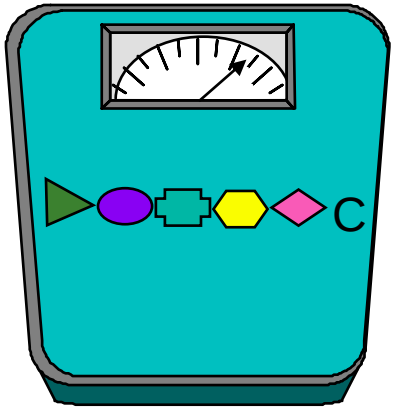
Espectrometria de Massas



O QUE FAZ UM ESPECTRÔMETRO DE MASSAS?

1. Ele mede a massa melhor do que qualquer outra técnica.
2. Ele pode dar informações sobre as estruturas químicas.

PARA QUE SERVEM AS MEDIÇÕES DE MASSA?

Para identificar, verificar e quantificar: metabólitos, proteínas recombinantes, proteínas isoladas de fontes naturais, oligonucleotídeos, candidatos a fármacos, peptídeos, produtos químicos orgânicos sintéticos, polímeros

Aplicações da Espectrometria de Massas

Análise Farmacêutica

- Estudos de biodisponibilidade
- Estudos do metabolismo de fármacos, farmacocinética
- Caracterização novos fármacos
- Análise de produtos de degradação em medicamentos
- Triagem de candidatos a medicamentos
- Identificação de alvos de drogas

Caracterização de biomoléculas

- Proteínas e peptídeos
- oligonucleotídeos

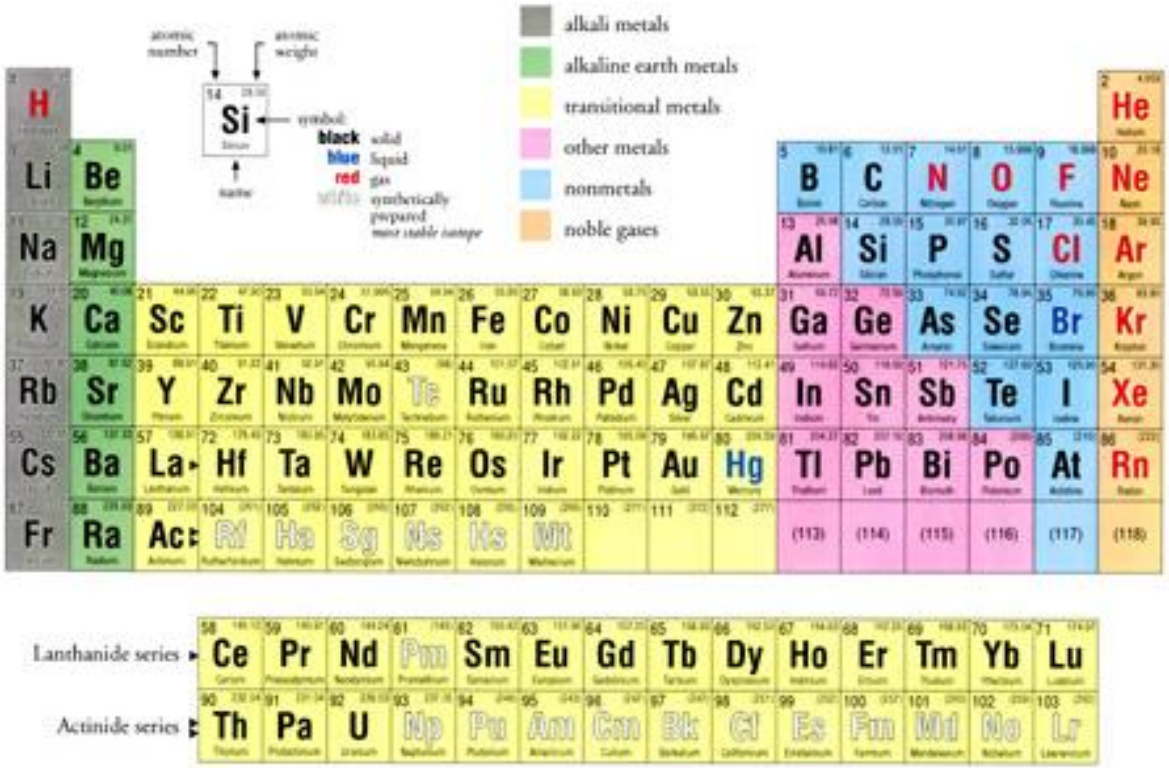
Análise ambiental

- Pesticidas em alimentos
- Contaminação do solo e águas subterrâneas

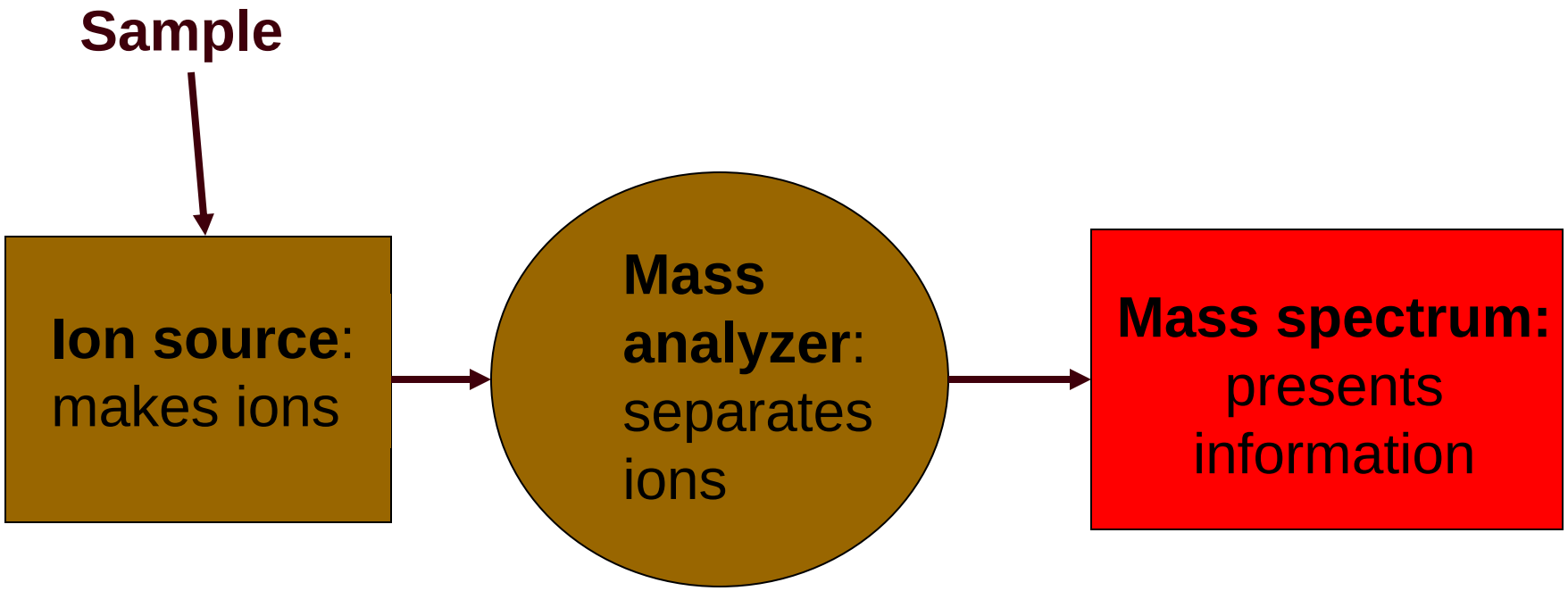
Análise forense / clínica

MS Principles

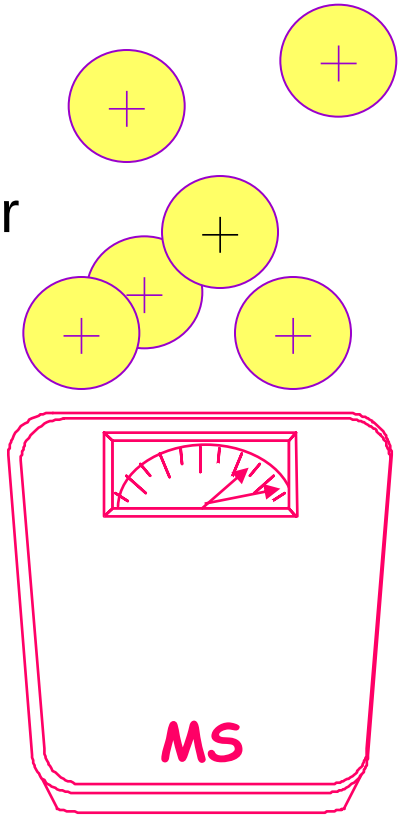
- Different elements can be uniquely identified by their mass



How does a mass spectrometer work?



- Only gaseous ions can be detected by MS:
  - MS provides molecular weight or fragmentations for structural information



  - according to their ***mass to charge ratio***
  - output signals : ***the relative abundance of each ionic species.***

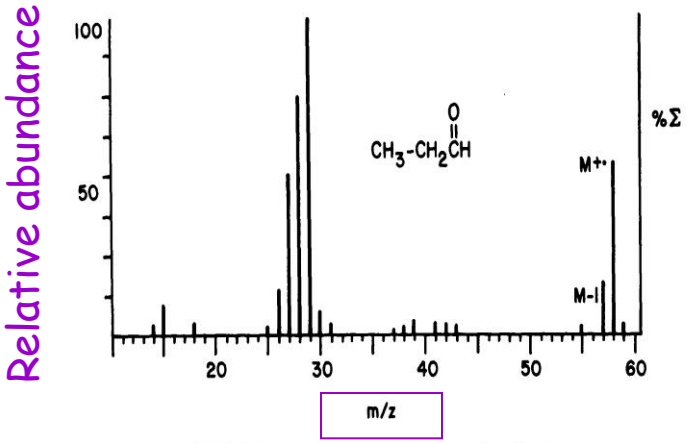


FIG. 1.7. Bar-graph mass spectrum of propionaldehyde.

F = ma (Newton's second law)

The force causes an acceleration that is mass-dependent.

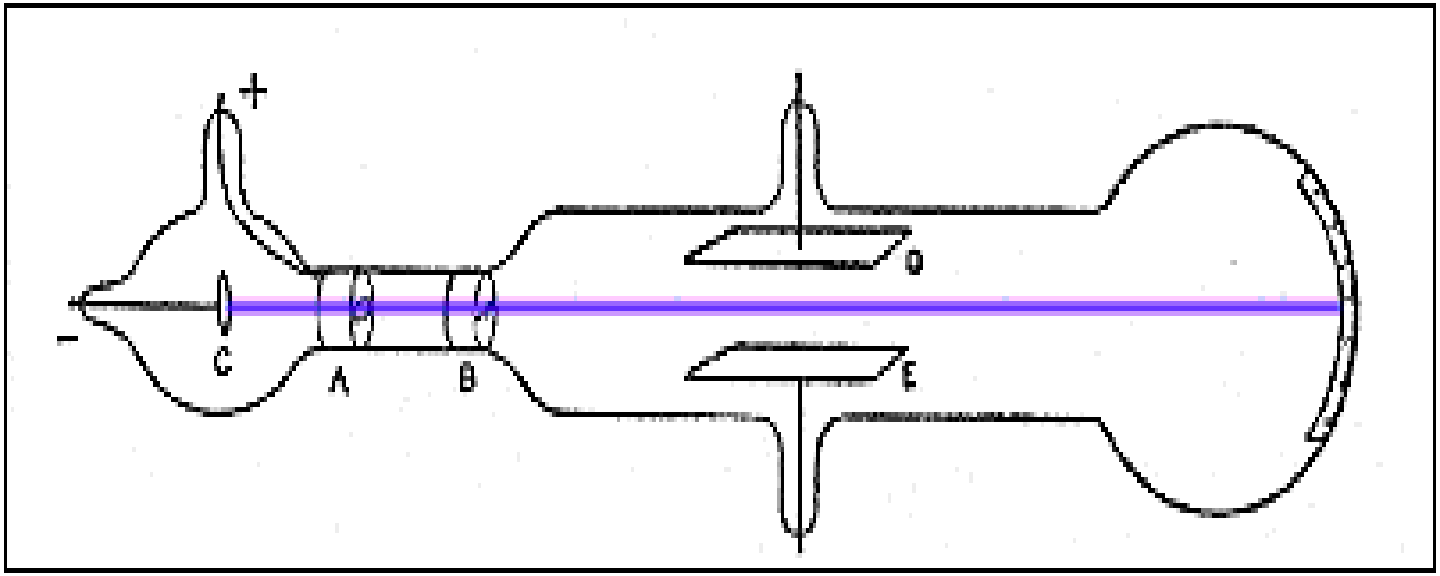
F= e(E+ v x B) (Lorentz force law)

where

F is the force applied to the ion,
m is the mass of the ion,
a is the acceleration,
e is the ionic charge,
E is the electric field
v x B is the vector cross product of the ion velocity and the applied magnetic field

The applied force is also dependent on the ionic charge.

