Mass Spectrometry

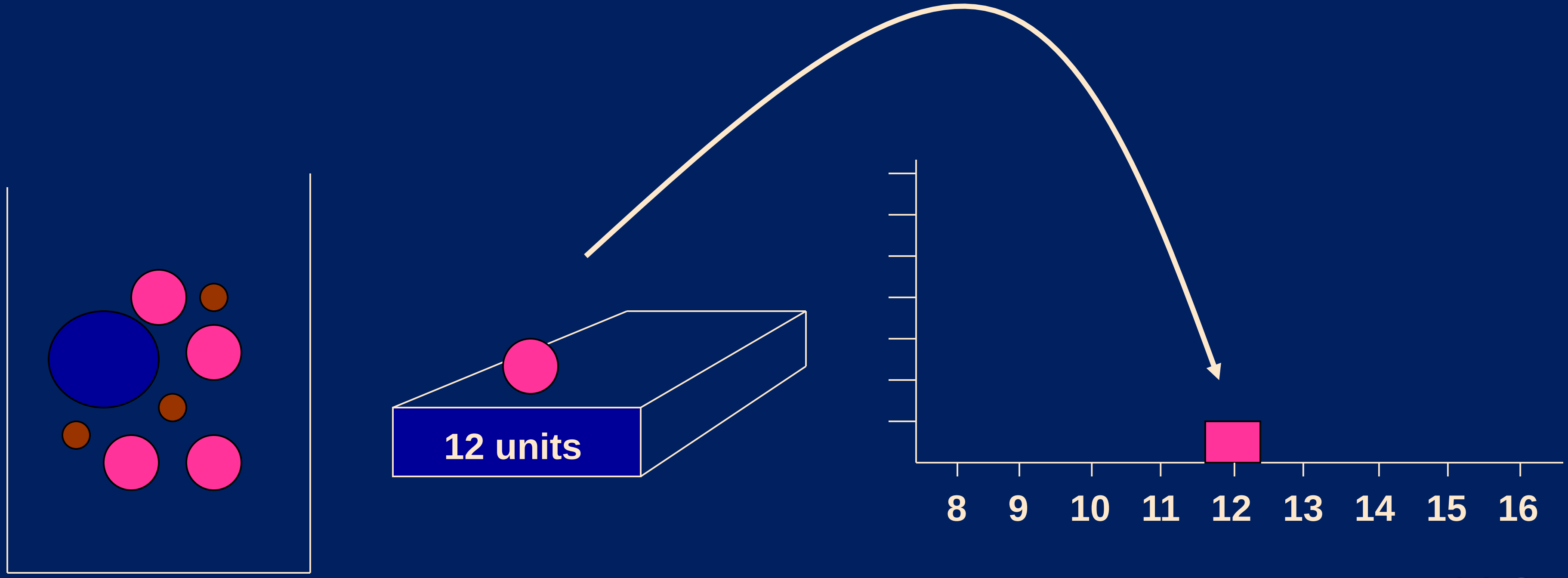
By: Sujatha K
KUD

Mass Spectrometry (MS)

- An analytical technique for measuring the mass-to-charge ratio (*m/z*) of ions in the gas phase.
  - Mass spectrometry is our most valuable analytical tool for determining accurate molecular masses.
  - Also can give information about structure.
  - Proteins can now be sequenced by MS.

What is a Mass Spectrometer?

- A Mass Spectrometer is a machine that weighs molecules !



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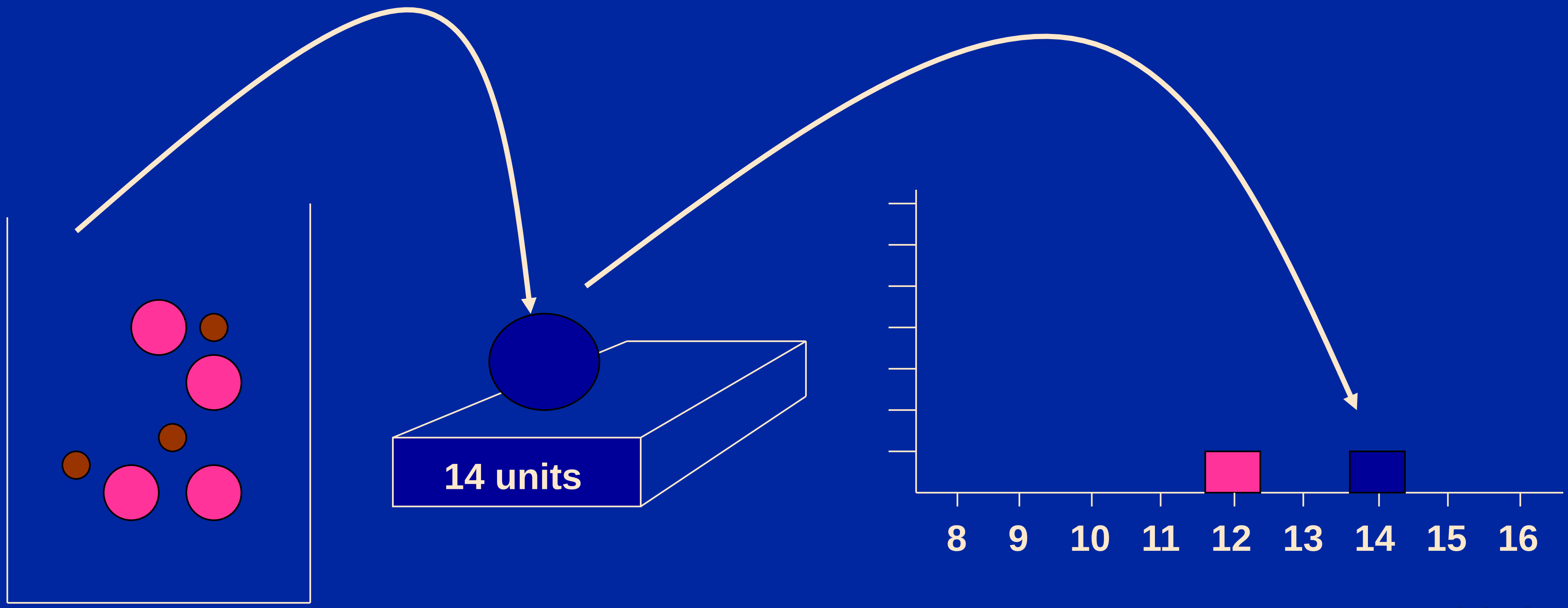
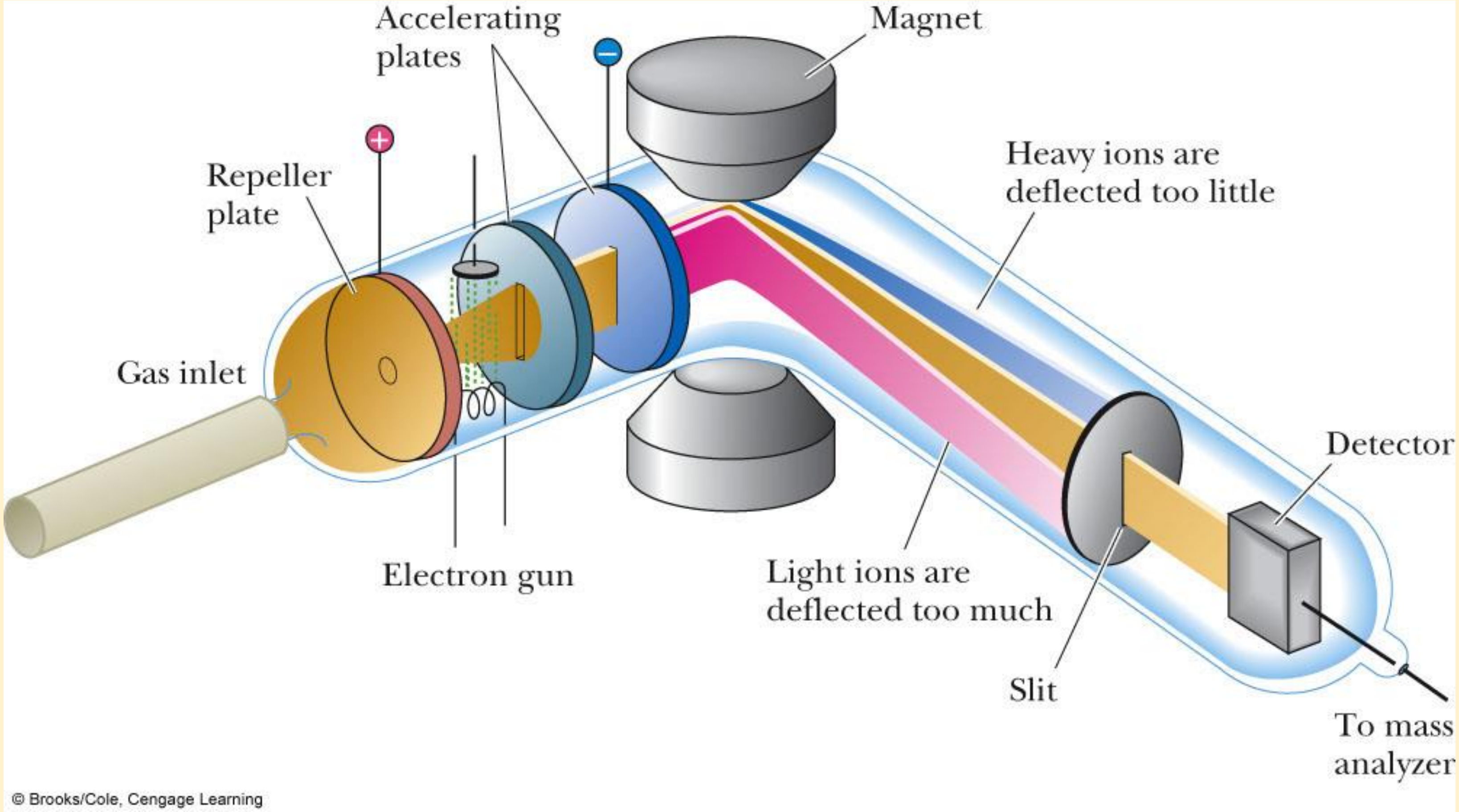
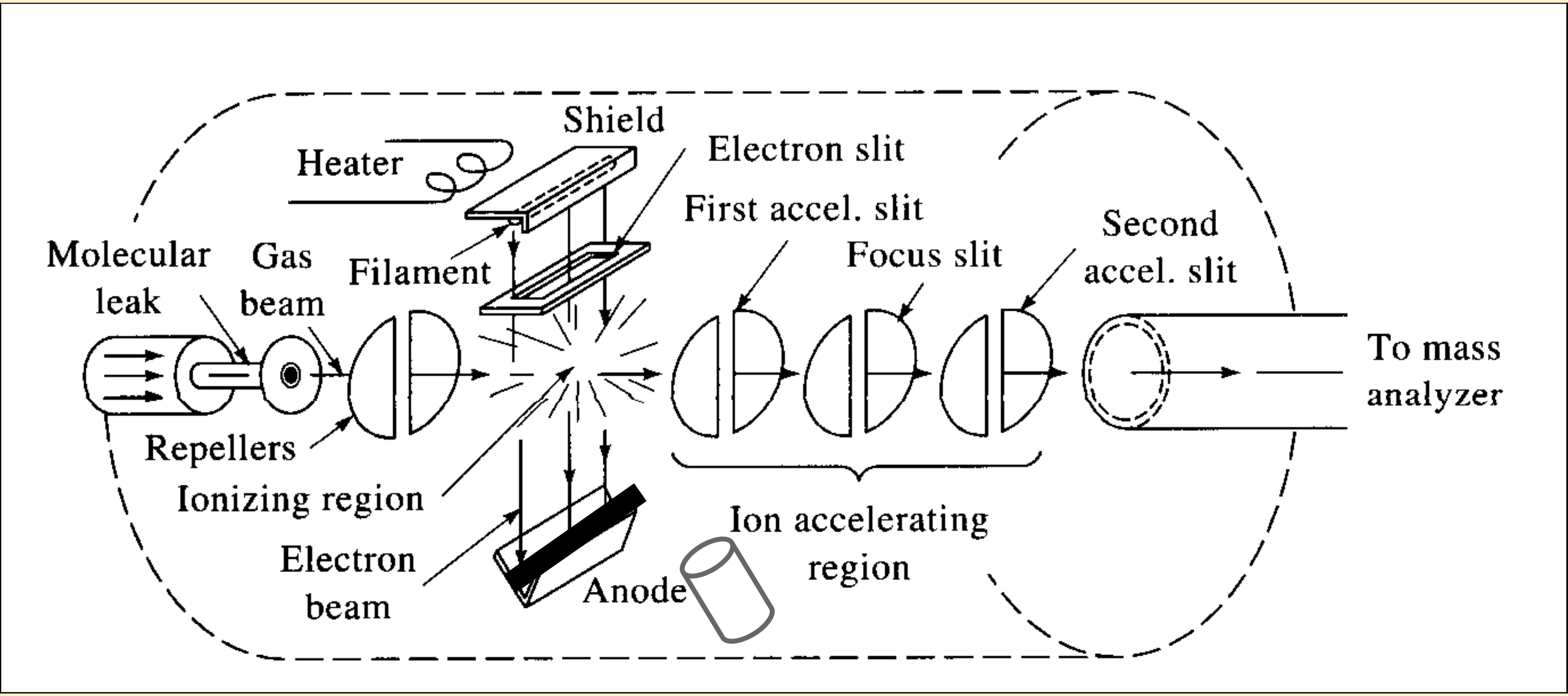


Mass Spectrometry (MS)

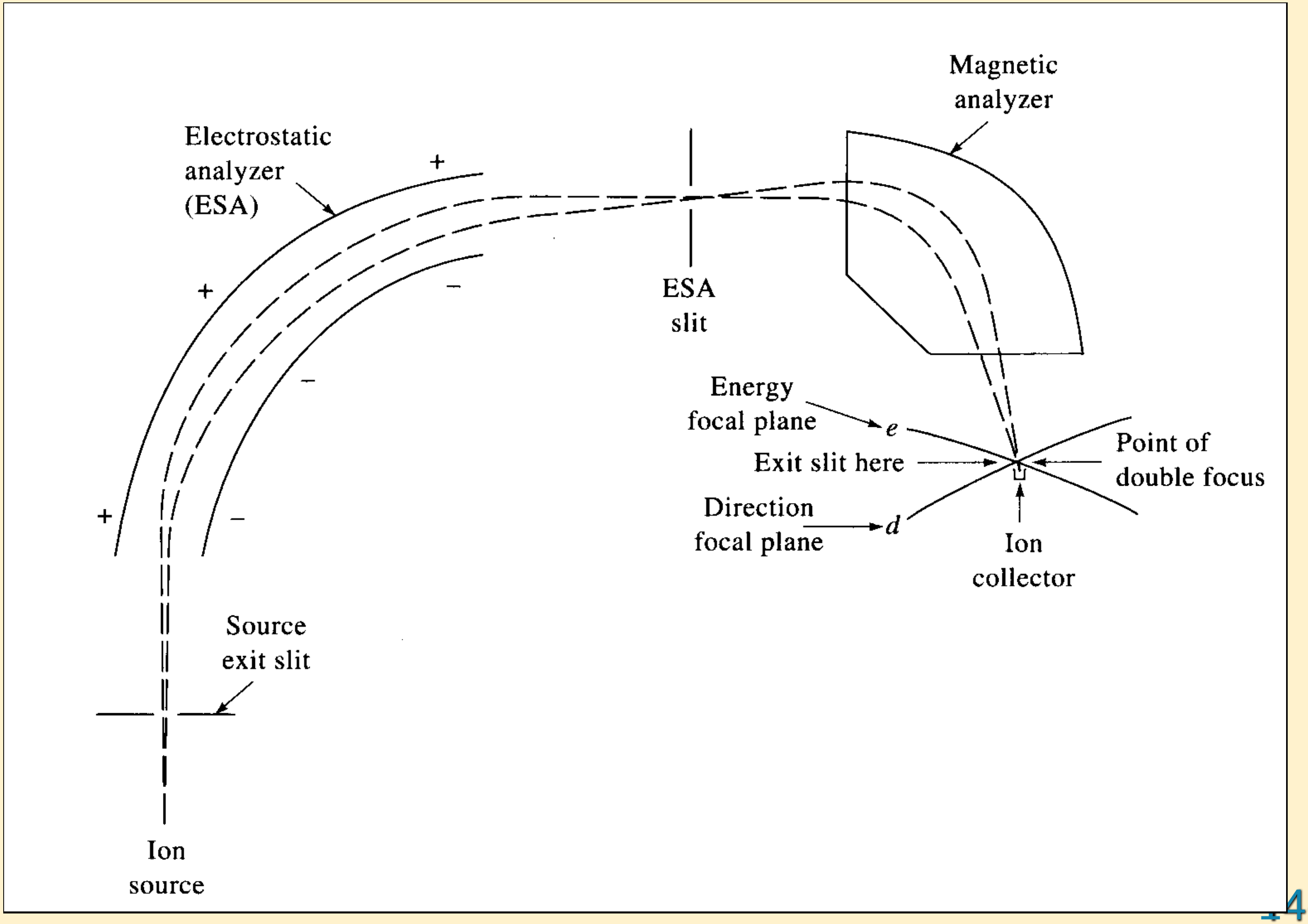
- **Schematic of an electron ionization mass spectrometer (EI-MS).**



