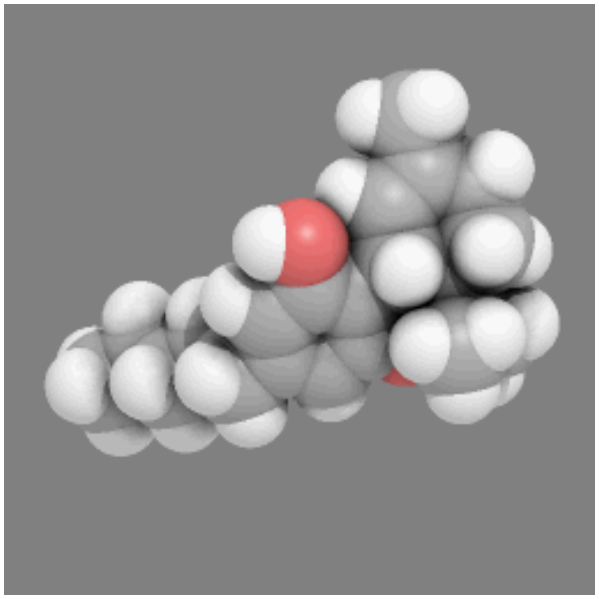
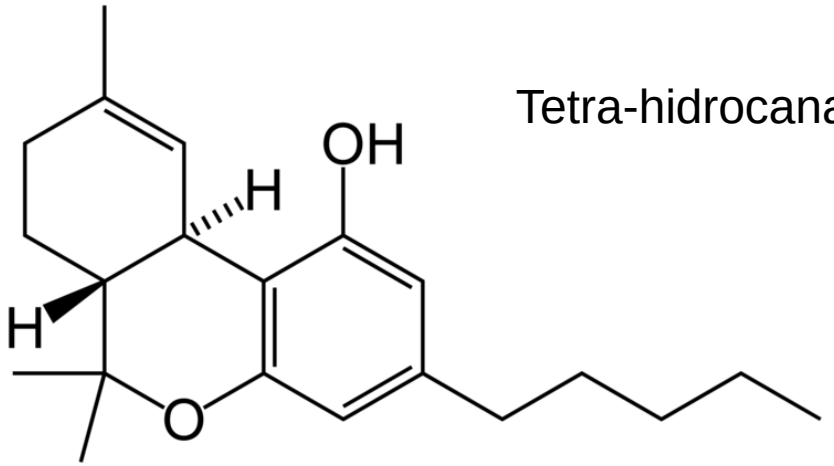
Grande parte dos Fármacos foi obtida, ou desenvolvida, a partir de produtos naturais

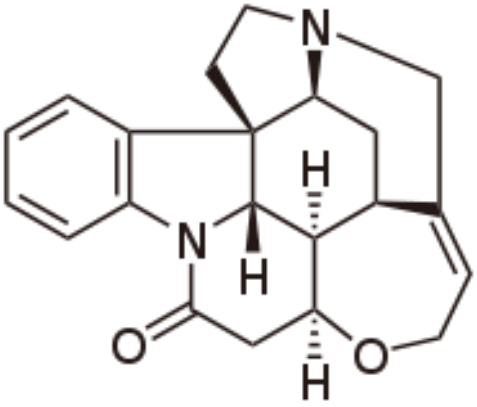




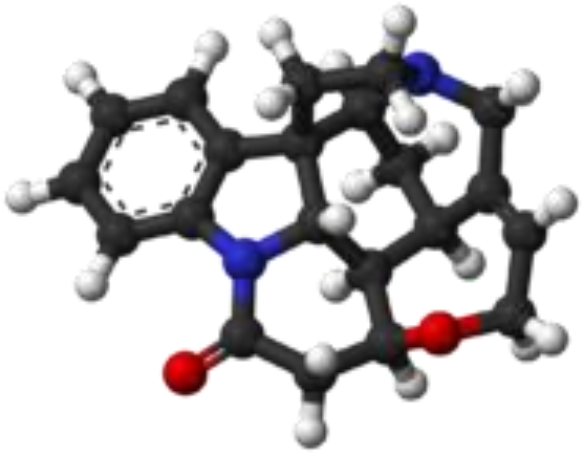
Tetra-hidrocanabinol

History

Structural elucidation of natural products used to be *very hard* and *take forever*.



Strychnine *alkaloid toxin*
Isolated by Pelletier & Caventou (1818)
Past: H. Leuchs worked on structure for 40 years until R. Woodward (1954) beat him to it.



Today: <1 mg sample needed; a weekend would be enough.

Etapas para elucidação estrutural

- Determinação da Fórmula Molecular
- Caracterizar Grupos funcionais
- Degradação da Molécula e Síntese de Derivados

Atualmente

- Métodos Espectroscópicos

STRUCTURAL ELUCIDATION

- ***Spectroscopic methods:***
- ***Infrared (IR)***

- indicates presence of functional groups:

C=O ~ 1670 – 1750 cm^{-1}

amide, ketone, ester

OH, NH/NH2 ~ 3100 cm^{-1} to 3500 cm^{-1}

Limitation; non polar and semi polar compounds only.

STRUCTURAL ELUCIDATION cont'd

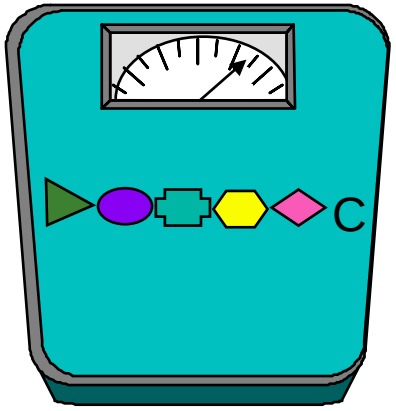
- ***Mass Spectrometry***

- **Enables the determination of molecular weight.**
- **Aids structural elucidation – fragmentation peaks: loss of CO $(M^+ - 28)$, loss of H_2O $(M^+ - 18)$.**
- **Enables identification of mixtures; MS-MS.**
- **Various ionization techniques – to accommodate different compounds; polar, ionic, non-polar, macromolecules.**
- **Various Analyzers; usage (MS-MS, HRMS), cost.**

NUCLEAR MAGNETIC RESONANCE

- ***Nuclear Magnetic resonance:***
  - **Permits the establishment of the structural skeleton of the compound investigated.**
  - **^{1}HNMR showed resonances of protons while ^{13}C NMR showed the C resonances.**
  - **Allows to establish the connectivity between carbons and protons.**
  - **One dimensional and two dimensional techniques available:**
    - **COSY, HMQC, HMBC, NOESY etc.**
  - **For ^{1}HNMR ~ 1-5 mg (pure) sufficient**
  - **For ^{13}C NMR ~ 20 mg sufficient.**

Espectrometria de Massas



What does a mass spectrometer do?

1. It measures mass better than any other technique.

2. It can give information about chemical structures.

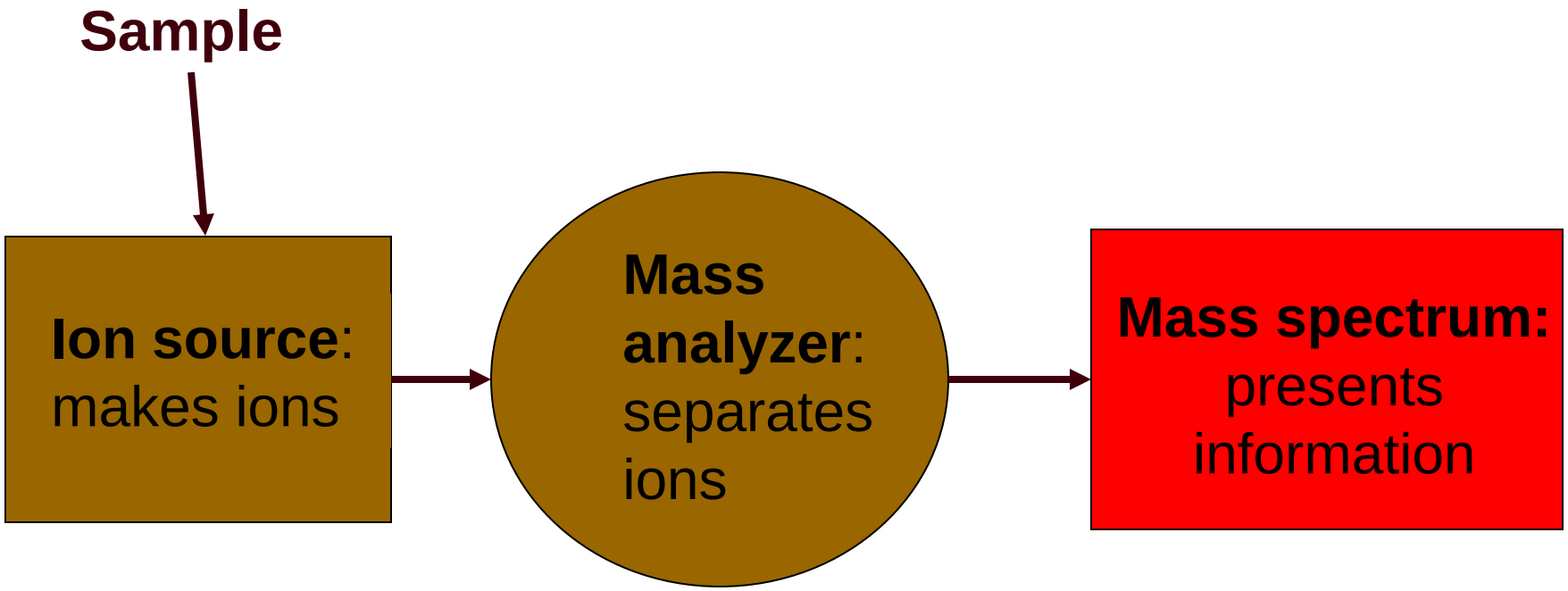
What are mass measurements good for?