Mass spectrometers separate ions according to their *mass-to-charge ratio* (*m/z*) rather than by their mass alone.



Schematic drawing of Thomson's apparatus in the experiment. Rays from the cathode (C) pass through a slit in the anode (A) and through a slit in a grounded metal plug (B). An electrical voltage is established between aluminum plates (D and E), and a scale pasted on the outside of the end of the tube measures the deflection of the rays.

Cathode Rays & Mass to Charge Ratio (m/z)

- **Cathode rays were deflected in magnetic fields as well as in electric fields.**
  - Thomson was able to calculate the ratio of the mass of the electron to its charge.
    - He could not measure either one separately. Millikane's oil drop experiment solved this problem. → **$e=1.6 \times 10^{-19}$ coulomb**

F = ma (Newton's second law)

F= e(E+ v x B) (Lorentz force law)

Thomson's Parabola Mass Spectrograph

1907 **for his work in *positive ray***

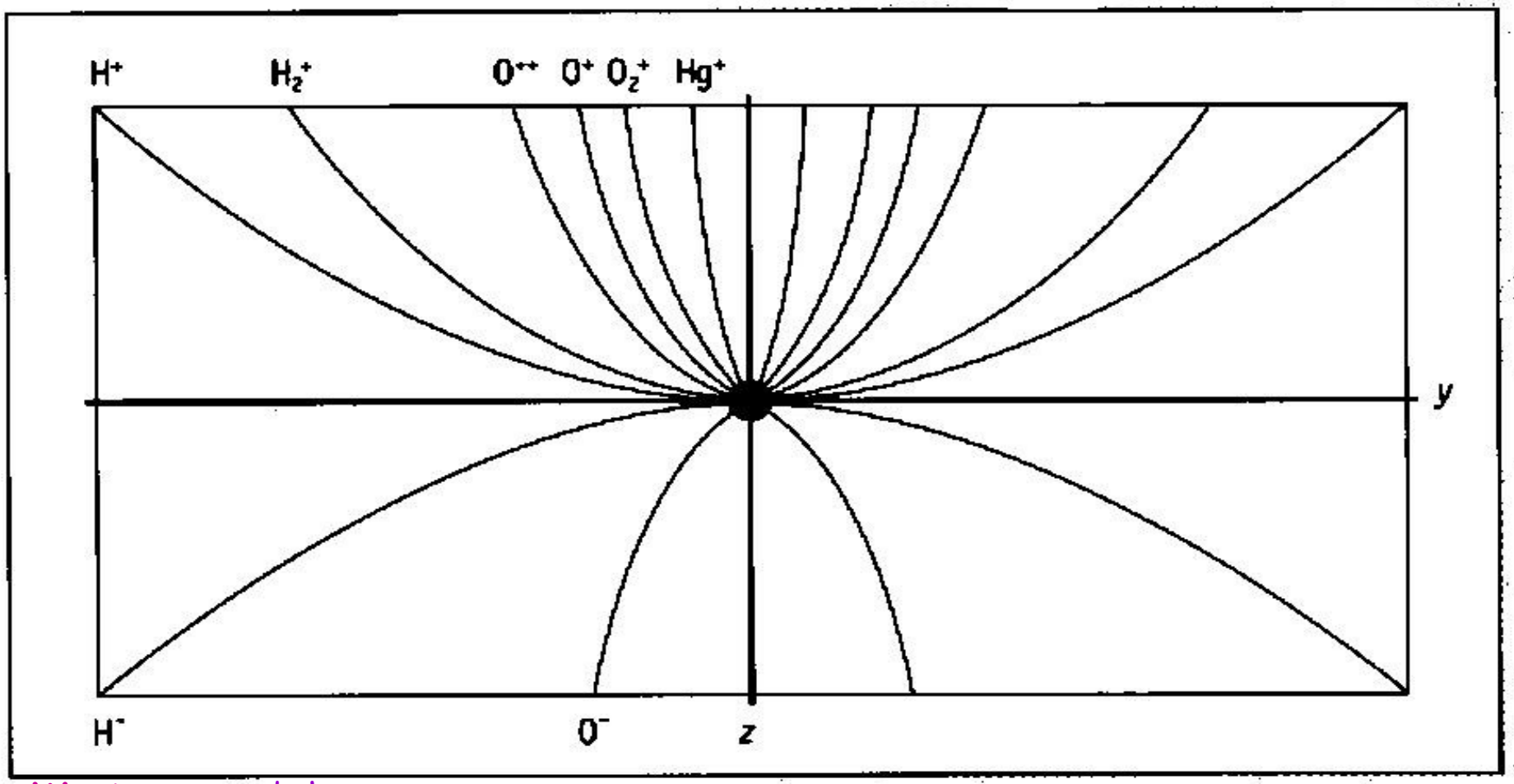


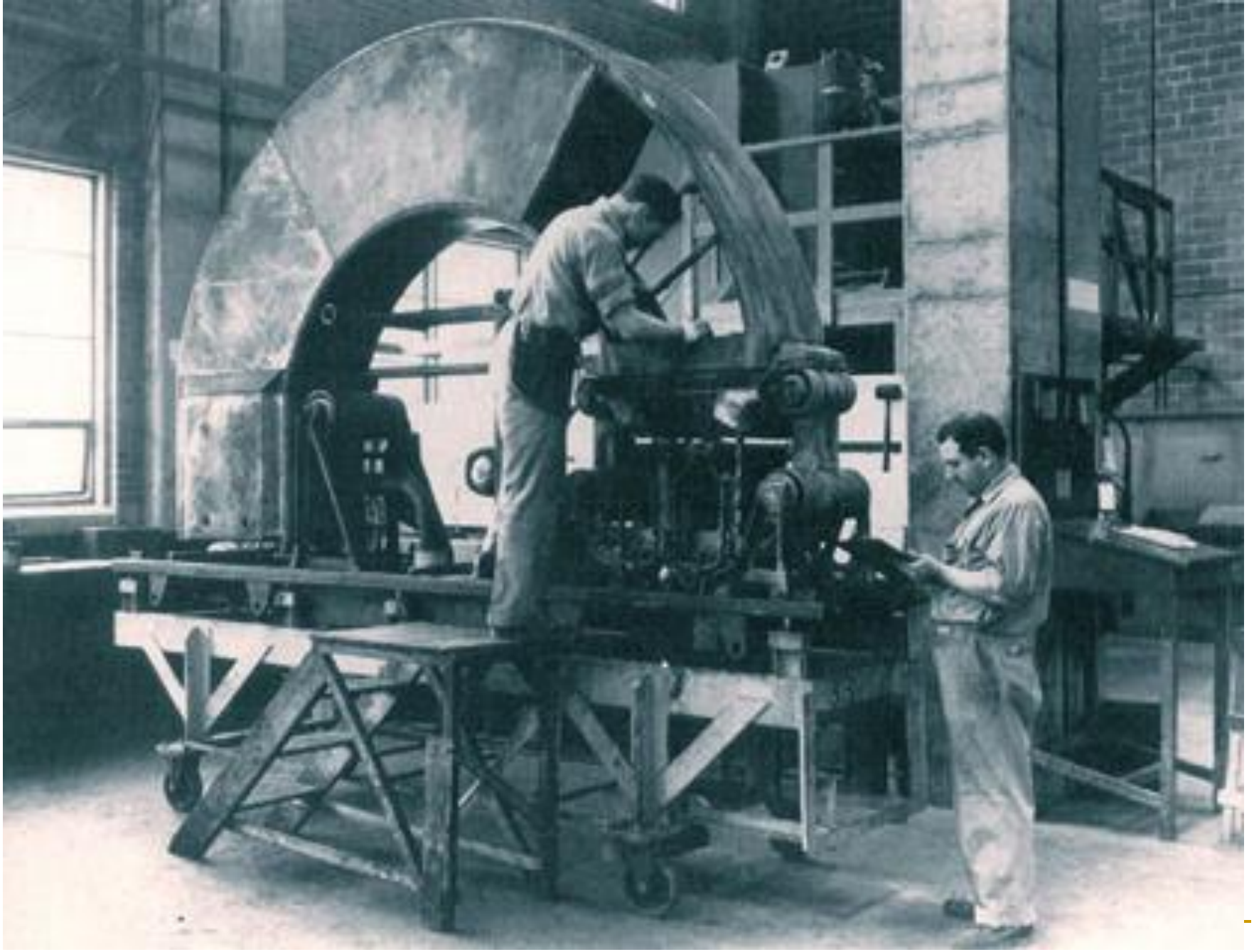
Figure 1. Hypothetical photographic record of a parabola mass spectrograph. Reproduced from R. W. Kiser, Introduction to Mass Spectrometry and Its Appucations *(Englewood Cliffs, N.J.: Prentice Hall. 1965).*

A. W. Aston used the mass spectrometry technology to discover the isotopes for many elements →

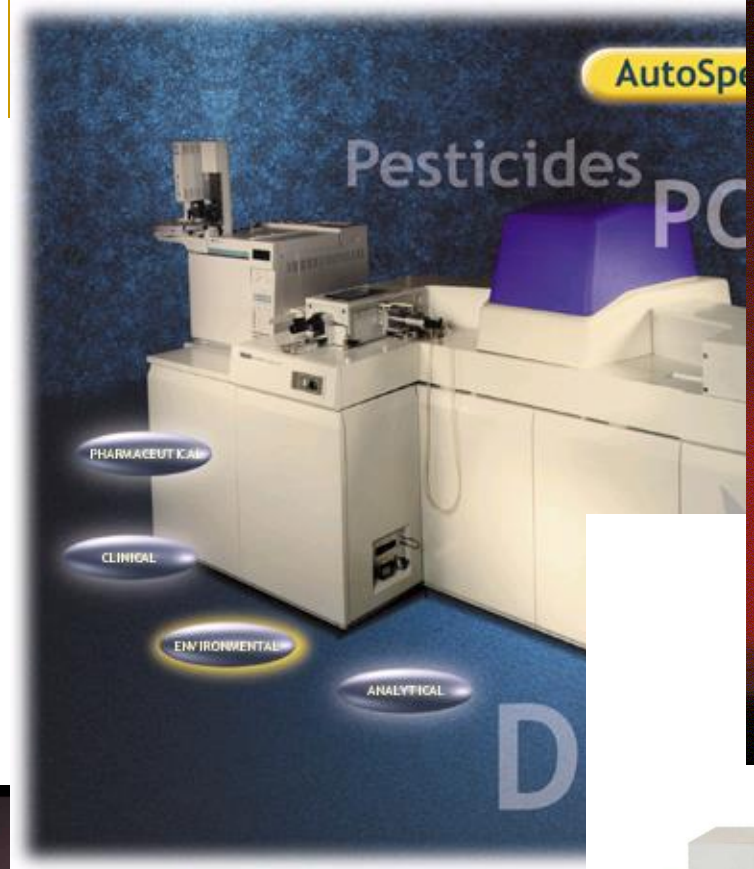
Inorganic chemist→ Petroleum chemists

Espectrometria de Massas
Resumo Histórico

- 1898 – Wien observou que um feixe de íons positivos poderiam ser desviados empregando campo elétrico ou magnético.
- 1912 – Thomsom provou a existência de dois isótopos de neônio usando um instrumento que desviava os íons em um campo elétrico.
- 1918 – Dempster e Aston desenharam instrumentos que foram utilizados nas medidas de abundâncias relativas de isótopos.
- 1940 – Espectrômetros de massas começaram a ser utilizados em indústrias de petróleo.
- 1960 – McLafferty, Beynon, Biemann, Djerassi e Budzikiewicz entre outros estudaram a fragmentação de compostos orgânicos no espectrômetro de massas.



