Techniques chimiques pour la biologie

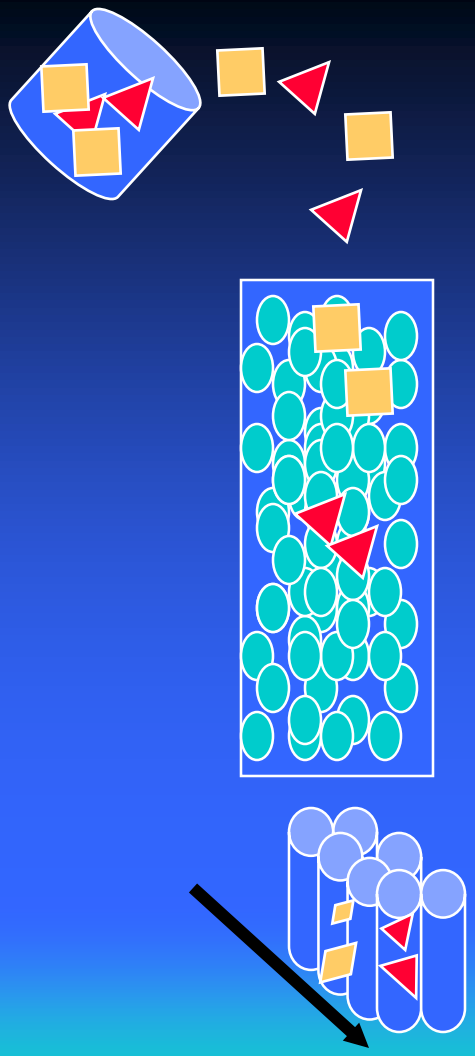
SCIENCES DE LA VIE

An Introduction to Chromatography

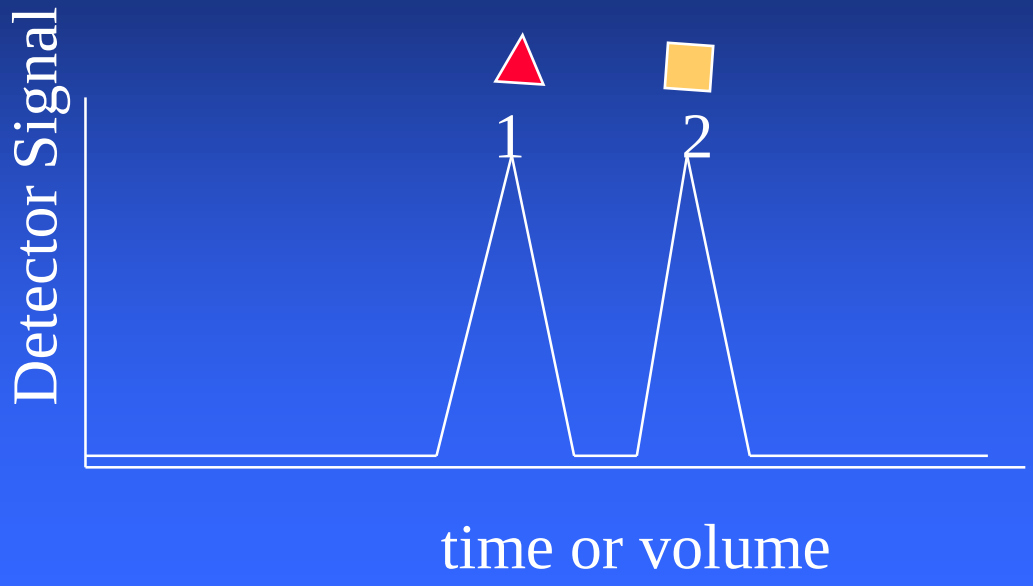
- What IS chromatography?