To identify, verify, and quantitate: metabolites, recombinant proteins, proteins isolated from natural sources, oligonucleotides, drug candidates, peptides, synthetic organic chemicals, polymers

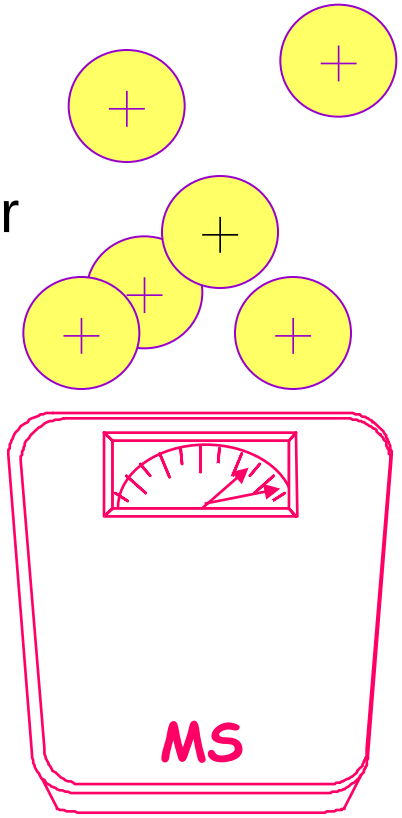
Applications of Mass Spectrometry

- Pharmaceutical analysis
  - Bioavailability studies
  - Drug metabolism studies, pharmacokinetics
  - Characterization of potential drugs
  - Drug degradation product analysis
  - Screening of drug candidates
  - Identifying drug targets
- Biomolecule characterization
  - Proteins and peptides
  - Oligonucleotides
- Environmental analysis
  - Pesticides on foods
  - Soil and groundwater contamination
- Forensic analysis/clinical

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*.**



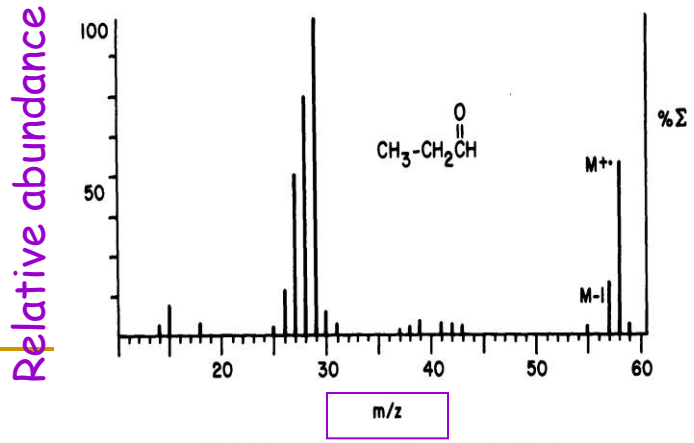
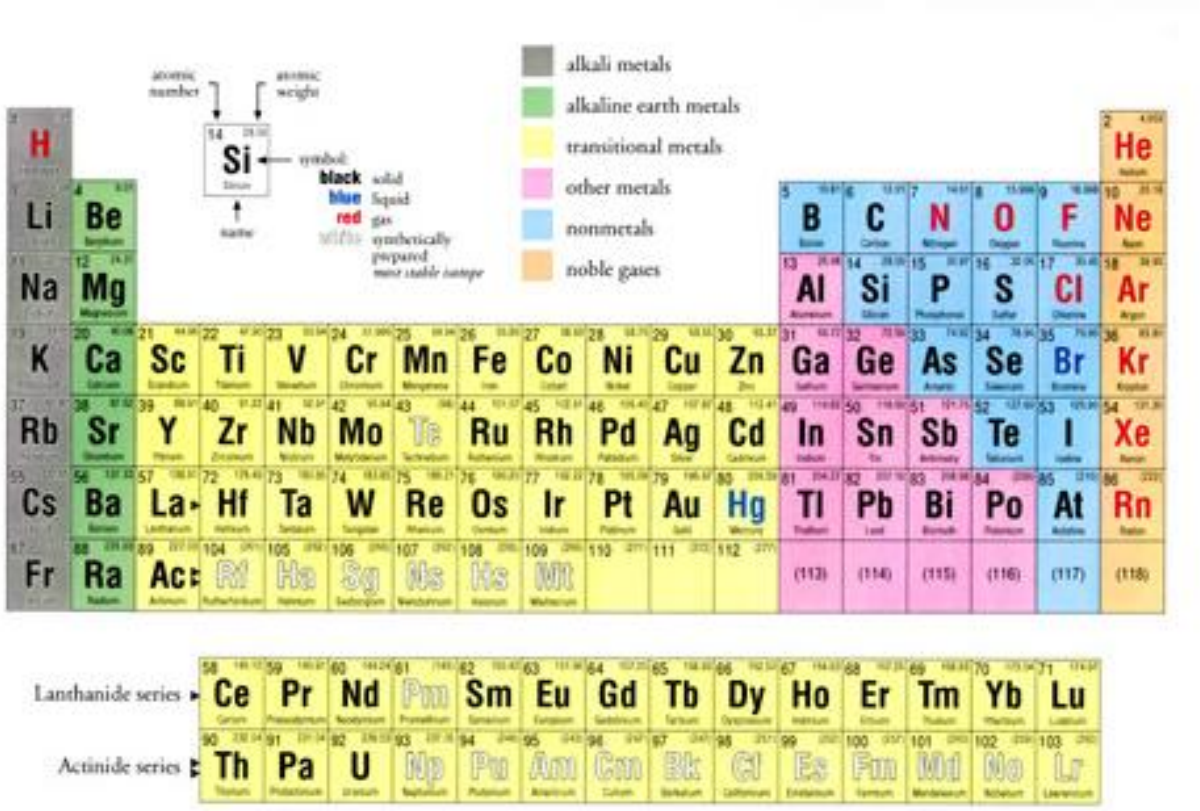
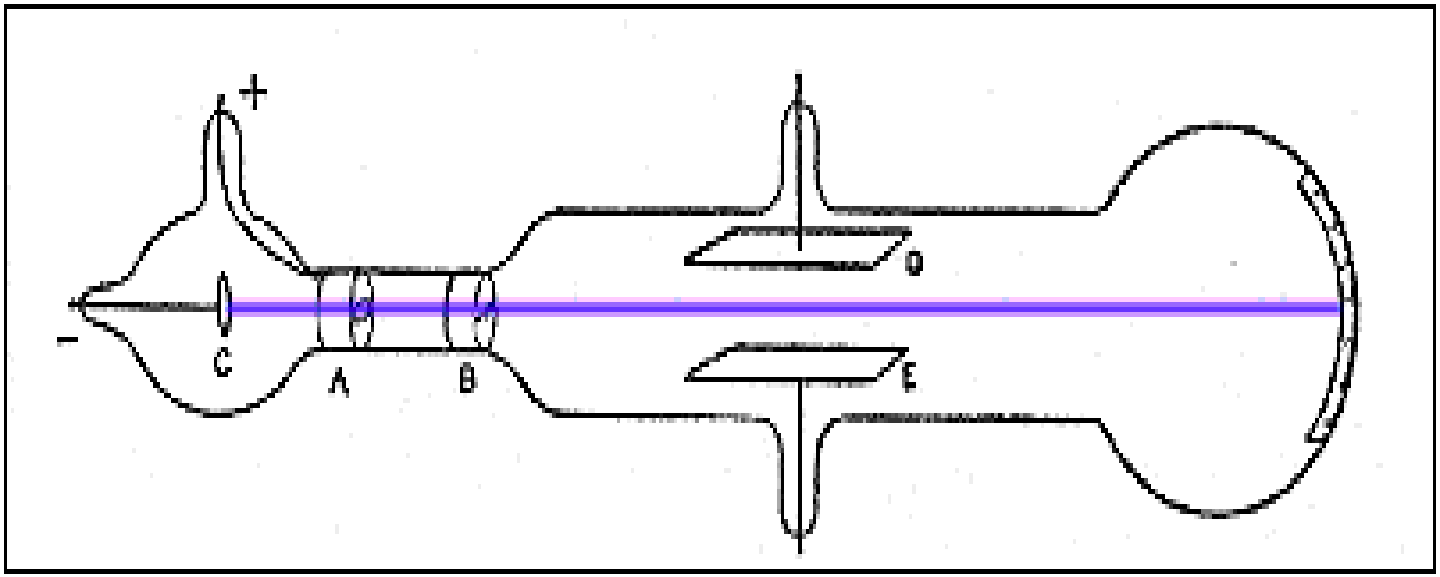


FIG. 1.7. Bar-graph mass spectrum of propionaldehyde.