http://masspec.scripps.edu/MSHistory/histpers.php


AutoSpe
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PHARMACEUTICAL
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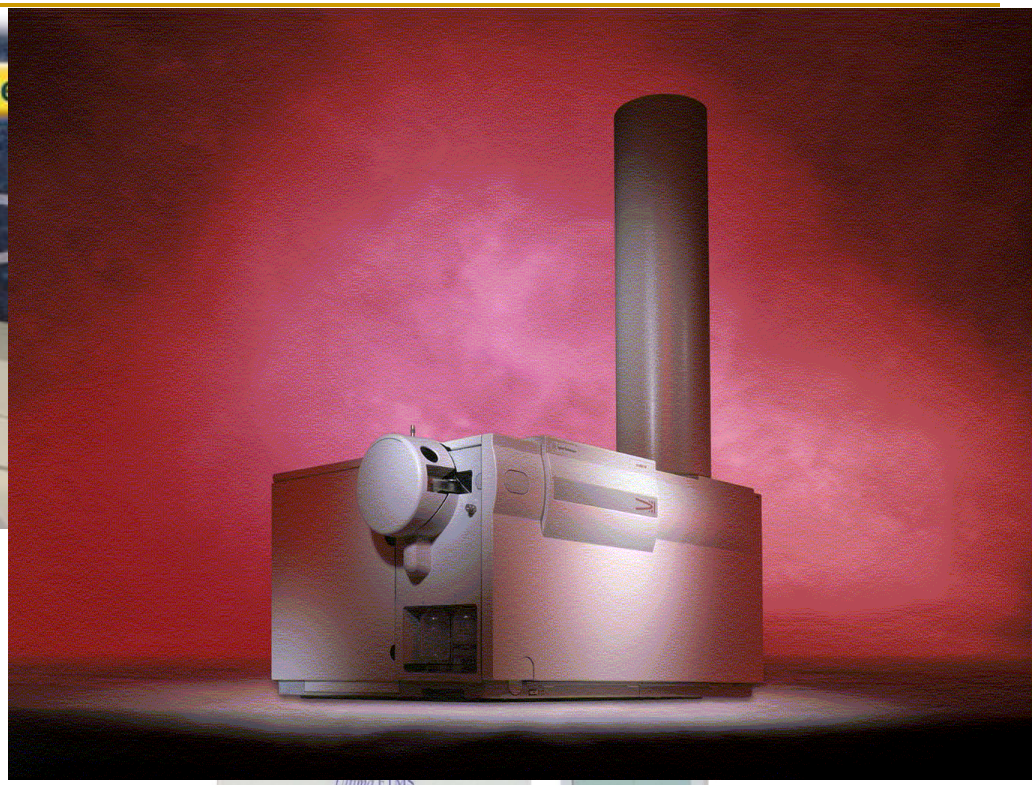
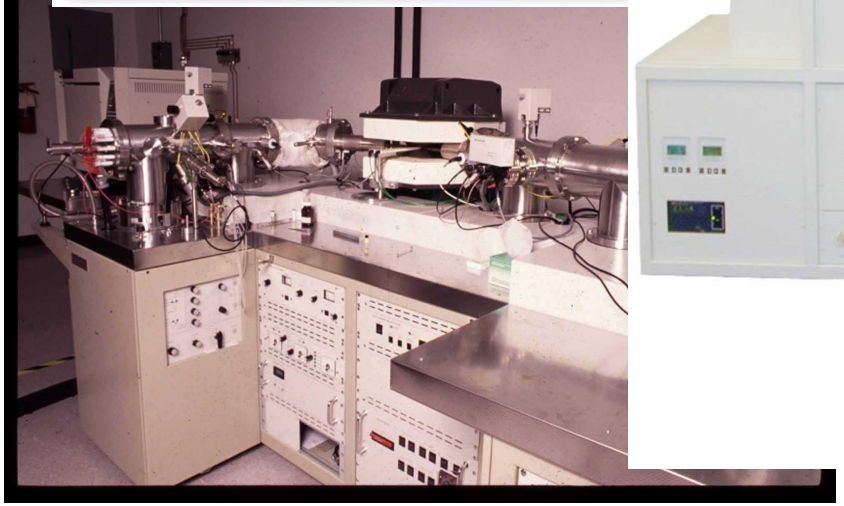
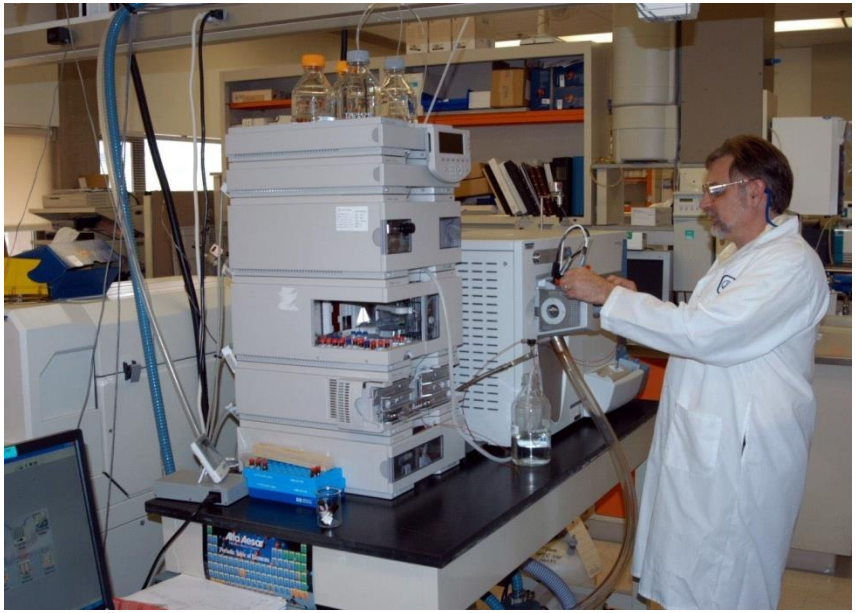


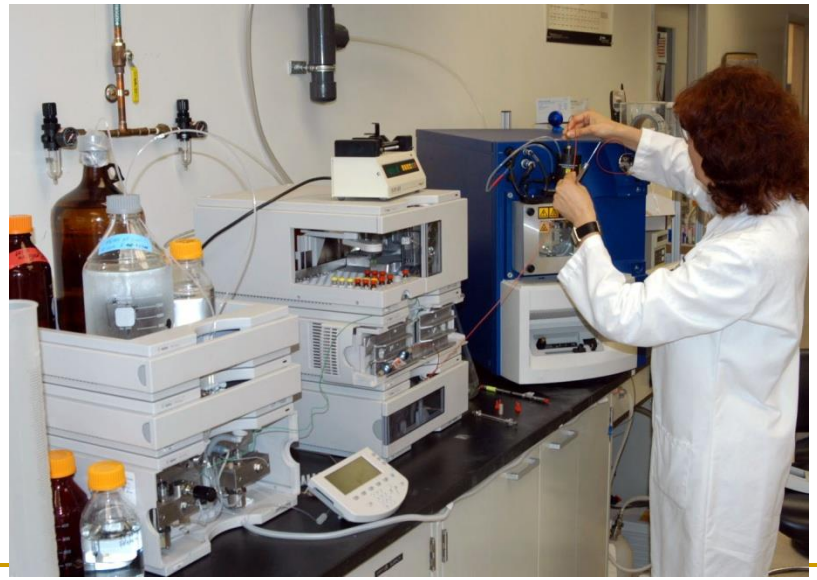


Ultima FTMS

GC/MS – Circa Late 1980s

“Bench-top” LC/MS Systems





Base da Espectrometria de Massas

Que informações podem ser obtidas?

- Peso molecular
- Fórmula molecular (HRMS)
- Estrutura (a partir de padrões de fragmentação)
- Incorporação isotópica / distribuição
- Seqüência de proteína (MS-MS)

Mass Spectrometry Basics

Mass spectrometry has 4 basic operations:

- Sample introduction (analyte must be in vapor phase)
- Ionization
- Mass analysis (separating ions by mass/charge ratio)
- Detection and quantitation

Sample Introduction

Method	Applications
Batch (reservoir)	gases, volatile liquids
Direct insertion probe	very low vapor pressure solids and liquids
Membrane	aqueous solutions, air samples
Chromatography eluent	LC-MS, GC-MS, etc.

Ionization Methods

1. Electron Ionization (EI)

   most common ionization technique, limited to relatively low MW compounds (<600 amu)

2. Chemical Ionization (CI)

   ionization with very little fragmentation, still for low MW compounds (<800 amu)

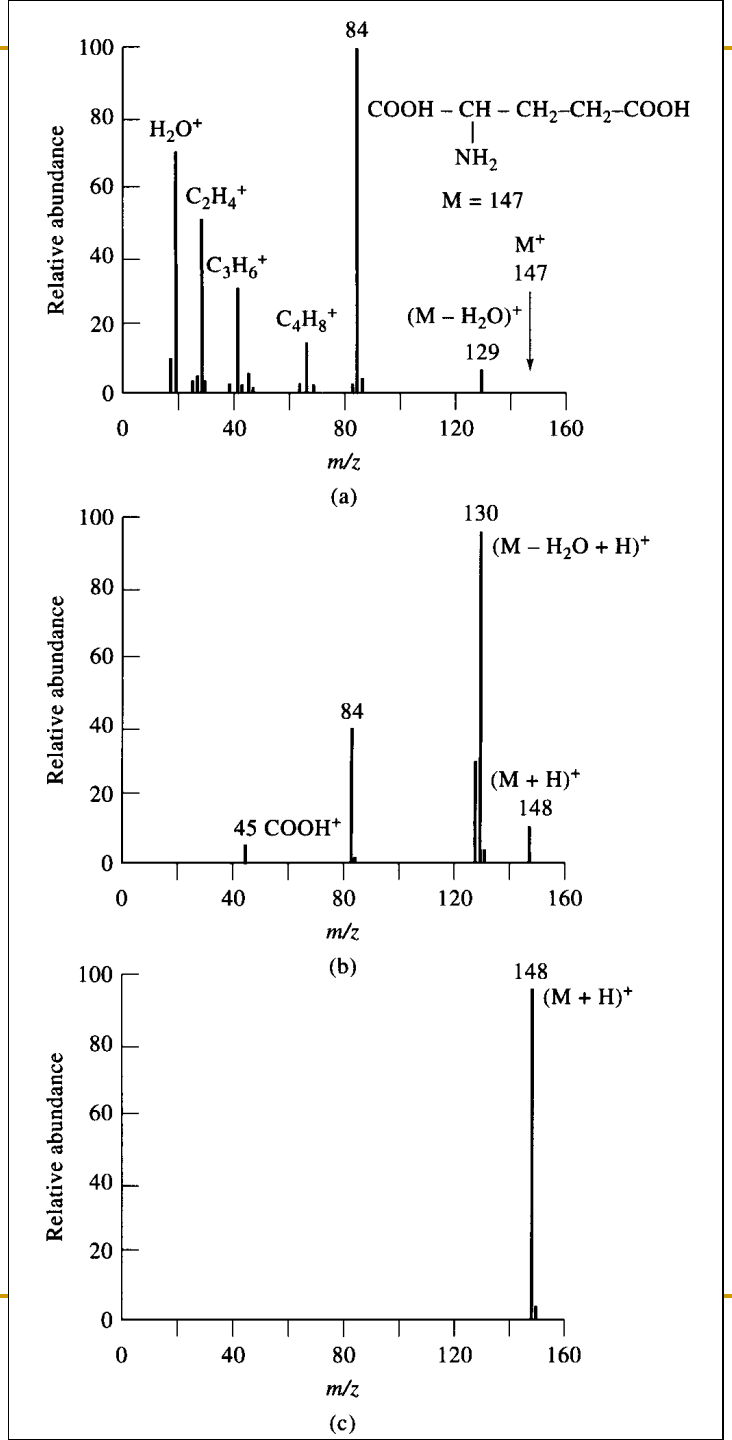
3. Desorption Ionization (DI)

   for higher MW or very labile compounds

4. Spray ionization (SI)

   for LC-MS, biomolecules, etc.