- 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

Chromatography



Chromatogram - Detector signal vs. retention time or volume



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)

Purpose of Chromatography

- **Analytical -** determine chemical composition of a sample

- **Preparative -** purify and collect one or more components of a sample

Classification of Methods

- There are two classification schemes:
  - mobile phase
  - attractive forces

Mobile Phase

- gas (GC)
- water (LC)
- organic solvent (LC)
- supercritical fluid (SCFC)

Classification based on Mobile Phase

Gas Chromatography

Gas - solid

Gas - liquid

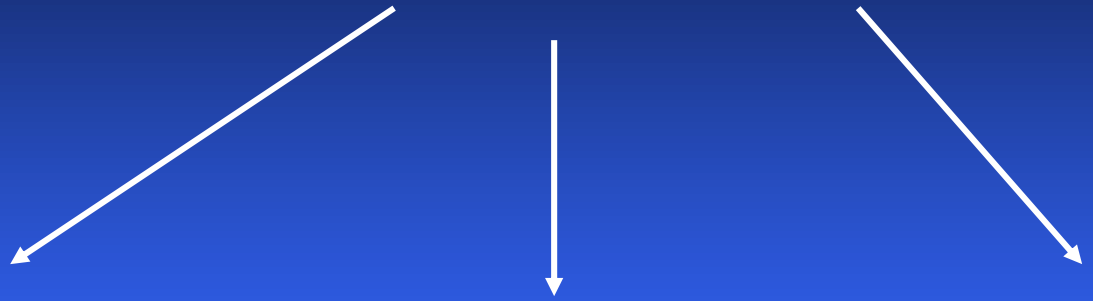
Stationary Phase

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

Sample MUST be volatile at temperatures BELOW 350^{0}C

Classification based on Mobile Phase

Liquid chromatography (LC)



Column (gravity flow)

High performance (pressure flow)

Thin layer (adsorption)

