GC/MS
A Very Useful Analytical Tool

- Introduction to Gas Chromatography
- Introduction to Mass Spectroscopy
- GC/MS

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Chromatography

The separation of a mixture by distribution of its components between a mobile and stationary phase over time

- mobile phase = solvent
- stationary phase = column packing material

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Milestones in Chromatography

- 1903 Tswett - plant pigments separated on chalk columns
- 1931 Lederer & Kuhn - LC of carotenoids
- 1938 TLC and ion exchange
- 1950 reverse phase LC
- 1954 Martin & Synge (Nobel Prize)
- 1959 Gel permeation
- 1965 instrumental LC (Waters)

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Purpose of Chromatography

- Analytical - determine chemical composition of a sample, and identify compounds via comparison of the retention time of known molecules
- Preparative - purify and collect one or more components of a sample

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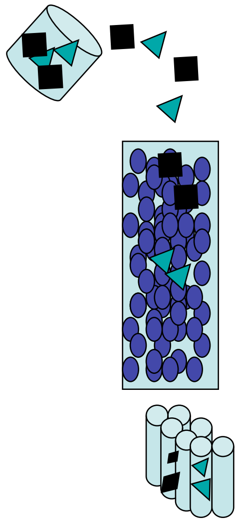
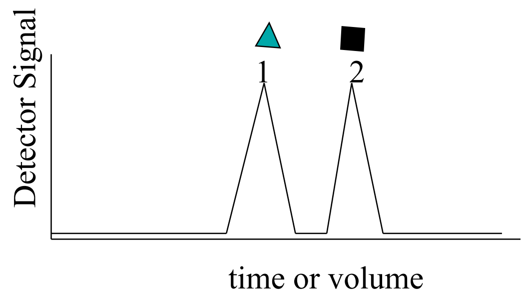
Chromatography



Chromatogram - Detector signal vs. retention time or volume



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Definitions

- Mobile phase - phase that moves through chromatograph
  - In GC - carrier gas is the mobile phase
- Stationary phase - column; phase that is stationary in chromatograph
- Bonded phase - reactive groups imparted to stationary phase in order to achieve selectivity

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Classification of Chromatography

- Mobile phase
- Attractive forces

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Types of Chromatography

Based on Mobile Phase

- Gas - Gas chromatography (GC)
  - 1951 Martin and James (fatty acids)
- Liquid - Liquid chromatography (LC)
  - 1964 Horvath (Yale) instrument
  - 1966 Horvath and Lipsky (nucleic acid components)
- Supercritical fluid - Supercritical fluid chromatography (SFC)
  - 1958 Lovelock (Yale)

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Classification based on Mobile Phase

Gas Chromatography

Gas - solid

Gas - liquid

Stationary Phase

Pyrolysis GC - heat solid materials to 500 - 1000^0C so they decompose into gaseous products

Sample MUST be volatile at temperatures BELOW 350^0C

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Classification based on Mobile Phase

Liquid chromatography (LC)

Column (gravity flow)

High performance (pressure flow)

Thin layer (adsorption)

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Types of Chromatography
Based on Attractive Forces

- Adsorption - for polar non-ionic compounds
- Ion Exchange - for ionic compounds
  - Anion - analyte is anion; bonded phase has positive charge
  - Cation – analyte is cation; bonded phase has negative charge
- Partition - based on the relative solubility of analyte in mobile and stationary phases
  - Normal – analyte is nonpolar organic; stationary phase MORE polar than the mobile phase
  - Reverse – analyte is polar organic; stationary phase LESS polar than the mobile phase
- Size Exclusion - stationary phase is a porous matrix; sieving

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Detectors

- UV-vis
- Refractive Index (RI)
- Mass spectrometry (MS)
- Electrochemical (EC)
  - amperometric
- NMR - novel

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Gas Chromatography

- Mobile phase is gas, the carrier
- Stationary phase is liquid or solid
- The analyzed compounds must be volatile, and stable under the column condition, primarily stable while heated.

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Principle of GC

- Theoretically, the GC is still a process of extraction, one phase is gas, the other is the stationary phase
- The separation efficiency of GC depends on the volatility of molecule and the interaction between the stationary phase and molecules

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GC Liquid Phase

- Low volatility
- High boiling point
- Chemically unreactive
- Examples:
  - 1-squalene
  - Tetrahydroxyethylenediamine
  - Carbowax (polyethylene glycol)
  - Silica gel coated with silane or hydrocarbon

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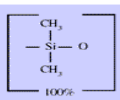
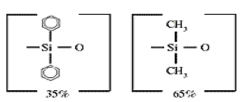
Phases



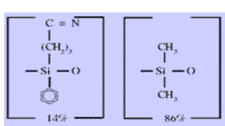
Rtx®/MXT®-1
100% dimethyl polysiloxane
Stable to 360°C
Polarity: non-polar

Uses: solvents, petroleum products, pharmaceutical samples, waxes



Rtx®/MXT®-35
35% diphenyl - 65% dimethyl polysiloxane
Stable to 300°C
Polarity: intermediately polar

Uses: pesticides, Aroclors, amines, nitrogen containing herbicides



Rtx®/MXT®-1701
14% cyanopropylphenyl - 86% dimethyl polysiloxane
Stable to 280°C
Polarity: intermediately polar

Uses: pesticides, Aroclors, alcohols, oxygenates

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Modes of GC Separation

- **Capillary (open tubular)**
  - **Inner wall modified with thin (1 μm) film of liquid**
  - **0.3 - 0.5 mm ID; 10 - 50 m length**
- **Packed**
  - **Solid particles either porous or non-porous coated with thin (1 μm) film of liquid**
  - **1 - 8 mm ID; 1 - 10 m length**

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Modes of GC Separation

- **Isothermal (GC)**
- **Programmed temperature (GC)**
  - **Raising column temperature (GC)**
    - Decreases retention time
    - Sharpens peaks

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Possible Detector

- High sensitivity - high ratio of response/concentration
- Universal or selective response
  - selectivity - ability to distinguish between species
- Rapid response
- Linearity - concentration range over which signal is proportional to concentration
- Stability with respect to noise (baseline noise) and time (drift)

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Detectors for GC

- Destructive
  - Mass Spectral (CI/EI)
  - Flame Ionization (FID)
  - Nitrogen-Phosphorus (NPD)
  - Flame Photometric (FPD)
  - Electrolytic Conductivity (Hall/ELCD)
- Non-destructive
  - Thermal Conductivity (TCD)
  - Electron Capture (ECD)
  - Photo Ionization (PID)
  - Fourier Transfer Infrared (FTIR)

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Detectors for GC

- Electron capture (ECD)
  - radioactive
  - good for X^-, NO_2^- and conjugated
- Thermal conductivity (TCD)
  - change in resistance of heated wire
- Flame ionization (FID)
  - destruction of combustible sample in flame produces measurable current
- Fourier transform infrared (FTIR)
- Mass spectrometry (MS)

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Instrumentation for GC

- Carrier gas
  - N_2, He, H_2
- Injector
- Column
- Detector
- Computer

(Injector, Column, Detector boxed ← oven)

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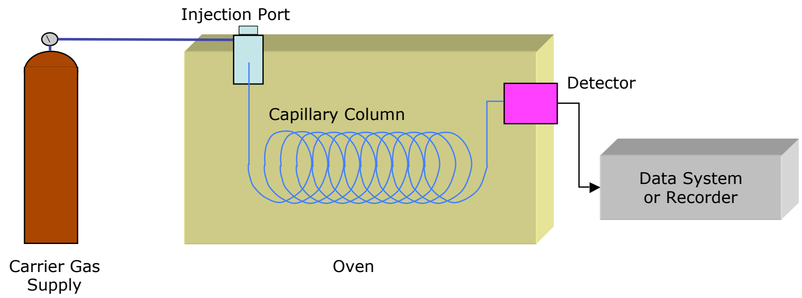
Gas Chromatograph