Glutamic Acid

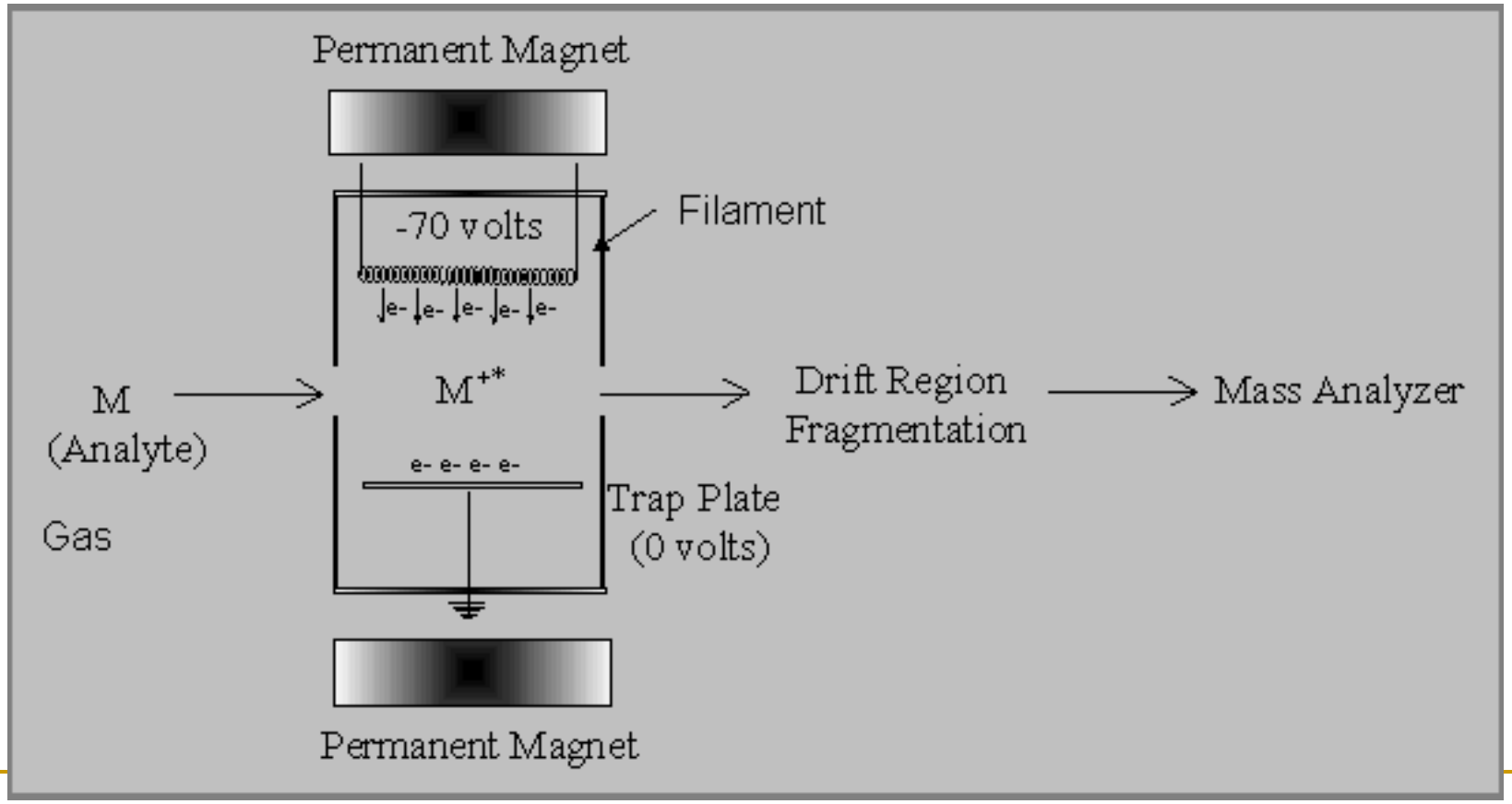


Electron Impact (EI)

Field Ionization

Field Desorption

Electron Impact



http://www.noble.org/PlantBio/MS/ion_tech_main.html

How to ionize neutral sample?

Positive ion mode

- Remove an electron → $M^{+\cdot}$
- Add one or more protons → $(M+nH)^{n+}$
- Fragmentation to produce ionized fragments," fragment ions"

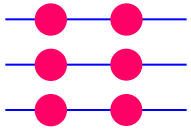
How does ionization occur?

Consider the Ionization potential

- a minimum amount of energy for ion formation to occur.

- the first ionization potential

the energy input required to remove an electron from the highest occupied atomic or molecular orbital of the neutral particle



$M^{+\cdot}$

- **First ionization potential**
  - **in the *5-15 eV* range for most elements**
  - **in the *8-12 eV* range for most organic molecules and radicals**

1 eV=1.6021 x10^{-19} Joules=3.8291 x10^{-20} calories

- **To remove a second, third, *etc* electron, additional energy is needed.**

- **When excess energy is available, fragmentation of the molecule may also occur during the process of ionization**

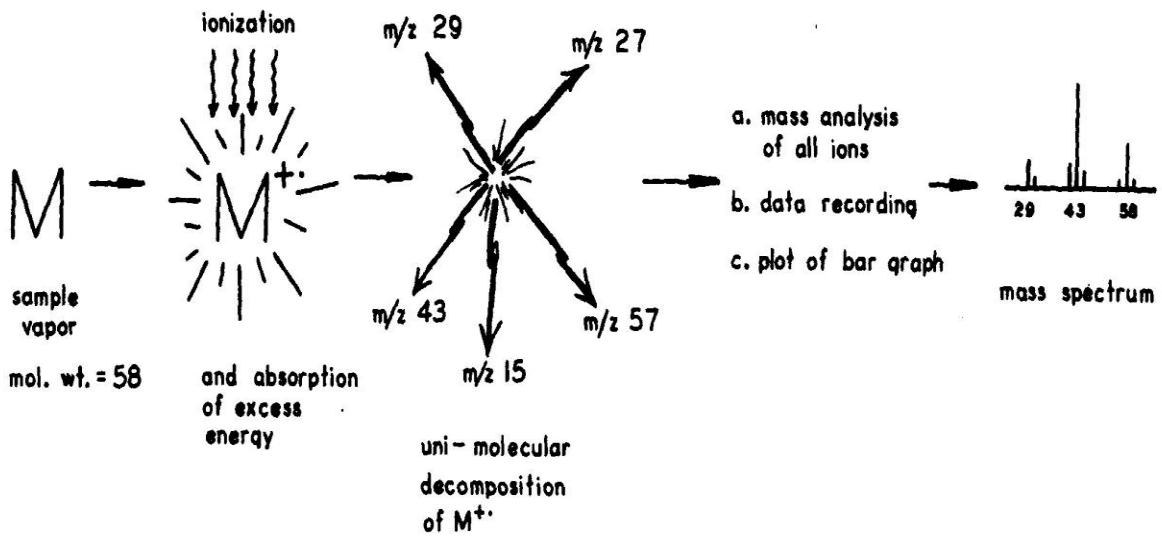


FIG. 1.1. General conceptual scheme for vapor-phase analysis by mass spectrometry.

Why chose 70 eV as ionization voltage?

***1 eV** is the energy gained (23 kcal/mole) by **an electron** in traversing an electric field maintained by **a potential difference of 1V**.*

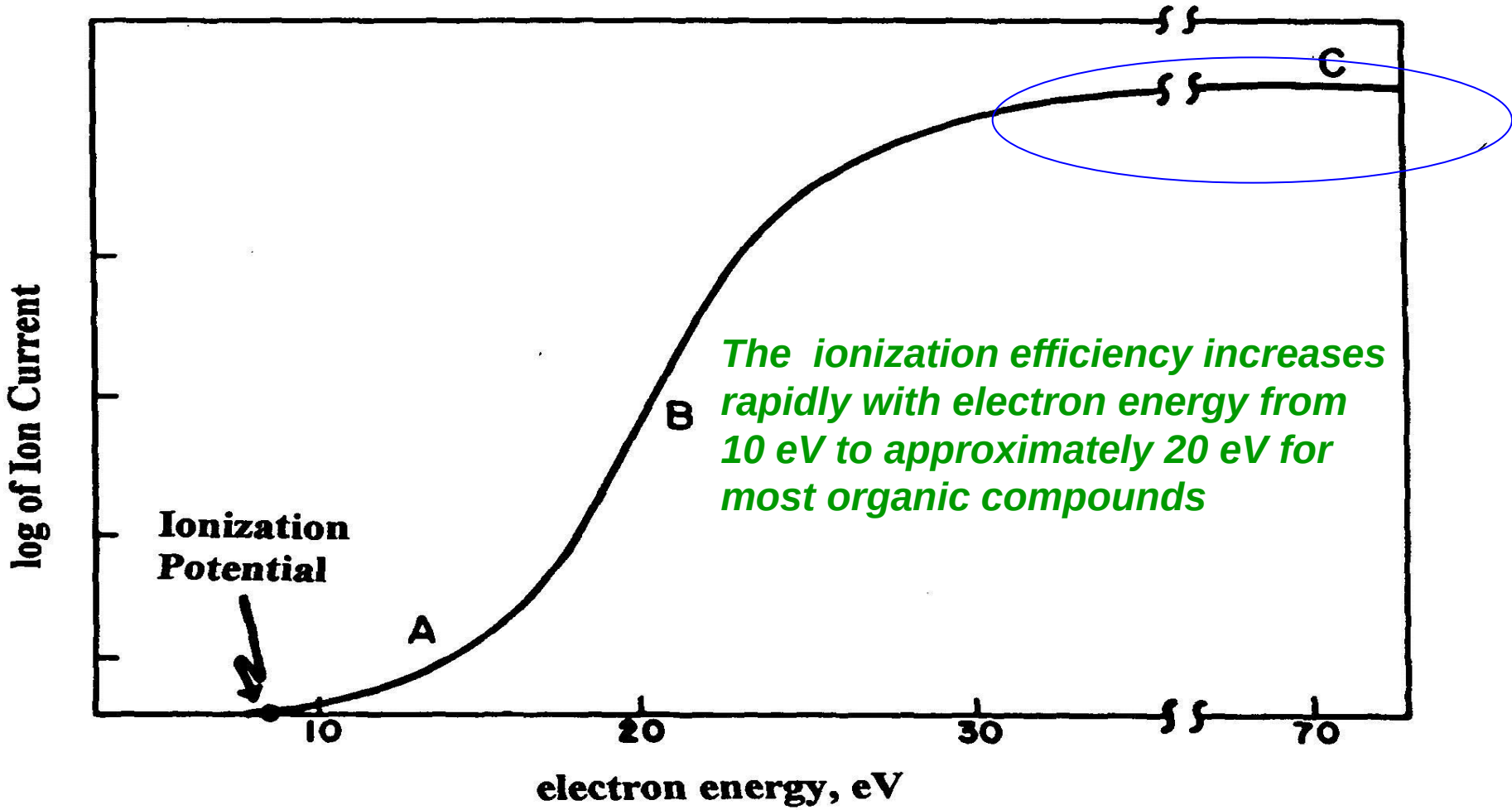


FIG. 7.3. Relationship between ion production and energy (electron volts) of ionizing electrons: *region A,* threshold region, principally molecular ions produced; *region B,* production of fragment ions becomes important; *region C,* routine operation, mostly fragment ions.