Classification 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

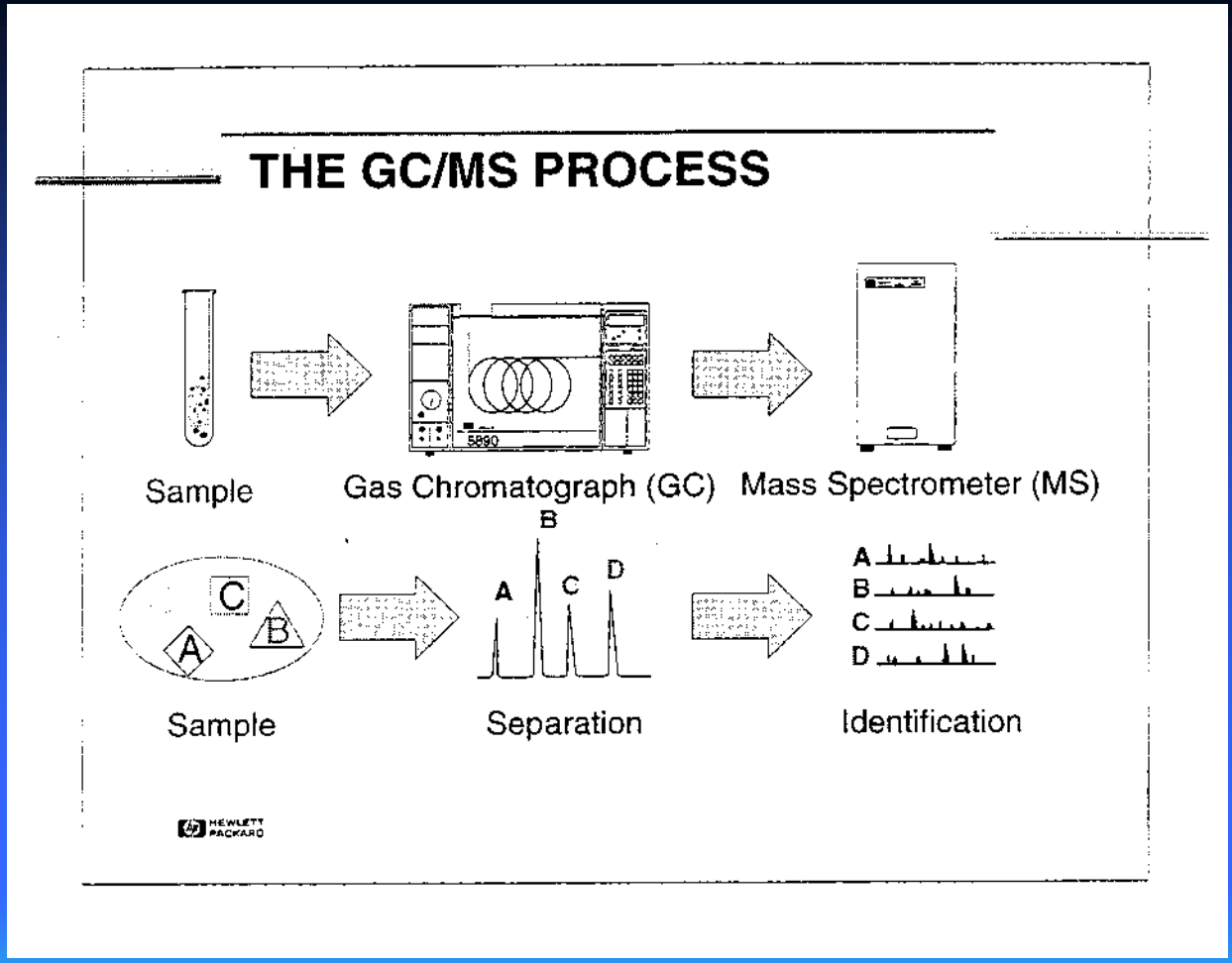
Detectors

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

Problem:

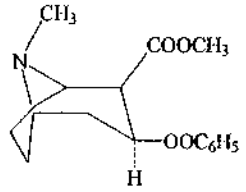
- What would be a good method for determining the following:
  - identity of accelerant at a suspected arson scene
  - amount of caffeine in Coca Cola
  - identifying active ingredient in an illicit drug preparation (LSD is heat sensitive)
  - purification and characterization of novel thermophilic plant enzyme from South America
  - identifying explosive materials used in Oklahoma bombing

The GC-MS Process

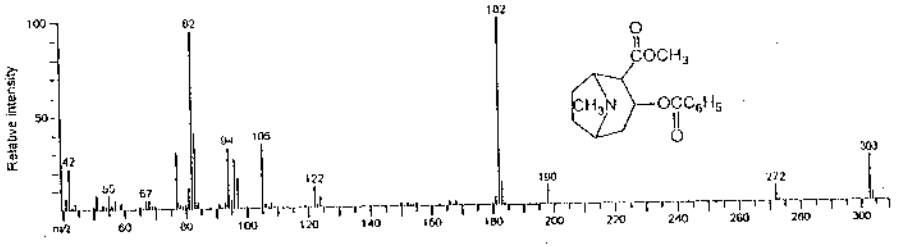


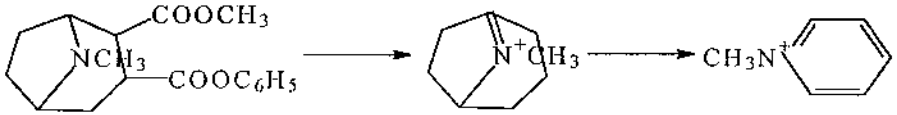
GC-MS Analysis of Money

Can An Innocent Person be Contaminated by Cocaine?



Cocaine





m/z 122 m/z 94

Courtesy of Agilent.

Food Chemistry

- Size Exclusion Chromatography to assess olive oil quality (authenticity, storage)
  - SEC - mode of separation
  - Detection
    - RI
    - UV (254 nm)

Dauwe, C.; Reinhold, G.; Okogeri, O. *Am. Lab.* **2001**, Dec., 22,24.

Evaluate

- Resolution
  - selectivity (Δt_r)
  - efficiency (w)
- Recovery
  - mass recovery
  - activity recovery
- Capacity
- Practicality (Robustness)

Instrumentation

Analytical Chemistry

Lecture #2

Basis of Chromatography

- Definition:

  $C_s = C_m \ K$

- Mechanism - selective retardation caused by interactions with bonded phase of stationary phase

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

Types of Chromatography

- Classification by 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)

Instrumentation for GC

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

oven

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

GC Liquid Phase

- Low volatility
- High bp
- Chemically unreactive
- Examples:
  - 1-squalene
  - Tetrahydroxyethylenediamine
  - Carbowax (polyethylene glycol)

GC - Modes of Separation

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

Properties of a Good Detector

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

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)