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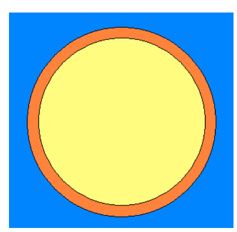
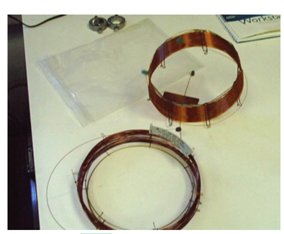
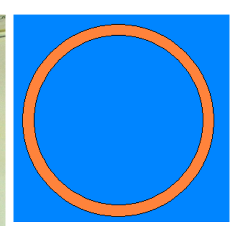
Columns

- Packed



- Capillary





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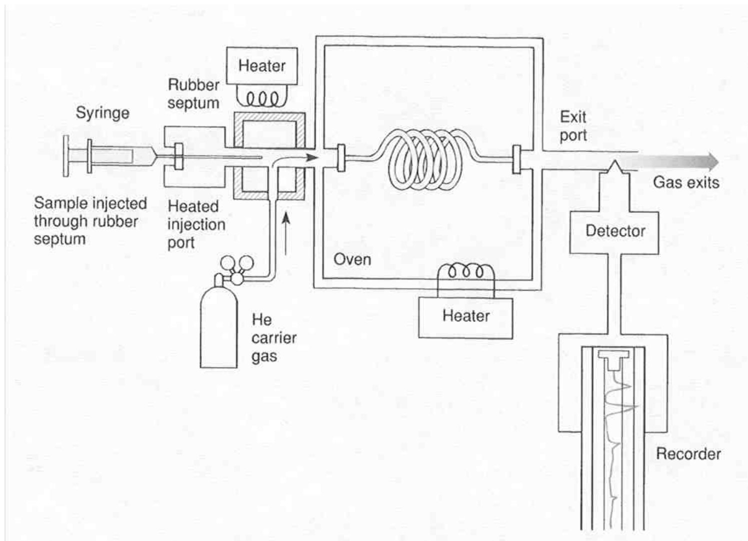
The Gas Chromatograph



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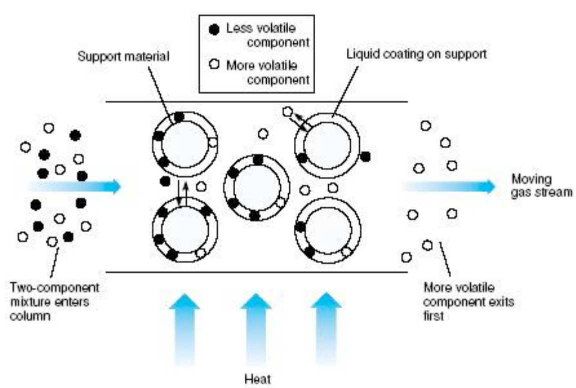
Separation of a Mixture



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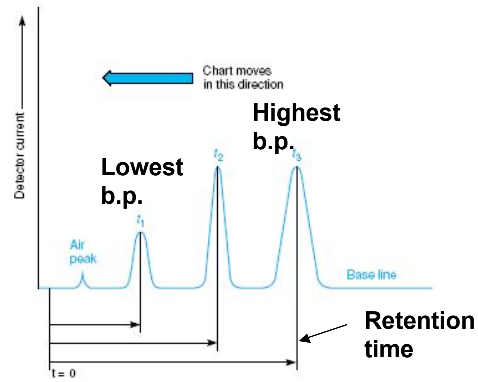
Gas Chromatogram



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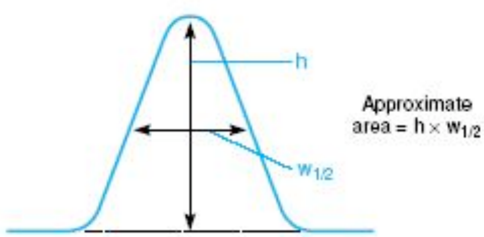
Triangulation of a Peak



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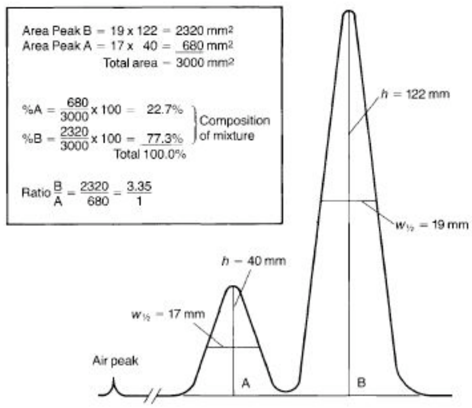
Sample Percentage Composition Calculation



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Gas Chromatography: Results

In a modern gas chromatography instrument, the results are displayed and analyzed using a computerized **data station**. It is no longer necessary to calculate peak areas by triangulation; this determination is made electronically.

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Hexanes (mixture of isomers) **68-70^{o}C**

Cyclohexane **80^{o}C**

Heptane **98^{o}C**

Toluene **110^{o}C**

Mixture separates by distillation according to the boiling point. Compounds with the lower boiling point come off first! The same is true on the gas chromatographic column; the lower boiling compound comes off first!

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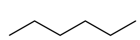
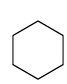
1) hexane has the lowest retention time
2) toluene has the highest retention time

The four compounds come off in the order of increasing boiling point.

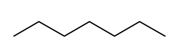
Hexane is actually a mixture of three compounds. It is usually called "hexanes"



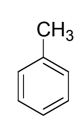
hexane



cyclohexane



heptane



toluene

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Retention Times vs Response Factors

Component	Retention Time (min)	Response Factor
Hexanes (mixture of isomers)	3.054	1.022
Cyclohexane	3.491	1.133
Heptane	3.812	1.000
Toluene	4.331	1.381

NOTE: These values are for illustration purposes. Your actual values will be different!

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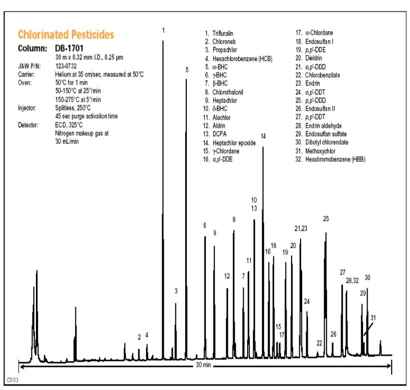
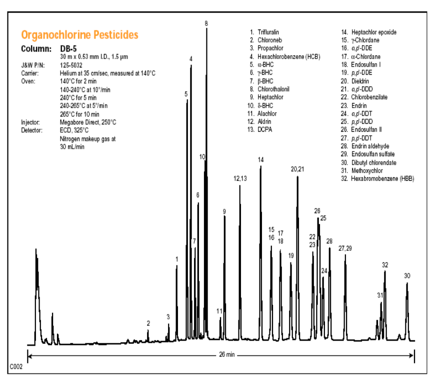
Examples of GC Spectra





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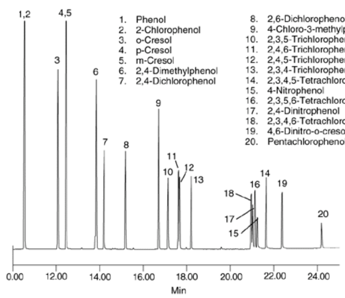
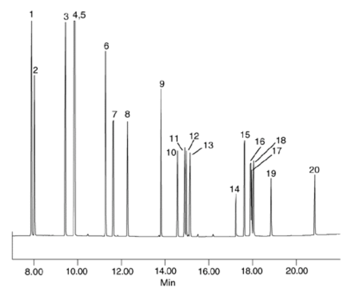
Separation by Different Column



Supelco® PTE-5



Supelco® SPB-50

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Usage and Limitation of GC

- ➢Quantitative Analysis
- ➢Qualitative Analysis (retention time)
- ➢High Separation Efficiency
- ➢Cannot determine the actual structure even if a standard compound is used for standard

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Mass Spectroscopy

- Molecules after ionized, carry charge
- Ionized particles move in either electric or magnetic field
- The distance the charged particles can move depends on the strength of magnetic (or electric field) and the mass-to-charge ratio
- Record of number of particles detected as a function of mass-to-charge ratio is the mass spectrum

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Mass Spectrometry

- Molecular weight can be obtained from a very small sample.
- It does not involve the absorption or emission of light.
- A beam of high-energy electrons breaks the molecule apart.
- The masses of the fragments and their relative abundance reveal information about the structure of the molecule.