Ionization Efficiency

- On average, one ion is produced for every 1000 molecules entering the source under the usual spectrometers conditions, at 70 eV.

1/1000

Negative ions are not produced under electron impact conditions.

The energy associated with the electron has to be about 1 eV for the capture to be possible.

- at that level the perturbations in electron energy have negligible effects on ion production
- Reproducible fragmentation pattern are obtained

Electron Impact

(low picomole)

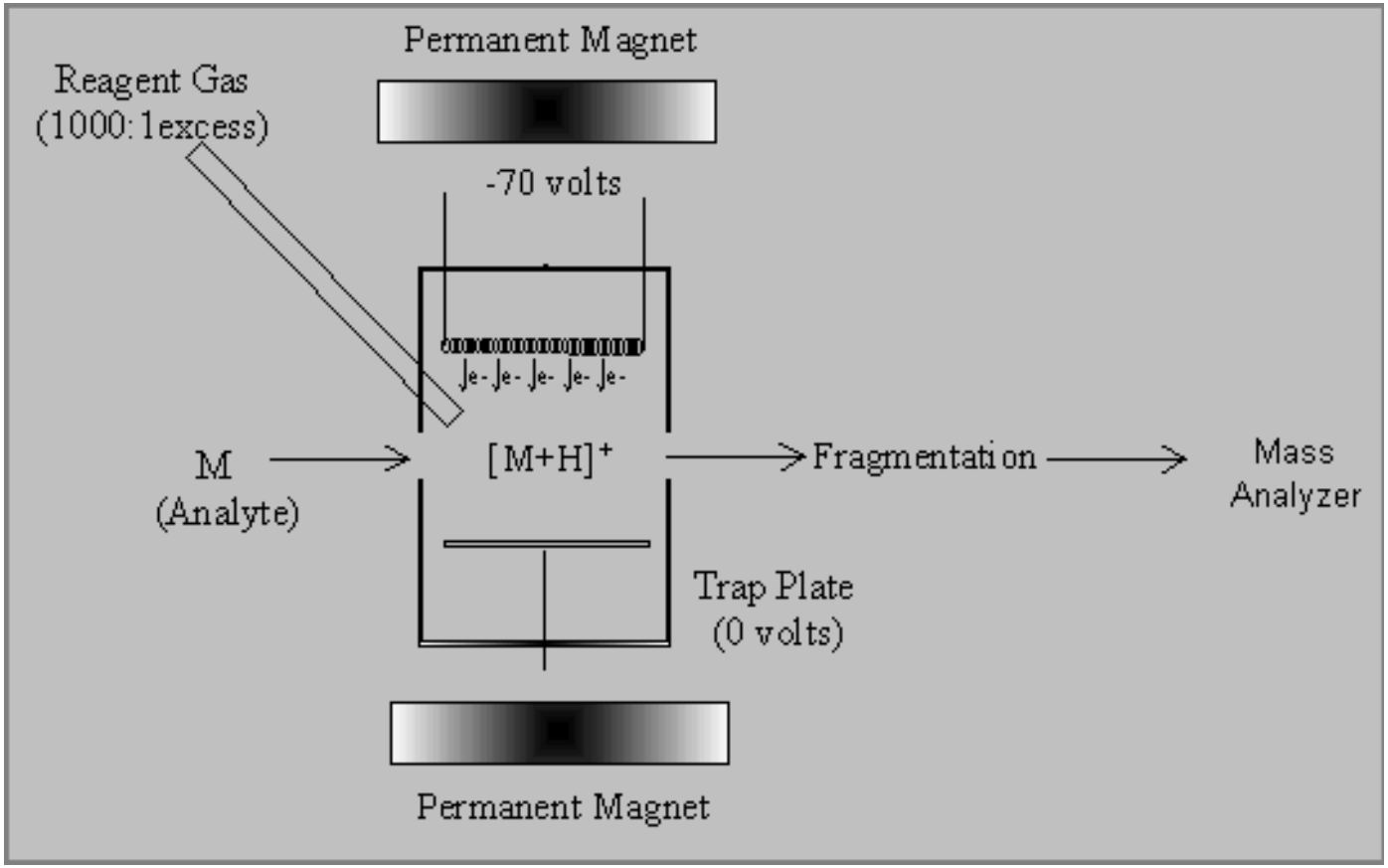
Advantages

- Well-Established
- Fragmentation Libraries
- No Supression
- Insoluble Samples
- Interface to GC
- Non-Polar Samples

Disadvantages

- Parent Identification
- Need Volatile Sample
- Need Thermal Stability
- No Interface to LC
- Low Mass Compounds (<1000 amu)
- Solids Probe Requires Skilled Operator

Chemical Ionization



http://www.noble.org/PlantBio/MS/ion_tech_main.html

Chemical Ionization MS Sources

$$CH_4$$

High Energy electrons ➔

$$\downarrow$$

$$CH_4 \quad CH_4^+ \quad CH_3^+ \quad CH_2^+$$

$$\downarrow$$

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$

$$\downarrow$$

$$CH_5^+ + MH \rightarrow MH_2^+ + CH_4$$

$$C_2H_5^+ + MH \rightarrow MH_2^+ + C_2H_4$$

$$C_2H_5^+ + MH \rightarrow M^+ + C_2H_6$$

Sample Molecule MH ➔

Molecule Ions ➔

Chemical Ionization

(low picomole)

Advantages	Disadvantages
Parent Ion	No Fragment Library
Interface to GC	Need Volatile Sample
Insoluble Samples	Need Thermal Stability
	Quantitation Difficult
	Low Mass Compounds (<1000 amu)
	Solids Probe Requires Skilled Operator

Mass Analyzers

- Double Focusing Magnetic Sector
- Quadrupole Mass Filter
- Quadrupole Ion Trap
- Linear Time-of-Flight (TOF)
- Reflectron TOF
- Fourier Transform Ion Cyclotron Resonance (FT-ICR-MS)

How is mass defined?

Assigning numerical value to the intrinsic property of “mass” is based on using carbon-12, ^{12}C, as a reference point.

One unit of mass is defined as a Dalton (Da).

One Dalton is defined as 1/12 the mass of a single carbon-12 atom.

Thus, one ^{12}C atom has a mass of 12.0000 Da.

Mass-to-Charge Ratio (m/z)

- **m: the mass number (m) of a given particle to the number (z) of electrostatic charge unit carried by the particle**

Unit

Dalton (Da) is used for the molecular weight natural isotope-averaged molecular mass (or often the integral mass number)

Alternatively, the symbol for a mass unit is u or amu.

The Da is *not* a unit of mass-to-charge ratio.

m/z ??

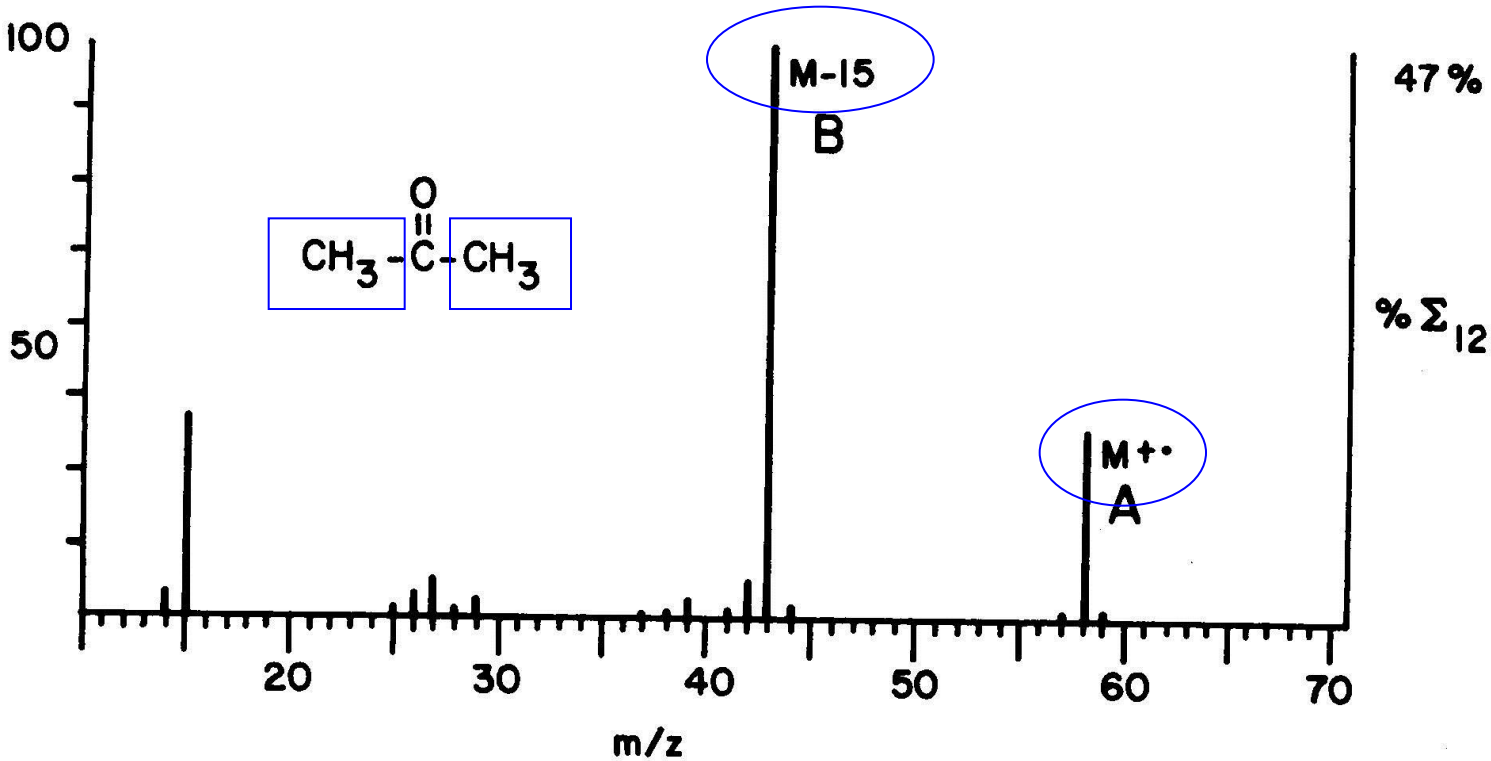


FIG. 1.6. Bar-graph format for the mass spectrum of acetone.

- m/z :

  Thomson (Th), symbolized by *m/z*.

The use of the abbreviation Da/e is not acceptable.

- The symbol u corresponds to 1/12 of ^{12}C, which has been assigned the value 12.000000 by IUPAC convention.

$1u = 1\ Da = 1.665402 \times 10^{-27}\ kg$

Isotopes

+Most elements have more than one stable isotope.

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da.

+Why do we care?