MS Components

- Ionization source
- Analyzer
- Detector

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

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

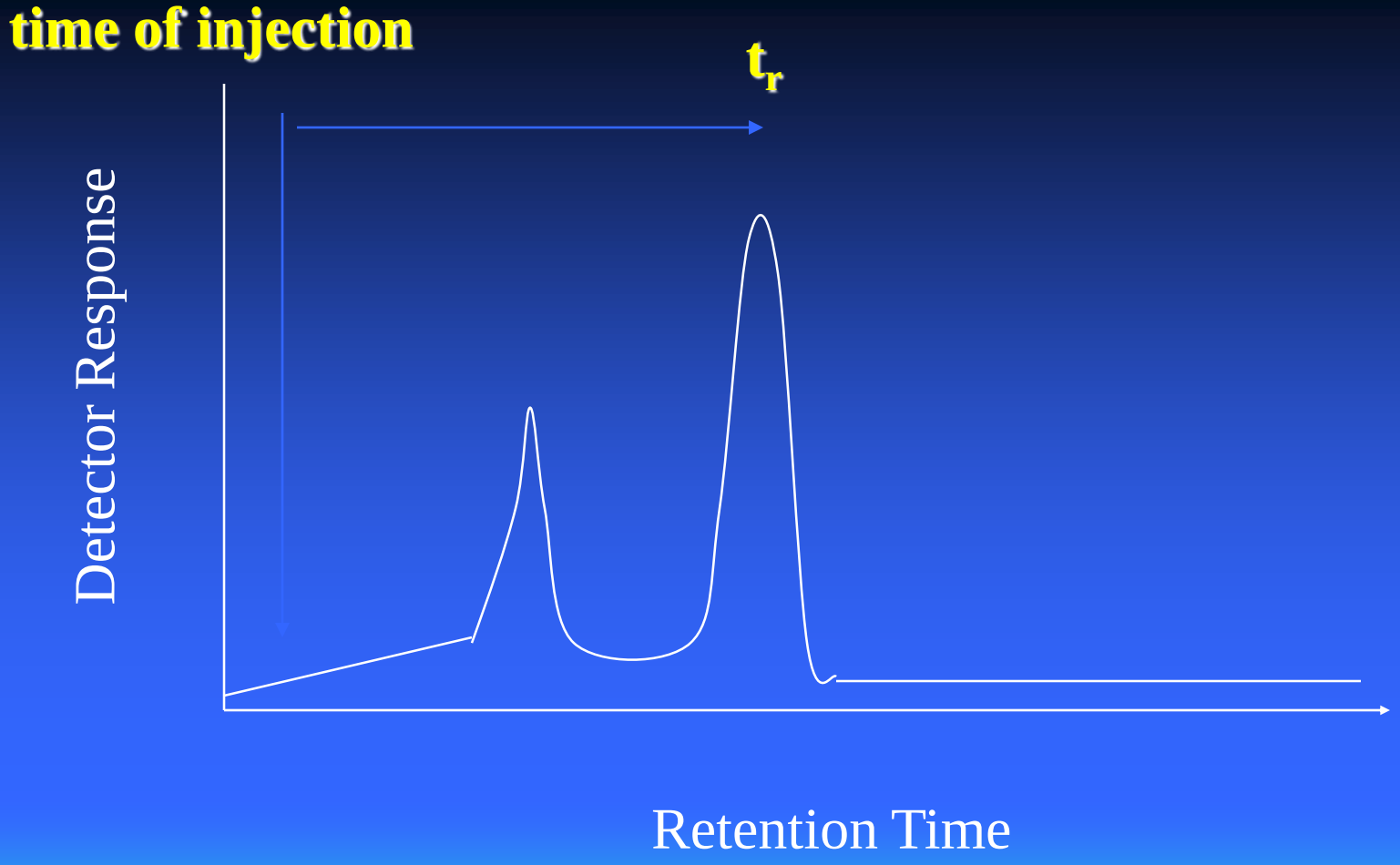
Types of MS Analyzers

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

Two Operational Modes

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

Total Ion Chromatogram



Mass Spectrum - GC-MS

- x-axis
  - GC-MS - m/z
  - LC - retention time or volume
- y-axis - detector response
  - GC-MS - % abundance
  - LC - Abs

