- Atmospheric-pressure chemical ionization (APCI)
- Matrix-assisted Laser Desorption (MALDI)



Ionization techniques



Hard ionization gas phase

Electron Ionization (EI)

Spark Ionization (SS)

Thermic Ionization (TI)

Glow discharge (GD)

Chemical Ionization (CI)

Atmospheric-pressure chemical ionization (APCI)

Atmospheric-pressure photo ionization (APPI)

Inductive coupled plasma (ICP)

Elements / isotopes

Intact small molecules

Intact macromolecules

Secondary ion mass spectrometry (SIMS)

Laser desorption/ionization (LDI)

Fast atom bombardment (FAB)

Matrix-assisted Laser Desorption (MALDI)

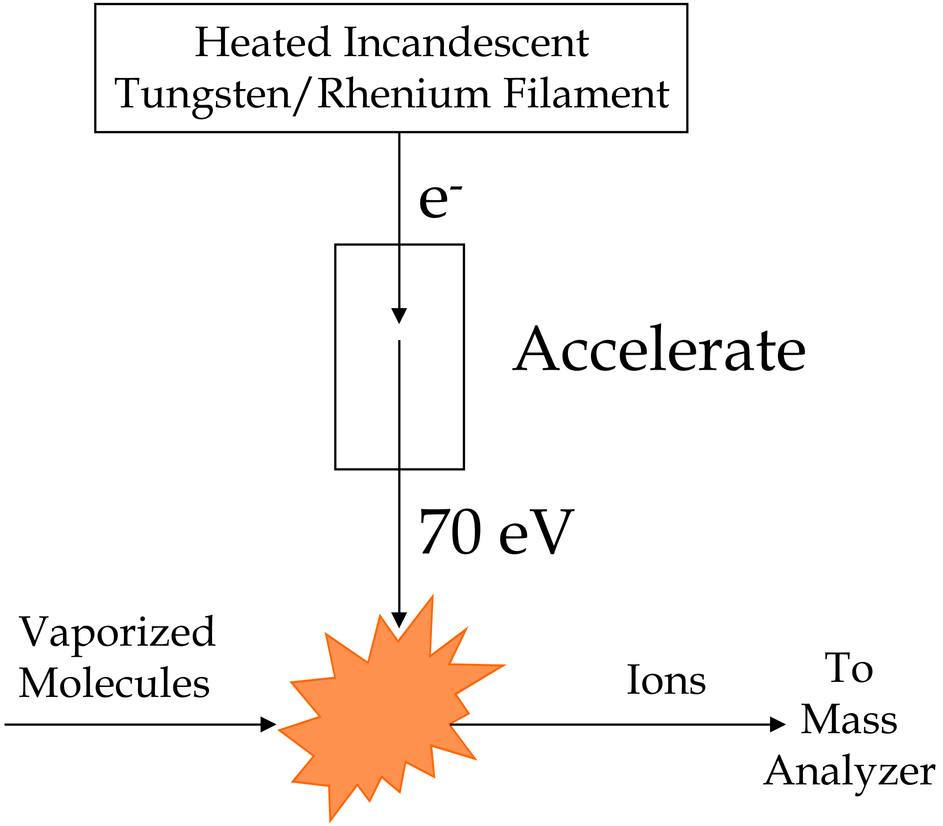
Electrospray ionization (ESI)

Soft ionization condensed phase

Electron ionization (EI) *(not electron impact!)*

- The electron ionization (EI) source is designed to produce gaseous ions for analysis.

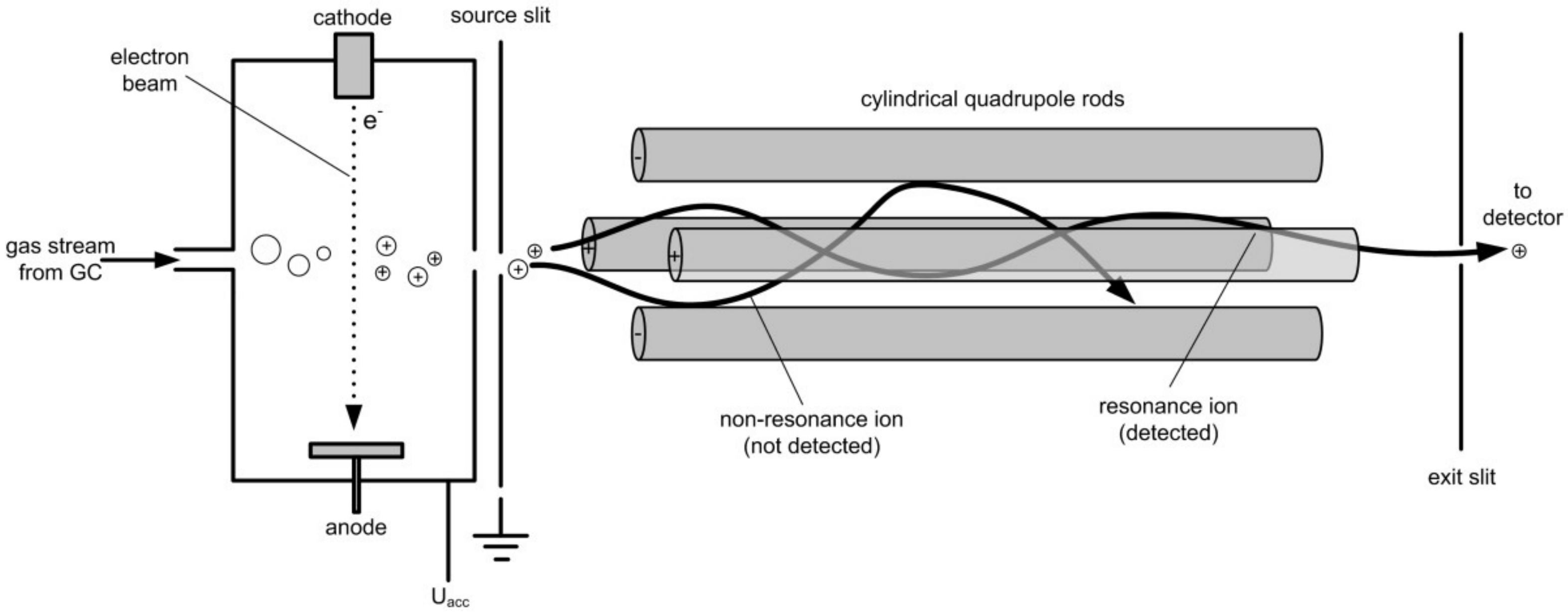
- EI was one of the earliest sources in wide use for MS, it usually operates on vapors (such as those from GC)



Electron ionization (EI)

How EI works:

- Electrons are emitted from a filament made of tungsten, rhenium…
- They are accelerated by a potential of 70 V.
- The electrons and molecules cross (usually at a right angle) and “collide”.
- The ions are primarily *singly-charged, positive ions*, that are extracted by a small potential (5V) through a source slit.



Electron ionization (EI)

- When electrons "hit" – the molecules undergo rotational-vibrational excitation (the mass of electrons is too small to "move" the molecules)

- About one in a million molecules undergo the reaction:

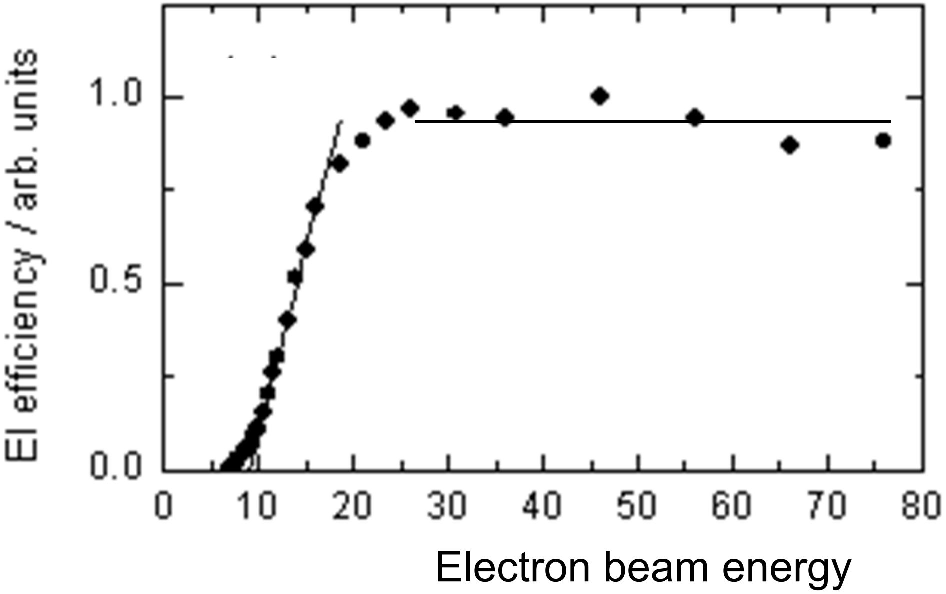
$$\mathbf{M + e^- \longrightarrow M^{\cdot +} + 2e^-}$$

- *Advantages:*
  - Results in complex mass spectra with fragment ions, useful for structural identification

- *Disadvantages:*
  - Can produce too much fragmentation, leading to no molecular ions! (makes structural identification often difficult!)

Electron ionization (EI)

Standardized electron beam energy of 70 eV in all systems



70 eV well above ~10 eV ionization energy for organic molecules → gives stable, instrument-independent mass spectra for database searching.

Chemical ionization (CI)

- Chemical ionization (CI) is a form of gas-phase chemistry that is "softer" (less energetic) than EI
  - Ionization via proton transfer reactions
- A gas (methane, isobutane, ammonia) is introduced into the source at ~1 torr.
- Example: CH_4 reagent gas

$$CH_4 \xrightarrow{EI} CH_4^{+\cdot}$$

$$CH_4^{+\cdot} + CH_4 \longrightarrow CH_5^{+} + CH_3^{\cdot}$$

$$A + CH_5^{+} \longrightarrow AH^{+} + CH_4$$

Strong acid

Chemical ionization (CI)

4 possibilities to generate positive ions by neutral analyte-molecule M :

$\mathbf{M + [BH]^+ \longrightarrow [M+H]^+ + B}$ *Protontransfer*

$\mathbf{M + X^+ \longrightarrow [M+X]^+}$ *Electrophilic addition*

$\mathbf{M + X^+ \longrightarrow [M-A]^+ + AX}$ *Anion abstraction*

$\mathbf{M + X^{+\cdot} \longrightarrow M^{+\cdot} + X}$ *Charge transfer / exchange*

$[M+H]^+$ protonated molecule

$[M-H]^+$ deprotonated molecule

$[M+Na]^+$ sodium cationized molecule

Chemical ionization (CI) – hard/soft sources

- The energy difference between EI and CI is apparent from the spectra:

- CI gases:
  - harshest (most fragments): methane
  - softest: ammonia

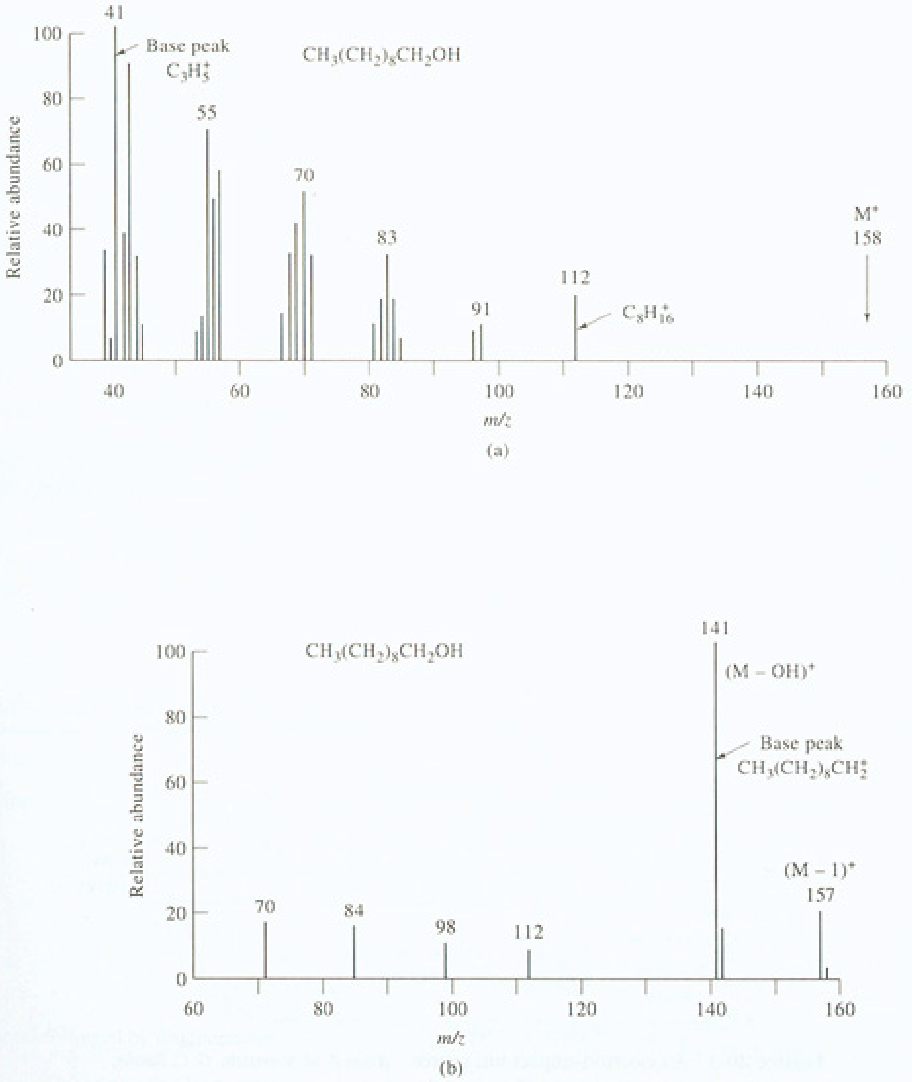
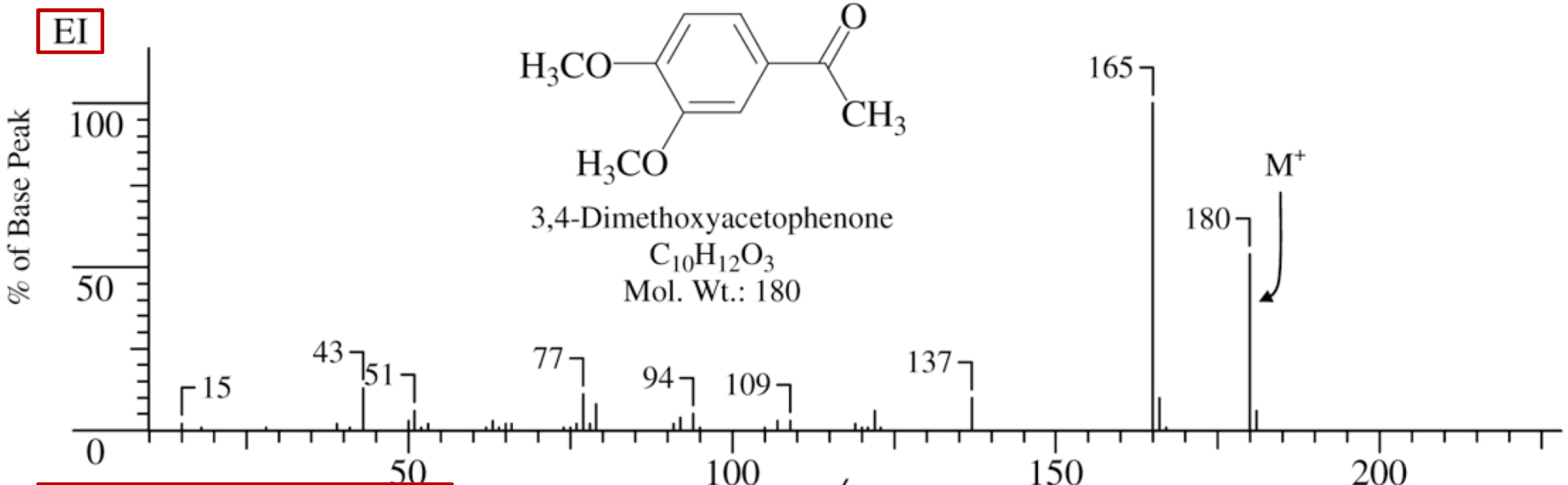


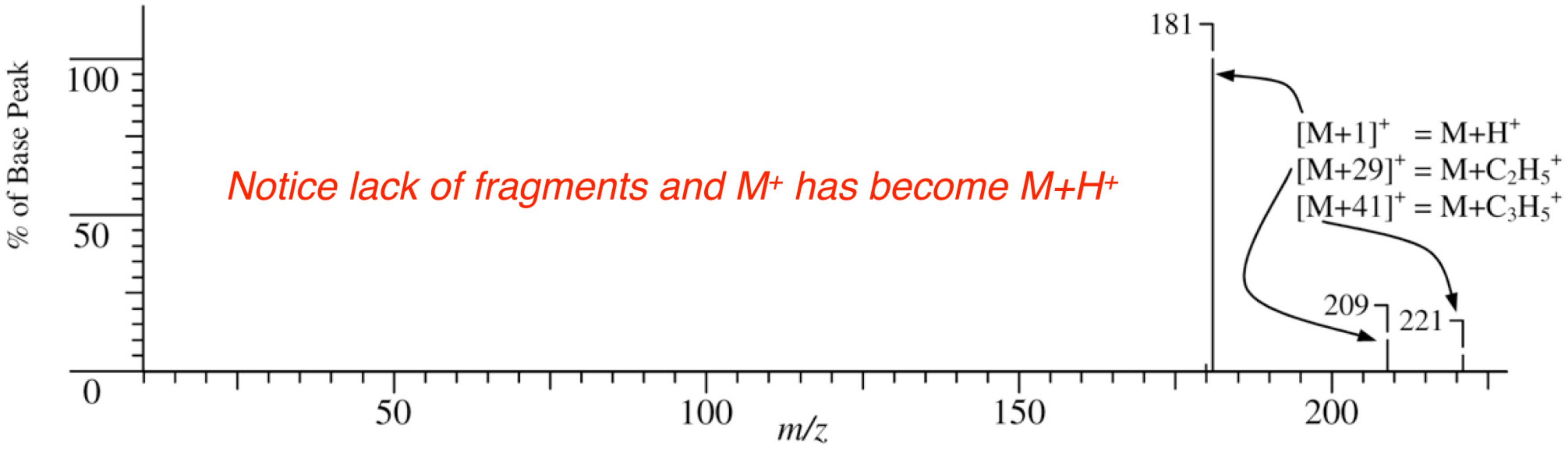
Figure 20-2 Mass spectrum of 1-decanol from (a) a hard source and (b) a soft source.

Comparison EI / CI

EI



CI Reagent Gas Methane



Notice lack of fragments and M^+ has become $M+H^+$

Unimolecular decompositions

Timescale for EI-MS

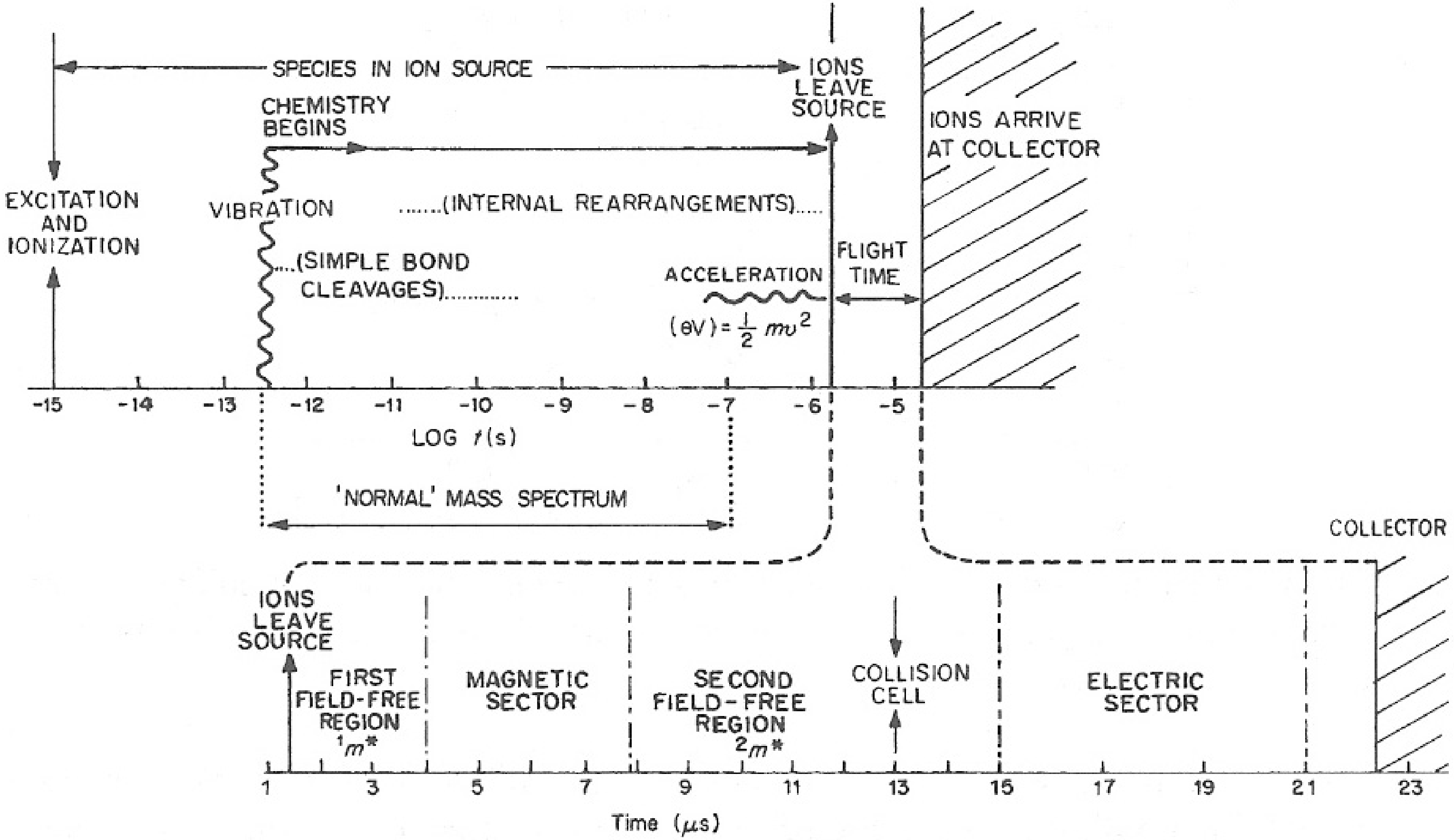


Fig. 2.9. The mass spectrometric time scale. It is important to note the logarithmic time scale for the ion source spanning over nine orders of magnitude. Reproduced from Ref. [9] with permission. © John Wiley & Sons Ltd., 1985.

Unimolecular Ion decompositions

→fragmentation of a gas phase ion in a reaction with a molecularity of one

Ionization (70 eV)→ molecular ions internal energy values (E)
(0 to > 20 eV) → average a few eV

Distribution described by probability function (PE) in upper part of Wahrhaftig diagramm

Range of energies is different from < 1 eV thermal-energy distribution in condensed-phase

1eV = 23 kcal/mol = 96.5 kJ/mol

Unimolecular decompositions

Important: only unimolecular decompositions are possible for gaseous ions formed by EI → *nature and extend of these reactions only depend on the ion's structure and internal energy, irrespective of the ionization method.*

Energy deposited in the ion from ionization causes ion to decompose or isomerize.

Probability of such reaction can be expressed as rate konstant k, which obviously changes with internal energy E.

Wahrhaftig diagram

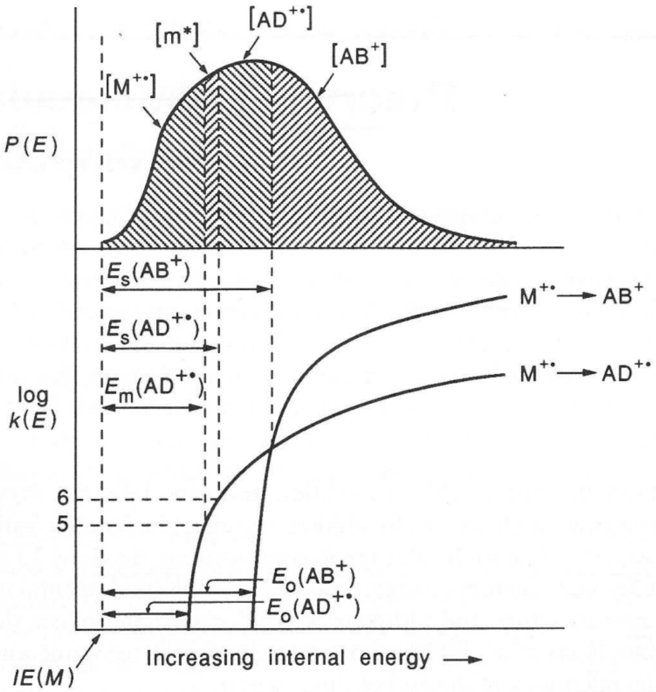


Figure 7.1. The Wahrhaftig diagram: relationship of $P(E)$ and $k(E)$ for unimolecular ion decompositions of $ABCD^{+\bullet}$. See text for definitions. (Wahrhaftig 1962, 1986.)

Unimolecular decompositions

Lower part of the diagram → logarithm of the rate constant k for unimolecular dissociation

Upper portion of the diagram → probability of forming a particular product ion

Red bubble → rate of the rearrangement reaction
$ABCD^+ \longrightarrow AD^+ + BC$

Green bubble → direct cleavage reaction
$ABCD^+ \longrightarrow AB^+ + CD$

Initial abundances $AD^{+\cdot}$ and AB^+
→ With sufficient internal energy further decompose

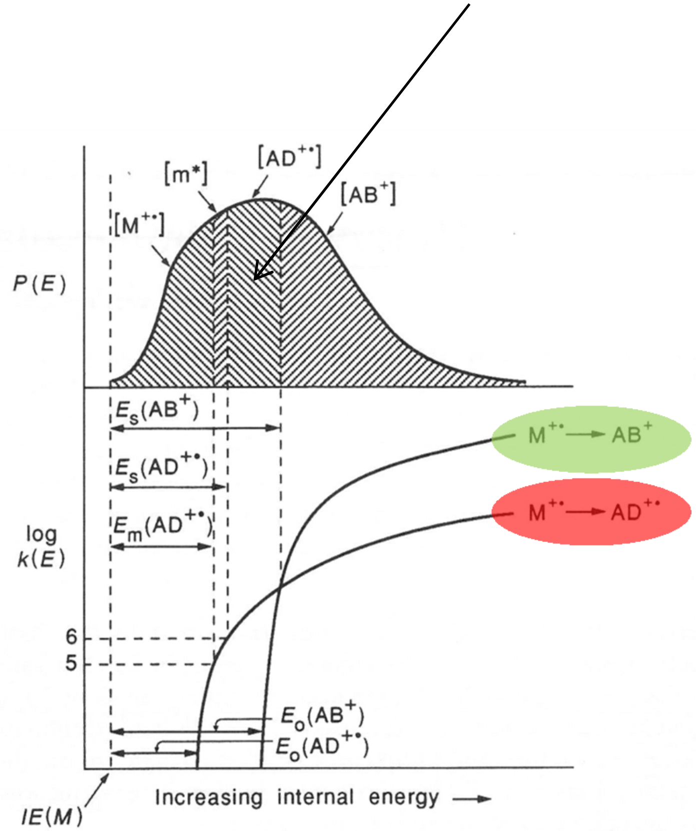


Figure 7.1. The Wahrhaftig diagram: relationship of $P(E)$ and $k(E)$ for unimolecular ion decompositions of $ABCD^{+\cdot}$. See text for definitions. (Wahrhaftig 1962, 1986.)

Unimolecular decompositions

- Collision of a high energy electron with a molecule not only causes the loss of a valence electron, it imparts some kinetic energy by collision into the remaining ion.
- This energy typically resides in increased vibrational/rotational energy states for the molecule – this energy can be lost by the molecule by breaking into *fragments.*
- The time between ionization and detection is **~10^{-5} sec**.
- If ionized molecule can "hold together" for >10^{-5} s, the $M^{+\cdot}$ ion reaches the detector and is observed intact in the spectrum.
- If the ionized molecule dissociates in less than 10^{-5} s, fragments will be observed in the spectrum.
- Special case: decomposition >10^{-6} s and <10^{-5} s → *metastable ions!*

QET = Quasi–equilibrium theory

Physical description of mass-spectral behavior → now generally accepted

Ionization 10^{-16} s → yields the excited molecular ion without change in bond length (Franck-Codon process)

Except for the smallest molecules, transitions between all possible energy states of this ion are sufficiently rapid, that a „quasi-equilibrium“ among these eneregy states is established before ion decomposition takes place

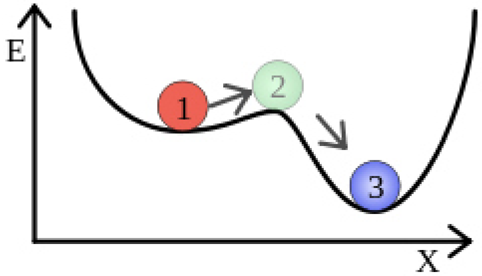
→ ***Possible decompositions of an ion depend only on ist structure and internal enery***

→ ***independ of initial ionization, structure of precursor or formation mechanism***

Metastable ions

„metastable“ state is thermodynamically unstable state that has not yet dissociated for kinetic reasons

(1) Metastable state of weaker bond
(2) Transitional 'saddle' configuration
(3) Stable state of stronger bond



„metastable ion“ is one that does dissociate but whose dissociation is delayed for kinetic reasons

Metastable ions

Internal energy of $ABCD^+$ is greater than $E_m(AD^+)$, the ions are metastable (indicated by m^*) → this occurs near log $k > 5$

A metastable ion has sufficient internal energy to dissociate prior to detection

Energy $E_s(AD^+)$ is defined as the internal energy of $ABCD^+$ that results in an equal probability that $ABCD^+$ and AD^+ leave the ion source, which occurs at near log k = 6

When the precursor ion has an internal energy equal to $E_s(AB^+)$, the rates of formation of AD^+ and AB^+ are equal.

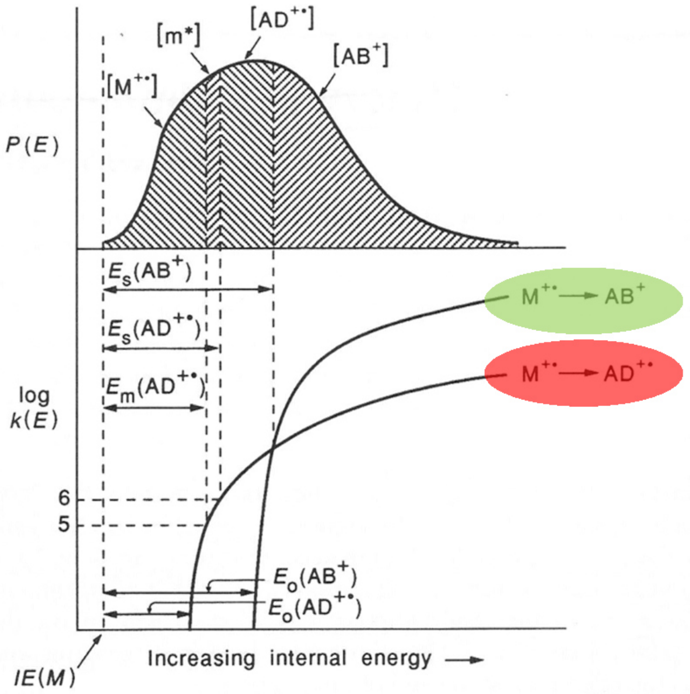


Figure 7.1. The Wahrhaftig diagram: relationship of $P(E)$ and $k(E)$ for unimolecular ion decompositions of $ABCD^{+\cdot}$. See text for definitions. (Wahrhaftig 1962, 1986.)

Detection of metastable ions

$$\boldsymbol{m_a^* = m_f^2 / m_i}$$

m_a^* = *apparent measured mass*
M_f = *fragment mass*
M_i = *precursor mass*

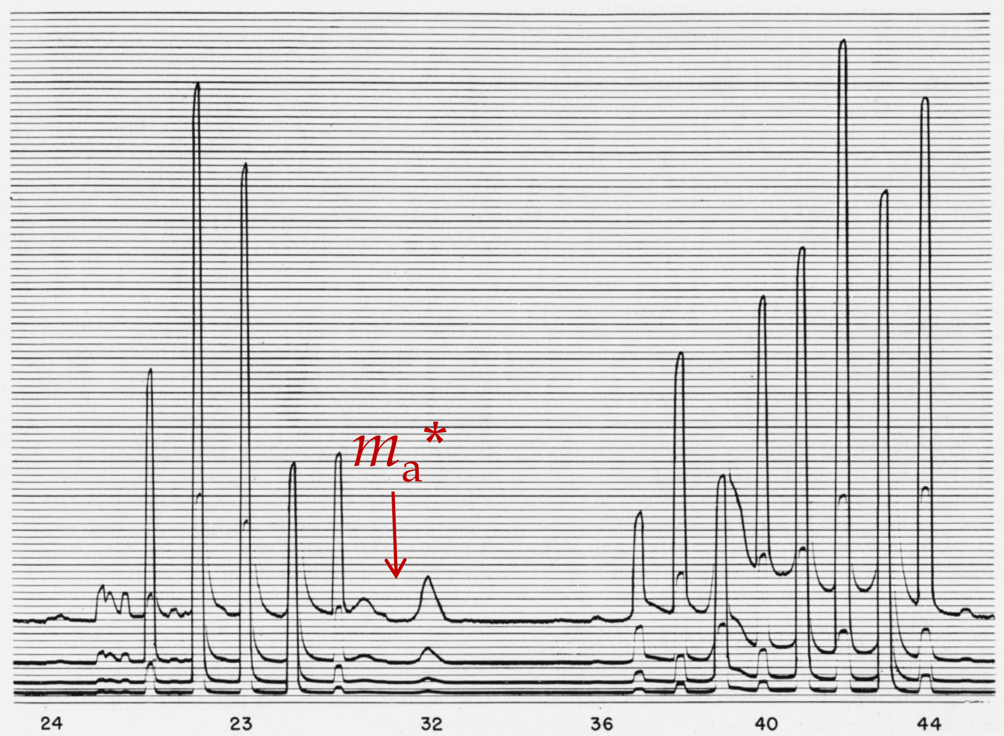


FIGURE 1. *Part of n-butane mass spectrum showing metastable transition peaks at mass numbers 25.1, 30.4, 31.9, and 39.2 and smaller peaks at 24.1 and 37.1.*

Example: peak at *m/z* 32

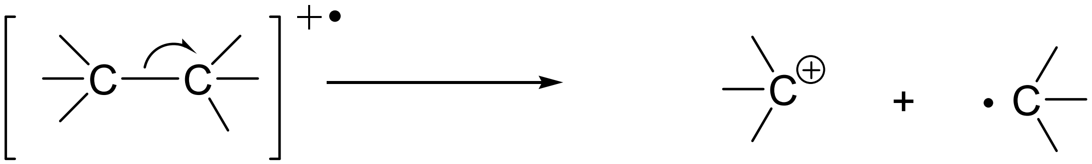
$m_a^* = 43^2 / 58 = 31.9$

$CH_3CH_2CH_2CH_3^+ \rightarrow CH_3CH_2CH_2^+ + CH_3^\cdot$

Because the initial ion does not dissociate at a definite point but over a range of positions, the resulting ion peak is broad and diffuse

Unimolecular decompositions

- Fragmentation of radical cation in most cases results in a covalent bond breaking homolytically – one fragment is then missing a full pair of electrons and has + charge; other fragment is neutral radical.



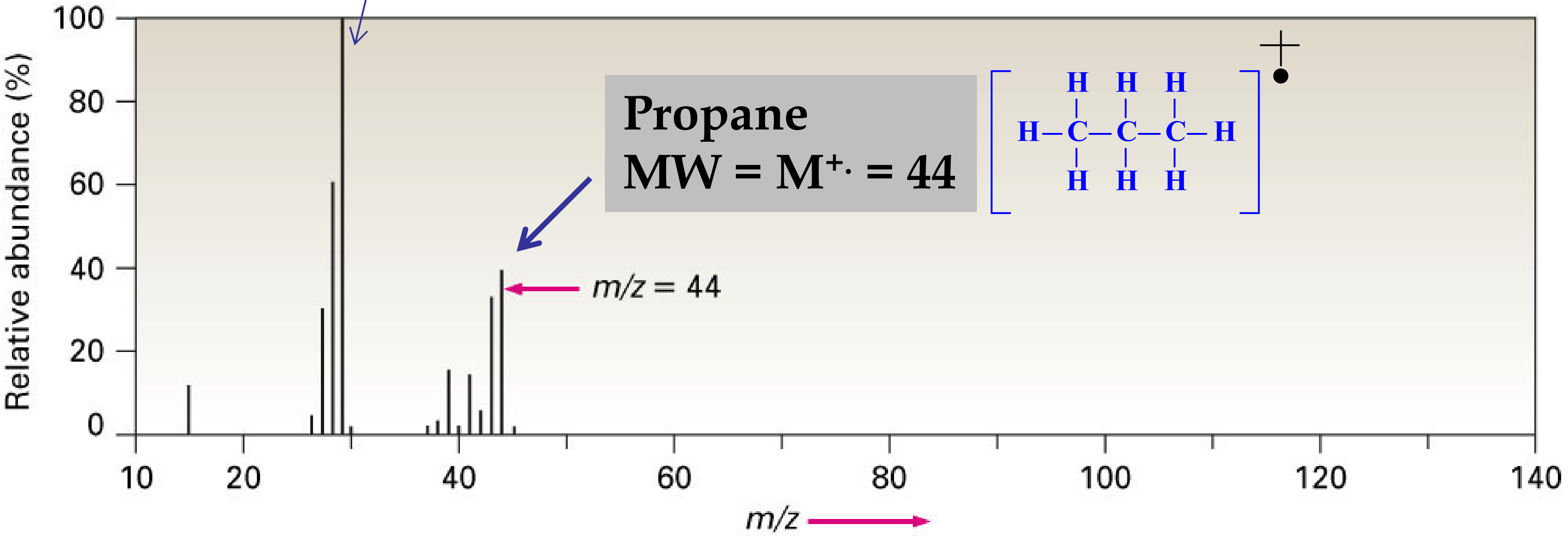
- Only charged molecules will be observed; <u>but</u> loss of neutral fragment is inferred by difference of $M^{+\cdot}$ and *m/z* of fragment.

- Fragmentations often follow trends seen in general organic chemistry – processes that give the most stable cations and radicals will occur with higher abundance.

The mass spectrum

- Plot *m/z* of ions versus the intensity of the signal (~ corresponding to **number of ions**)
- Tallest peak is ***base peak*** (100%)

  → other peaks listed as % of that peak (relative abundance in %)
- Peak that corresponds to unfragmented **radical cation** is **precursor peak** or ***molecular ion*** ($\mathbf{M^{+\cdot}}$)



Propane
MW = $\mathbf{M^{+\cdot}}$ = 44

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First steps in mass spectral interpretation

Three initial steps:

1. Run the spectra against a ***database*** (if available).

2. Obtain a ***high resolution*** (HR) mass spectrum if possible. This will help constrain the elemental compositions.

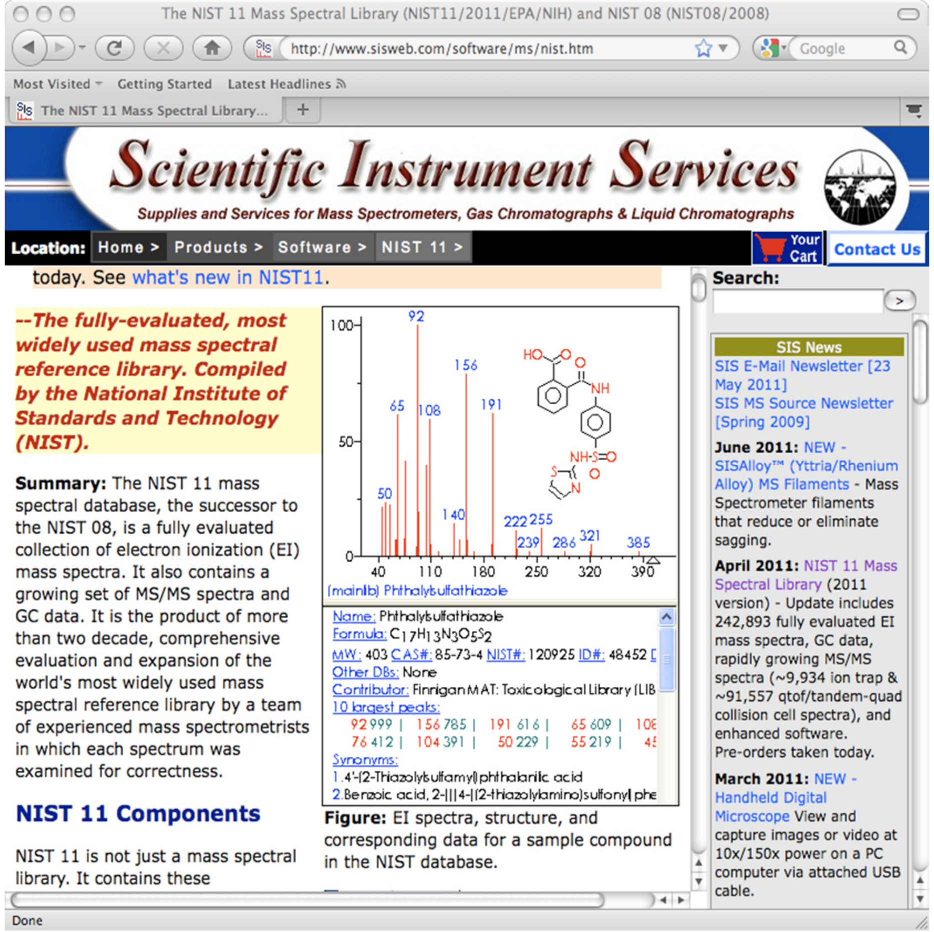
3. Then follow ***standard interpretation procedure*** and make sure that the identification is self-consistent.

1. Databases

- *Databases are critical even for the most experienced mass spectrometrists!*
- EI databases: NIST, Wiley, Palisades…
- DB usually included with GC-MS instrument.

NIST 11 Mass Spectrometry database

- 243,893 EI spectra of 212,961 compounds
- 9934 ion trap MS for 4649 compounds
- 91,557 QqTOF and QqQ spectra for 3774 compounds
- 224,038 RI values for 21,847 compounds



today. See what's new in NIST11.

--The fully-evaluated, most widely used mass spectral reference library. Compiled by the National Institute of Standards and Technology (NIST).

Summary: The NIST 11 mass spectral database, the successor to the NIST 08, is a fully evaluated collection of electron ionization (EI) mass spectra. It also contains a growing set of MS/MS spectra and GC data. It is the product of more than two decade, comprehensive evaluation and expansion of the world's most widely used mass spectral reference library by a team of experienced mass spectrometrists in which each spectrum was examined for correctness.

Name: Phthalysulfathiazole
Formula: $C_{17}H_{13}N_3O_5S_2$
MW: 403 CAS#: 85-73-4 NIST#: 120925 ID#: 48452
Other DBs: None
Contributor: Finnigan MAT: Toxicological Library (LIB
10 largest peaks:
92 999 | 156 785 | 191 616 | 65 609 | 10
76 412 | 104 391 | 50 229 | 55 219 | 4
Synonyms:
1.4'-(2-Thiazolylsulfamyl)phthalanilic acid
2.Benzoic acid, 2-[[[4-[(2-thiazolylamino)sulfonyl]phe

Figure: EI spectra, structure, and corresponding data for a sample compound in the NIST database.

NIST 11 Components

NIST 11 is not just a mass spectral library. It contains these

SIS News

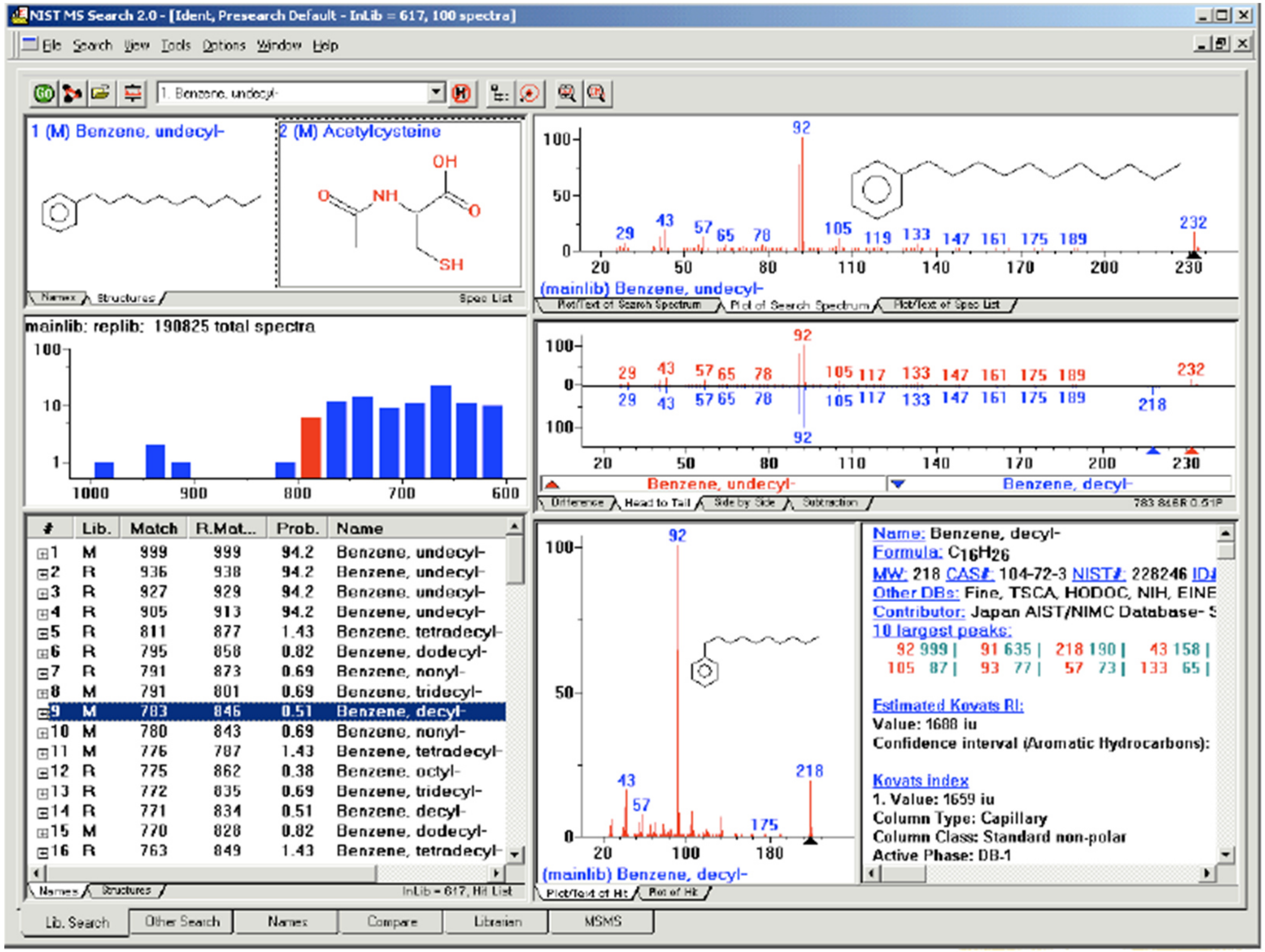
SIS E-Mail Newsletter [23 May 2011]
SIS MS Source Newsletter [Spring 2009]

June 2011: NEW - SISAlloy™ (Yttria/Rhenium Alloy) MS Filaments - Mass Spectrometer filaments that reduce or eliminate sagging.