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MS Components

- Ionization source
- Analyzer
- Detector

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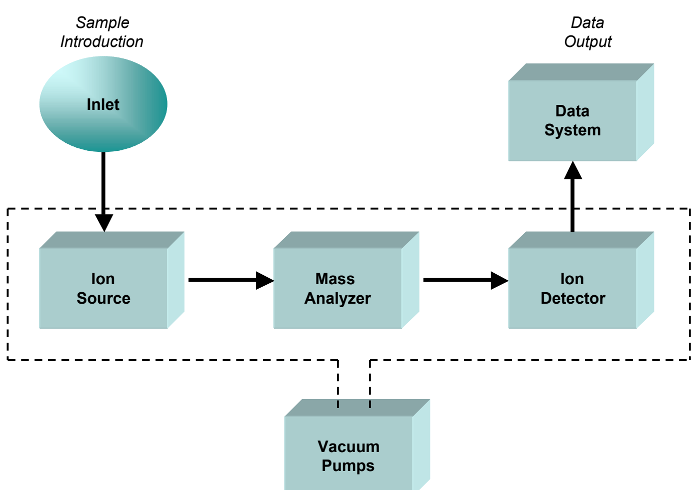
Mass Spectrometer



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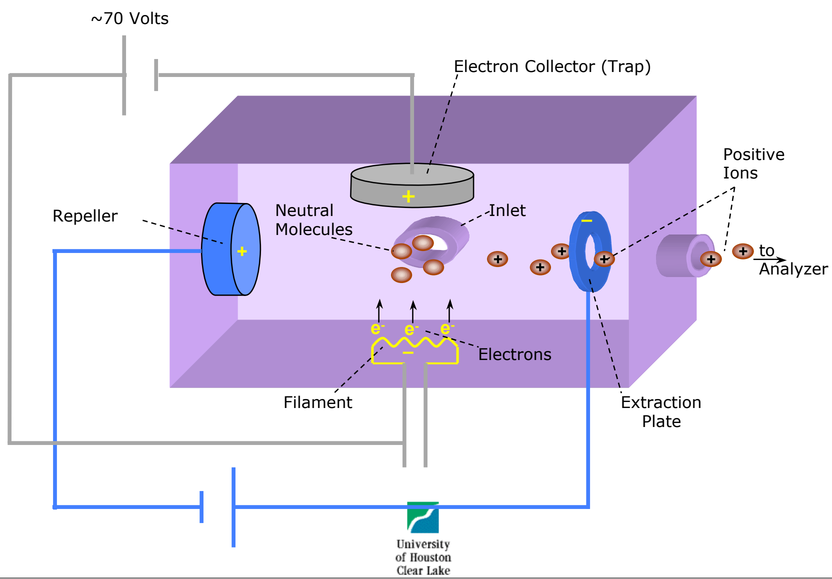
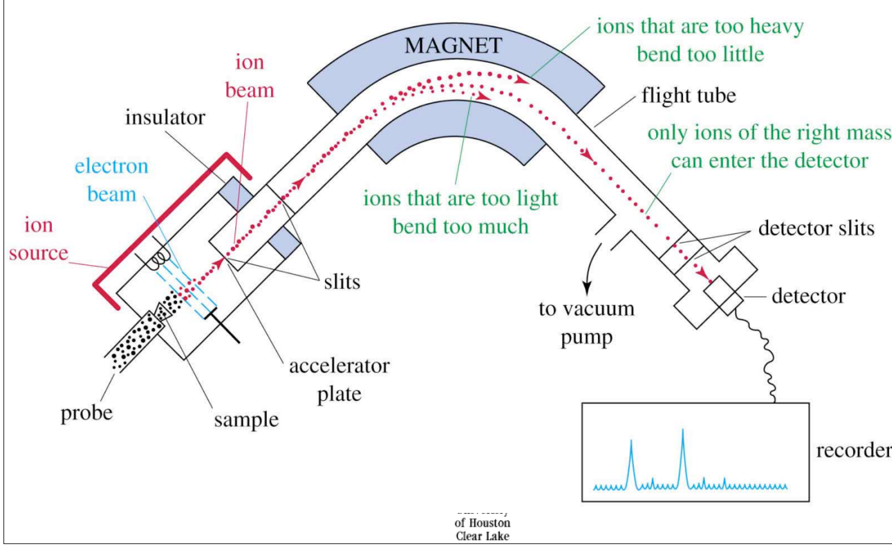
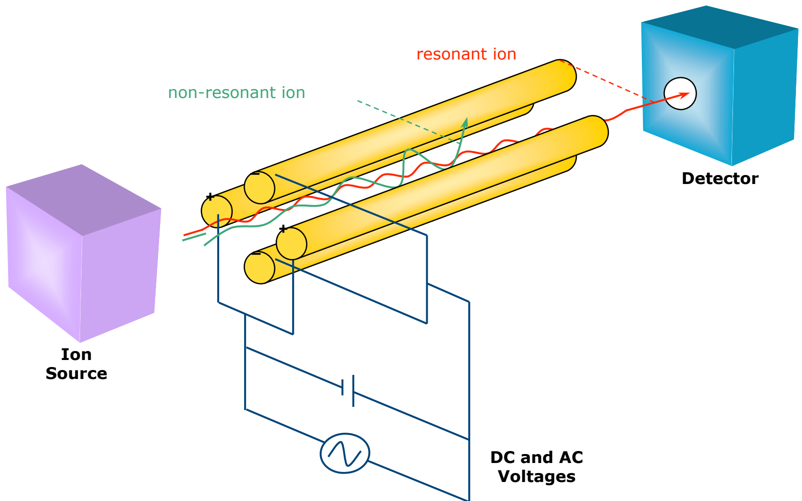
Electron Impact Ionization



Mass Spectrometer



Quadrupole Ion Filter



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Separation of Ions

- Only the cations are deflected by the magnetic field.
- Amount of deflection depends on *m/z*.
- The detector signal is proportional to the number of ions hitting it.
- By varying the magnetic field, ions of all masses are collected and counted.

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Ionization Methods

- **Electron capture (EC)**
  - 70 eV e^- → neutral molecule → energetic molecular ion
  - hard; fragmentation
- **Chemical ionization (CI)**
  - Reagent ion + molecule → molecular ion + reagent ion
  - Reagent ion = He, OH^- (water), CH_5^+ or CH_3^+ (CH_4)
  - soft; less fragmentation

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Ionization Methods

- **Electrospray (ESI)**
  - generation of ions by desolvation or desorption of charged liquid droplets
- **Matrix Assisted Laser Desorption (MALDI)**
  - ionization facilitated by laser irradiation of sample dissolved in an organic matrix
  - EX: sinapinic acid

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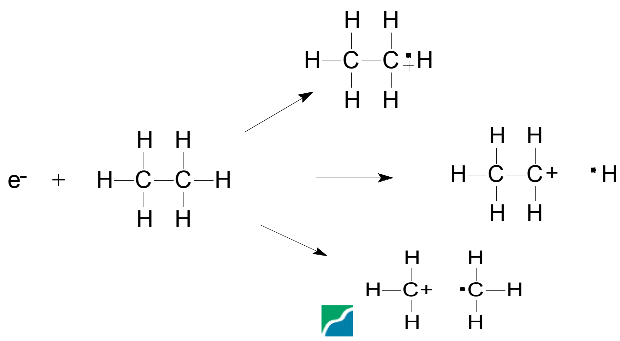
Electron Impact Ionization

A high-energy electron can dislodge an electron from a bond, creating a radical cation (a positive ion with an unpaired e⁻).