Analysis of Organic Mass Spectral Data

Analytical Chemistry

Lecture Topic #3

Mass Spectrum

- X - axis: m/z
- mass - based on $^{12}C \equiv 12.0000$

- Y - axis: relative abundance
  - usually normalized wrt largest line (base peak)
  - 0 - 100 %

Major Steps in Analysis of Mass Spectral Data

- 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

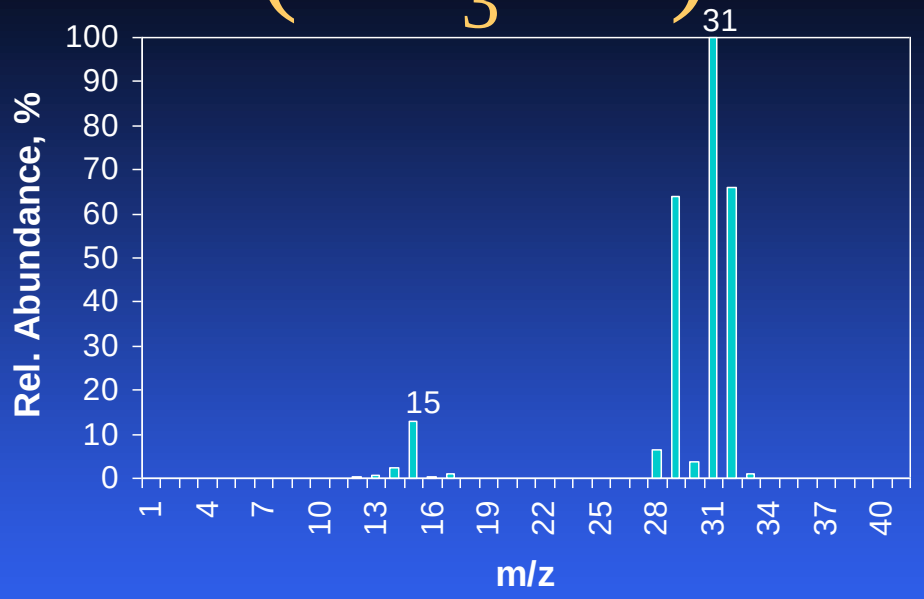
Molecular Ion

- 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!

Example: Mass Spectrum of Methanol (CH_3OH)

m/z	Rel. Abundance
12	0.33
13	0.72
14	2.4
15	13.
16	0.21
17	1.0
28	6.3
29	64
30	3.8
31	100.
32	66.
33	0.98
34	0.14



$CH_3OH + e^- \rightarrow CH_3OH^{+\bullet} + 2e^-$

$CH_3OH^{+\bullet} \rightarrow CH_2OH^+ + H^\bullet$

$CH_3OH^{+\bullet} \rightarrow CH_3^+ + {}^\bullet OH$

$CH_2OH^+ \rightarrow H_2 + CHO^+$

Example 2: Mass spectra for cyclophosphamide

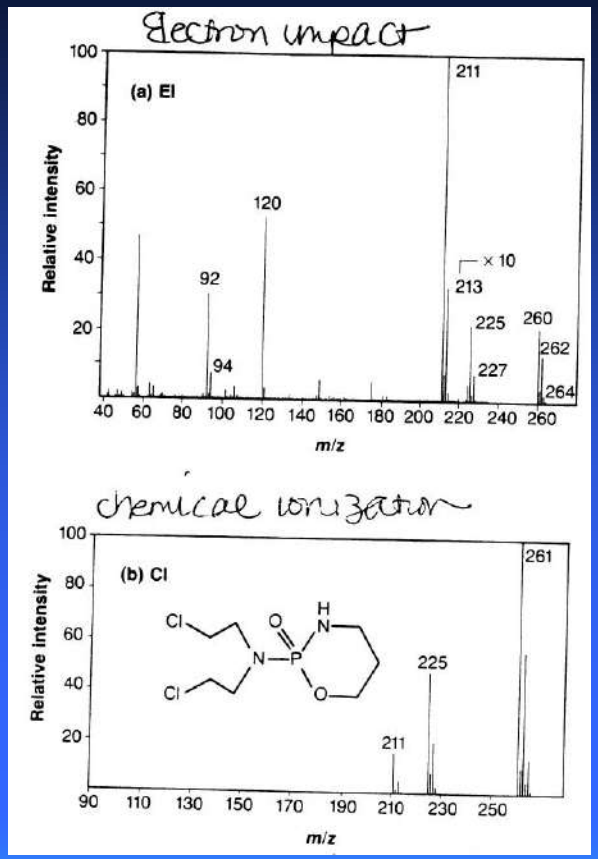


Figure taken from Rubinson, K.A. *Chemical Analysis* Boston: Little, Brown, 1987.

