Mass Spec

CYL703

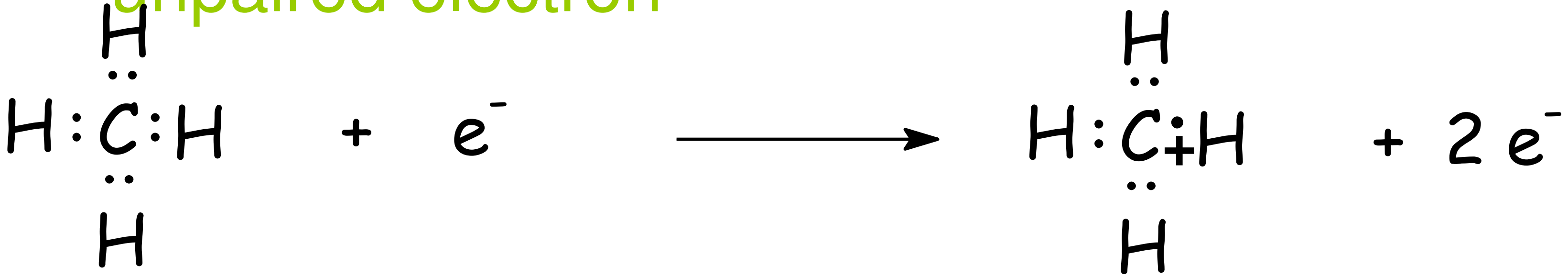
Background

- Mass spectrometry (Mass Spec or MS) uses high energy electrons to break a molecule into fragments.

- Separation and analysis of the fragments provides information about:
  - Molecular weight
  - Structure

Background

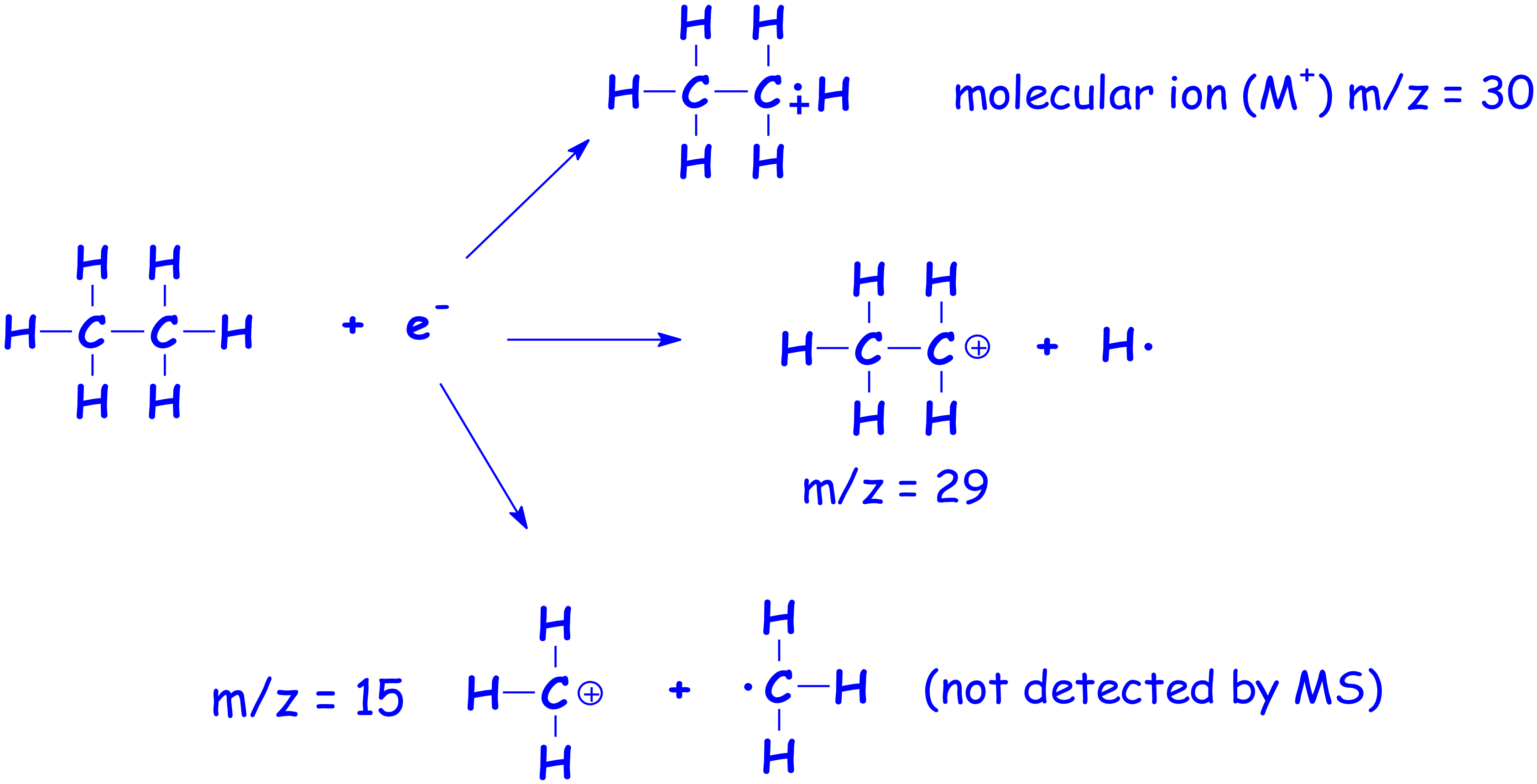
- The impact of a stream of high energy electrons causes the molecule to lose an electron forming a radical cation.
  - A species with a positive charge and one unpaired electron



Molecular ion (M^+)
$m/z = 16$

Background

- The impact of the stream of high energy electrons can also break the molecule or the radical cation into fragments.



molecular ion (M^+) m/z = 30

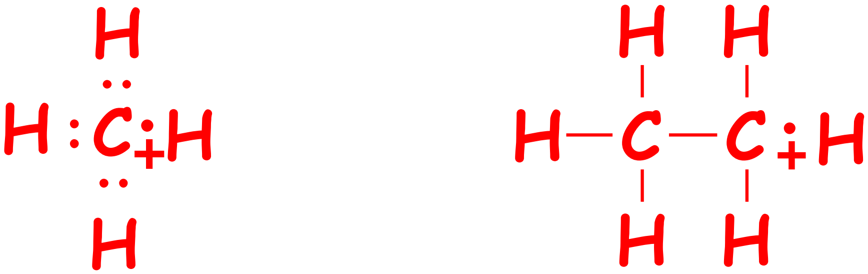
m/z = 29

m/z = 15

(not detected by MS)

Background

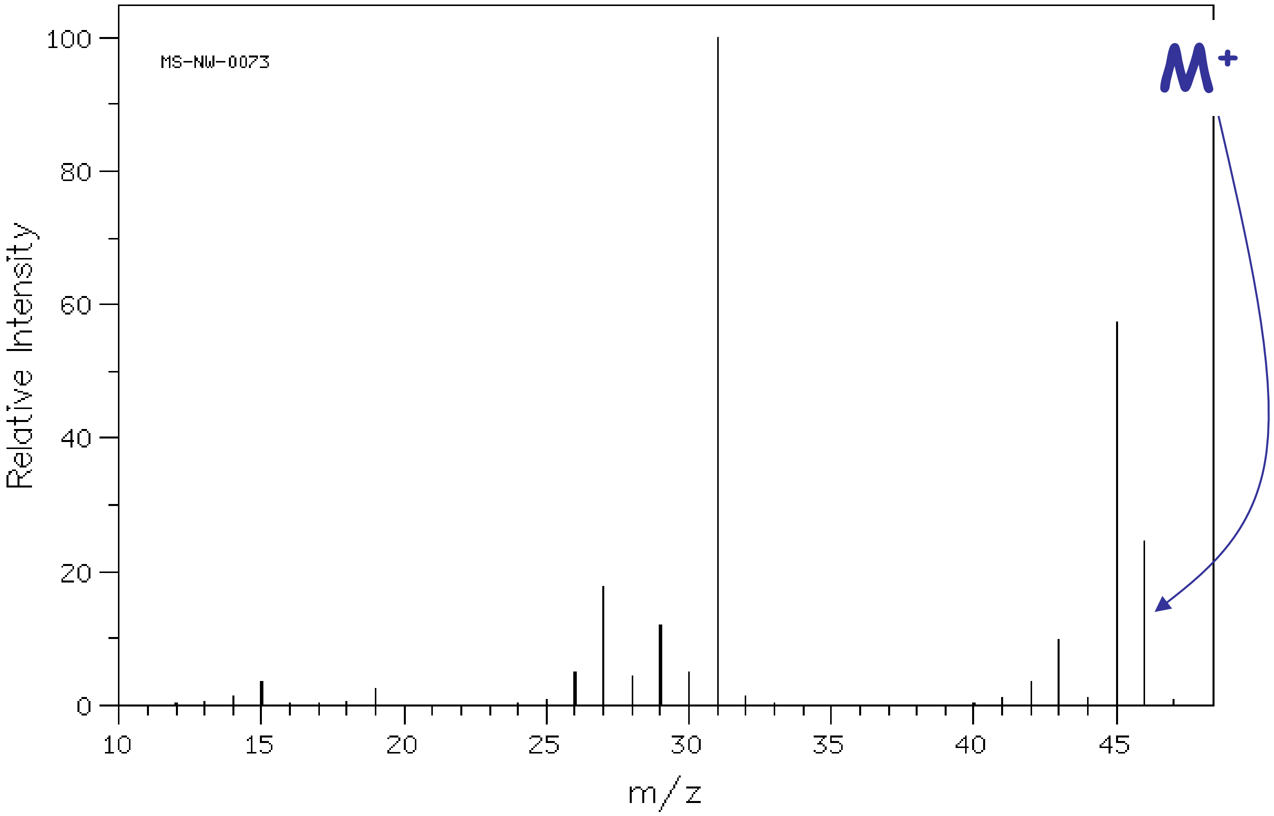
- Molecular ion (parent ion):
  - The radical cation corresponding to the mass of the original molecule



- The molecular ion is usually the highest mass in the spectrum
  - Some exceptions w/specific isotopes
  - Some molecular ion peaks are absent.

Background

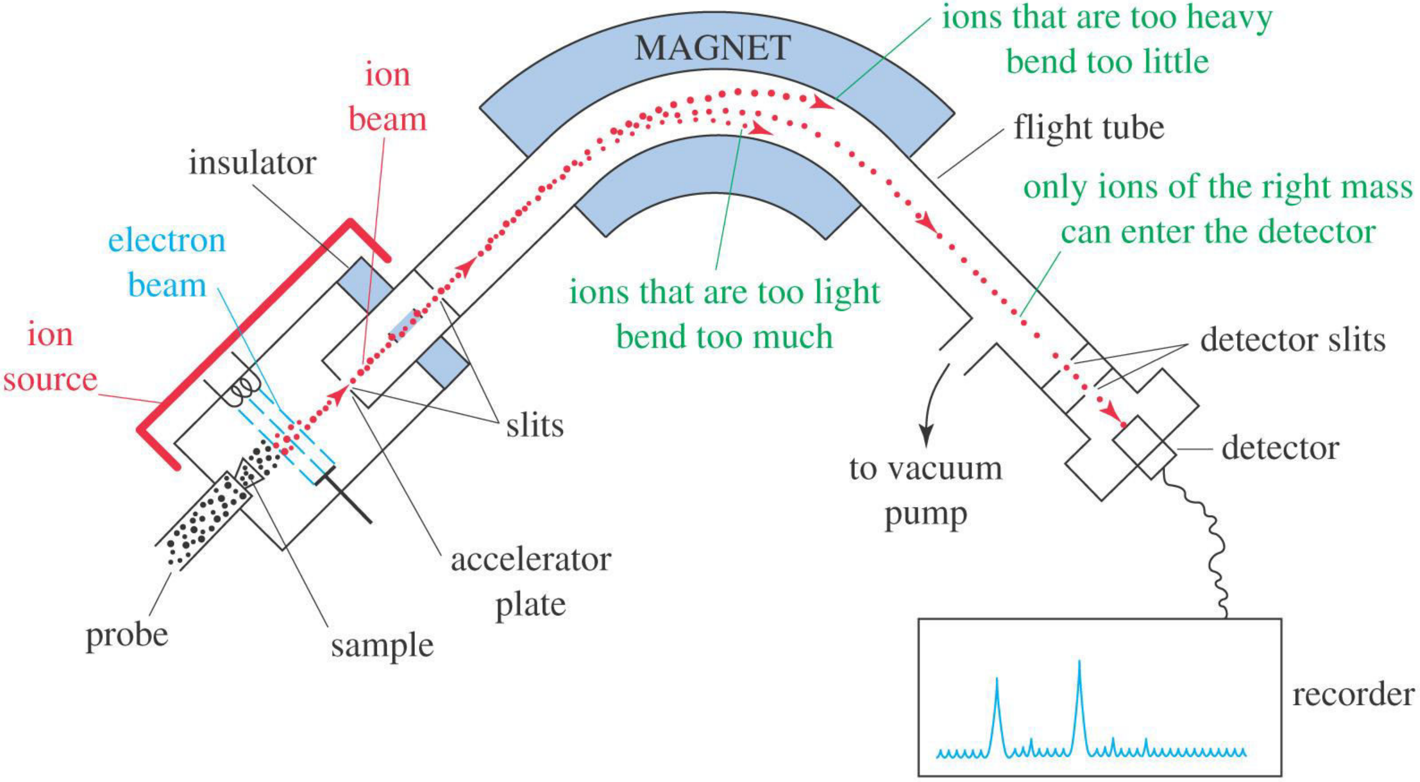
- Mass spectrum of ethanol (MW = 46)



SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

Background

- The cations that are formed are separated by magnetic deflection.



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Background

- Only cations are detected.
  - Radicals are “invisible” in MS.

- The amount of deflection observed depends on the mass to charge ratio (m/z).
  - Most cations formed have a charge of +1 so the amount of deflection observed is usually dependent on the mass of the ion.