MS Principles

- Different elements can be uniquely identified by their mass

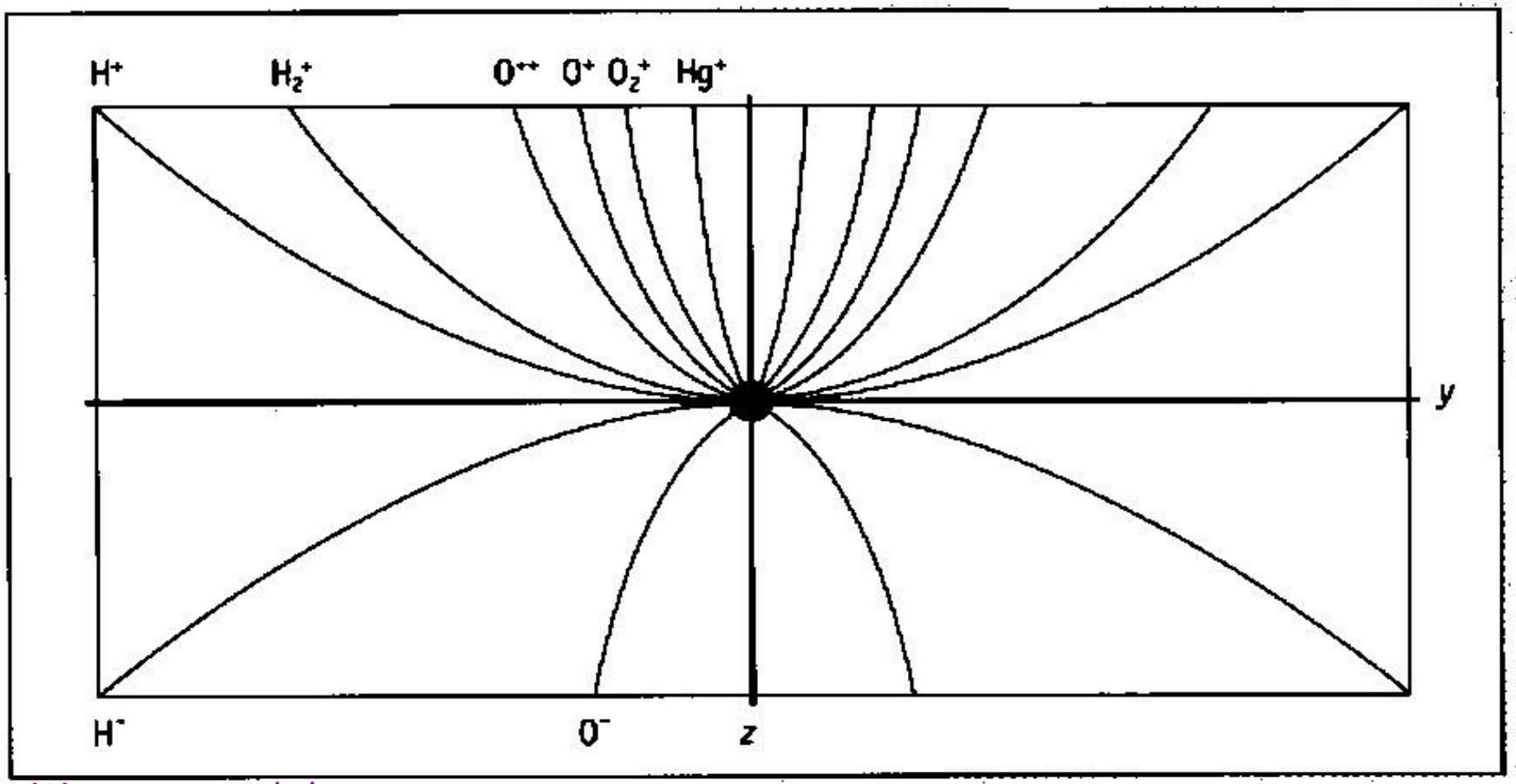




Schematic drawing of Thomson's apparatus in the experiment. Rays from the cathode (C) pass through a slit in the anode (A) and through a slit in a grounded metal plug (B). An electrical voltage is established between aluminum plates (D and E), and a scale pasted on the outside of the end of the tube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

Thomson's Parabola Mass Spectrograph

1907 for his work in *positive ray*



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

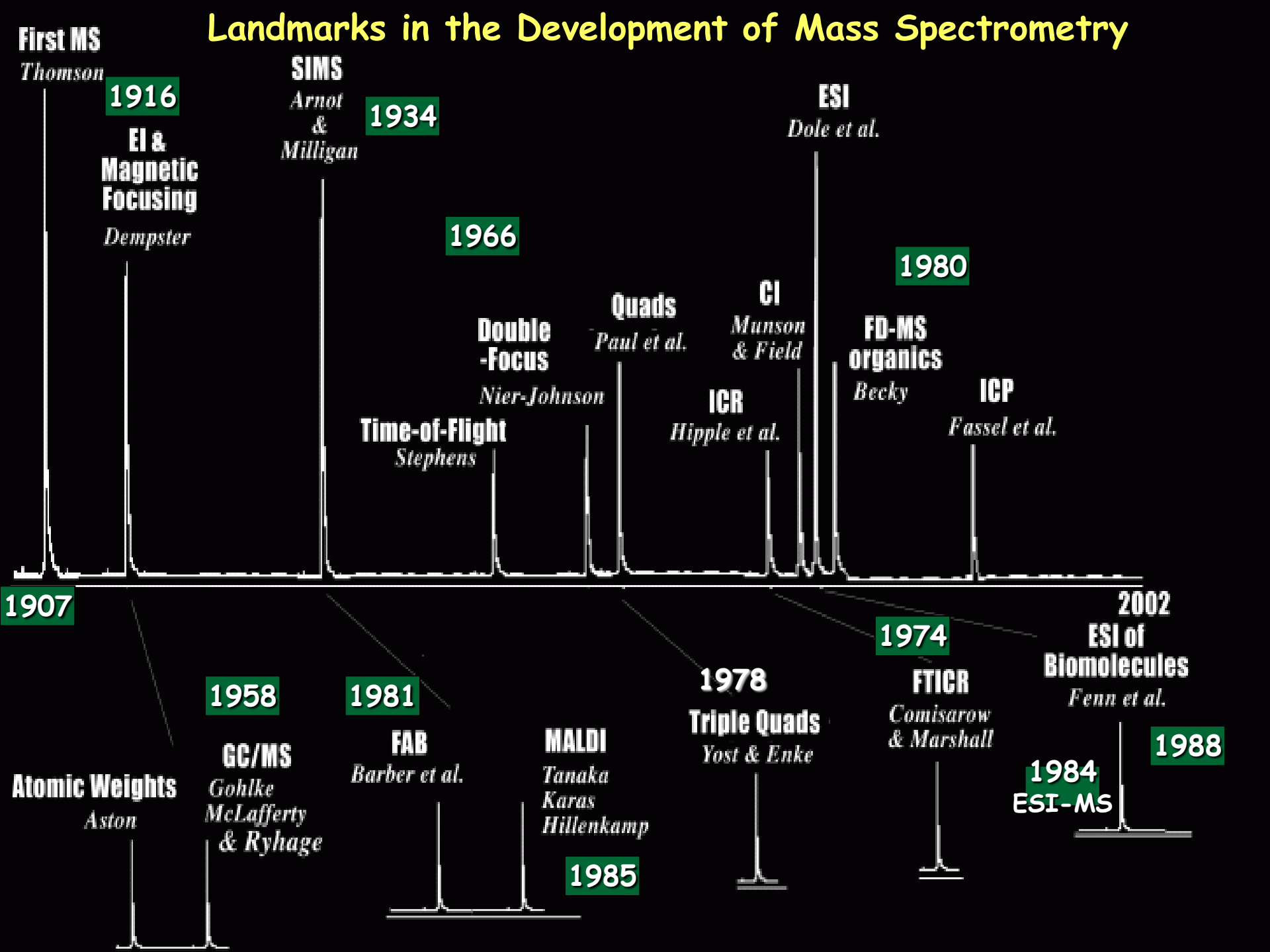
Inorganic chemist→ Petroleum chemists

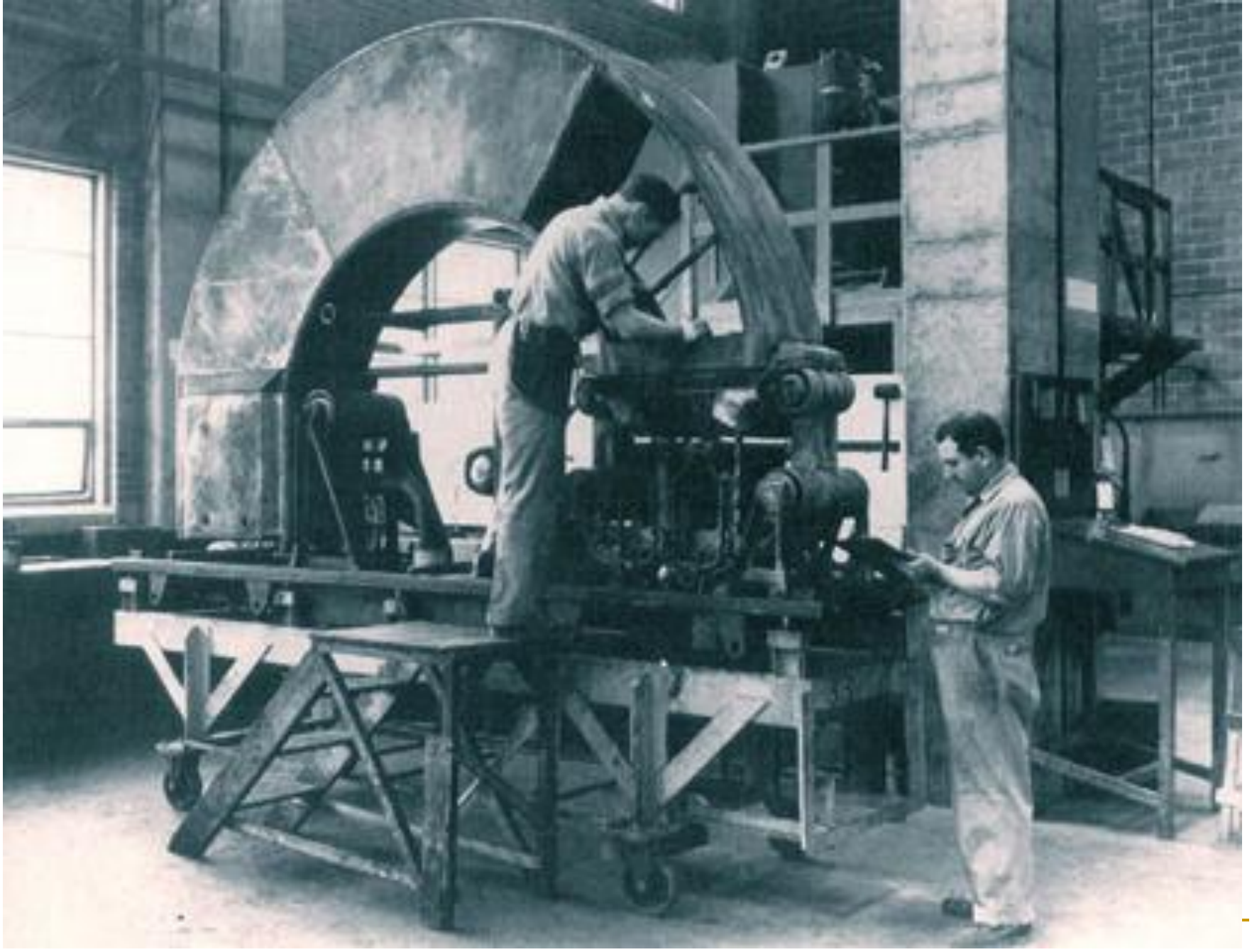
Figure 1. *Hypothetical photographic record of a parabola mass spectrograph. Reproduced from R. W. Kiser,* Introduction to Mass Spectrometry and Its Appucations *(Englewood Cliffs, N.J.: Prentice Hall. 1965).*