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Types of MS Analyzers

- **Quadrupole - most common**
- **Ion trap**
- **Time of Flight (TOF)**

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Two Operational Modes

- Scan
  - Collect mass data over known range
  - Slow
- Selective ion monitoring (SIM)
  - Sample mass at predetermined values
  - Fast

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Mass Spectrum

- X - axis: m/z
- mass - based on $^{12}C \equiv 12.0000$
- Y - axis: relative abundance
  - usually normalized with largest line (base peak)
  - 0 - 100 %

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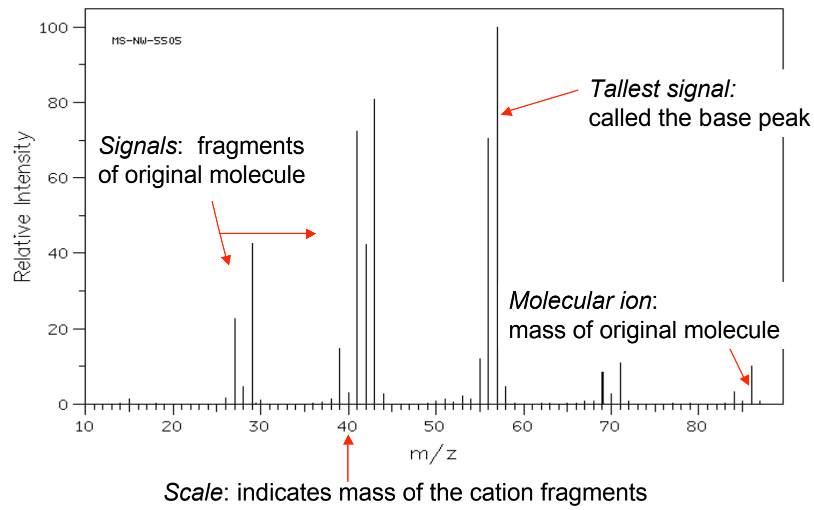
Features of a Basic Spectrum



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Molecular Ion

- Ion whose mass equals that calculated from the molecular formula using the masses for each element which have the highest natural abundance; often tallest peak in highest m/z group
- **Base peak** - most intense peak in spectrum; not necessarily the molecular ion peak!

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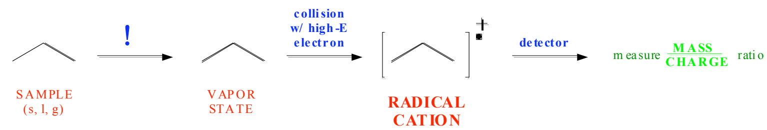
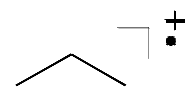
How are Ions Generated in Mass Spec Experiments?

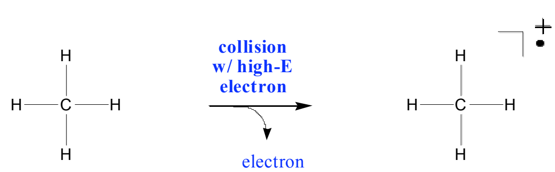


- Radical cations are often abbreviated as:
  - Notice the brackets have been replaced by a corner-brace symbol



What is a Radical Cation?

- **Radical Cations** are *molecules* that have lost one electron
  - As a result the molecule becomes positively charged



1 C 6 (+) 4 H 4 (+)	1 C 6 e⁻ 4 H 4 e⁻	→	1 C 6 (+) 4 H 4 (+)	*ONLY* 9 e⁻ still 10 (+)
10 (+)	10 e⁻		10 (+)	1 (+) charge

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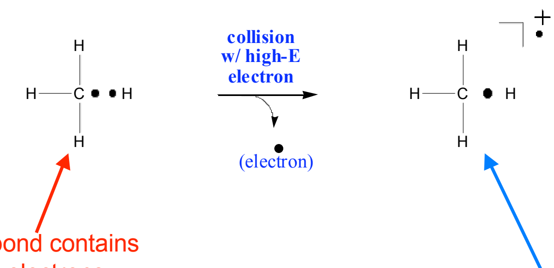
Why is it a Radical *Cation*?



Each bond contains
two electrons
8 total valence electrons

Somewhere in the molecule
a **"one-electron bond"** exists

- **A "one-electron bond" creates an unpaired electron --- which is a *radical***
- **More protons (+) than electrons (-) creates a cation**

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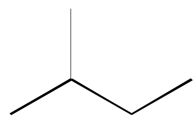
Structure of a Radical Cation Predictable?

- ***YES!!!***
- **Let's discuss the radical cation structures possible for our favorite molecule**



- **Consider: *The stability of carbocations***
- **Question: *Is the stability of carbocations related to radicals?***

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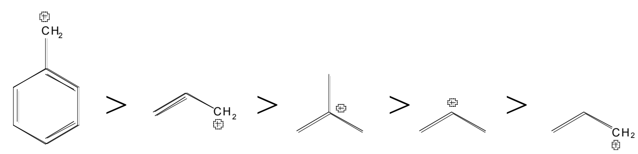
Radical Cation Stability

- Cation stability can be used to predict fragmentation of a molecule
- Cation stability:



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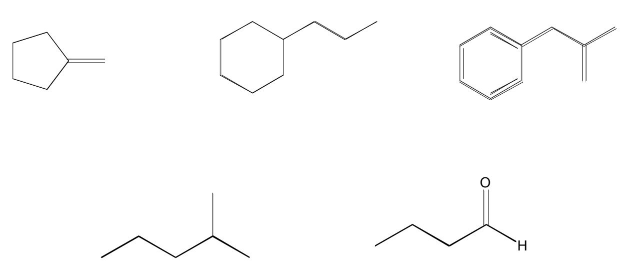
Application of Cation Stability

- Predict the radical cations that are most likely formed from the following molecules



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Mass Spectroscopy

Common Fragmentation Patterns

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General Trends

- Fragments with most stable cation are preferred
  - Form radical stabilized cations before tertiary cations
- Main types of radical cation fragmentation
  - One bond cleavage – forms cation and radical
  - Two bond cleavage – forms neutral and a new radical cation
  - Benzylic rearrangements
  - McLafferty Rearrangement

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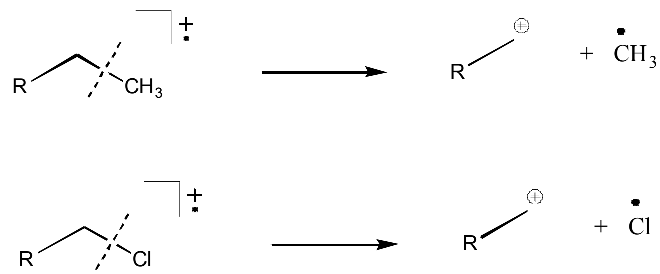
One Bond Cleavage

- Examples



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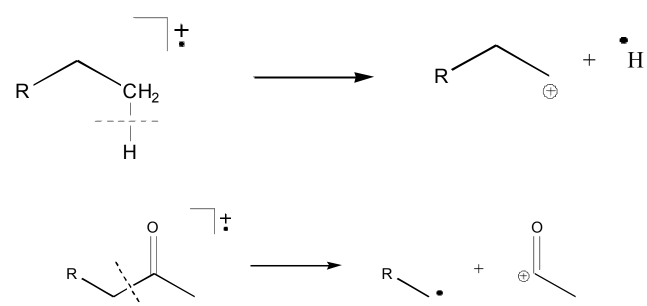
One Bond Cleavage

- Examples



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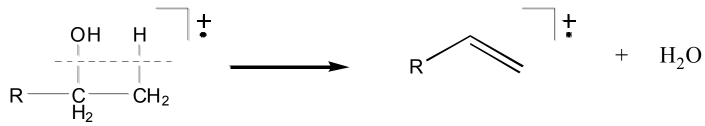
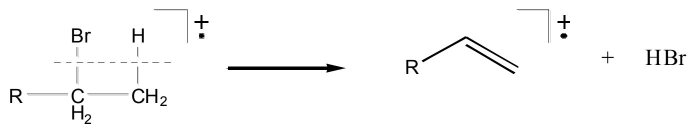
Two Bond Cleavage