April 2011: NIST 11 Mass Spectral Library (2011 version) - Update includes 242,893 fully evaluated EI mass spectra, GC data, rapidly growing MS/MS spectra (~9,934 ion trap & ~91,557 qtof/tandem-quad collision cell spectra), and enhanced software. Pre-orders taken today.

March 2011: NEW - Handheld Digital Microscope View and capture images or video at 10x/150x power on a PC computer via attached USB cable.

NIST database search



#	Lib.	Match	R.Mat...	Prob.	Name
1	M	999	999	94.2	Benzene, undecyl-
2	R	936	938	94.2	Benzene, undecyl-
3	R	927	929	94.2	Benzene, undecyl-
4	R	905	913	94.2	Benzene, undecyl-
5	R	811	877	1.43	Benzene, tetradecyl-
6	R	795	858	0.82	Benzene, dodecyl-
7	R	791	873	0.69	Benzene, nonyl-
8	M	791	801	0.69	Benzene, tridecyl-
9	M	783	846	0.51	Benzene, decyl-
10	M	780	843	0.69	Benzene, nonyl-
11	M	776	787	1.43	Benzene, tetradecyl-
12	R	775	862	0.38	Benzene, octyl-
13	R	772	835	0.69	Benzene, tridecyl-
14	R	771	834	0.51	Benzene, decyl-
15	M	770	828	0.82	Benzene, dodecyl-
16	R	763	849	1.43	Benzene, tetradecyl-

Name: Benzene, decyl-
Formula: $C_{16}H_{26}$
MW: 218 CAS#: 104-72-3 NIST#: 228246 ID
Other DBs: Fine, TSCA, HODOC, NIH, EINE
Contributor: Japan AIST/NIMC Database-
10 largest peaks:
92 999 | 91 635 | 218 190 | 43 158 |
105 87 | 93 77 | 57 73 | 133 65 |

Estimated Kovats RI:
Value: 1688 iu
Confidence interval (Aromatic Hydrocarbons):

Kovats index
1. Value: 1659 iu
Column Type: Capillary
Column Class: Standard non-polar
Active Phase: DB-1

2. High resolution mass spectra

- **Molecular weight** from *m/z* of the molecular ion.
- TOF, FTICR and orbitrap instruments provide high-resolution and "*accurate mass*" of the ion.
  → ~0.0001 u – **can distinguish specific atoms**
- Example: MW = 72 is ambiguous, either C_5H_{12} or C_4H_8O, but:
  - C_5H_{12} 72.0939 u exact mass, C_4H_8O 72.0575 u exact mass.
  - Result from fractional mass differences of atoms:
    $^{16}O = 15.99491$, $^{12}C = 12.0000$, $^{1}H = 1.00783$
- Software allows computation of formulas for each peak.
- If precursor ion is not present because EI causes decomposition, softer methods such as CI can be implemented.

Exact mass measurements

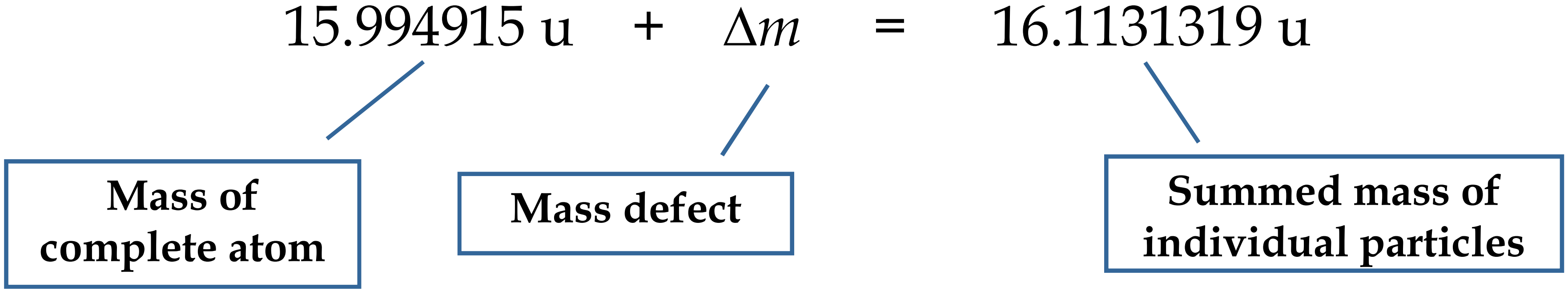
Theory ^{16}O: **16.131919**

Proton	1.0072765
Neutron	1.0086649
Elektron	0.0005458

Observed ^{16}O: **15.994915**

Difference between observed and theoretical mass = mass defect

Every isotope → unique , characteristic „mass defect“



$$15.994915\ \mathrm{u} \quad + \quad \Delta m \quad = \quad 16.1131319\ \mathrm{u}$$

Mass of complete atom

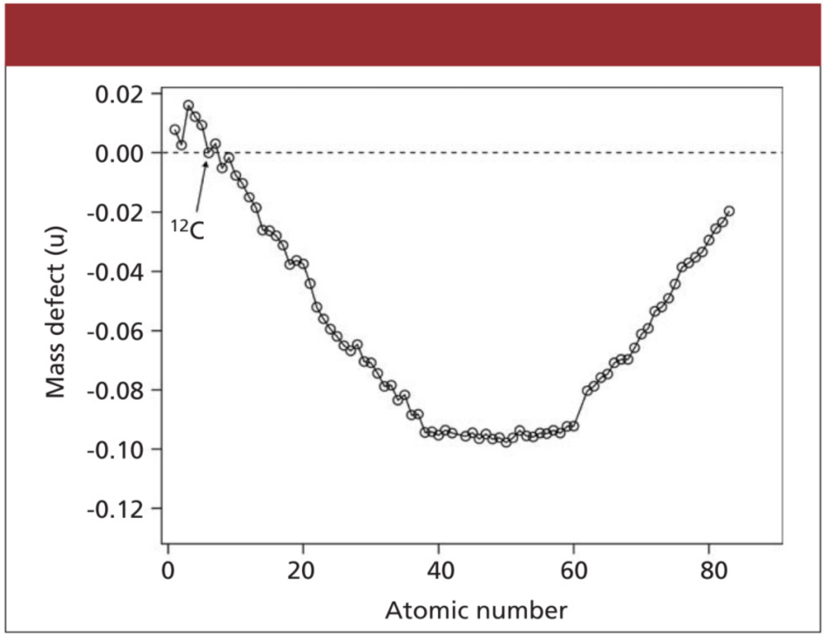
Mass defect

Summed mass of individual particles

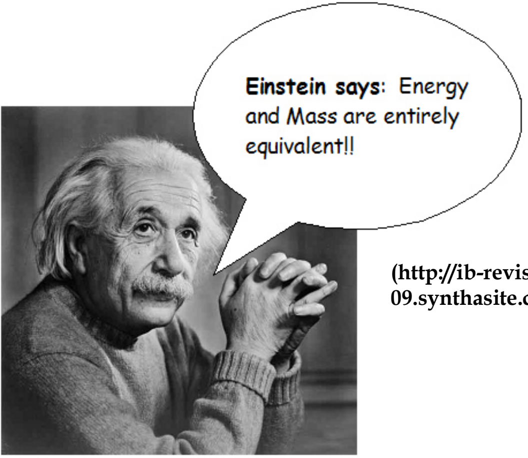
$$\Delta m = 16.0131319 - 15.994915 = 0.137004\ \mathrm{u}$$

Mass defect

$$\Delta E = \Delta mc^2$$



Calculated mass defect for the first 90 elements (Leslie, Volmer. *Spectroscopy* 2007, 22, 38).



(http://ib-revision-09.synthasite.com)

Mass defect = Exact mass – nominal mass

Two different chemical formulae never have the same exact mass!

Mass of the ion which shows total mass defect, identifies its isotopic and elemental composition

Relative carbon mass scale

- IUPAC → ^{12}C standardization in 1960.
- 1 u = 1/12 of the mass of a ^{12}C atom.
- ^{12}C reference refers to the specific isotope only.
- Definition: mass defect ^{12}C = zero!

^{12}C	**12.00000**
^{13}C	**13.00336**
^{1}H	**1.007825**
^{14}N	**14.00307**
^{16}O	**15.99491**
^{18}O	**17.99916**
^{19}F	**18.9984**
^{32}S	**31.9721**
^{34}S	**33.96787**
^{35}Cl	**34.9689**
^{37}Cl	**36.9659**

P. DeBievre, I.L. Barnes. *Int. J. Mass Spectrom. Ion Proc*. 1985, 65, 211-230.

Example for molecular formulae

Possible formulas can be predicted from *m/z* (M$^{+\bullet}$)

Predict possible molecular formulas containing C, H and O if :

M$^{+\bullet}$, *m/z* 86

M$^{+\bullet}$, *m/z* 156

Example for molecular formulae (*1*)

Possible formulas can be predicted from *m/z* ($M^{+\bullet}$)

Predict possible molecular formulas containing C, H and O if :

$M^{+\bullet}$, *m/z* 86

C_6H_{14}
$C_5H_{10}O$
$C_4H_6O_2$
$C_3H_2O_3$

$M^{+\bullet}$, *m/z* 156

$C_{11}H_{24}$
$C_{12}H_{12}$
$C_{11}H_8O$
$C_{10}H_{20}O$

$C_{10}H_4O_2$
$C_9H_{16}O_2$
$C_8H_{12}O_3$
$C_7H_8O_4$
$C_6H_4O_5$

Determining elemental formulae (2)

For a molecule of MW = 44 g/mol, the following formulae are possible (for C, H, O, N):

C_3H_8 C_2H_4O CO_2 CN_2H_4

Now we measure the *accurate mass* at *m/z* 44.02652

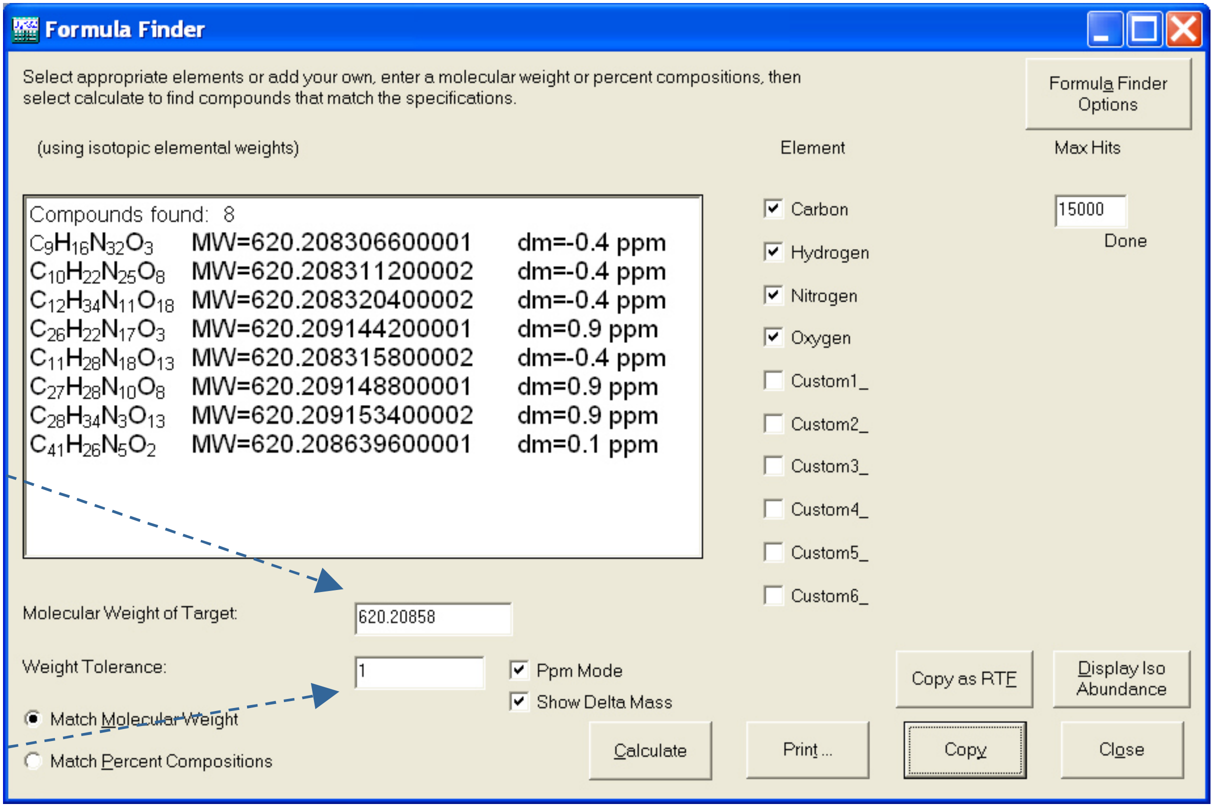
→ Empirical formula can be assigned:

C_3H_8	C_2H_4O	CO_2	CN_2H_4
44.06260	44.02620	43.98983	44.03740

Calculated (*exact*) mass

Determining elemental formulae

If the measurement is accurate enough, the elemental formula can be readily determined from a mass spectrum.



Select appropriate elements or add your own, enter a molecular weight or percent compositions, then select calculate to find compounds that match the specifications.

(using isotopic elemental weights)

Compounds found: 8

$C_9H_{16}N_{32}O_3$ MW=620.208306600001 dm=-0.4 ppm

$C_{10}H_{22}N_{25}O_8$ MW=620.208311200002 dm=-0.4 ppm

$C_{12}H_{34}N_{11}O_{18}$ MW=620.208320400002 dm=-0.4 ppm

$C_{26}H_{22}N_{17}O_3$ MW=620.209144200001 dm=0.9 ppm

$C_{11}H_{28}N_{18}O_{13}$ MW=620.208315800002 dm=-0.4 ppm

$C_{27}H_{28}N_{10}O_8$ MW=620.209148800001 dm=0.9 ppm

$C_{28}H_{34}N_3O_{13}$ MW=620.209153400002 dm=0.9 ppm

$C_{41}H_{26}N_5O_2$ MW=620.208639600001 dm=0.1 ppm

☑ Carbon
☑ Hydrogen
☑ Nitrogen
☑ Oxygen
☐ Custom1_
☐ Custom2_
☐ Custom3_
☐ Custom4_
☐ Custom5_
☐ Custom6_

Max Hits: 15000

Molecular Weight of Target: 620.20858

Weight Tolerance: 1

☑ Ppm Mode
☑ Show Delta Mass

● Match Molecular Weight
○ Match Percent Compositions

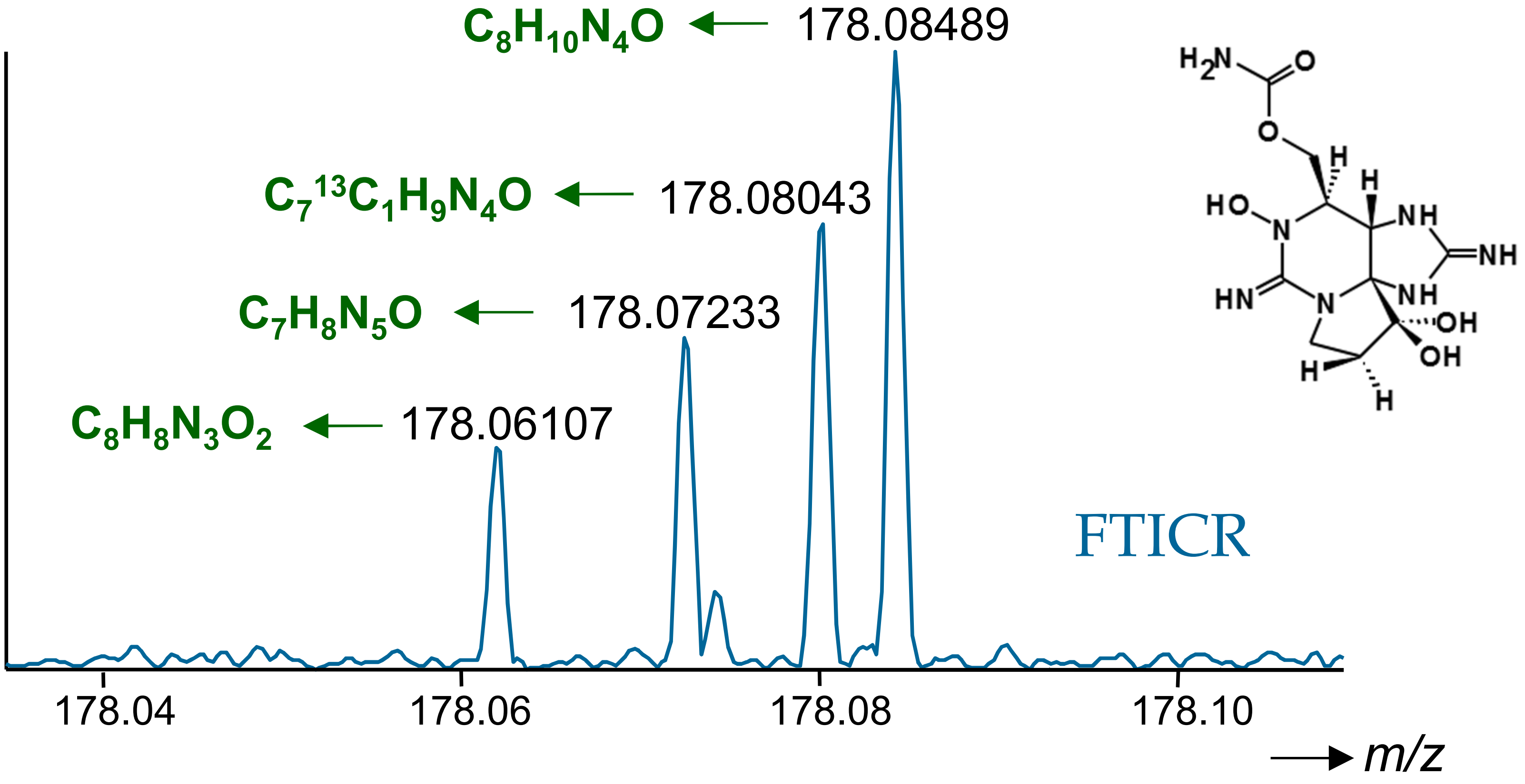
Accurate mass

Mass error

(http:// omics.pnl.gov/software/molecular-weight-calculator)

ppm = parts per million (1 ppm = 0.0001%)

Determining elemental formulae



FTICR

Important properties of mass spectra

Resolution (*R*): $R = m/\Delta m$

Δm = mass difference of two adjacent resolved peaks

(typically m = mass of first peak or average)

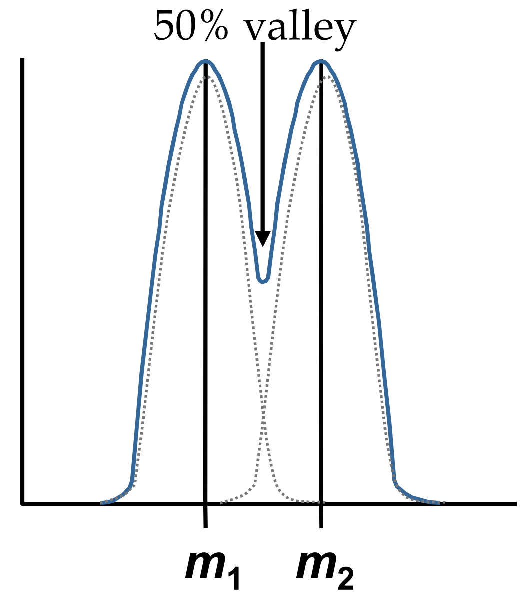
Example: *R* = 500-2000 ("low" resolution)

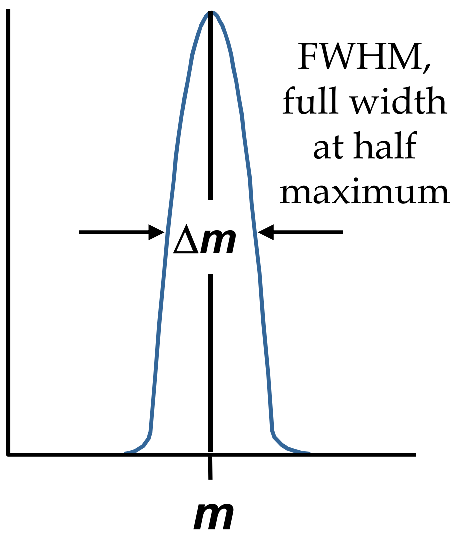
resolves *m/z* 50 and 50.1, and *m/z* 500 and 501

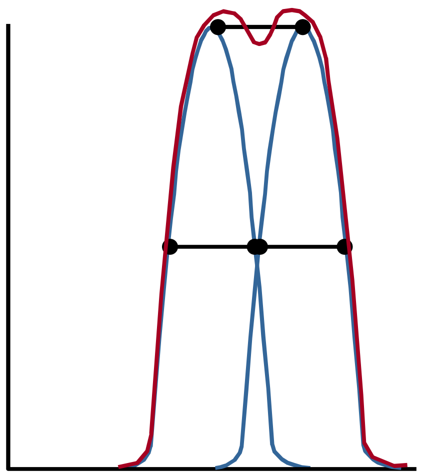
Example: $R > 5000$ ("high" resolution)

resolves *m/z* 50 and 50.0003 and *m/z* 500 and 500.0033

Resolving power







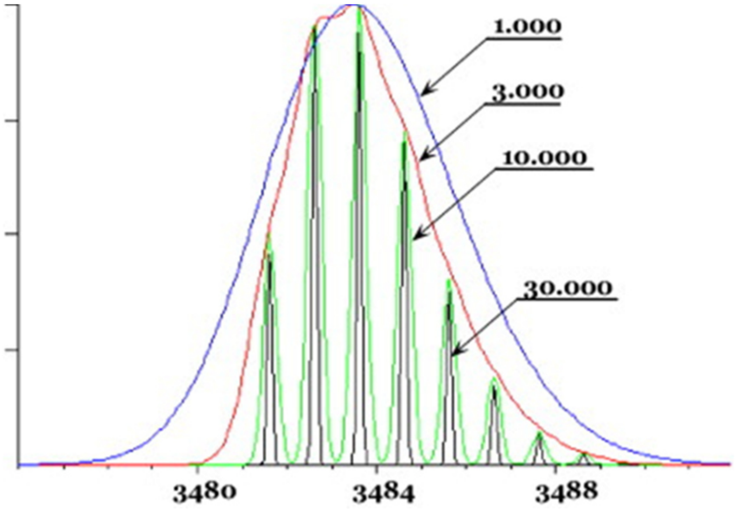
$$R_{50\%} = \frac{m_1}{m_2 - m_1} = \frac{m_1}{\Delta m}$$

$$R_{FWHM} = \frac{m}{\Delta m}$$

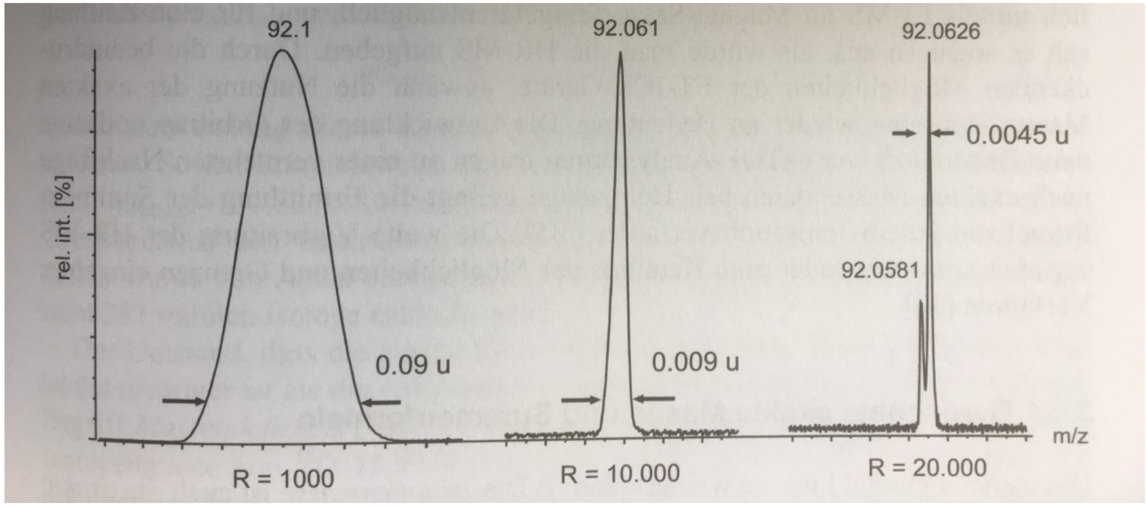
$R_{50\%}$= 1000 → 1000 and 1001 are resolved with a 50 % valley

R_{FWHM}= 1000 → 1000 and 1001 are barely resolved, the required 50% valley resolving power would be ~ R_{FWHM} 1700

Different resolving powers



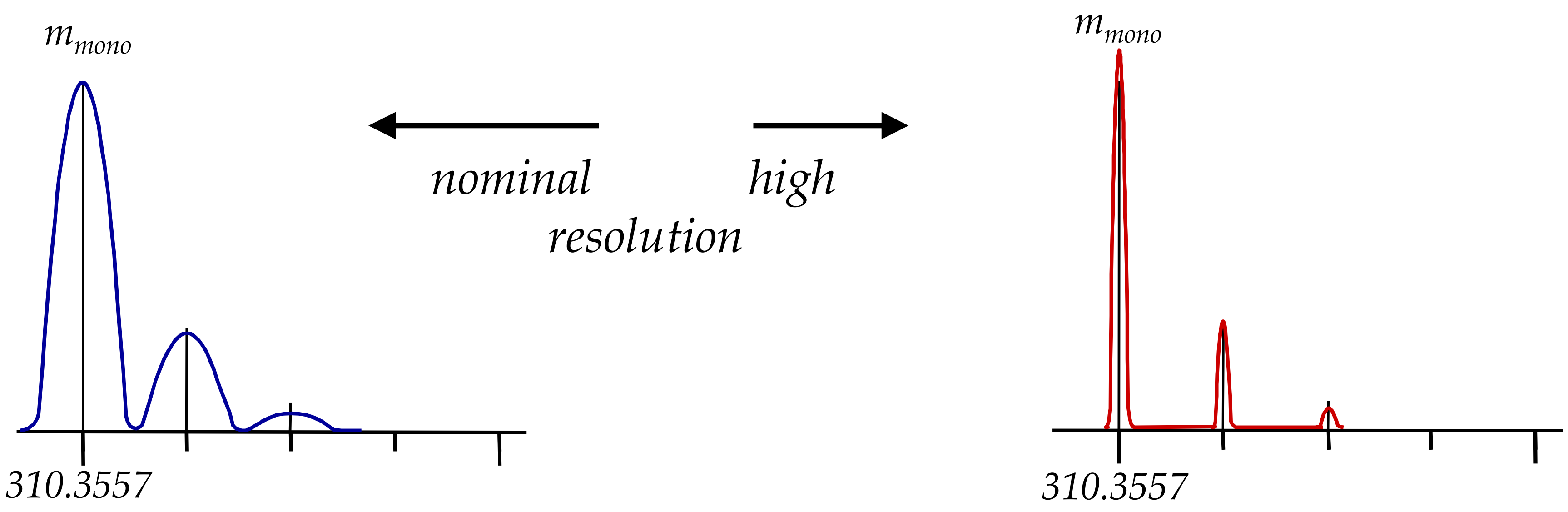
Mixture of Xylol and Toluol



Mass accuracy

Mass accuracy $\Delta\, m = m_{\text{experimental}} - m_{\text{theoretical}}$

Often given as relative measure $\Delta\, m/m$ in ppm



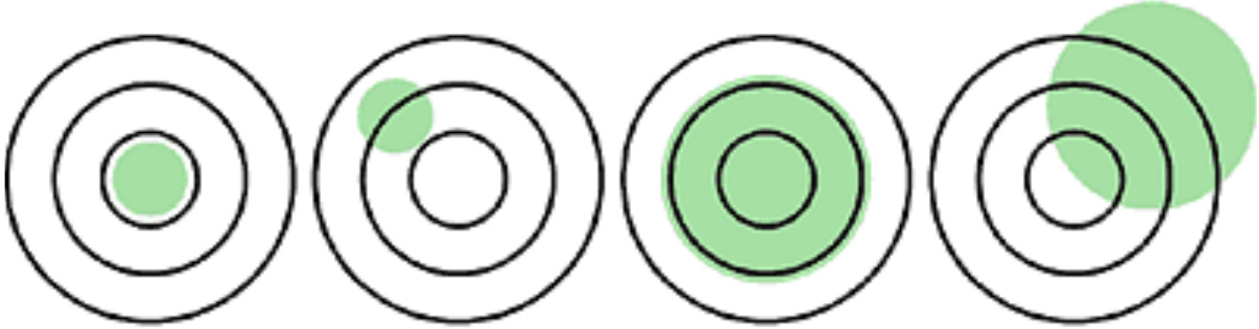
nominal *high*
resolution

$C_{22}H_{46}$	**$m_{\text{theoretical}}$:**	**310.3599**	**$\Delta m/m$=-13.53 ppm**
$C_{21}H_{44}N$	**$m_{\text{theoretical}}$:**	**310.34737**	**$\Delta m/m$= 26.84 ppm**

Accuracy (A) / Precision (P)

Accuracy = agreement between the (individual) measurement result and the true value of the measured variable

Precision = agreement between independent measurement results under fixed conditions



A + P + **A - P +** **A + P -** **A - P -**

3. Typical standard interpretation procedure

1. Confirm molecular ion (M+$^{\cdot}$) identity (if possible), must be highest mass in spectrum and odd-electron ion! Confirm with CI or other soft ionization if required.
2. Use isotopic abundances (where possible) and deduce elemental composition of each peak in the spectrum (HRMS); calculate rings plus double bonds.
3. Mark ‘important’ ions: odd/even-electron and those of highest abundance, highest mass and/or highest in a group of peaks.
4. Evaluate secondary sub-structural assignments for:

    a) Important low-mass ion series.
    b) Important primary neutral fragments from $M^{+\cdot}$ indicated by high-mass ions (loss of largest alkyl favored) plus fragmentations from MS/MS (if available).
    c) Important characteristic ions.

5. Postulate molecular structure; test against reference spectrum, or against spectra of similar compounds.

The molecular ion $M^{+\bullet}$

Requirements for *useful* molecular ion

- It must be the ion of highest mass in the spectrum.
- It must be an odd-electron ion (cannot be even-electron!)
- It must be capable of yielding important ions in the high mass region by logical neutral losses.

Odd-electron ion

In EI, molecules become ionized by losing an electron, leaving one electron unpaired → odd-electron ion (OE) $M^{+\bullet}$, for example $CH_4^{+\bullet}$

Ease of ionization $n > \pi > \sigma$

The molecular ion $M^{+\bullet}$

Even-electron ion

Only paired electrons, even-electron (EE) ion

Generally more stable → often fragment ions in spectra

For example

Cleavage of C–H bond in $CH_4^{+\bullet}$ gives stable EE ion CH_3^+ and $H^\bullet$.

Ionization techniques such as ESI or APCI almost always only give primary EE ions (protonated molecules $[M+H]^+$).

The nitrogen rule

If a compound contains **no** (or an even number) of nitrogen atoms, its molecular ion will be at an even mass:

H_2O	*m/z* 18
CH_4	*m/z* 16
CH_3OH	*m/z* 32
$H_2N\text{-}NH_2$	*m/z* 32
$C_5H_6N_2$	*m/z* 94

An odd number of nitrogens causes $M^{+\bullet}$ to be at an odd mass number:

NH_3	*m/z* 17
$C_2H_5NH_2$	*m/z* 45
C_9H_7N	*m/z* 129

→ If molecular ion mass is odd, it must contain an odd number of N atoms

→ An odd-electron ion will be at an even mass number if it contains an even number of nitrogen atoms.

The nitrogen rule

- A molecule with even numbered MW must contain either no N or even number of N.
- A molecule with odd numbered MW must contain an odd number of N.
- Holds for all compounds with C, N, O, S, X, P, B, Si, As & alkaline earths

- As a consequence: for fragmentation at a single bond:
  - even number $M^{+\cdot}$ will give odd no. fragment ion
  - odd number $M^{+\cdot}$ will give even no. fragment ion
  - fragment ion must contain all N if any

The nitrogen rule

Example for fragment ions:

$$H_3C{-}CH_3^{+\cdot} \longrightarrow CH_3^{+} + CH_3^{\cdot}$$

m/z 30 *m/z* 15

$$CH_3NHCH_3 \longrightarrow CH_3NH^{+} + CH_3^{\cdot}$$

m/z 45 *m/z* 30

or $CH_3NH^{\cdot}$ + CH_3^{+}

m/z 15

“The rule of 13”

Assumes C_nH_{n+r} is present in the investigated molecular ions

Step 1: Divide $M^{+\cdot}$ Mass by 13, this gives *n*.

Step 2: Any remainder represents additional H atoms.

Example 1: ***m/z* 78**

$78/13 = 6 \rightarrow n = 6 \rightarrow C_6H_6$

Example 2: ***m/z* 92**

$92/13 = 7.077 \rightarrow n = 7$

$7 \times 13 = 91 \rightarrow$ 1 extra is present

Formula is $C_7H_{7+1} = C_7H_8$

The procedure is based on chemical logic: only carbon and hydrogen are present in the molecule, so the number "13“ then represents the sum of the atomic weights of one carbon atom and one hydrogen atom.

Typical mass spectra with characteristic ions

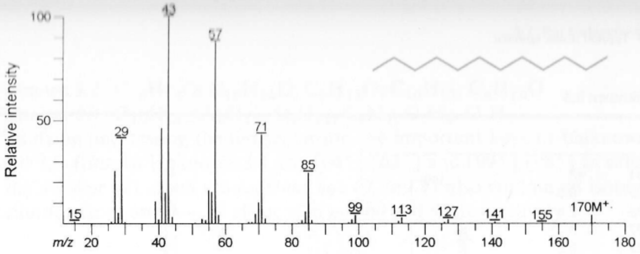


Figure 3.2. Mass spectrum of dodecane.

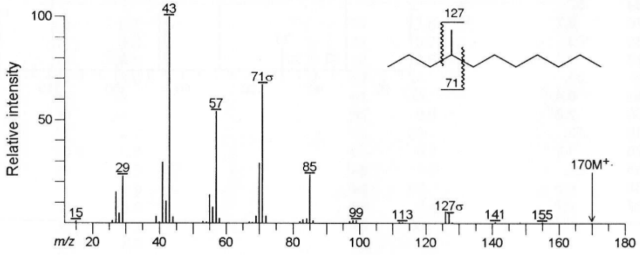


Figure 3.3. Mass spectrum of 4-methylundecane.

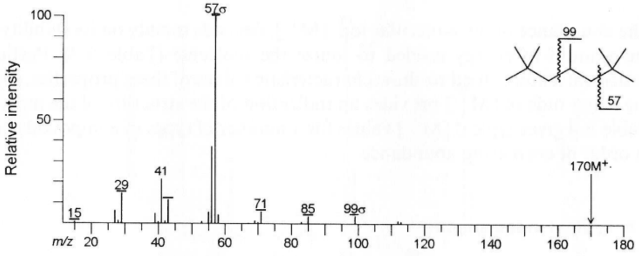
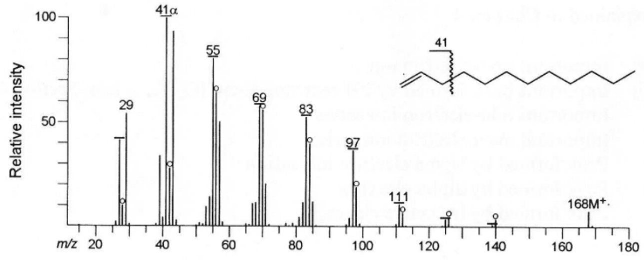


Figure 3.4. Mass spectrum of 2,2,4,6,6-pentamethylheptane.



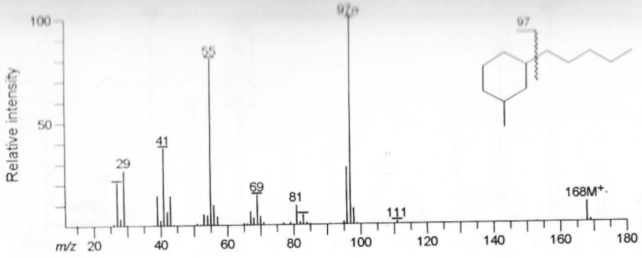


Figure 3.6. Mass spectrum of 1-methyl-3-pentylcyclohexane.

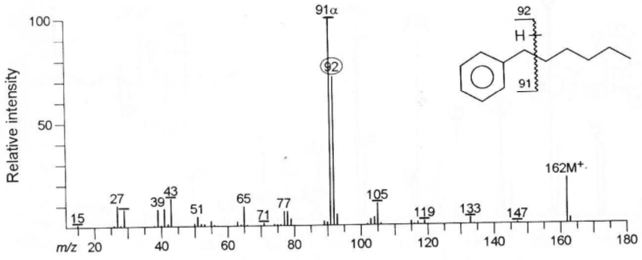


Figure 3.7. Mass spectrum of 1-phenylhexane.

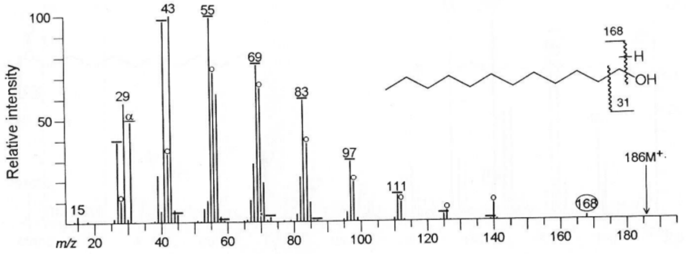
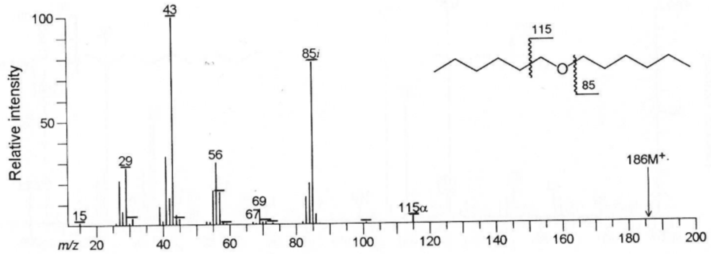


Figure 3.8. Mass spectrum of 1-dodecanol.



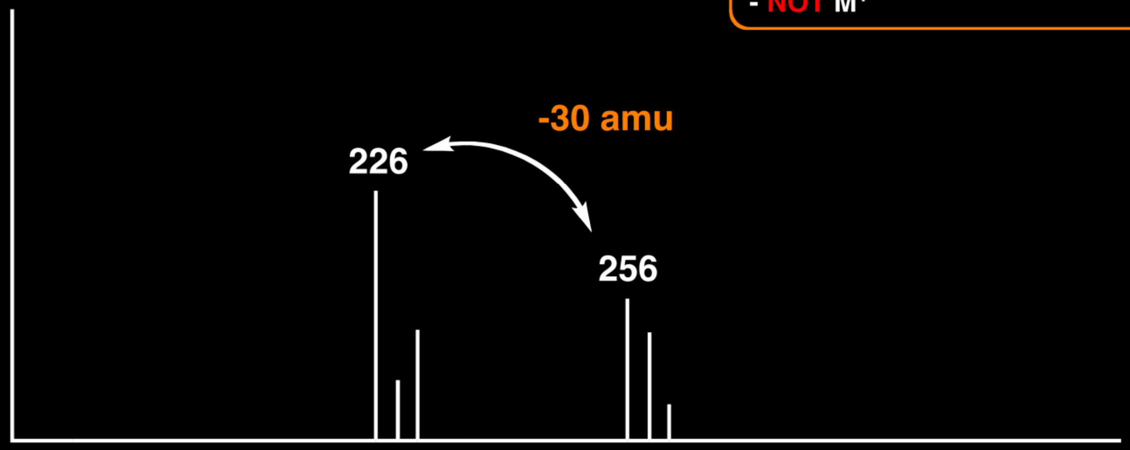
Example 1: determining molecular ion $M^{+\bullet}$

How do we know if we have the molecular ion or a fragment ion?

1. Must obey nitrogen rule!
2. Must generate lower mass ions by logical neutral losses

Unlikely losses
4 – 14
21 – 25
33, 37, 38

- ok mass loss
- even # "M^{+}", even # fragment
- NOT M^{+}



Example 2: determining molecular ion $M^{+\bullet}$

How do we know if we have the molecular ion or a fragment ion?

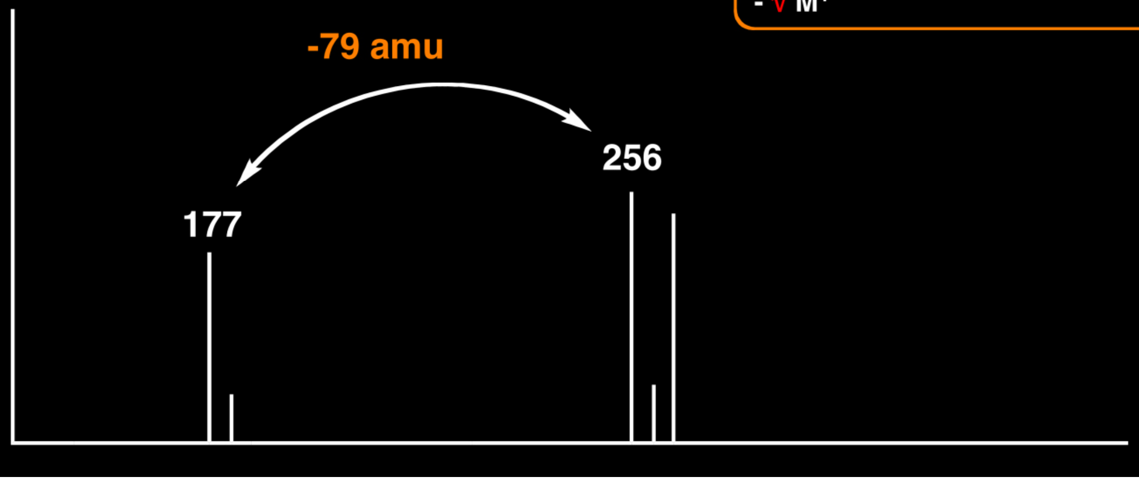
1. Must obey nitrogen rule!
2. Must generate lower mass ions by logical neutral losses

Unlikely losses

4 – 14

21 – 25

33, 37, 38

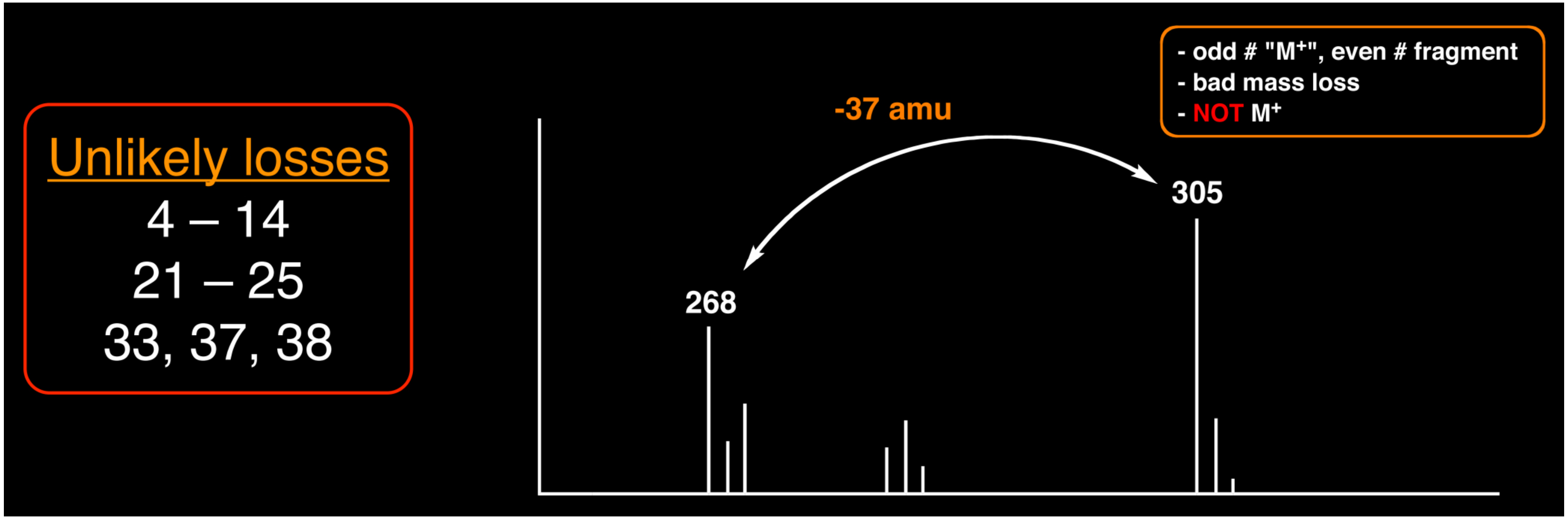


- ok mass loss
- even # "M^+", odd # fragment
- √ M^+

Example 3: determining molecular ion $M^{+\bullet}$

How do we know if we have the molecular ion or a fragment ion?

1. Must obey nitrogen rule!
2. Must generate lower mass ions by logical neutral losses



Unlikely losses

4 – 14
21 – 25
33, 37, 38

- odd # "M^+", even # fragment
- bad mass loss
- NOT M^+

The molecular ion $M^{+\bullet}$

How do we know if we have the molecular ion or a fragment ion?