Background

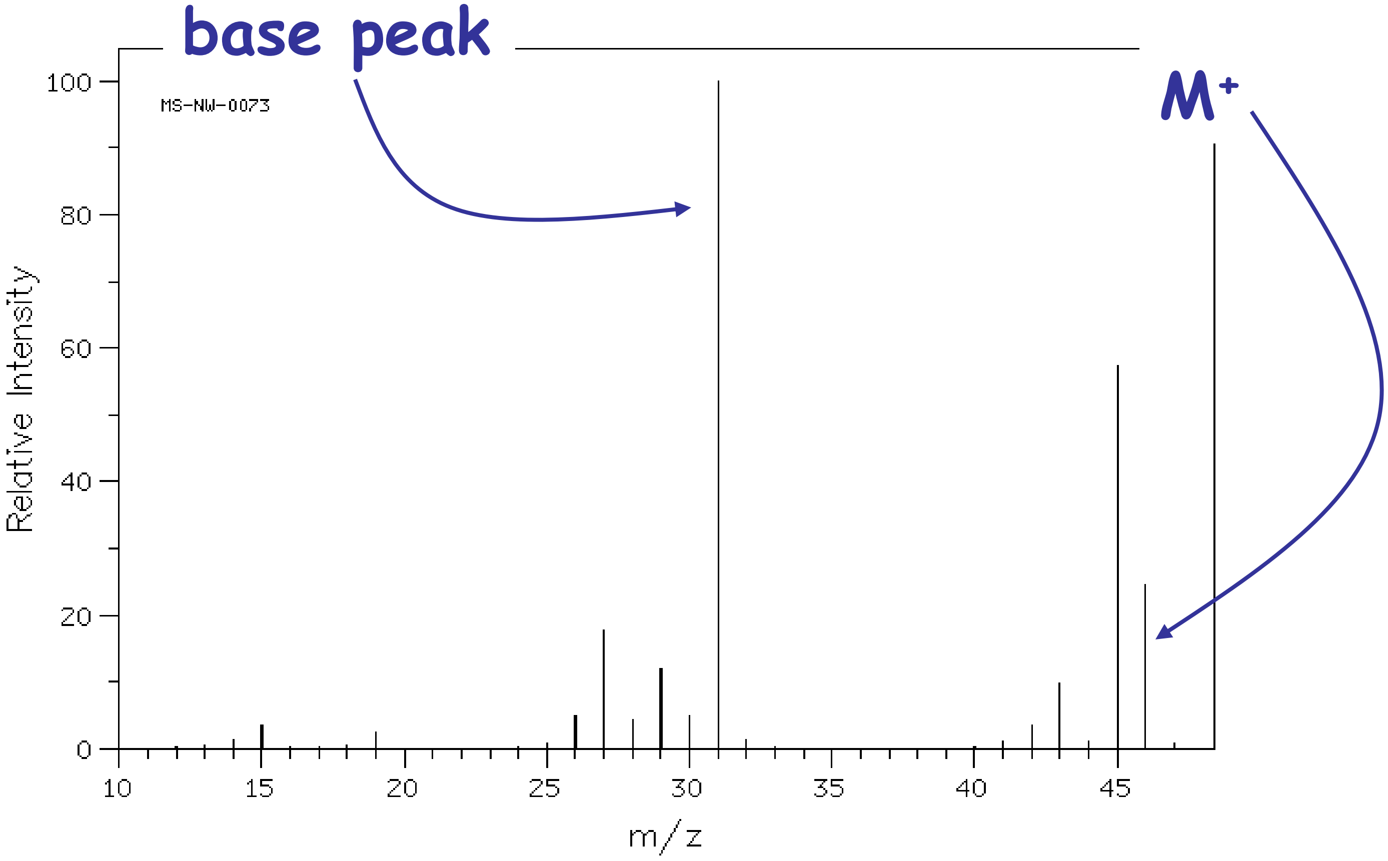
- The resulting mass spectrum is a graph of the mass of each cation vs. its relative abundance.

- The peaks are assigned an abundance as a percentage of the base peak.
  - the most intense peak in the spectrum

- The base peak is not necessarily the same as the parent ion peak.

Background

The mass spectrum of ethanol



base peak

M^+

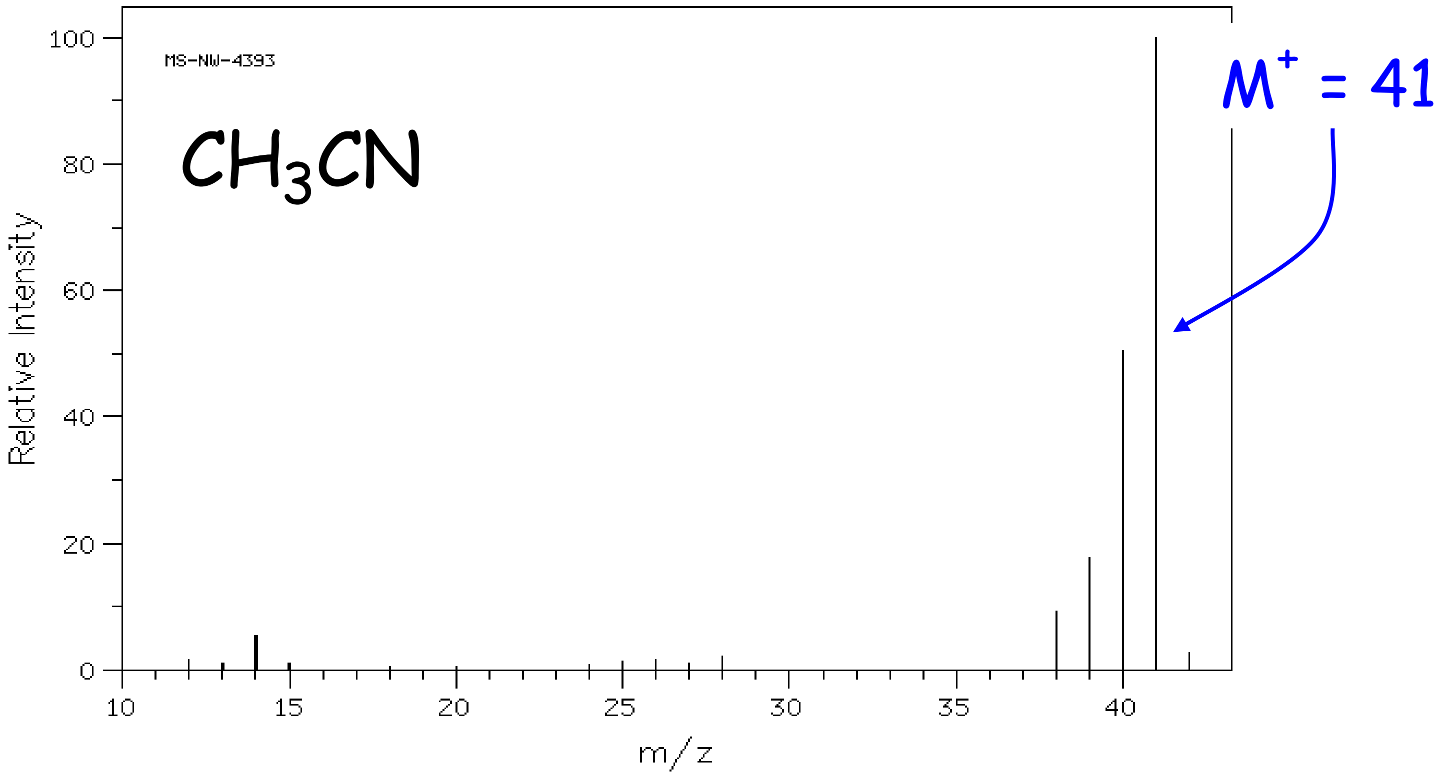
SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

Background

- Most elements occur naturally as a mixture of isotopes.
  - The presence of significant amounts of heavier isotopes leads to small peaks that have masses that are higher than the parent ion peak.
- M+1 = a peak that is one mass unit higher than M^+
- M+2 = a peak that is two mass units higher than M^+

Easily Recognized Elements in MS

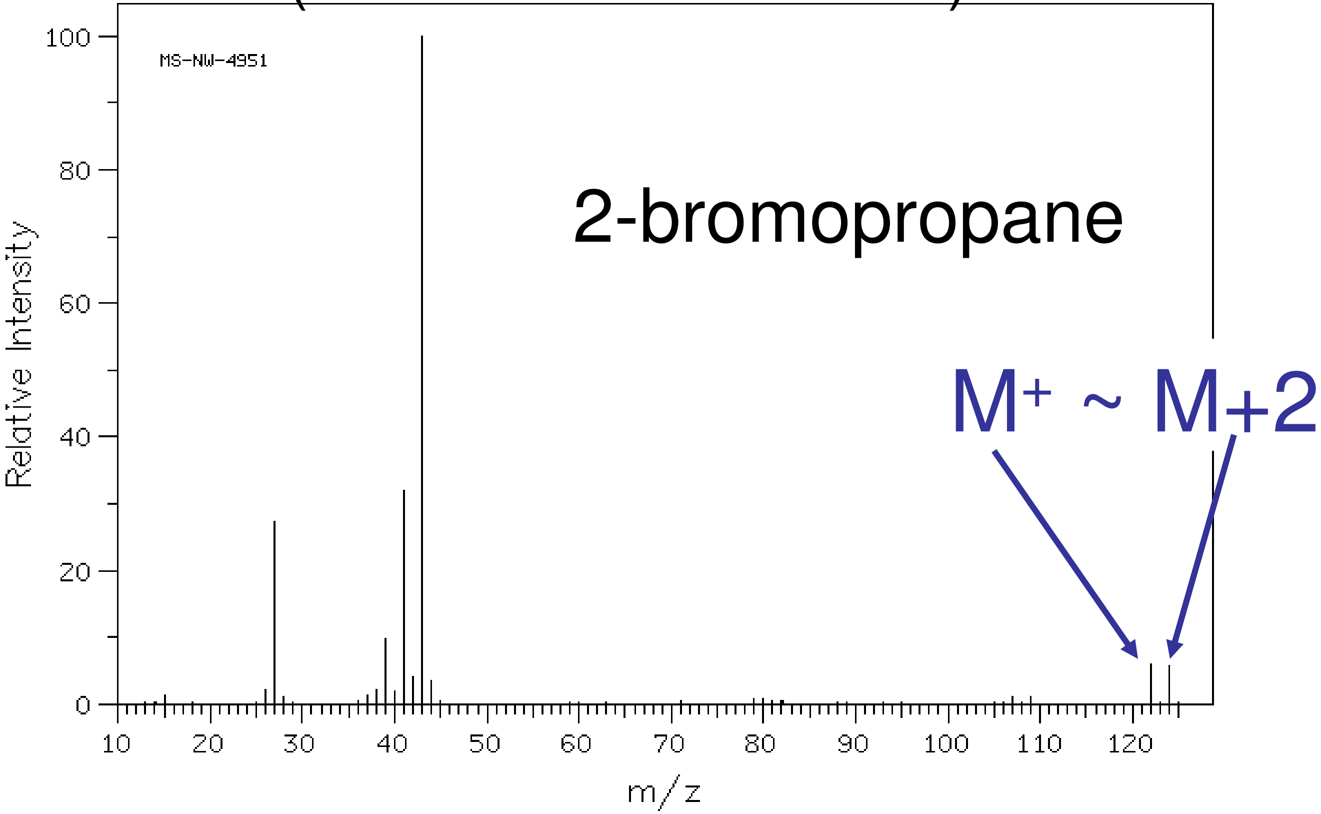
- Nitrogen:
  - Odd number of N = odd MW



SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/2/09)

Easily Recognized Elements in MS

- Bromine:
  - $M^+ \sim M+2$ (50.5% ^{79}Br/49.5% ^{81}Br)



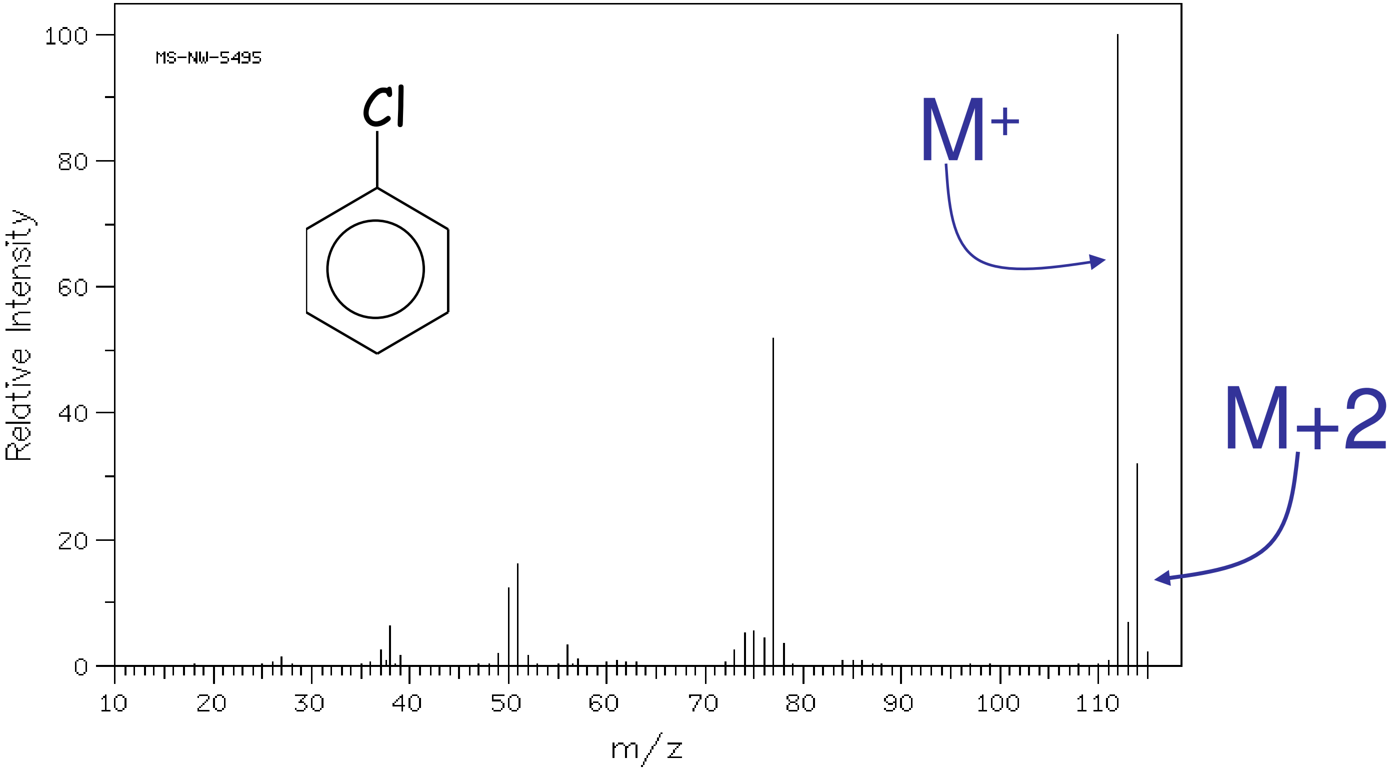
2-bromopropane

$M^+ \sim M+2$

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

Easily Recognized Elements in MS

- Chlorine:
  - M+2 is ~ 1/3 as large as M^+



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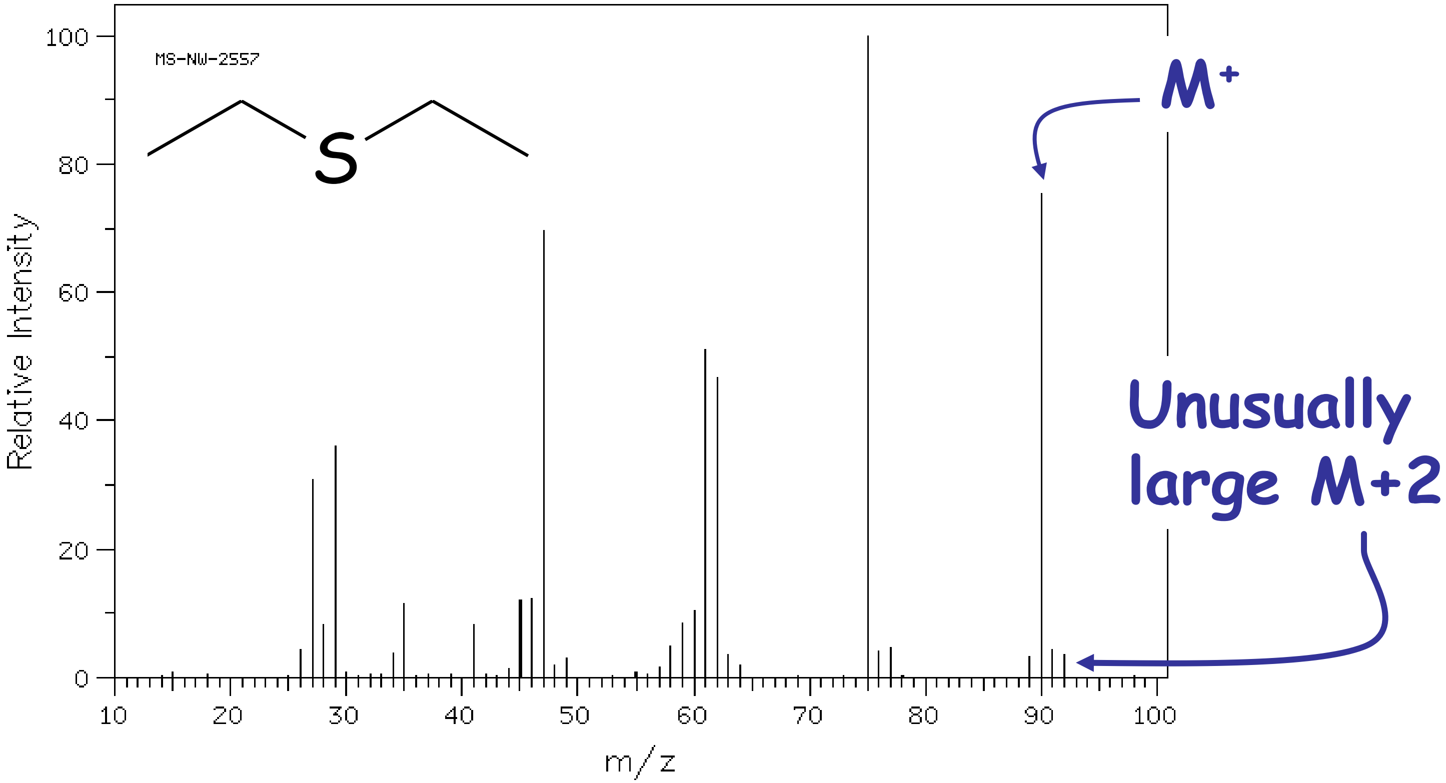
Easily Recognized Elements in MS