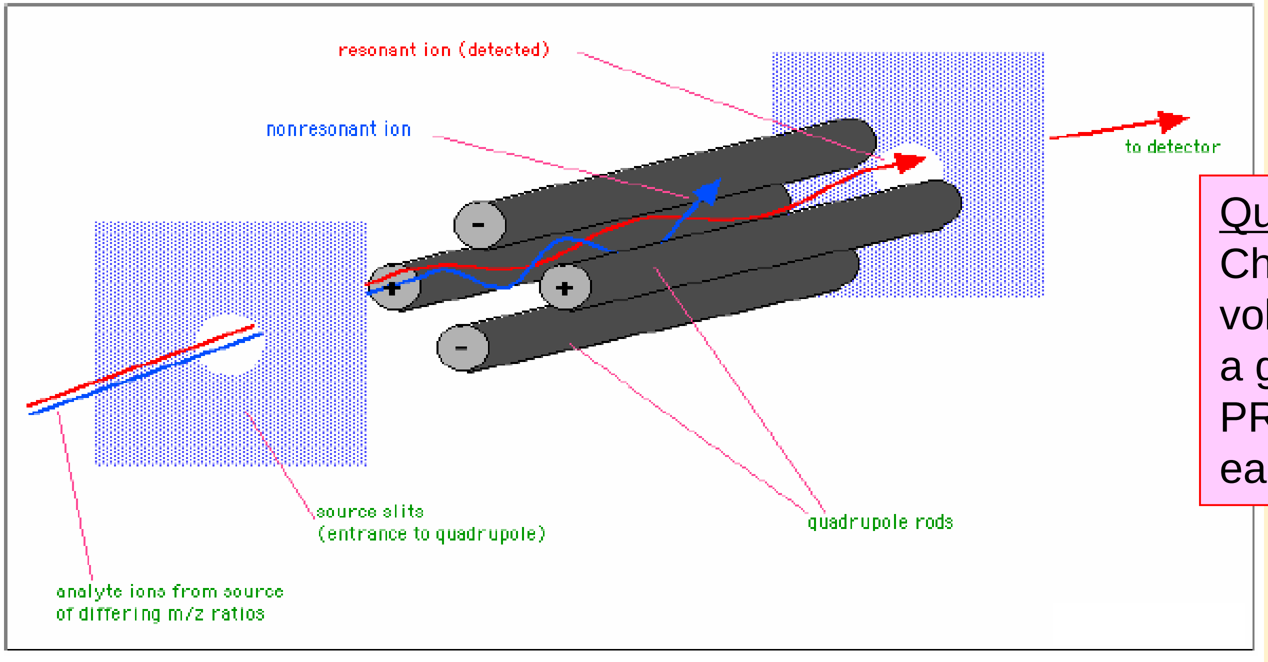


CI REAGENT GAS

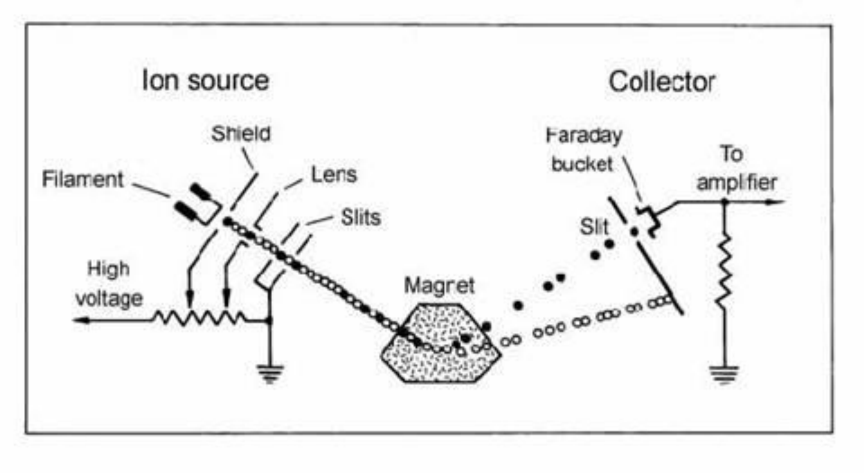
Double Focusing Mass Spectrometer



Mass Analyzers - the quadrupole vs. magnetic sector



<u>Quadrupole</u>:
Changes DC and RF voltages to isolate a given m/z ion.
PRO: cheap, fast, easy



<u>Magnetic Sector:</u>
Changes B and V to focus a given m/z into detector.
PRO: turn in geometry means less ‘dark noise’, higher precision

MS-MS

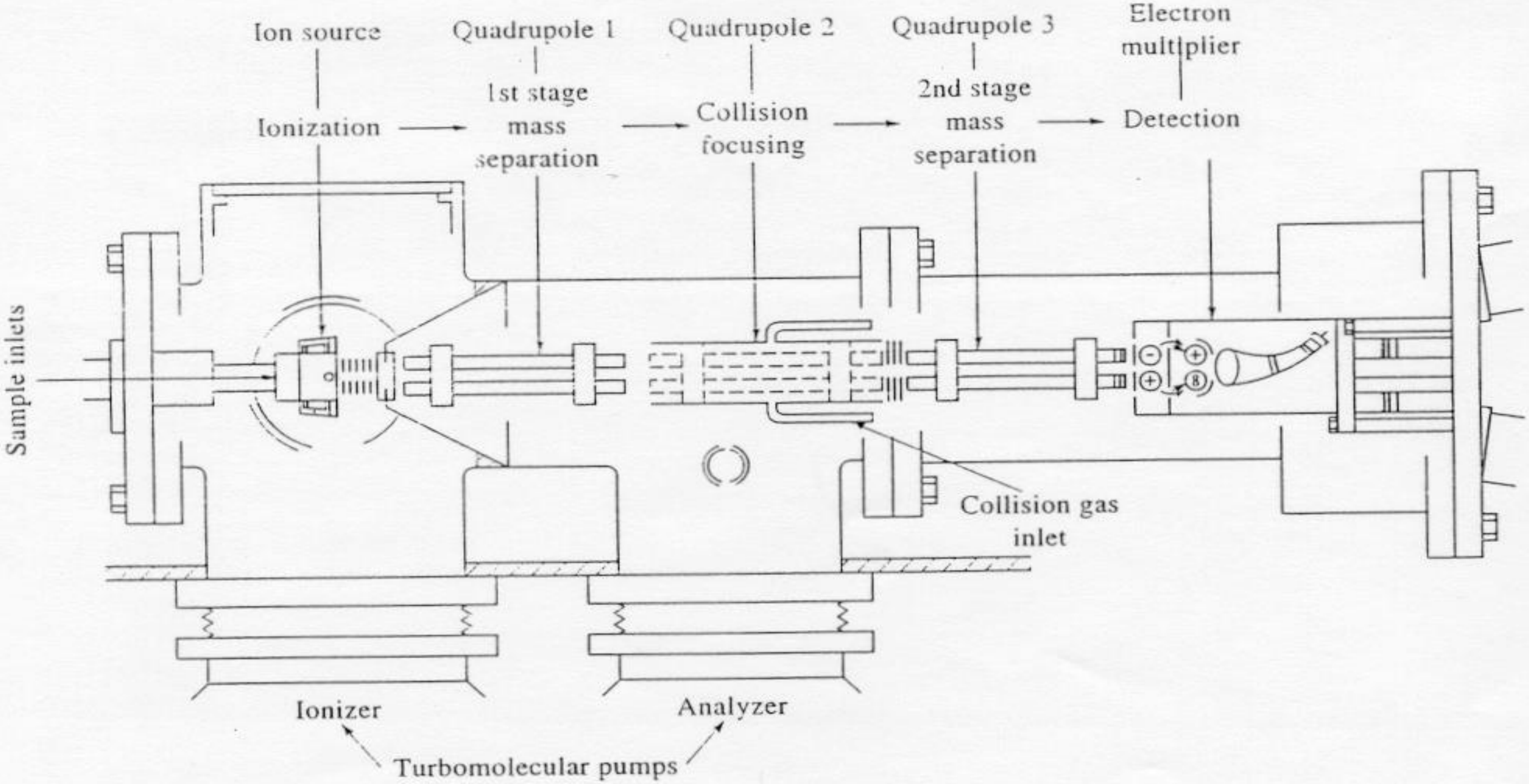
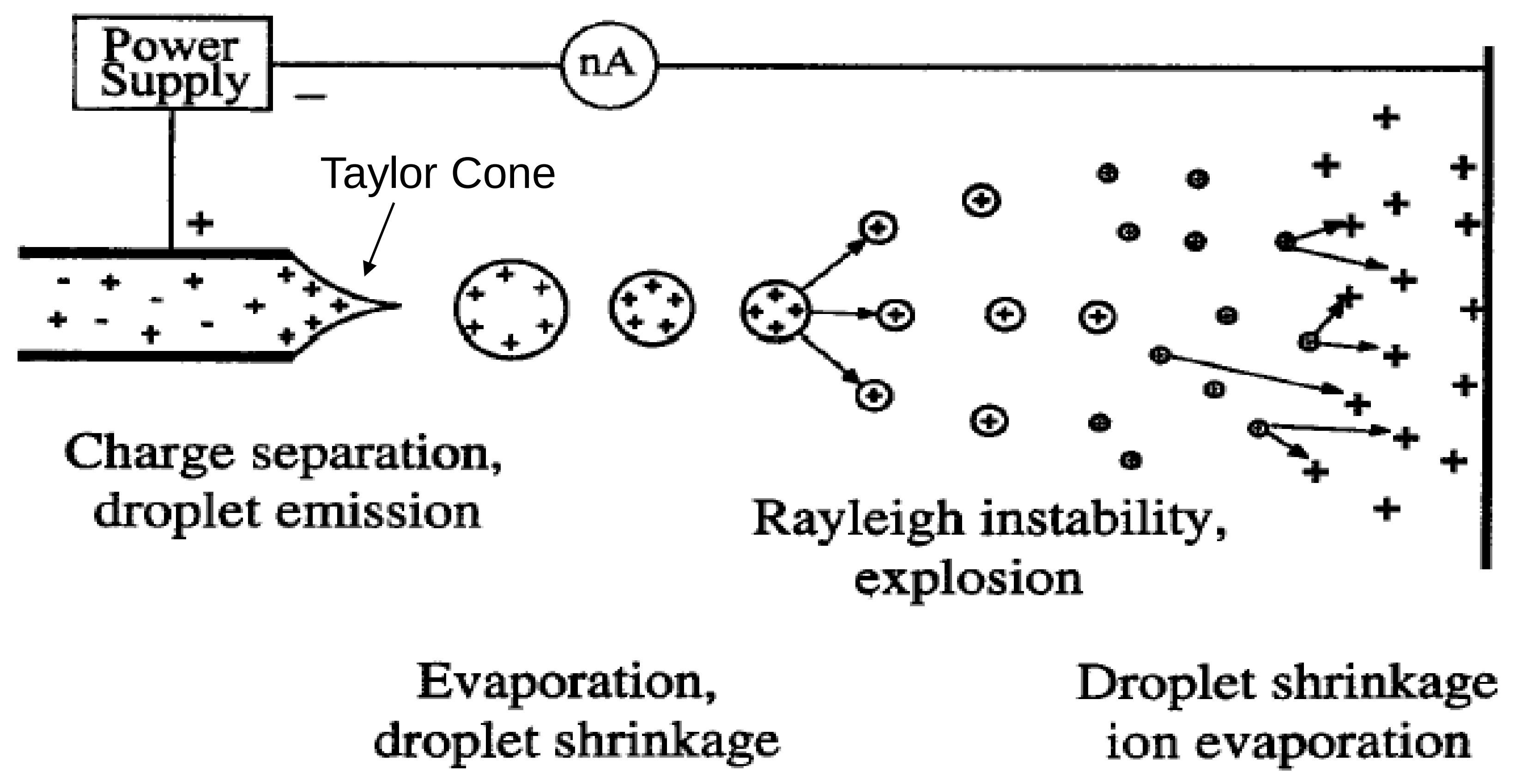


Figure 20-24 Schematic of a tandem quadrupole MS/MS instrument. *(Courtesy of Finnigan MAT, San Jose, CA.)*

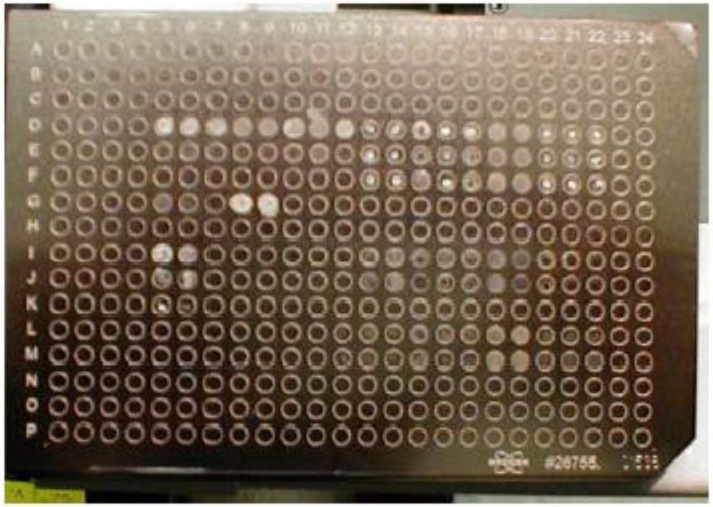


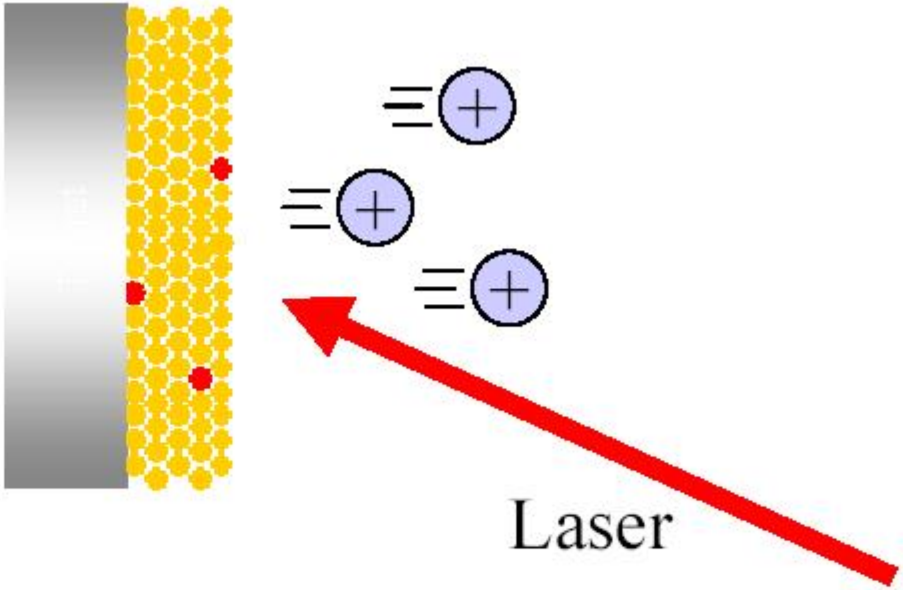
Three Step Process

1) Droplet formation
2) Droplet Shrinkage
3) Gaseous Ion Formation

Electrospray process does not ionize samples !