Espectrometria de Massas
Resumo Histórico

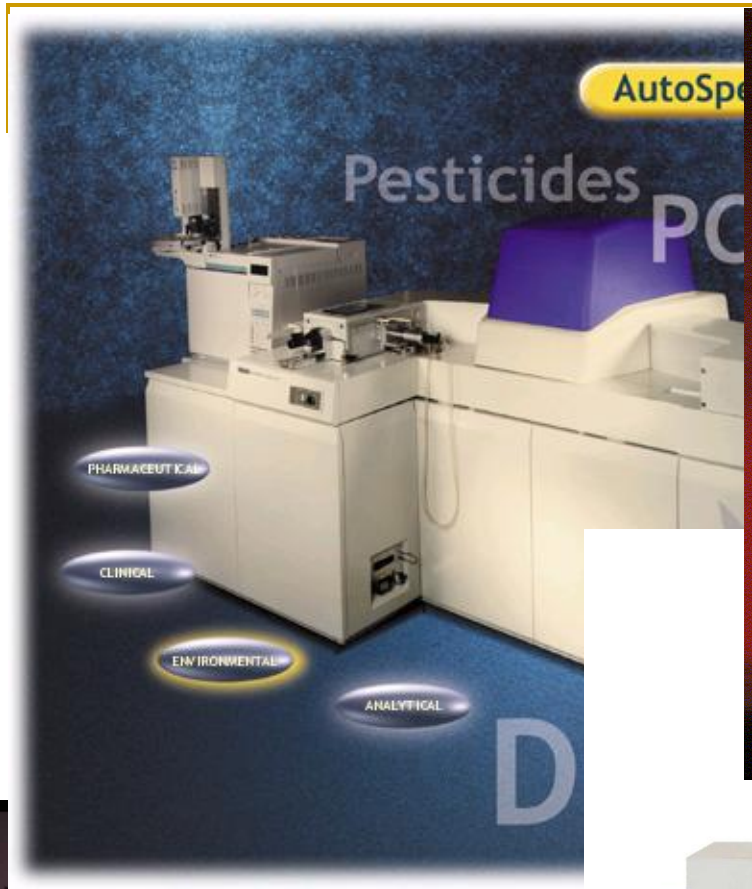
- 1898 – Wien observou que um feixe de íons positivos poderiam ser desviados empregando campo elétrico ou magnético.
- 1912 – Thomsom provou a existência de dois isótopos de neônio usando um instrumento que desviava os íons em um campo elétrico.
- 1918 – Dempster e Aston desenharam instrumentos que foram 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.

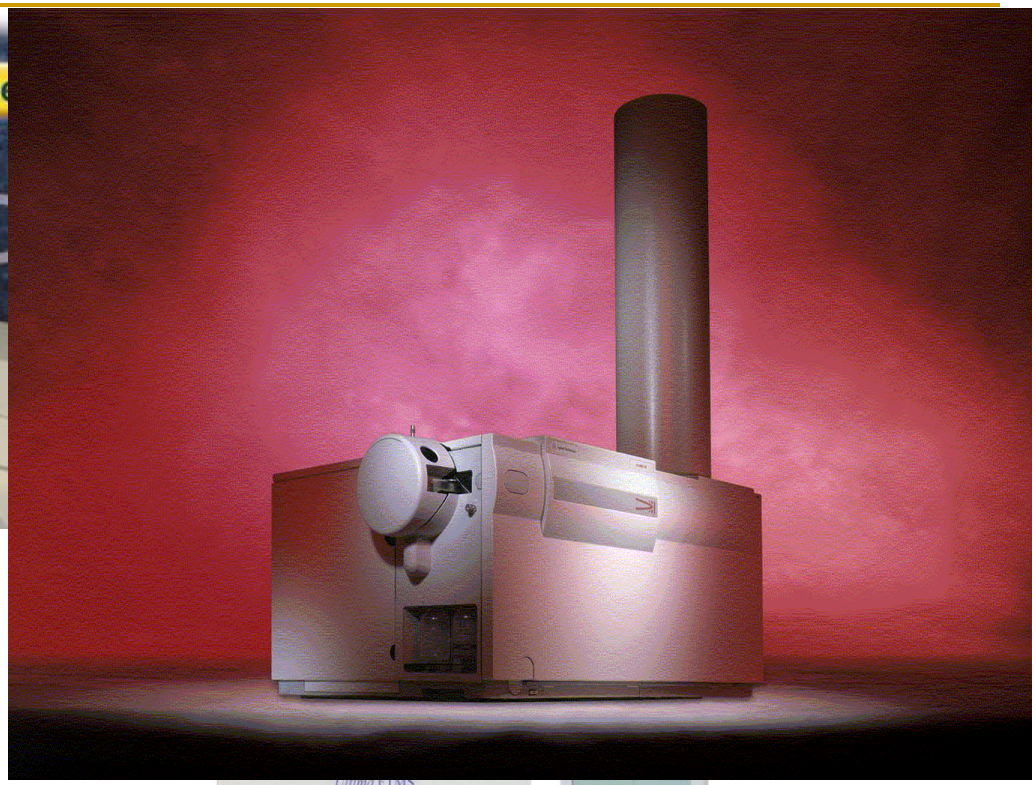
Landmarks in the Development of Mass Spectrometry





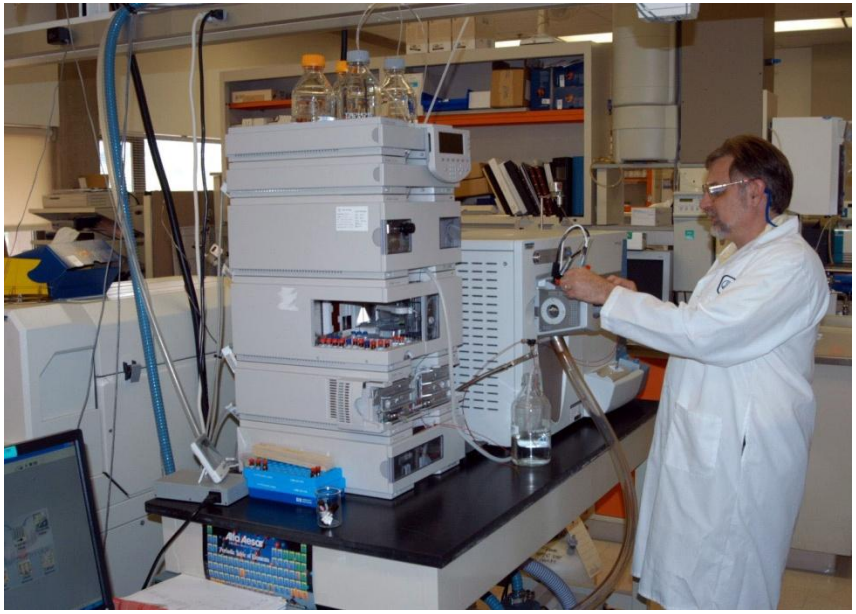
http://masspec.scripps.edu/MSHistory/histpers.php


AutoSpe
Pesticides
PC
PHARMACEUTICAL
CLINICAL
ENVIRONMENTAL
ANALYTICAL
D



GC/MS – Circa Late 1980s

“Bench-top” LC/MS Systems



Mass Spectrometry Basics

What information can be determined?