- Sulfur:
  - M+2 larger than usual (4% of M^+)



M^+

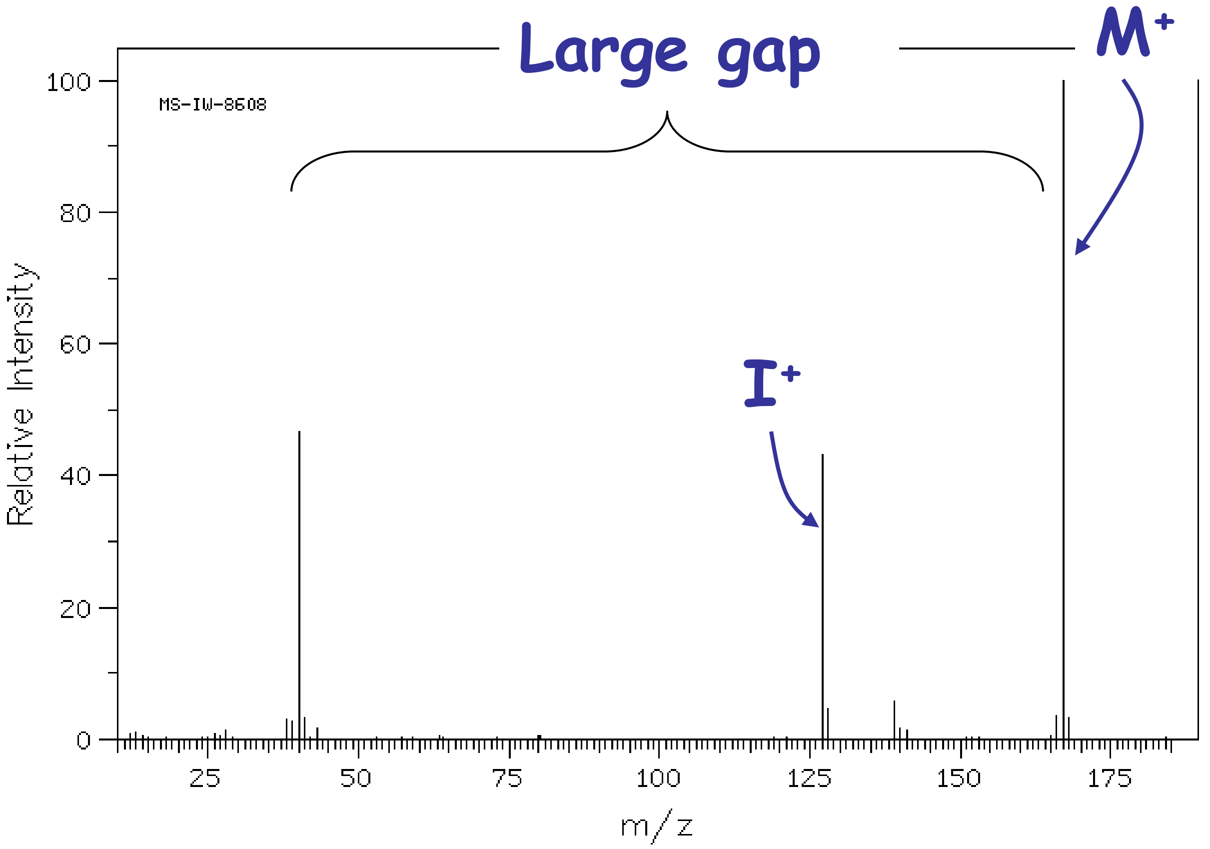
Unusually large M+2

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

Easily Recognized Elements in MS

- Iodine
  - I^+ at 127
  - Large gap

ICH_2CN



SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/2/09)

Fragmentation Patterns

- The impact of the stream of high energy electrons often breaks the molecule into fragments, commonly a cation and a radical.
    - Bonds break to give the most stable cation.
    - Stability of the radical is less important.

Fragmentation Patterns

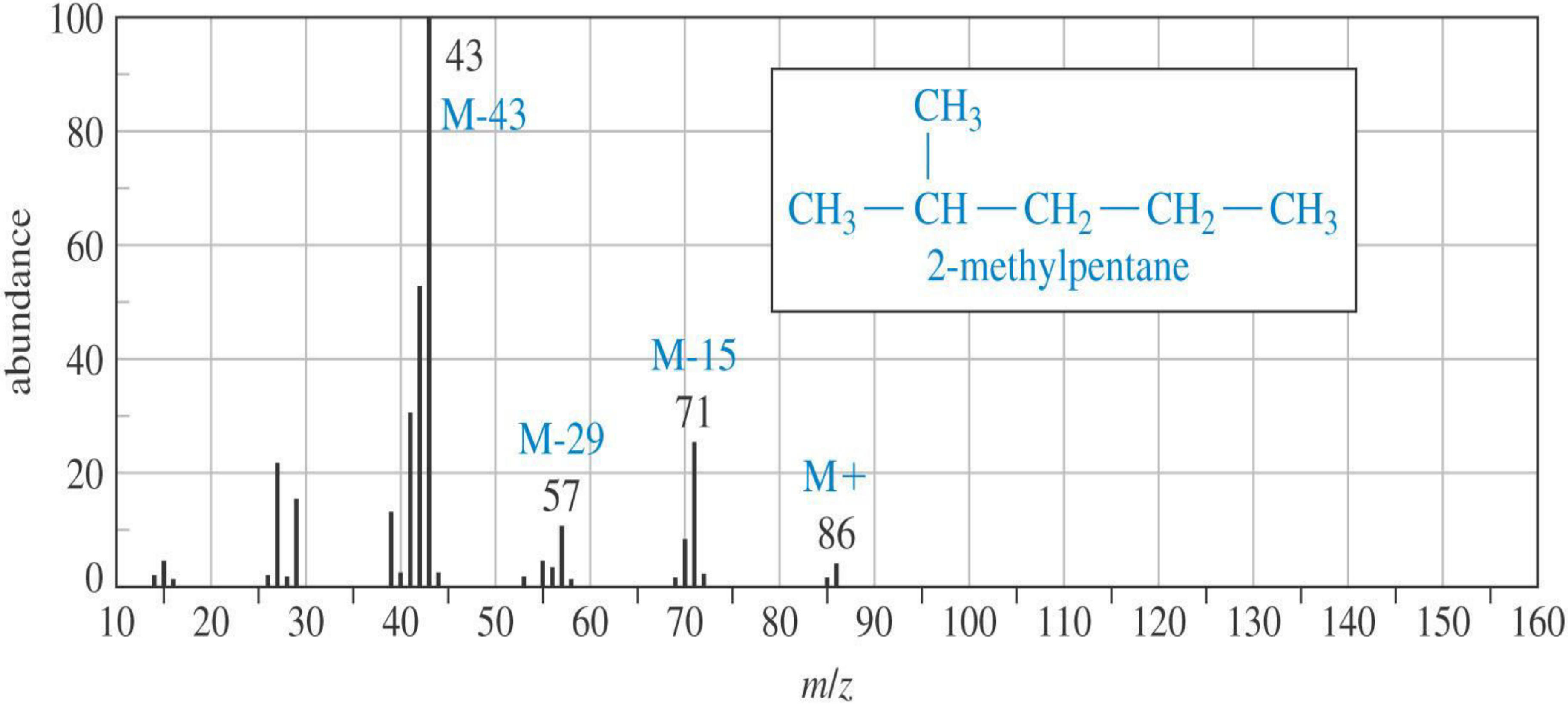
- Alkanes
  - Fragmentation often splits off simple alkyl groups:

Loss of methyl	$M^+ - 15$
Loss of ethyl	$M^+ - 29$
Loss of propyl	$M^+ - 43$
Loss of butyl	$M^+ - 57$

  - Branched alkanes tend to fragment forming the most stable carbocations.

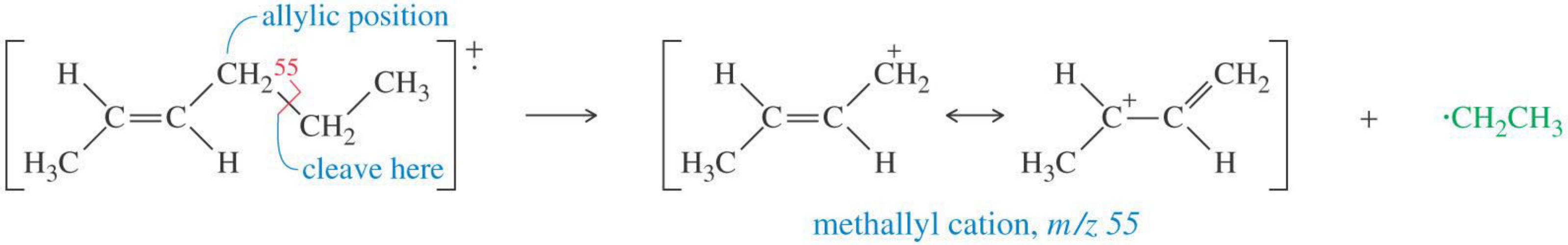
Fragmentation Patterns

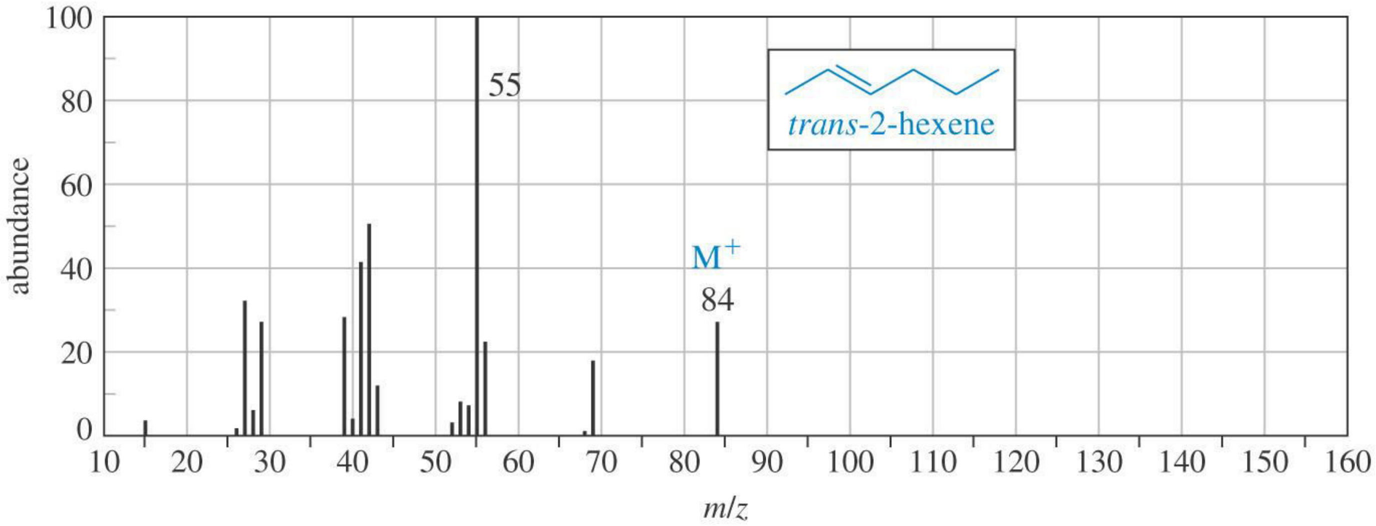
- Mass spectrum of 2-methylpentane



Fragmentation Patterns

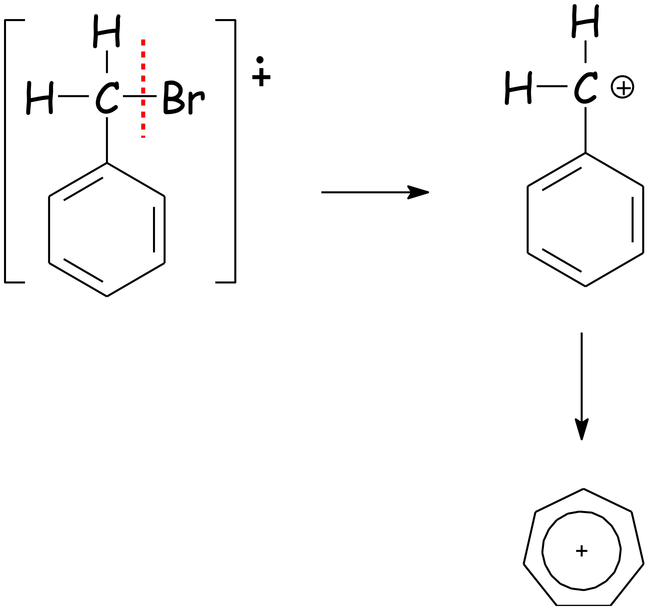
- Alkenes:
  - Fragmentation typically forms resonance stabilized allylic carbocations