MALDI





- Matrix Assisted Laser Desorption Ionization
- Analyte co-deposited with Matrix
- Laser excites matrix which transfers energy to analyte
- Produces singly charged species
- Typically used for large biomolecules / polymers
- MALDI is a high mass/pulsed source so usually combined with TOF

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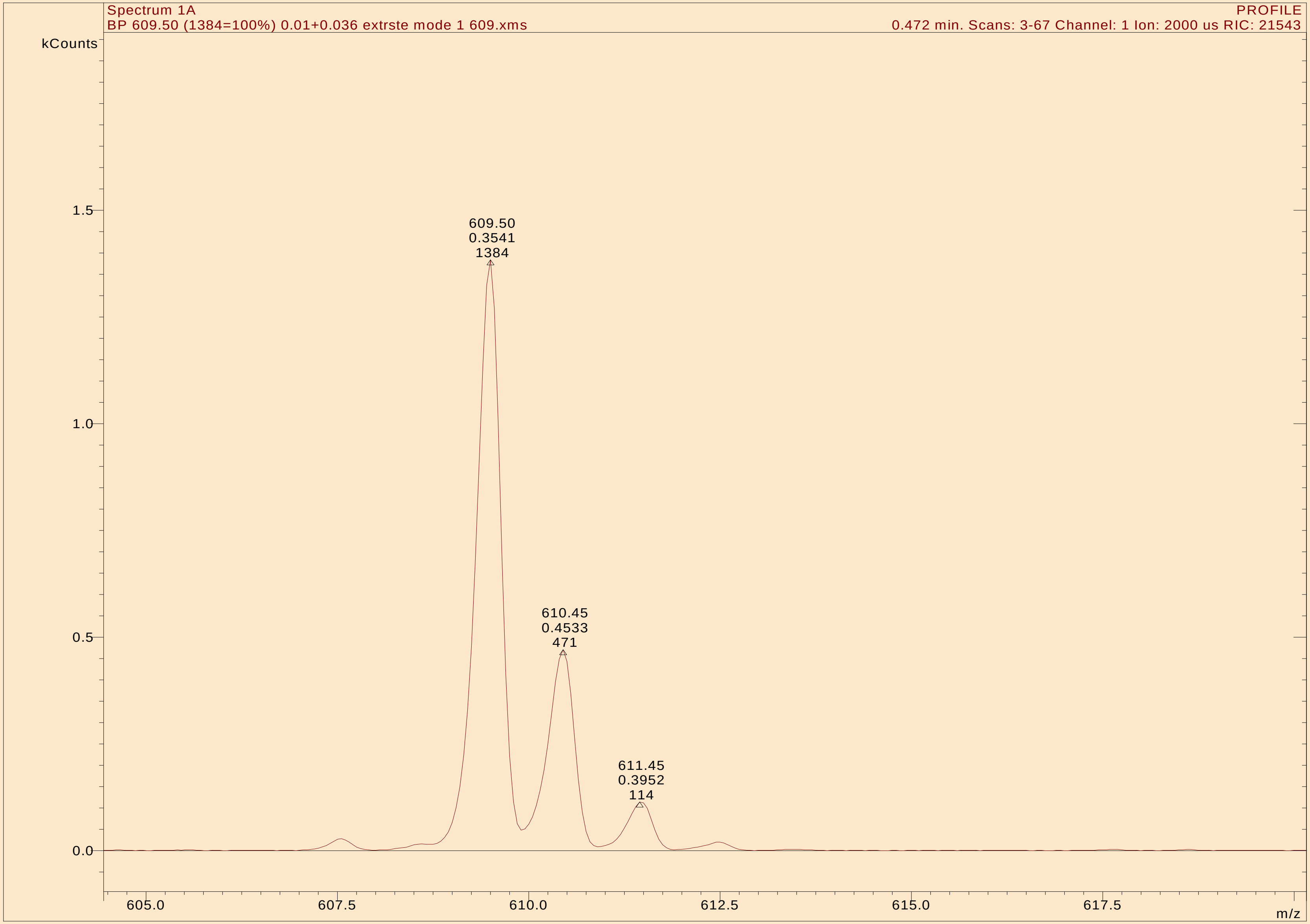

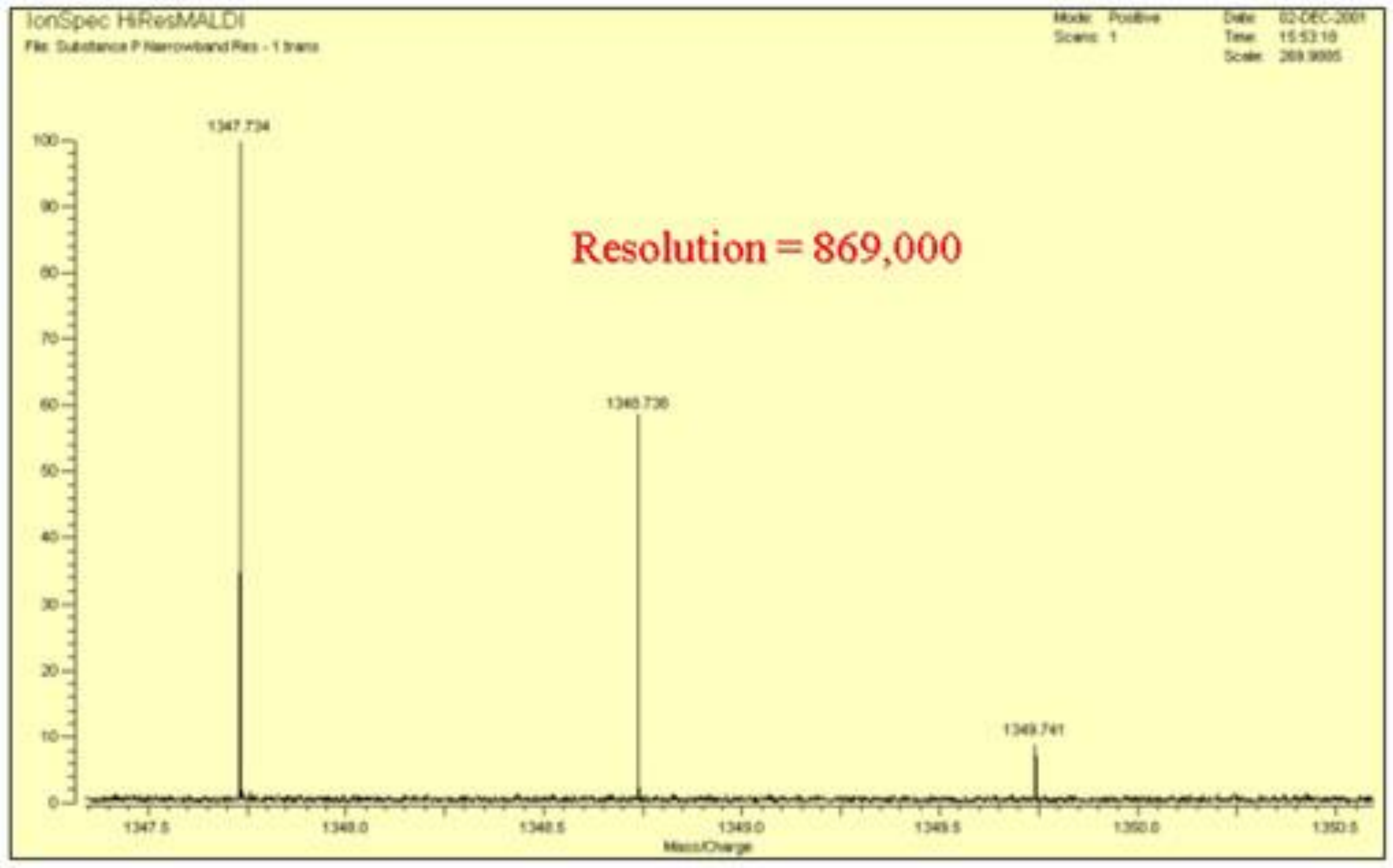
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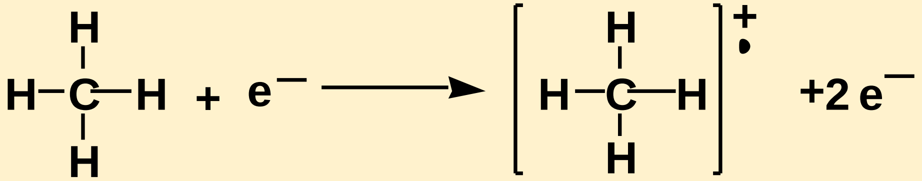
1350.5
Mass/Charge

A Mass Spectrometer

- A mass spectrometer is designed to do three things
    - Convert neutral atoms or molecules into a beam of positive (or rarely negative) ions.
    - Separate the ions on the basis of their mass-to-charge (*m/z*) ratio.
    - Measure the relative abundance of each ion.

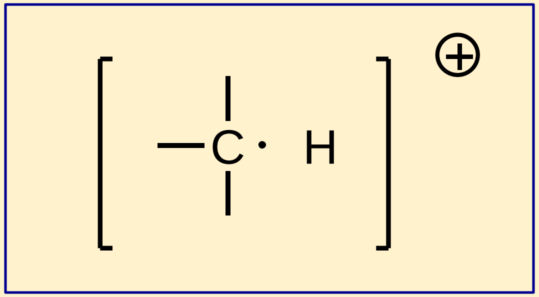
A Mass Spectrometer

- **Electron Ionization MS**
  - In the ionization chamber, the sample is bombarded with a beam of high-energy electrons.
  - Collisions between these electrons and the sample result in loss of electrons from sample molecules and formation of positive ions.



$$CH_4 + e^- \longrightarrow [CH_4]^{+\bullet} + 2e^-$$

Molecular ion
(a radical cation)



Molecular Ion

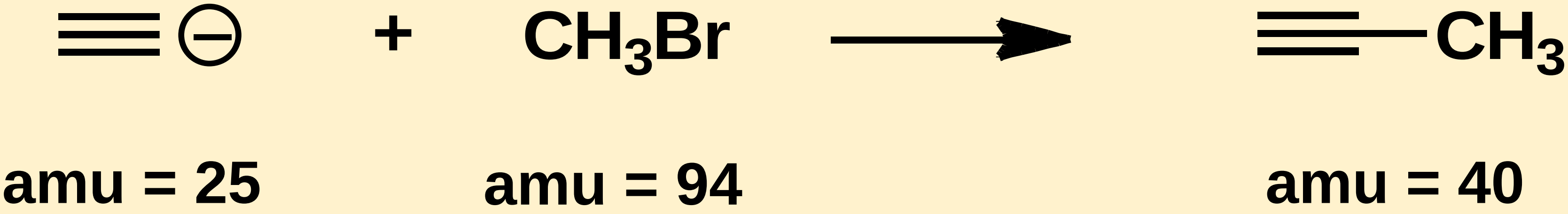
- **Molecular ion (M^+):** A radical cation formed by removal of a single electron from a parent molecule in a mass spectrometer.
- For our purposes, it does not matter which electron is lost; radical cation character is delocalized throughout the molecule; therefore, we write the molecular formula of the parent molecule in brackets with
  - a plus sign to show that it is a cation.
  - a dot to show that it has an odd number of electrons.

Other MS techniques

- What we have described is called electron ionization mass spectrometry (EI-MS).
- Other mass spectrometry techniques include
  - fast atom bombardment (FAB).
  - matrix-assisted laser desorption ionization (MALDI).
  - chemical ionization (CI).
  - electrospray ionization (ESI).

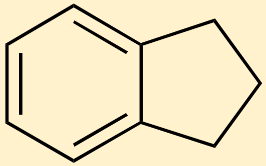
Resolution

- **Resolution:** A measure of how well a mass spectrometer separates ions of different mass.
  - **Low resolution:** Refers to instruments capable of separating only ions that differ in nominal mass; that is ions that differ by at least 1 or more atomic mass units (amu).



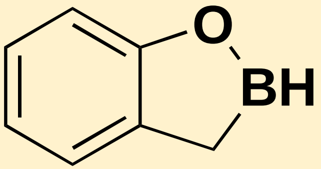
Resolution

- **Resolution:** A measure of how well a mass spectrometer separates ions of different mass.
  - **High resolution:** Refers to instruments capable of separating ions that differ in mass by as little as 0.0001 amu. Can Help Determine Molecular Formula



amu = 118

exact mass = 118.0783



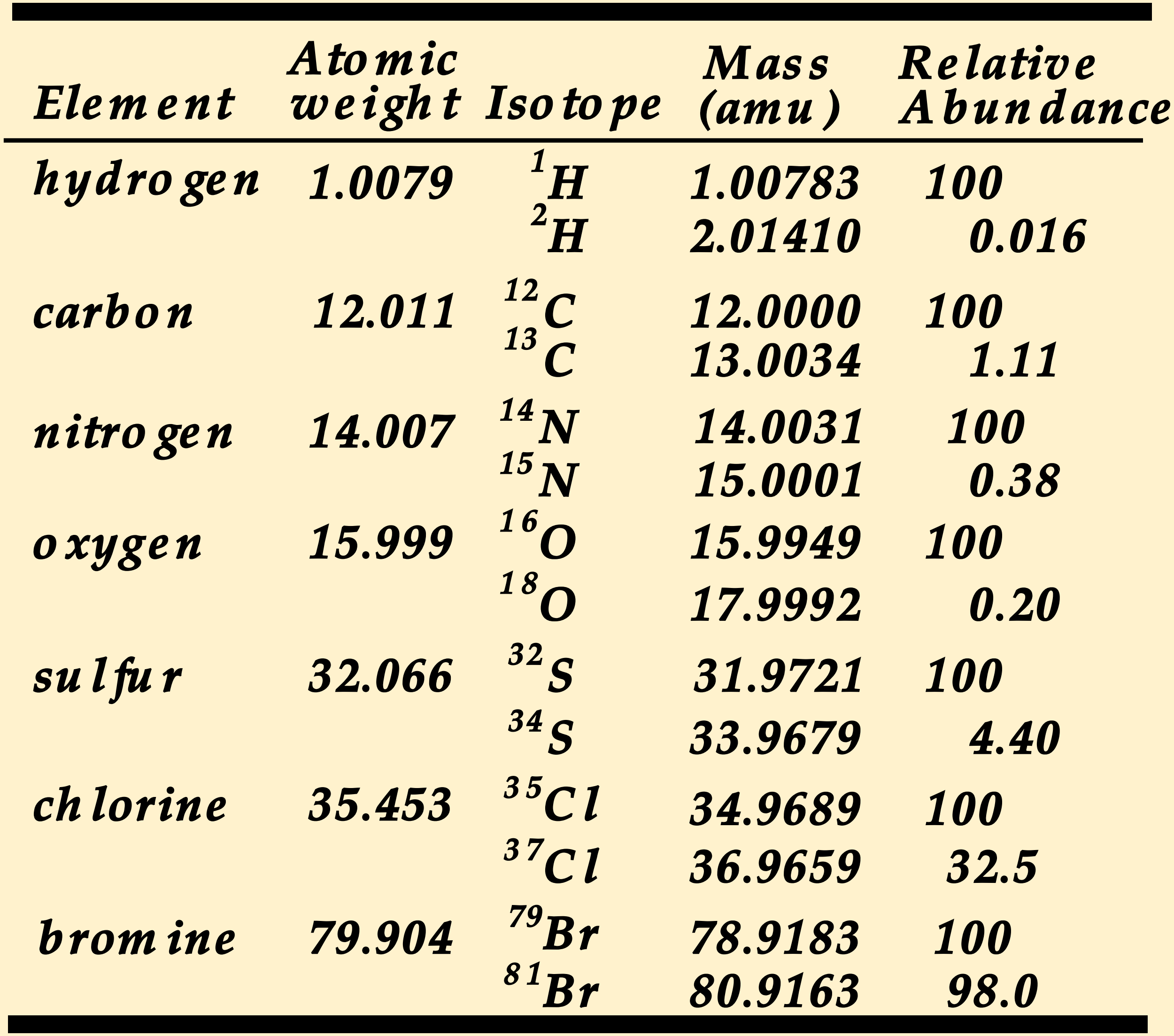
amu = 118

exact mass = 118.0590

Exact Mass takes into account mass of major isotopes to several decimal points

Isotopes

- Virtually all elements common to organic compounds are mixtures of isotopes.



Element	Atomic weight	Isotope	Mass (amu)	Relative Abundance
hydrogen	1.0079	^{1}H	1.00783	100
		^{2}H	2.01410	0.016
carbon	12.011	^{12}C	12.0000	100
		^{13}C	13.0034	1.11
nitrogen	14.007	^{14}N	14.0031	100
		^{15}N	15.0001	0.38
oxygen	15.999	^{16}O	15.9949	100
		^{18}O	17.9992	0.20
sulfur	32.066	^{32}S	31.9721	100
		^{34}S	33.9679	4.40
chlorine	35.453	^{35}Cl	34.9689	100
		^{37}Cl	36.9659	32.5
bromine	79.904	^{79}Br	78.9183	100
		^{81}Br	80.9163	98.0

Resolution

- C_3H_6O and C_3H_8O have nominal masses of 58 and 60, and can be distinguished by low-resolution MS.
- C_3H_8O and $C_2H_4O_2$ both have nominal masses of 60.
- distinguish between them by high-resolution MS.