- **Examples**





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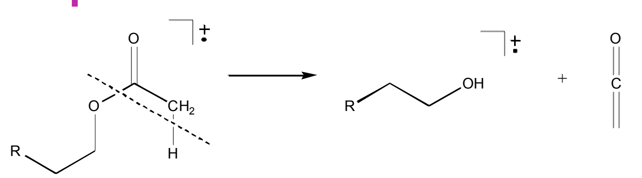
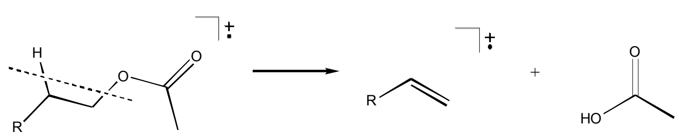
Two Bond Cleavage

- **Examples**





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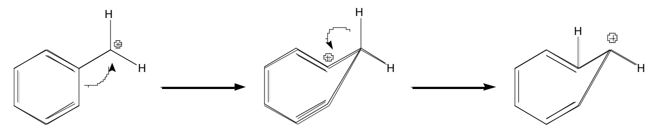
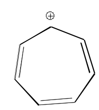
Benzylic Rearrangments

➢ **Tropylium ion formation**



➢ **Tropylium ion structure:** 

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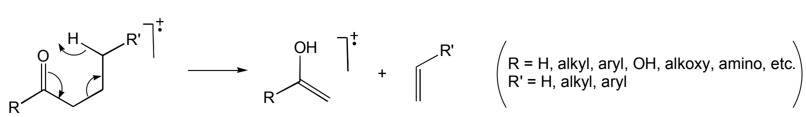
McLafferty Rearrangement

➢ the rearrangement of mono-unsaturated molecular ion or radical ion with the cleavage of α,β-bond of the unsaturated system along with the concomitant transfer of a γ-hydrogen in a six-membered transition state by the formation of a pair of unsaturated fragments regardless of which fragment hold the charge.



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Isotopes

- Most abundant isotope of an element is set to 100%
- Abundance of other isotopes are normalized with respect to it

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Three Classes of Isotopes

- A - only a single isotope
  - Examples: F, P, I
- A+1 - two isotopes with significant relative abundance differing by 1 mass unit
  - Examples: H, C, N
- A+2 - two isotopes with significant relative abundance differing by 2 mass units
  - Examples: Br, Cl, O, S

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Isotopic Abundance

TABLE 12-4 Isotopic Composition of Some Common Elements

Element	*M*⁺		*M+1*		*M+2*	
hydrogen	^{1}H	100.0%				
carbon	^{12}C	98.9%	^{13}C	1.1%		
nitrogen	^{14}N	99.6%	^{15}N	0.4%		
oxygen	^{16}O	99.8%			^{18}O	0.2%
sulfur	^{32}S	95.0%	^{33}S	0.8%	^{34}S	4.2%
chlorine	^{35}Cl	75.5%			^{37}Cl	24.5%
bromine	^{79}Br	50.5%			^{81}Br	49.5%
iodine	^{127}I	100.0%				

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Molecules with Heteroatoms

- Isotopes: present in their usual abundance.
- Hydrocarbons contain 1.1% C-13, so there will be a small M+1 peak.
- If Br is present, M+2 is equal to M^+.
- If Cl is present, M+2 is one-third of M^+.
- If iodine is present, peak at 127, large gap.
- If N is present, M^+ will be an odd number.
- If S is present, M+2 will be 4% of M^+.

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Cl_2 Revisited

- Two isotopes: ^{35}Cl and ^{37}Cl
- Three possible species formed: $^{35}Cl^{35}Cl$, $^{37}Cl^{35}Cl$, and $^{37}Cl^{37}Cl$
- Relative abundance:
  - $^{35}Cl^{35}Cl$: 1.0 x 1.0 = 1.0
  - $^{37}Cl^{35}Cl$ and $^{35}Cl^{37}Cl$: 1.0 x 0.325 = 0.325 each or 0.66
  - $^{37}Cl^{37}Cl$: 0.325 x 0.325 = 0.106
- So, answer: 3 peaks at 70, 72, and 74 with relative intensities of 100, 32.5, and 10.6 %

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Major Steps in Analysis of Mass Spectra

- Identification of molecular ion
  - Base peak
- Examination of isotopic distribution pattern
  - Negative information
  - Determine elemental composition
- Analysis of fragmentation pattern
  - Propose possible structures
  - Compare postulated species to available reference spectra

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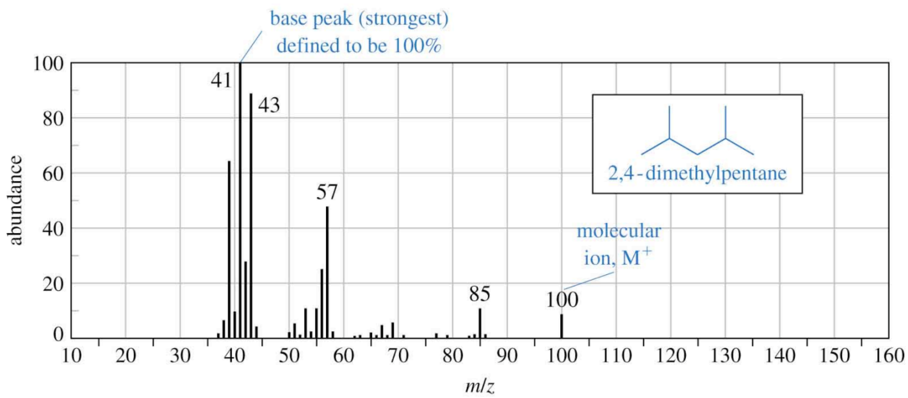
Masses are graphed or tabulated according to their relative abundance.



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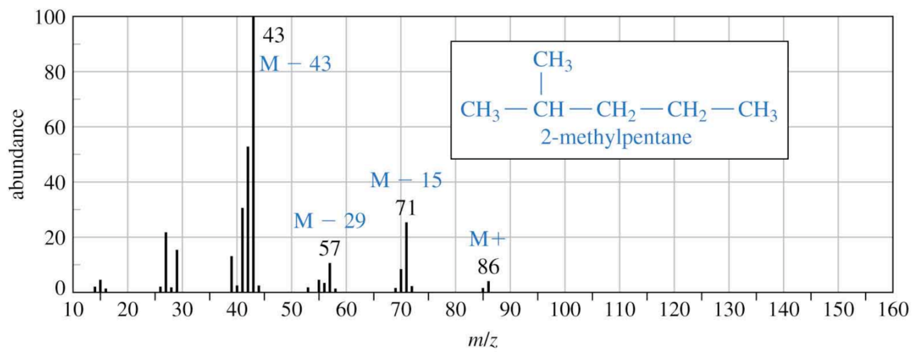
Mass Spectra of Alkanes

More stable carbocations will be more abundant.