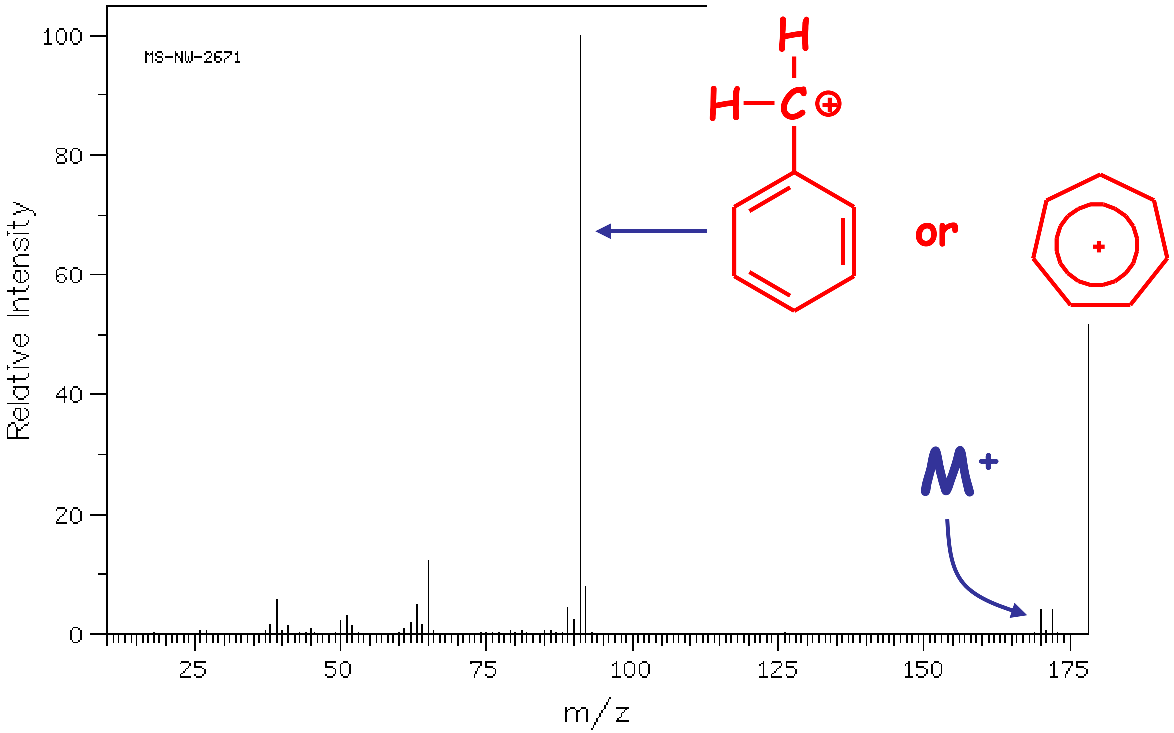




Fragmentation Patterns

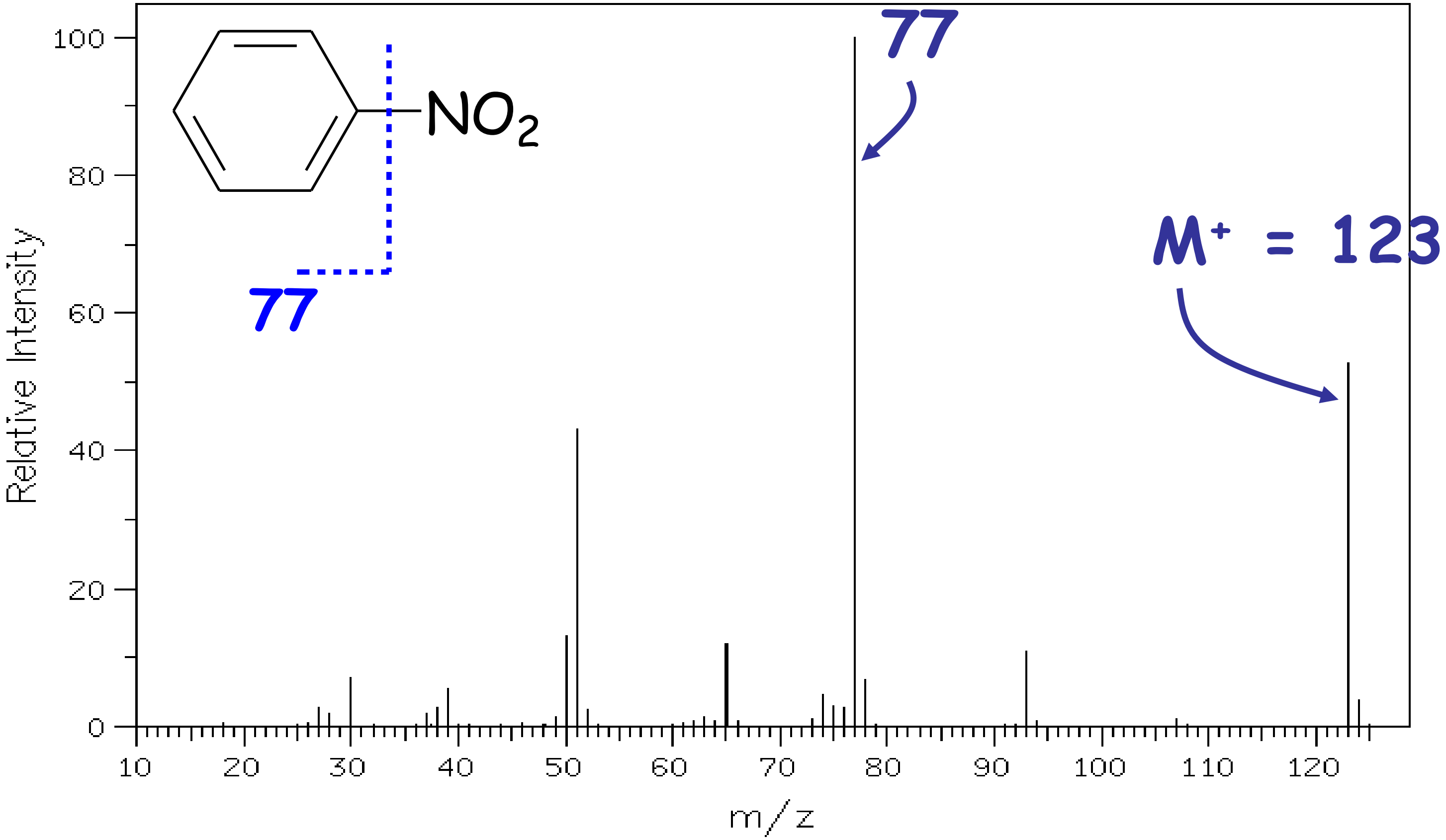
- Aromatics:
  - Fragment at the benzylic carbon, forming a resonance stabilized benzylic carbocation (which rearranges to the tropylium ion)





Fragmentation Patterns

Aromatics may also have a peak at m/z = 77 for the benzene ring.

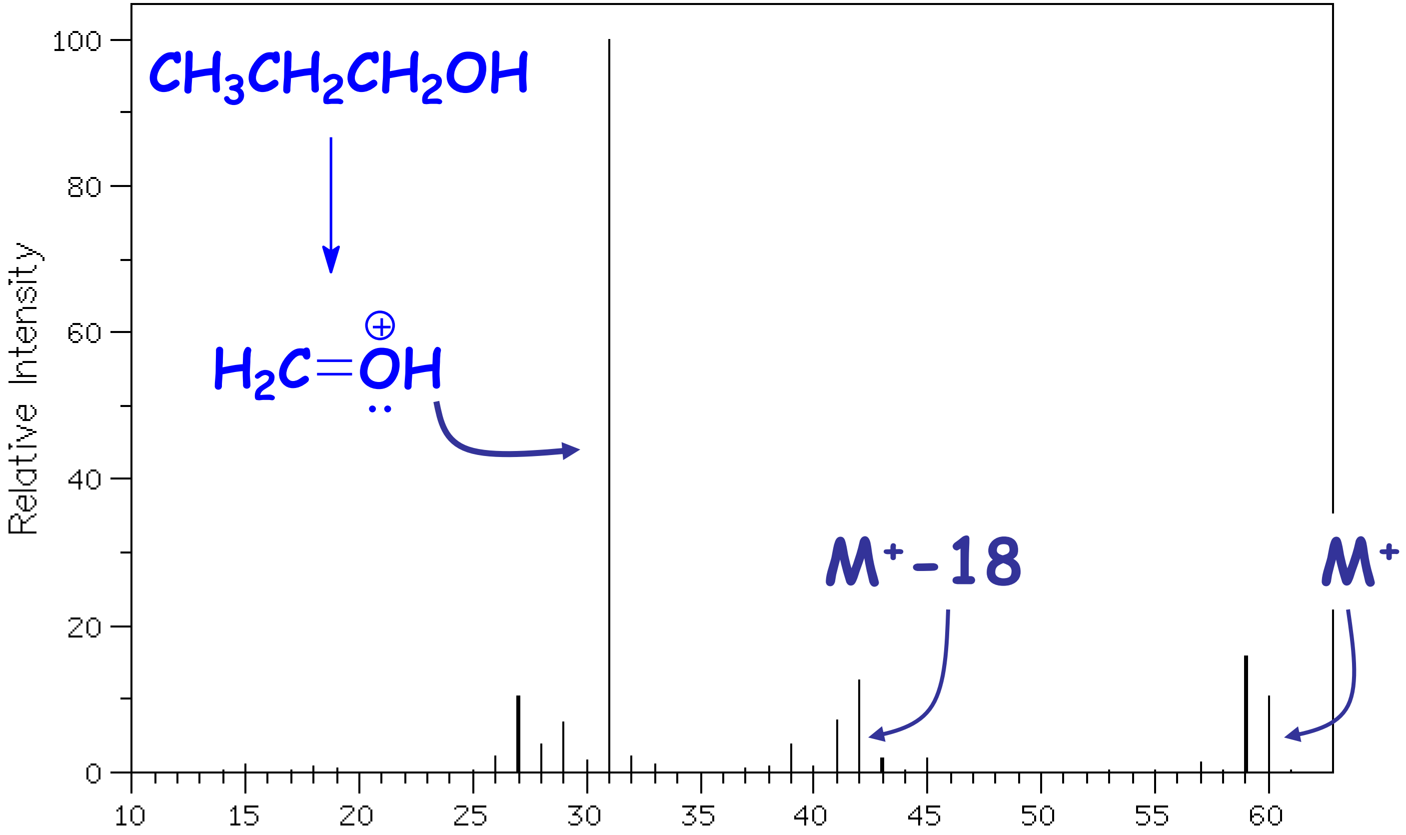


Fragmentation Patterns

- Alcohols
  - Fragment easily resulting in very small or missing parent ion peak
  - May lose hydroxyl radical or water
    - $M^+ - 17$ or $M^+ - 18$
  - Commonly lose an alkyl group attached to the carbinol carbon forming an oxonium ion.
    - 1° alcohol usually has prominent peak at $m/z = 31$ corresponding to $H_2C{=}OH^+$

Fragmentation Patterns

- MS for 1-propanol



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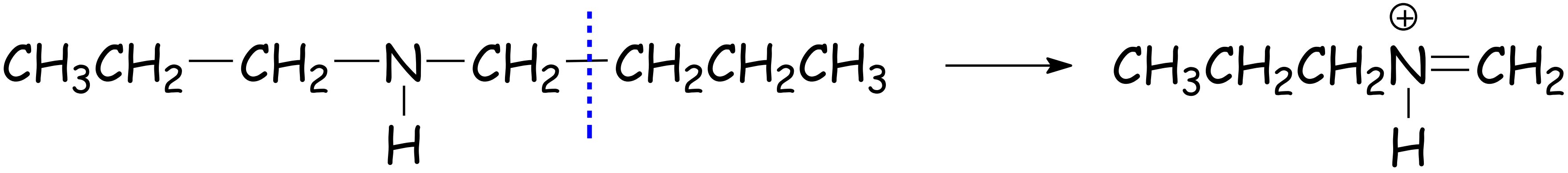
Fragmentation Patterns

- Amines
  - Odd M^+ (assuming an odd number of nitrogens are present)
  - □ α-cleavage dominates forming an iminium ion

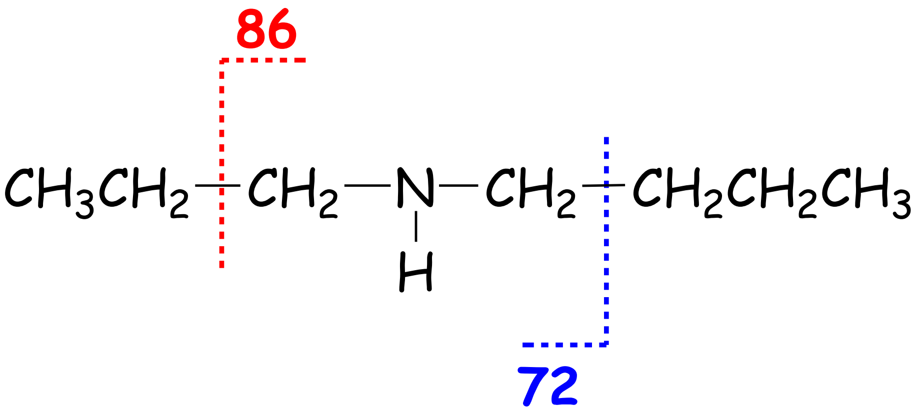


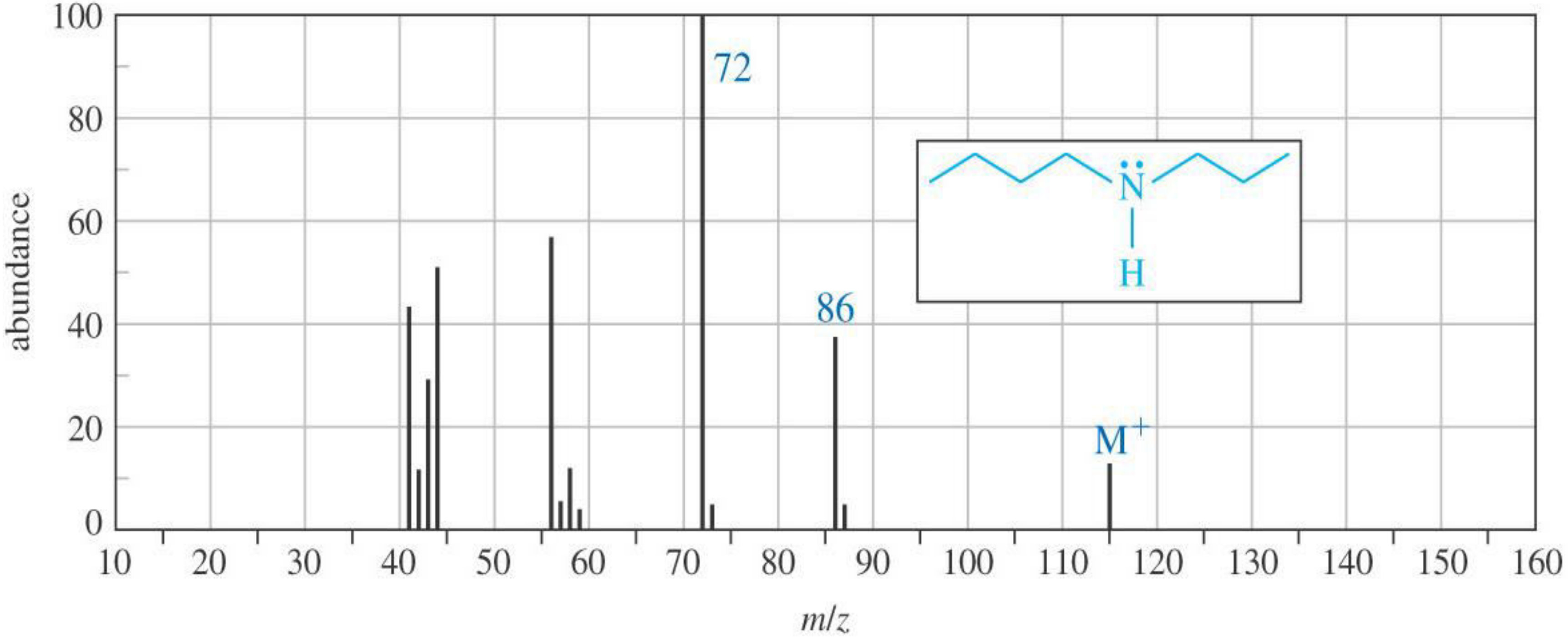
$$CH_3CH_2-CH_2-\underset{H}{\underset{|}{N}}-CH_2 \vdots CH_2CH_2CH_3 \longrightarrow CH_3CH_2CH_2\overset{\oplus}{\underset{H}{\underset{|}{N}}}=CH_2$$