Molecular Formula	*Nominal Mass*	*Precise Mass*
C_3H_8O	*60*	*60.05754*
$C_2H_4O_2$	*60*	*60.02112*

Isotopes

- Some elements have isotopic ratios that can lead to distinctive patterns in the ms.

Element	Atomic weight	Isotope	Mass (amu)	Relative Abundance
hydrogen	1.0079	^{1}H	1.00783	100
		^{2}H	2.01410	0.016
carbon	12.011	^{12}C	12.0000	100
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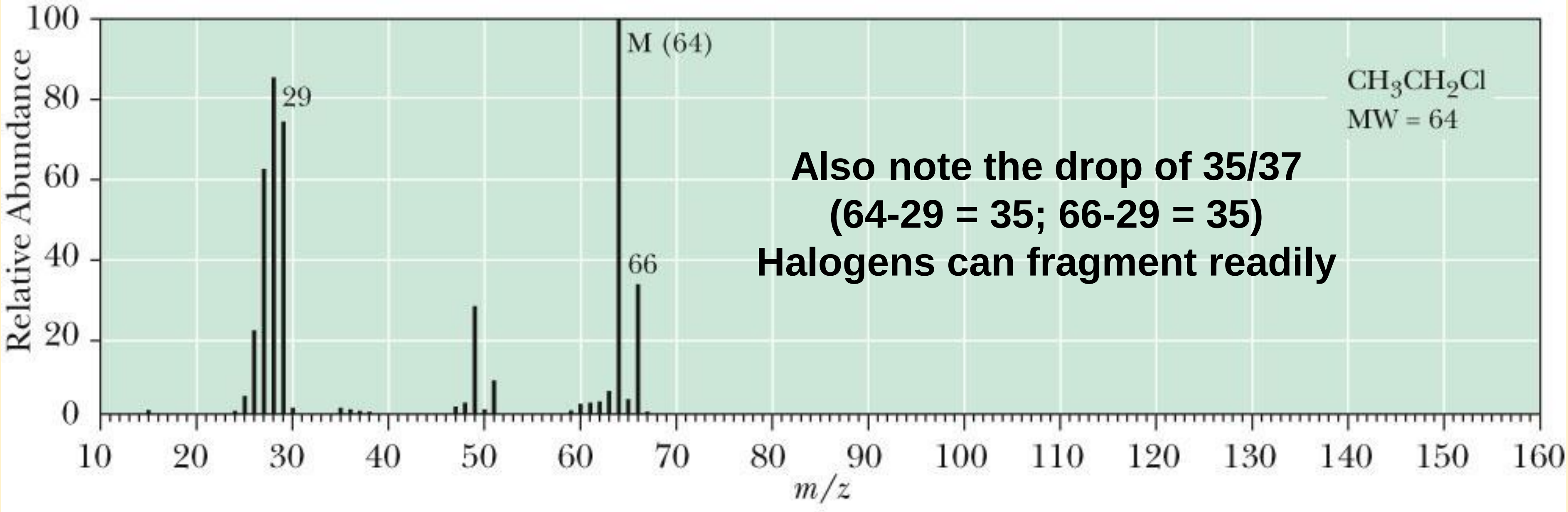
		^{37}Cl	36.9659	32.5
bromine	79.904	^{79}Br	78.9183	100
		^{81}Br	80.9163	98.0

M+2 and M+1 Peaks

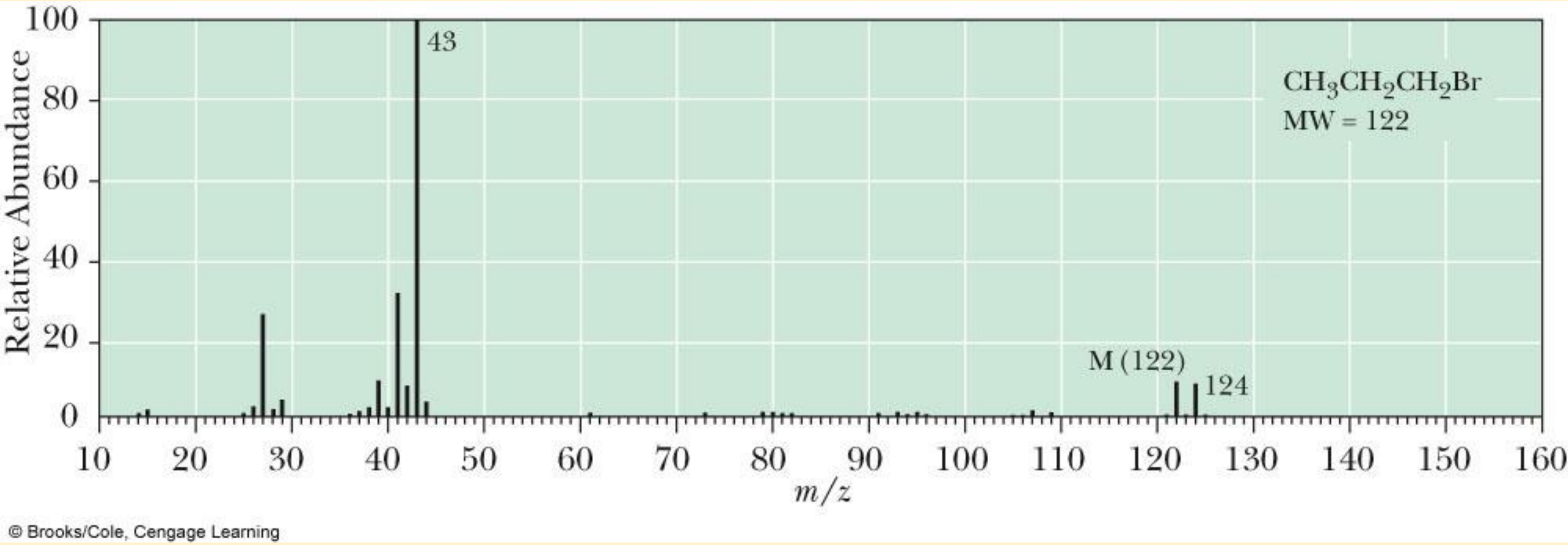
- The most common elements giving rise to significant M + 2 peaks are chlorine and bromine.
  - Chlorine in nature is 75.77% ^{35}Cl and 24.23% ^{37}Cl.
  - A ratio of M to M + 2 of approximately 3:1 indicates the presence of a single chlorine in a compound, as seen in the MS of chloroethane.



Also note the drop of 35/37
(64-29 = 35; 66-29 = 35)
Halogens can fragment readily

M+2 and M+1 Peaks

- Bromine in nature is 50.7% ^{79}Br and 49.3% ^{81}Br.
- A ratio of M to M + 2 of approximately 1:1 indicates the presence of a single bromine atom in a compound, as seen in the MS of 1-bromopropane.



M+2 and M+1 Peaks

- Sulfur is the only other element common to organic compounds that gives a significant M + 2 peak
  - ^{32}S = 95.02% and ^{34}S = 4.21%
- Because M + 1 peaks are relatively low in intensity compared to the molecular ion and often difficult to measure with any precision, they are generally not useful for accurate determinations of molecular weight.

Molecular Ions and Interpreting a mass spectrum

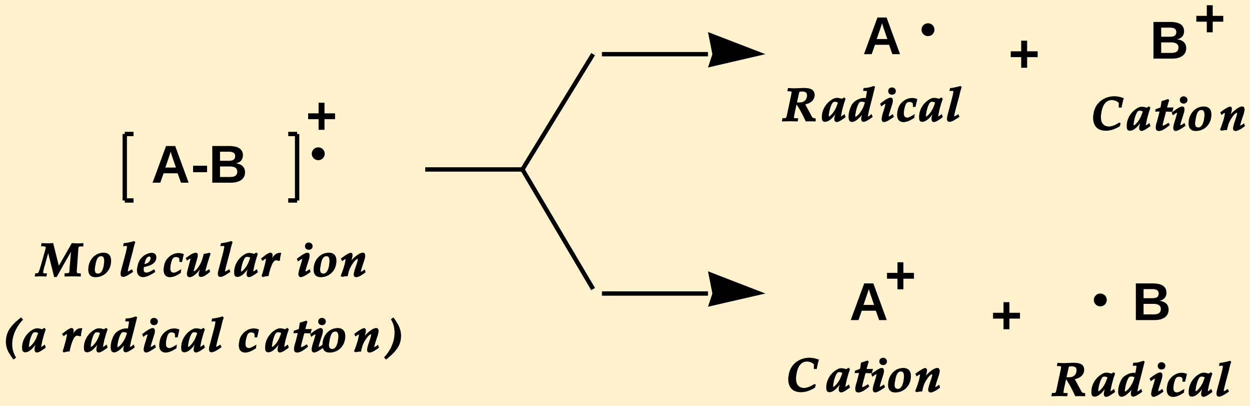
- The only elements to give significant M + 2 peaks are Cl and Br.
  - If no large M + 2 peak is present, these elements are absent.
- Is the mass of the molecular ion odd or even?
- **Nitrogen Rule:** If a compound has
  - zero or an even number of nitrogen atoms, its molecular ion will have an even *m*/*z* value.
  - an odd number of nitrogen atoms, its molecular ion will have an odd *m*/*z* value.

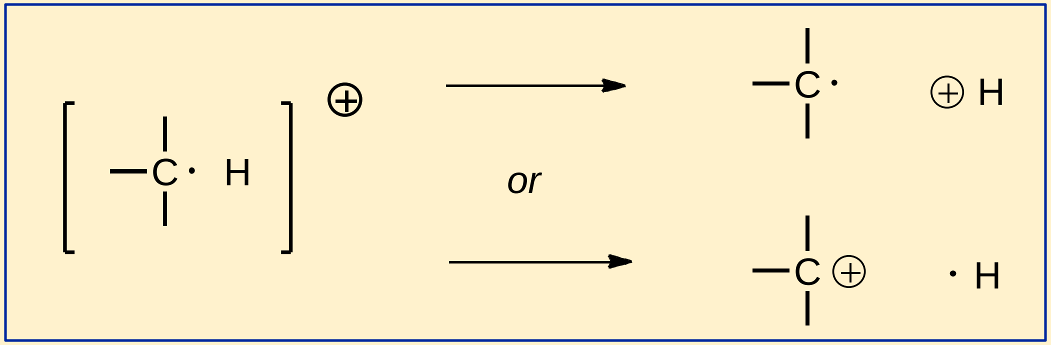
Fragmentation of the Molecular Ion

- To attain high efficiency of molecular ion formation and give reproducible mass spectra, it is common to use electrons with energies of approximately 70 eV [6750 kJ (1600 kcal)/mol].
  - This energy is sufficient not only to dislodge one or more electrons from a molecule, but also to cause extensive fragmentation.
  - These fragments may be unstable as well and, in turn, break apart to even smaller fragments.

Fragmentation of M

- Fragmentation of a molecular ion, M, produces a radical and a cation.
  - Only the cation is detected by MS.





Fragmentation of M

- **A great deal of the chemistry of ion fragmentation can be understood in terms of the formation and relative stabilities of carbocations in solution.**
  - **Where fragmentation occurs to form new cations, the mode that gives the most stable cation is favored.**
  - **The probability of fragmentation to form new carbocations increases in the order.**

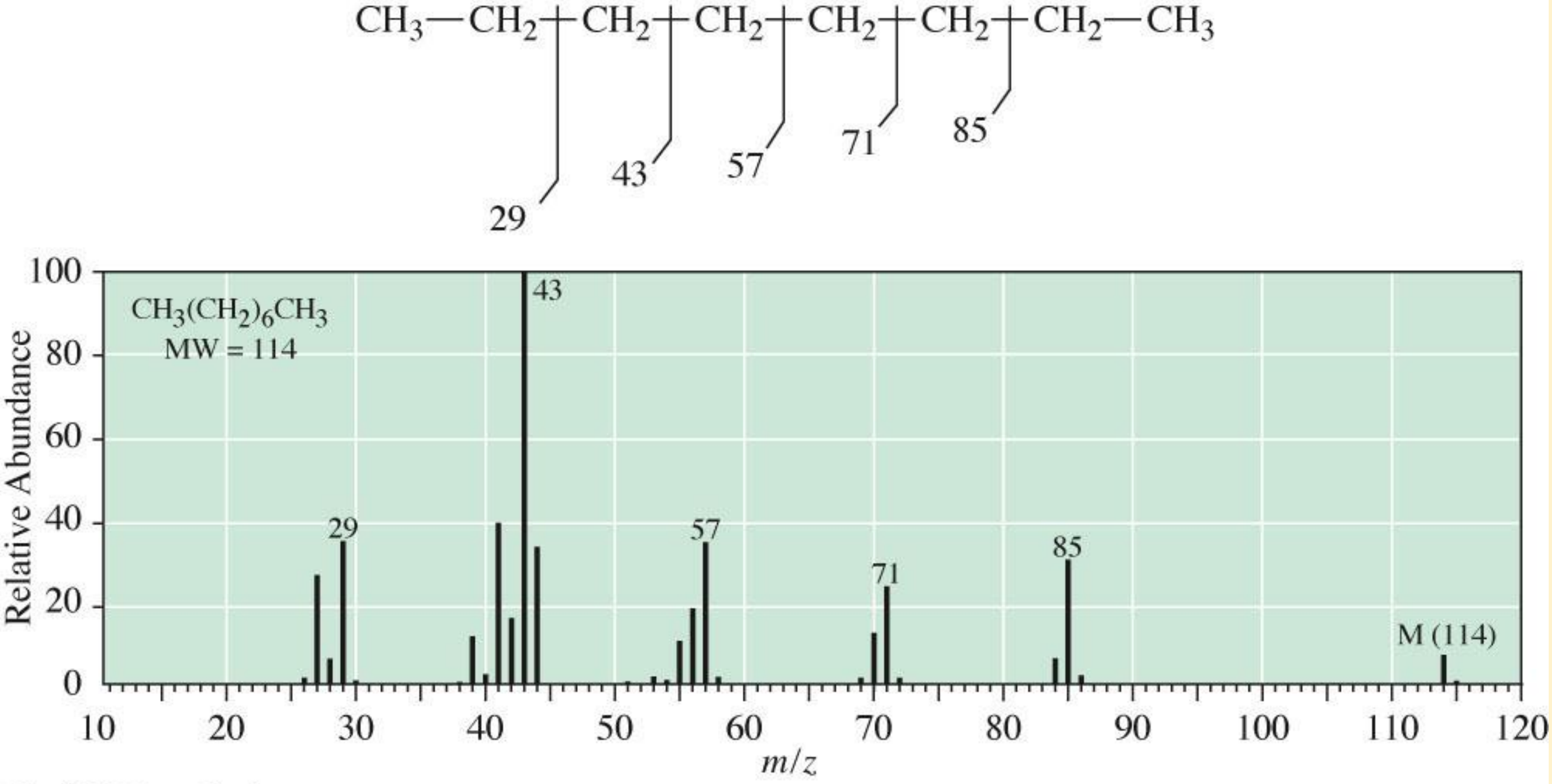
CH_3^+ < 1° < 2°, 1° allylic, 1° benzylic < 3°, 2° allylic, 2° benzylic < 3° allylic, 3° benzylic

Alkanes

- Fragmentation tends to occur in the middle of unbranched chains rather than at the ends.
- The difference in energy among allylic, benzylic, 3°, 2°, 1°, and methyl cations is much greater than the difference among comparable radicals.
  - Where alternative modes of fragmentation are possible, the more stable carbocation tends to form in preference to the more stable radical.