- Molecular weight
- Molecular formula (HRMS)
- Structure (from fragmentation fingerprint)
- Isotopic incorporation / distribution
- Protein sequence (MS-MS)

Mass Spectrometry Basics

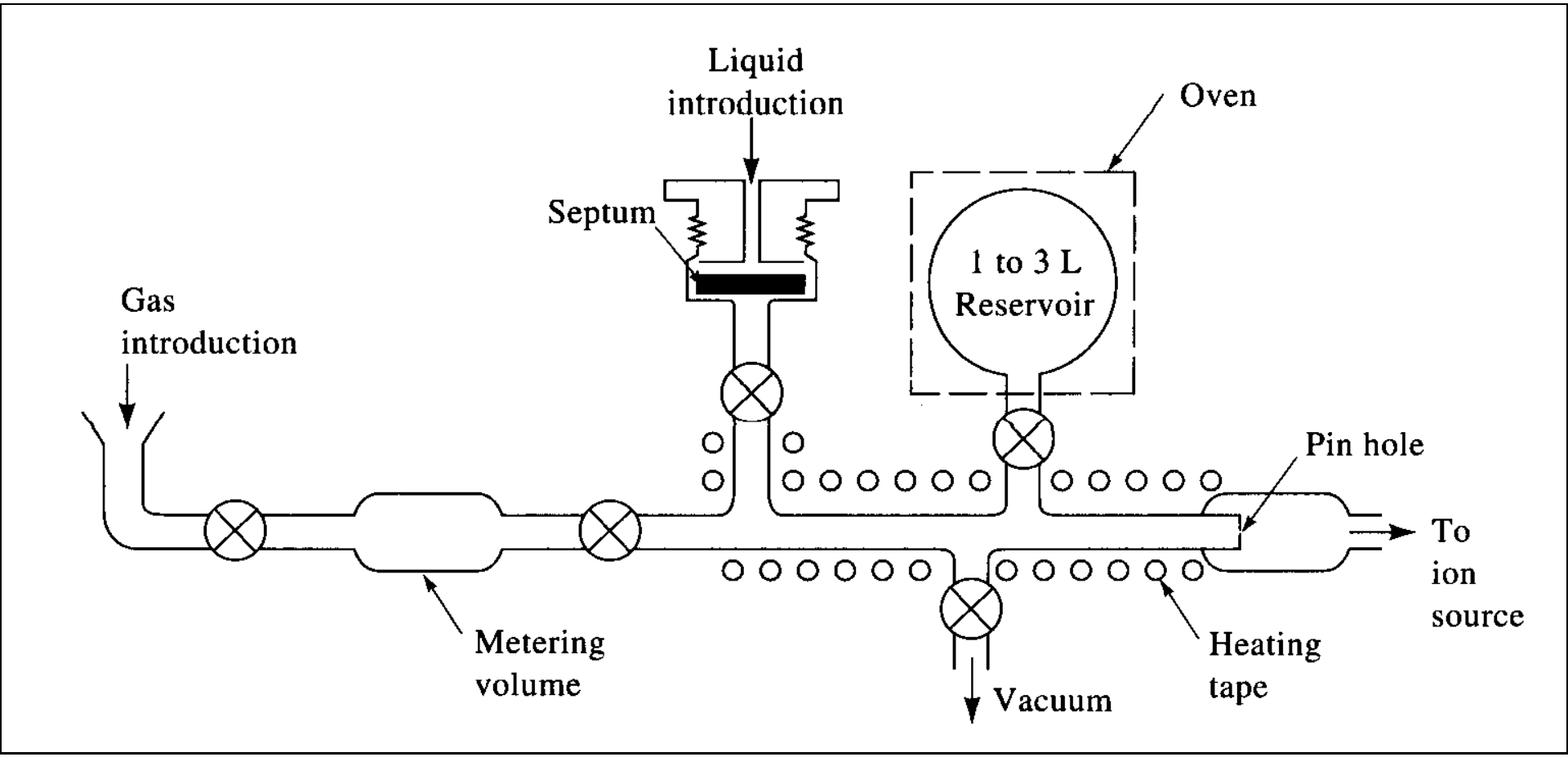
Mass spectrometry has 4 basic operations:

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

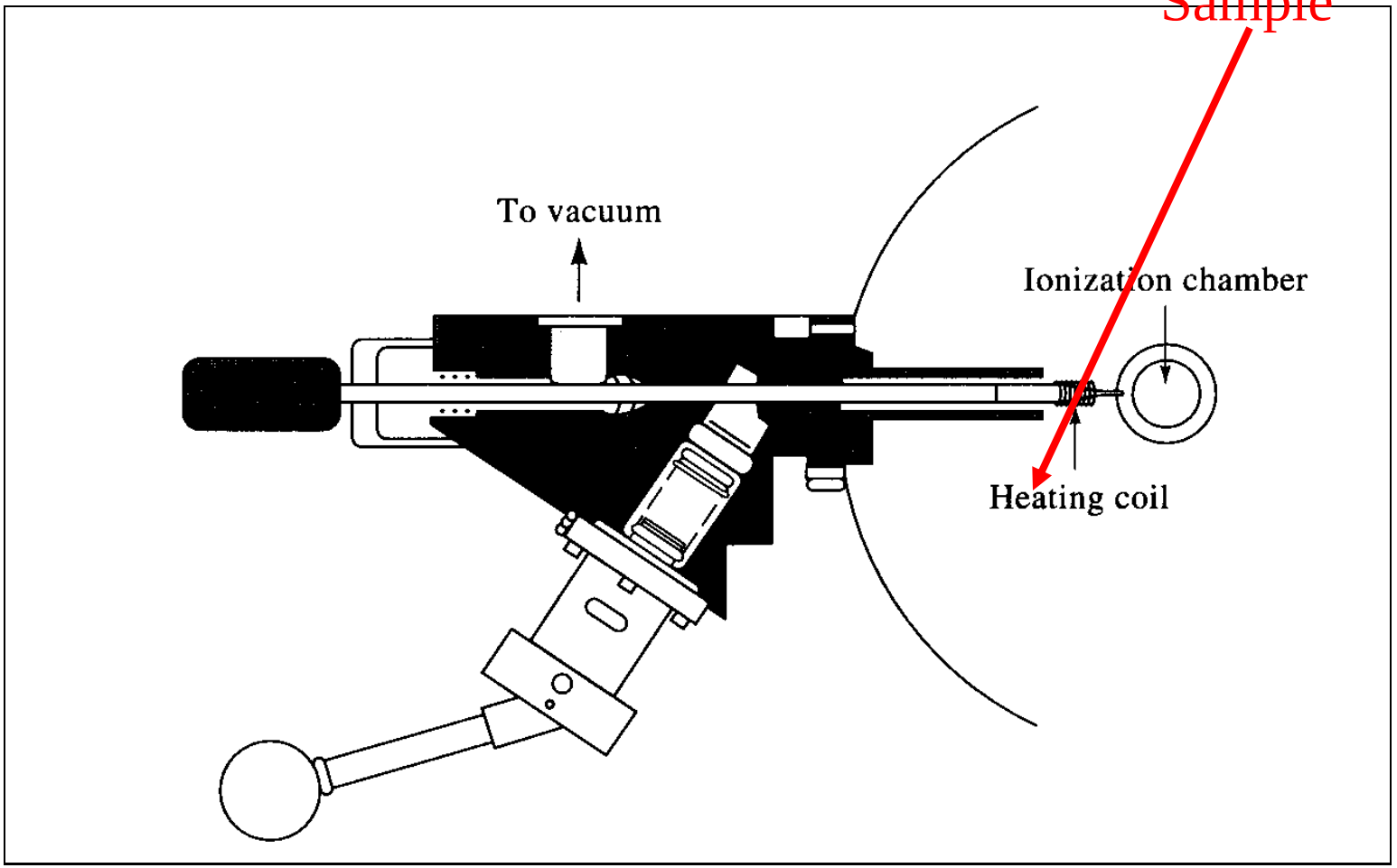
Sample Introduction

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.

Gas/Liquid Inlet System



Solid/Matrix Inlet Systems



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.

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"

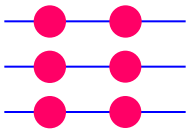
How does ionization occur?

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**

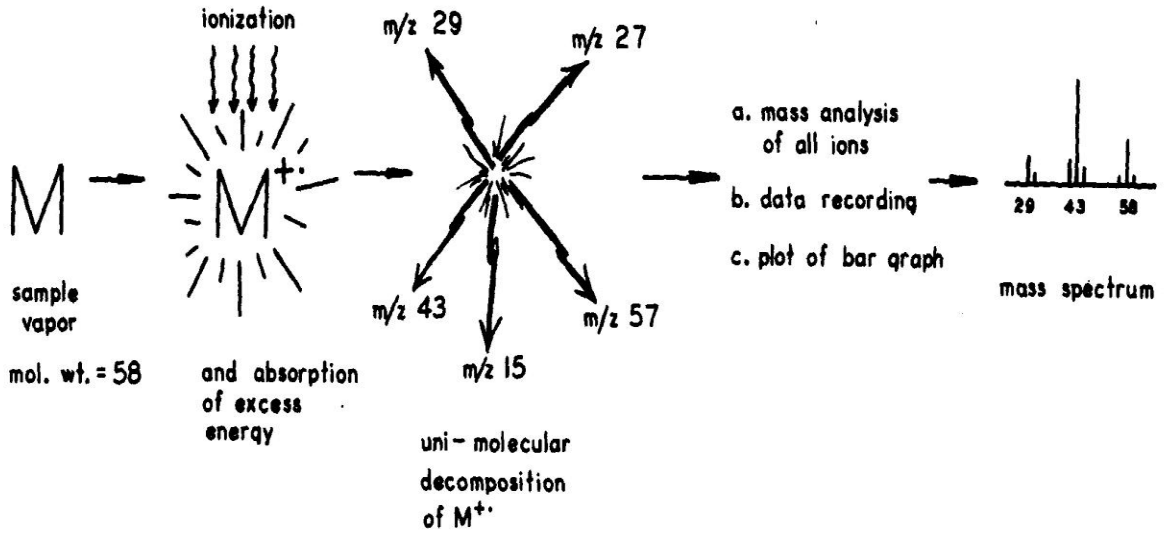


FIG. 1.1. General conceptual scheme for vapor-phase analysis by mass spectrometry.