m/z =72

iminium ion

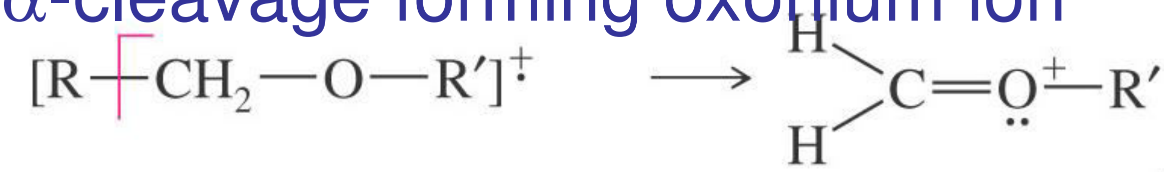
Fragmentation Patterns


$$CH_3CH_2-CH_2-\underset{|\atop H}{N}-CH_2-CH_2CH_2CH_3$$



Fragmentation Patterns

- Ethers
  - α-cleavage forming oxonium ion

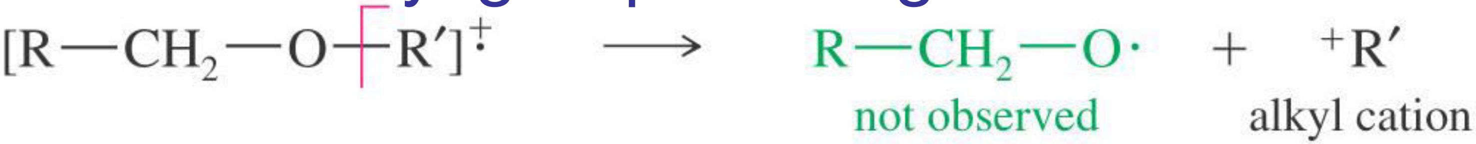


$[R-CH_2-O-R']^{+\cdot} \longrightarrow H_2C=\overset{+}{O}-R'$

  - Loss of alkyl group forming oxonium ion

$[R-CH_2-O-R']^{+\cdot} \xrightarrow{\sim H} R-CH=\overset{+}{O}-H$

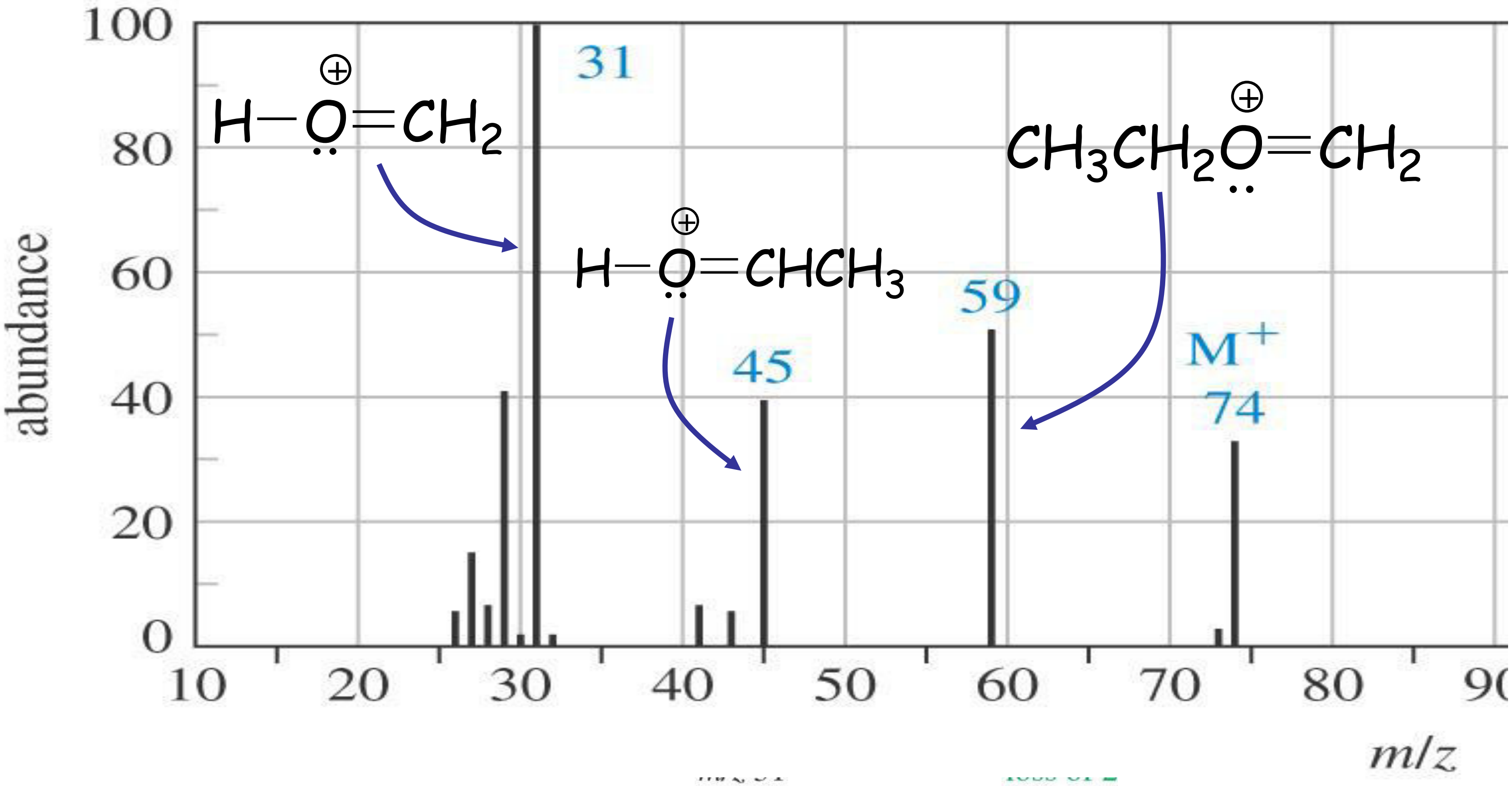
  - Loss of alkyl group forming a carbocation



$[R-CH_2-O-R']^{+\cdot} \longrightarrow R-CH_2-O\cdot + {}^{+}R'$

Fragmentation Patterns

MS of diethylether ($CH_3CH_2OCH_2CH_3$)



Fragmentation Patterns

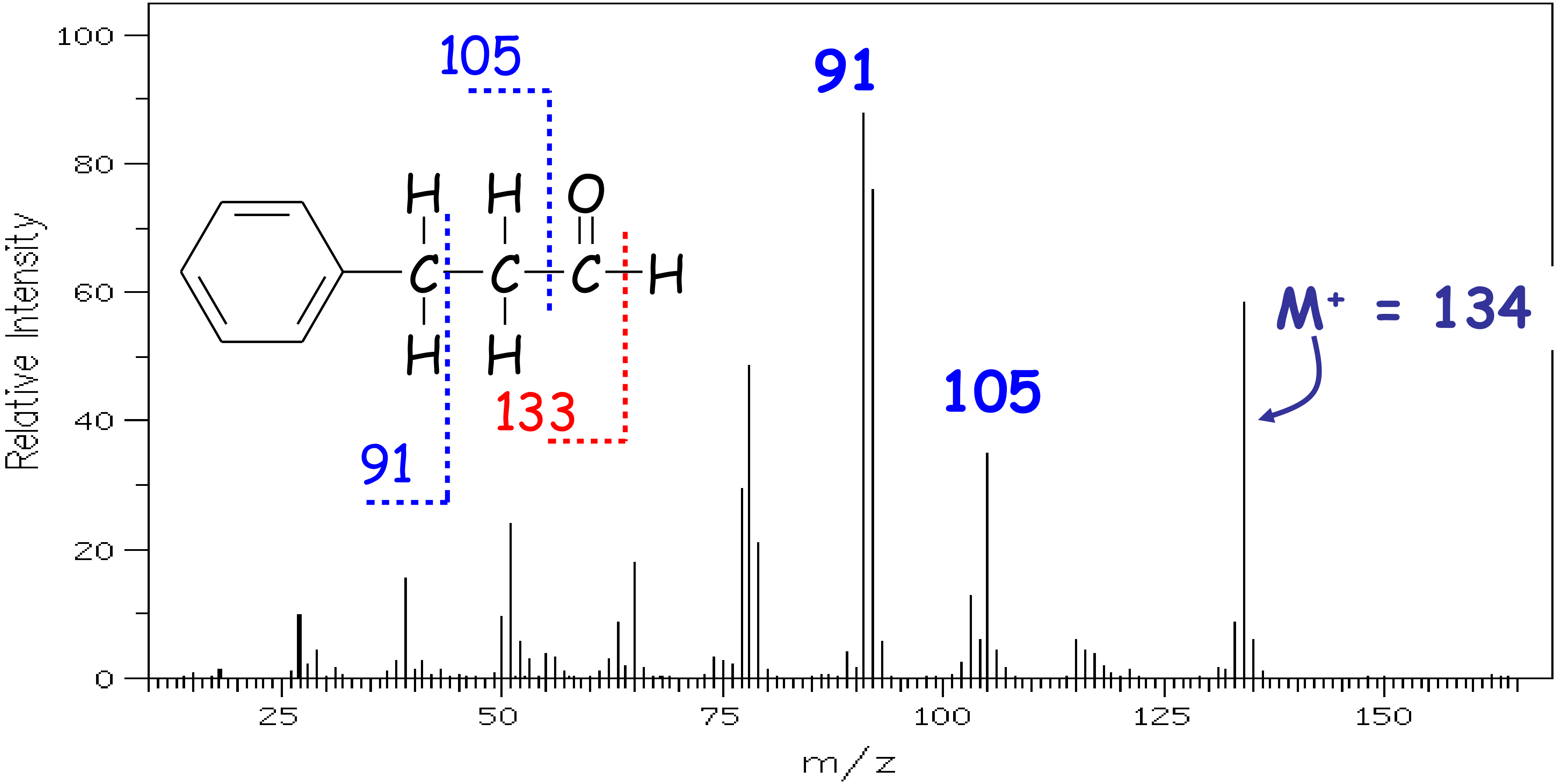
- Aldehydes (RCHO)
  - Fragmentation may form acylium ion

$$RC\equiv\overset{\oplus}{O}:$$

  - Common fragments:
    - M^+ - 1 for $RC\equiv\overset{\oplus}{O}:$
    - M^+ - 29 for $R^{\oplus}$ (i.e. RCHO - CHO)

Fragmentation Patterns

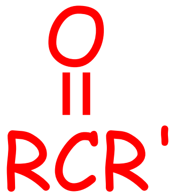
- MS for hydrocinnamaldehyde



SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/28/09)