Mass spectrometers can “see” isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

The Mass Spectrum

Origin of Relative Ion Abundances

M contributors		M+1 contributors		M+2 contributors	
Isotope	**Natural Abundance**	**Isotope**	**Natural Abundance**	**Isotope**	**Natural Abundance**
^{1}H	99.9855%	^{2}H	0.015%	^{3}H	ppm
^{12}C	98.893	^{13}C	1.107	^{14}C	ppm
^{14}N	99.634	^{15}N	0.366		
^{16}O	99.759	^{17}O	0.037	^{18}O	0.204
^{19}F	100.0				
^{32}S	95.0	^{33}S	0.76	^{34}S	4.22
^{35}Cl	75.77			^{37}Cl	24.23
^{79}Br	50.69			^{81}Br	49.31
^{127}I	100.0				

The Mass Spectrum

Relative Intensity of Molecular Ion Peaks

Imagine a sample containing 10,000 methane molecules...

Molecule	# in sample	m/z	Relative abundance
$^{12}C^{1}H_4$	9889	12 + (4 x 1) = 16	100%
$^{13}C^{1}H_4$	110	13 + (4 x 1) = 17	(110/9889) x 100% = 1.1%*
$^{14}C^{1}H_4$	~1	14 + (4 x 1) = 18	(1/9889) x 100% = < 0.1%*

*Contributions from ions with ^{2}H are ignored because of its very small natural abundance

CH_4 mass spectrum
m/z = 16 (M; 100%), m/z = 17 (M+1; 1.1%), m/z = 18 (M+2; < 0.1%)

Formula from Mass Spectrum

M+1 Contributors

Comparing many mass spectra reveals M+1 intensity ↑ ~1.1% per C in formula

•Examples: C_2H_6 M = 100%; M+1 = ~2.2%

C_6H_6 M = 100%; M+1 = ~6.6%

Working backwards gives a useful observation...

When relative contribution of M = 100% then relative abundance of M+1/1.1% gives the approximate number of carbon atoms in the molecular formula

Other M+1 contributors

•^{15}N (0.37%) and ^{33}S (0.76%) should be considered

•^{2}H (0.015%) and ^{17}O (0.037%) can be ignored

Formula from Mass Spectrum

M+2 Contributors

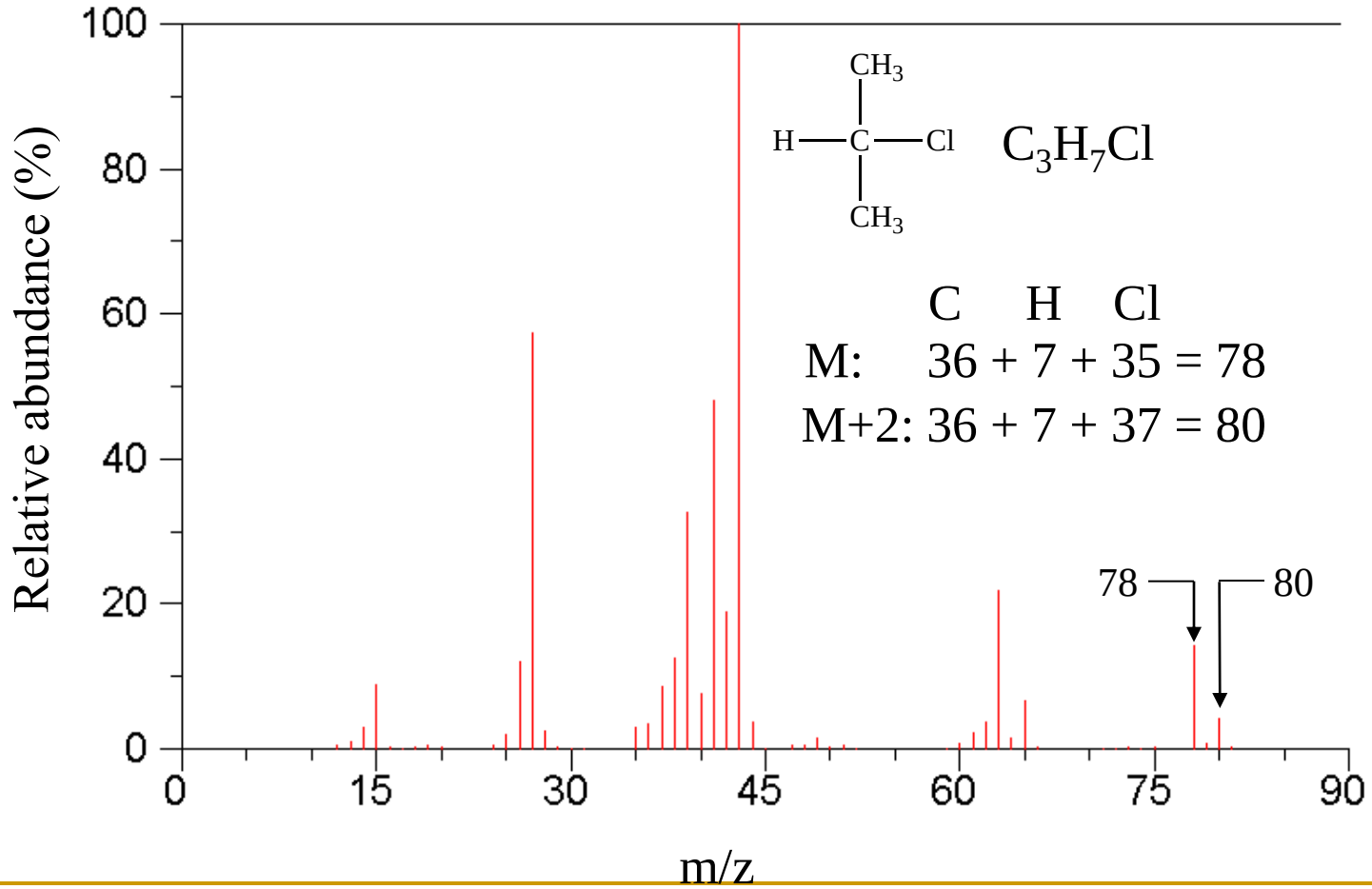
Anything useful from intensity of M+2?

Isotopes	Natural abundances	Intensity M : M+2
^{32}S : ^{34}S	95.0 : 4.2	100 : 4.4
^{35}Cl : ^{37}Cl	75.8 : 24.2	100 : 31.9
^{79}Br : ^{81}Br	50.7 : 49.3	100 : 97.2

Conclusion: *Mass spectra of molecules with S, Cl, or Br have significant M+2 peaks*