Stability plays a factor in whether or not we see molecular ion.

Decreasing ability to give prominent $M^{+\cdot}$:
aromatics > conjugated alkenes > cyclic compounds >
organic sulfides > alkanes > mercaptans

Decreasing ability to give recognizable $M^{+\cdot}$:
ketones > amines > esters > ethers >
carboxylic acids ~ aldehydes ~ amides ~ halides

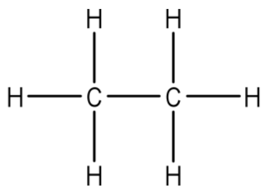
$M^{+\cdot}$ is frequently not detectable from:
aliphatic alcohols, nitrites, nitrates, nitro compounds,
nitriles, highly branched compounds

Double bond equivalent (DBE)

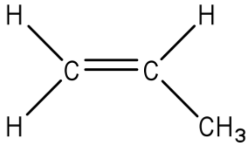
DBE is also called degree of unsaturation. From the chemical structure, each π bond or ring will generate one DBE.

$$DBE = C - H/2 + N/2 + 1$$

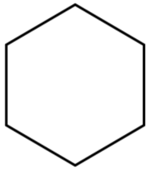
- Double bonds contain 1 DBE
- Triple bonds ontain 2 DBE
- A benzene ring contains four DBE



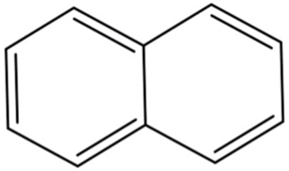
Ethylene
DBE=0



Propylene
DBE=1

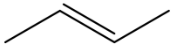


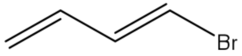
Cyclohexane
DBE=1

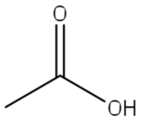


Only count two rings in this structure
5 pi bonds+ 2 rings => DBE= 5+2=7

Examples:

1)


2)


3)


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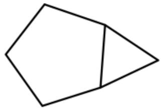

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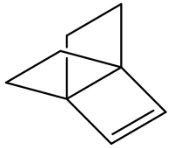

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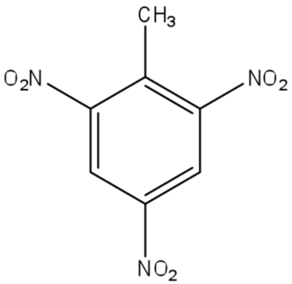
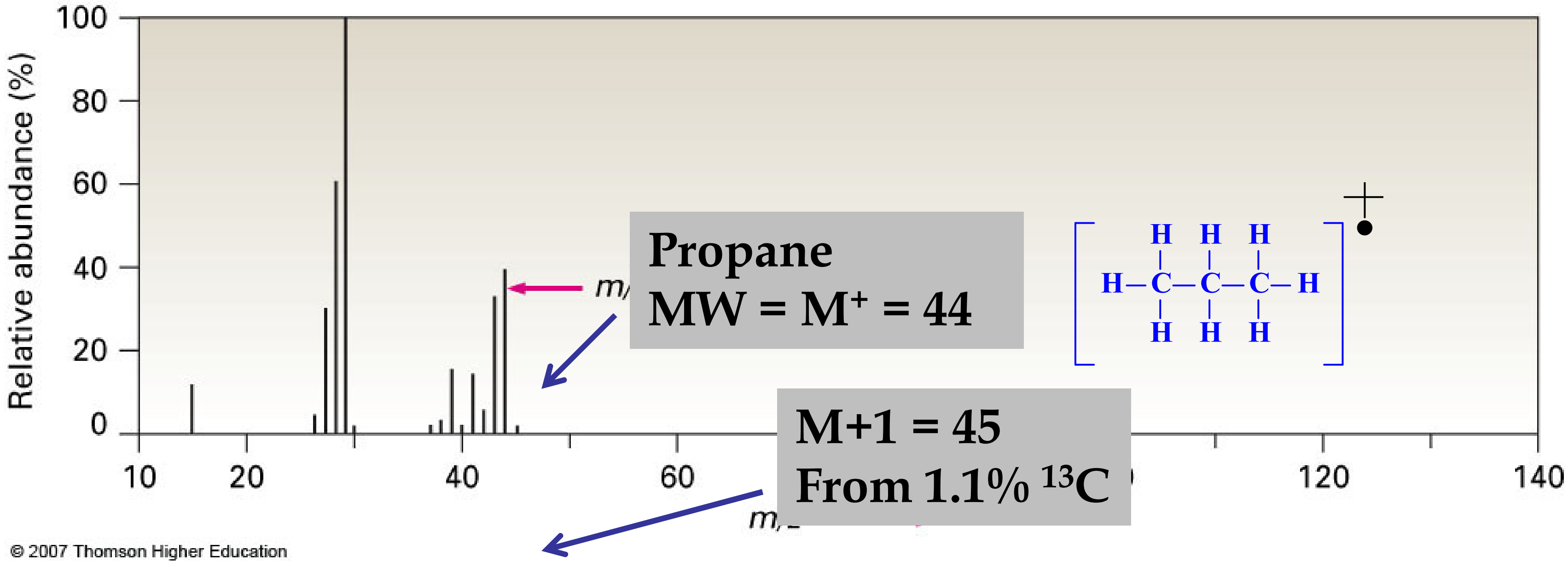


Isotopes (M+1 elements)

- **Peaks above the molecular weight appear as a result of naturally occurring heavier isotopes**
  - (M+1) from 1.1% ^{13}C in nature



Propane
MW = M^+ = 44

M+1 = 45
From 1.1% ^{13}C

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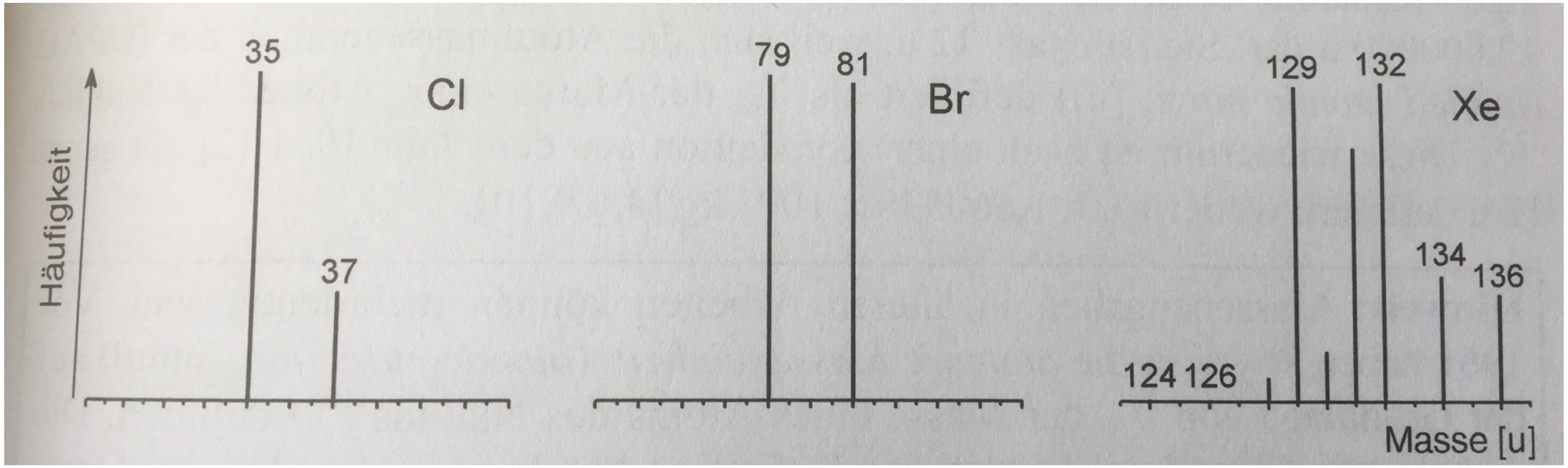
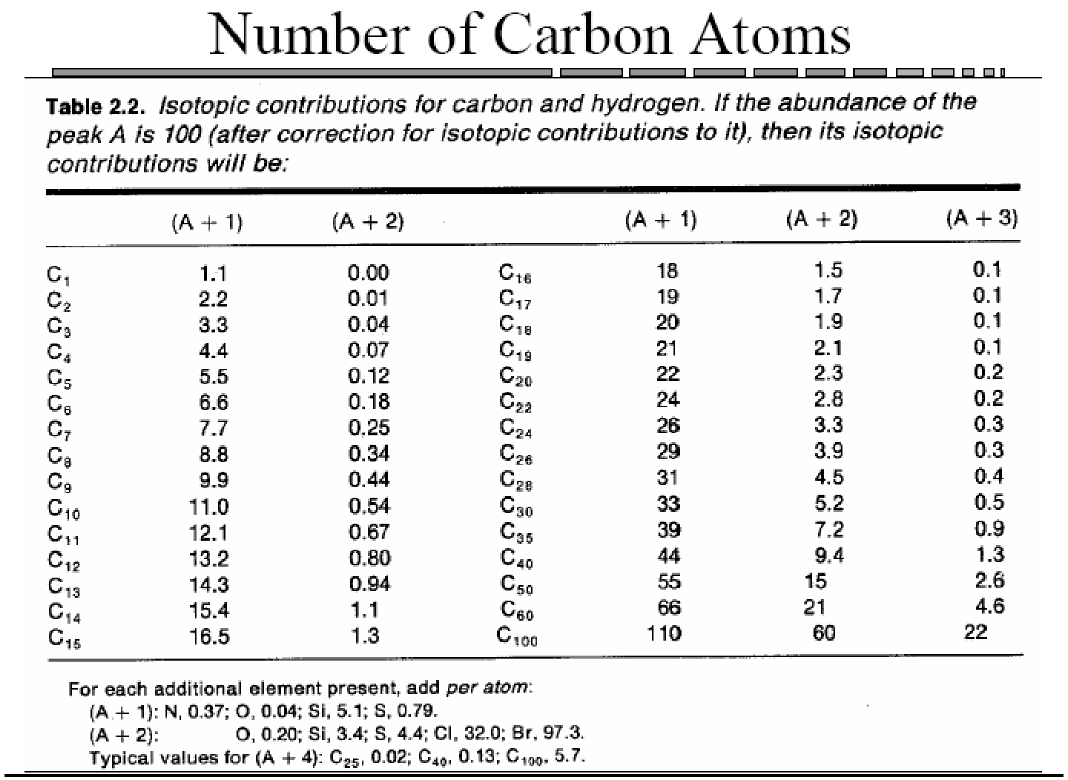
Isotopes (M+2 elements)

- (M and M+2) in 75.8%/24.2% ratio = ^{35}Cl and ^{37}Cl
- (M and M+2) in 50.7%/49.3% ratio = ^{79}Br and ^{81}Br



Isotopes (M+1 elements)

- Increasing number of C atoms linearly increases the probability that one of them is ^{13}C atom
- A way to deduce the number of carbon atoms is to determine the relative abundance of ^{13}C peak



Number of Carbon Atoms

Table 2.2. *Isotopic contributions for carbon and hydrogen. If the abundance of the peak A is 100 (after correction for isotopic contributions to it), then its isotopic contributions will be:*

	(A + 1)	(A + 2)		(A + 1)	(A + 2)	(A + 3)
C_1	1.1	0.00	C_{16}	18	1.5	0.1
C_2	2.2	0.01	C_{17}	19	1.7	0.1
C_3	3.3	0.04	C_{18}	20	1.9	0.1
C_4	4.4	0.07	C_{19}	21	2.1	0.1
C_5	5.5	0.12	C_{20}	22	2.3	0.2
C_6	6.6	0.18	C_{22}	24	2.8	0.2
C_7	7.7	0.25	C_{24}	26	3.3	0.3
C_8	8.8	0.34	C_{26}	29	3.9	0.3
C_9	9.9	0.44	C_{28}	31	4.5	0.4
C_{10}	11.0	0.54	C_{30}	33	5.2	0.5
C_{11}	12.1	0.67	C_{35}	39	7.2	0.9
C_{12}	13.2	0.80	C_{40}	44	9.4	1.3
C_{13}	14.3	0.94	C_{50}	55	15	2.6
C_{14}	15.4	1.1	C_{60}	66	21	4.6
C_{15}	16.5	1.3	C_{100}	110	60	22

For each additional element present, add *per atom*:
(A + 1): N, 0.37; O, 0.04; Si, 5.1; S, 0.79.
(A + 2): O, 0.20; Si, 3.4; S, 4.4; Cl, 32.0; Br, 97.3.
Typical values for (A + 4): C_{25}, 0.02; C_{40}, 0.13; C_{100}, 5.7.

Isotopes (M+1 elements)

To calculate expected M+1 peak for known molecular formula:

$$\begin{aligned}\%(M+1) = 100\,\frac{(M+1)}{M} &= 1.1 \text{ x } \#\text{ of carbon atoms}\\ &\quad + 0.016 \text{ x } \#\text{ of hydrogen atoms}\\ &\quad + 0.38 \text{ x } \#\text{ of nitrogen atoms}\ldots\textit{etc.}\end{aligned}$$

Example: $M^{+\cdot}$ peak at *m/z* 78 has a M+1 at *m/z* 79 with 7% intensity:

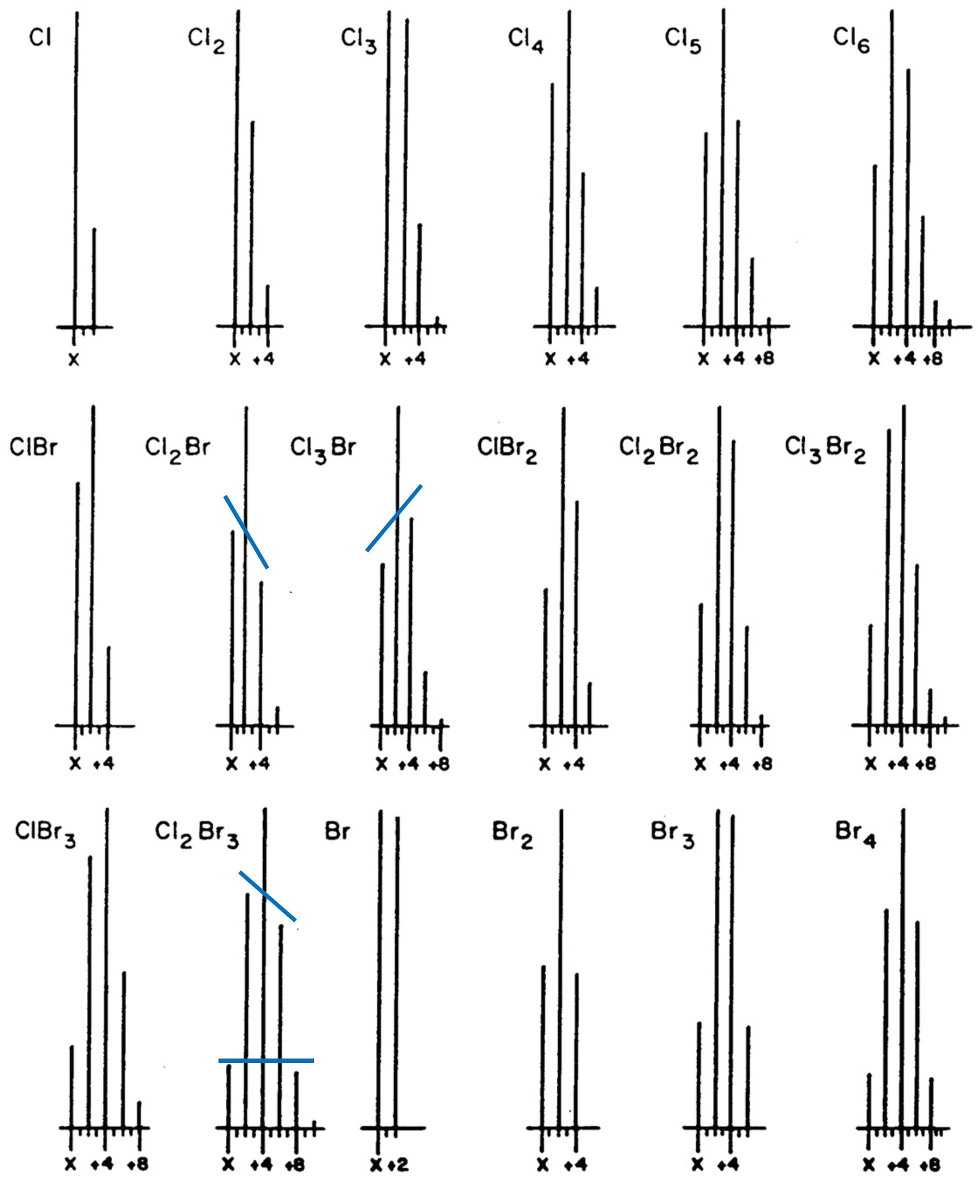
no. C x 1.1 = 7%

no. C = 7%/1.1 = ~6

Isotope patterns for multiple halogens

M^+ and M^{+2} in
75.8%:24.2% (~ 3 :1) ratio
= ^{35}Cl and ^{37}Cl

M^+ and M^{+2} in
50.7%:49.3% (~ 1 :1) ratio
= ^{79}Br and ^{81}Br



Factors that influence ion abundance (1)

- Most important: Stability of the product ion
  - *Electron sharing stabilization*

    From non-bonding orbital of heteroatom (*n*)

    $CH_3\text{-}C^+\text{=}O \leftrightarrow CH_3\text{-}C\equiv O^+$ (m/z 43)
  - *Resonance stabilization*

    $CH_2\text{=}CH\text{-}{}^+CH_2 \quad \leftrightarrow \quad {}^+CH_2\text{-}CH\text{=}CH_2$ (m/z 41)

    Benzyl $C_6H_5CH_2^+$ >> phenyl $C_6H_5^+$
  - *"Distonic" radical ions*

    Separation of charge and radical sites

    $CH_3CH_2CH_2CH\text{=}O^{+\cdot} \rightarrow {}^{\cdot}CH_2CH_2CH_2CH\text{=}O^+H$

    $CH_3NH_2^{+\cdot} \rightarrow {}^{\cdot}CH_2N^+H_3$

Factors that influence ion abundance (2)

- **Stevenson’s Rule**

Immediately after the ionization of a molecule in the ion source, it tries to give as much energy as possible. The simplest way to do this is by splitting off a radical as large as possible (larger the radical → more vibration-free degrees and more energy it can absorb). Stevenson recognized this in the early 1950s, which is why the following rule is also called the Stevenson rule:

- In the case of fragmentation, the largest radical always cleaves.
- The remaining fragment usually forms the base peak

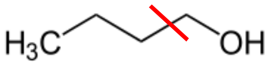
Factors that influence ion abundance (2)

- **Stevenson's Rule**

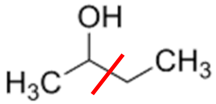
Immediately after the ionization of a molecule in the ion source, it tries to give as much energy as possible. The simplest way to do this is by splitting off a radical as large as possible (larger the radical → more vibration-free degrees and more energy it can absorb). Stevenson recognized this in the early 1950s, which is why the following rule is also called the Stevenson rule:

- In the case of fragmentation, the largest radical always cleaves
- The remaining fragment usually forms the base peak

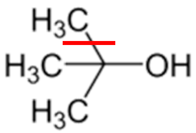
Isomers of butanol



→ 1-Butanol : base peak : mass 31 (dissociation C_3H_7 radical)



→ 2-butanol : mass 45 (dissociation ethyl group)



→ t-butanol (dissociation CH_3 radical)

Factors that influence ion abundance (2)

- **Stevenson’s Rule**

  – In a cleavage of a single bond in an OE $^{+\cdot}$ ion

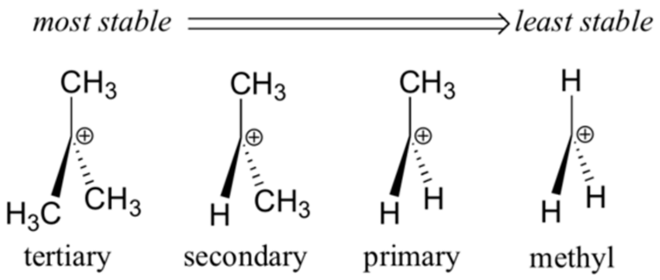
  $ABCD^{+\cdot}$ can give $A^{+} + BCD^{\cdot}$ or $A^{\cdot} + BCD^{+}$

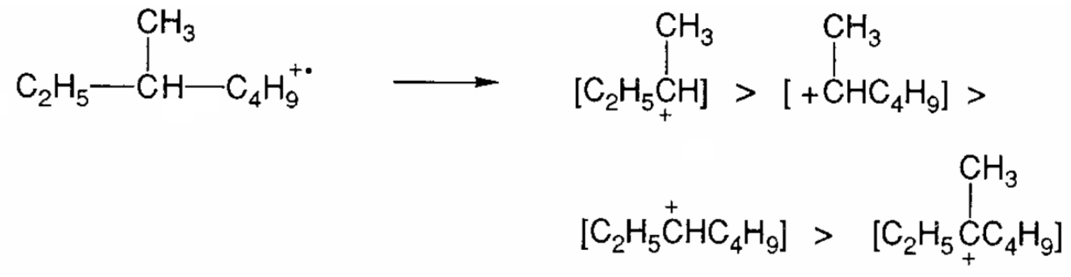
  – The fragment with the higher tendency to retain the unpaired electron should have the higher ionization energy.

    - It will be the less abundant ion in the spectrum

  - Loss of the largest alkyl (C_nH2_{n+1})

  – <u>Exception to Stevenson’s rule</u>: abundance decreases with increasing ion stability





Factors that influence ion abundance (3)

Stability of the neutral product

- *Stability of the ion is much more important!*

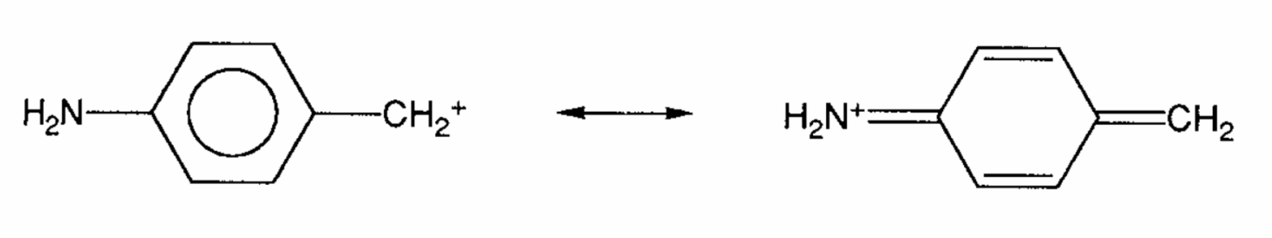
- A favorable product site for the unpaired electron can provide additional influence
  - Electronegative sites such as oxygen (·OR)

- The neutral product can be a molecule
  - Small stable molecules of high ionization energy are favored
  - H_2, CH_4, H_2O, C_2H_4, CO, NO, CH_3OH, H_2S, HCl, $CH_2{=}C{=}O$, CO_2
  - Losses of 2, 16, 18, 28, 30, 32, 34, 36, 42, 44 u.

Factors that influence ion abundance (4)

- **Entropy / Steric Effects**
  - Most favored reactions for enthalpy often have steric restrictions (e.g. rearrangement)
  - Dissociation favors products with less restrictive entropy requirements even if the enthalpy barrier is higher (*i.e.*, simple bond cleavage)

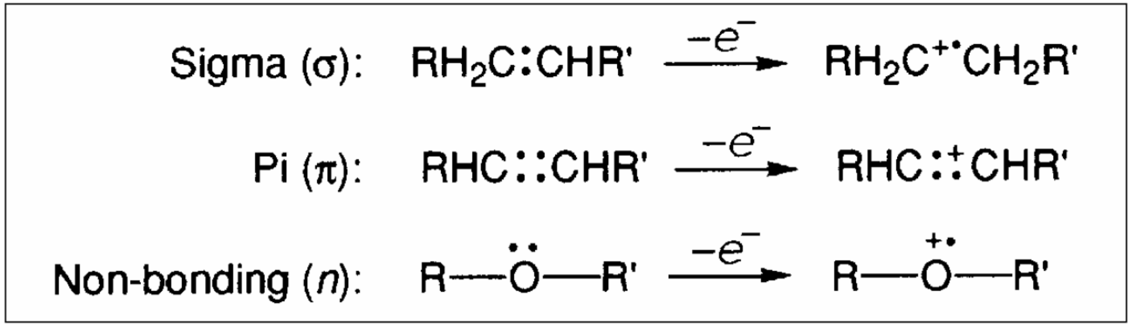
– Predict the *most abundant ion* in the spectrum of:



- Cleaving CH_2-CH_2 bond is sterically easiest
- *p*-aminobenzyl cation is very stable *via* resonance

Reaction initiation at *radical or charge site*

- Fragmentation reactions are often initiated at the favored sites for the unpaired electron or the charge
- The most favored radical and charge sites in the molecular ion are assumed to arise from loss of the molecule’s electron of lowest ionization energy
- Favorability σ < π < *n* electrons:



Basic fragmentation mechanisms

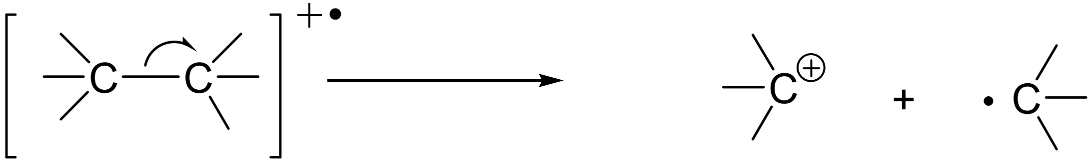
- Sigma (σ) cleavage
- Homolytic alpha (α) cleavage (radical site initiated)
- Inductive (*i*) cleavage (charge site initiated, heterolytic)
- Multiple bond cleavages, McLafferty rearrangements

σ-cleavage

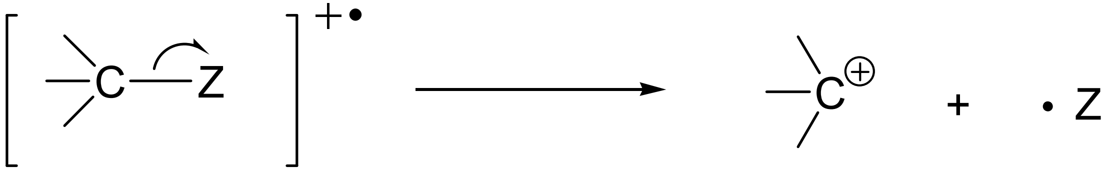
- Removal of an electron from a σ bond via EI weakens it.
- Cleavage of single bond in the odd-electron molecular ion $M^{+\bullet}$ must produce an even electron (EE+) fragment.
- As bond breaks, one fragment gets the remaining electron, and is neutral ($R^{\bullet}$).
- The other fragment is a charged, ***even electron*** species (R^{+})
- Highly substituted carbocations are more stable (Stevenson's Rule).
  - Cleavage of the C_1-C_2 bond in long *n*-alkanes is not favored
  - Lower IE fragments are favored
- Long *n*-alkane chains tend to produce many fragments separated by 14 u in the range *m/z* 20-90.

One bond σ-cleavage

a. cleavage of C-C



b. cleavage of C-heteroatom

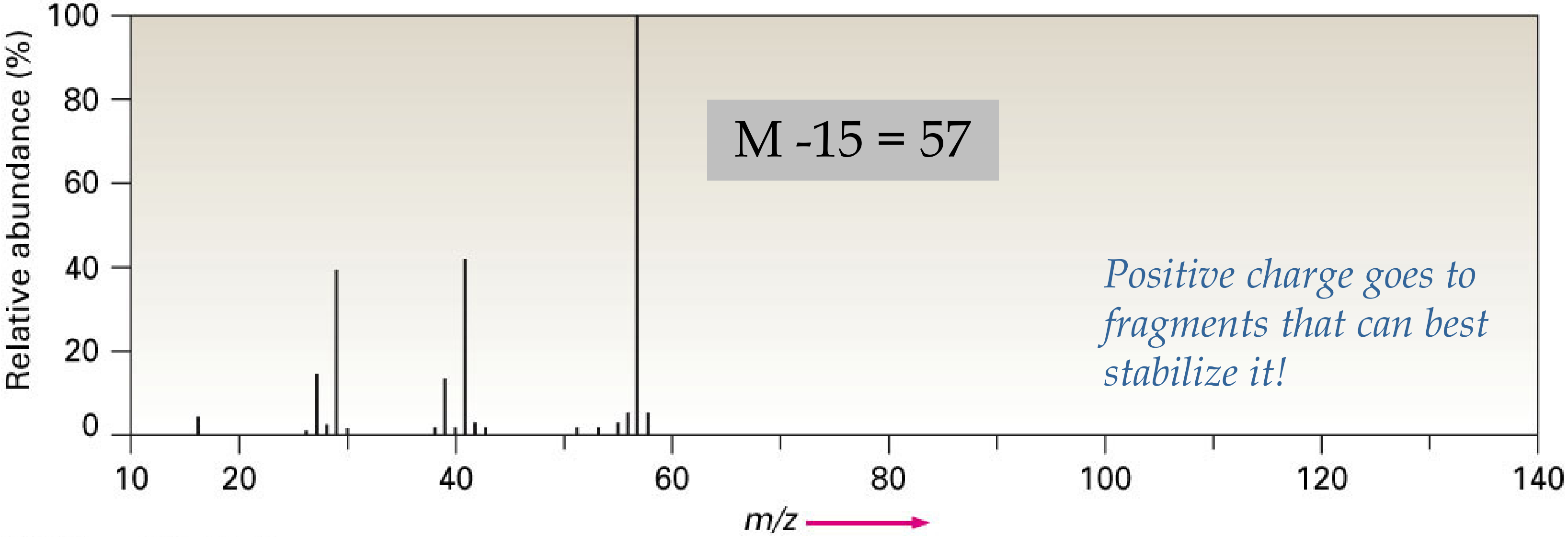


Notice "fishhook" arrows! **→ single electron movement**

curved, two-barbed arrow:
two electron movement

curved, single-barbed ('fish-hook') arrow:
single electron movement

Example 1: one bond σ-cleavage



M -15 = 57

Positive charge goes to fragments that can best stabilize it!

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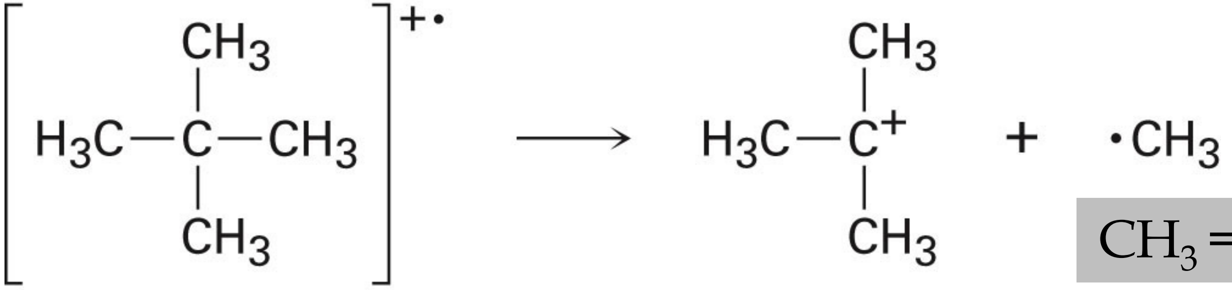
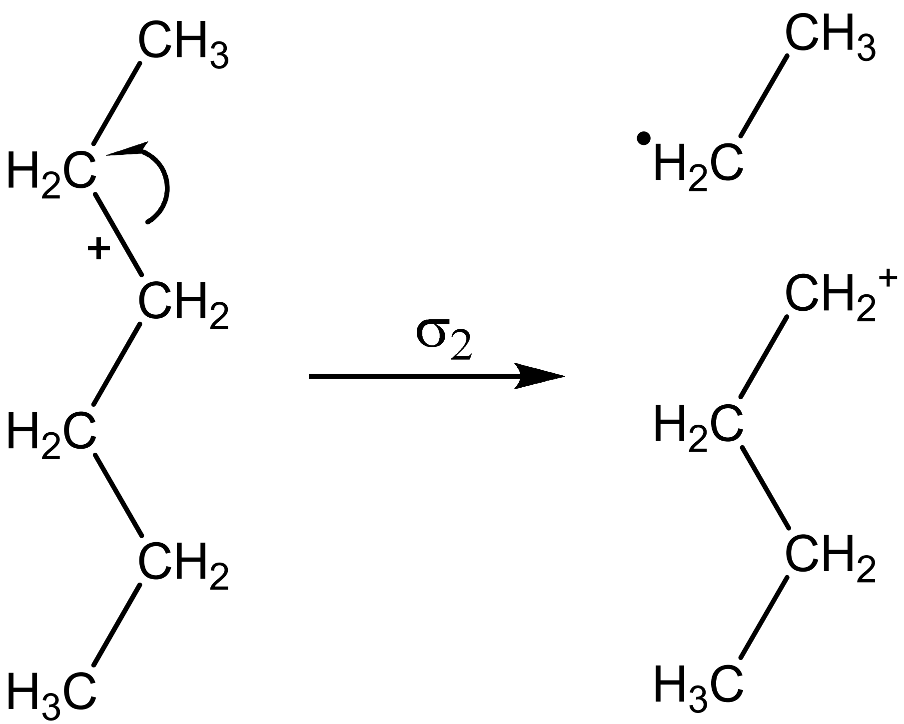


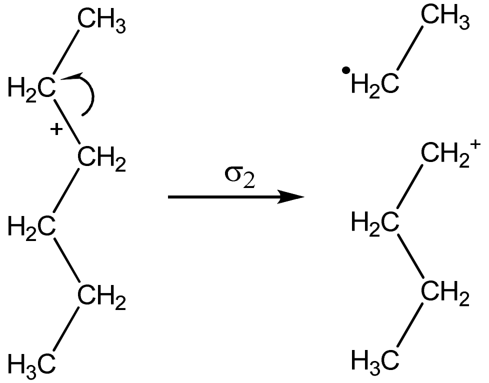
$CH_3 = 15$

$m/z = 57$

MW = 72;
$M^{+\cdot}$ peak not seen

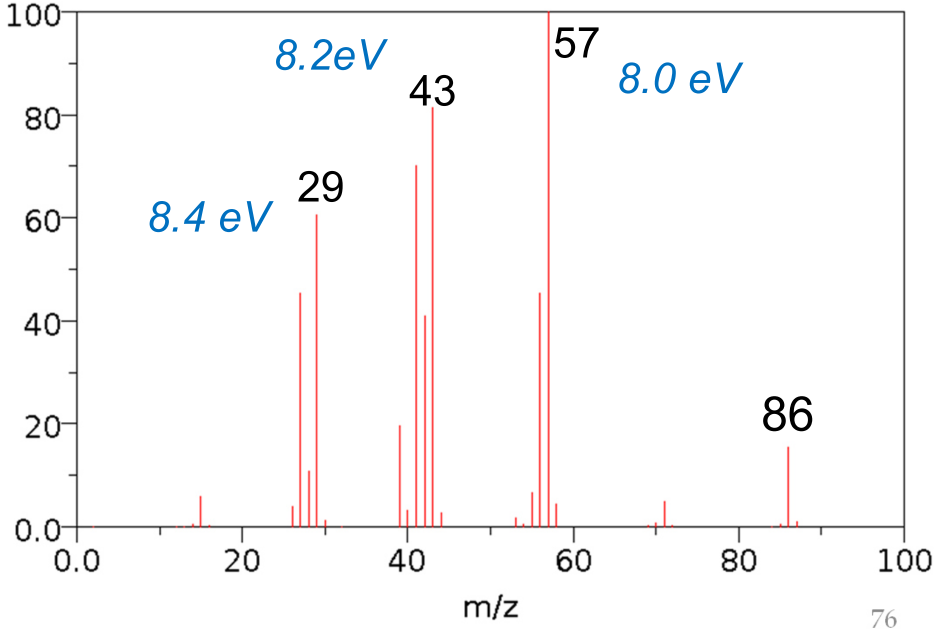
Example 2: σ-cleavage of hexane



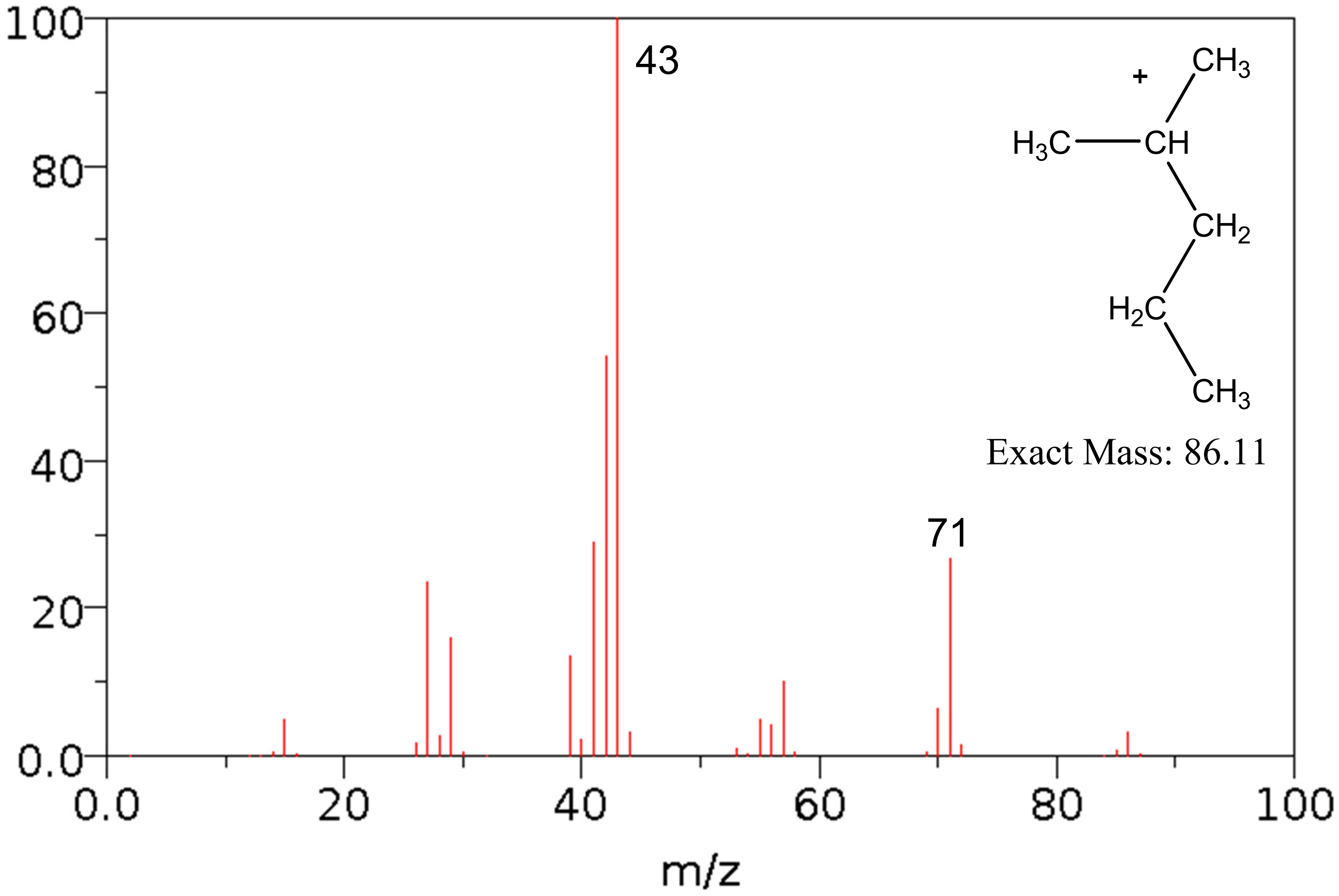


Exact Mass: 86.11

Exact Mass: 57.07

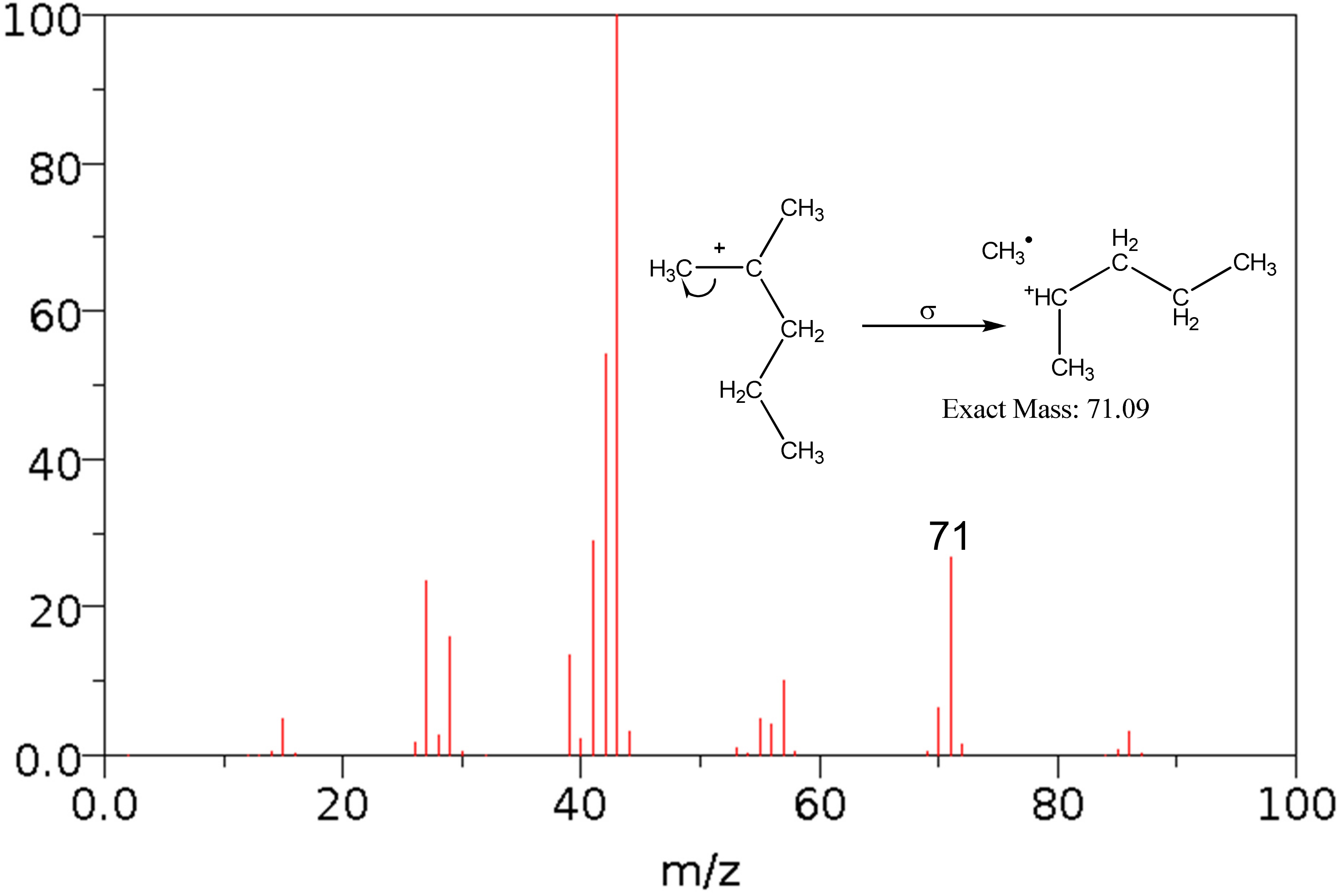


Example 3: σ-cleavage of 2-methyl-pentane

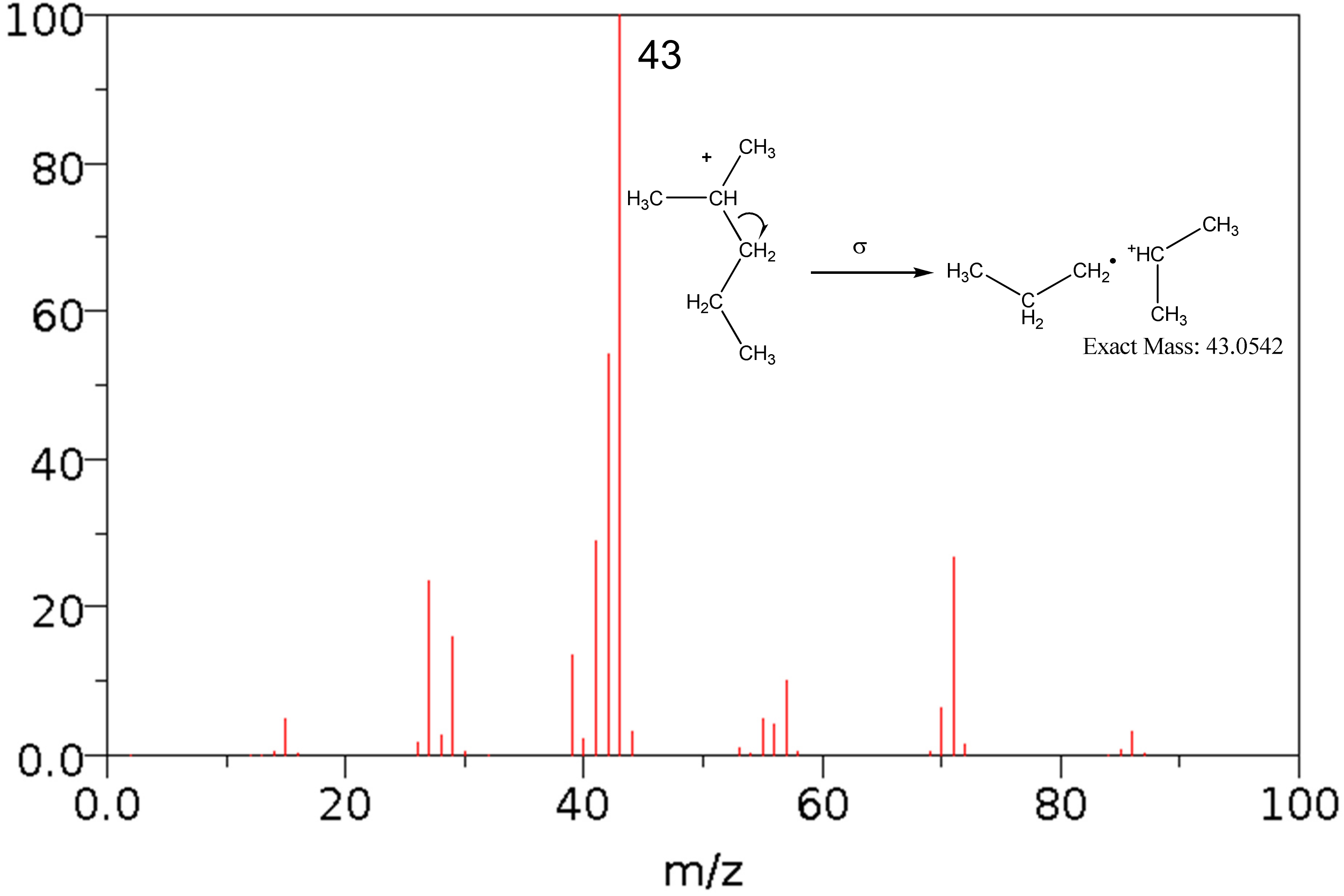


Exact Mass: 86.11

Example 3: σ-cleavage of 2-methyl-pentane



Example 3: σ-cleavage of 2-methyl-pentane

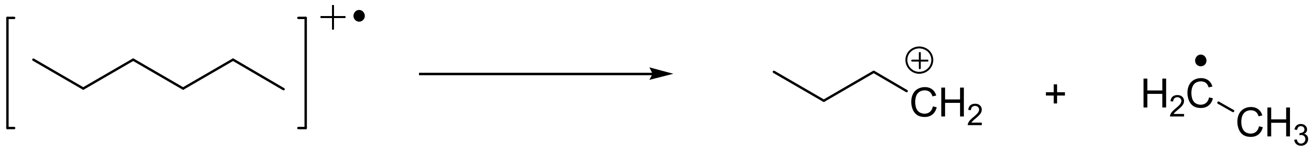


Some helpful rules for fragmentation schemes

- The **even-odd electron rule** applies: “thermodynamics predicts that even electron ions rarely cleave to a pair of odd electron fragments”.
- Mass losses of 14 u are rare.
- The order of carbocation/radical stability is benzyl/*tert.* > allyl/*secondary* > *primary* > methyl > H.
- The loss of the longest carbon chain is preferred.
- Fragment ion stability is more important than fragment radical stability.