Fragmentation Patterns

- Ketones



$$R\overset{\overset{\displaystyle O}{\|}}{C}R'$$

  - Fragmentation leads to formation of acylium ion:
    - Loss of R forming

$$R'C\equiv\overset{\oplus}{O}:$$

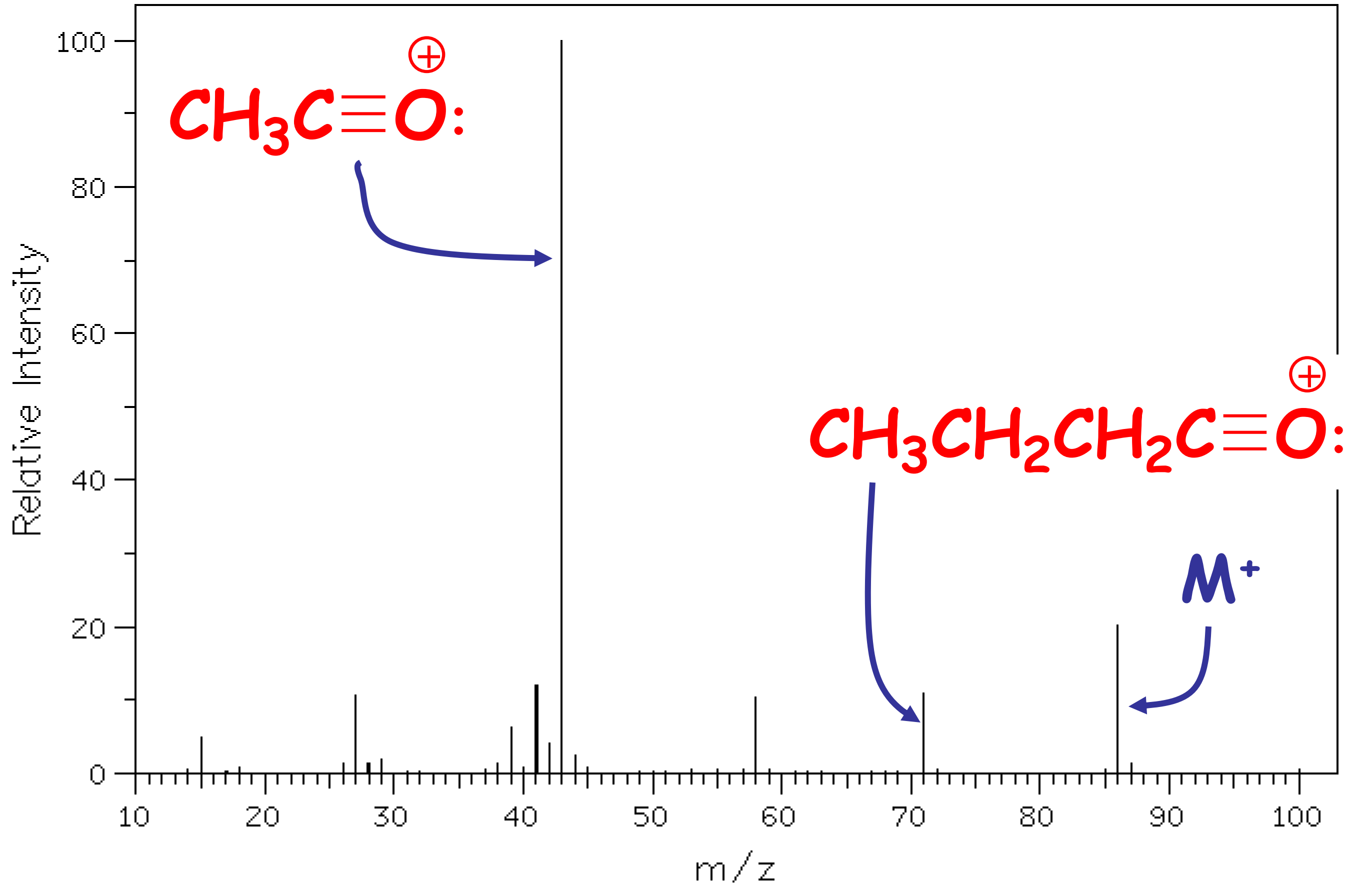
    - Loss of R’ forming

$$RC\equiv\overset{\oplus}{O}:$$

Fragmentation Pat

$$CH_3\overset{\overset{O}{||}}{C}CH_2CH_2CH_3$$

- MS for 2-pentanone

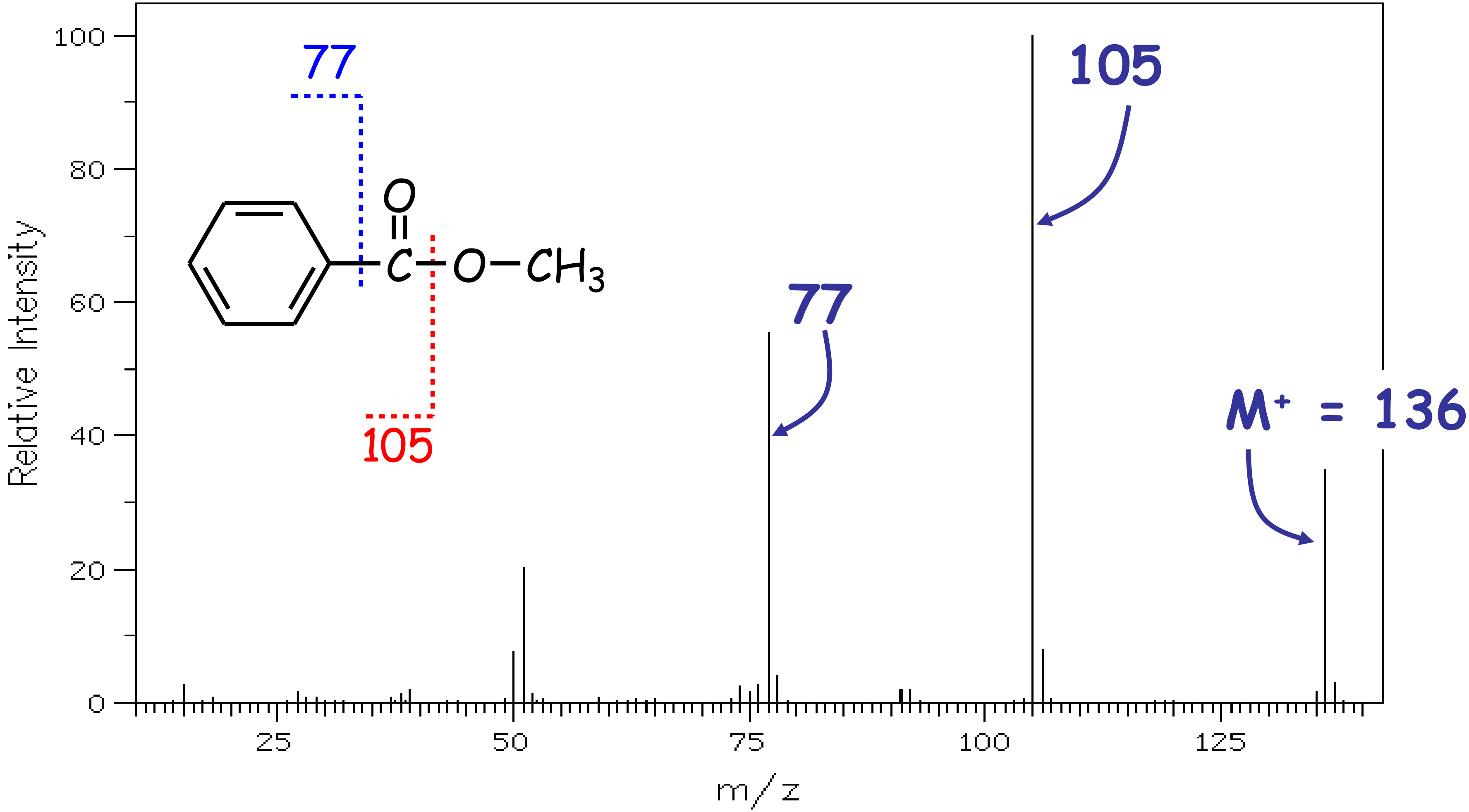


SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/28/09)

Fragmentation Patterns

- Esters (RCO_2R')
  - Common fragmentation patterns include:
    - Loss of OR’
      - peak at M^+ - OR’
    - Loss of R’
      - peak at M^+ - R’

Frgamentation Patterns



SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/28/09)

Rule of Thirteen

- The “Rule of Thirteen” can be used to identify possible molecular formulas for an unknown hydrocarbon, C_nH_m.
  - Step 1: n = $M^+/13$ (integer only, use remainder in step 2)
  - Step 2: m = n + remainder from step 1

Rule of Thirteen

- Example: The formula for a hydrocarbon with $M^+ = 106$ can be found:
  - Step 1: $n = 106/13 = 8$ $(R = 2)$
  - Step 2: $m = 8 + 2 = 10$
  - Formula: C_8H_{10}

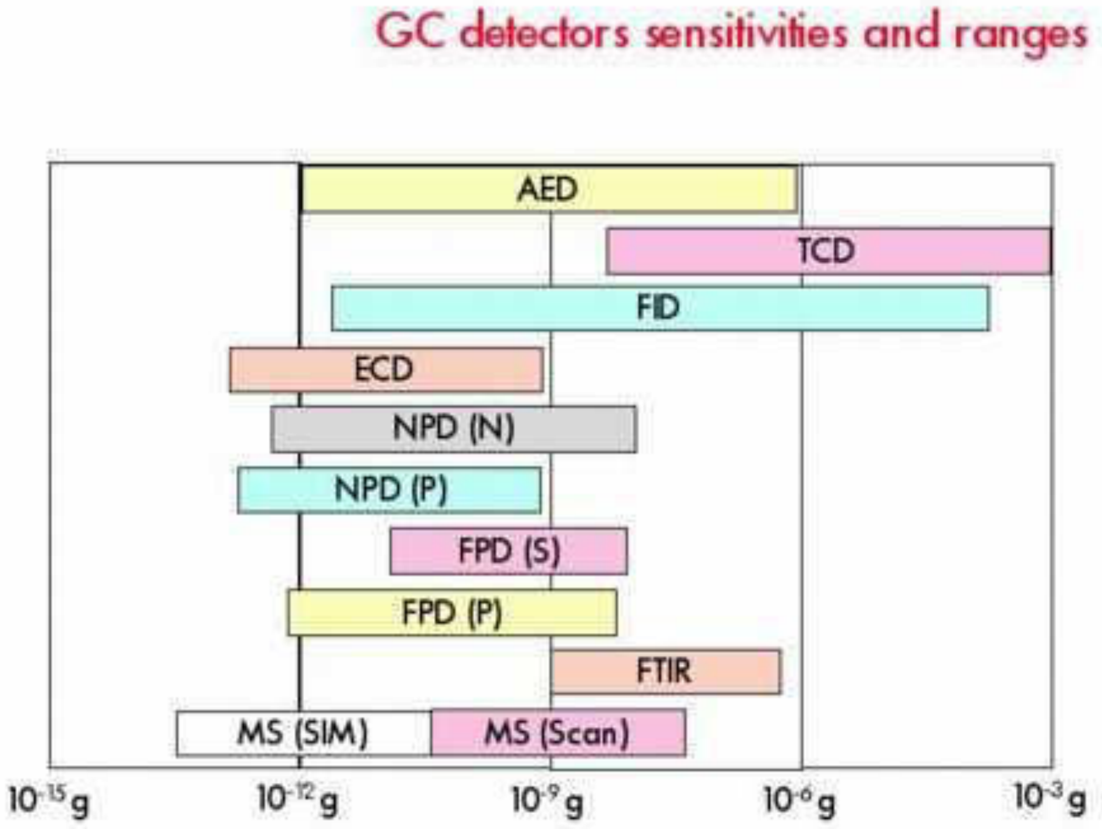
Rule of Thirteen

- If a heteroatom is present,
  - Subtract the mass of each heteroatom from the MW
  - Calculate the formula for the corresponding hydrocarbon
  - Add the heteroatoms to the formula

Outline

- General principles of mass spectrometry
- Ionization techniques
- Mass analyzers for GC/MS Detectors
- Scan and SIM mode
- Tuning
- Common problems with GC-MS systems

GC detectors

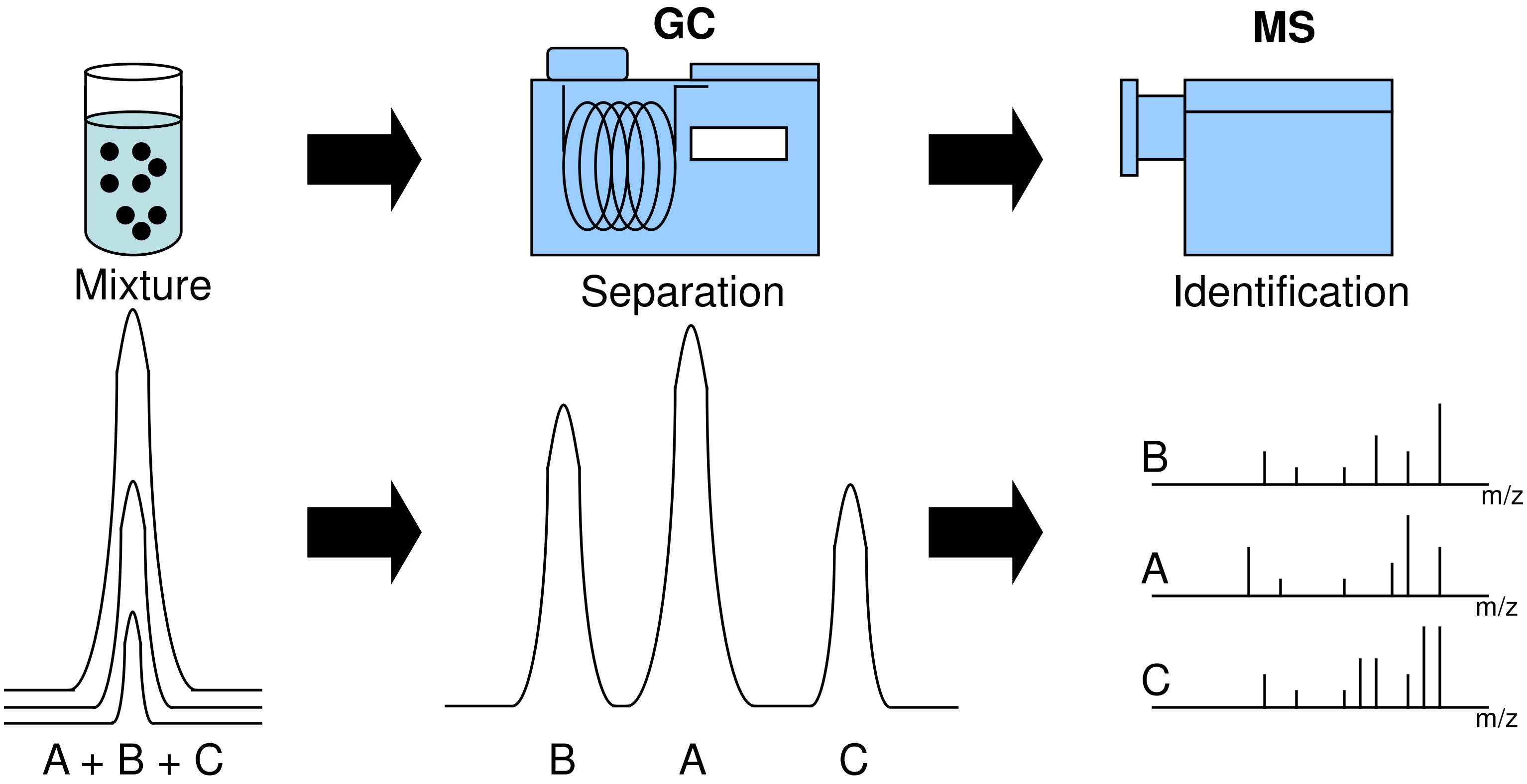


http://elchem.kaist.ac.kr/jhkwak/analchem/09/03/36.gif

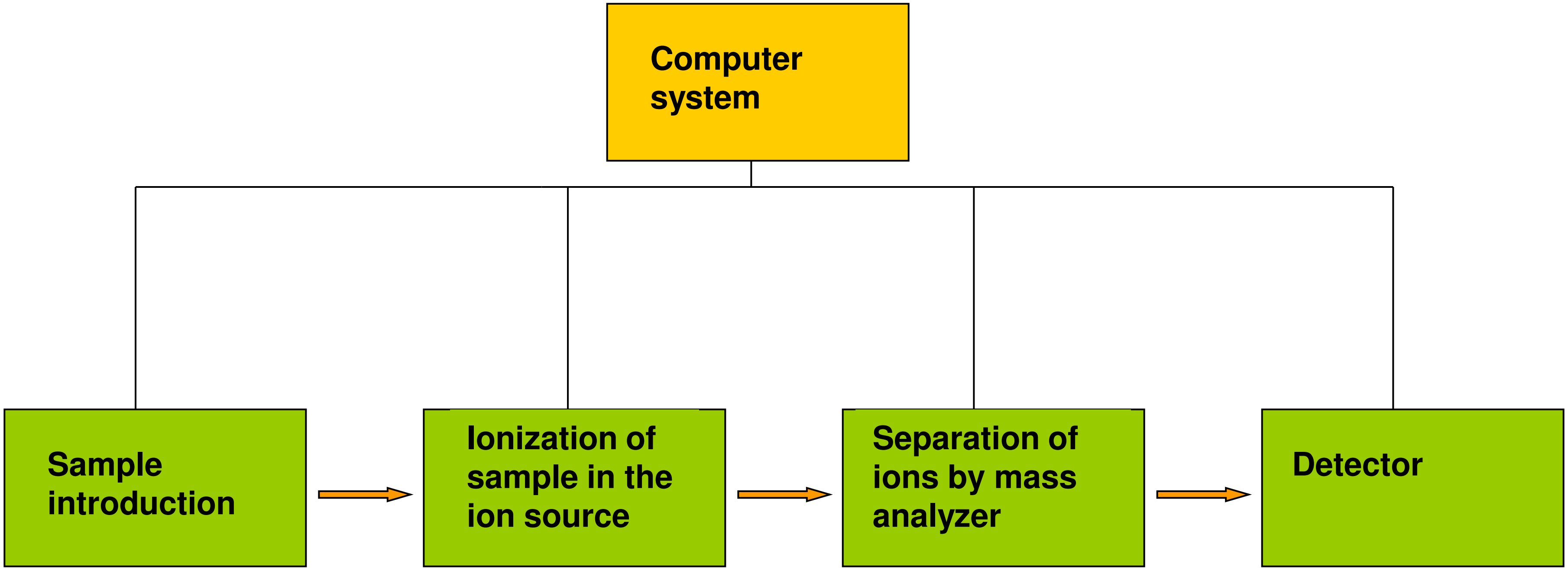
Some useful definitions

- Analytes = The compounds we would like to to analyze.
- Isotopes = Atoms with the same number of electrons and protons, but different number of neutrons. Isotopes have the same chemical properties but differ in molecular mass.