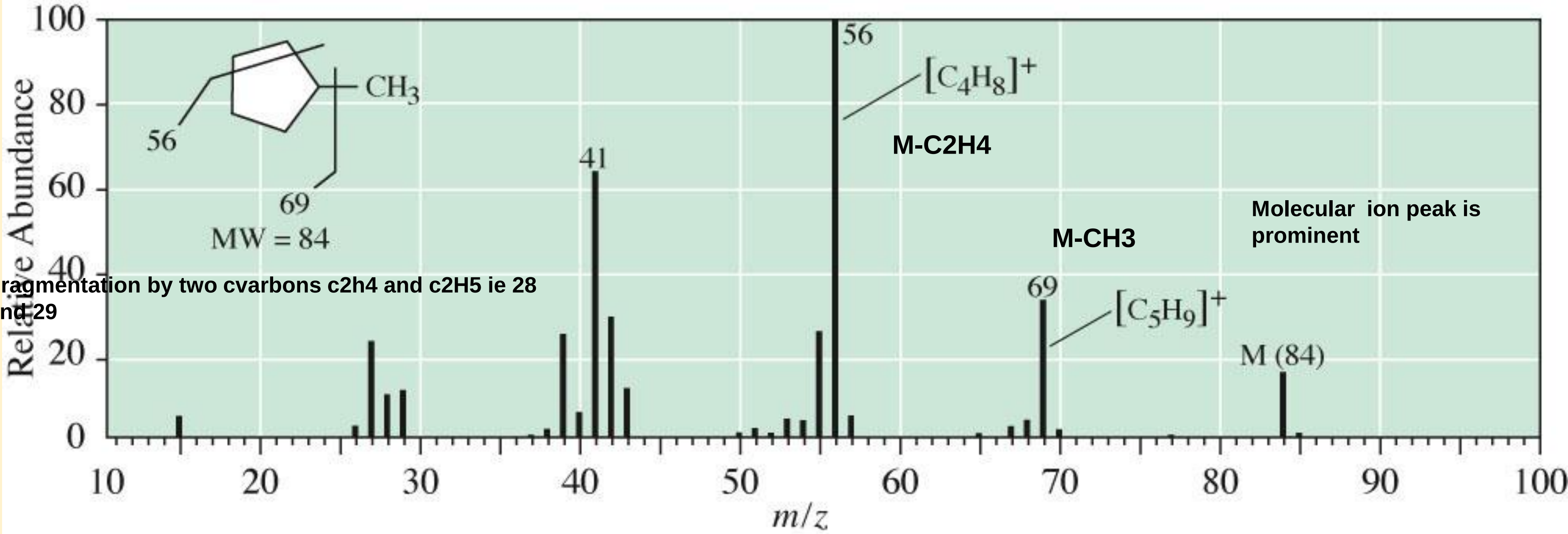
Alkanes

- **Mass spectrum of octane.**



Alkanes

- **Mass spectrum of methylcyclopentane.**



M-C2H4

M-CH3

Molecular ion peak is prominent

Fragmentation by two cvarbons c2h4 and c2H5 ie 28 and 29

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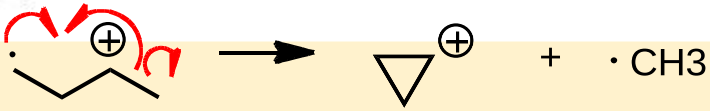
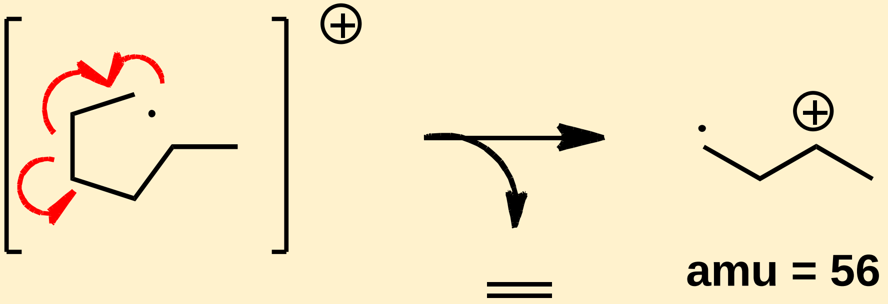


amu = 41

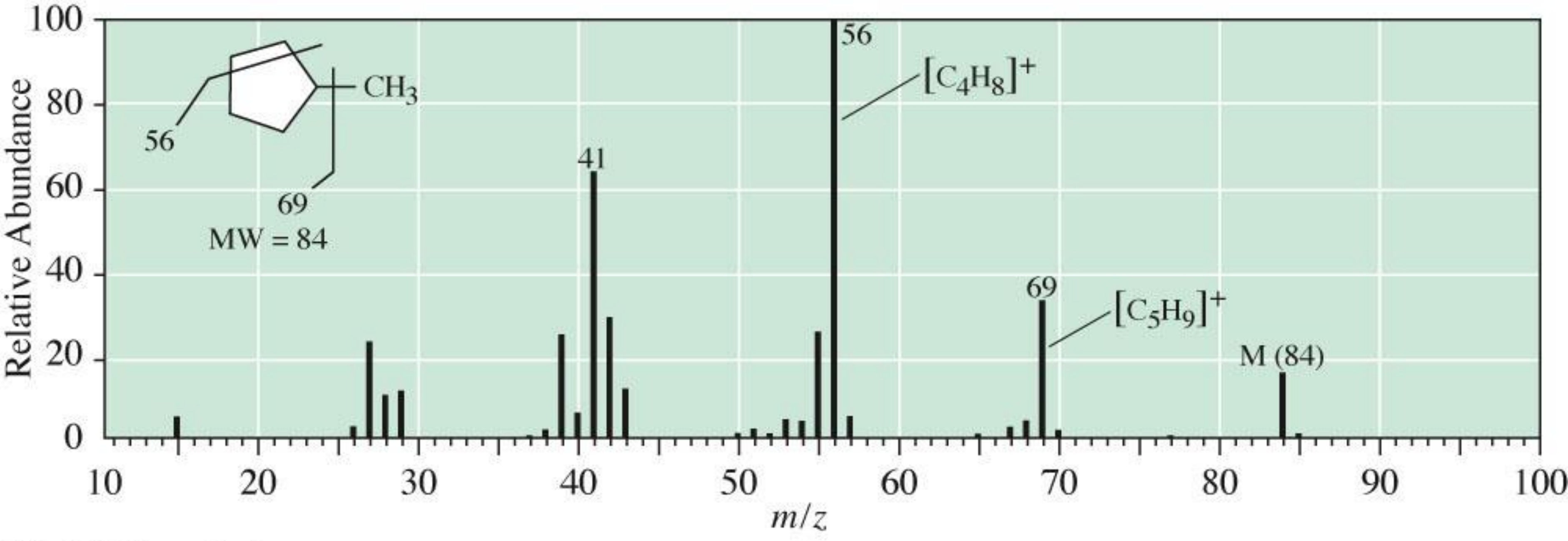
What about 41?



amu = 56

Alkanes

- **Mass spectrum of methylcyclopentane.**



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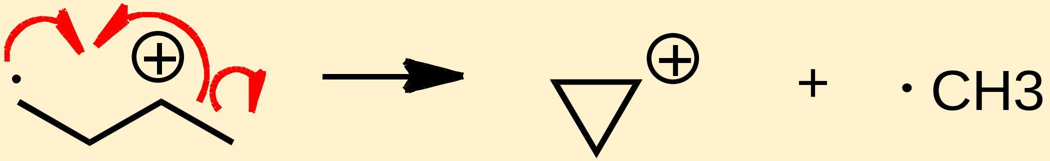
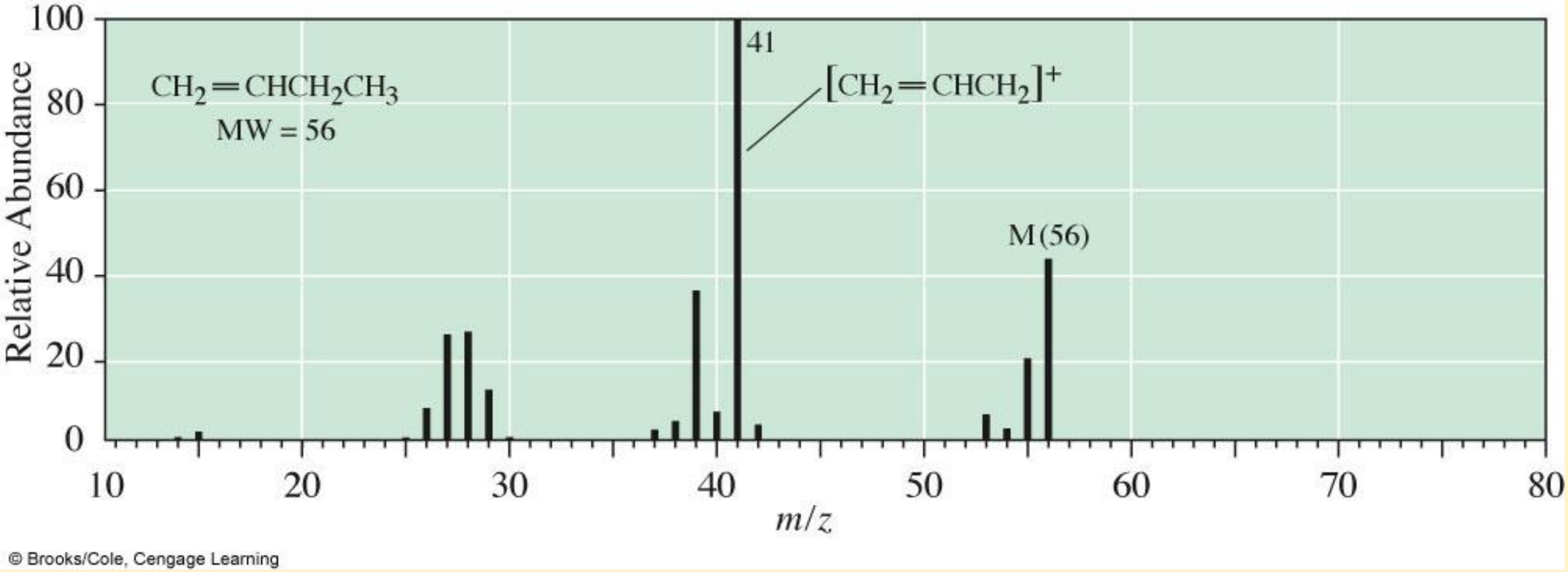


amu = 41

Alkenes

- **Alkenes characteristically**
  - **show a strong molecular ion peak.**
  - **cleave readily to form resonance-stabilized allylic cations.**

$$[CH_2{=}CHCH_2CH_2CH_3]^{\bullet+} \longrightarrow CH_2{=}CHCH_2^{\,+} + \cdot CH_2CH_3$$



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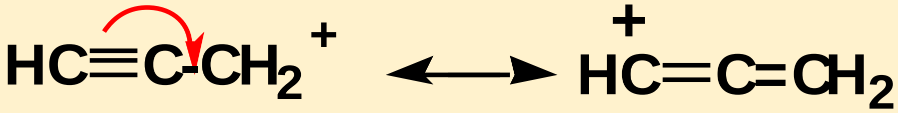
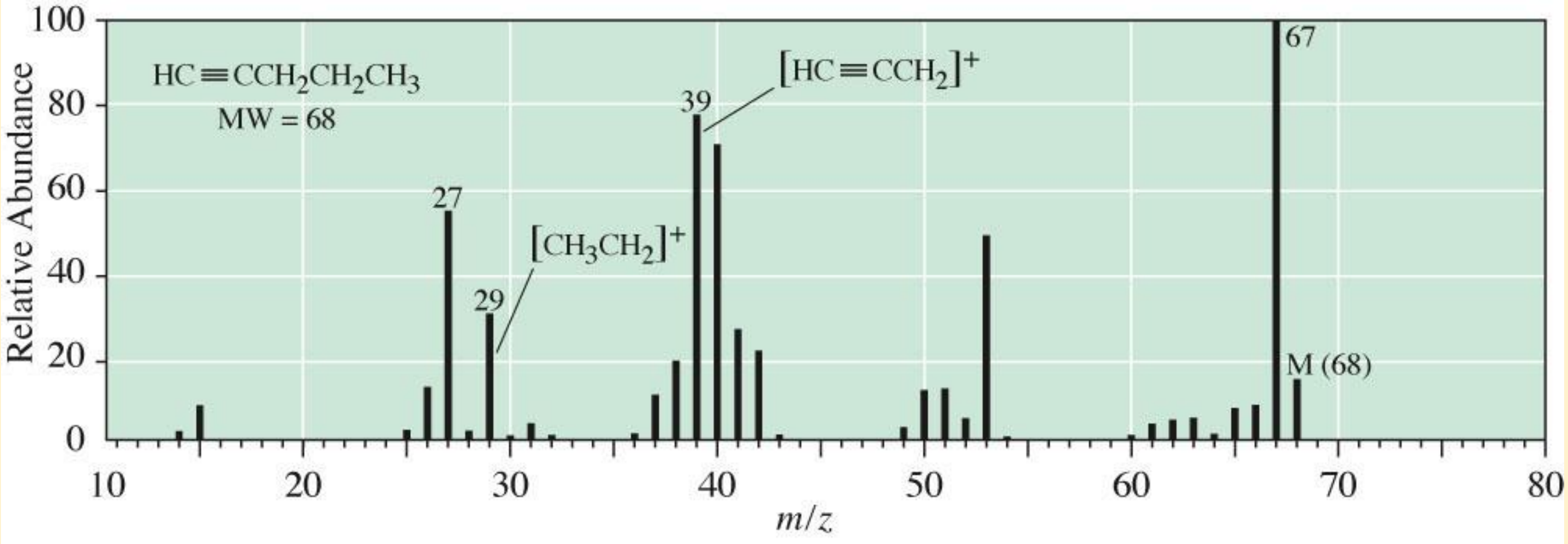
Alkynes

- **Alkynes characteristically**
  - **show a strong molecular ion peak.**
  - **cleave readily to form the resonance-stabilized propargyl cation or substituted propargyl cations.**

3-Propynyl cation (Propargyl cation)

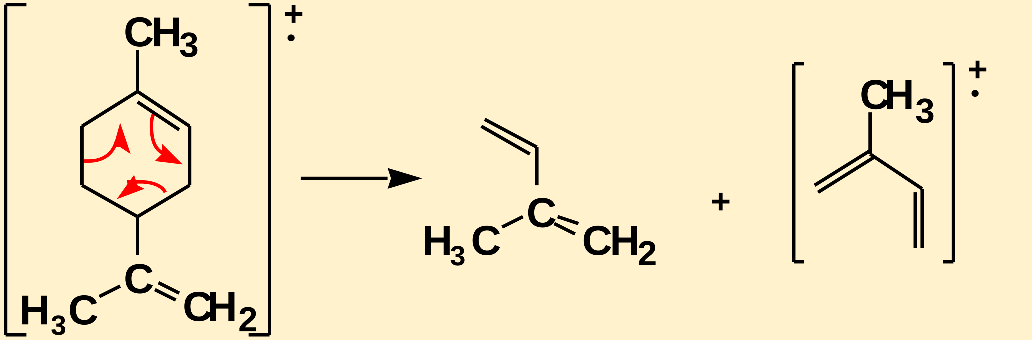


$$HC{\equiv}C{-}CH_2^{\,+} \longleftrightarrow H\overset{+}{C}{=}C{=}CH_2$$



Cyclohexenes

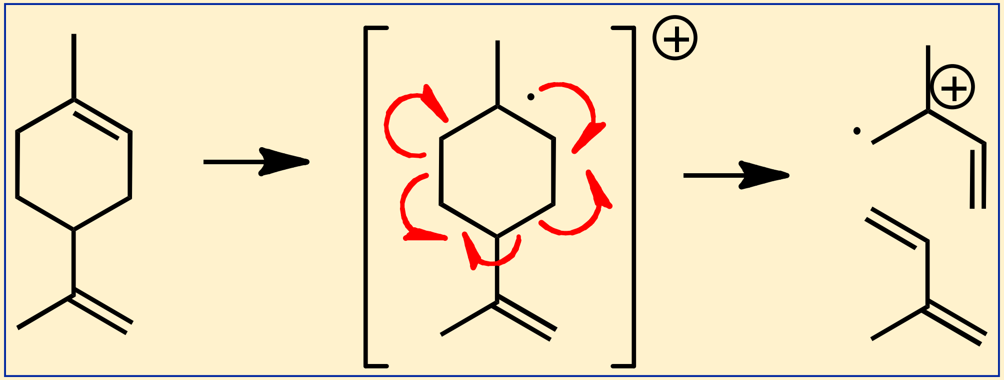
- Cyclohexenes give a 1,3-diene and an alkene, a process that is the reverse of a Diels-Alder reaction (Chapter 24).