Formula from Mass Spectrum

M+2 Contributors



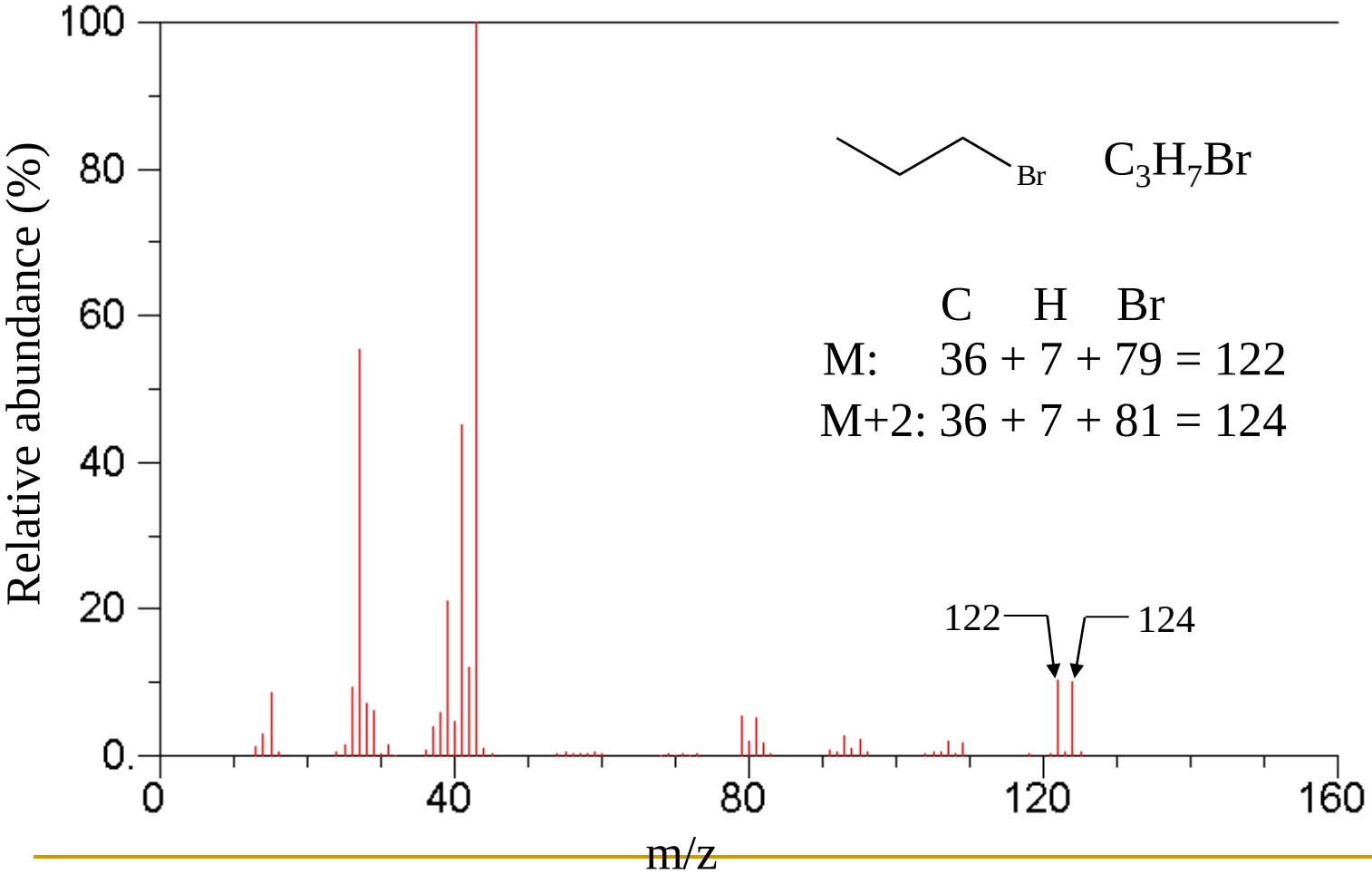
C_3H_7Cl

	C	H	Cl	
M:	36 +	7 +	35	= 78
M+2:	36 +	7 +	37	= 80

M:M+2 abundance ~3:1

Formula from Mass Spectrum

M+2 Contributors

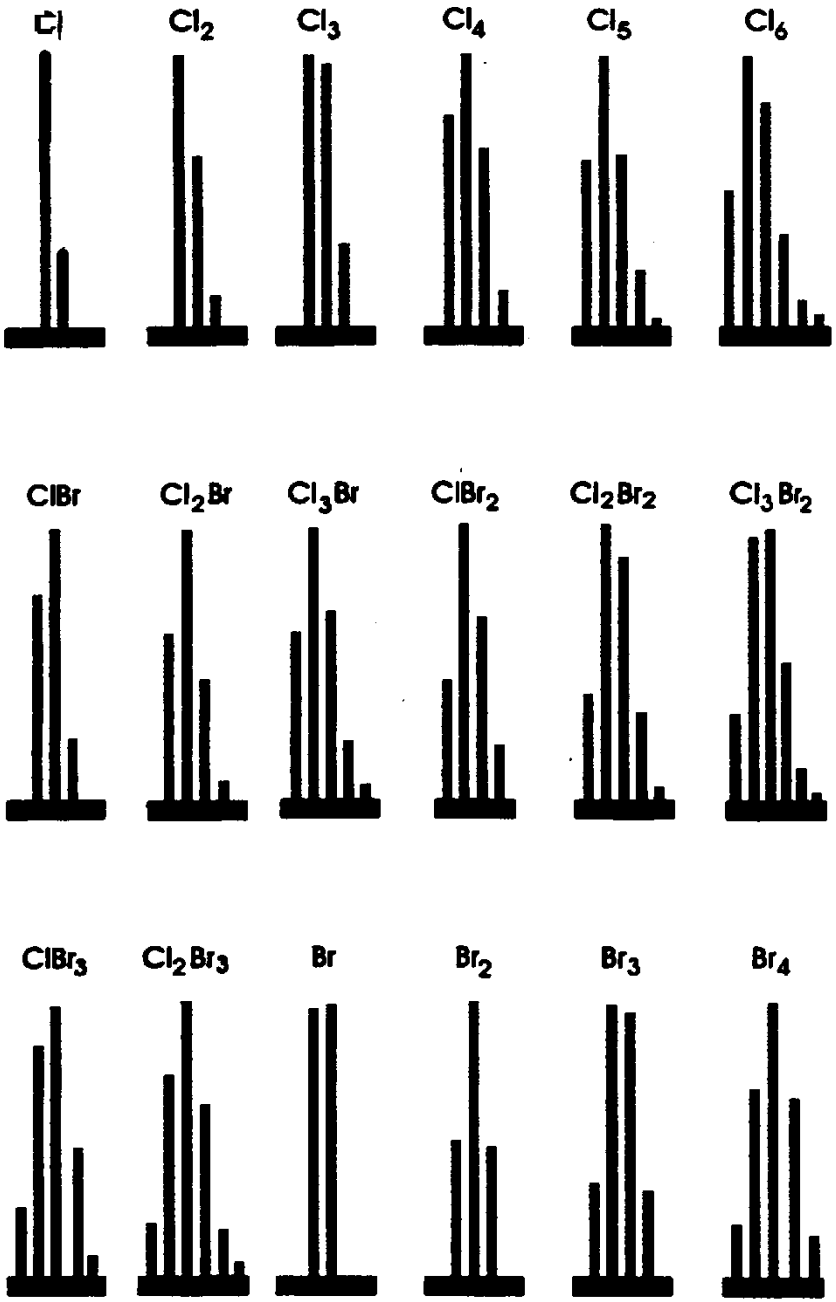


C_3H_7Br

	C	H	Br	
M:	36 +	7 +	79	= 122
M+2:	36 +	7 +	81	= 124

M:M+2 abundance ~1:1

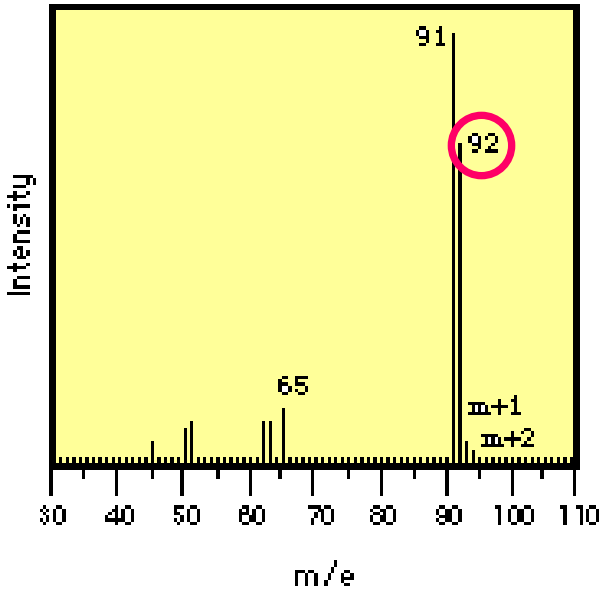
Halogen Isotope Clusters

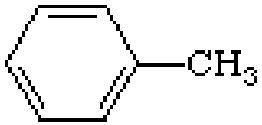


Molecular Ion

- The molecular ion results from ionization of the analyte molecule.
- The molecular ion peak appears at an m/z value numerically equal to the nominal molecular weight (MW) of the compound.
  - The nominal molecular weight is calculated by summation of the atomic masses of the lightest isotope of each element composing the molecule.

Molecular ion ⟶ $MW=M^{+\cdot}$

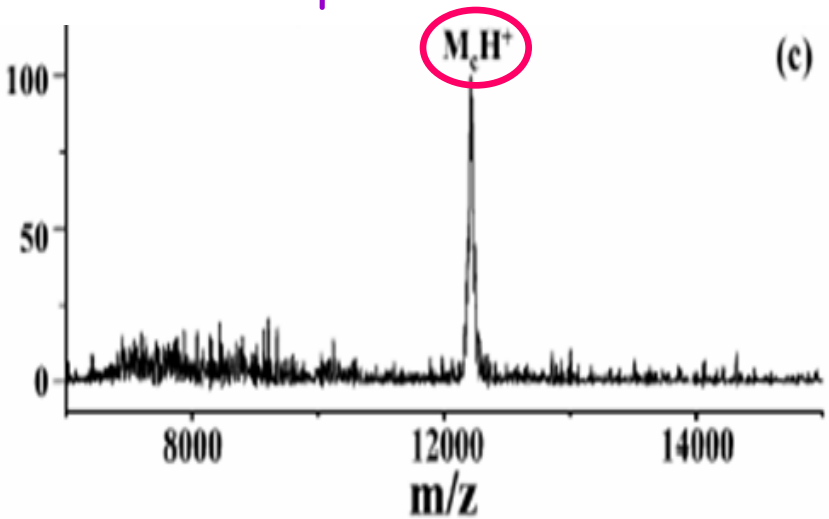




Toluene

$MH^{+}=MW+1$

called pseudomolecular ion

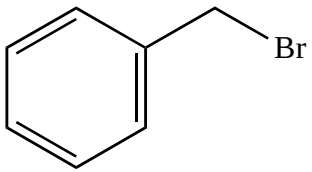


Identifying the Molecular Ions

Which peaks are molecular ions?

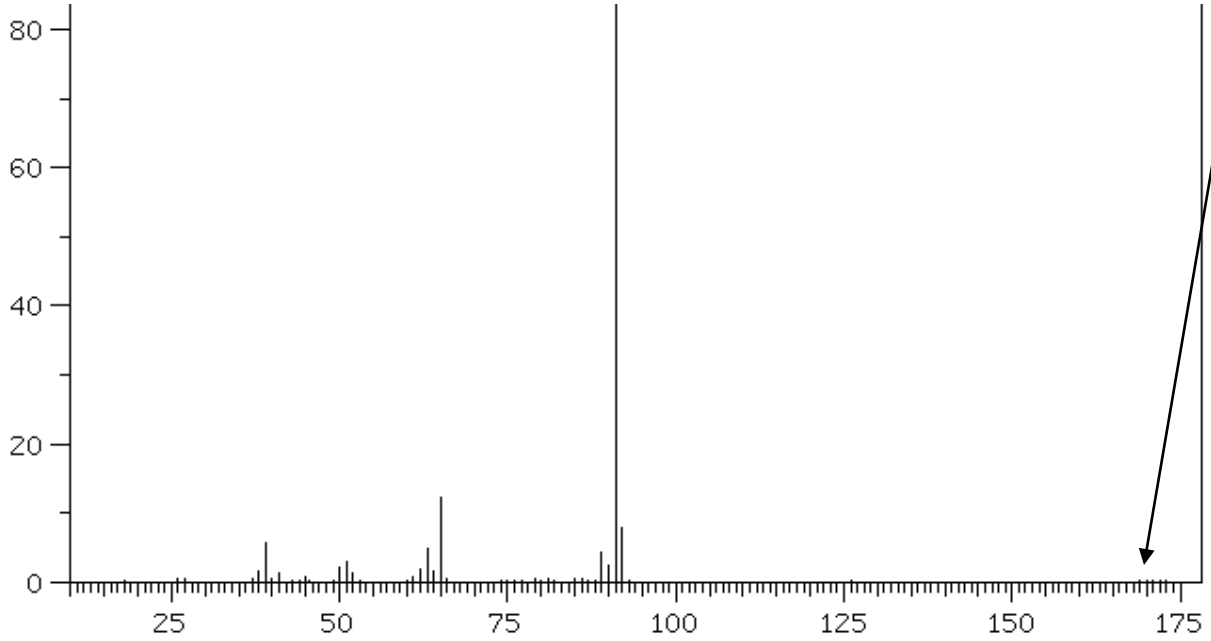
•Highest m/z not always M

•M+1 has m/z one more than m/z of M



C_7H_7Br

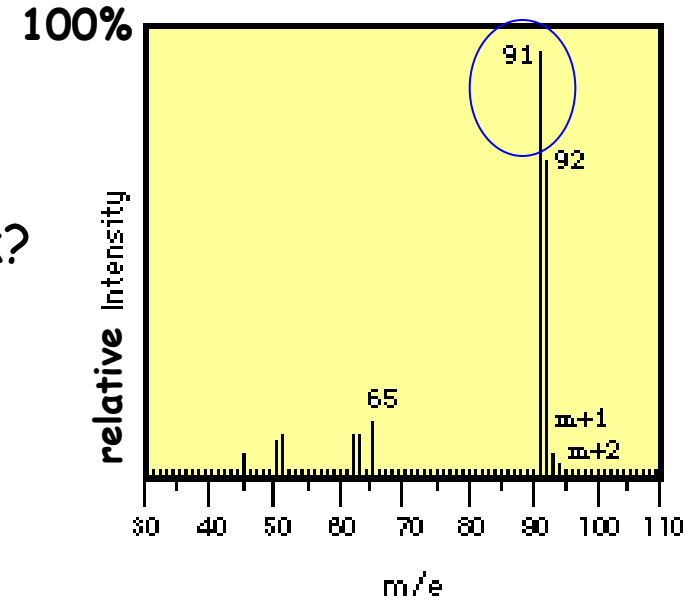
M: m/z = 170

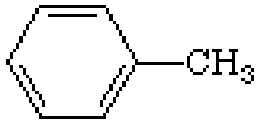


Base peak

- The base peak is the most intense peak in the mass spectrum.
- It is used as the base against which the intensities of all other peaks are normalized.

Where is the base peak?

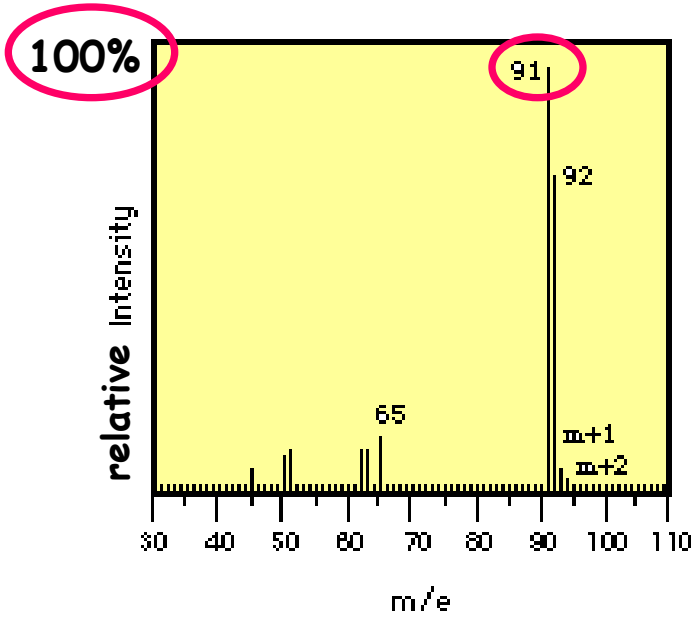


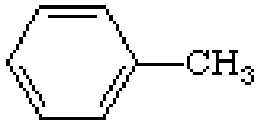


Toluene

Relative Intensity

- The relative intensity of a given peak expresses its intensity **relative to that of the base peak**, the most intense peak in the mass spectrum.