How to abbreviate these fragmentation patterns

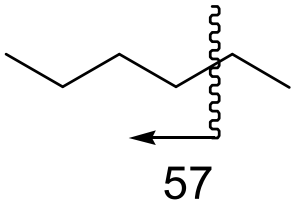
<u>Nomenclature</u>: rather than writing out single bond cleavages each time, use graphical representation:



Fragment obs. by MS

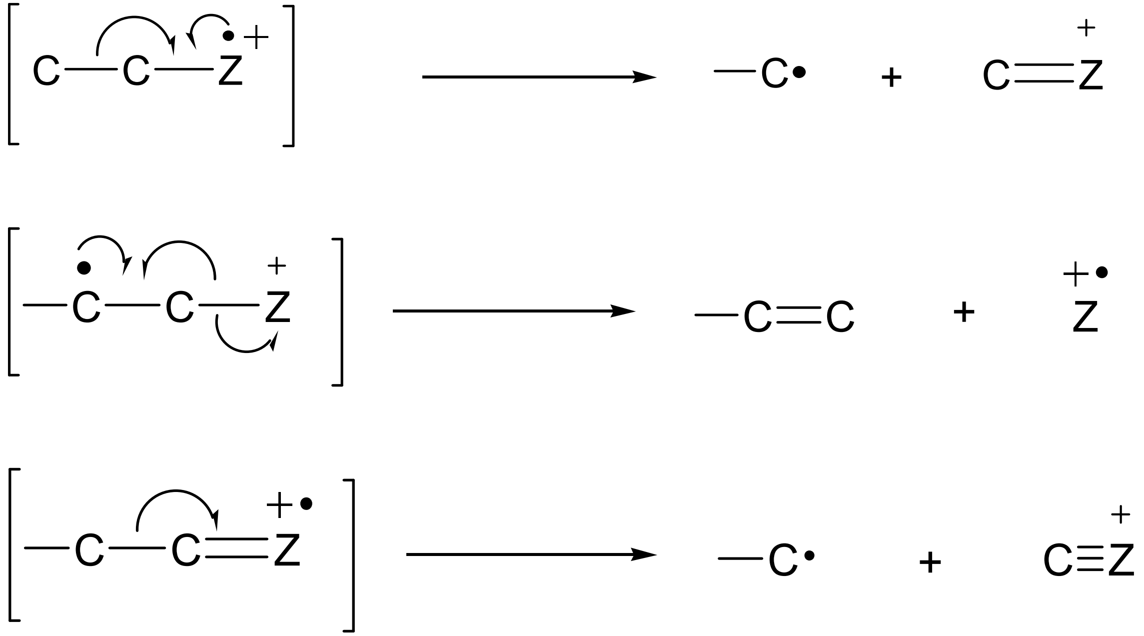
Neutral fragment inferred by its loss – not observed

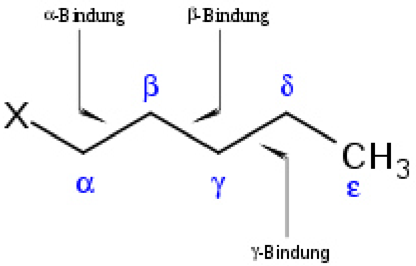
Can be written as:



α-cleavage – homolytic cleavage

Homolytic cleavage – *radical site driven*





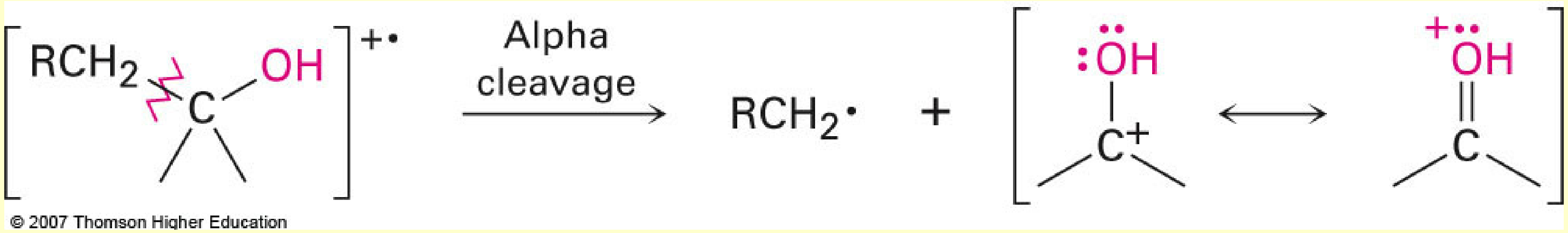
Reaction initiation at the radical site arises from its strong tendency for electron pairing; the odd electron is donated to form a new bond to an adjacent atom, followed by cleavage of another bond adjacent to that α atom.

α-cleavage

- Cleavage is caused when an electron from a bond to an atom adjacent to the charge site pairs up with the radical
  - Weakened α-sigma bond breaks!
  - This mechanism is also called *radical site initiated cleavage*.
- The charge does not move in this reaction!
- Charged product is usually an even electron species.
- α-cleavage directing atoms: N > S, O, π, $R^\bullet$ > Cl, Br > H
  - Loss of longer alkyl chains is often favored.
  - Energetics of both products (charged and neutral) are important.

Example 1: α-cleavage of alcohols

Example: alcohols undergo α-cleavage (at bond next to the C-OH)



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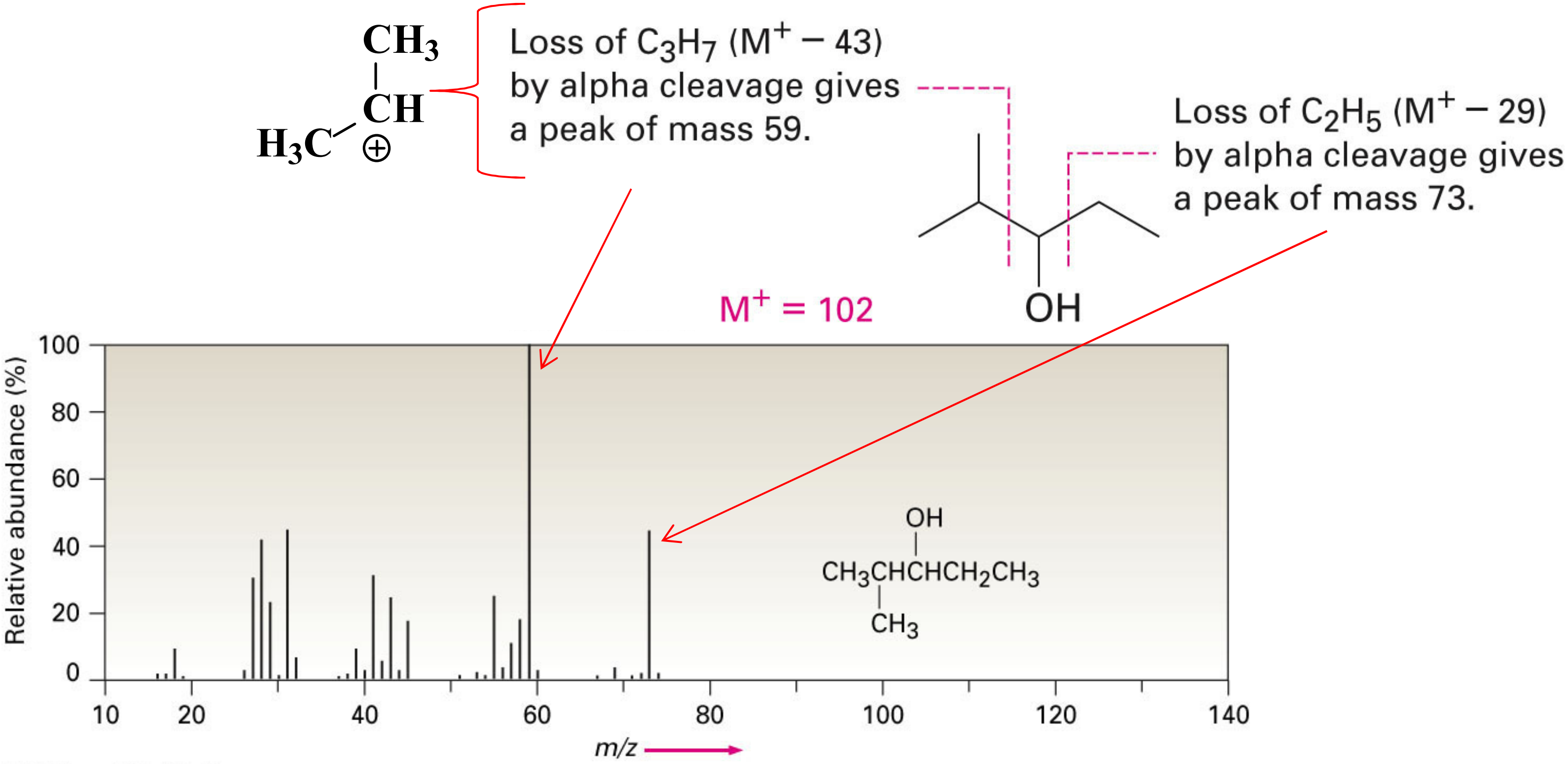

Loss of C_3H_7 ($M^+ - 43$) by alpha cleavage gives a peak of mass 59.

Loss of C_2H_5 ($M^+ - 29$) by alpha cleavage gives a peak of mass 73.

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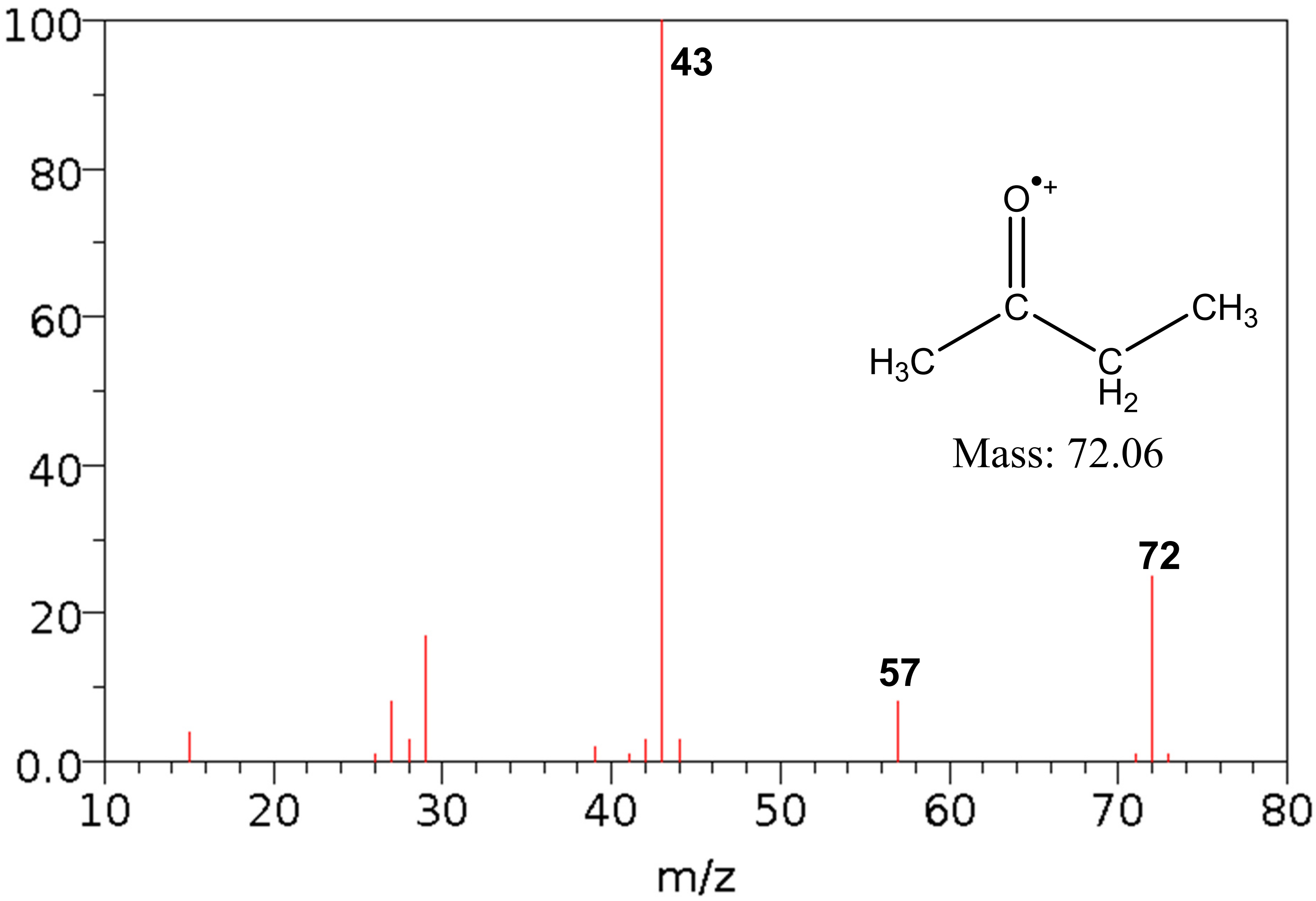
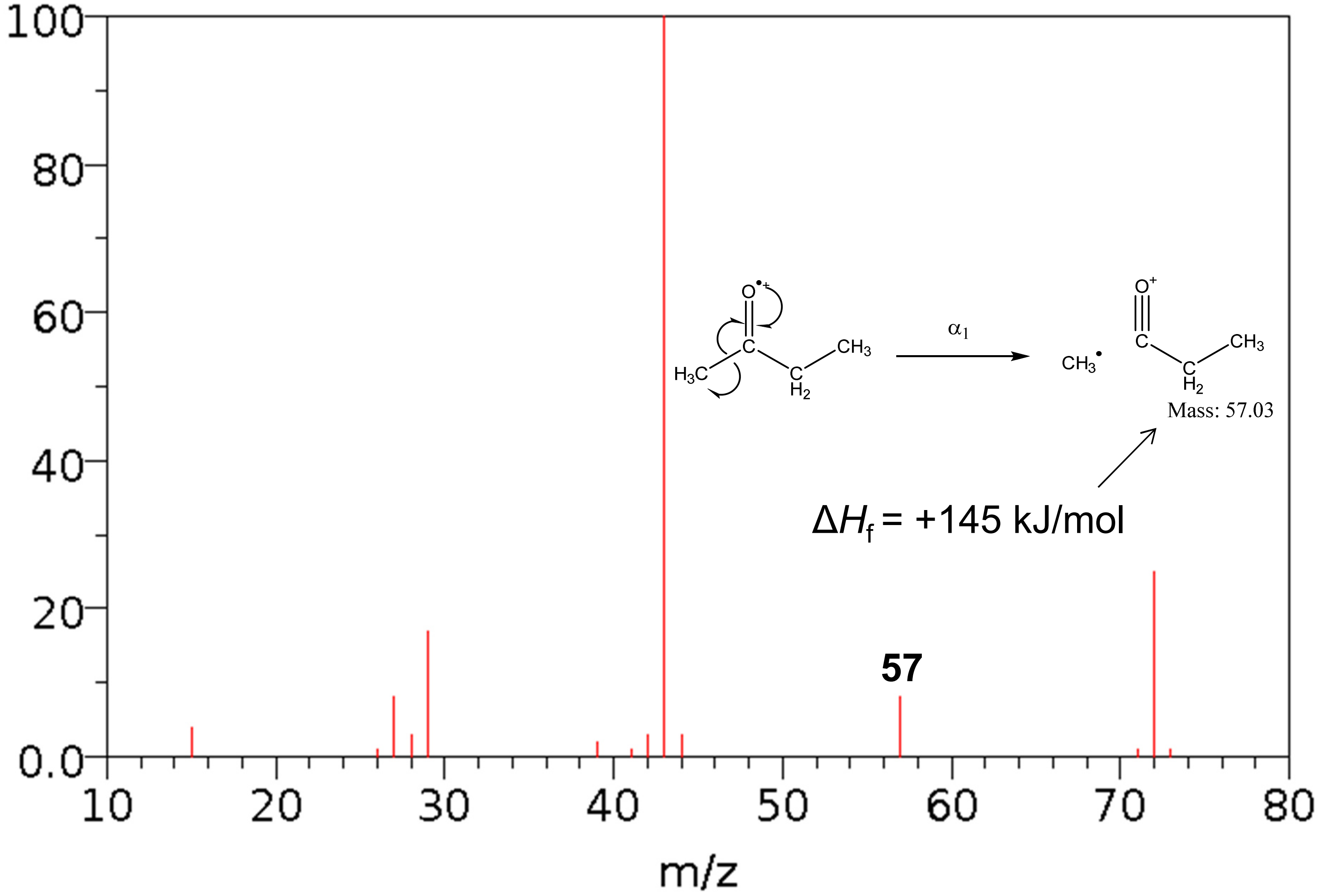
Example 2: α-cleavage of 2-butanone

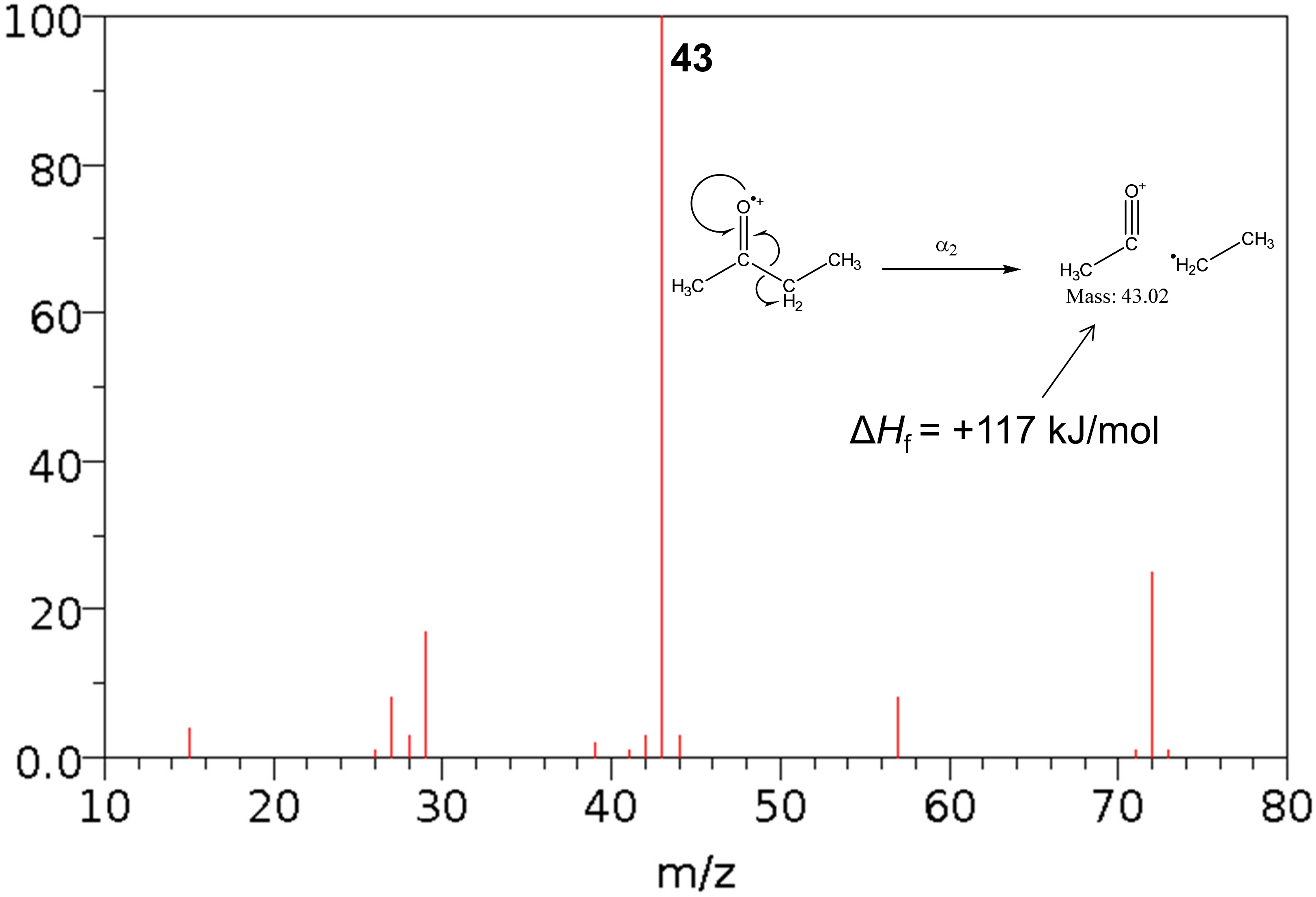


Example 2: α-cleavage of 2-butanone

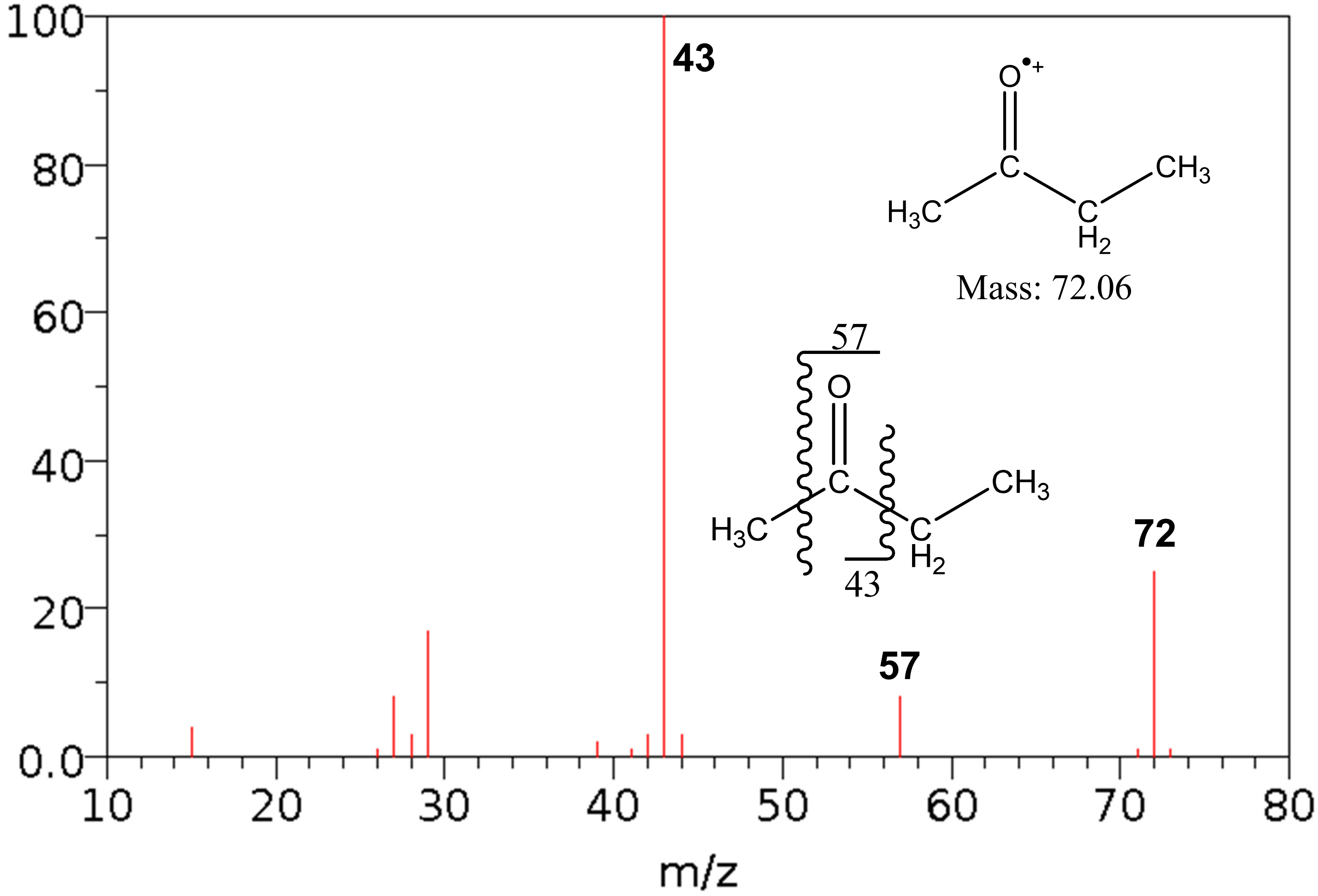


ΔH_f = +145 kJ/mol

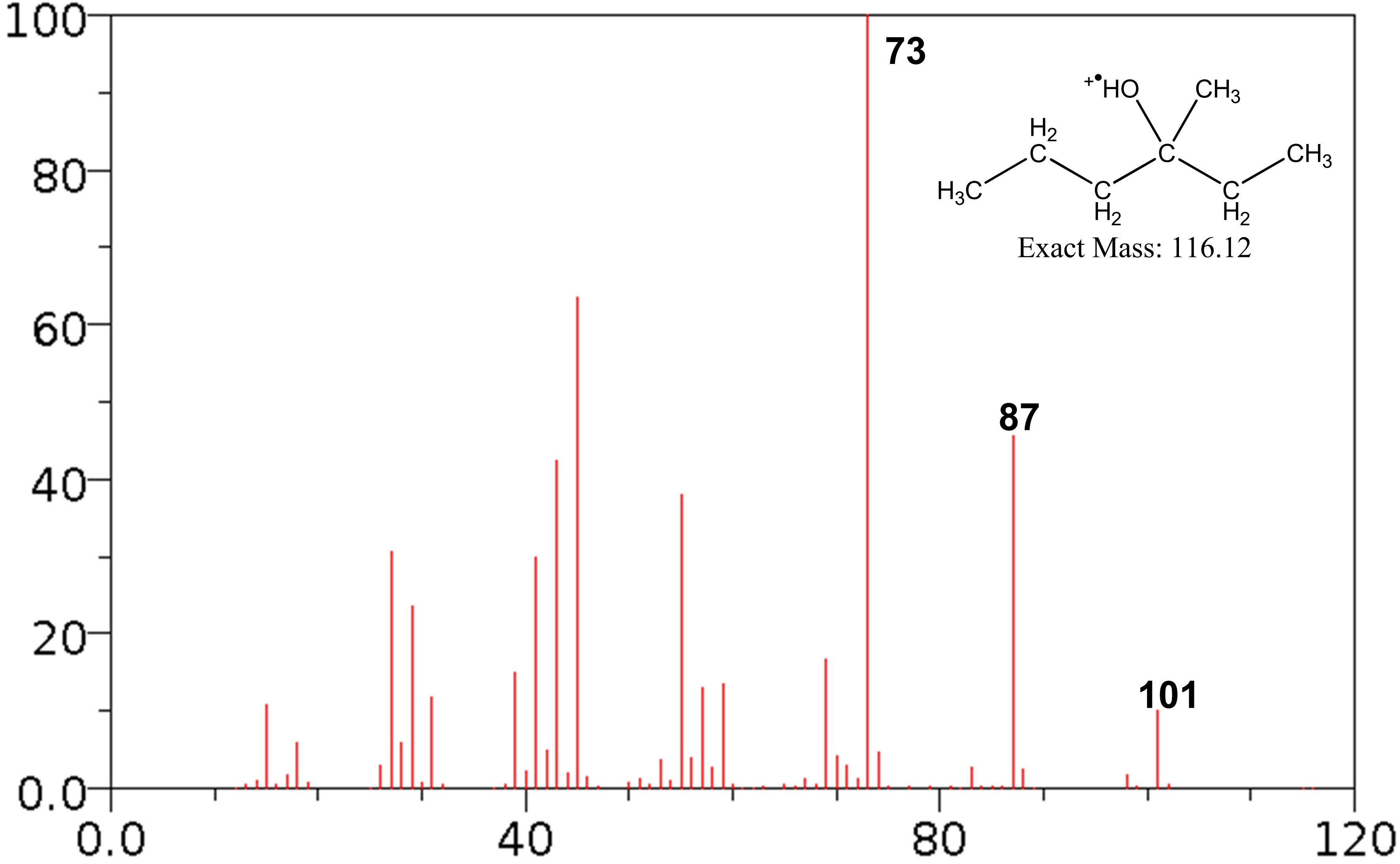
Example 2: α-cleavage of 2-butanone



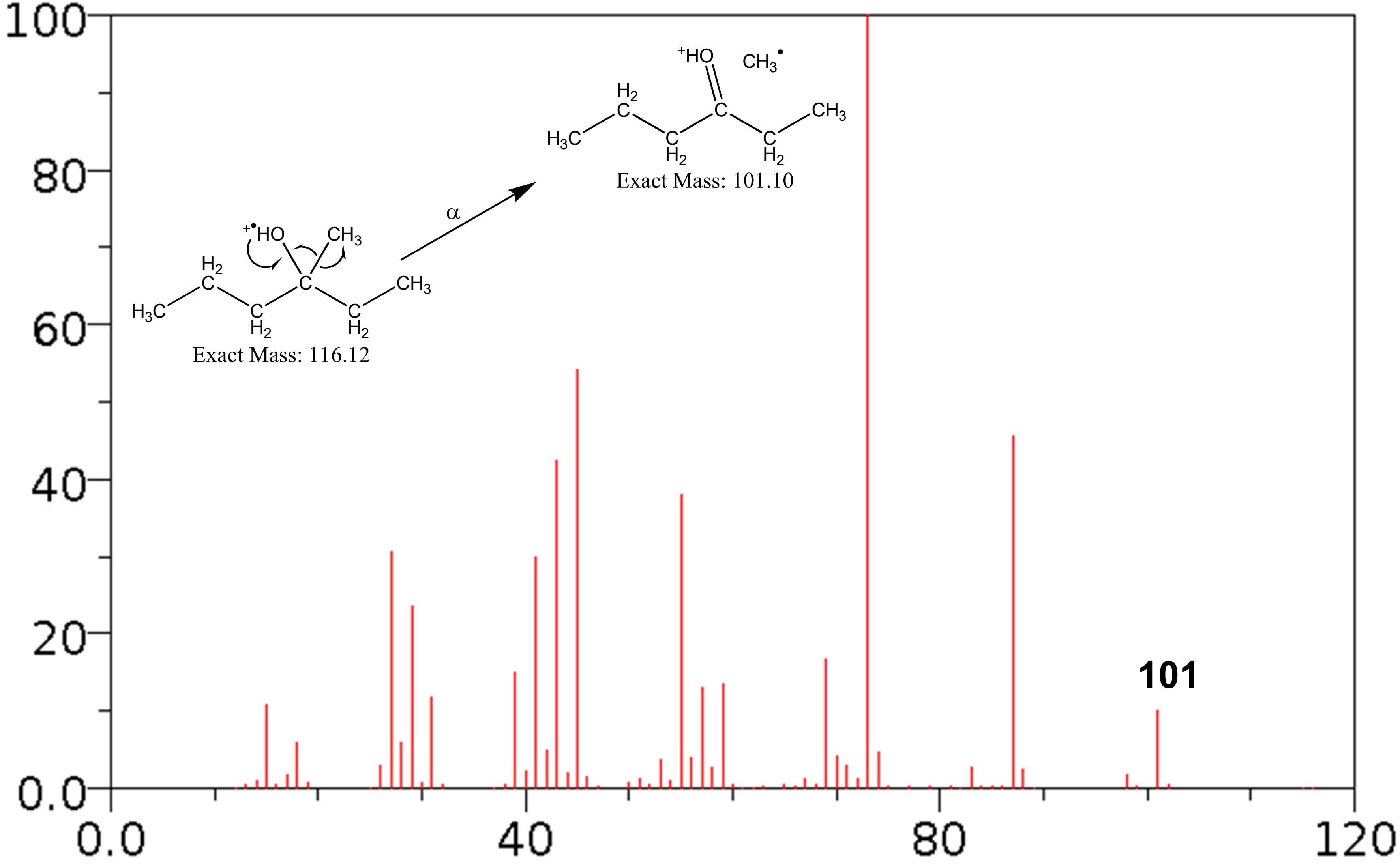
Example 2: α-cleavage of 2-butanone



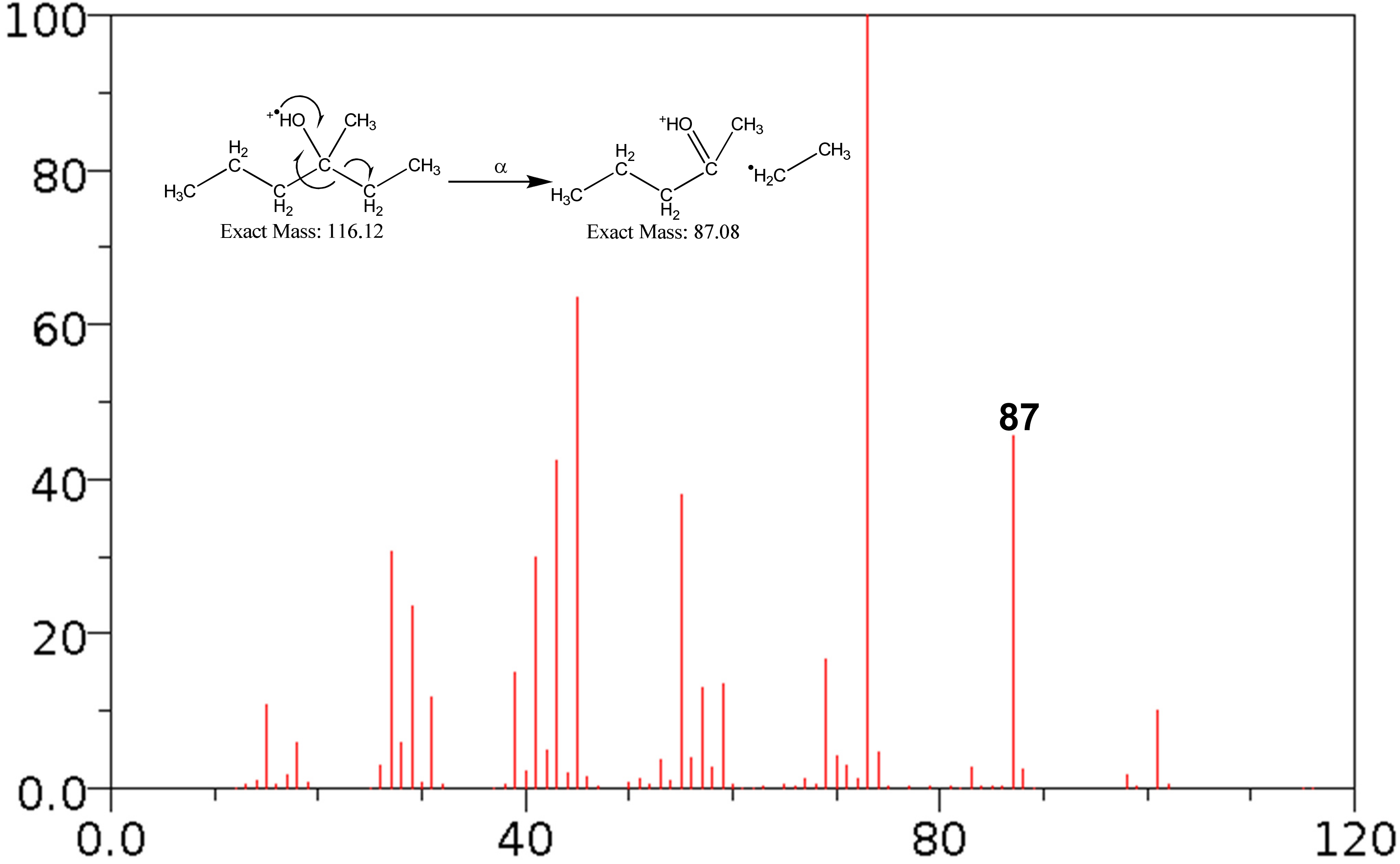
Example 3: α-cleavage of 3-methyl-3-hexanol



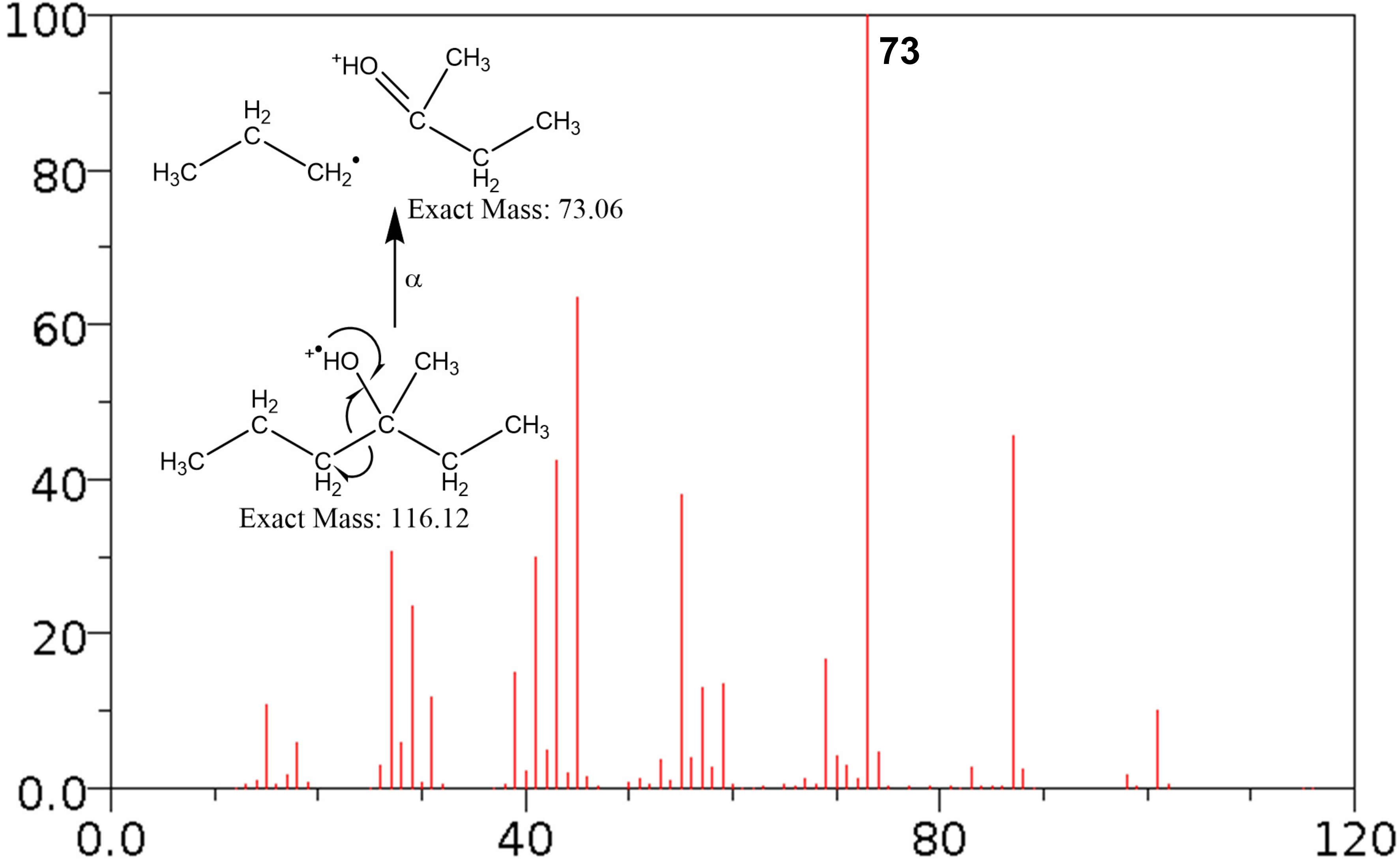
Example 3: α-cleavage of 3-methyl-3-hexanol



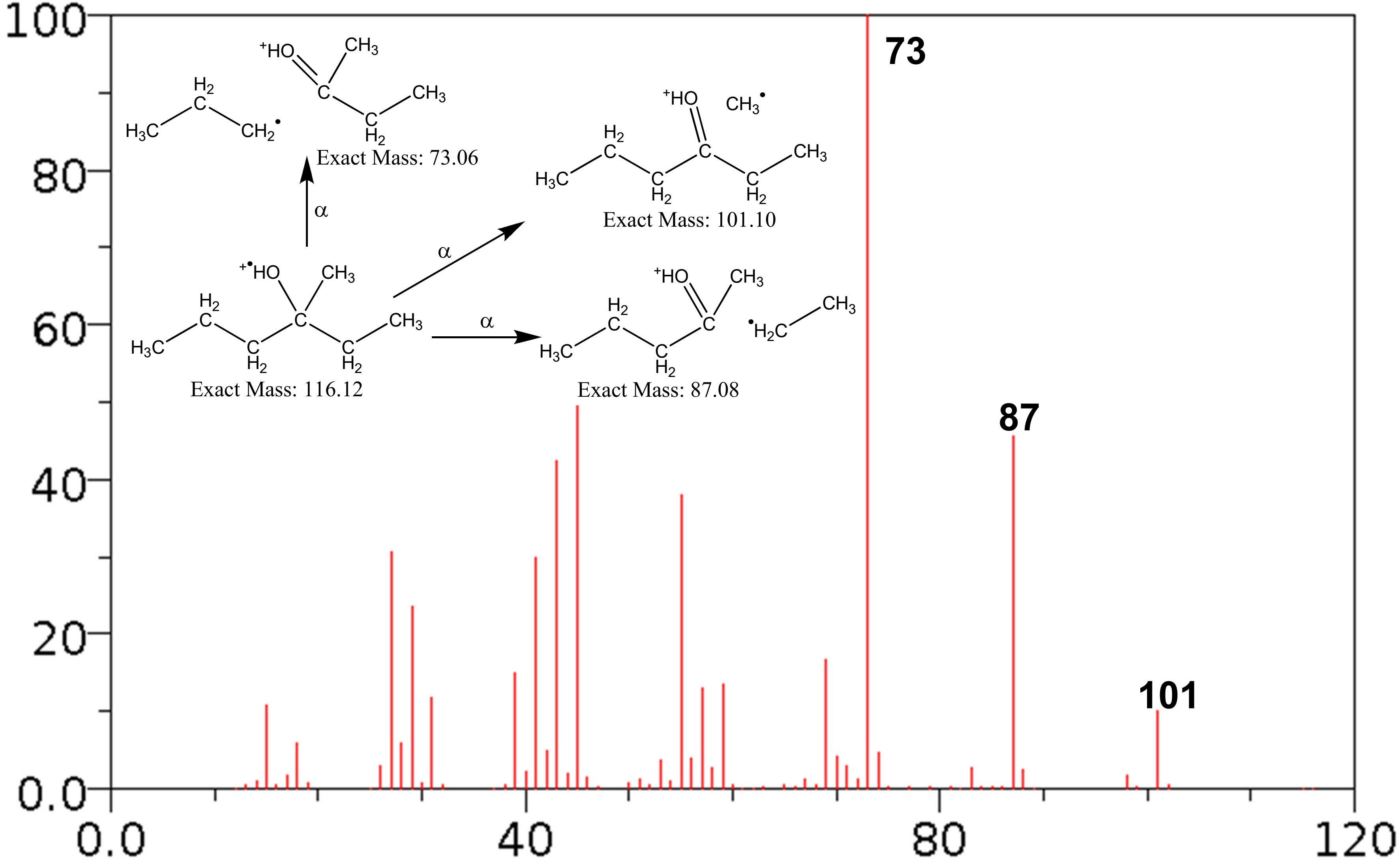
Example 3: α-cleavage of 3-methyl-3-hexanol