Ionization Techniques

Gas-Phase Methods

- Electron Impact (EI)
- Chemical Ionization (CI)

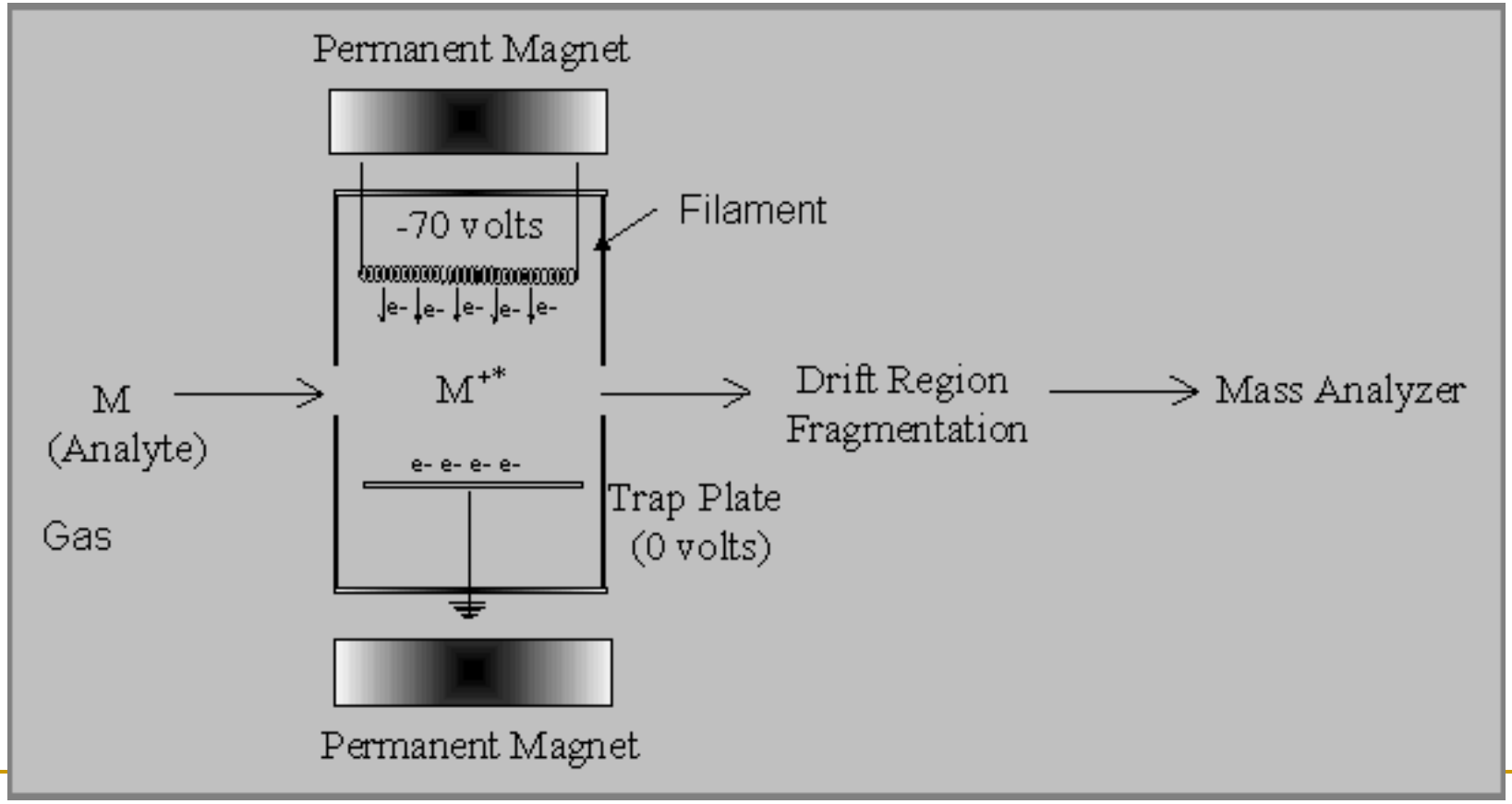
Desorption Methods

- Matrix-Assisted Laser Desorption Ionization (MALDI)
- Fast Atom Bombardment (FAB)

Spray Methods

- Electrospray (ESI)
- Atmospheric Pressure Chemical Ionization (APCI)

Electron Impact



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

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**.*

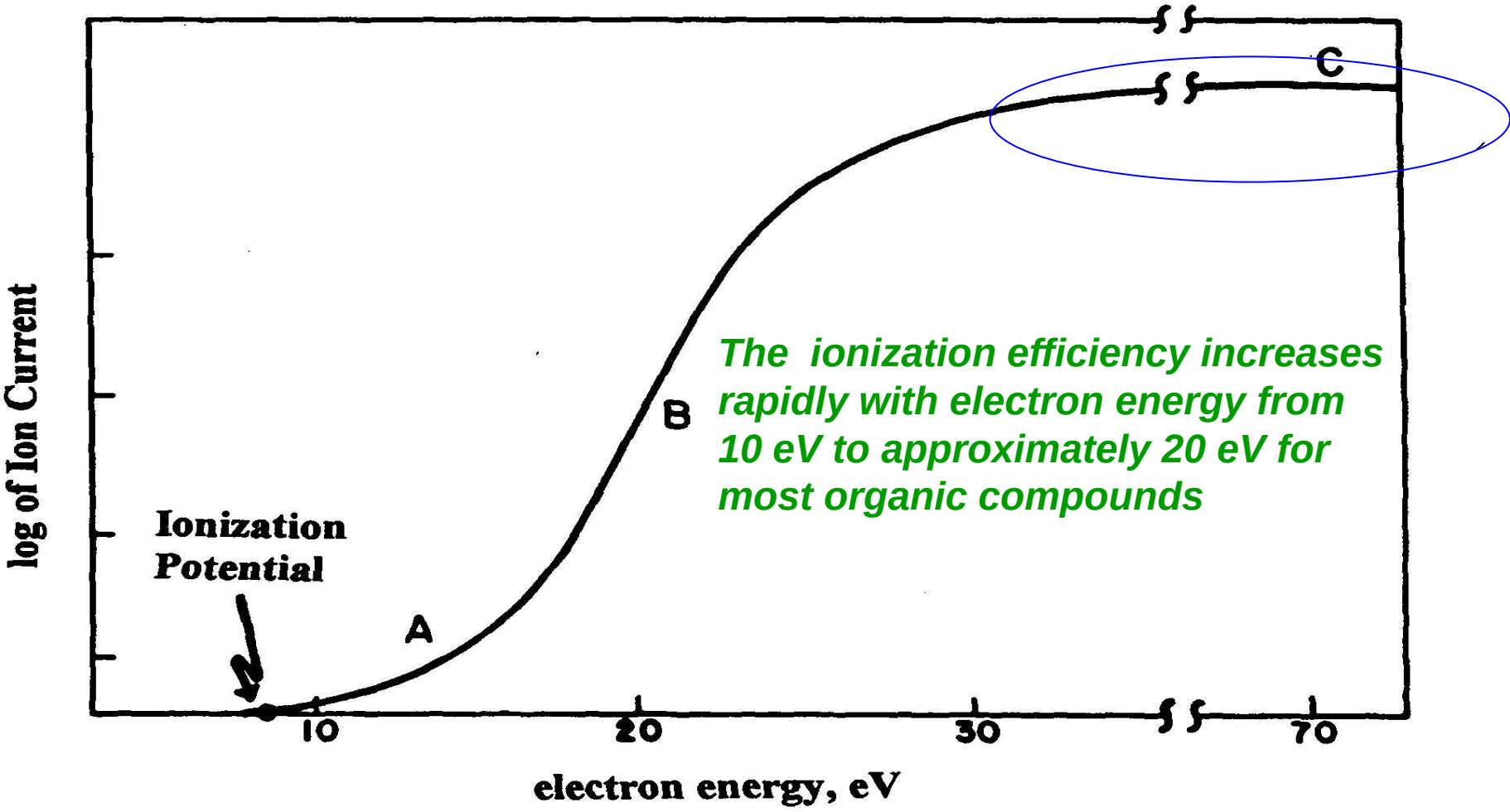


FIG. 7.3. Relationship between ion production and energy (electron volts) of ionizing electrons: *region A,* threshold region, principally molecular ions produced; *region B,* production of fragment ions becomes important; *region C,* routine operation, mostly fragment ions.