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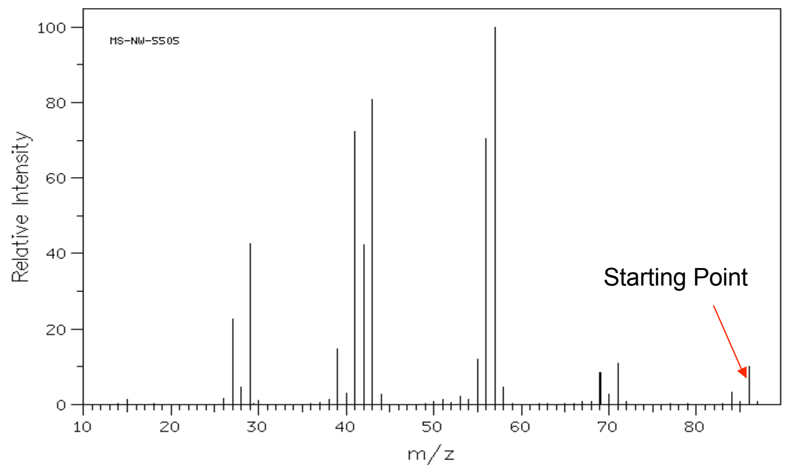
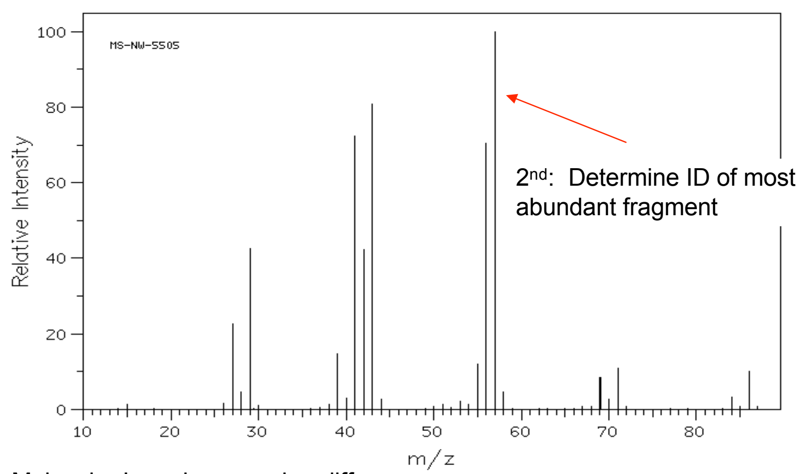
Fragmentation of Hexane



M.W. of Hexanes = 86.15 g/mol

Fragmentation of Hexane

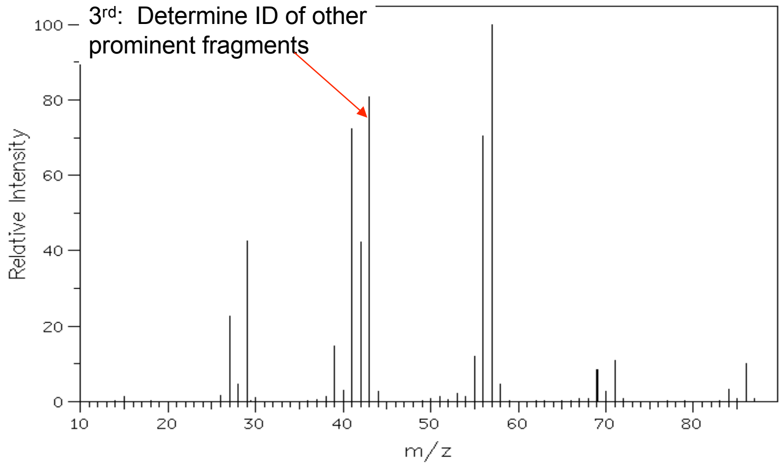


Molecular Ion – base peak = difference

86 – 57 = 29

Difference = $CH_3CH_2^{\bullet}$

Fragmentation of Hexane



Peak = 43 $CH_3CH_2CH_2^{\bullet}$

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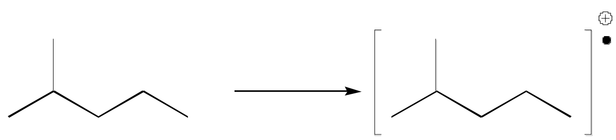

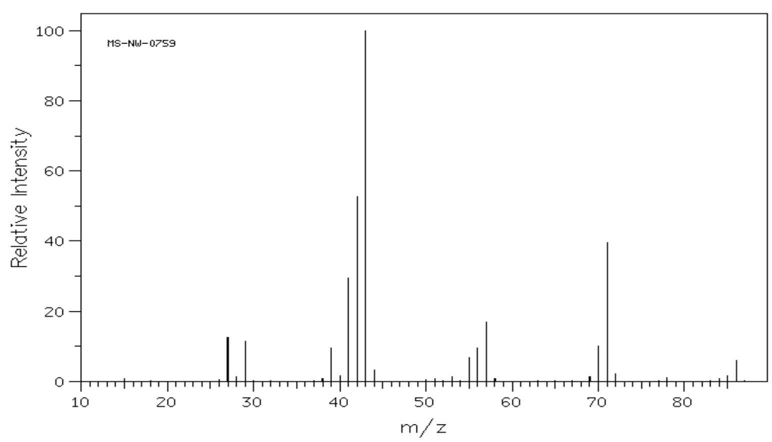

MW = 86

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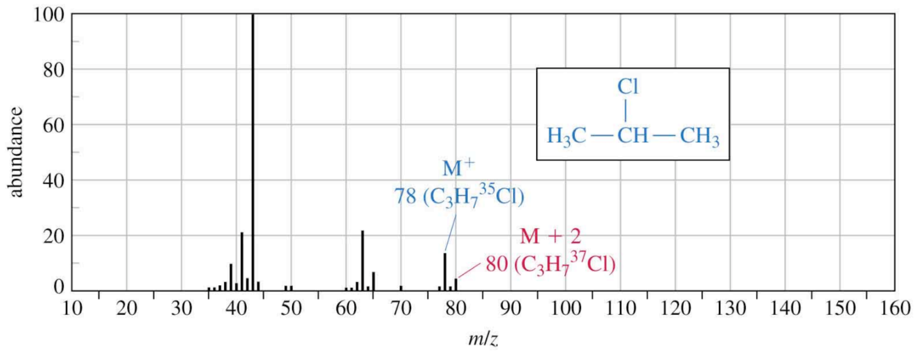
Mass Spectrum with Chlorine



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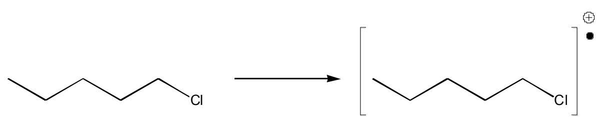
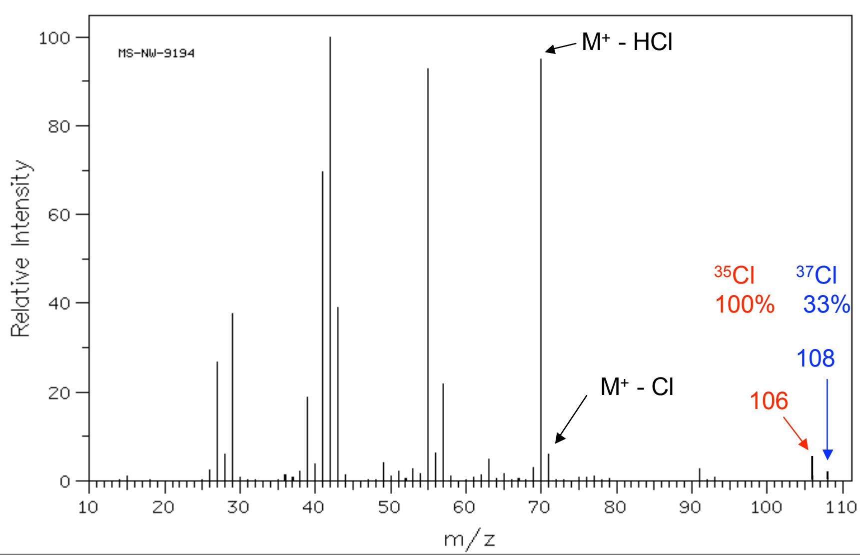
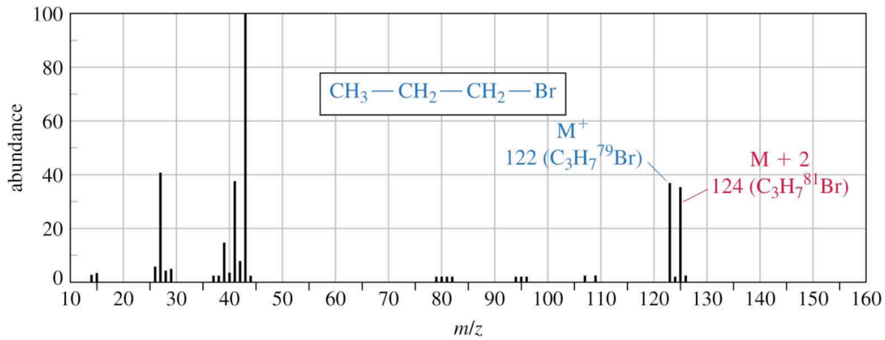