Analysis by GC-MS



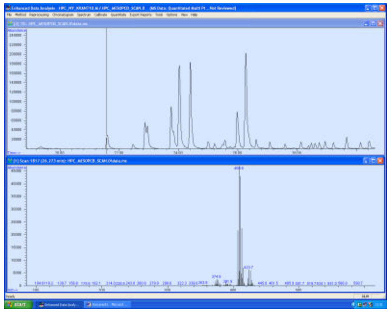
General principles on mass spectrometry



Computer system

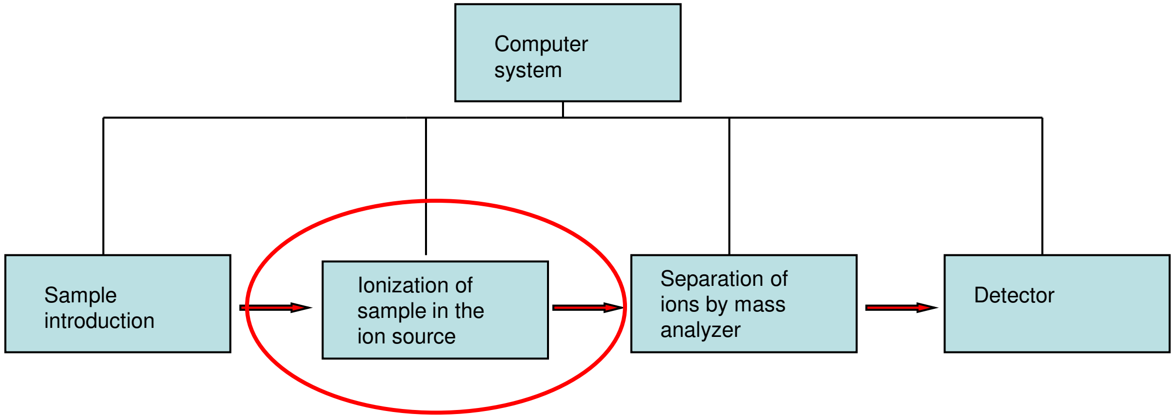
Sample introduction → **Ionization of sample in the ion source** → **Separation of ions by mass analyzer** → **Detector**

Resulting chromatogram



Ionization techniques

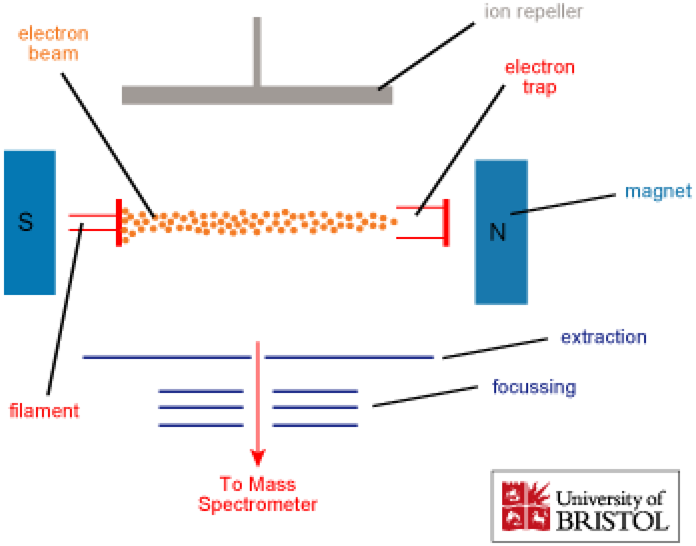
- The ionization takes place in the ion source.
- Samples from GC interface are in gas-phase when introduced into the MS. The MS is operated in vacuum to make sure analytes are evaporized and to avoid collisions between analytes and other compounds.
- Two different ionization methods
  - Electron Impact Ionization (EI)
  - Chemical Ionization (CI)



Electron Impact Ionization (EI)

- Most widely used method
- Analytes are bombared with high-energy electrons (usually 70eV)
- As a result of collision, an electron is removed from the analytes (M), generating a molecular ion M+ (radical cation)

$$M + e^- \xrightarrow{\quad} M^+ + 2e^-$$



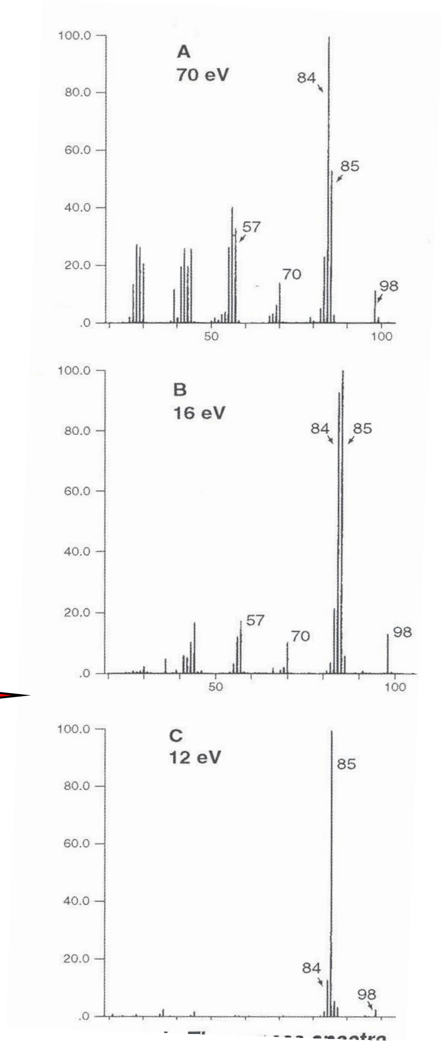
Electron Impact Ionization (EI)

- Due to excess internal energy, fragmentation of the molecular ion will occur.
- The fragmentation is reproducible and characteristic of the compund.
- It is also possible to predict the fragmentation on the basis of chemical structures which is why MS is good tool for structure elucidation of unknown compounds

Drawbacks with EI

- Sometimes the fragmentation is too great (depending on the stability of the sample molecules) and the molecular ion will not show up in the mass spectra. It is then possible to reduce the ionization voltage, but the disadvantage is that the fragmentation pattern will change and the obtained spectra cannot be compared to ”standard” litterature spectra.

- Another solution is to use chemical ionization (CI)!



Watson, Introduction to Mass Spectrometry, 4th ed

Chemical Ionization (CI)

- Softer ionization technique →Less fragmentation → Easier to find molecular ions.
- Two different modes: Negative chemical ionization (NCI) and Positive Chemical Ionization (PCI).
- NCI is used for analytes that are able to form stable negative ions, for example samples containing acidic groups or halogens. NCI is often used to analyze pesticides (contains Cl or Br).
- PCI is used for samples that can form positive ions (most compounds).

Chemical Ionization (CI)

- The principle for NCI and PCI is similar:
- Reagent gas (usually methane, isobutane or ammonia) is introduced into the source where it is ionized:

  PCI (simplified): $CH_4 + e^- \rightarrow CH_4^+ + 2e^-$
  $CH_4 + CH_4^+ \rightarrow CH_3 + CH_5^+$

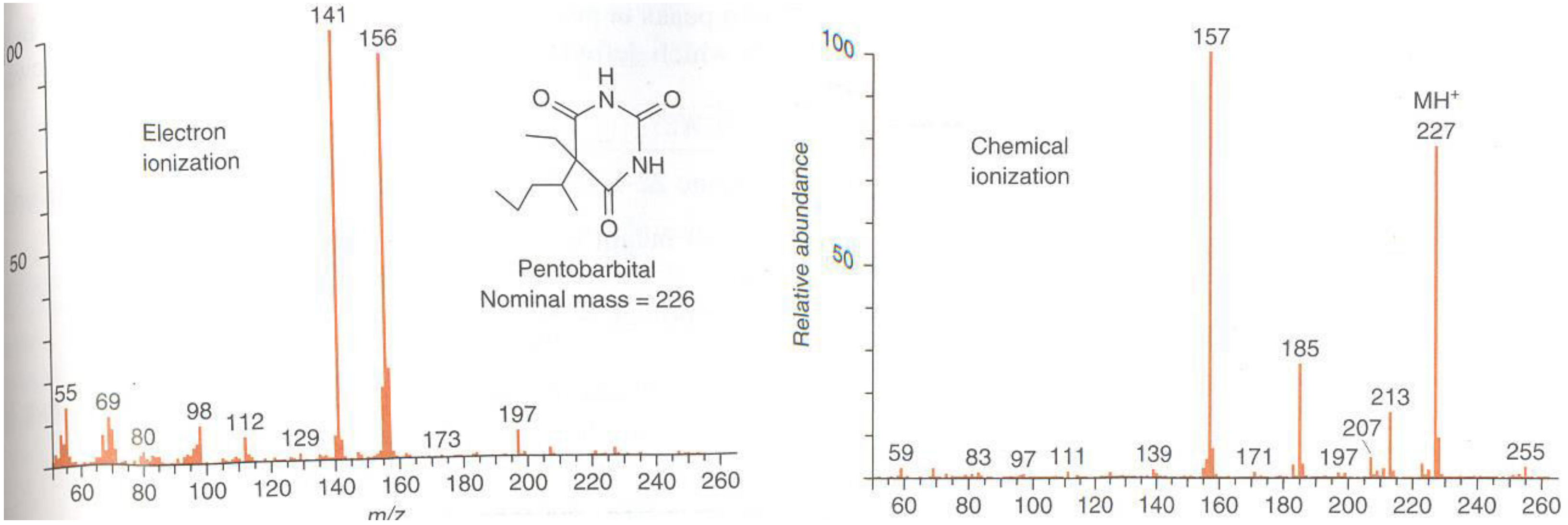
  NCI: $CH_4 + e^- \rightarrow CH_4^-$

- The ionized gas collide with the sample molecules generating a $[M+H]^+$ or $[M+H]^-$ ion that is detected:

  PCI: $CH_5^+ + M \rightarrow [M+H]^+ + CH_4$

  NCI: $CH_4^- + M \rightarrow [M+H]^- + CH_4$

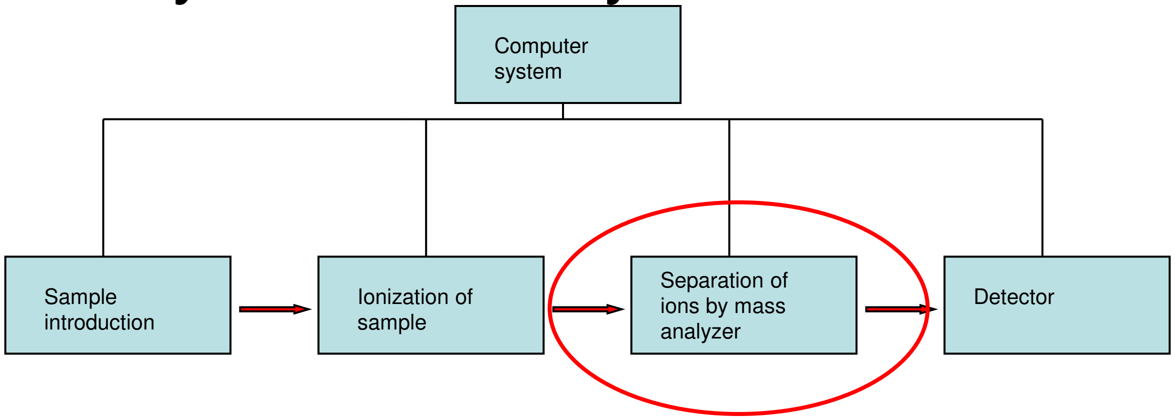
EI spectra/ PCI Spectra



Harris. Quantitative Chemical Analysis, 6th ed.

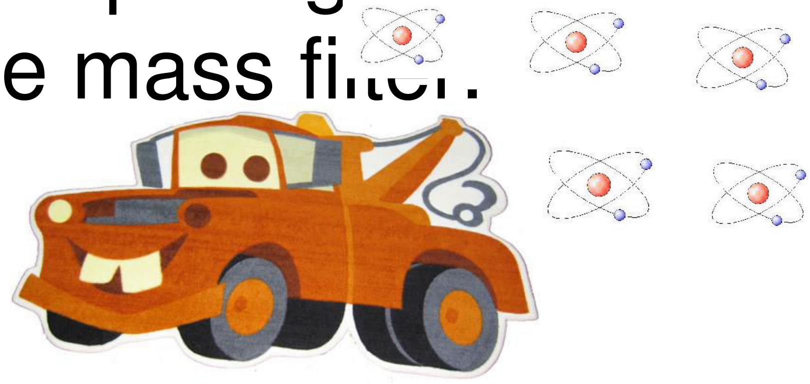
Mass analyzers

- After analytes have been ionized they are separated according to their mass-to-charge ratio (m/z) in a mass analyzer (mass filter).
- Quadrupoles and ion traps are common mass filters in GC-MS systems.
- Time of flight (TOF) mass filter is very much used nowadays in LC-MS systems.



Transport of ions to the mass filter

- The ionization takes place in the ion source.
- Ions are then transported to the mass filter by focusing lenses. These have a voltage running through them and by either attracting or repelling the ions they guide them into the mass filter.

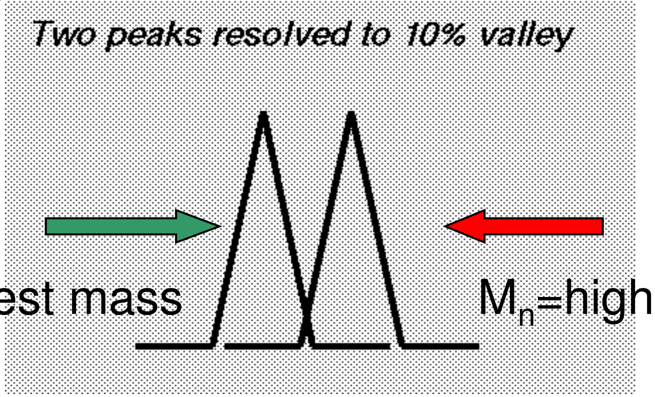


High and low resolution MS

- Resolution = difference in m/z values of ions that can be separate from another.
- Quadrupoles and ion traps have constant resolution, meaning that ions that differ with 1 m/z unit will have the same separation at m/z 150 an 151 as they do at m/z 1000 and 1001.
- High resolution instruments do not have constant resolution, but constant *resolving power.*

Resolving power (R)

$$\boxed{R = \frac{M_n}{M_n - M_m}}$$

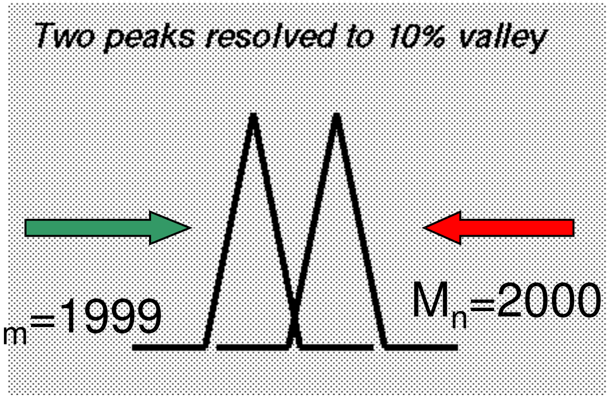


M_m=lowest mass

M_n=highest mass

Low resolution MS instruments have a resolving power of 1000-2000. Quadrupoles and ion traps has constant resolution (ΔM=1) and R will then vary with m/z. On the other hand TOF has contant resolving power, meaning that at lower m/z the resolution will increase.

$$R_{low} = \frac{2000}{2000-1999} = 2000$$

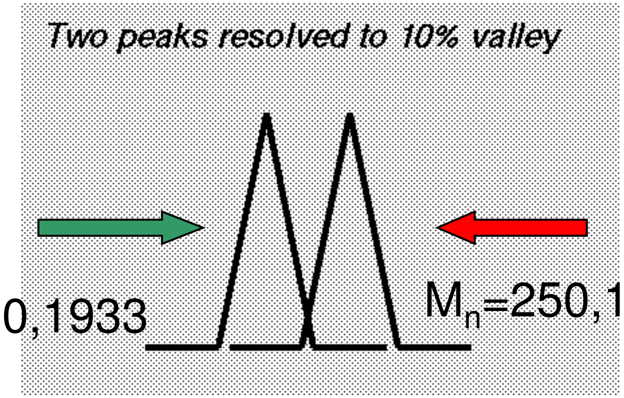


M_m=1999

M_n=2000

High resolution MS ha a resolving power of up to 20 000, making it possible to distinguish between very similar masses.