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)

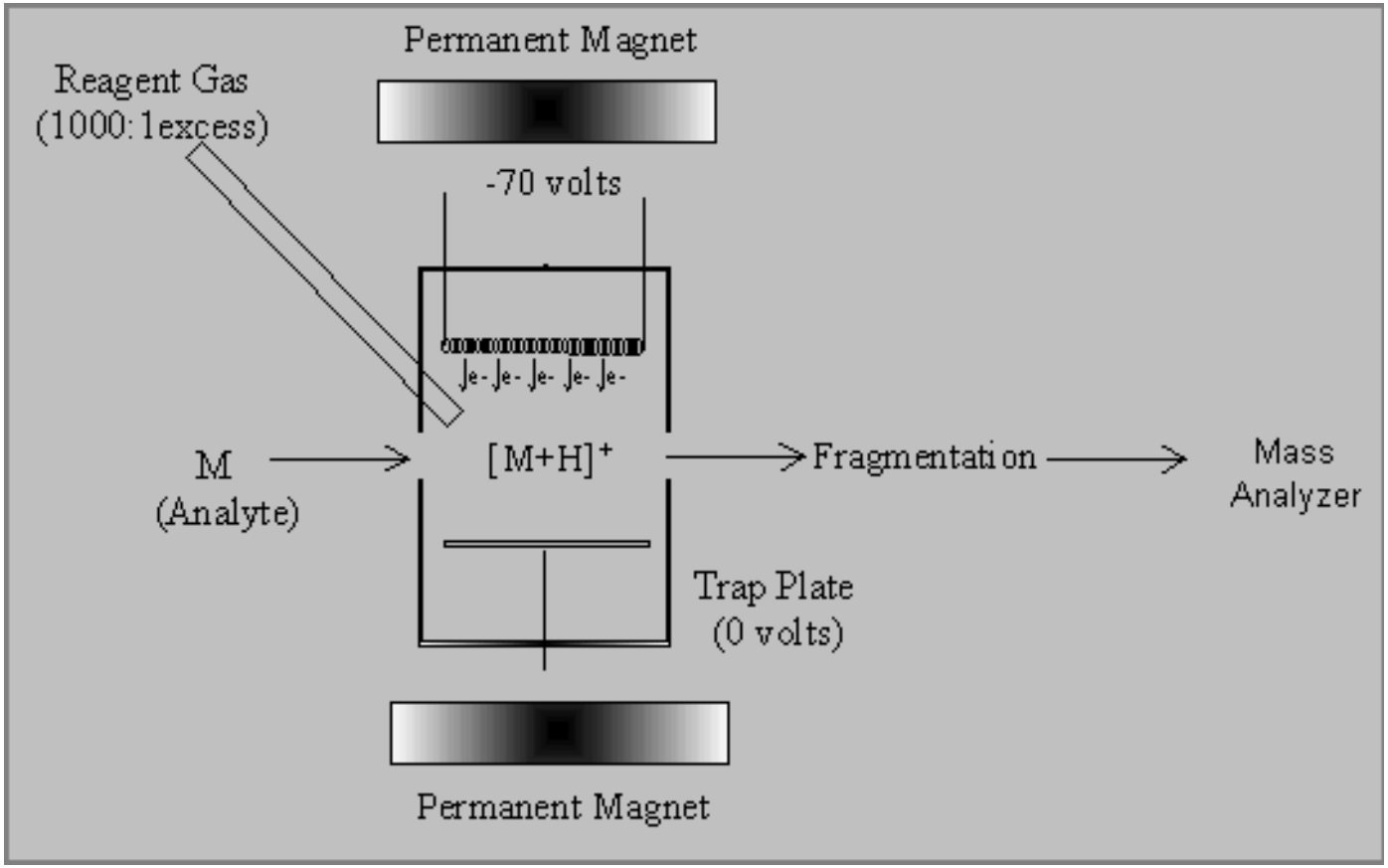
Advantages

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

Disadvantages

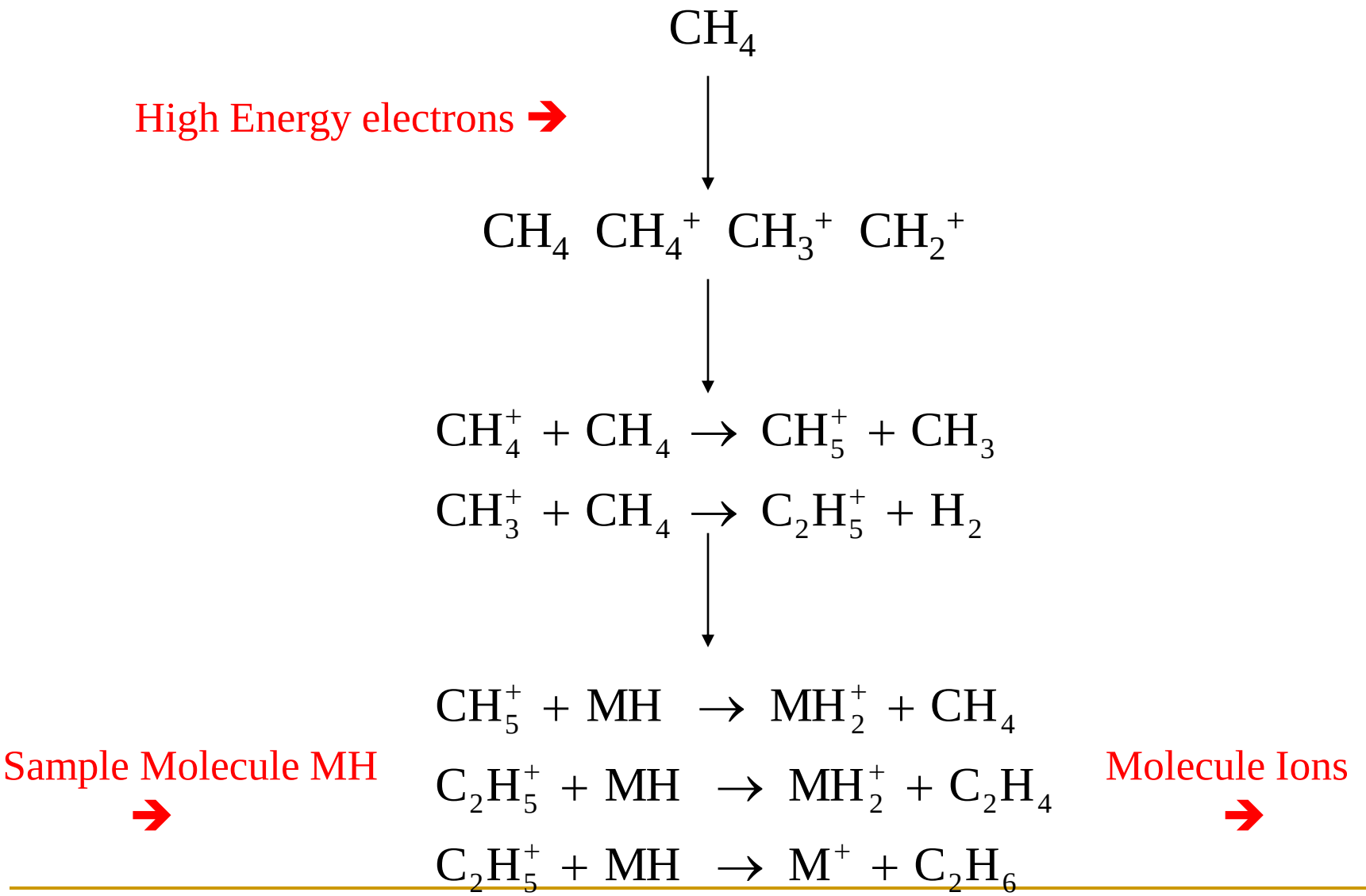
- Parent Identification
- Need Volatile Sample
- Need Thermal Stability
- No Interface to LC
- Low Mass Compounds (<1000 amu)
- Solids Probe Requires Skilled Operator

Chemical Ionization



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

Chemical Ionization MS Sources



Chemical Ionization

(low picomole)

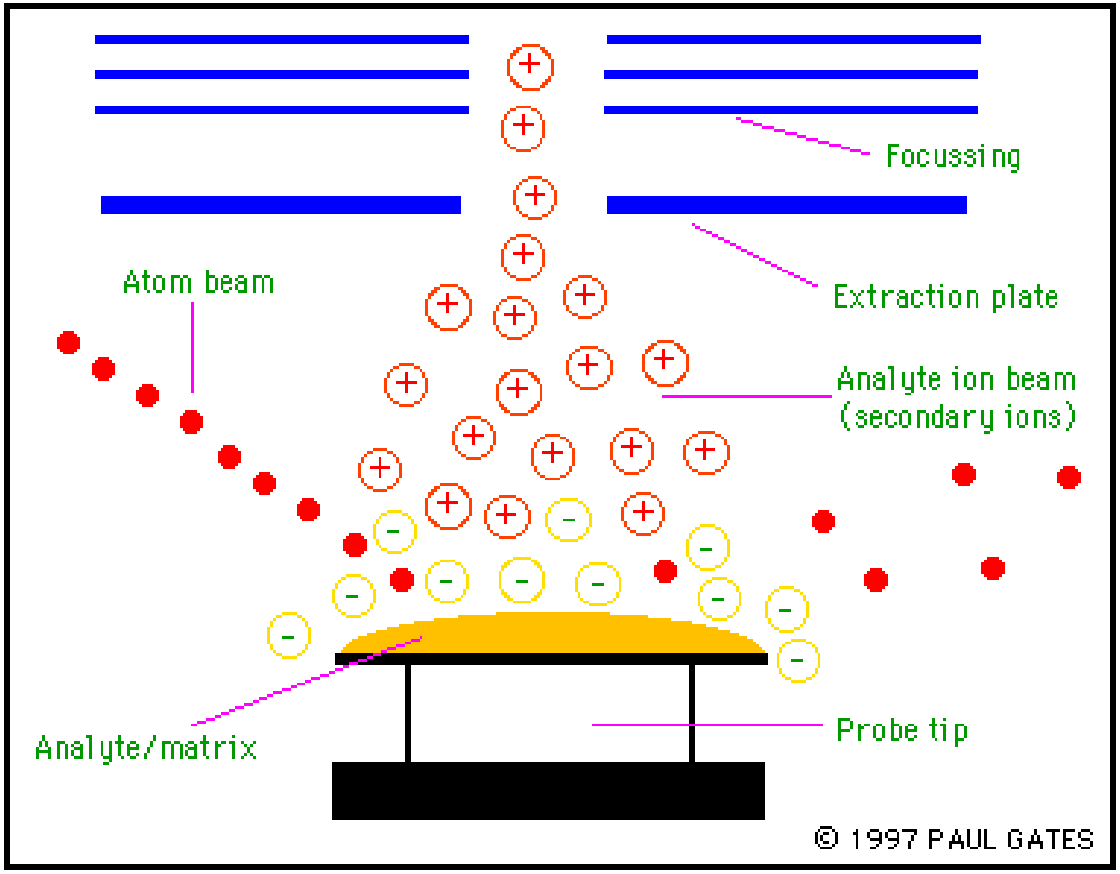
Advantages

- Parent Ion
- Interface to GC
- Insoluble Samples

Disadvantages

- No Fragment Library
- Need Volatile Sample
- Need Thermal Stability
- Quantitation Difficult
- Low Mass Compounds (<1000 amu)
- Solids Probe Requires Skilled Operator

FAB


Focussing
Extraction plate
Atom beam
Analyte ion beam
(secondary ions)
Analyte/matrix
Probe tip
© 1997 PAUL GATES

FAB

(nanomole)

Advantages

- Parent Ion
- High Mass Compounds (10,000 amu)
- Thermally Labile Compounds (R.T.)