MW = 106



Mass Spectrum with Bromine



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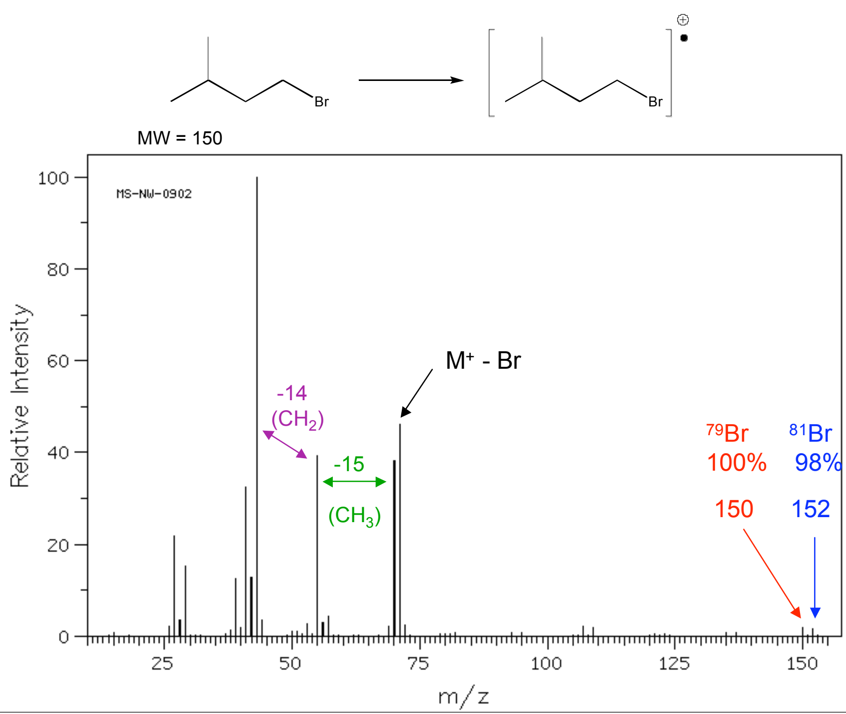
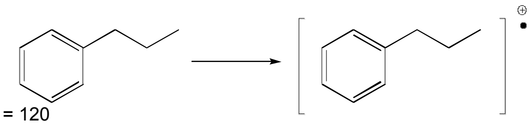
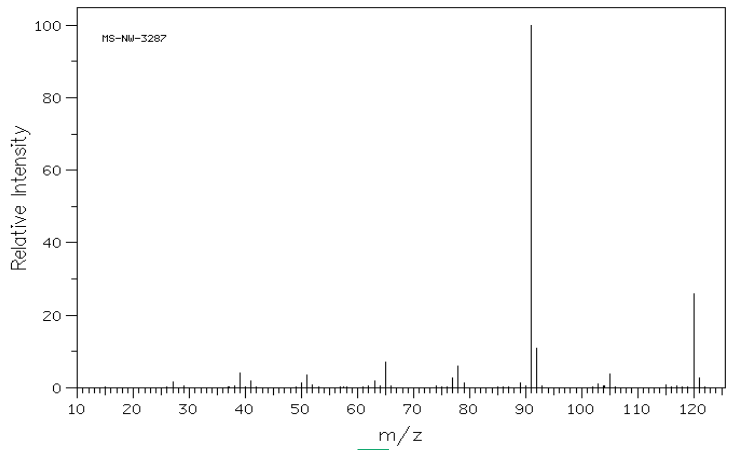




MW = 120



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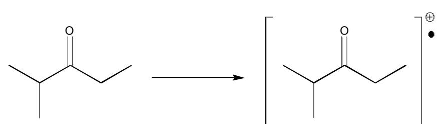
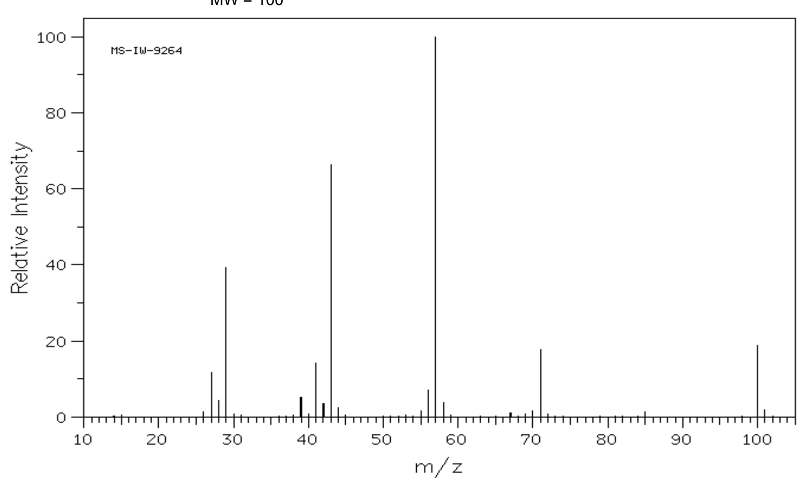


MW = 100



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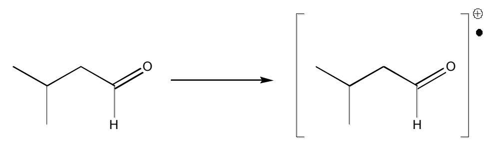
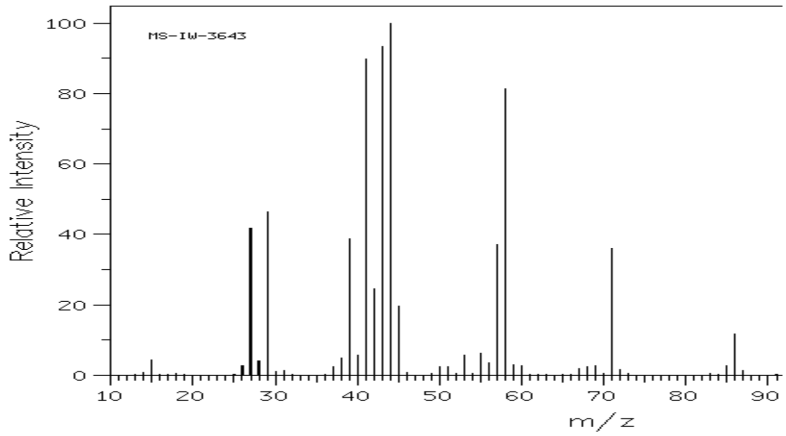


MW = 86



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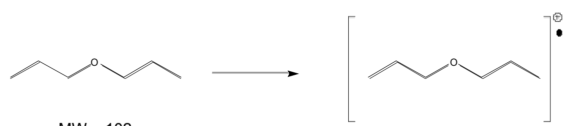
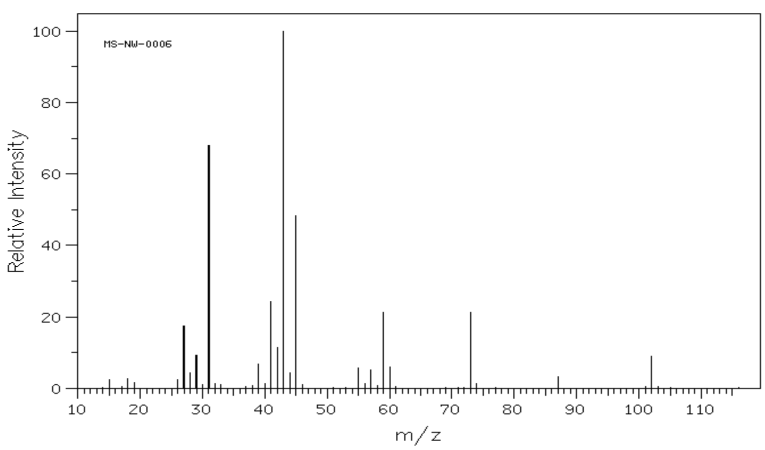