Example 3: α-cleavage of 3-methyl-3-hexanol



Example 3: α-cleavage of 3-methyl-3-hexanol

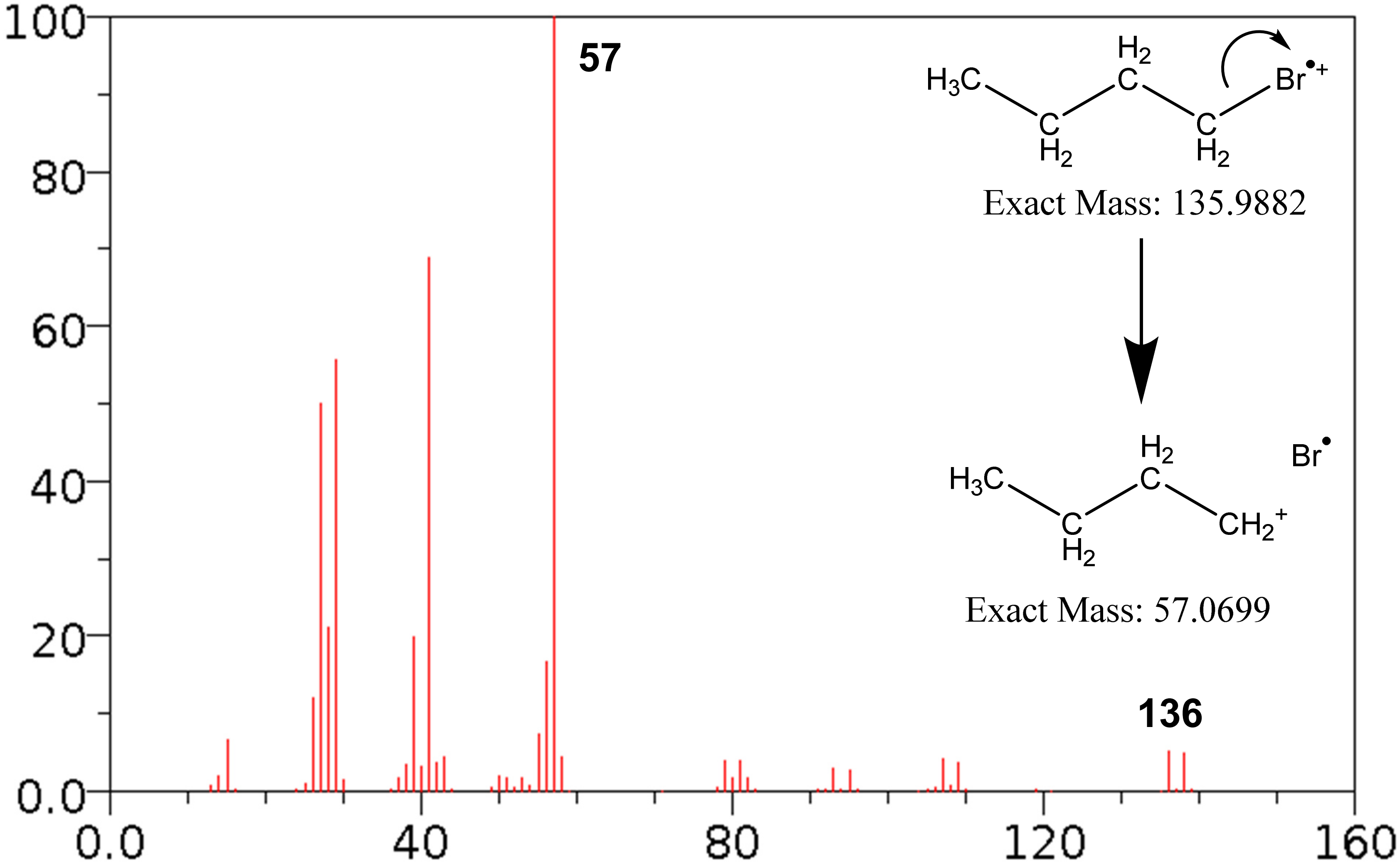


Heterolytic charge site initiated cleavage (*i*)

Heterolytic cleavage - charge driven

- Charged site induces a pair of electrons to migrate from an adjacent bond or atom
    - This breaks a sigma (σ) bond
- Also called *inductive cleavage* (*i*)
- The charge migrates to the electron pair donor
    - The electron pair neutralizes the original charge
- Even electron fragments can further dissociate by this mechanism
- Inductive cleavage directing atoms: Halogens > O, S, >> N, C

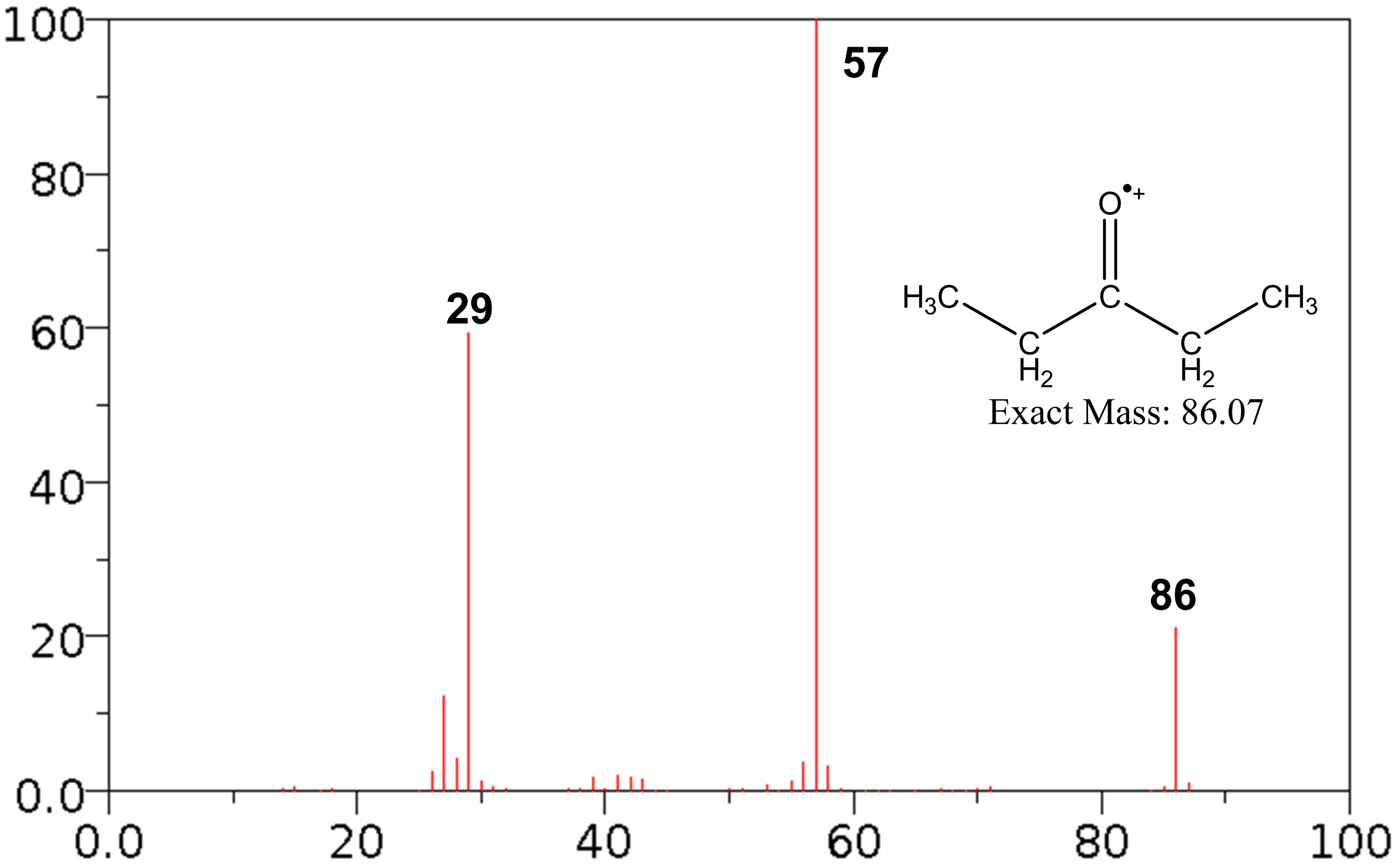
Example 1: *i*-cleavage of 1-bromo-butane



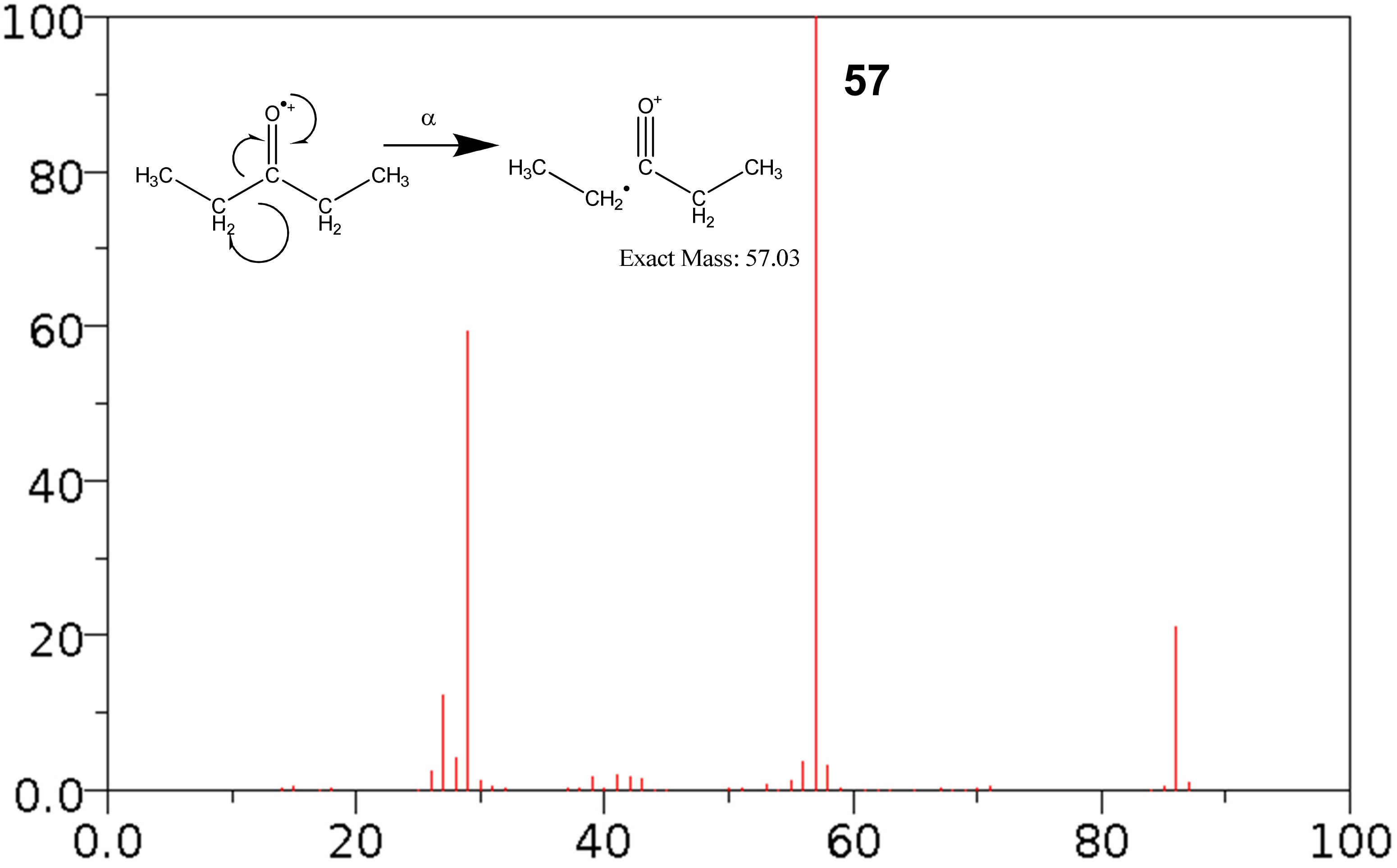
Exact Mass: 135.9882

Exact Mass: 57.0699

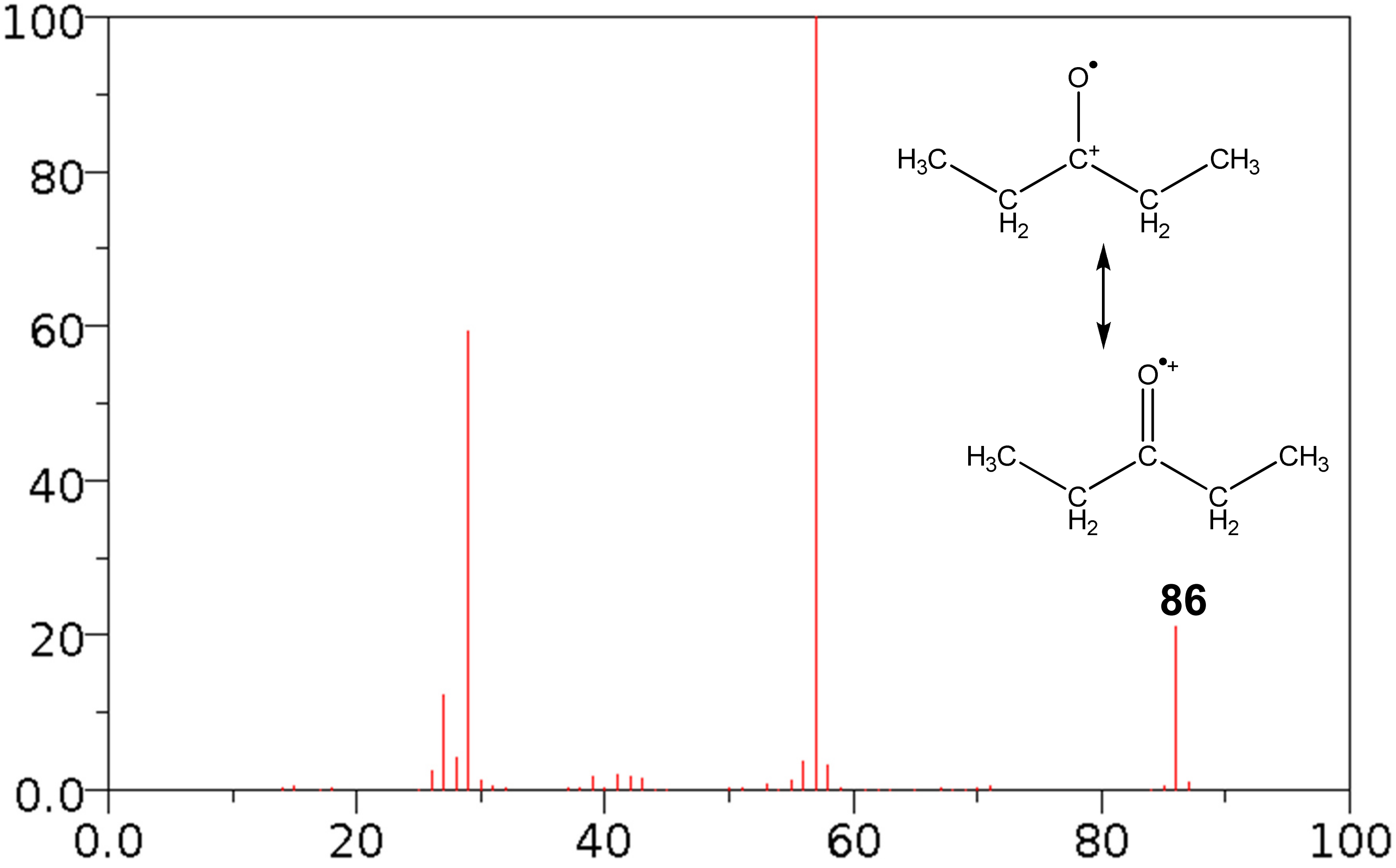
Example 2: *i*-cleavage of 3-pentanone



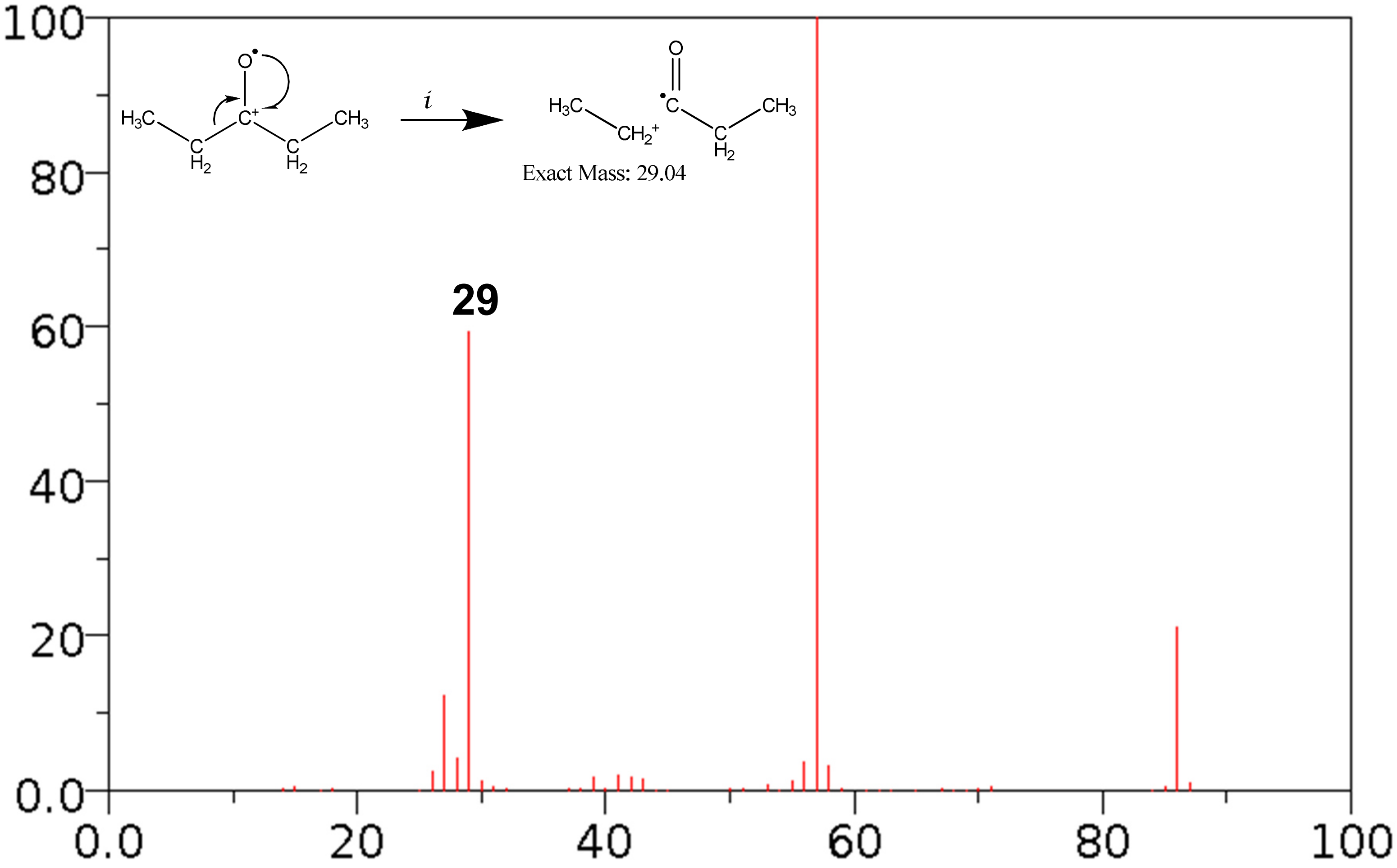
Example 2: *i*-cleavage of 3-pentanone



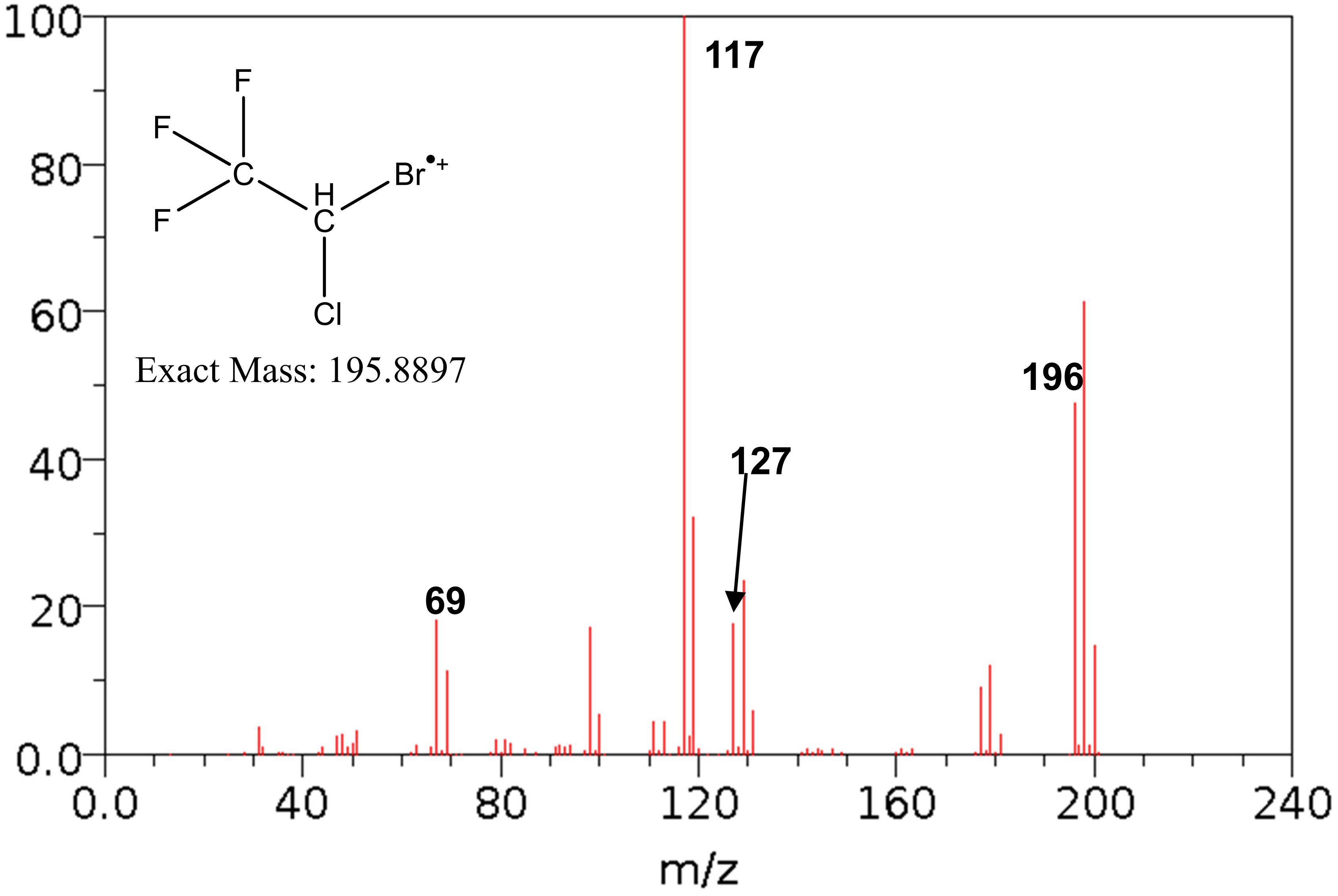
Example 2: *i*-cleavage of 3-pentanone



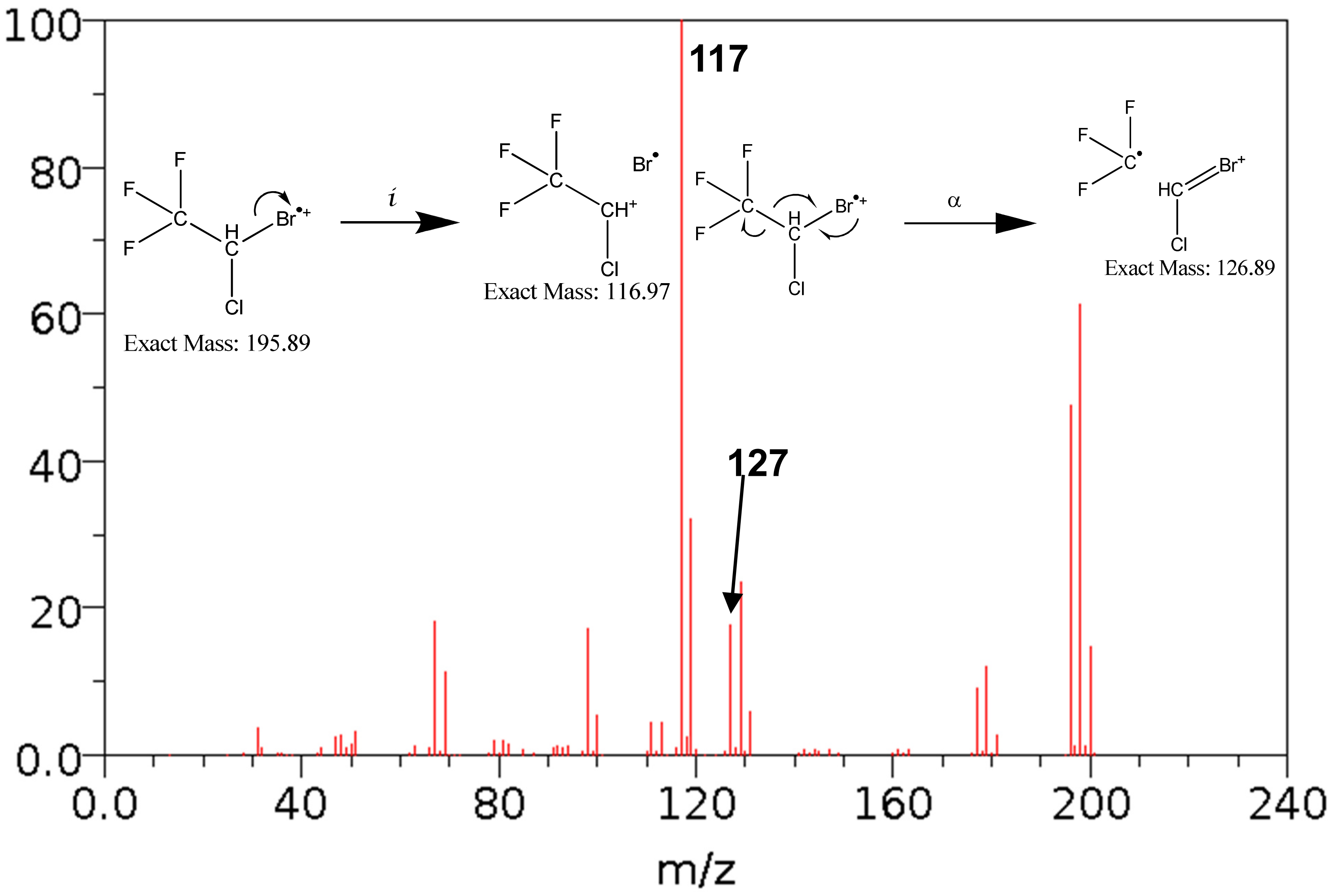
Example 2: *i*-cleavage of 3-pentanone



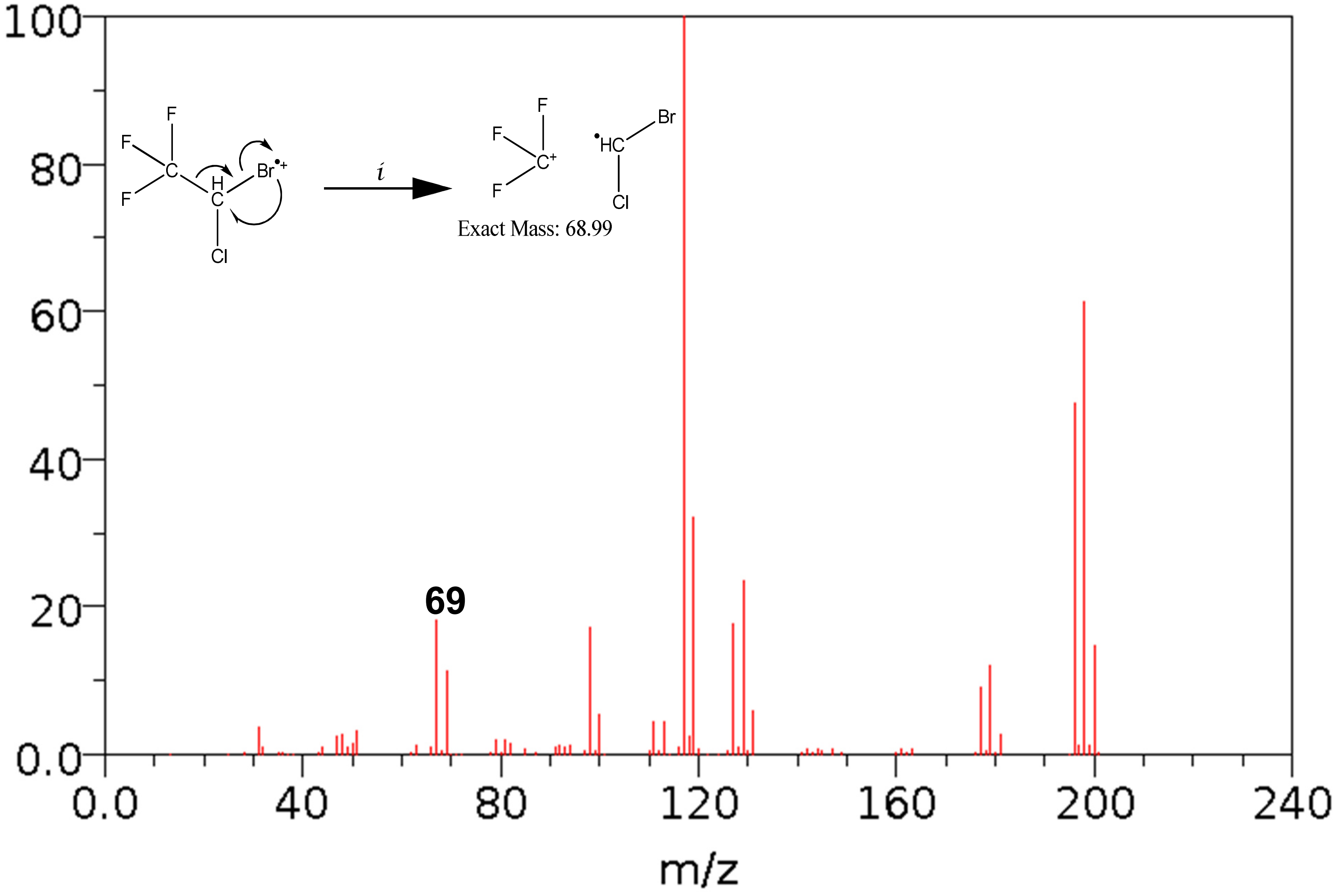
Example 3: *i*-cleavage of halothane



Example 3: *i*-cleavage of halothane

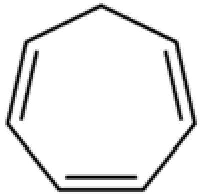


Example 3: *i*-cleavage of halothane



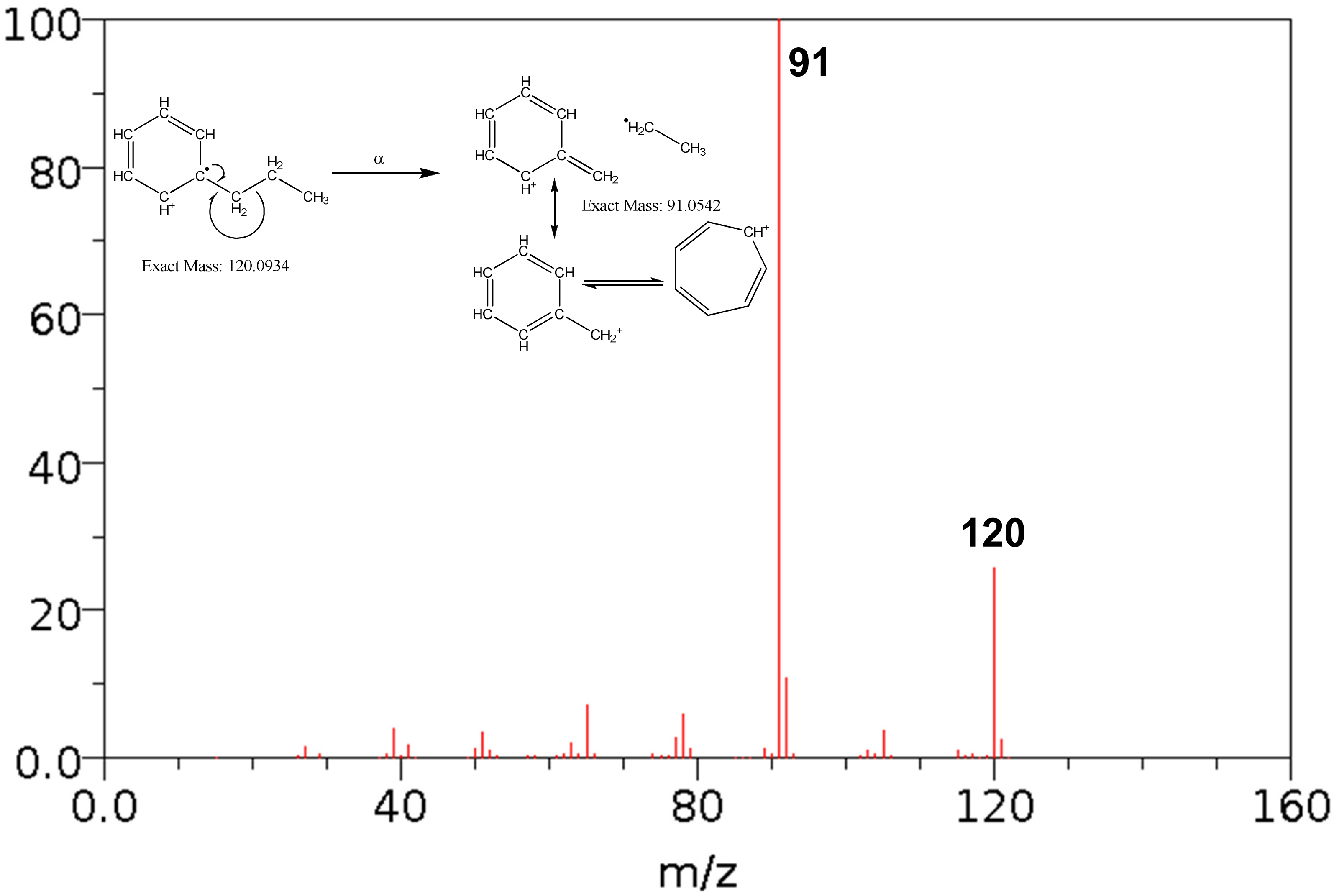
Special case: benzylic bond cleavages

- The charge-stabilizing ability of the aromatic group can dominate EI spectra.
- Alkylbenzenes will often form intense ions at *m/z* 91.
  - Tropylium ion (Hückel's rule 4*n* +2 electrons).
  - 7-membered ring favored by >11 kJ/mol.

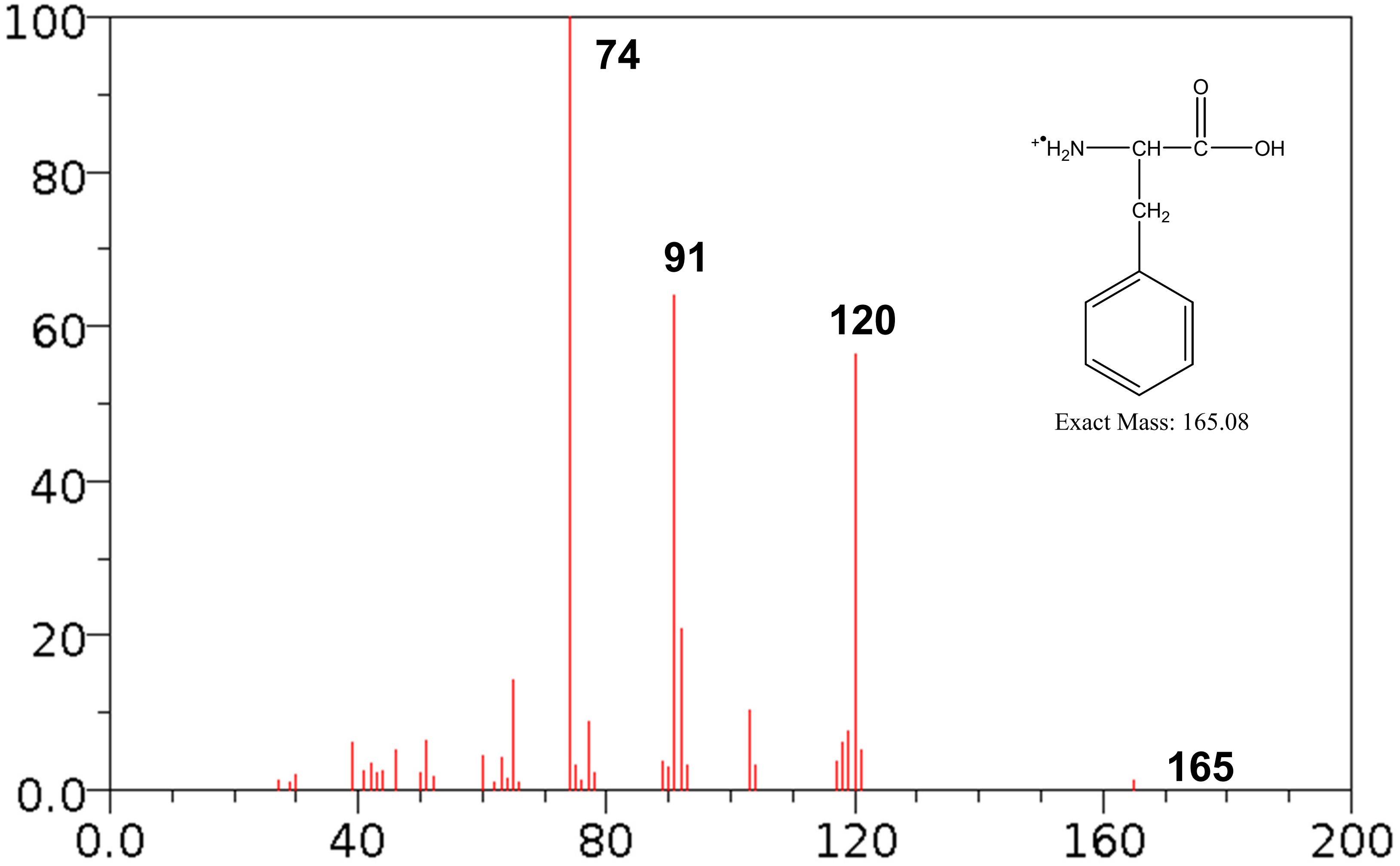


- Tropylium ion can fragment by successive losses of acetylene.
  - *m/z* 91 → 65 → 39

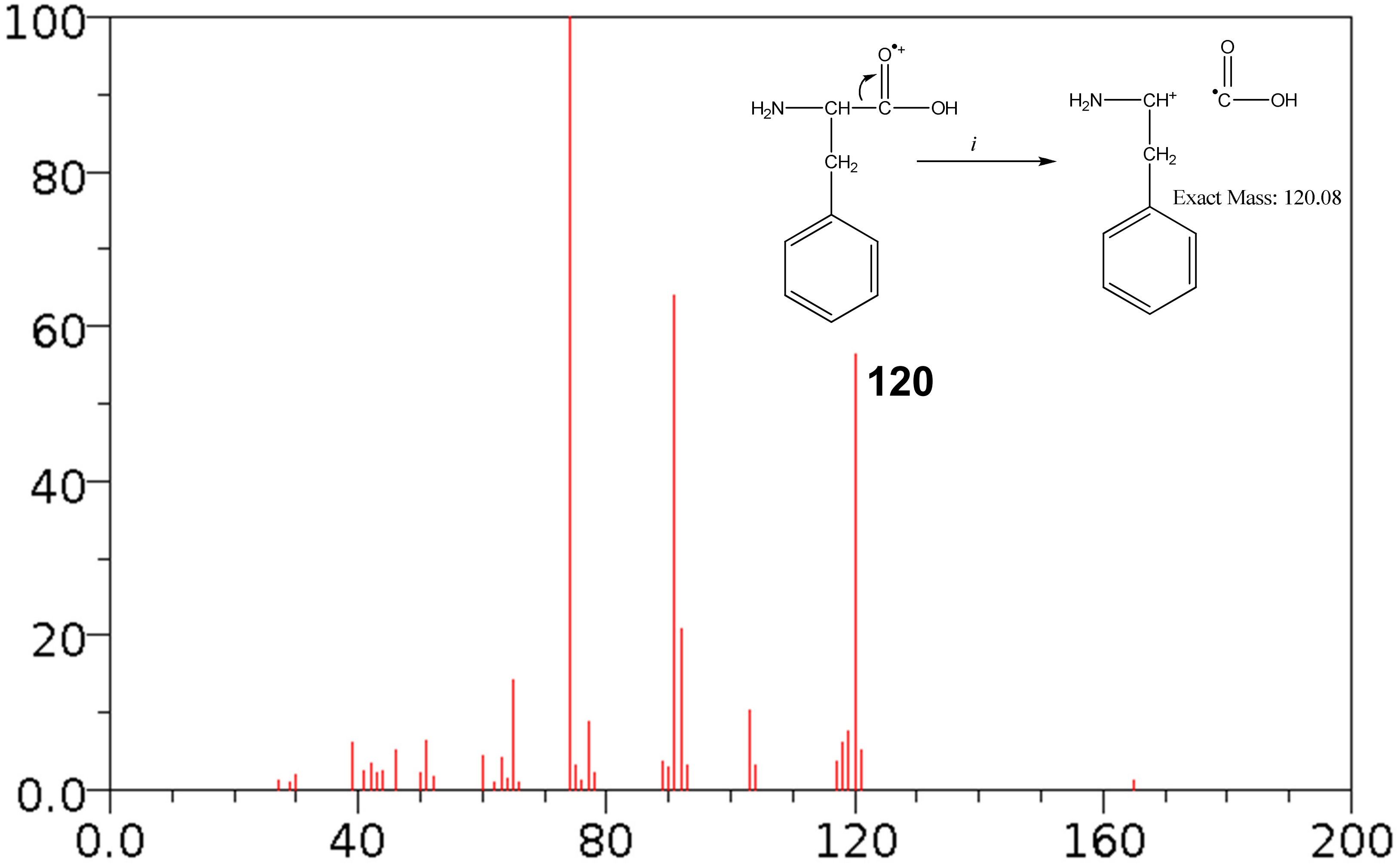
Example 1: benzylic cleavage of propylbenzene



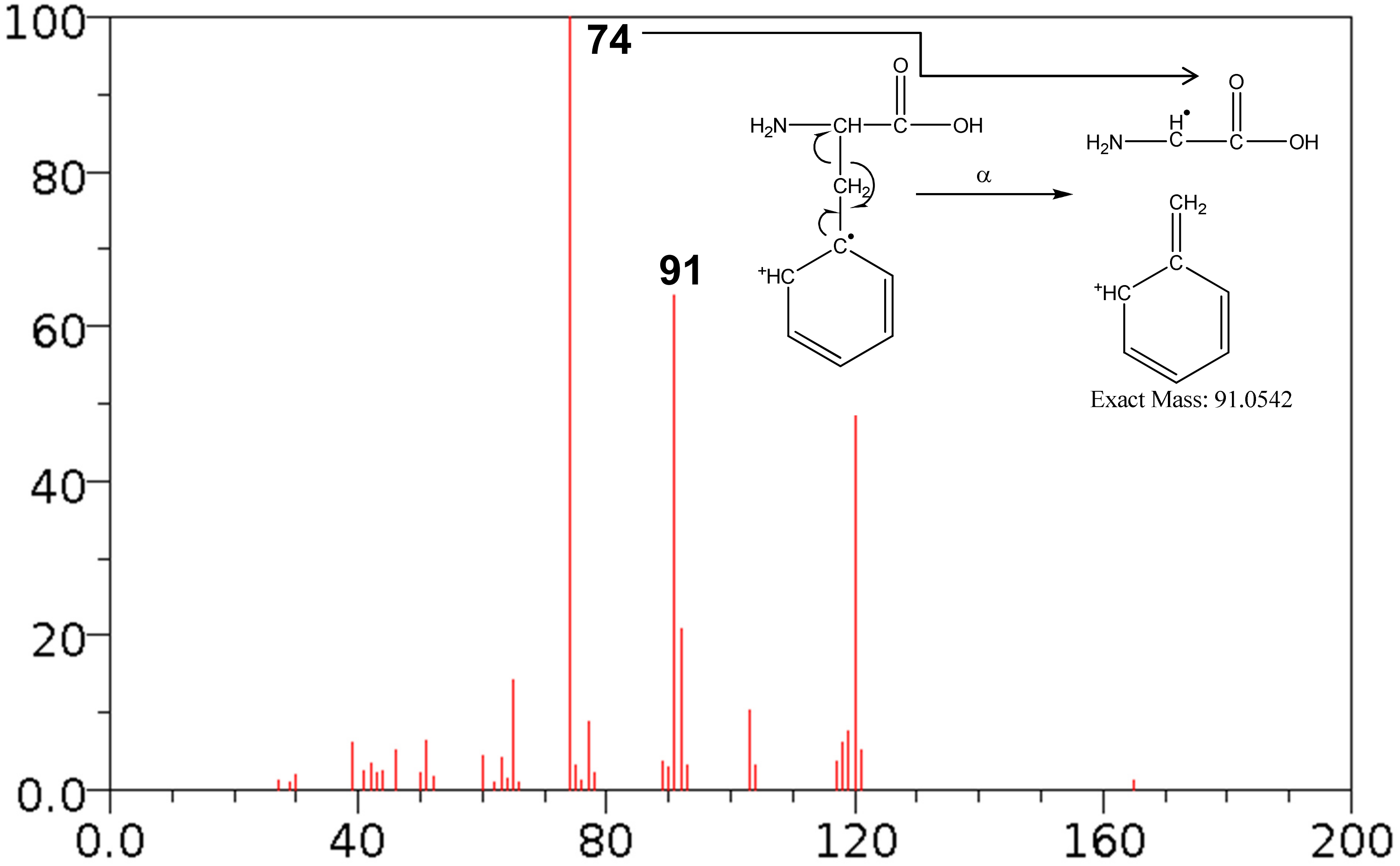
Example 2: benzylic cleavage of phenylalanine



Example 2: benzylic cleavage of phenylalanine

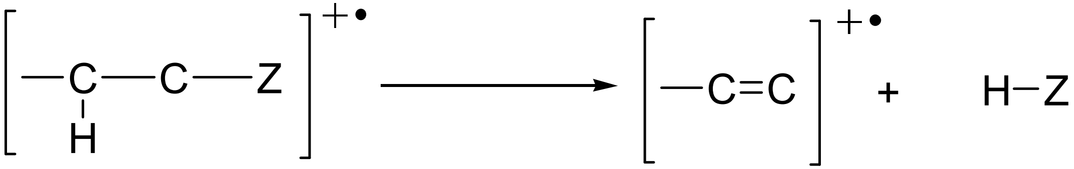


Example 2: benzylic cleavage of phenylalanine



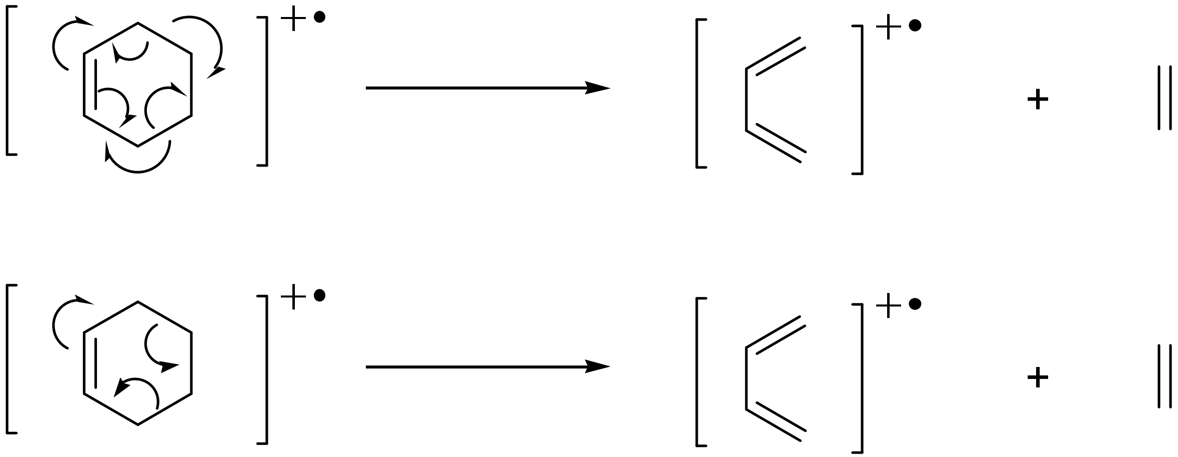
Multiple bonds cleavages

Elimination of a vicinal H and heteroatom:

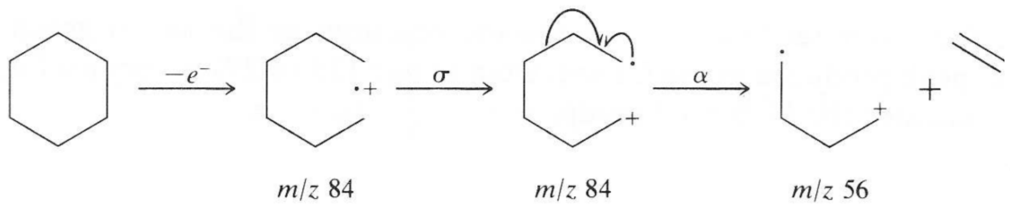


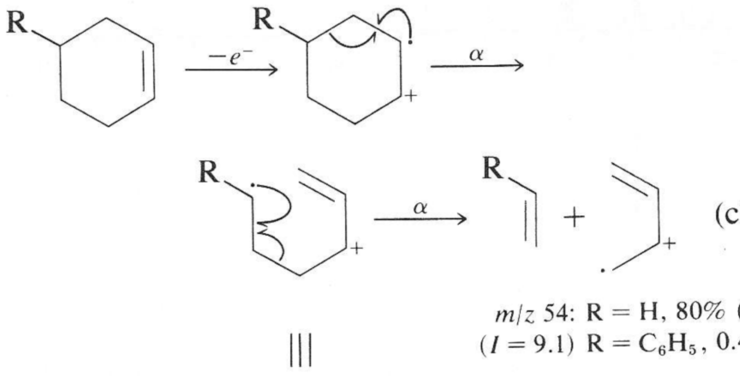
$$\left[-\underset{\mathrm{H}}{\underset{|}{\mathrm{C}}}-\mathrm{C}-\mathrm{Z}\right]^{+\bullet} \longrightarrow \left[-\mathrm{C}{=}\mathrm{C}\right]^{+\bullet} + \mathrm{H}-\mathrm{Z}$$

Retro-Diels-Alder



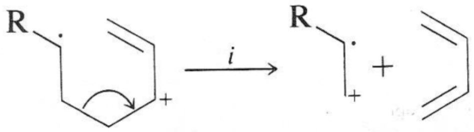
Multiple bonds cleavages, cyclic structures





(charge retention)

m/z 54: R = H, 80% ($C_5H_7^+$ = 100%)
(I = 9.1) R = C_6H_5, 0.4%



(charge migration)

R = H (I = 10.5), < 5%
R = C_6H_5 (I = 8.4), 100%

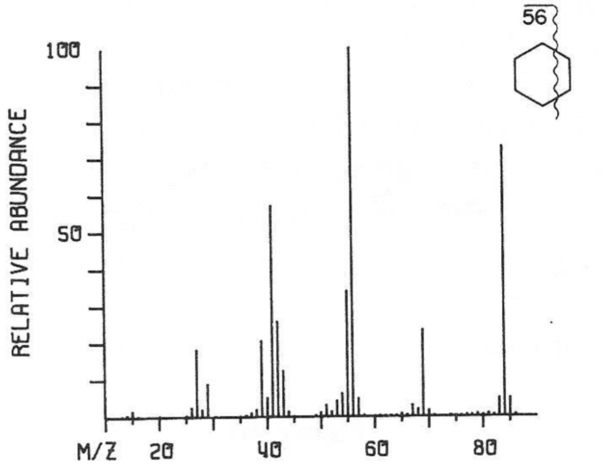
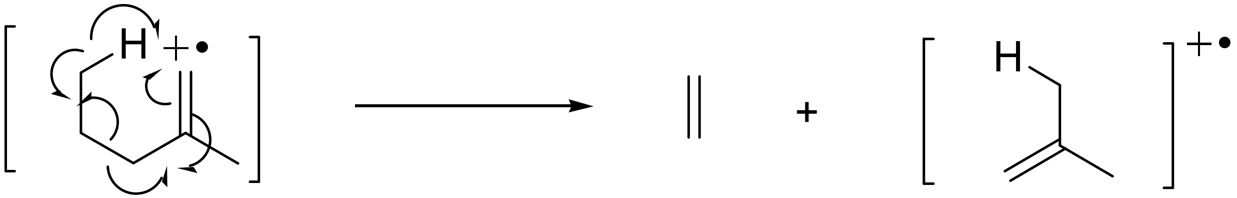


Figure 4F. Mass spectrum of cyclohexane.