Toluene

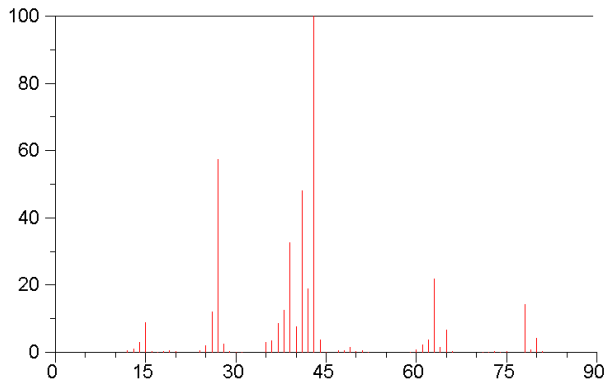
Formula from Mass Spectrum

Summary of Information from Mass Spectrum

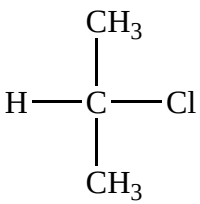
M: Reveals mass of molecule composed of lowest mass isotopes
M+1: Intensity of M+1 / 1.1% = number of carbons
M+2: Intensity reveals presence of sulfur, chlorine, and bromine

Mass Spectrum → Formula → Structure

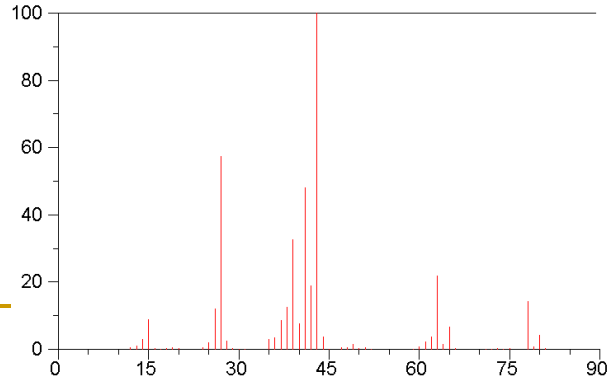
How do we derive structure from the mass spectrum?



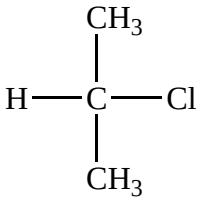
$\xrightarrow{?}$



- Not trivial to do this directly
- Structure comes from formula; formula comes from mass spectrum



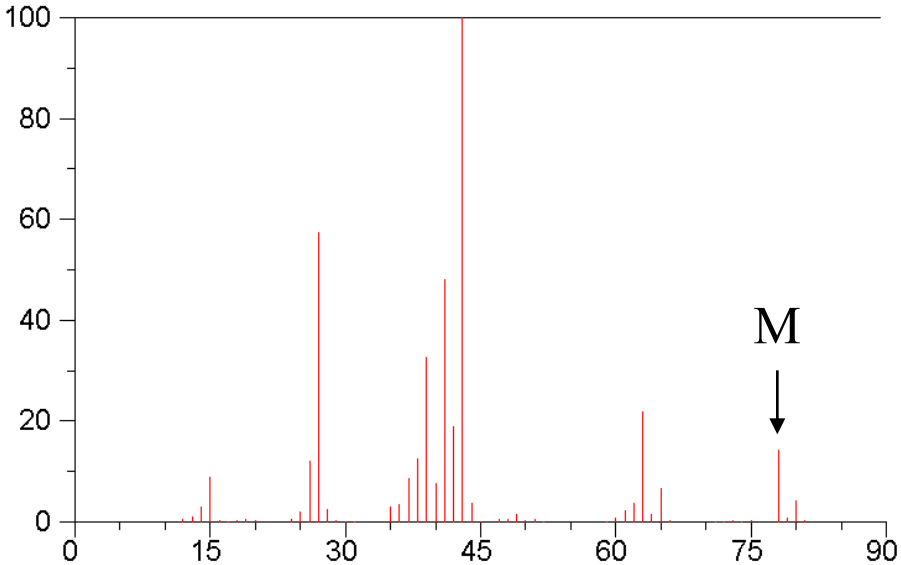
→ C_3H_7Cl →



Mass Spectrum → Formula → Structure

How do we derive formula from the mass spectrum?

•m/z and relative intensities of M, M+1, and M+2



M: m/z = 78

$C_2H_6O_3$

C_3H_7Cl

C_5H_4N

C_6H_6

etc.

•A few useful rules to narrow the choices

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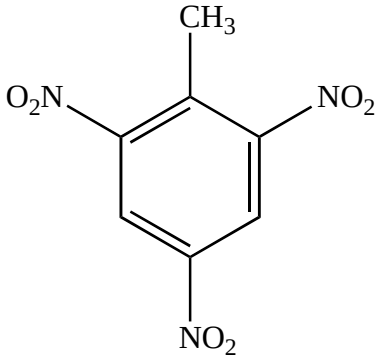
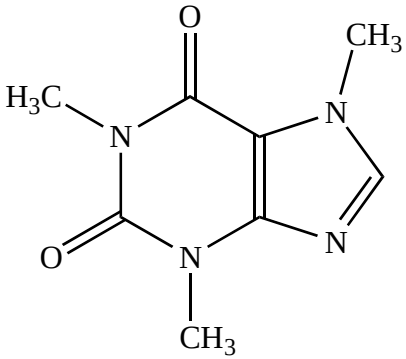
How Many Nitrogen Atoms?

Consider these molecules:

NH_3 H_2NNH_2





Formula:	NH_3	N_2H_4	$C_7H_5N_3O_6$	$C_8H_{10}N_4O_2$
m/z (M):	17	32	227	194

Conclusion

•When m/z (M) = even, number of N in formula is even
•When m/z (M) = odd, number of N in formula is odd

} **The Nitrogen Rule**

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How Many Nitrogen Atoms?

A Nitrogen Rule Example

Example: Formula choices from previous mass spectrum

M: m/z = 78 ← m/z even

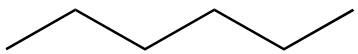
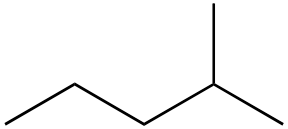
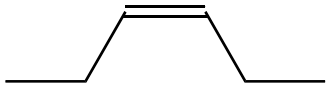
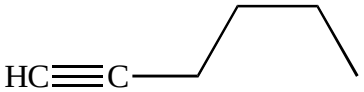
$C_2H_6O_3$ ← even nitrogen count

C_3H_7Cl ← even nitrogen count

discarded ~~C_5H_4N~~ ← odd nitrogen count

C_6H_6 ← even nitrogen count

How Many Hydrogen Atoms?

	One pi bond	Two pi bonds
		
		
C_6H_{14}	C_6H_{12}	C_6H_{10}
max H for 6 C	*H count = max - 2*	*H count = max - 4*

Conclusion: Each pi bond reduces max hydrogen count by two

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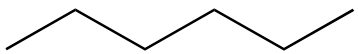
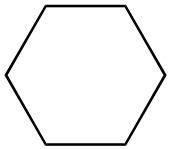
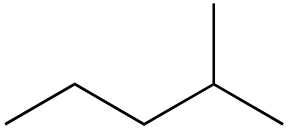
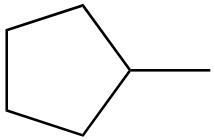
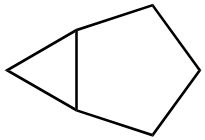
How Many Hydrogen Atoms?

	One ring	Two rings
		
		
C_6H_{14}	C_6H_{12}	C_6H_{10}
max H for 6 C	*H count = max - 2*	*H count = max - 4*

Conclusion: Each ring reduces max hydrogen count by two

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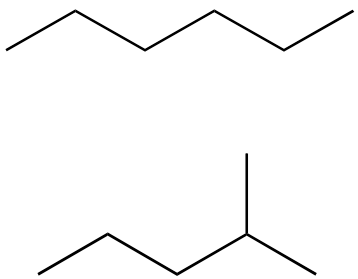
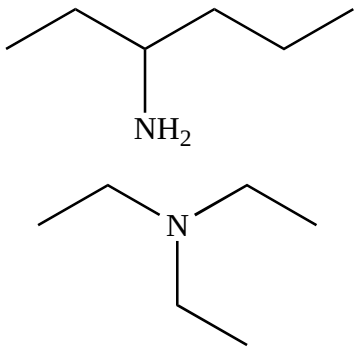
How Many Hydrogen Atoms?



C_6H_{14}

max H for 6 C

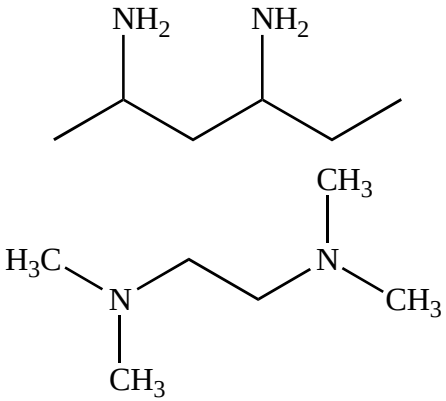
One nitrogen



$C_6H_{15}N$

H count = max + 1

Two nitrogens



$C_6H_{16}N_2$

H count = max + 2

Conclusion:

- Each nitrogen increases max H count by one
- For C carbons and N nitrogens, max number of H = 2C + N + 2

The Hydrogen Rule

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Mass Spectrum → Formula

Procedure

•Chem 14C atoms: H C N O F S Cl Br I

•M = molecular weight (lowest mass isotopes)

•M+1: gives carbon count

•M+2: presence of S, Cl, or Br

•No mass spec indicator for F, I *Assume absent unless otherwise specified*

•Accounts for all atoms except O, N, and H

•MW - mass due to C, S, Cl, Br, F, and I = mass due to O, N, and H

•Systematically vary O and N to get formula candidates

•Trim candidate list with nitrogen rule and hydrogen rule

Mass Spectrum → Formula

Example #1

	m/z	Molecular ion	Relative abundance	Conclusions
Given information	102	M	100%	*Mass (lowest isotopes) = 102*
				Even number of nitrogens
	103	M+1	6.9%	6.9 / 1.1 = 6.3 *Six carbons**
	104	M+2	0.38%	*< 4% so no S, Cl, or Br*
				~~*Oxygen?*~~

*Rounding: 6.00 to 6.33 = 6; 6.34 to 6.66 = 6 or 7; 6.67 to 7.00 = 7

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Mass Spectrum → Formula

Example #1

Mass (M) - mass (C, S, Cl, Br, F, and I) = mass (N, O, and H)

102 - C_6 = 102 - (6 x 12) = 30 amu for N, O, and H

Oxygens	Nitrogens	30 - O - N = H	Formula	Notes
0	0	30 - 0 - 0 = 30	~~C_6H_{30}~~	Violates hydrogen rule
1	0	30 - 16 - 0 = 14	$C_6H_{14}O$	Reasonable
2	0	30 - 32 - 0 = -2	~~$C_6H_{-2}O_2$~~	Not possible
0	2*	30 - 0 - 28 = 2	$C_6H_2N_2$	Reasonable

*Nitrogen rule!

•Other data (functional groups from IR, NMR integration, etc.) further trims the list

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Mass Spectrum → Formula

Example #2

m/z	Molecular ion	Relative abundance	Conclusions
157	M	100%	*Mass (lowest isotopes) = 157* *Odd number of nitrogens*
158	M+1	9.39%	9.39 / 1.1 = 8.5 *Eight or nine carbons*
159	M+2	34%	*One Cl; no S or Br*

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Mass Spectrum → Formula

Example #2

Try eight carbons: M - C_8 - Cl = 157 - (8 x 12) - 35 = 26 amu for O, N, and H

Oxygens	Nitrogens	26 - O - N = H	Formula	Notes
0	1*	26 - 0 - 14 = 12	$C_8H_{12}ClN$	Reasonable

*Nitrogen rule!

Not enough amu available for one oxygen/one nitrogen or no oxygen/three nitrogens