Limonene *(m/z 136)*

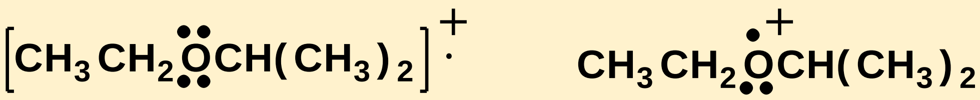
A neutral diene *(m/z 68)*

A radical cation *(m/z 68)*



Ionization of Lone Pair-Containing Molecules

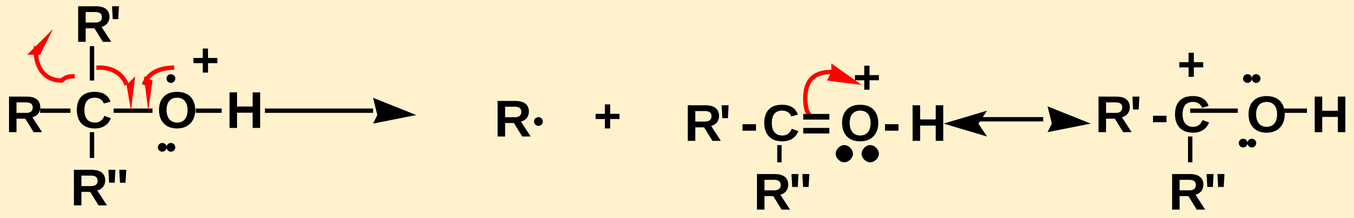
- **Perhaps not surprisingly, when radicals will be taken out of lone-pair containing molecules, it is the lone pair that can often lose the electron.**



$\left[CH_3CH_2\overset{\cdot\cdot}{\underset{\cdot\cdot}{O}}CH(CH_3)_2\right]^{+\cdot}$ $CH_3CH_2\overset{\cdot\,+}{\underset{\cdot\cdot}{O}}CH(CH_3)_2$

Alcohols

- One of the most common fragmentation patterns of alcohols is loss of H_2O to give a peak which corresponds to M-18.
- Another common pattern is loss of an alkyl group from the carbon bearing the OH to give a resonance-stabilized oxonium ion and an alkyl radical.

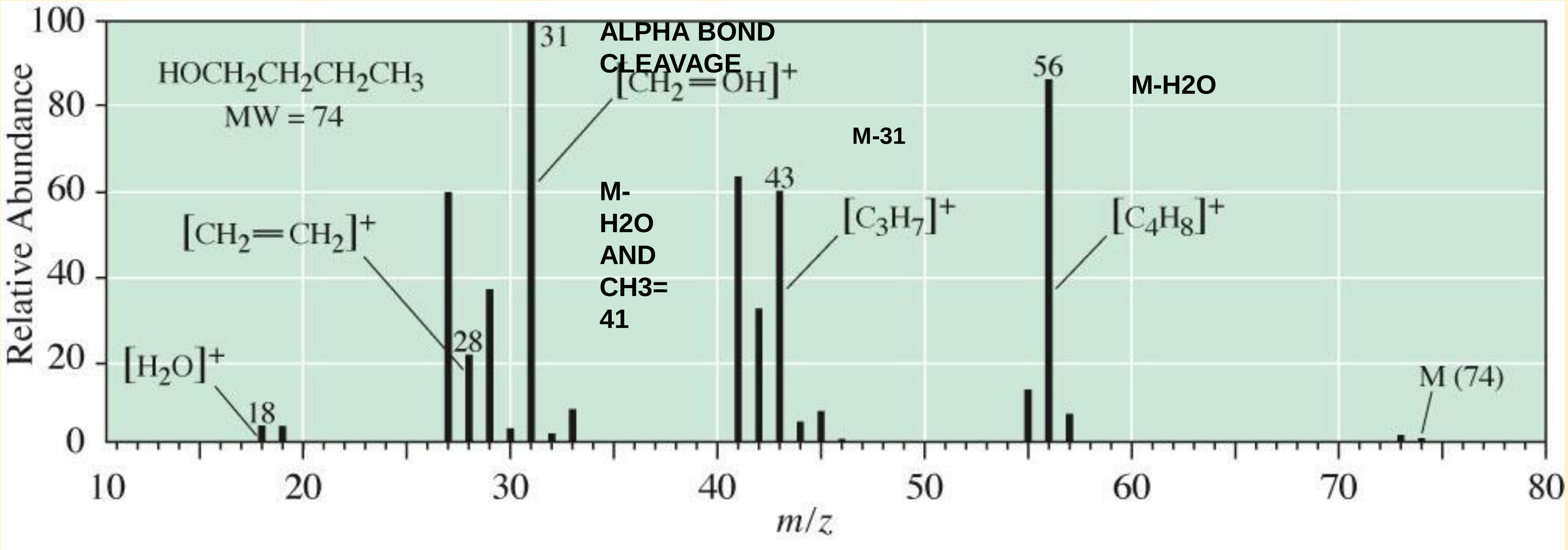


Molecular ion (a radical cation)   *A radical*   *A resonance-stabilized oxonium ion*

Similar Rearrangements for Amines

Alcohols

- **Mass spectrum of 1-butanol.**



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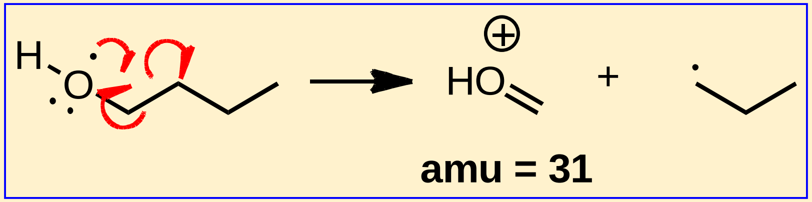
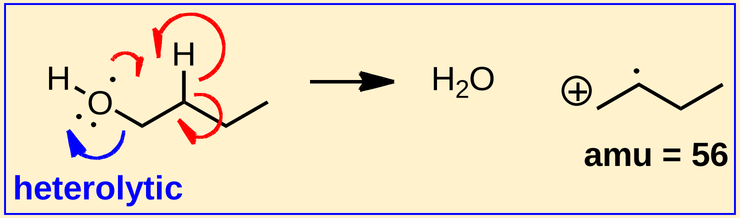


amu = 31



heterolytic

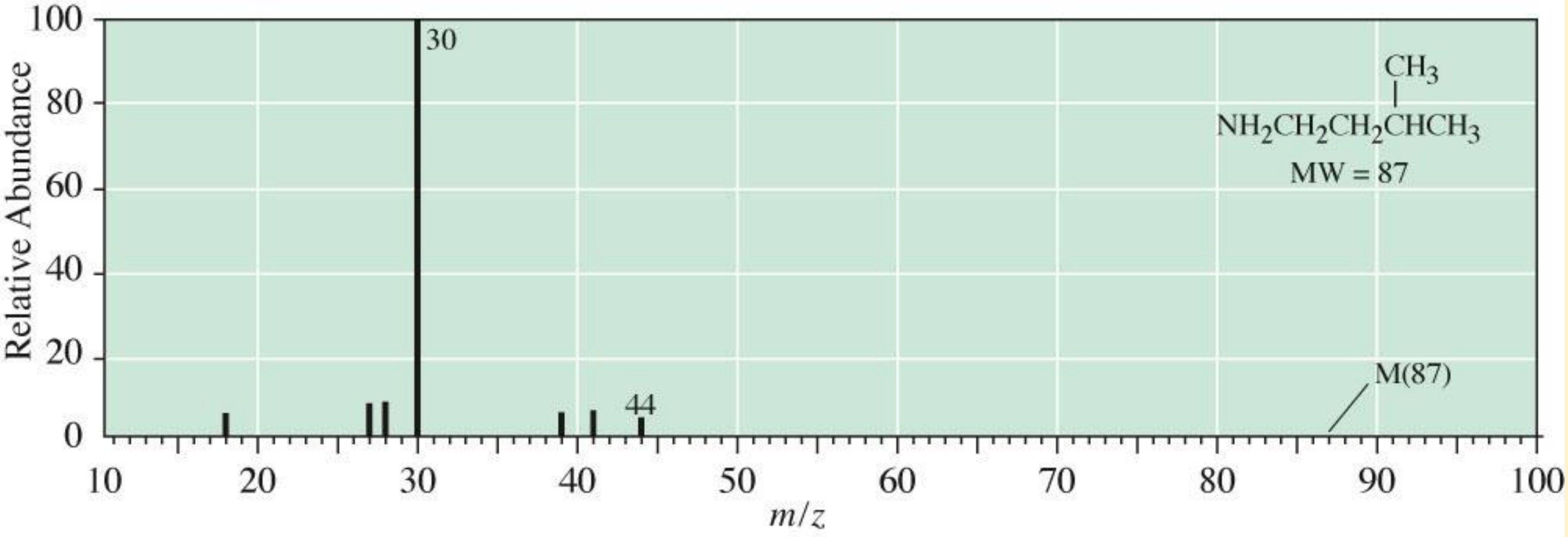
amu = 56

Amines

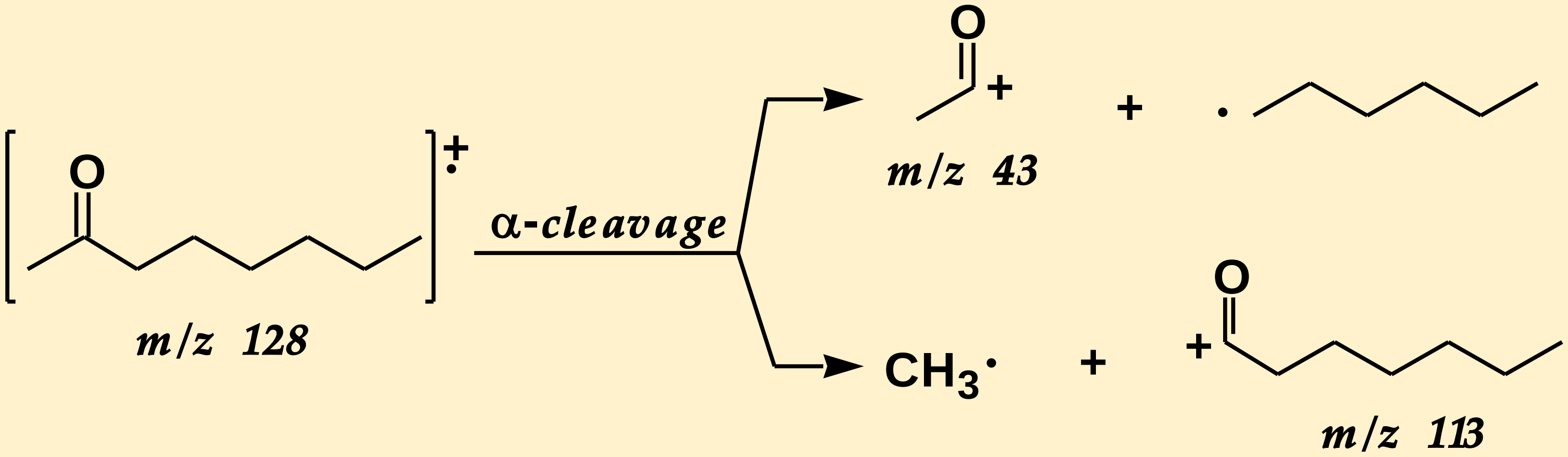
- The most characteristic fragmentation pattern of 1°, 2°, and 3° aliphatic amines is β-cleavage.

$$\mathrm{CH_3-\overset{CH_3}{\overset{|}{C}}H-CH_2-CH_2-\overset{\bullet\ +}{N}H_2 \xrightarrow{\beta\text{-cleavage}} CH_3-\overset{CH_3}{\overset{|}{C}}H-CH_2^{\bullet} + CH_2=\overset{+}{N}H_2}$$