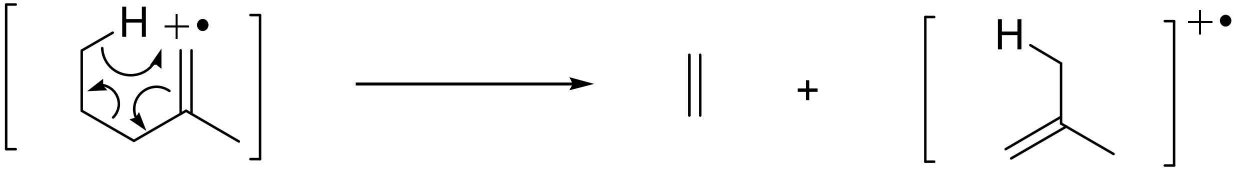
Multiple bonds cleavages

Two bond s-cleavages/rearrangements:
McLafferty Rearrangement

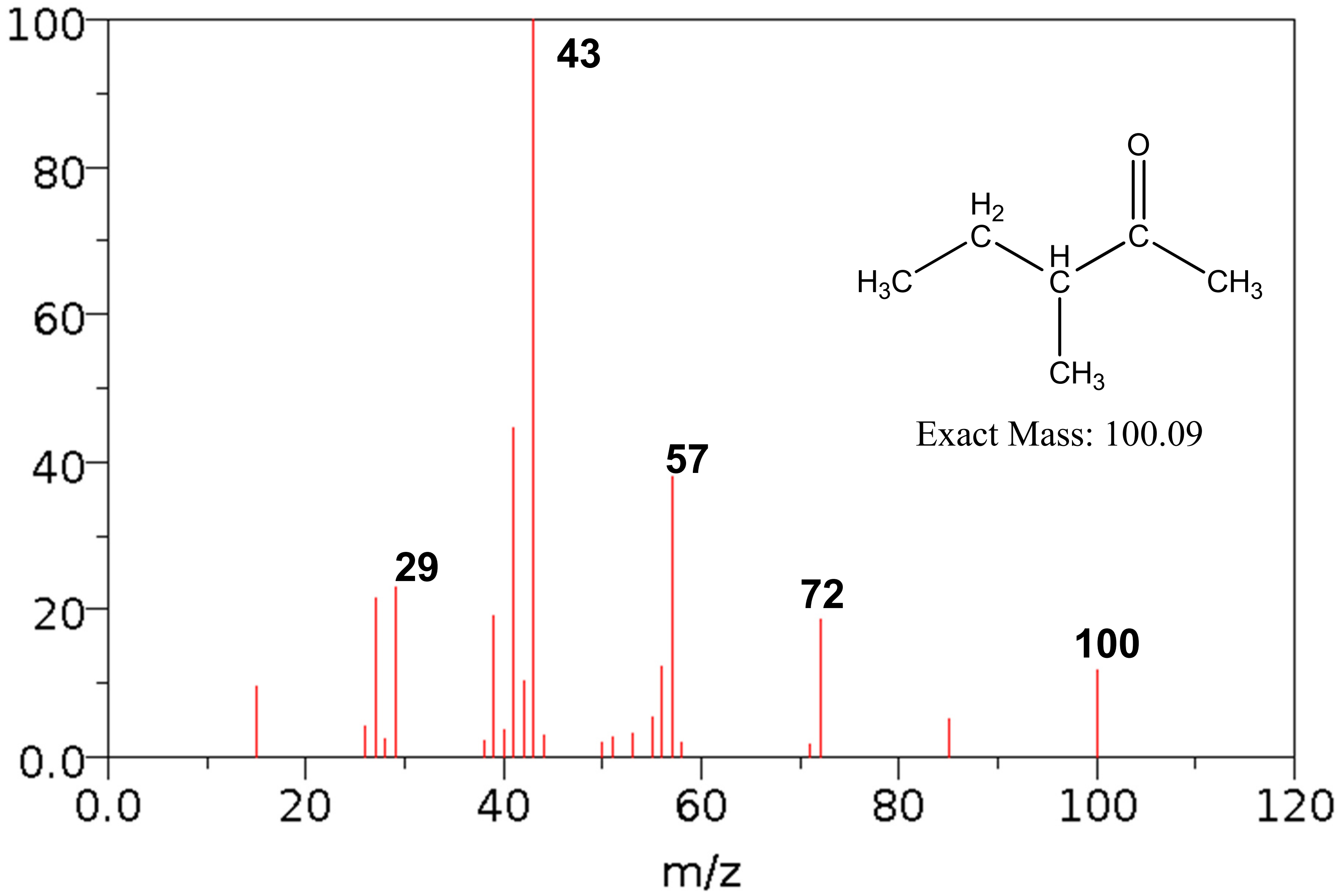
Full mechanism



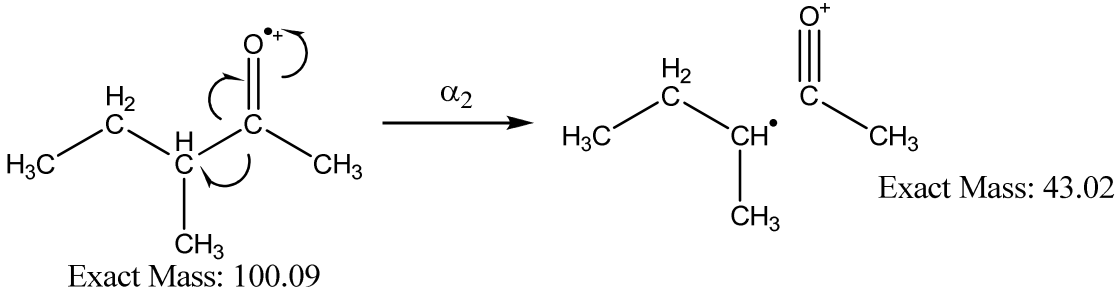
Abbreviated:

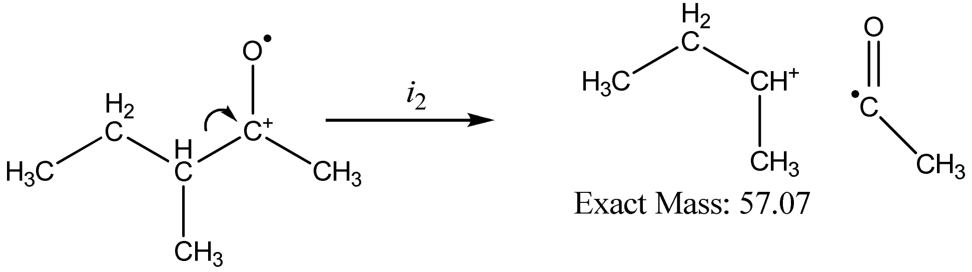


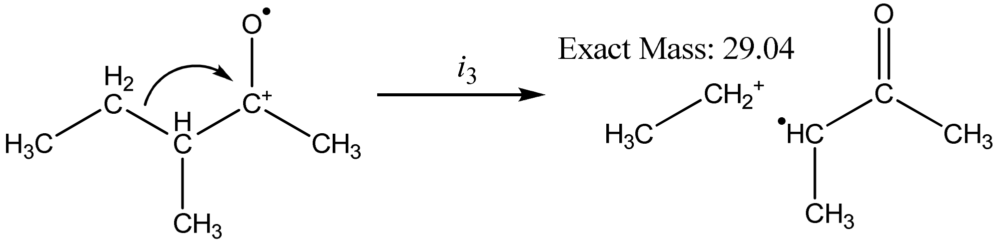
Example: EI spectrum of 3-methyl-2-pentanone



3-methyl-2-pentanone ions





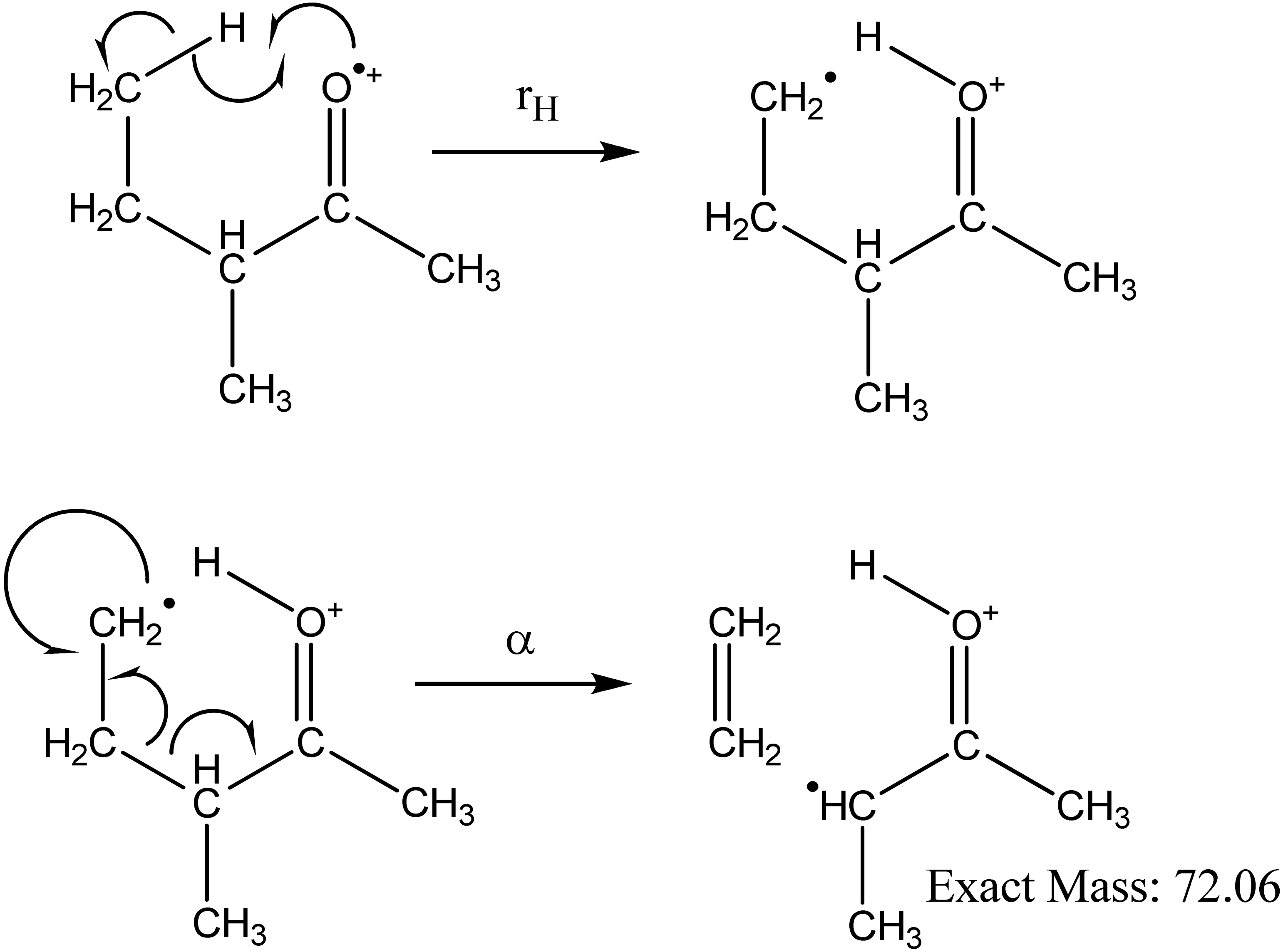


What about m/z 72?

McLafferty rearrangement

- 72 u fragment requires elimination of ethene
- A hydrogen on a carbon 4 atoms away from the carbonyl oxygen is transferred
  - The “1,5 shift” in carbonyl-containing ions is called the *McLafferty rearrangement*
  - Creates a distonic radical cation (charge and radical separate)
  - 6-membered intermediate is sterically favorable
  - Such rearrangements are common
- Once the rearrangement is complete, molecule can fragment by any previously described mechanism

McLafferty rearrangement



Exact Mass: 72.06

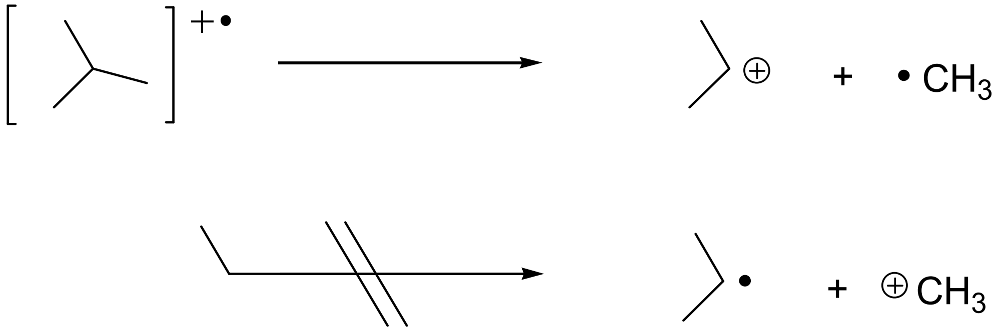
Examples: ***n*-alkanes**

a) Very predictable – apply the lessons of the stability of carbocations (or radicals) to predict or explain the observation of the fragments.

b) Method of fragmentation is single bond **σ-cleavage** in most cases.

c) This is governed by **Stevenson' s Rule** – the fragment with the lowest ionization energy will take on the + charge – the other fragment will still have an unpaired electron.

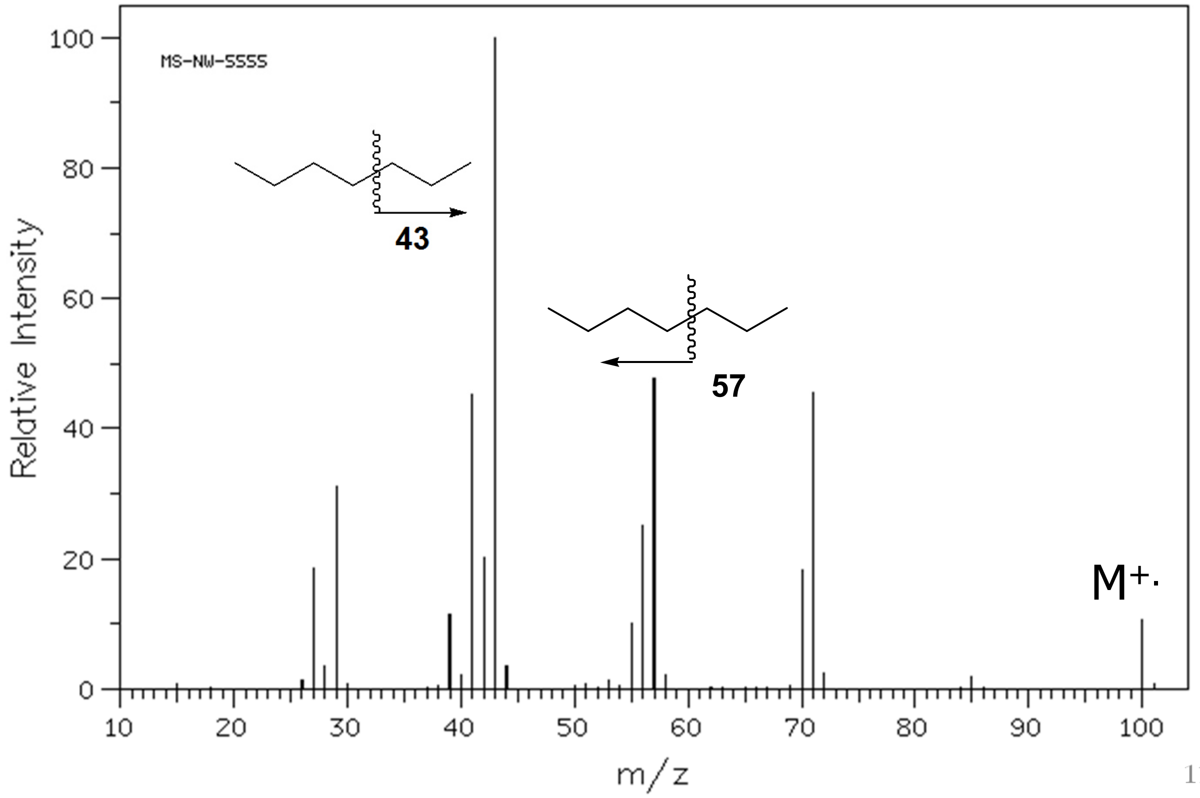
Example: *iso*-butane



Examples: ***n*-alkanes**

- For straight chain alkanes, a $M^{+\cdot}$ is often observed.
- Ions observed: clusters of peaks C_nH_{2n+1} apart from the loss of $–CH_3$, $-C_2H_5$, $-C_3H_7$ etc.
- Fragments lost: $^{\cdot}CH_3$, $^{\cdot}C_2H_5$, $^{\cdot}C_3H_7$ etc.
- In longer chains – peaks at 43 and 57 are the most common.

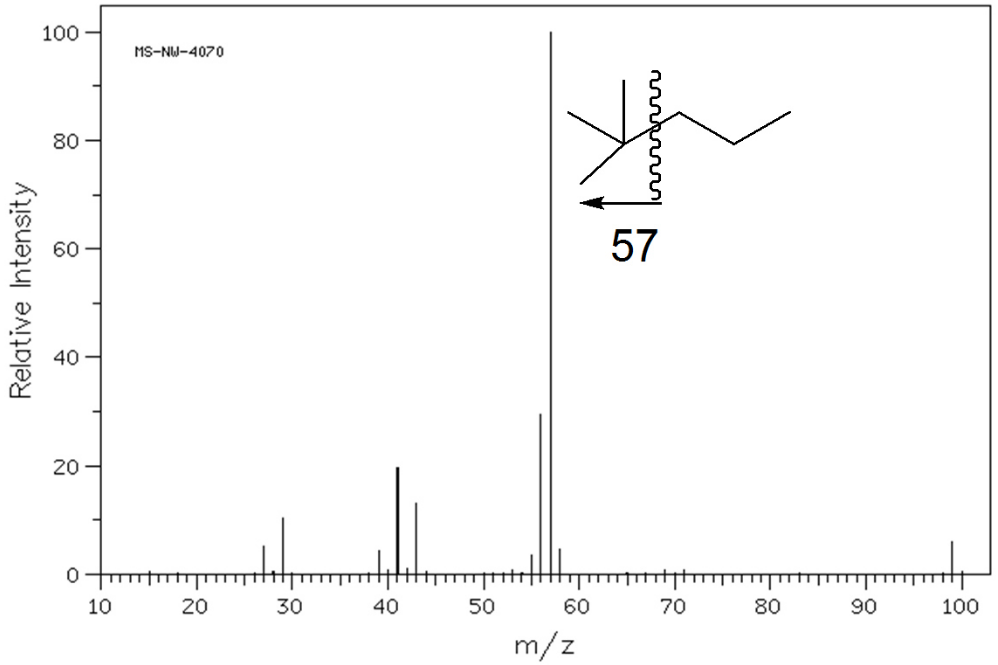
***n*-heptane**



Examples: **branched alkanes**

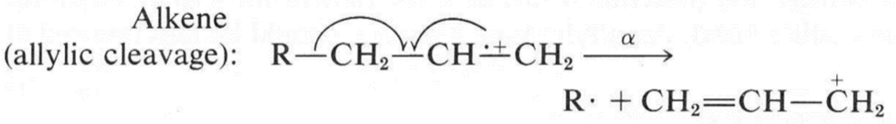
- Where the possibility of forming secondary or tertiary carbocations is high, the molecule is susceptible to fragmentation.
- Whereas in straight chain alkanes, a primary carbocation is always formed, its appearance is of lowered intensity in branched structures
- $M^{+\cdot}$ peaks become weak to non-existent as the size and branching of the molecule increase.
- Peaks at *m/z* 43 and 57 are the most common as these are *iso*-propyl and *tert*-butyl cations.

2,2-dimethylhexane

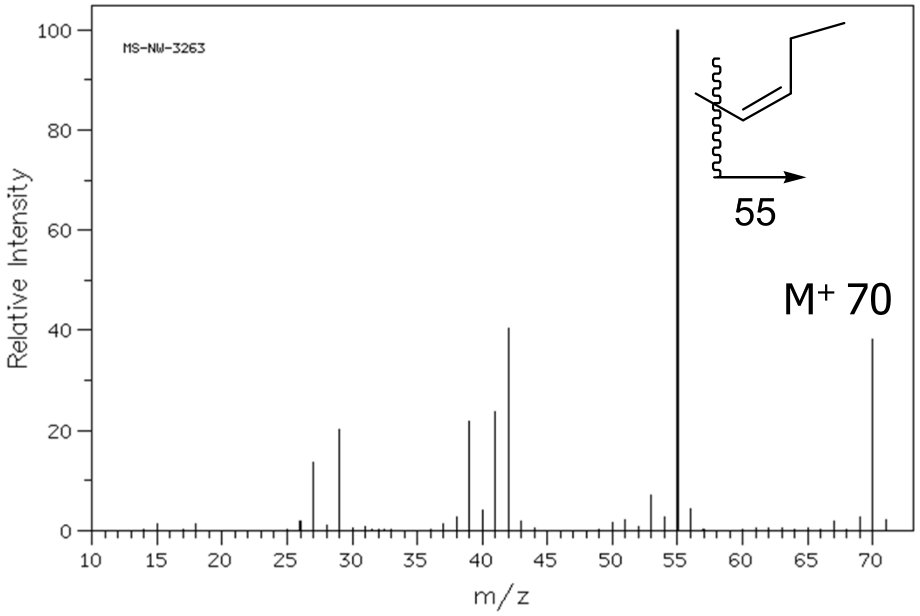


Examples: **alkenes**

- The π-bond of an alkene can absorb substantial energy – molecular ions are commonly observed
- After ionization, double bonds can migrate readily – determination of isomers is often not possible
- Ions observed: clusters of peaks C_nH_{2n-1} from **α-cleavages** apart from $-C_3H_5$, $-C_4H_7$, $-C_5H_9$ etc. at *m/z* 41, 55, 69, etc.
- Terminal alkenes readily form the allyl carbocation, *m/z* 41.

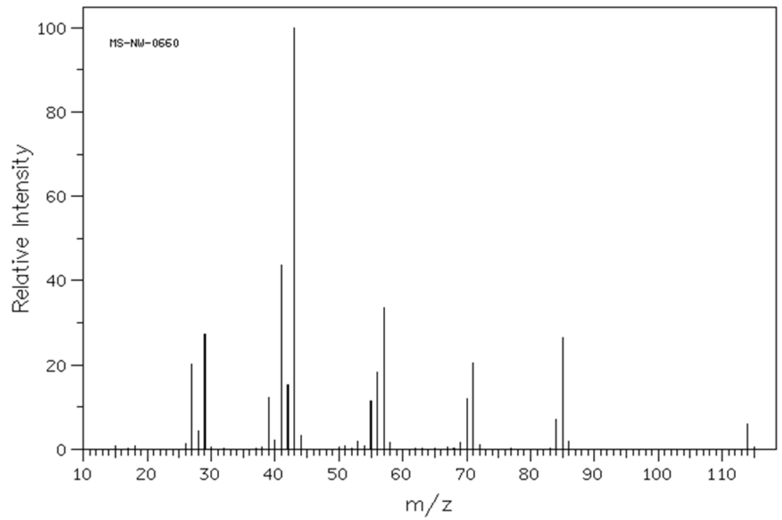


Alkene (allylic cleavage): $\text{R}{-}\text{CH}_2{-}\text{CH}\overset{\cdot+}{-}\text{CH}_2 \xrightarrow{\alpha} \text{R}\cdot + \text{CH}_2{=}\text{CH}{-}\overset{+}{\text{C}}\text{H}_2$



***cis-* 2-pentene**

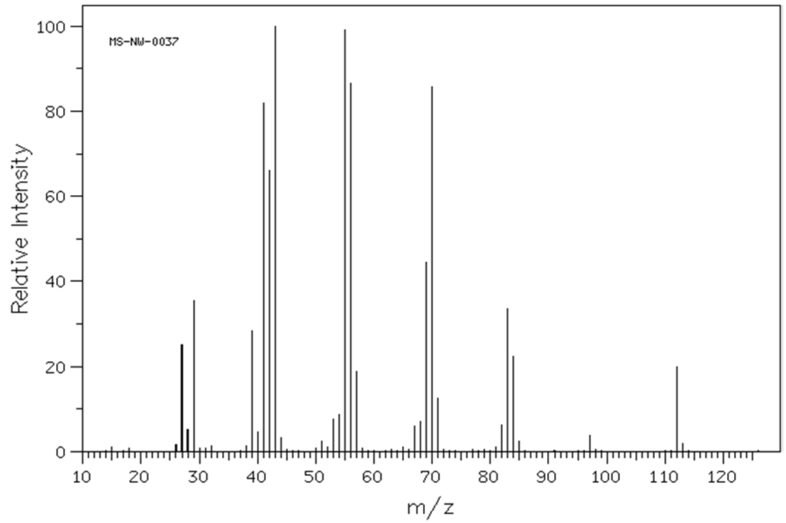
Examples: **alkanes *vs* alkenes**



Octane,

$M^{+\cdot}$ at m/z 114

m/z 85, 71, 57, 43 (base), 29

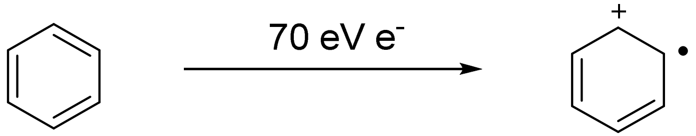


Octene
$M^{+\cdot}$ at *m/z* 112
(stronger than octane)

m/z 83, 69, 55, 41 (base), 29

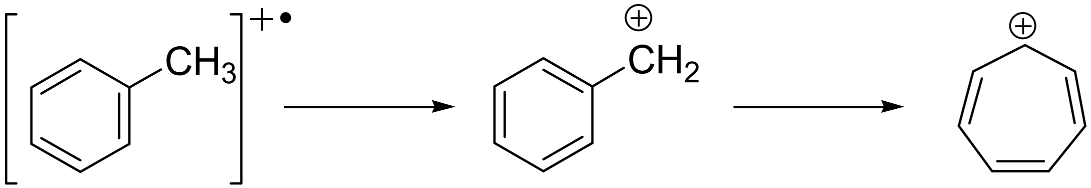
Examples: **aromatic hydrocarbones**

a) Very intense molecular ion peaks and limited fragmentation of the ring system are observed



b) Where alkyl groups are attached to the ring, a favorable mode of cleavage is to lose a H· radical to form the $C_7H_7^+$ ion (*m/z* 91)

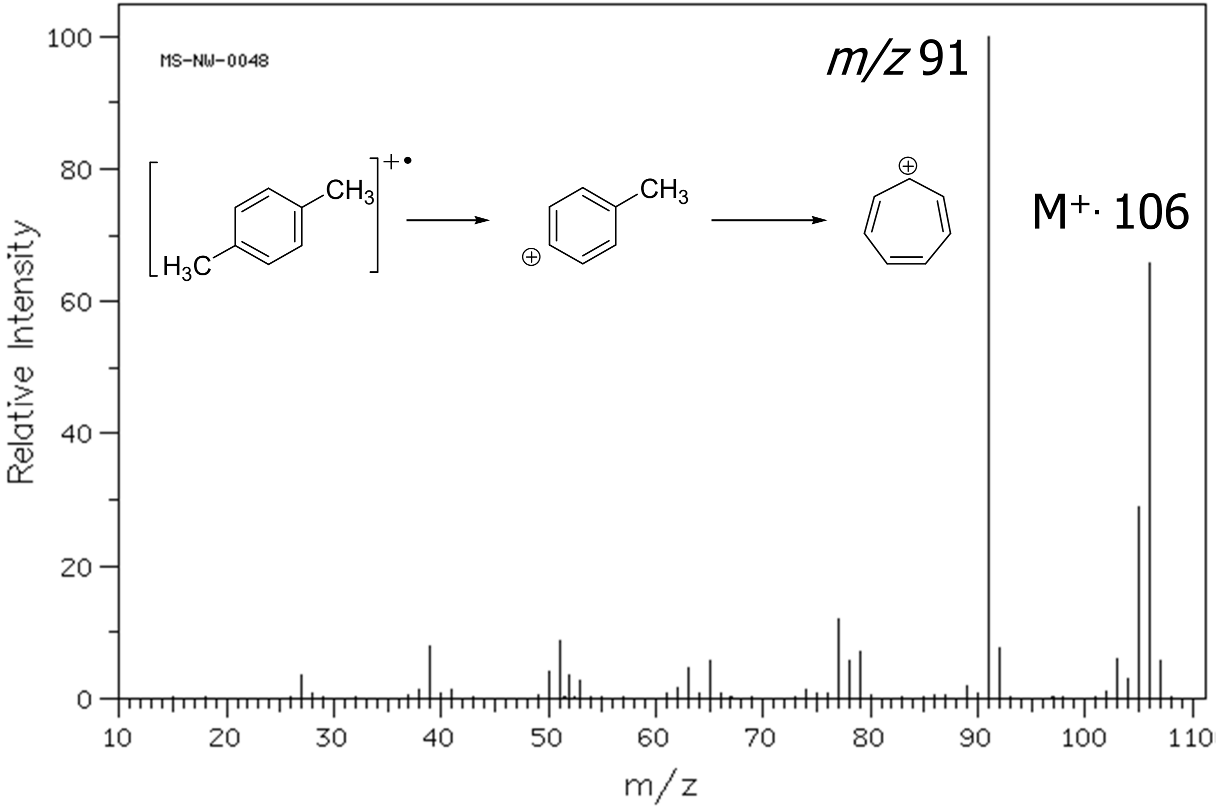
c) This ion is believed to be the tropylium ion; formed from rearrangement of the benzyl cation



Examples: **aromatic hydrocarbones**

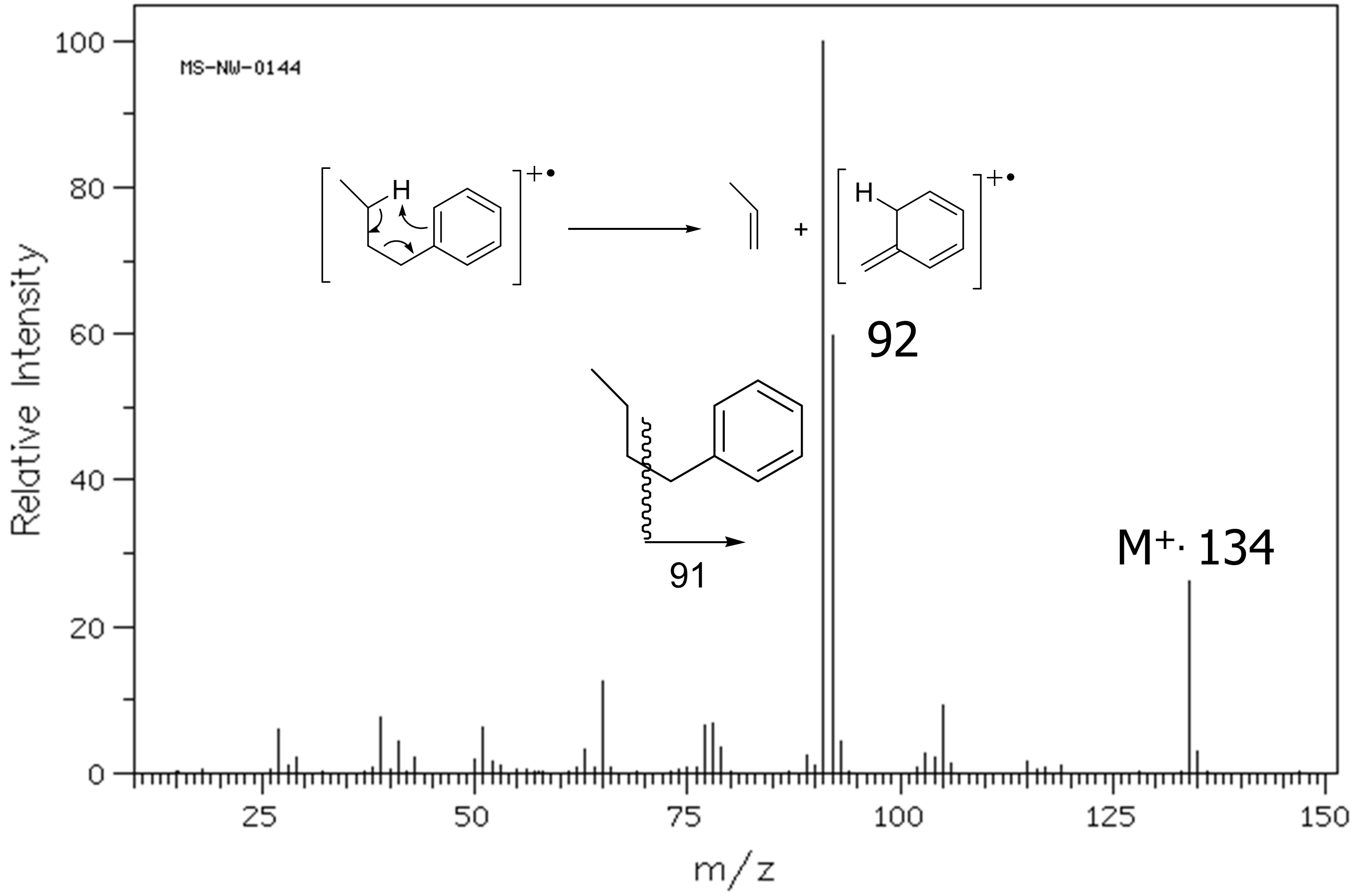
d) If a chain from the aromatic ring is sufficiently long, a McLafferty rearrangement is possible

e) Substitution patterns for aromatic rings are able to be determined by MS – with the exception of groups that have other ion chemistry



***p*-xylene**

Examples: **aromatic hydrocarbones**



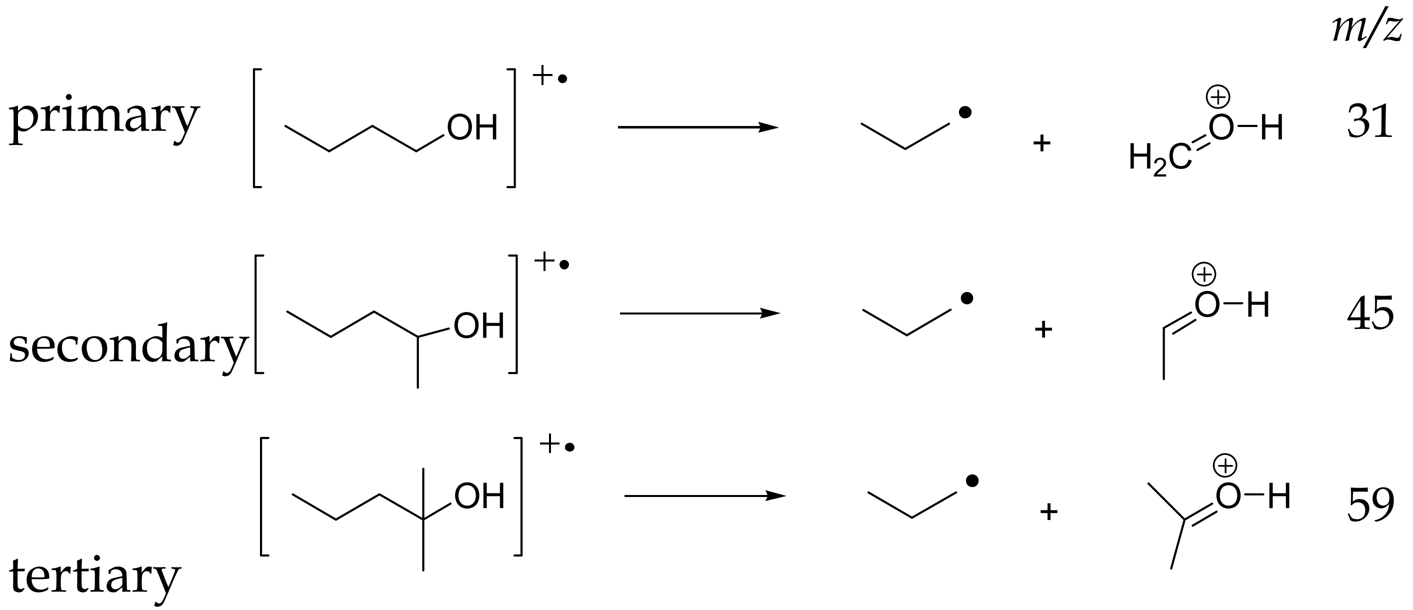
n **-butylbenzene**

Examples: **alcohols**

a) Additional modes of fragmentation will cause lower $M^{+\cdot}$ than for the corresponding alkanes

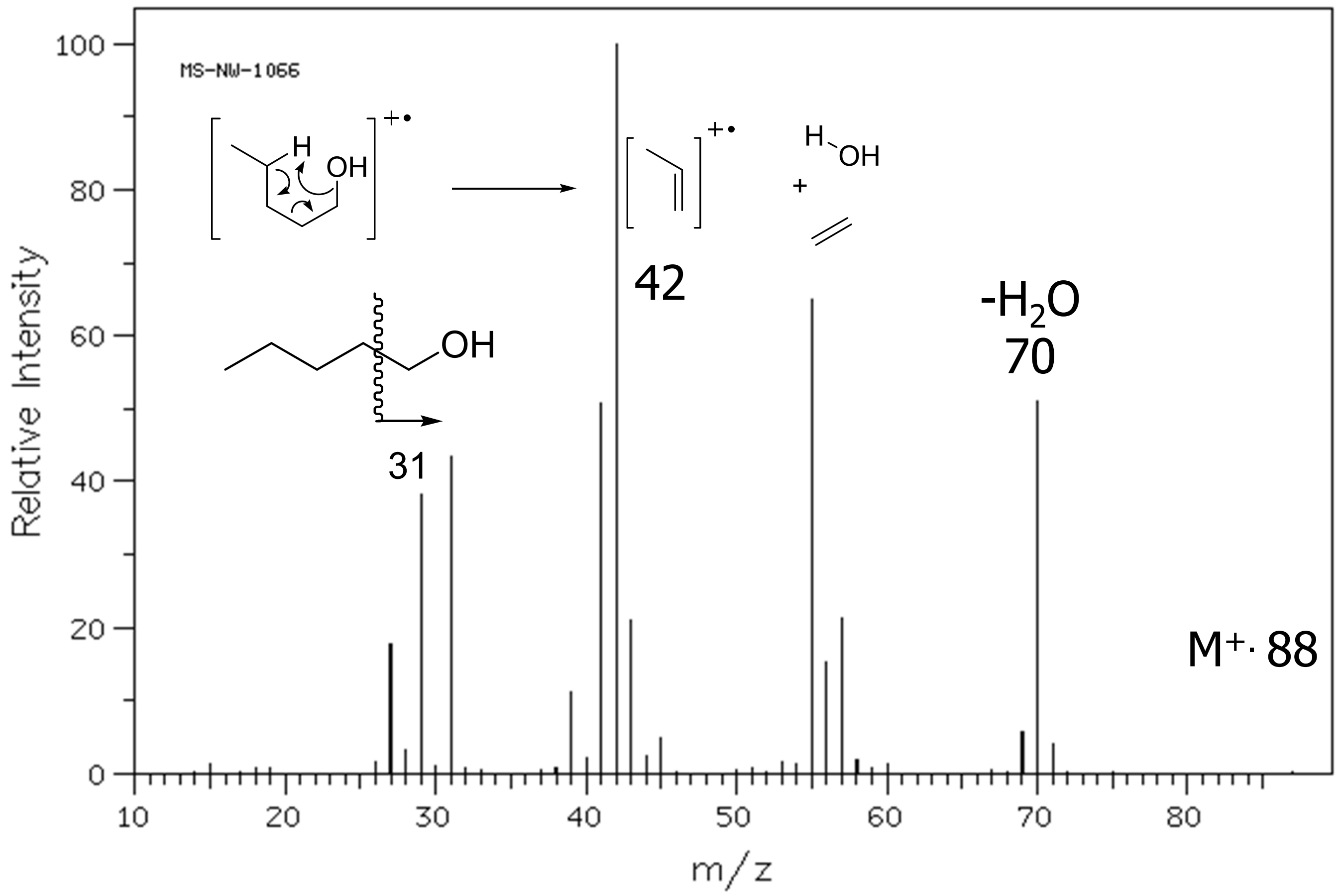
primary and secondary alcohols have a low $M^{+\cdot}$, tertiary may be absent

b) The largest alkyl group is usually lost **via α-cleavage**; the mode of cleavage typically is similar for all alcohols:



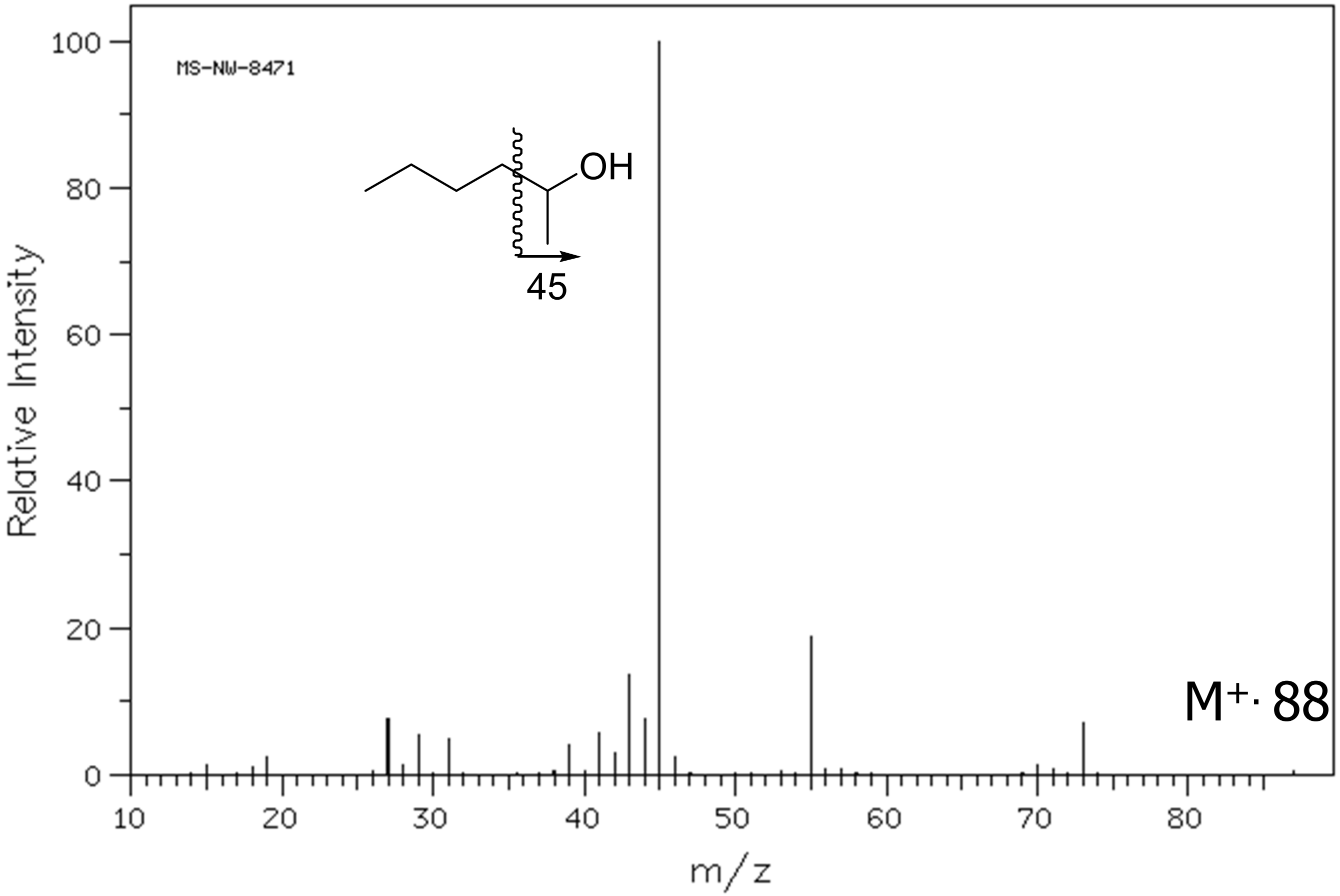
Examples: **primary alcohols**

n **-pentanol**



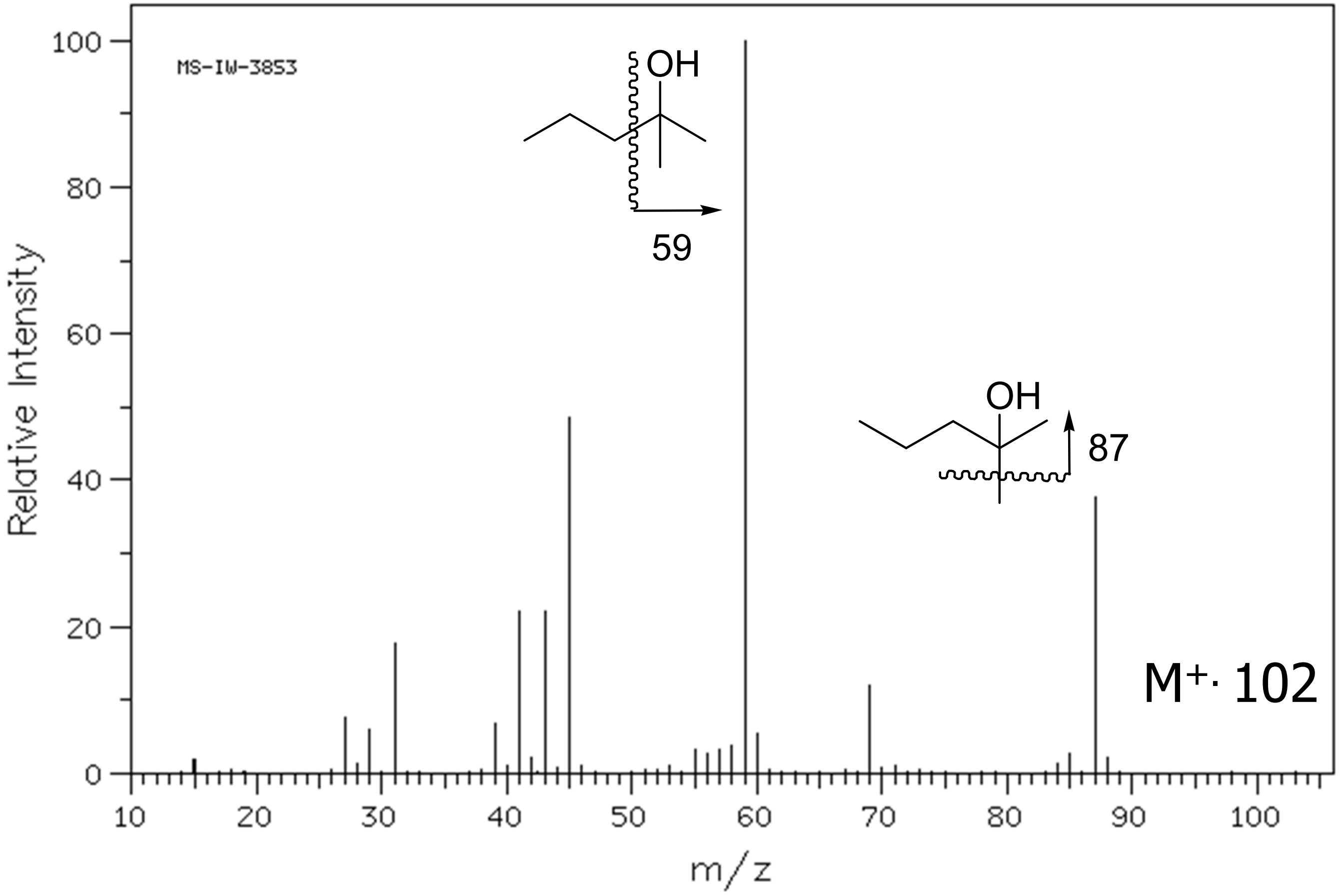
Examples: **secondary alcohols**

2-pentanol



Examples: **tertiary alcohols**

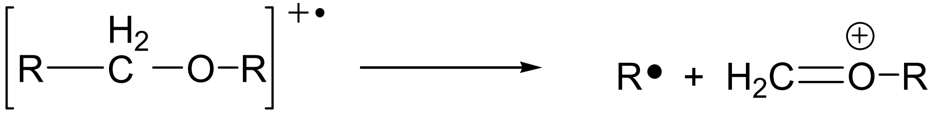
2-methyl-2-pentanol



Examples: **ethers**

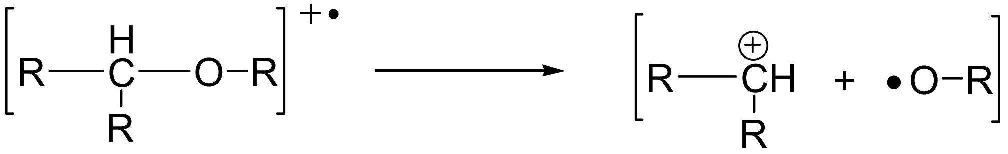
a) Slightly more intense $M^{+\cdot}$ than for the corresponding alcohols or alkanes

b) The largest alkyl group is usually lost to **a-cleavage**; the mode of cleavage typically is similar to alcohols:



$$\left[R-\overset{H_2}{C}-O-R\right]^{+\bullet} \longrightarrow R^{\bullet} + H_2C=\overset{\oplus}{O}-R$$

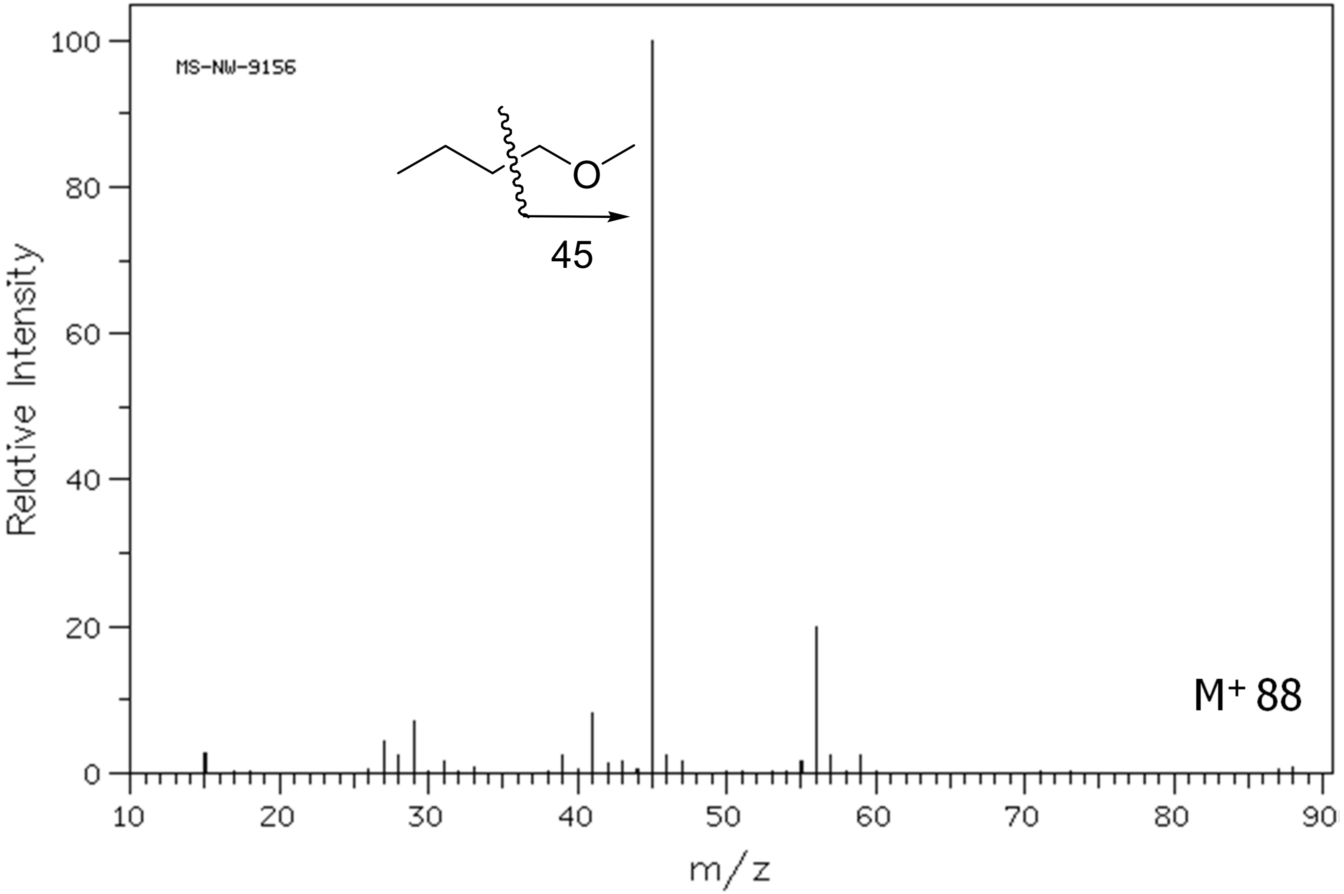
c) Cleavage of the C-O bond to give carbocations is observed where favorable



$$\left[R-\underset{R}{\overset{H}{C}}-O-R\right]^{+\bullet} \longrightarrow \left[R-\underset{R}{\overset{\oplus}{C}H} + {}^{\bullet}O-R\right]$$

Examples: **ethers**

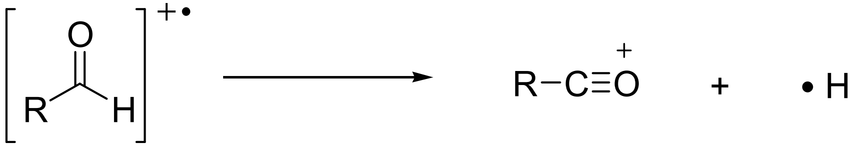
butyl methyl ether



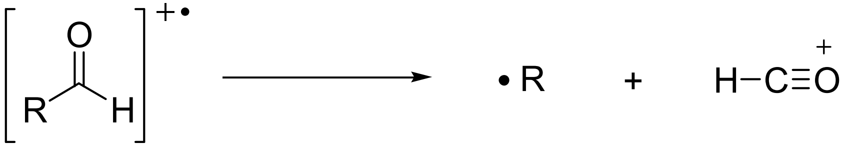
Examples: **aldehydes**

a) Weak $M^{+\cdot}$ for aliphatic, strong $M^{+\cdot}$ for aromatic aldehydes

b) **a-cleavage** is characteristic and often diagnostic for aldehydes – can occur on either side of the carbonyl

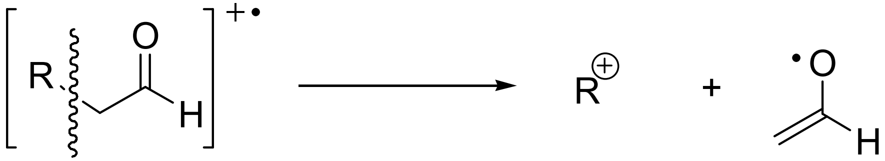


M-1 peak



m/z 29

c) β-cleavage is an additional mode of fragmentation

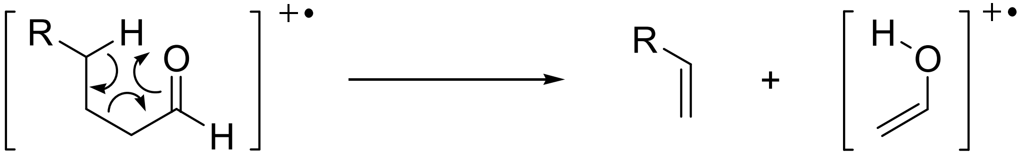


m/z R^+

M - 41

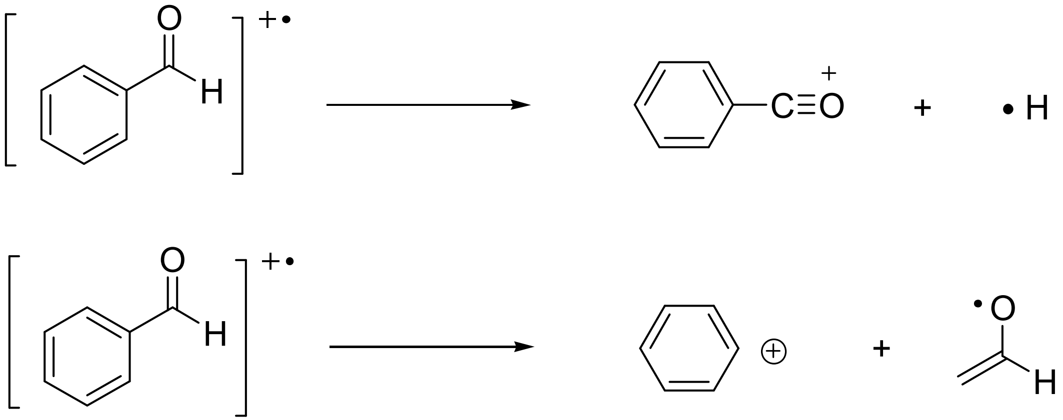
Examples: **aldehydes**

d) McLafferty rearrangement observed if γ-H present



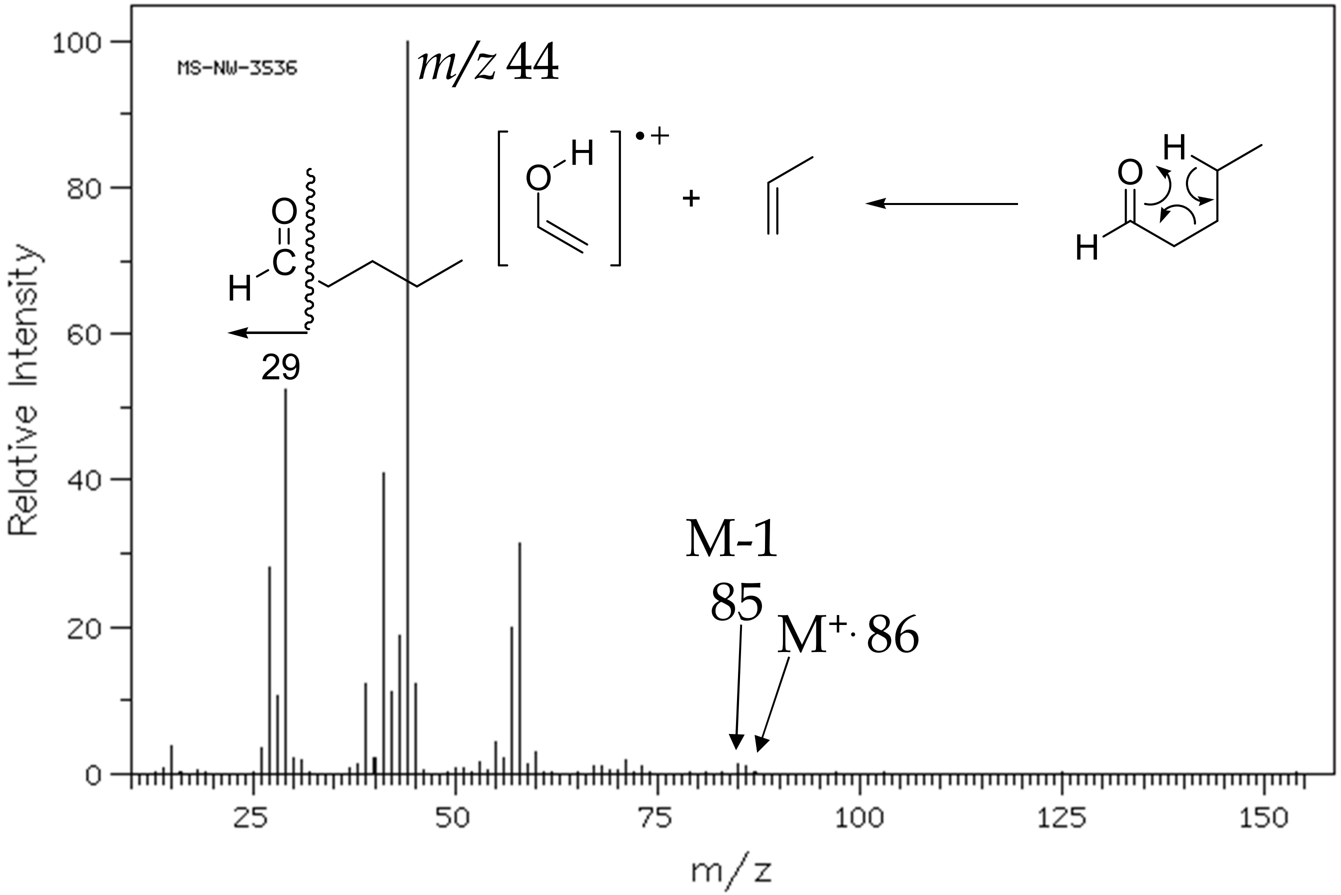
m/z 44

e) Aromatic aldehydes – a-cleavages are more favorable, both to lose H· (M - 1) and HCO · (M – 29)



Examples: **aldehydes**

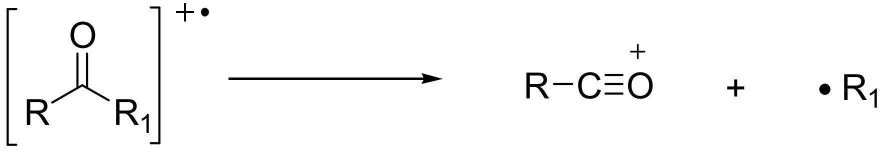
pentanal



Examples: **ketones**

a) Strong $M^{+\cdot}$ for aliphatic and aromatic ketones

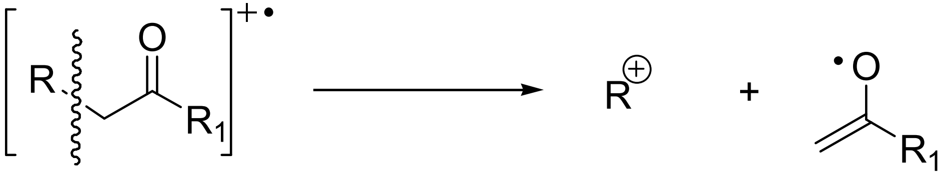
b) **a-cleavage** can occur on either side of the carbonyl group – the larger alkyl group is lost more often



M – 15, 29, 43…
m/z 43, 58, 72, etc.

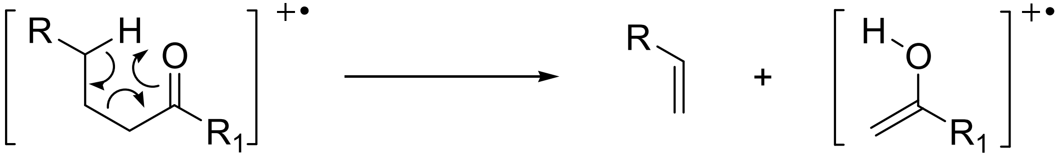
R_1 is larger than R

c) β-cleavage is not as important of a fragmentation mode for ketones compared to aldehydes – but sometimes observed

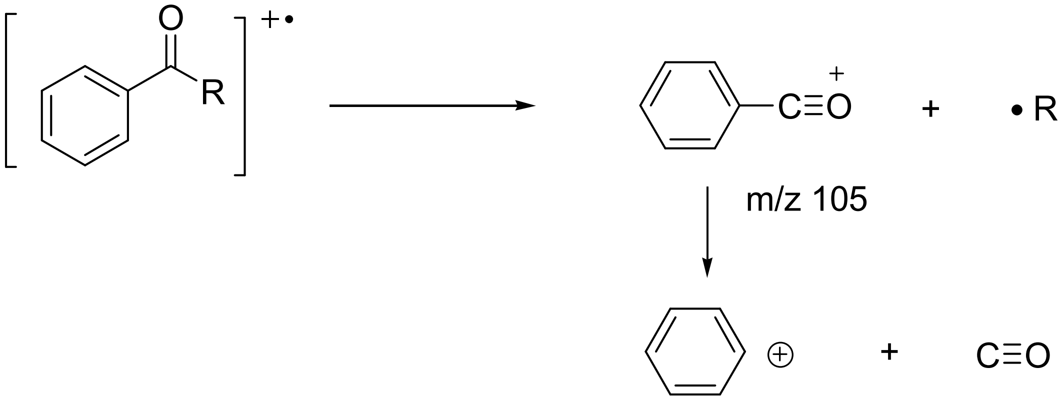


Examples: **ketones**

d) McLafferty rearrangement observed if γ-H present – if both alkyl chains are sufficiently long – both can be observed



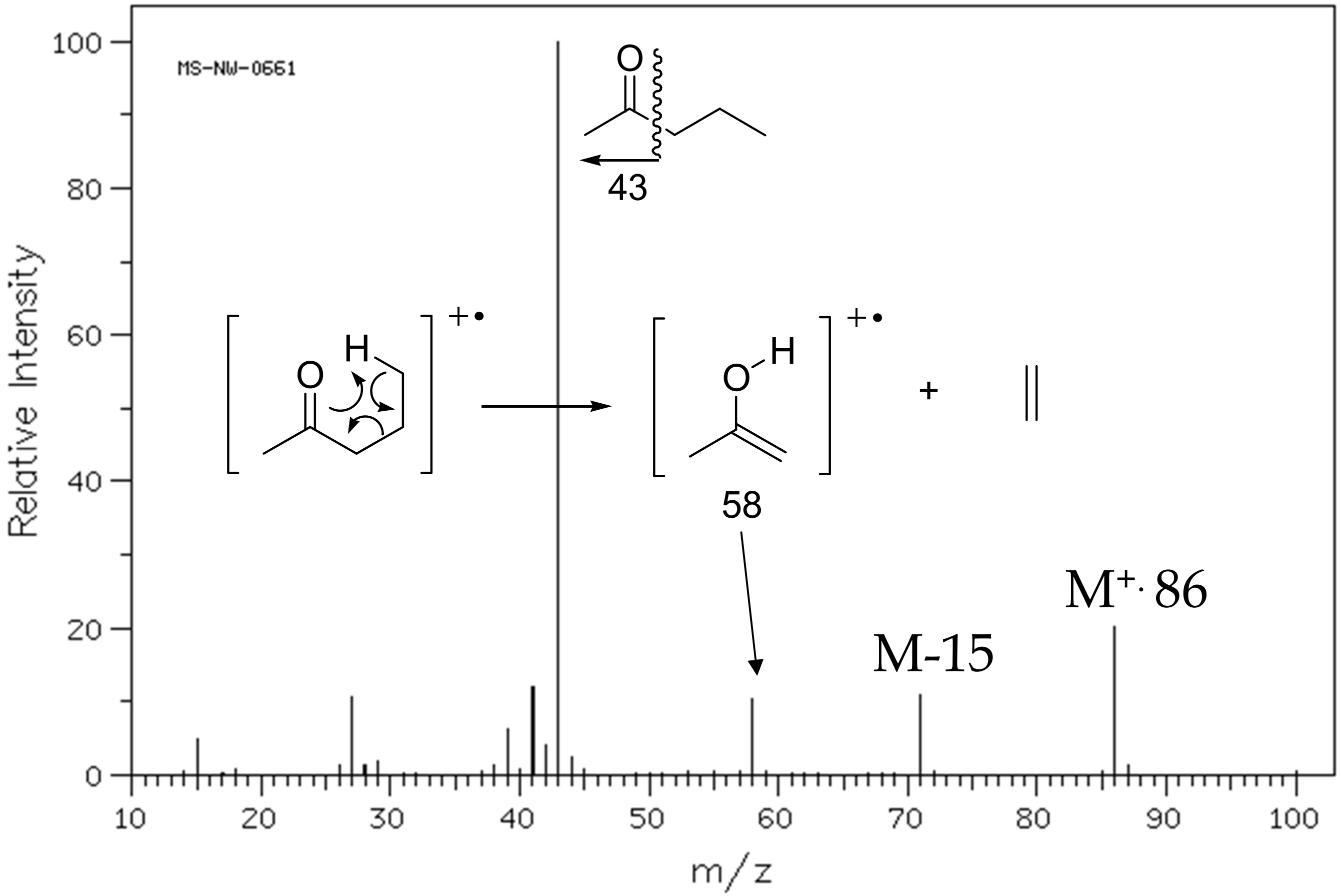
e) Aromatic ketones – α-cleavages are favorable primarily to lose R · (M – 15, 29…) to form the $C_6H_5CO^+$ ion, which can lose C≡O



m/z 77

Examples: **ketones**

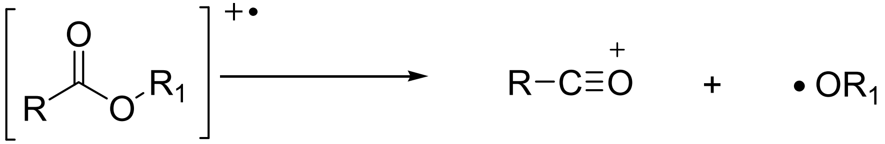
2-pentanone



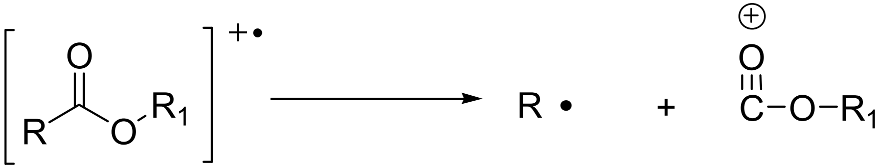
Examples: **esters**

a) $M^{+\cdot}$ weak in most cases, aromatic esters give a stronger peak

b) Most important α-cleavage reactions involve loss of the alkoxy-radical to leave the acylium ion

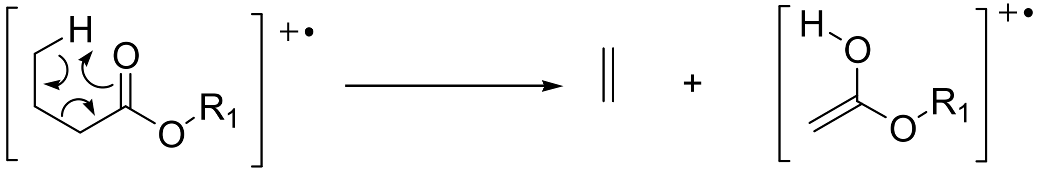


c) The other α-cleavage (most common with methyl esters, *m/z* 59) involves the loss of the alkyl group

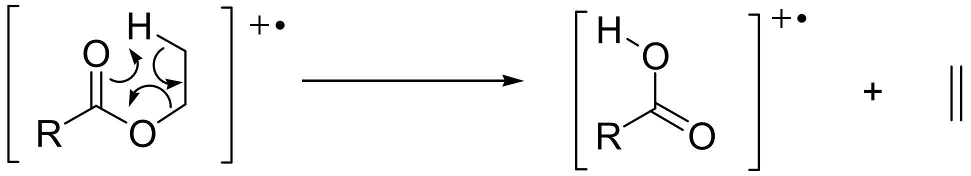


Examples: **esters**

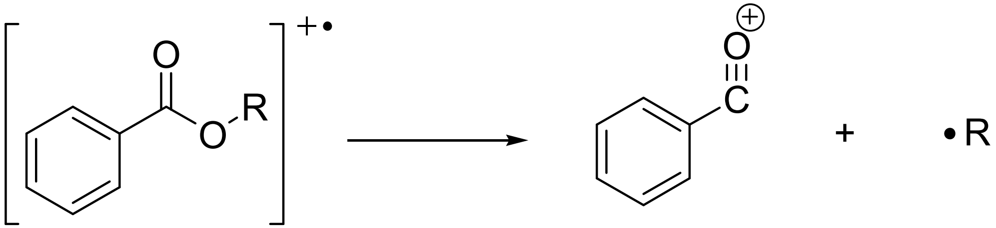
d) McLafferty occurs with sufficiently long esters



e) Ethyl and longer (alkoxy chain) esters can also undergo the McLafferty rearrangement

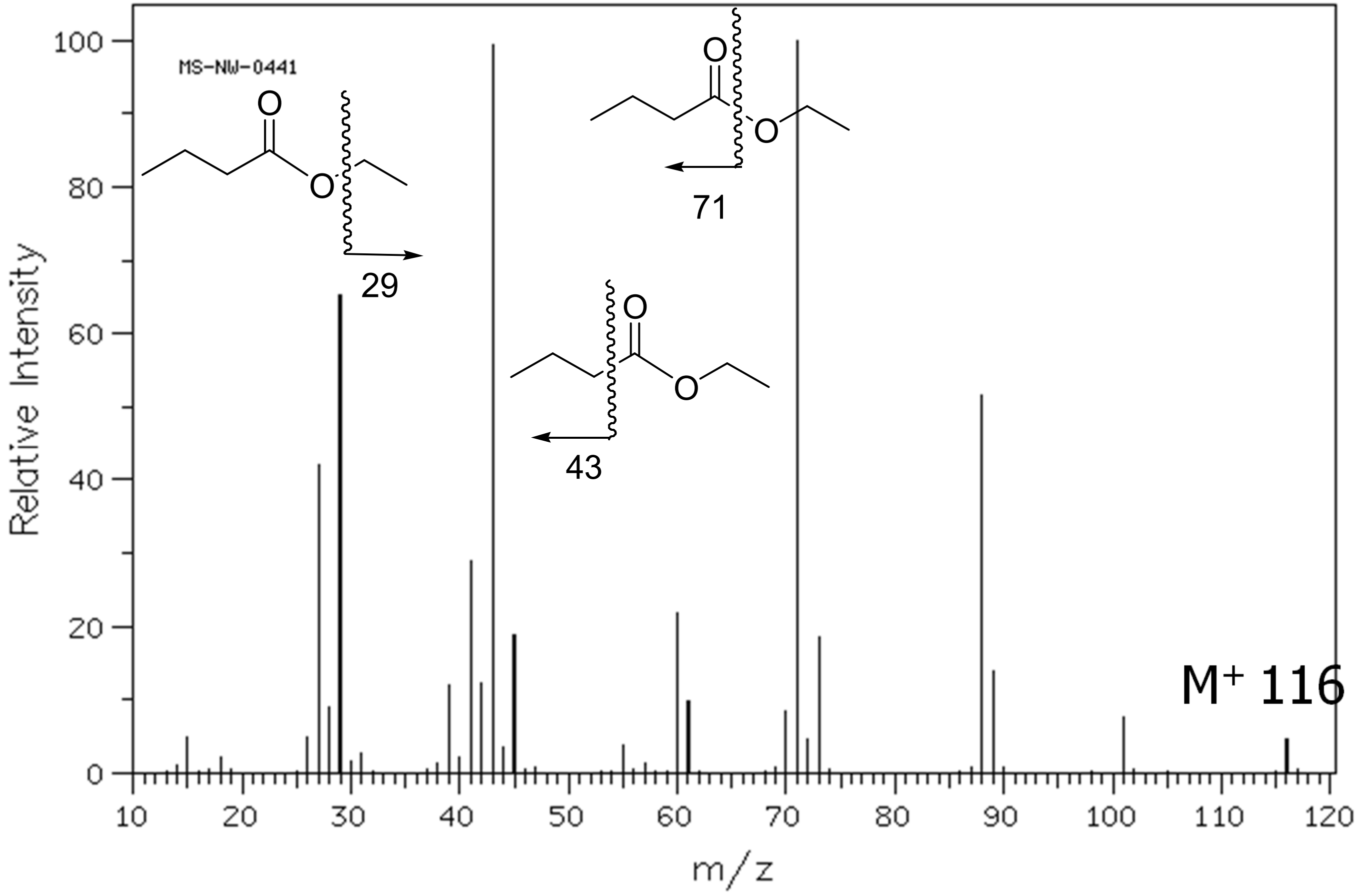


f) The most common fragmentation route is to lose the alkyl group by α-cleavage, to form the $C_6H_5CO^+$ ion (*m/z* 105)



Examples: **esters**

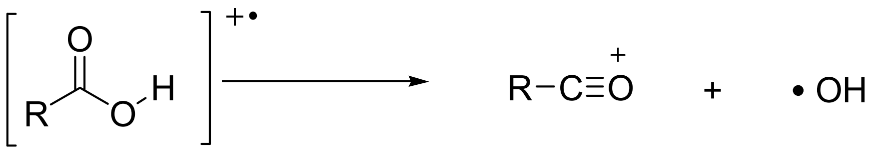
ethyl butyrate



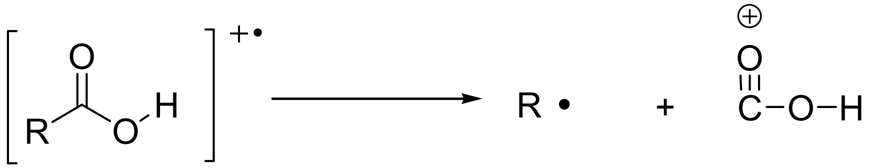
Examples: **carboxylic acids**

a) As with esters, $M^{+\cdot}$ weak in most cases, aromatic acids give a stronger peak

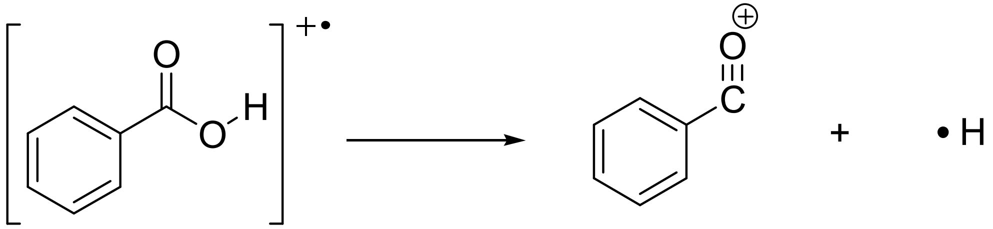
b) Most important α-cleavage reactions involve loss of the hydroxyl-radical to leave the acylium ion



c) The other α-cleavage (less common) involves the loss of the alkyl radical. Although less common, the *m/z* 45 peak is somewhat diagnostic for acids.



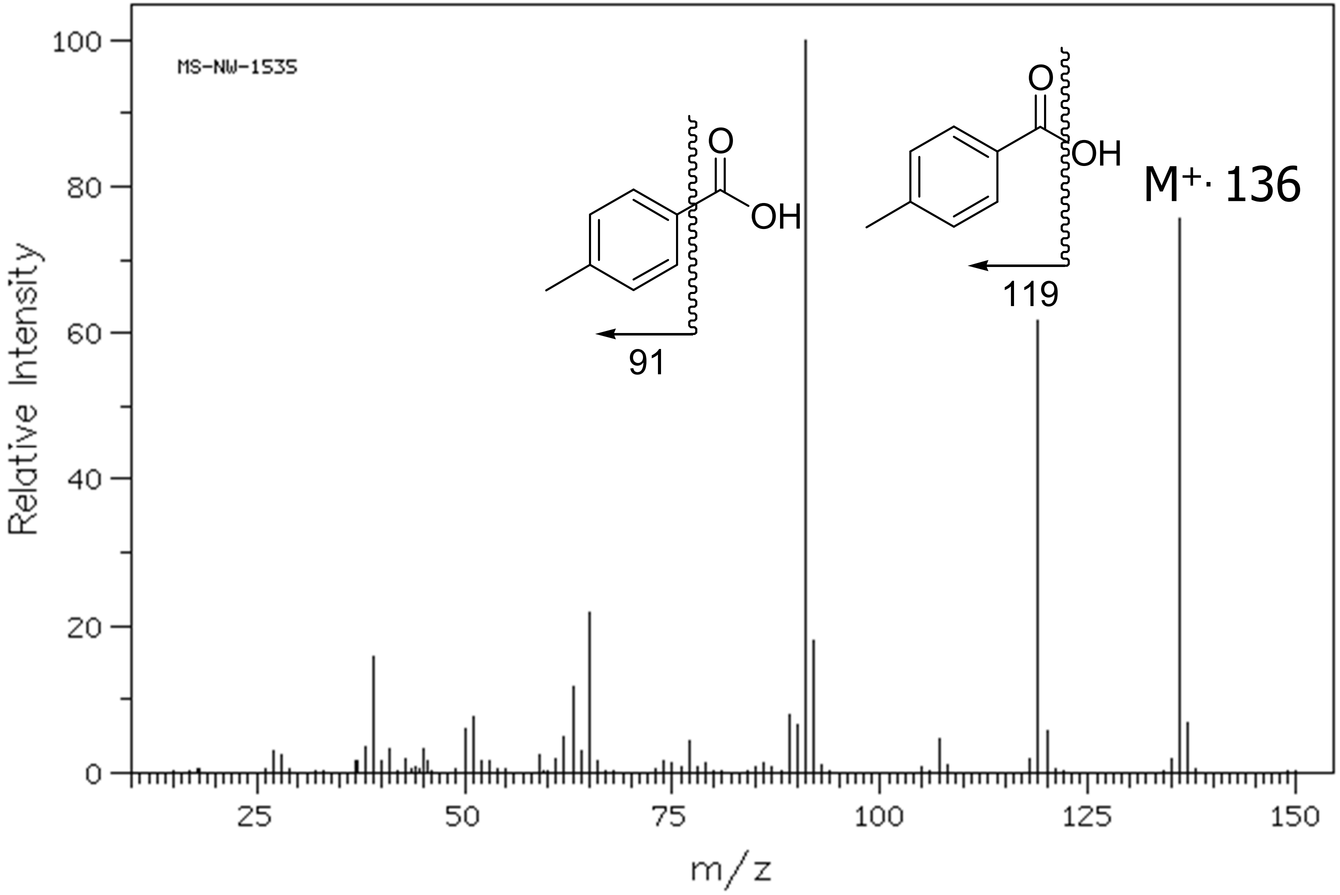
d) McLafferty occurs with sufficiently long acids



+ further loss of CO to m/z 77

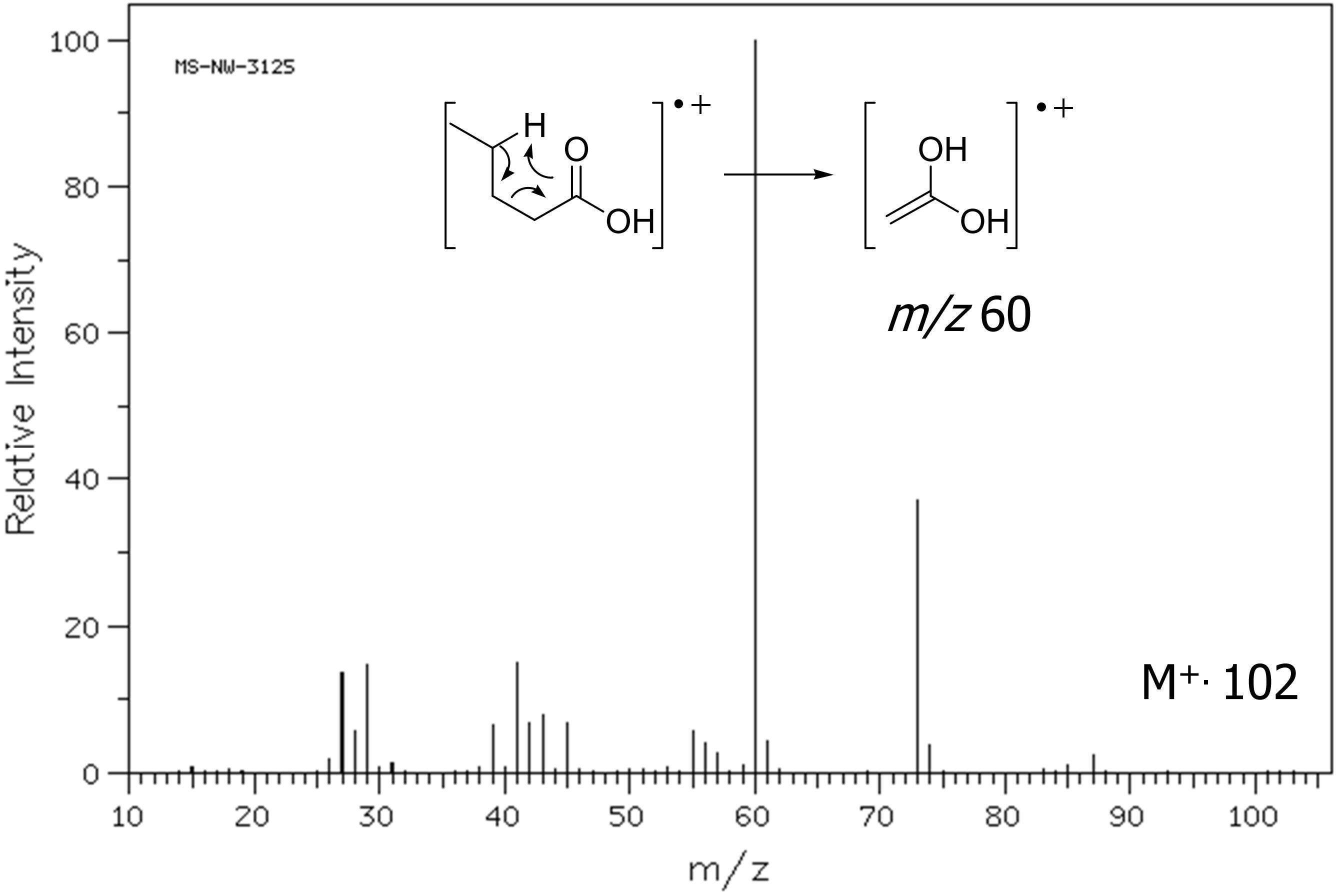
Examples: **carboxylic acids**

***p*-toluic acid**



Examples: **carboxylic acids**

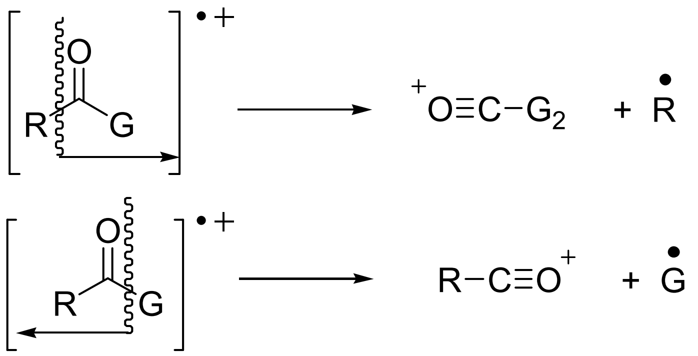
pentanoic acid



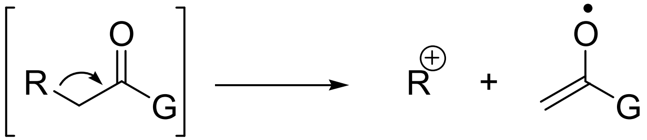
Summary for carbonyl compounds

There are 4 common modes of fragmentation:

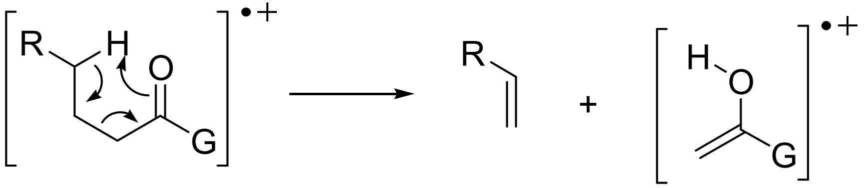
- **A_1 & A_2** → two α-cleavages



- **B** → β-cleavage



- **C** → McLafferty rearrangement



Summary for carbonyl compounds

Summary as table:

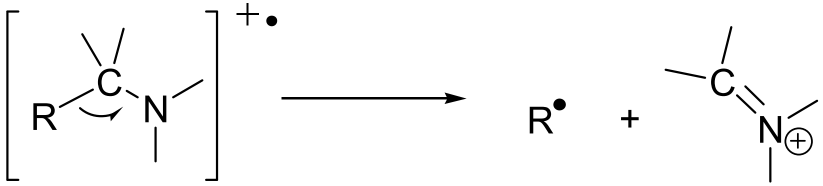
		m/z of ion observed				
Fragmentation	Path	Aldehydes G = H	Ketones G = R	Esters G = OR’	Acids G = OH	Amides G = NH_2
A$_1$ α-cleavage	- R	**29**	43[b]	59[b]	**45**	44[d]
A$_2$ α-cleavage	- G	43[b]	43[b]	43[b]	43[b]	43[b]
B β-cleavage	- G	43[a]	57[b]	73[b]	59[a]	58[a]
C McLafferty		44[a]	58[b,c]	74[b,c]	60[a]	59[a]