MW = 102



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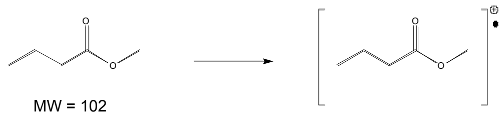
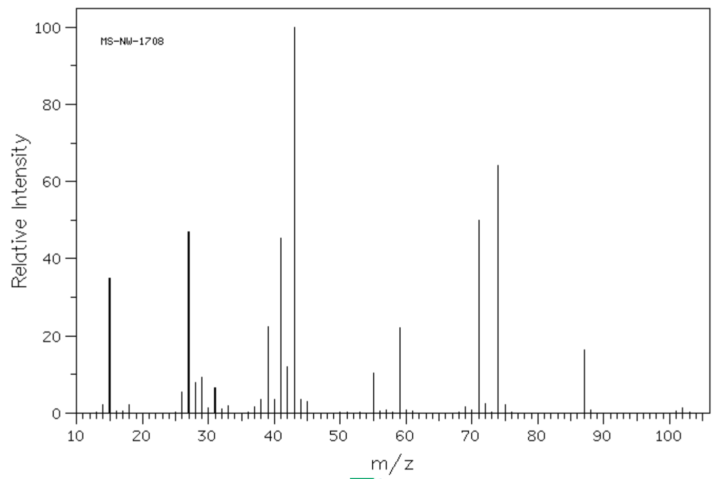


MW = 102



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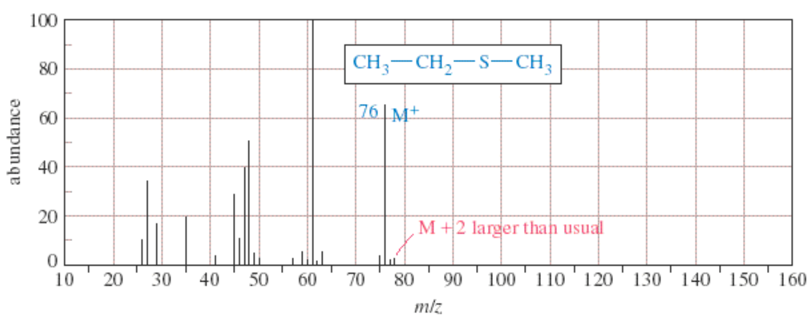
Mass Spectrum with Sulfur



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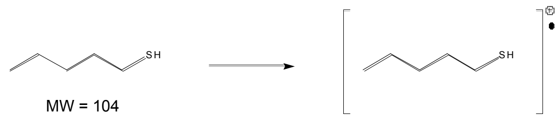
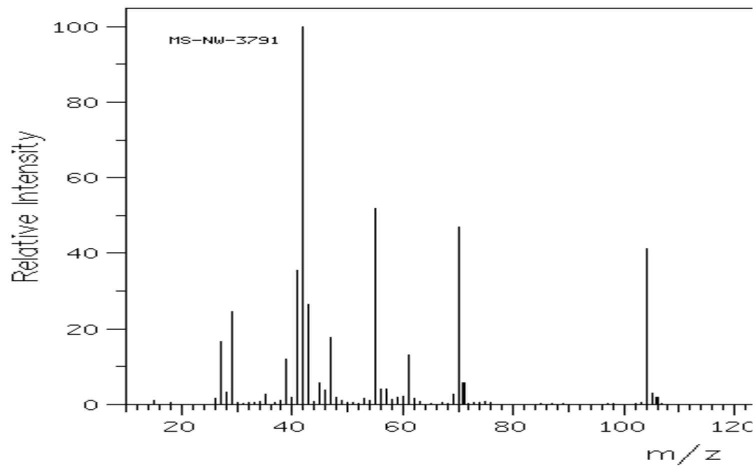


MW = 104



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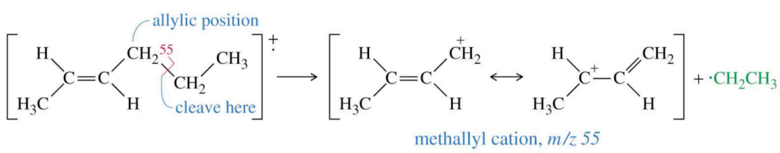
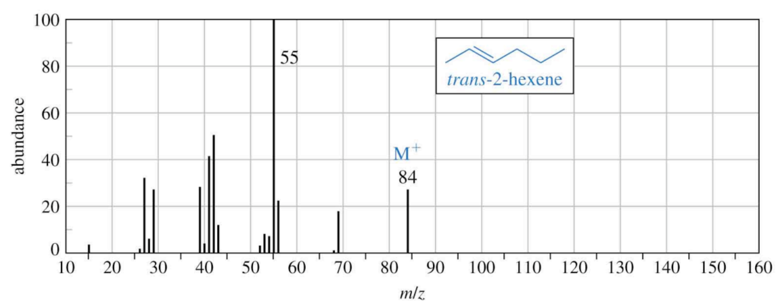
Mass Spectra of Alkenes

Resonance-stabilized cations favored.





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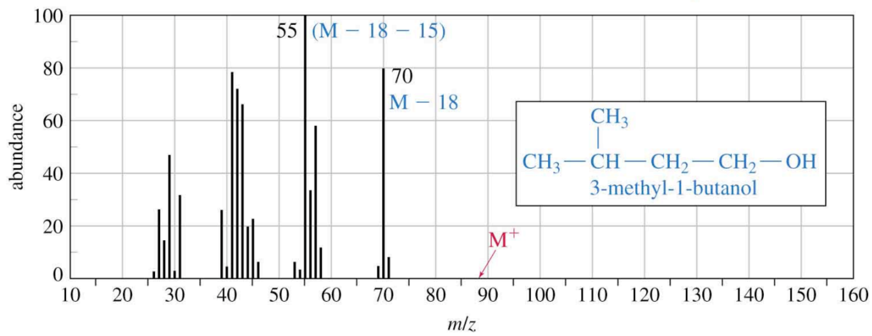
Mass Spectra of Alcohols

- Alcohols usually lose a water molecule.
- M^+ may not be visible.



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High Resolution MS

- Masses measured to 1 part in 20,000.
- A molecule with mass of 44 could be C_3H_8, C_2H_4O, CO_2, or CN_2H_4.
- If a more exact mass is 44.029, pick the correct structure from the table:

C_3H_8	C_2H_4O	CO_2	CN_2H_4
44.06260	44.02620	43.98983	44.03740

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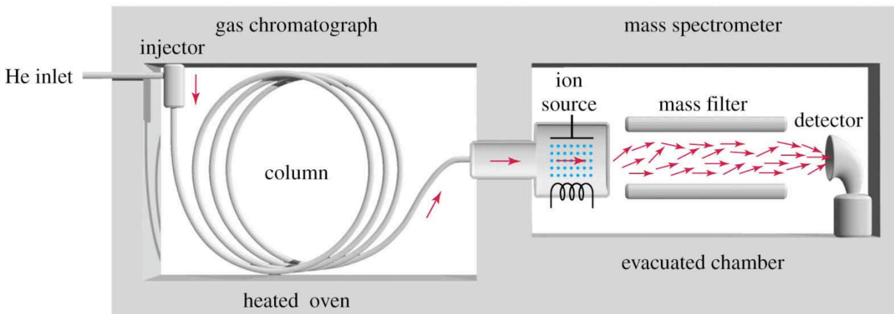
The GC-MS

A mixture of compounds is separated by gas chromatography, then identified by mass spectrometry.



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GC/MS

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