- Method of sample ionization may also change molecular ion
  - EI: $M^{+\bullet}$
  - CI: MH^{+}

Lessons:

- Single charge most common so m/z usually equates to mass (EI/CI)
- Ions may fragment
  - EX: CH_3OH loses H^+ readily
  - Observe: $CH_3O^{+\bullet}$

Isotopes

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

3 Classes of Isotopes

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

Natural Isotopic Abundance of Common Elements in Organic Compounds

Element	Mass	Relative Abundance
F	19	100
P	31	100
I	127	100

A

Natural Isotopic Abundance of Common Elements in Organic Compounds

Element	Mass	Rel. Abundance	Mass+1	Rel. Abundance	Mass+2	Rel. Abundance
H	1	100	2	0.016		
C	12	100	13	1.08		
N	14	100	15	0.36		
Cl	35	100			37	32.5

A+1

A+2

Isotopic Distribution Patterns

- If Cl^- present then two peaks with ratio 100:32.5

Question:

- What Will the Mass Spectrum of Cl_2 Look Like? (Relative Abundance and m/z for all species)

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 %

Natural Isotopic Abundance of Common Elements in Organic Compounds

Element	Mass	Rel. Abundance	Mass+1	Rel. Abundance	Mass+2	Rel. Abundance
O	16	100	17	0.04	18	0.20
S	32	100	33	0.80	34	4.40

A+2

Problem 1:

m/z	Rel. Abundance	m/z	Rel. Abundance
35	1.8	57	1.5
36	1.3	83	2.1
43	2.1	84	64.2
47	13.7	85	2.3
48	5.8	86	42.8
49	100	87	0.8
50	3.5	88	7.1
51	32		

Step 1: Identify Molecular Ion

m/z	Rel. Abundance	m/z	Rel. Abundance
35	1.8	57	1.5
36	1.3	83	2.1
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47	13.7	85	2.3
48	5.8	86	42.8
49	100	87	0.8
50	3.5	88	7.1
51	32		

?

?

Step 2: Normalize Intensity with Respect to the Molecular Ion

Q: Does pattern look familiar?

m/z	Rel. Abundance	m/z	Rel. Abundance	
35		57		
36		83		
43		84	100.	A +2
47		85	3.6	
48		86	66.7	A +2
49		87	1.2	
50		88	11.	A +2
51				

Step 3: Identify Possible Species

- 84 - 2*(35) = 14
- Remaining species must be A-type (C, H, etc)
- 14 - 12 = 2
- Suggests: 2H, 1 C, and 2 Cl → CH_2Cl_2

Step 3: Identify Possible Species (cont'd)

Q: Does pattern look familiar?

m/z	Rel. Abundance	m/z	Rel. Abundance
35	1.8	57	1.5
36	1.3	83	2.1
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47	13.7	85	2.3
48	5.8	86	42.8
49	100	87	0.8
50	3.5	88	7.1
51	32		

A+2

A+2

Step 3: Identify Possible Species

- 49 - (35) = 14
- Remaining species must be A-type (C, H, etc.)
- 14 - 12 = 2
- Suggests: 2H, 1 C, and 1 Cl → CH_2Cl^+

Bon courage

LIENS UTILES ☝

Visiter :

1. https://biologie-maroc.com
   - Télécharger des cours, TD, TP et examens résolus (PDF Gratuit)

2. https://biologie-maroc.com/shop/
   - Acheter des cahiers personnalisés + Lexiques et notions.
   - Trouver des cadeaux et accessoires pour biologistes et géologues.
   - Trouver des bourses et des écoles privées

3. https://biologie-maroc.com/emploi/
   - Télécharger des exemples des CV, lettres de motivation, demandes de ...
   - Trouver des offres d'emploi et de stage