m/z 30

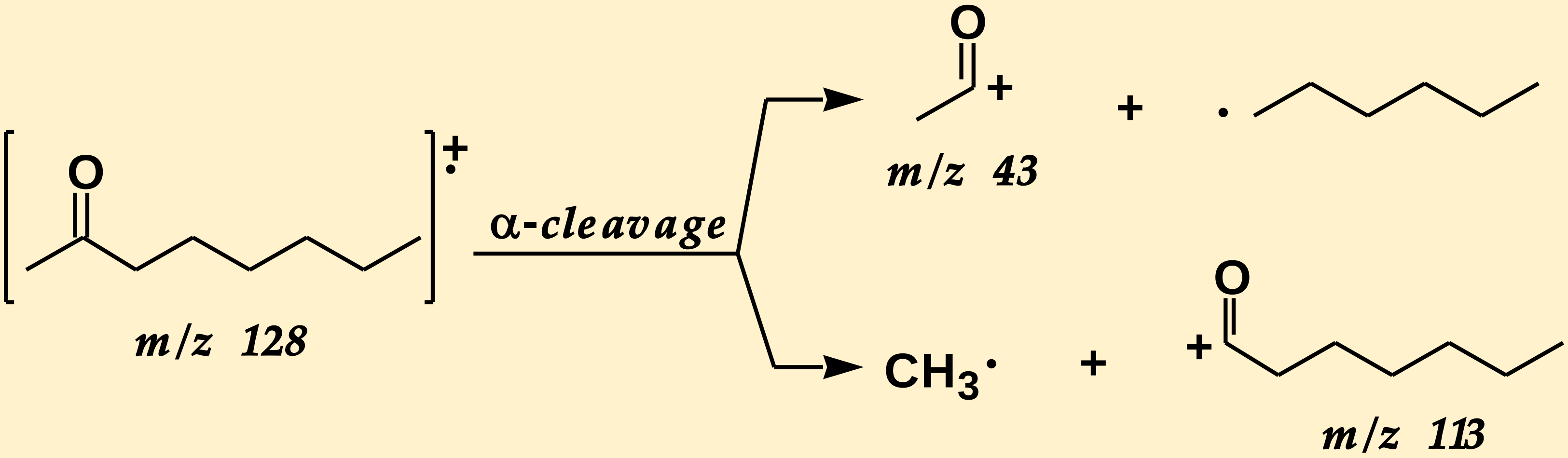


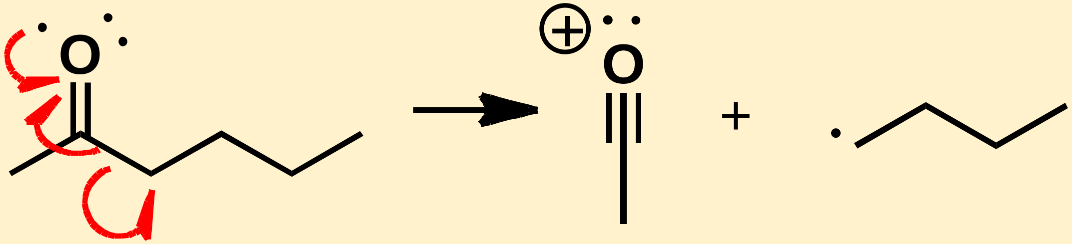
Carbonyls



- Carbonyls can also fragment. Propose a mechanism for this transformation

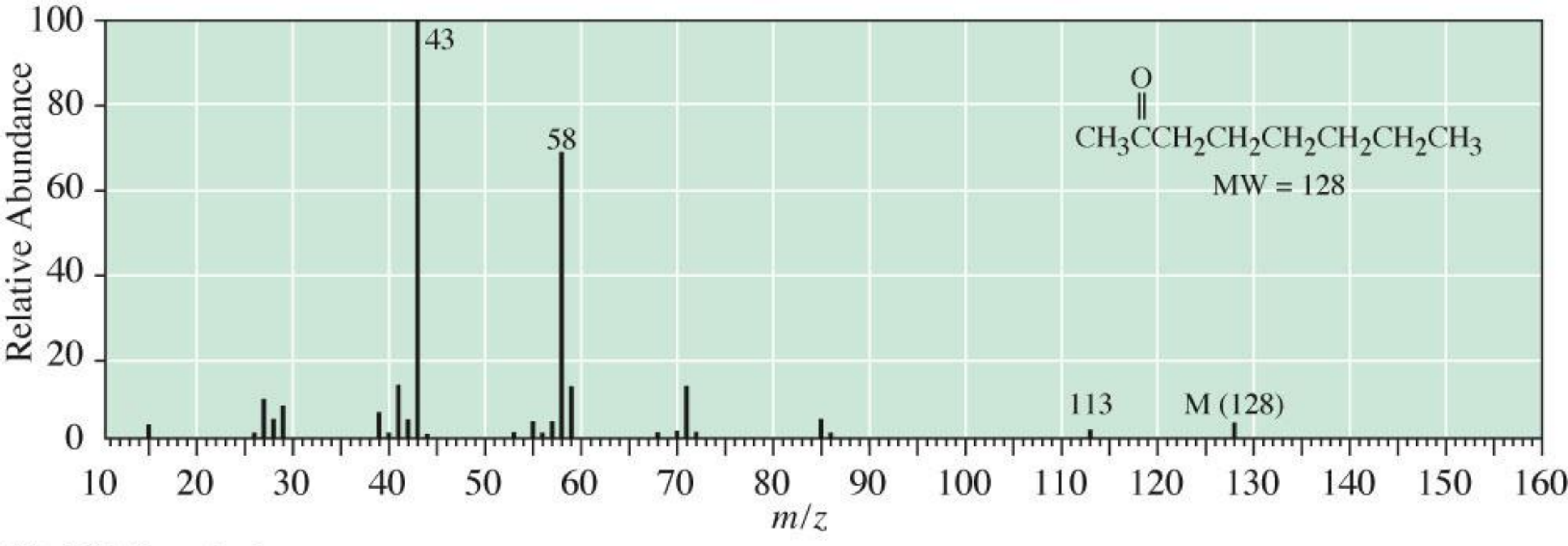
Carbonyls





Or alternatively, you could push arrows other way to methyl radical

Aldehydes and Ketones



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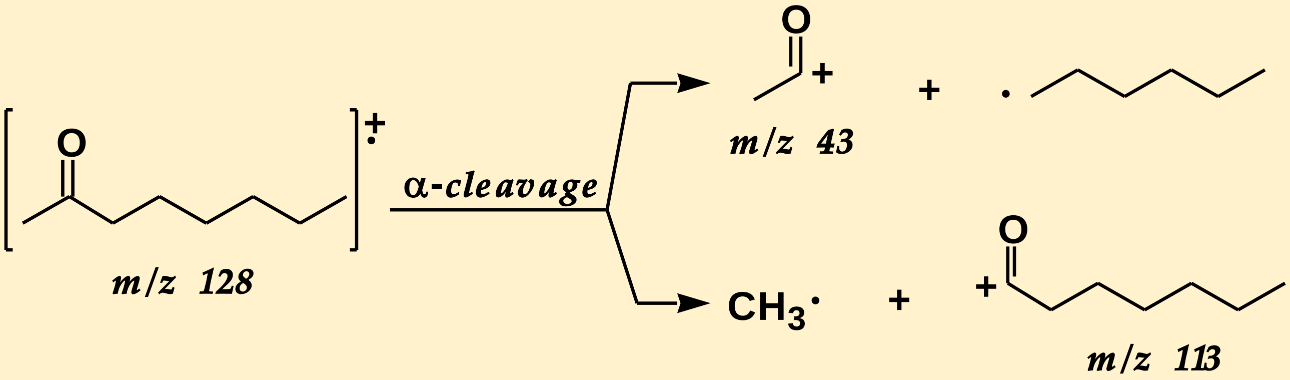
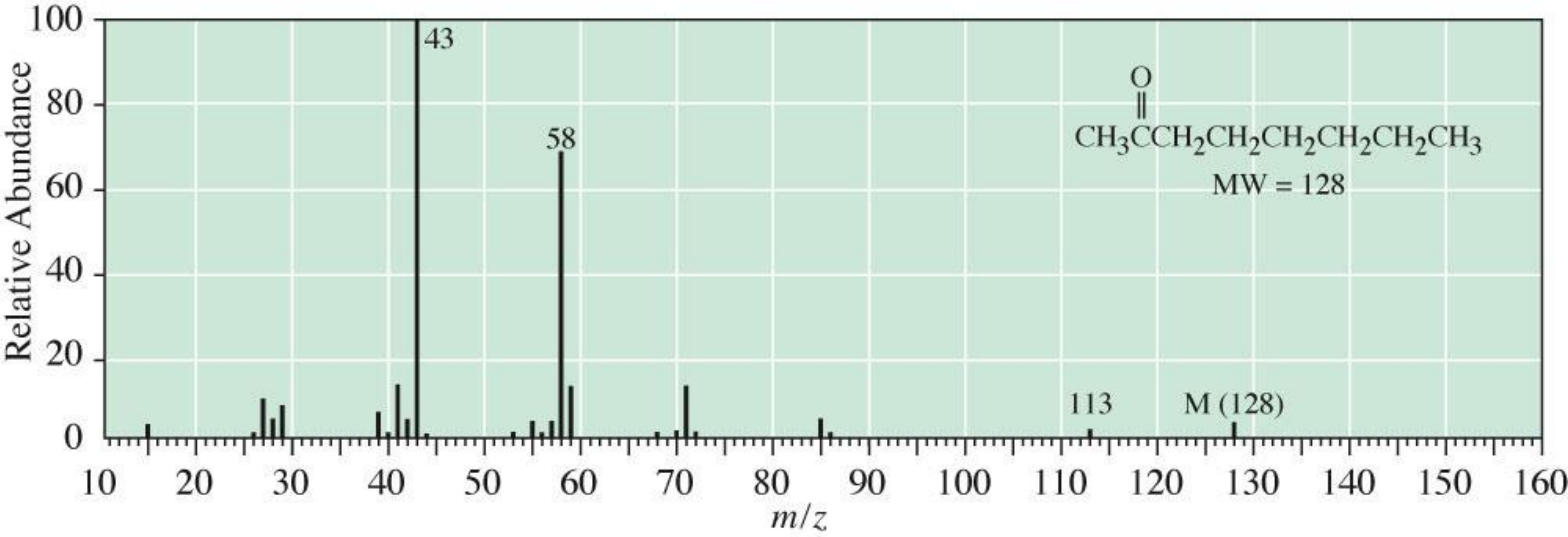


Why might you expect 43 be larger then 113?

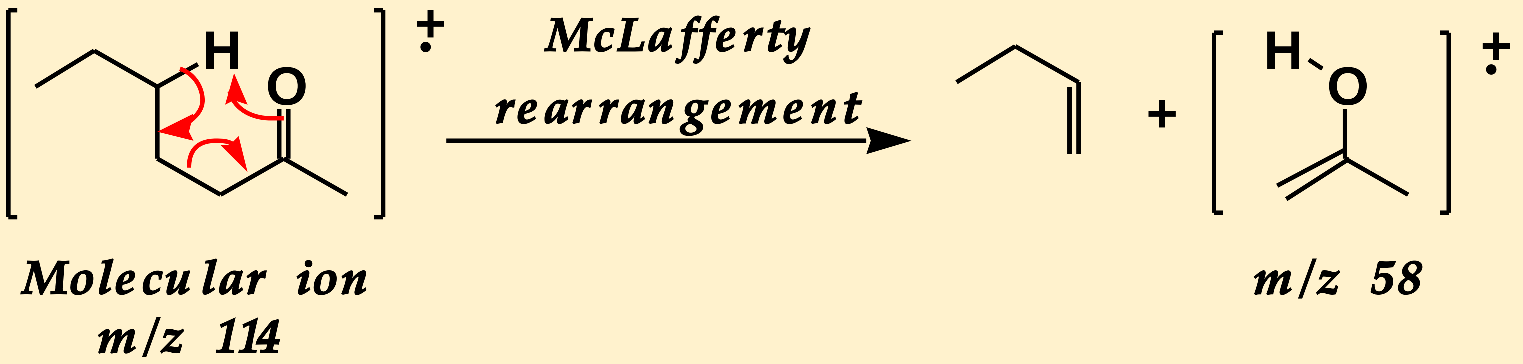
Aldehydes and Ketones



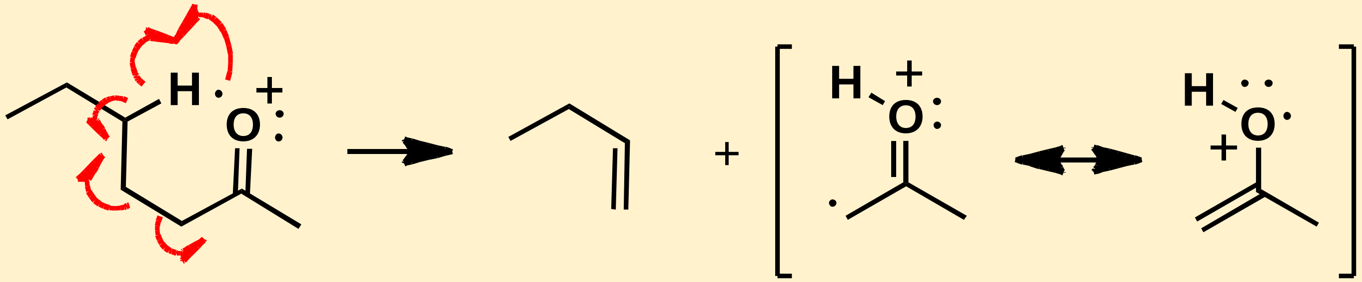
Molecular ion
m/z 114

m/z 58

Aldehydes and Ketones

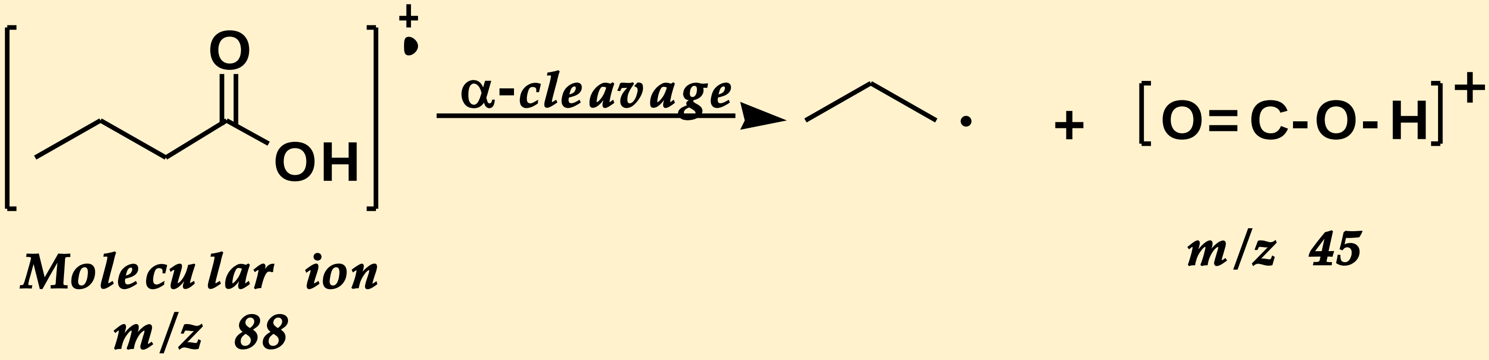


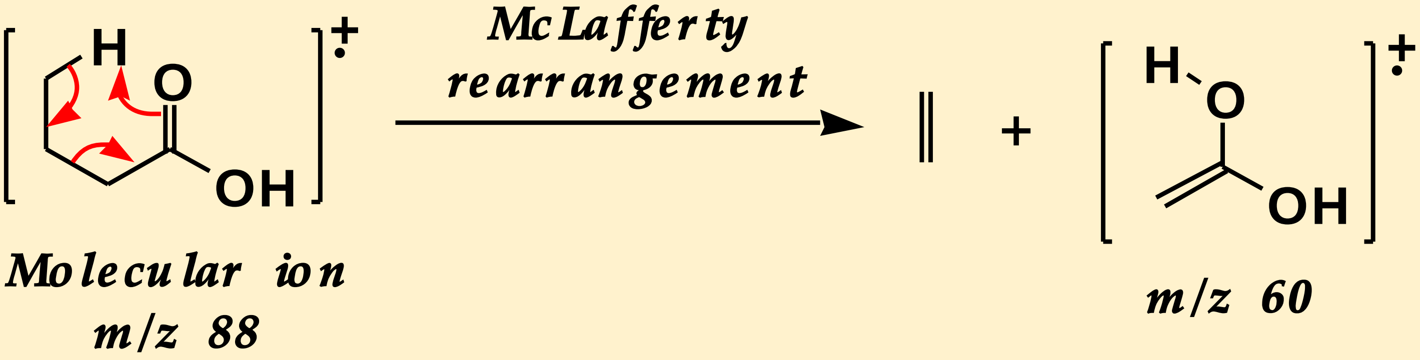
Who’s to say you can’t draw it as a radical mechanism too?



Carboxylic Acids

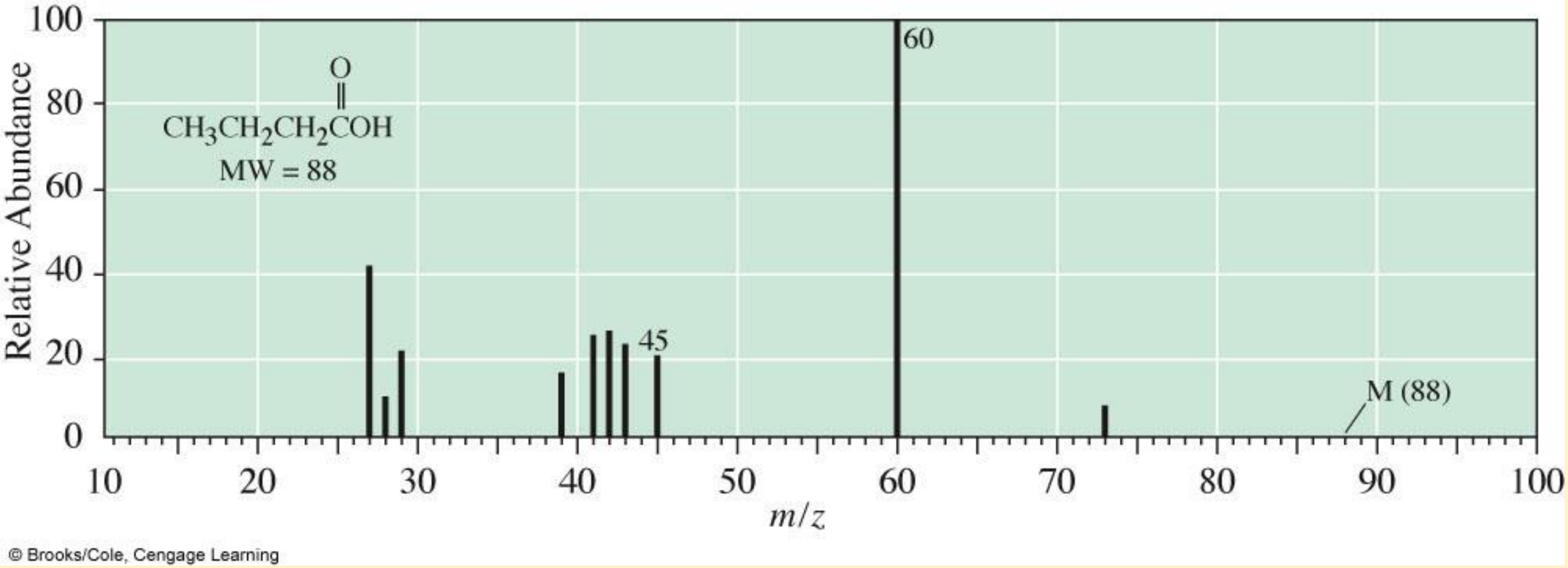
- **Characteristic fragmentation patterns are**
  - **α-cleavage to give the ion $[CO_2H]^+$ with *m/z* 45.**
  - **McLafferty rearrangement.**





Carboxylic Acids

- **Mass spectrum of butanoic acid.**



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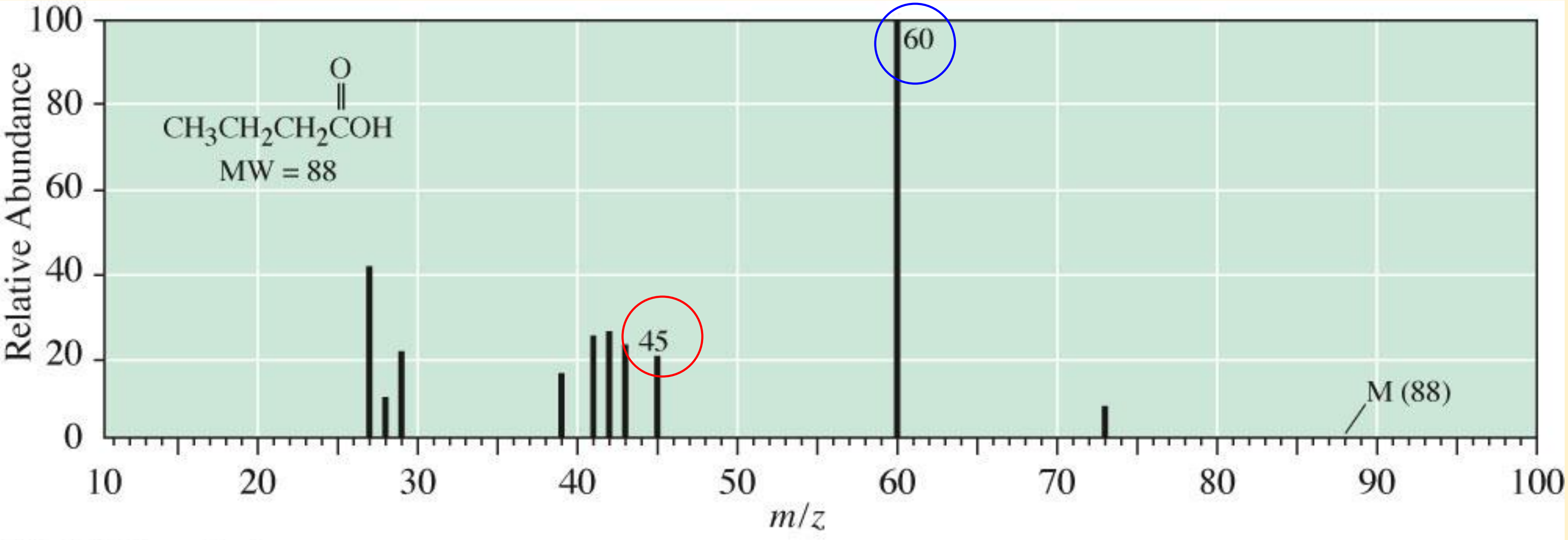
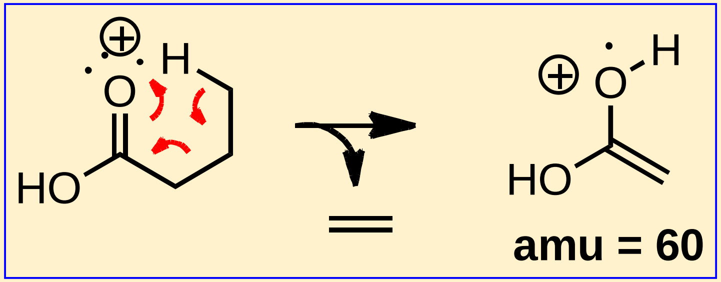
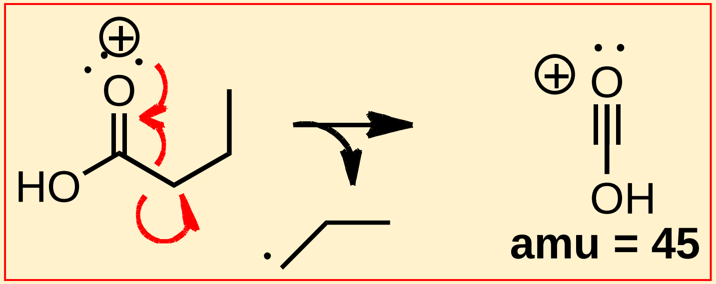
What are the fragmentations at 60 and 45?