[b] = *base,* add other mass attached to this chain
[a] = *base,* if α-carbon branched, add appropriate mass
[c] = sufficiently long structures can undergo on either side of C=O
[d] = if *N*-substituted, add appropriate mass

Examples: **amines**

a) Follow nitrogen rule – odd $M^{+\cdot}$, odd # of nitrogens; nonetheless, $M^{+\cdot}$ weak in aliphatic amines

b) α-cleavage reactions are the most important fragmentations for amines; for primary *n*-aliphatic amines *m/z* 30 is diagnostic

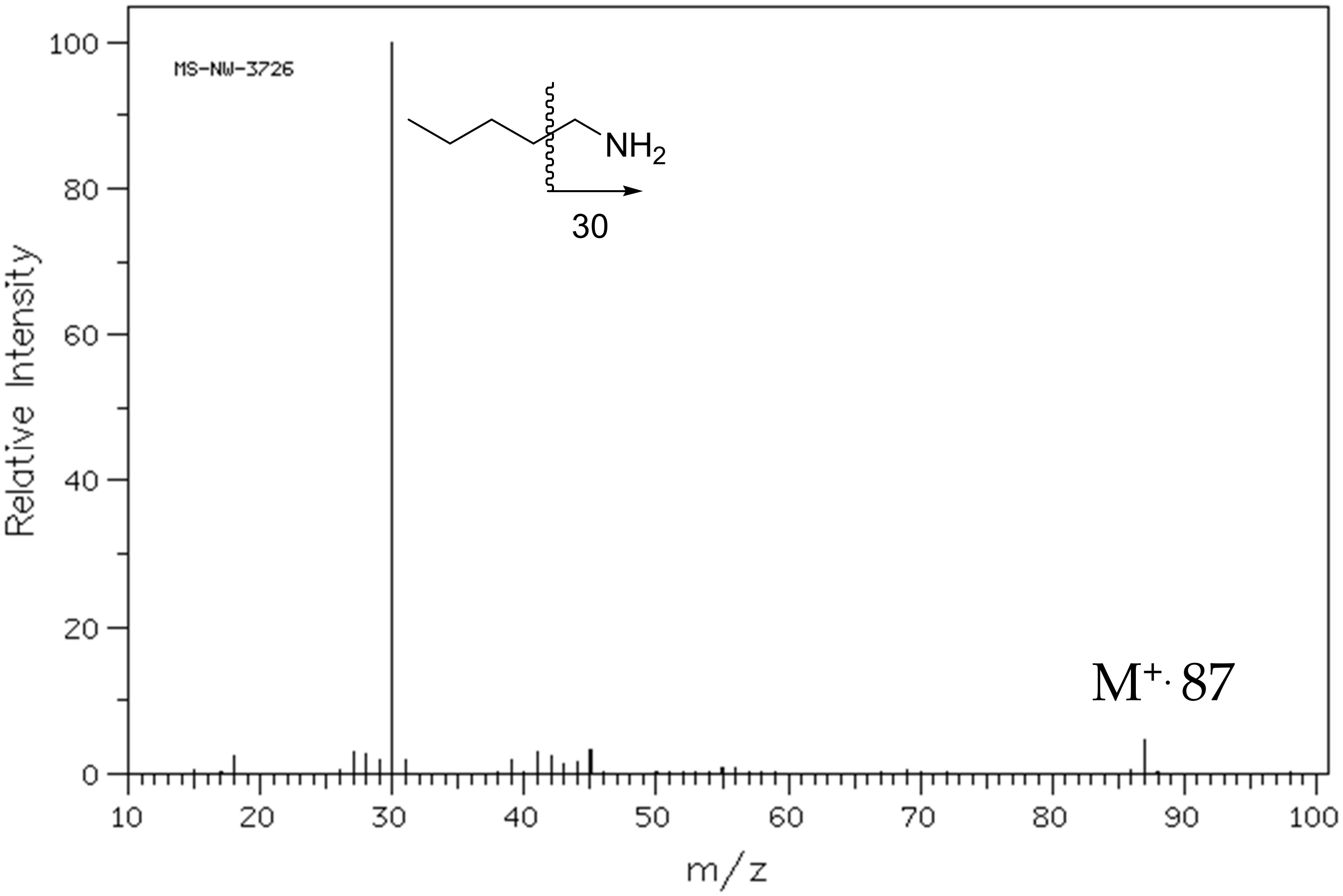


c) McLafferty not often observed with amines, even with sufficiently long alkyl chains

d) Loss of ammonia (M – 17) is not typically observed

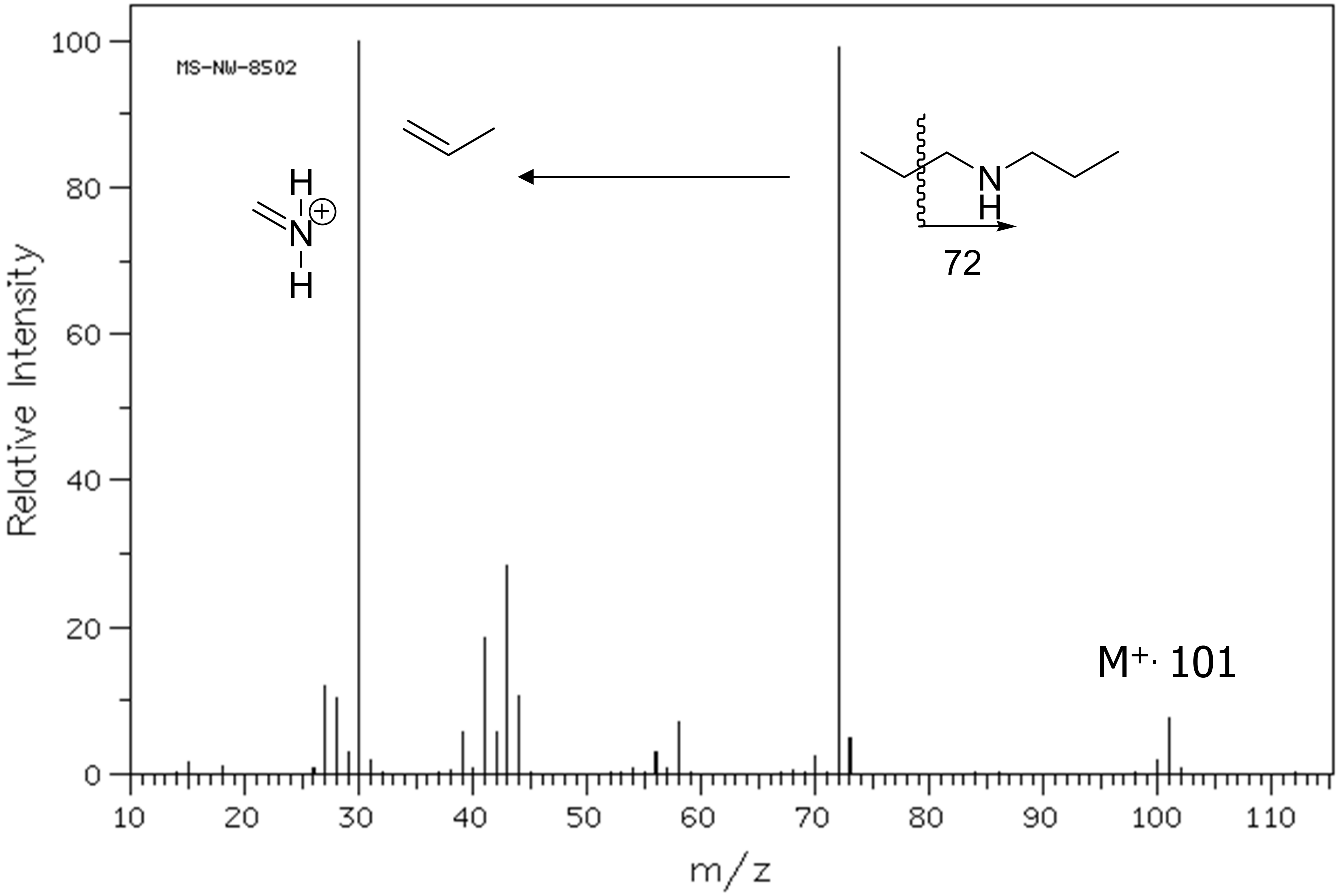
Examples: **primary amines**

pentylamine



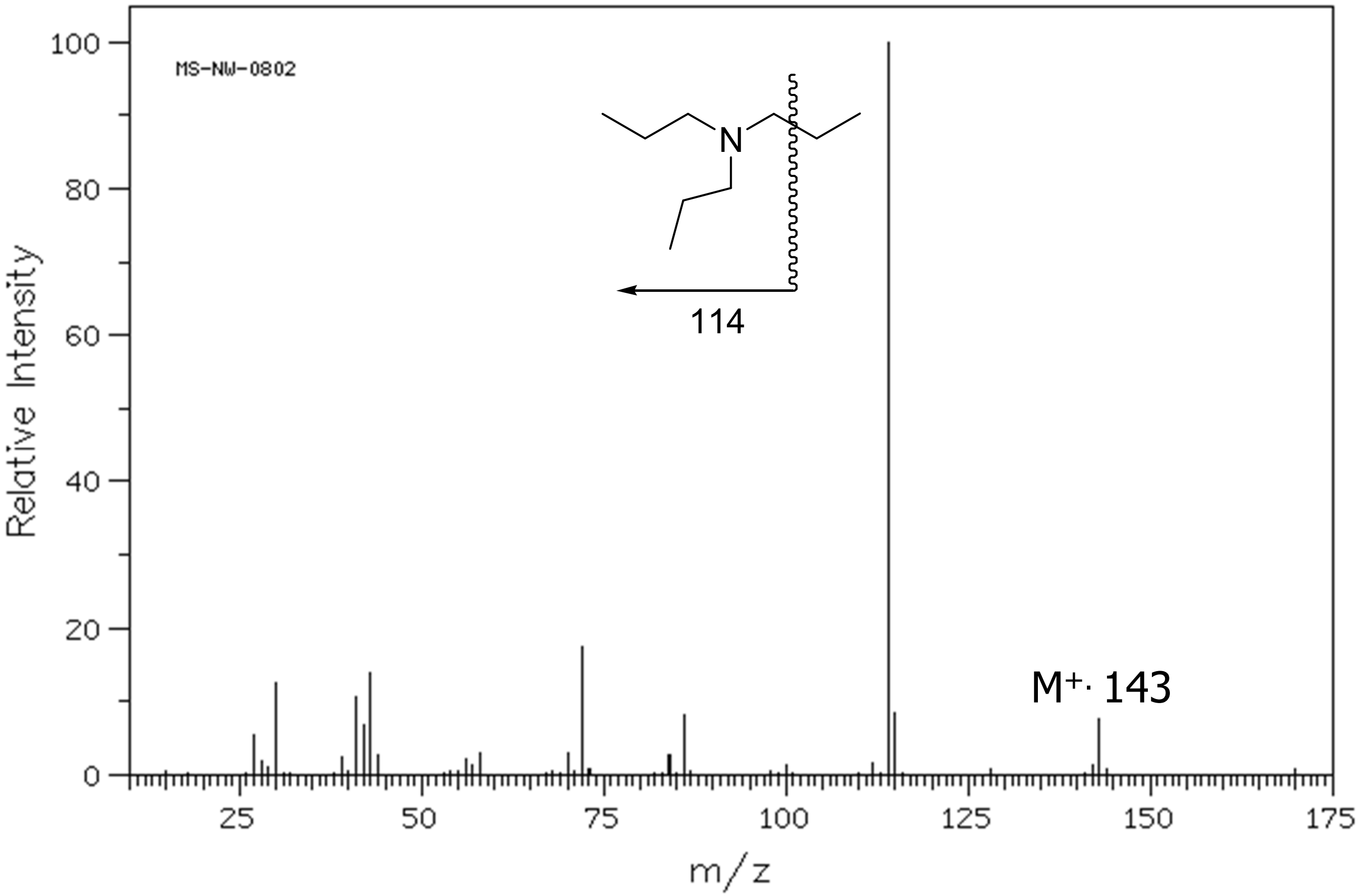
Examples: **secondary amines**

dipropylamine



Examples: **tertiary amines**

tripropylamine



Examples: **halogens**

a) Halogenated compounds often give good $M^{+\cdot}$

b) Fluoro and iodo compounds do not have appreciable contribution from isotopes.

c) Chloro and bromo compounds are unique in that they will show strong M+2 peaks for the contribution of higher isotopes.

d) For chlorinated compounds, the ratio of M to M+2 is about 3:1

e) For brominated compounds, the ratio of M to M+2 is 1:1

f) An appreciable M+4, M+6, … peak is indicative of a combination of these two halogens – use template to determine number of each

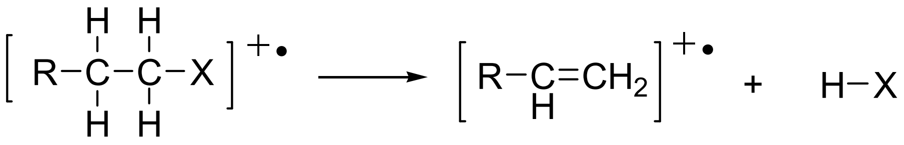
Examples: **halogens**

g) Principle fragmentation mode is to lose halogen atom via inductive cleavage, leaving a carbocation – the intensity of the peak will increase with cation stability

$$\left[\mathrm{R{-}X}\right]^{+\bullet} \longrightarrow \mathrm{R}^{\oplus} + \mathrm{X}^{\bullet}$$

h) Leaving group ability contributes to the loss of halogen most strongly for -I and -Br less so for -Cl, and least for –F

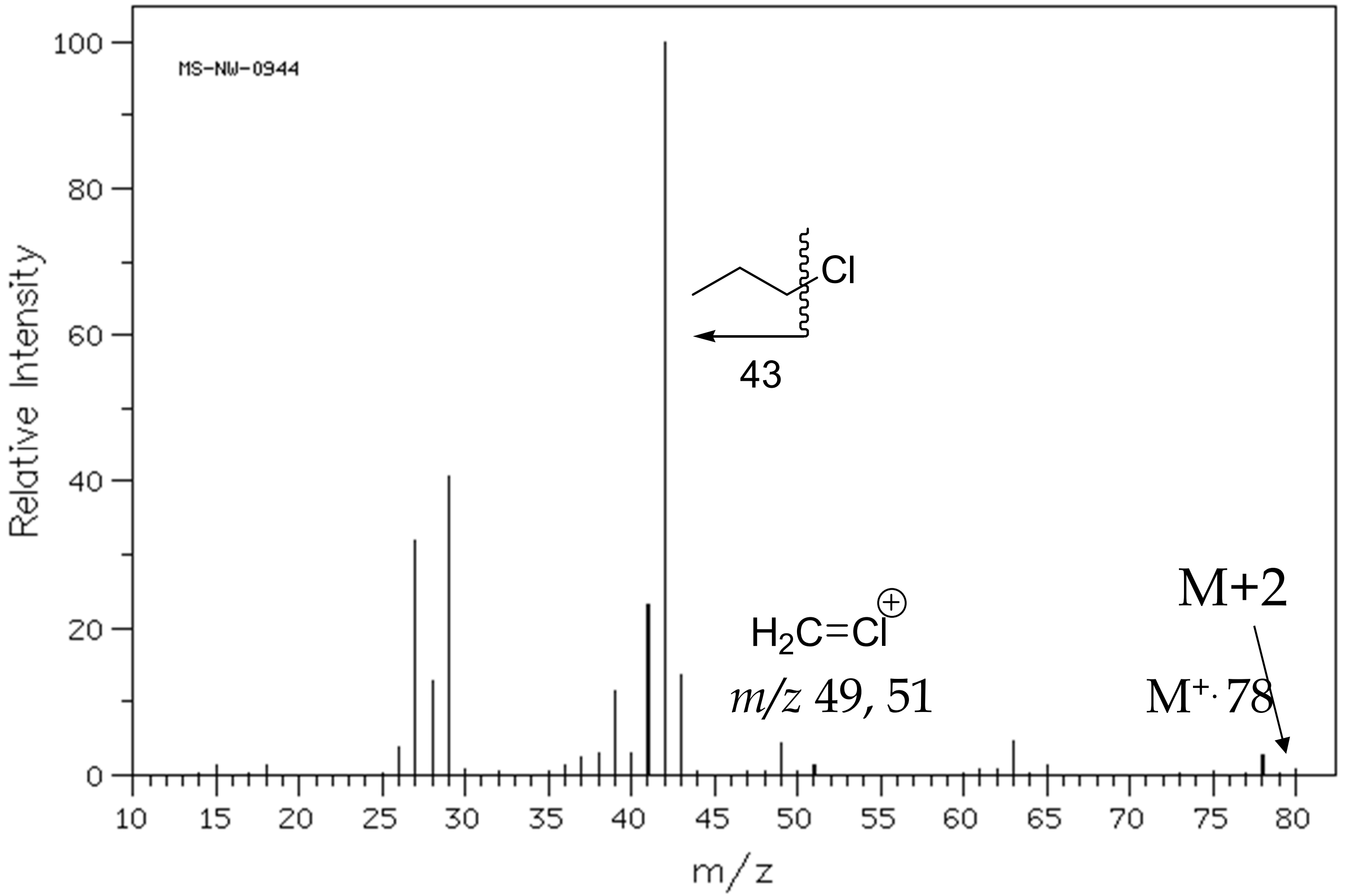
i) Loss of HX is the second most common mode of fragmentation – here the conjugate basicity of the halogen contributes (HF > HCl > HBr > HI)



$$\left[\mathrm{R{-}CH_2{-}CH_2{-}X}\right]^{+\bullet} \longrightarrow \left[\mathrm{R{-}CH{=}CH_2}\right]^{+\bullet} + \mathrm{H{-}X}$$

Examples: **halogens**

1-chloropropane



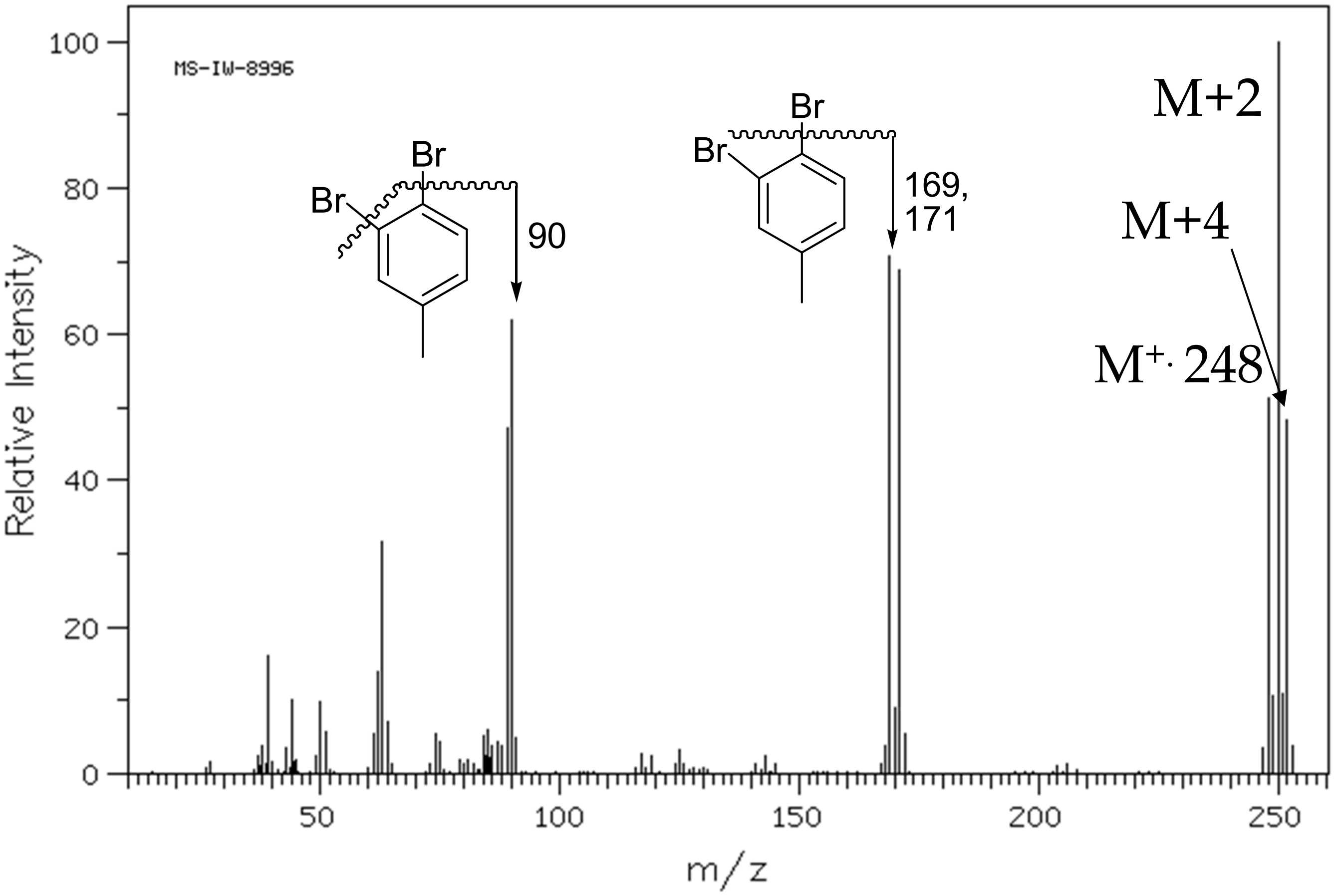
$H_2C{=}Cl^{\oplus}$

m/z 49, 51

$M^{+\cdot}$ 78

Examples: **halogens**

3,4-dibromotoluene



Approach to analyzing a spectrum (*1*)

1. **Squeeze everything you can out of the $M^{+\cdot}$ peak (once you have confirmed it is $M^{+\cdot}$)**
   - Strong or weak?
   - Isotopes? M+1? M+2, M+4, …
   - Use ^{13}C to determine number of carbons.
   - Apply the nitrogen rule.
   - Apply "Rule of 13" to generate possible formulas (you can quickly dispose of possibilities based on the absence of isotopic peaks or the inference of the nitrogen rule).
   - Use DBE to further reduce the possibilities.
   - Is there an M-1 peak? → aldehydes

Approach to analyzing a spectrum (2)

2. **Squeeze everything you can out of the base peak:**
   - What ions could give this peak? (*m/z* 43 doesn't help much)
   - What was lost from $M^{+\cdot}$ to give this peak?
   - When considering the base peak initially, only think of the *most common cleavages for each group*

3. **Look for the loss of small neutral molecules from $M^{+\cdot}$**
   - $H_2C{=}CH_2$, $HC{\equiv}CH$, H_2O, HOR, HCN, HX

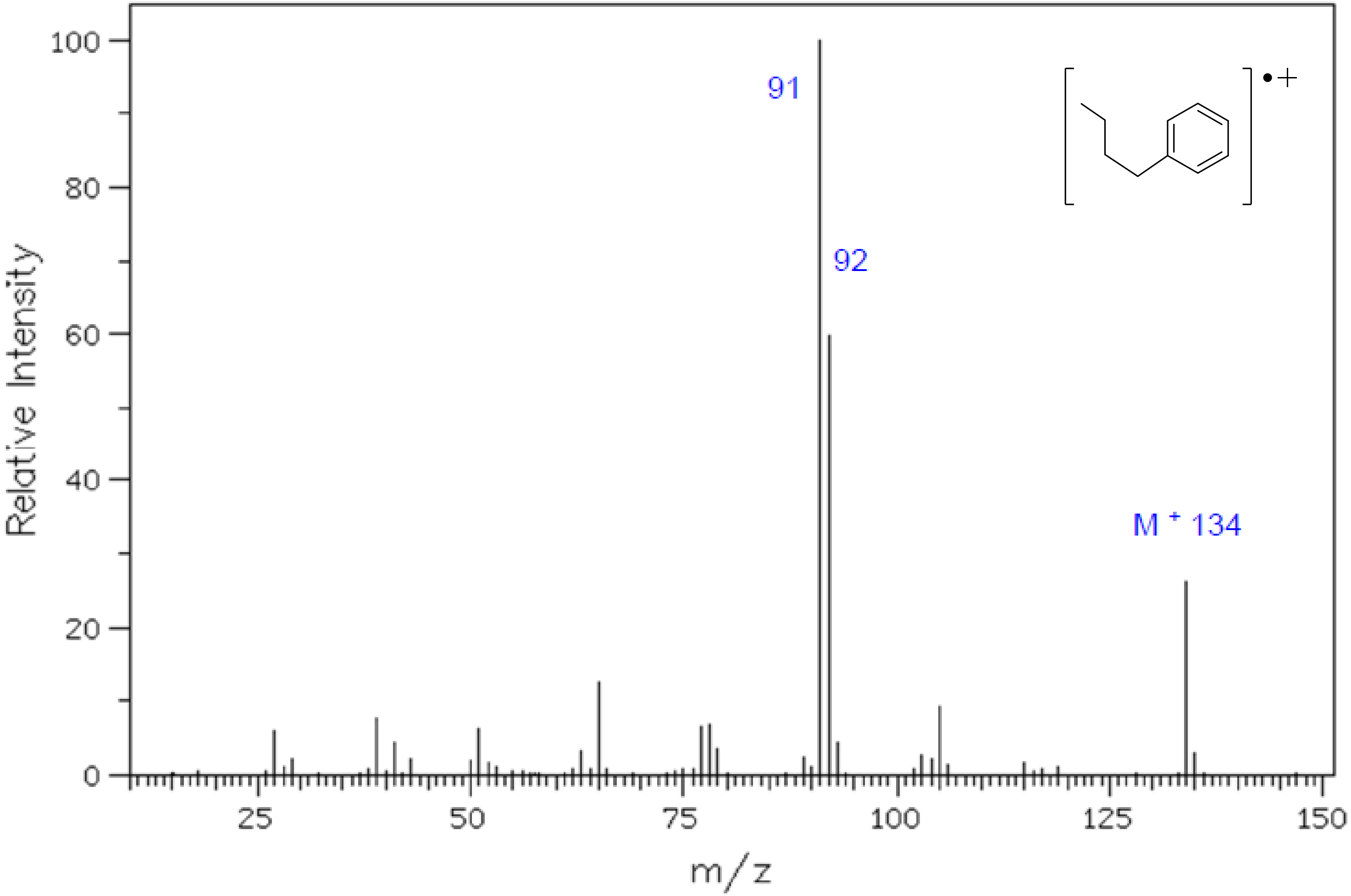
4. **Now consider possible diagnostic peaks in the spectrum** (e.g.: 29, 30, 31, 45, 59, 77, 91, 105 etc.)

5. **Lastly, once you have a hypothetical molecule that explains the data, see if you can verify it by use of other less intense peaks on the spectrum.**

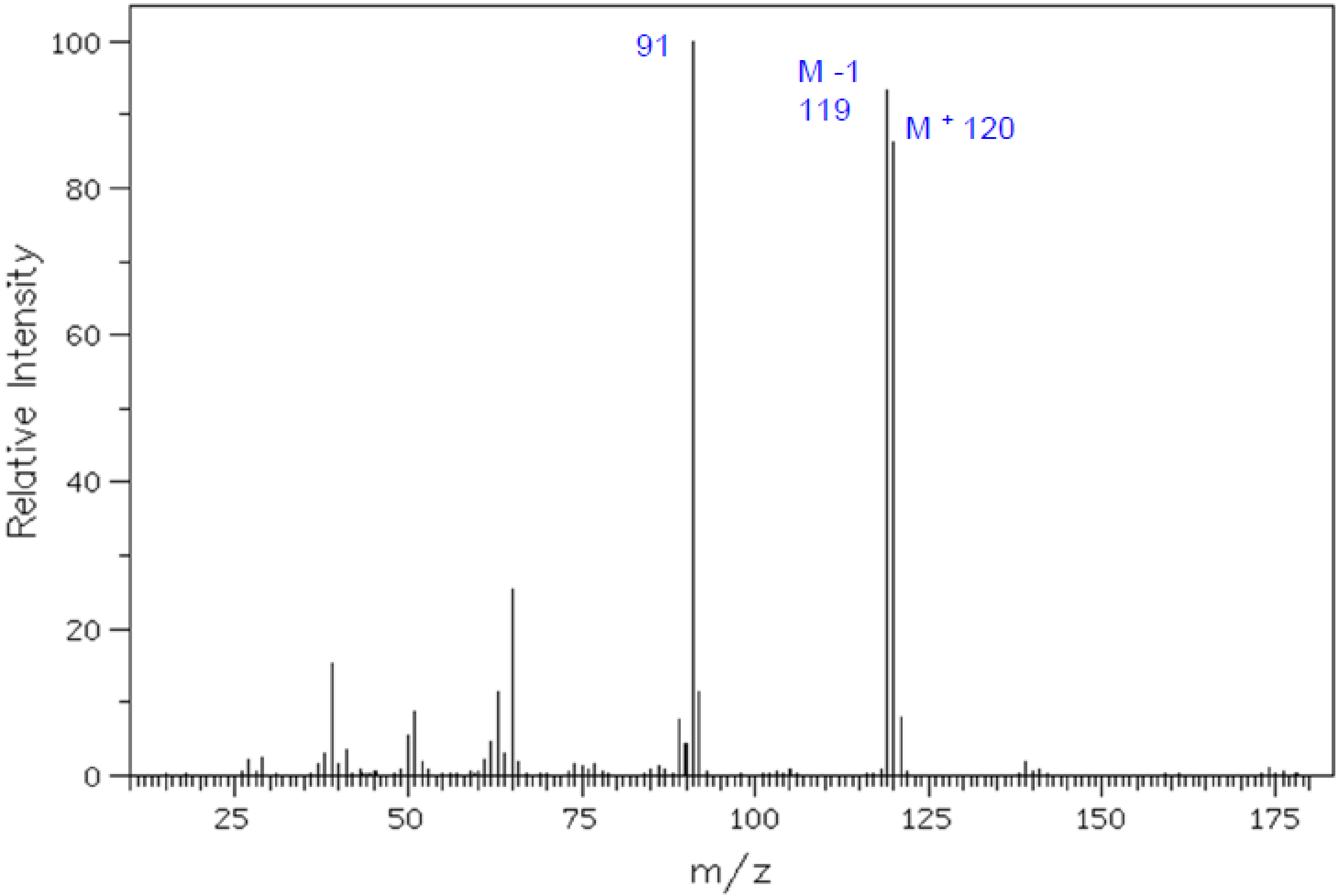
Theorie und Basic´s

EI allgemein; was ist das; was ist besonders ?
70 eV ?
QET ?
Metastabile Ionen ?
Mass Defect ?
Genauigkeit /Präzision ?
OE und EE ?
Auflösungsvermögen ?
Stickstoffregel ?
Regel der 13 ?
DBE ?
Isotope ?
Stevenson Regel ?
Fragmentierungsmechanismen

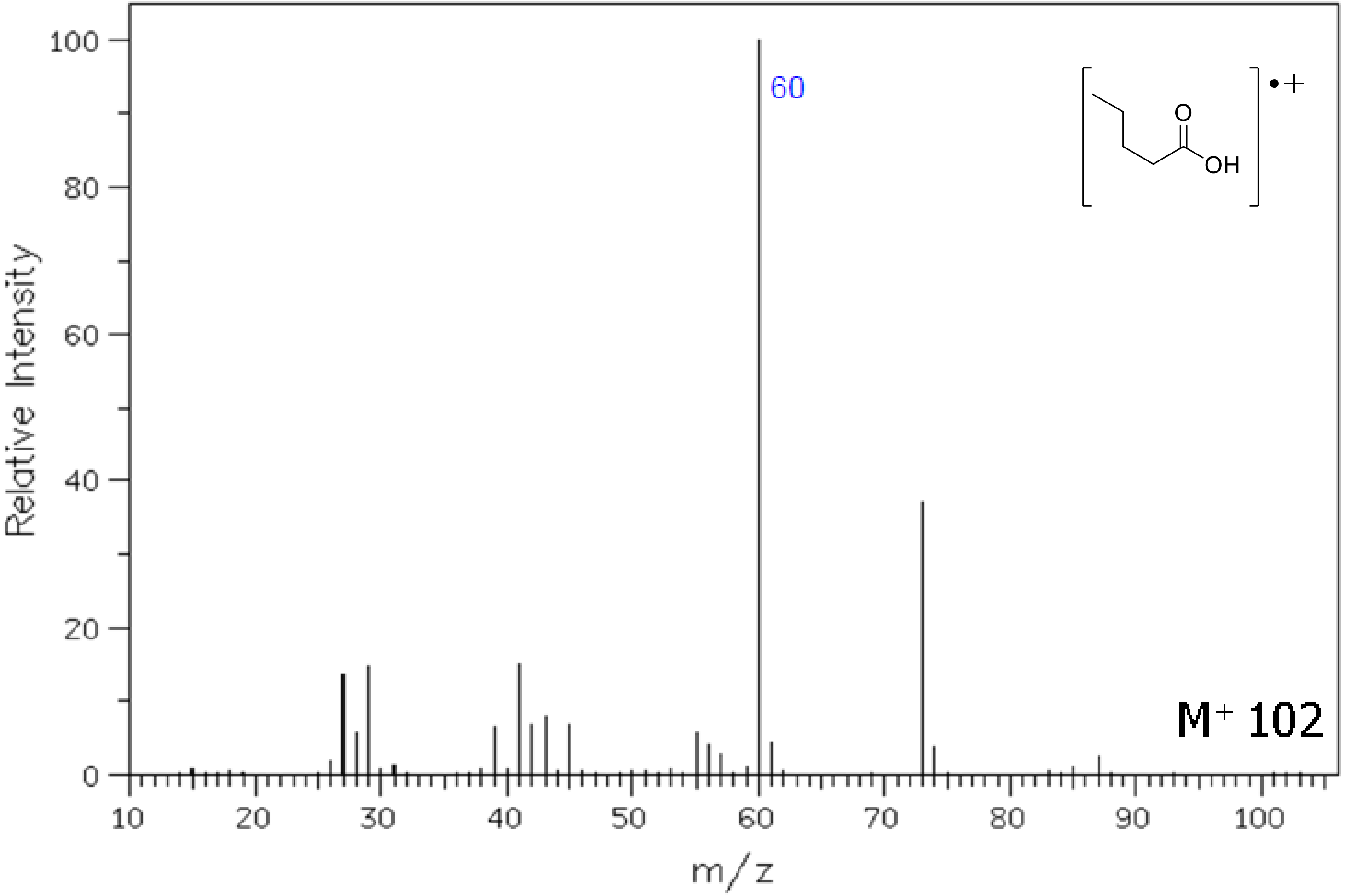
Aufgabe 1: Erklären Sie die Fragmente, welcher Fragmentierungstyp liegt vor ?



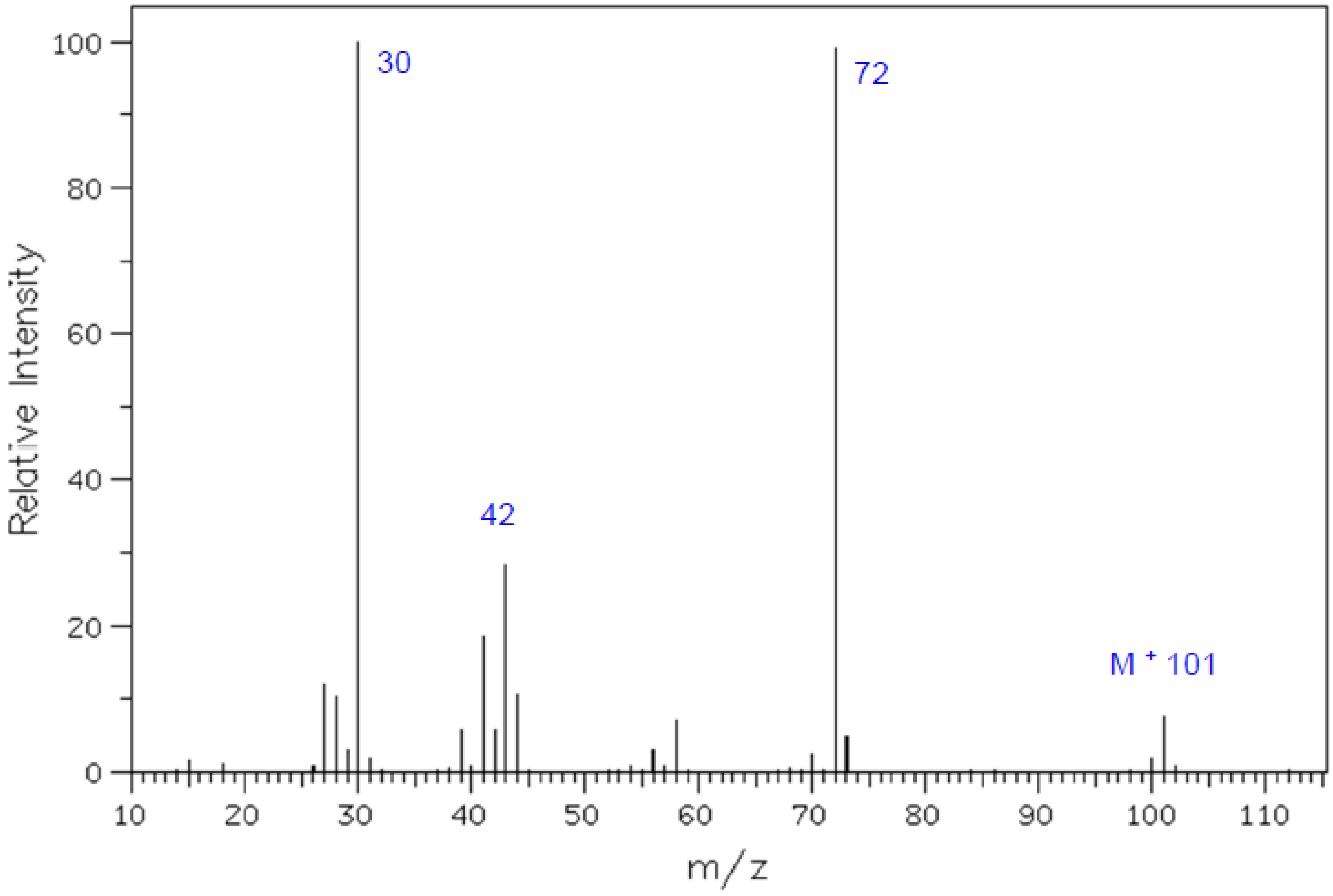
Aufgabe 2: Um welches aromatisches Aldehyd handelt es sich ?



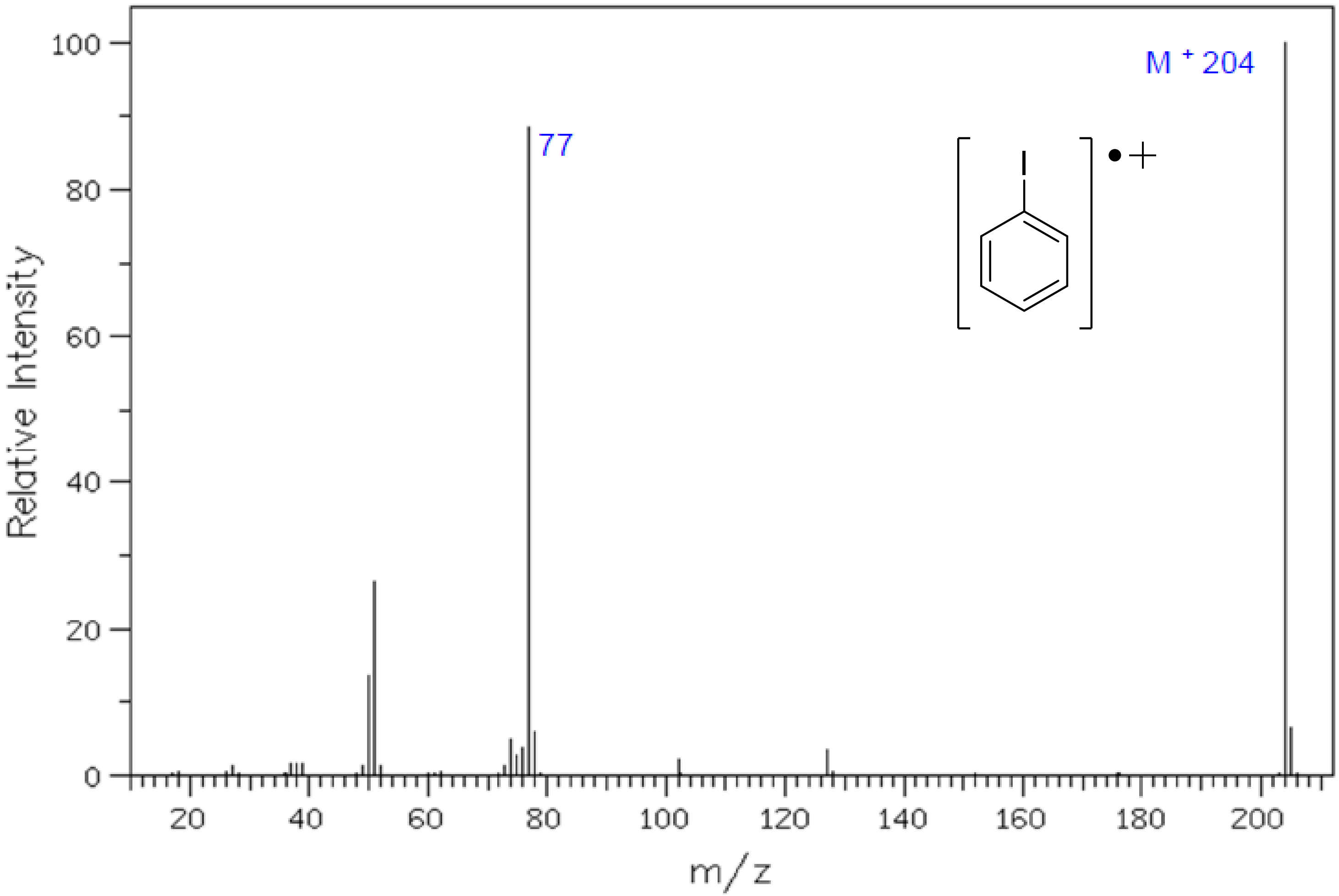
Aufgabe 3: Erklären Sie die Fragmente, welcher Fragmentierungstyp liegt vor ?



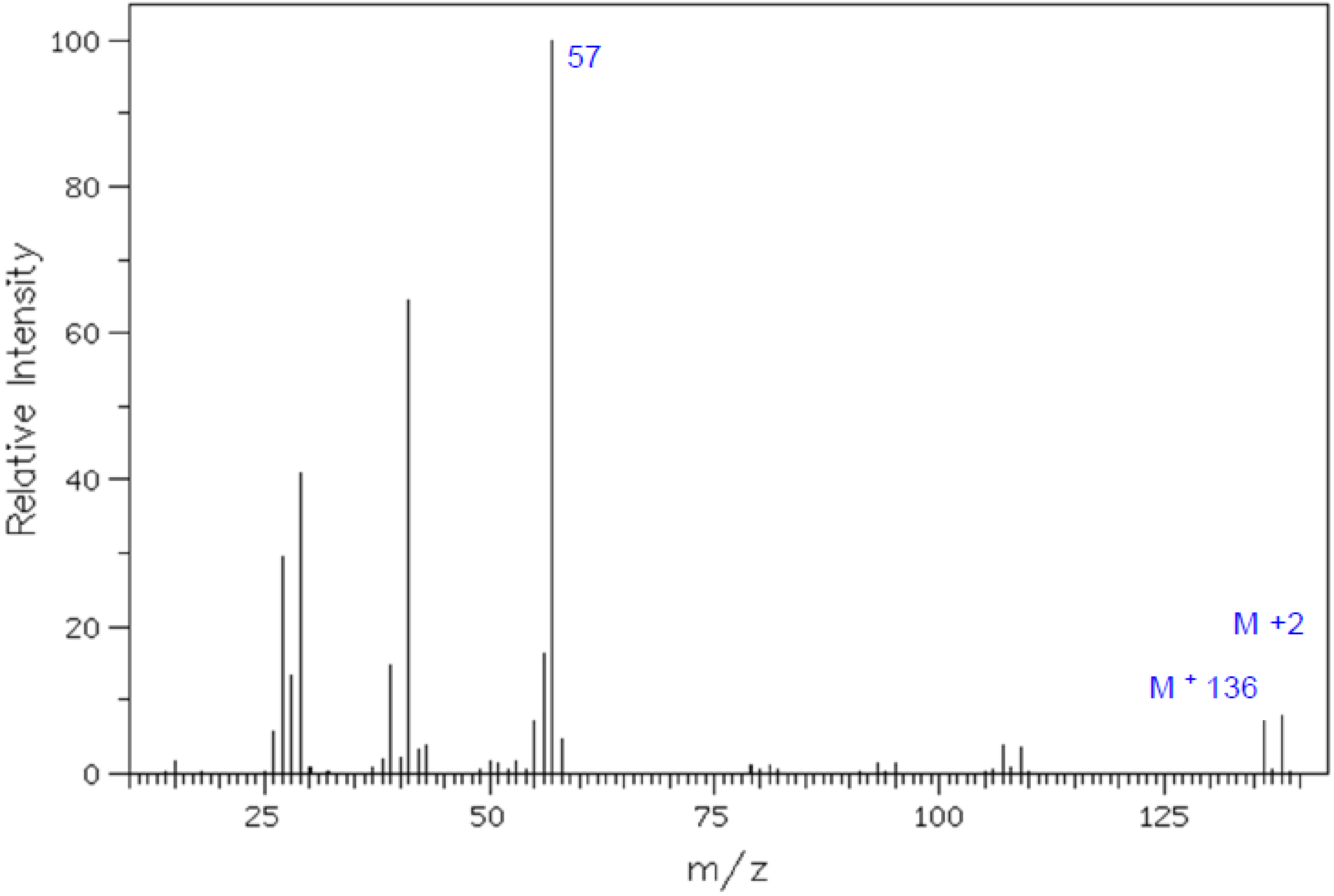
Aufgabe 4: Um welches sekundäre Amine handelt es sich ?



Aufgabe 5: Welcher Fragmentierungstyp liegt vor ?



Aufgabe 6: Um welche aliphatische Halogenverbindung handelt es sich ?



Aufgabe 7: Bei welchen Massen handelt es sich um eine Mc-Lafferty-Umlagerung