$$R_{high} = \frac{250{,}1933}{250{,}1933-250{,}1807} = 19857$$



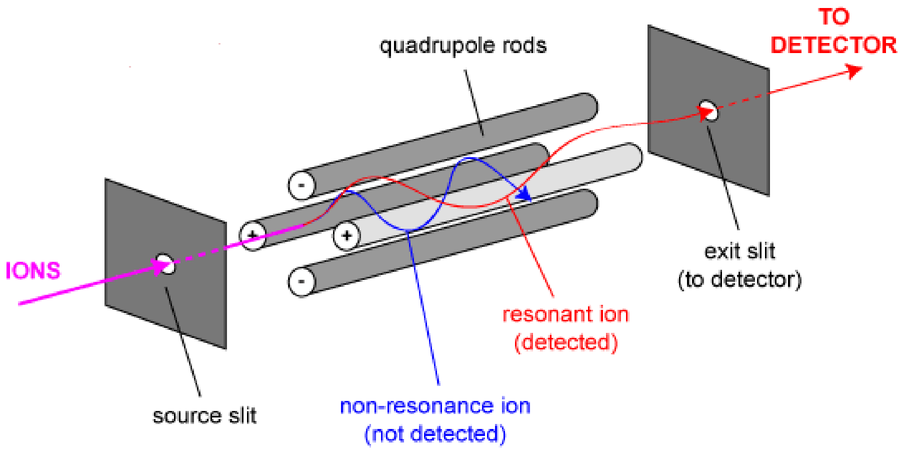
M_m=250,1933

M_n=250,1807

http://www.ivv.fraunhofer.de/ms/resolve10.gif

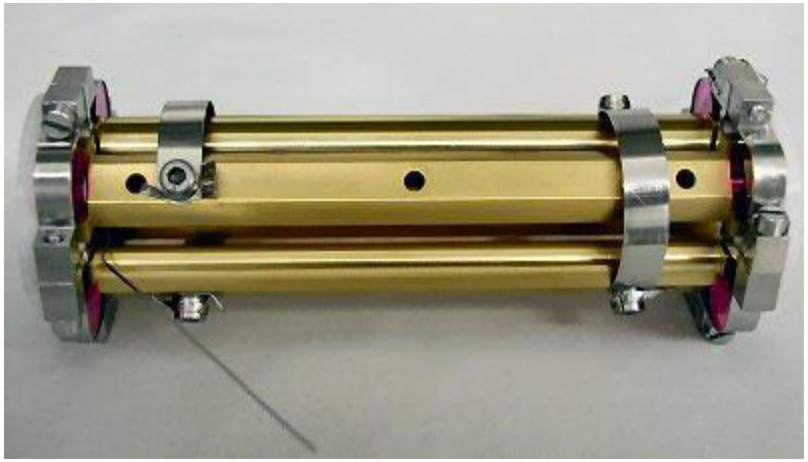
Quadrupole mass spectrometers

- A quadrupole consists of four cylindrical rods, all parallel to each other.
- Ions are introduced into the tunnel in between the four rods.
- A direct current field is applied to two rods in the quadrupole and a radio frequency to the others. The rods generate an electric field which ions can travel through.
- For a given DC/RF potential only ions with a specific m/z value are able to pass through the quadropole and reach the detector.
- All other ions will either collide with the rods or travel outside the qudrupole. Therefore they will never reach the detector.



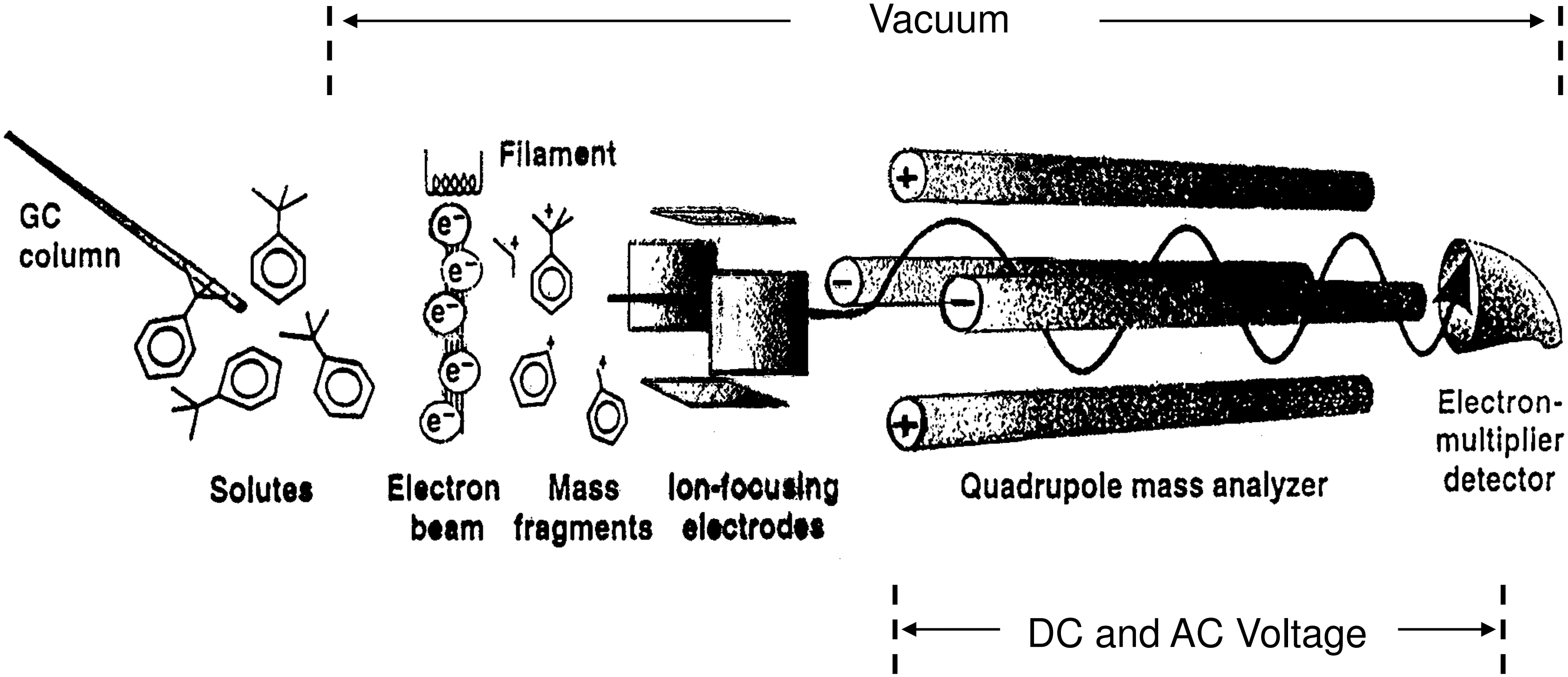
http://www.bris.ac.uk/nerclsmsf/images/quadrupole.gif

Quadrupole



http://ael.gsfc.nasa.gov/images/saturn/quadrupole.jpg

The quadrupole instrument

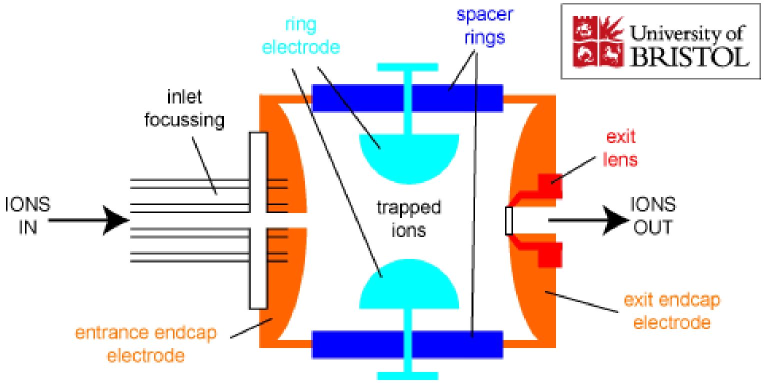


Vacuum

DC and AC Voltage

Ion trap mass spectrometer

- The ion trap uses three electrodes to trap ions in small volumes.
- Various voltages are applied to the ring electrodes as well as to the entrance and exit endcap electrodes. A cavity is created were the ions are trapped.
- Depending on different voltage settings, ions at a specific m/z is ejected and detected.



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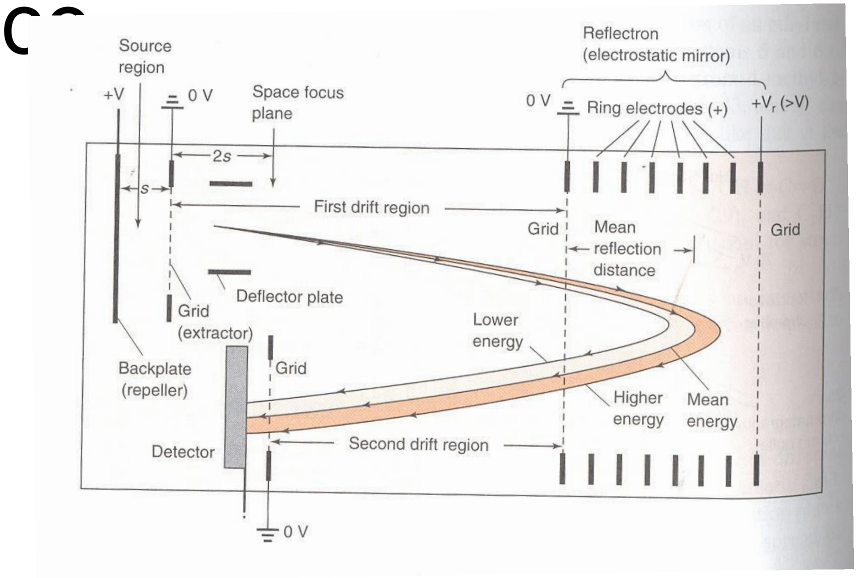
Time of flight (TOF)

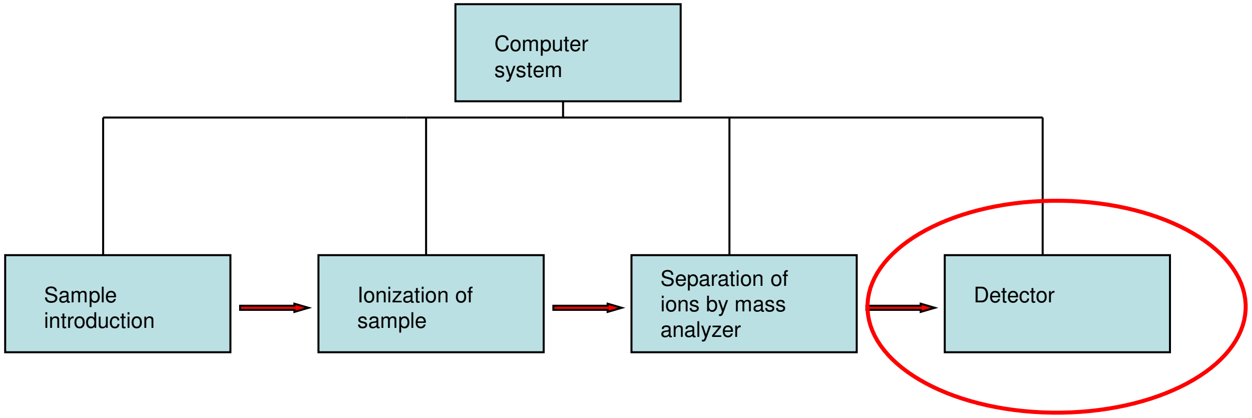
- High resolution MS.
- Separates ion with the same kinetic energy but different m/z, because heavier ions require more time to travel a fixed distanc



Harris. Quantitative Chemical Analysis, 6th ed.

MS detectors

- Many different types available
- Electron multipliers (EM) are often used
  - Continuous –Dynode Version mainly in GC-MS



Computer system

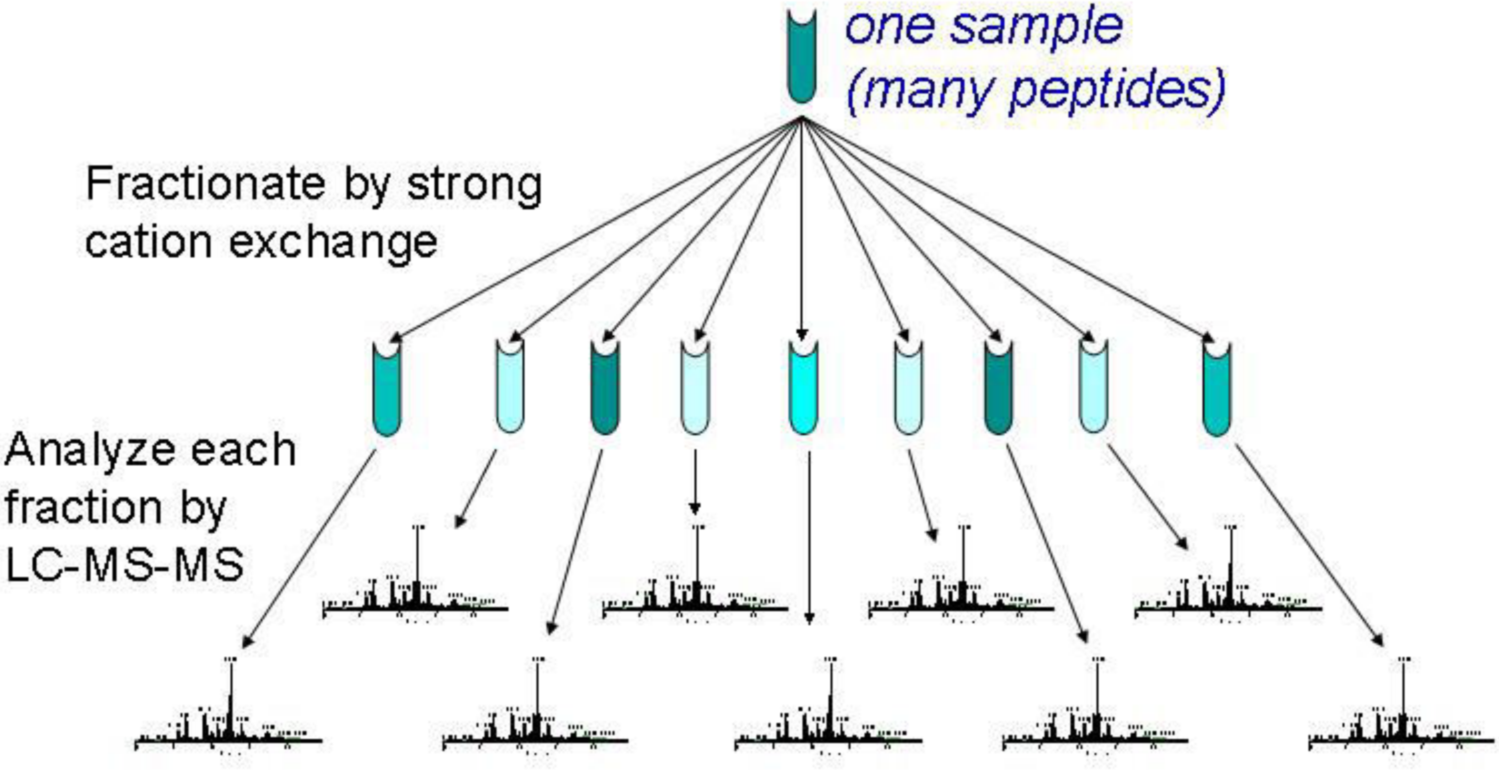
Sample introduction

Ionization of sample

Separation of ions by mass analyzer

Detector

Multidimensional LC-MS



one sample
(many peptides)

Fractionate by strong cation exchange

Analyze each fraction by LC-MS-MS

Multiple LC-MS-MS datasets