Carboxylic Acids

- **Mass spectrum of butanoic acid.**

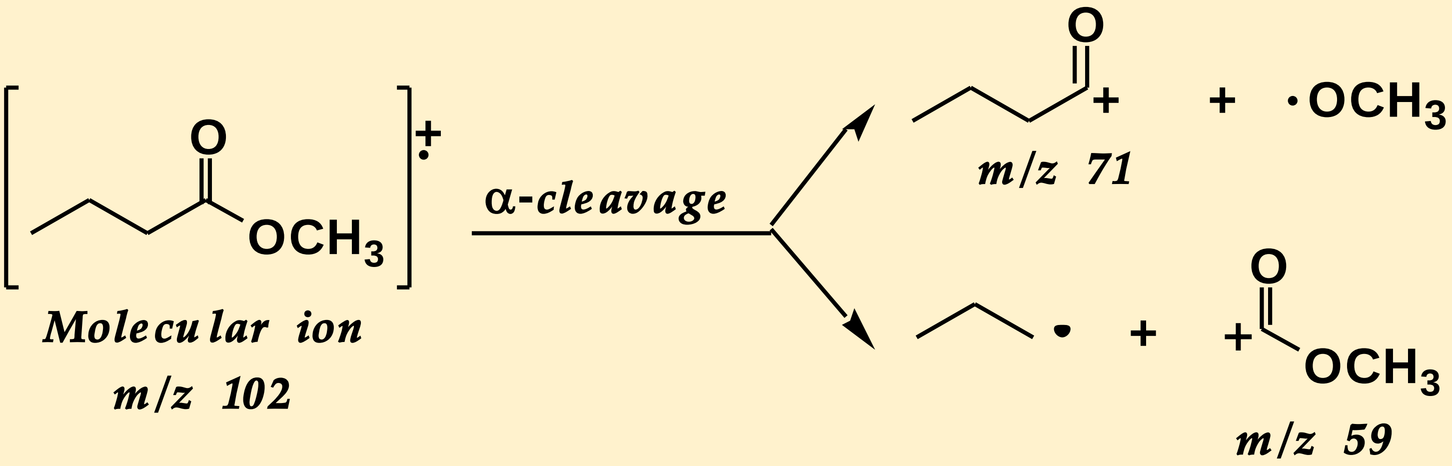


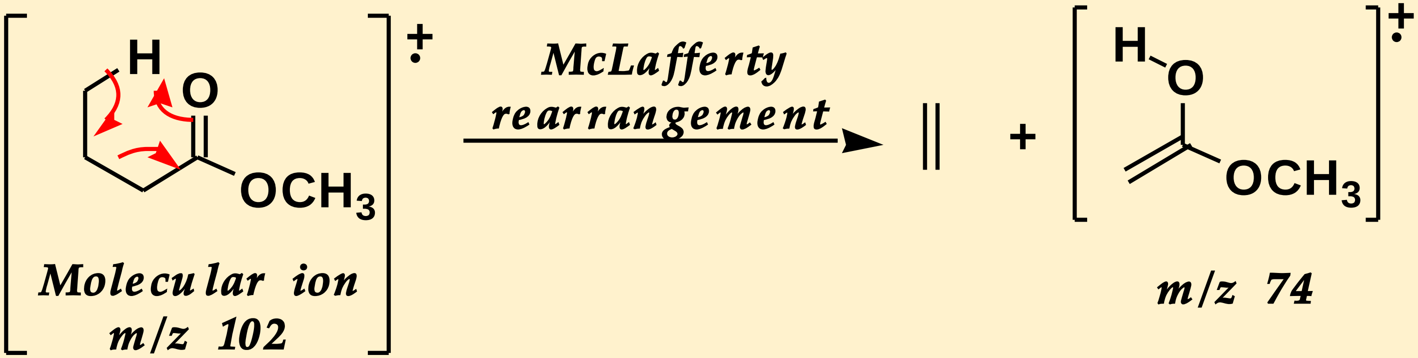




Esters

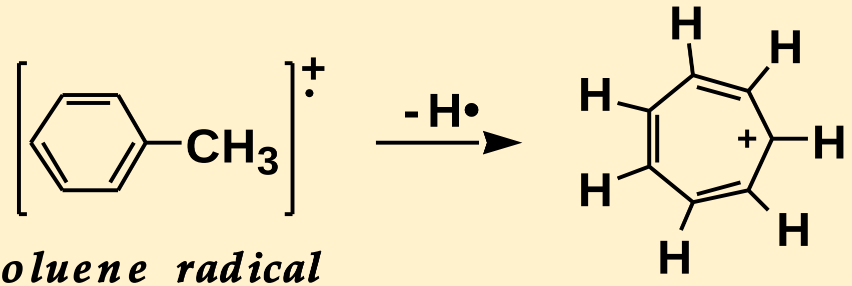
- α-cleavage and McLafferty rearrangement





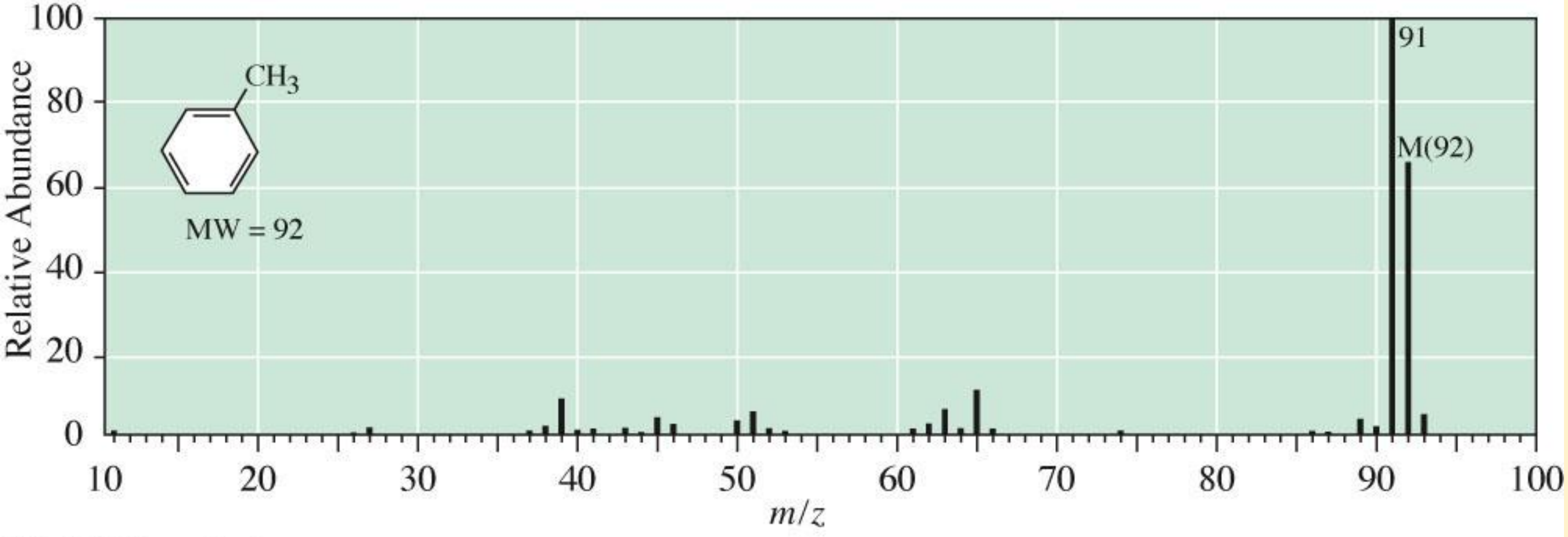
Aromatic Hydrocarbons

- **Most show an intense molecular ion peak.**
- **Most alkylbenzenes show a fragment ion of *m/z* 91.**



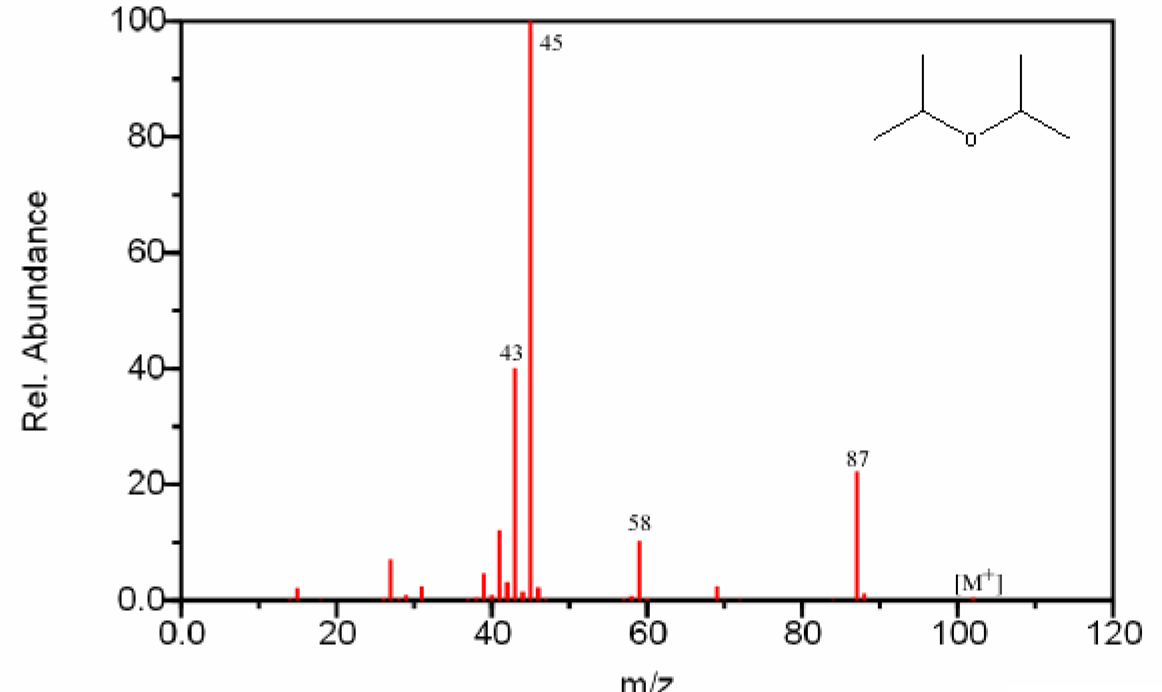
Toluene radical cation

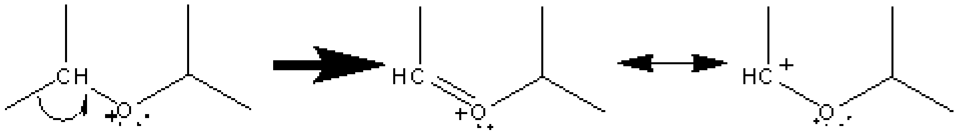
Tropylium cation (m/z 91)



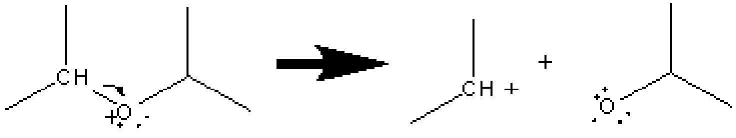
- **13 Fragmentation of Ethers**
- **The molecular ion peak is usually weak in ethers. The oxygen atom mediates the major fragment and creates a β cleavage that results in a resonance stabilized cation. This peak is prominent and sometimes is the base peak.**
- **The fragment can also undergo a subsequent rearrangement which typically creates the base peak when the α carbon is substituted.**
- **Ethers also produce prominent alkyl fragments when the C–O bond (α bond) is broken and the fragment containing oxygen is a radical.**

ethers

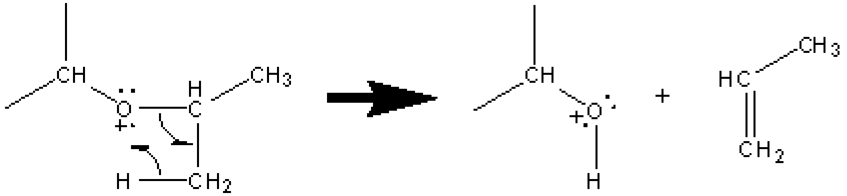




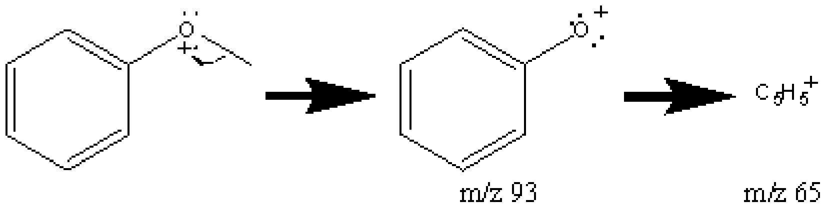
m/z 87

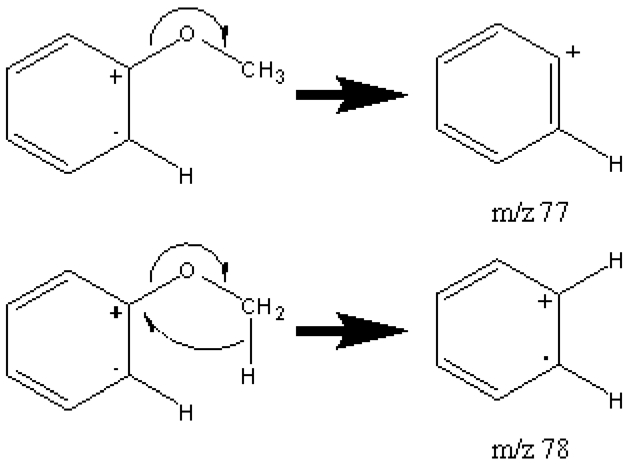


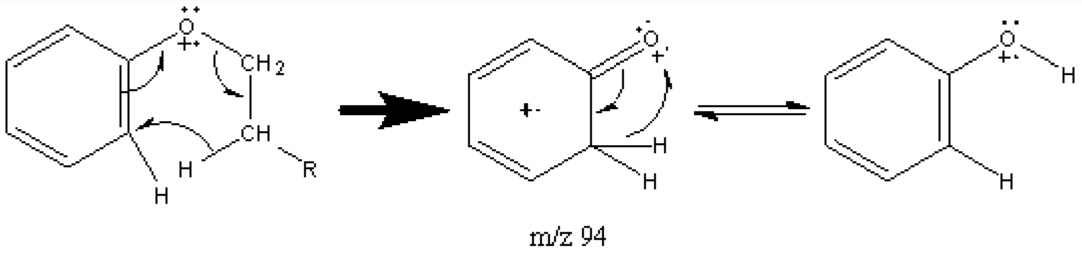
m/z 43



m/z 58


m/z 93
C5H5+
m/z 65


CH3
H
m/z 77
CH2
H
H
H
H
m/z 78


CH2
CH
H
H
R
H
H
m/z 94
H
H

THANK YOU