Disadvantages

- No Fragment Library
- Solubility in Matrix (MNBA, Glycerol)
- Quantitation Difficult
- Needs Highly Skilled Operator
- Relatively Low Sensitivity

Matrix-assisted Laser Desorption/Ionization Mass Spectrometry (MALDI)

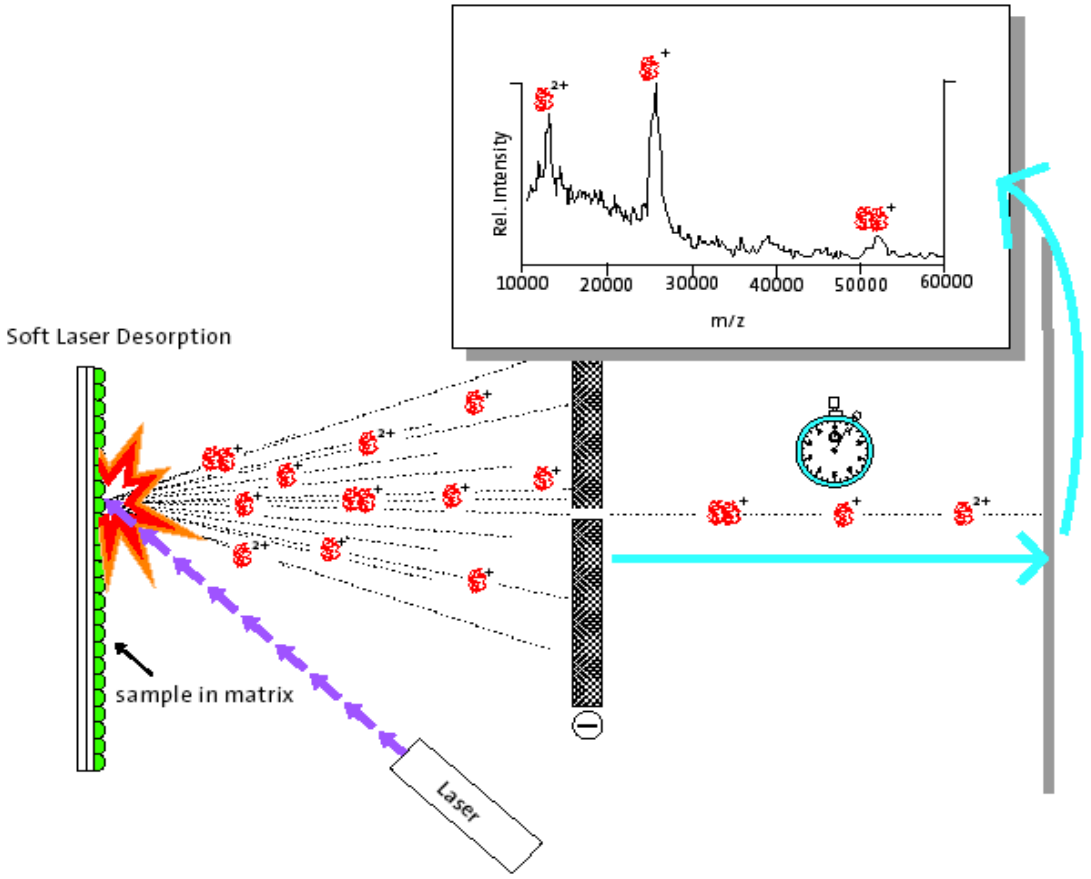
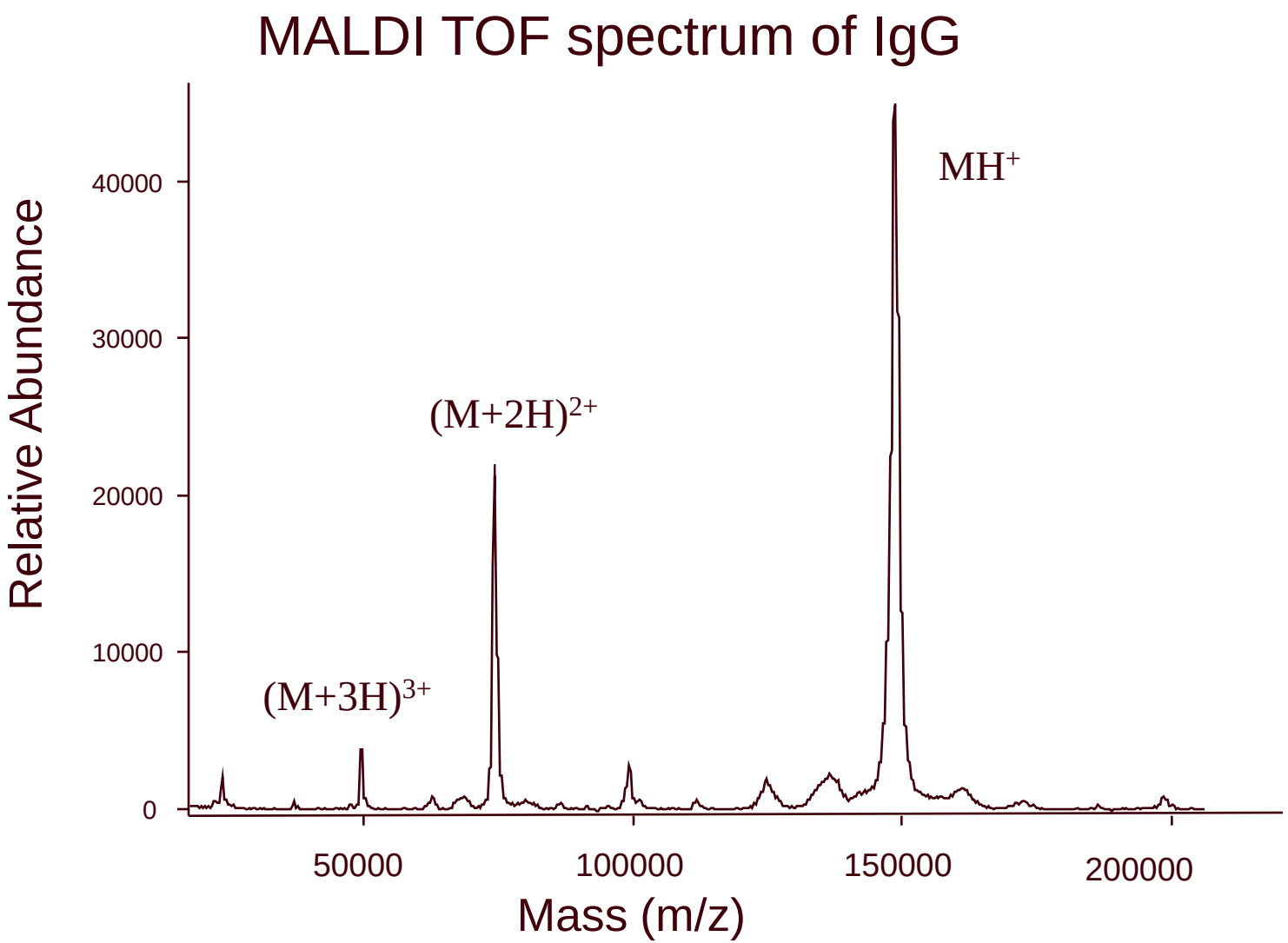


Figure 2. The soft laser desorption process.

From lectures by Vineet Bafna (UCSD)

The mass spectrum shows the results



MALDI

(low femtomole)

Advantages

- Parent Ion
- High Mass Compounds (>100,000 amu)
- Thermally Labile Compounds (R.T.)
- Easy to Operate

Disadvantages

- No Fragment Library
- Wide variety of matrices
- Quantitation Difficult

Método de Ionização por Nebulização

Ionização por *Electrospray* (*ESI*)

A amostra é nebulizada a partir de uma agulha que se encontra sob uma diferença de potencial (0 a 5 kV) que ajuda na ionização e separação dos íons, enquanto o calor e o fluxo de gás (N_2) dessolvatam os íons gerados.

Vantagens:

- bom p/ substâncias polares e íons
- detecção de substâncias de alto peso molecular pela razão m/z
- melhor método p/ múltiplas cargas
- baixo *background*
- controle de fragmentação

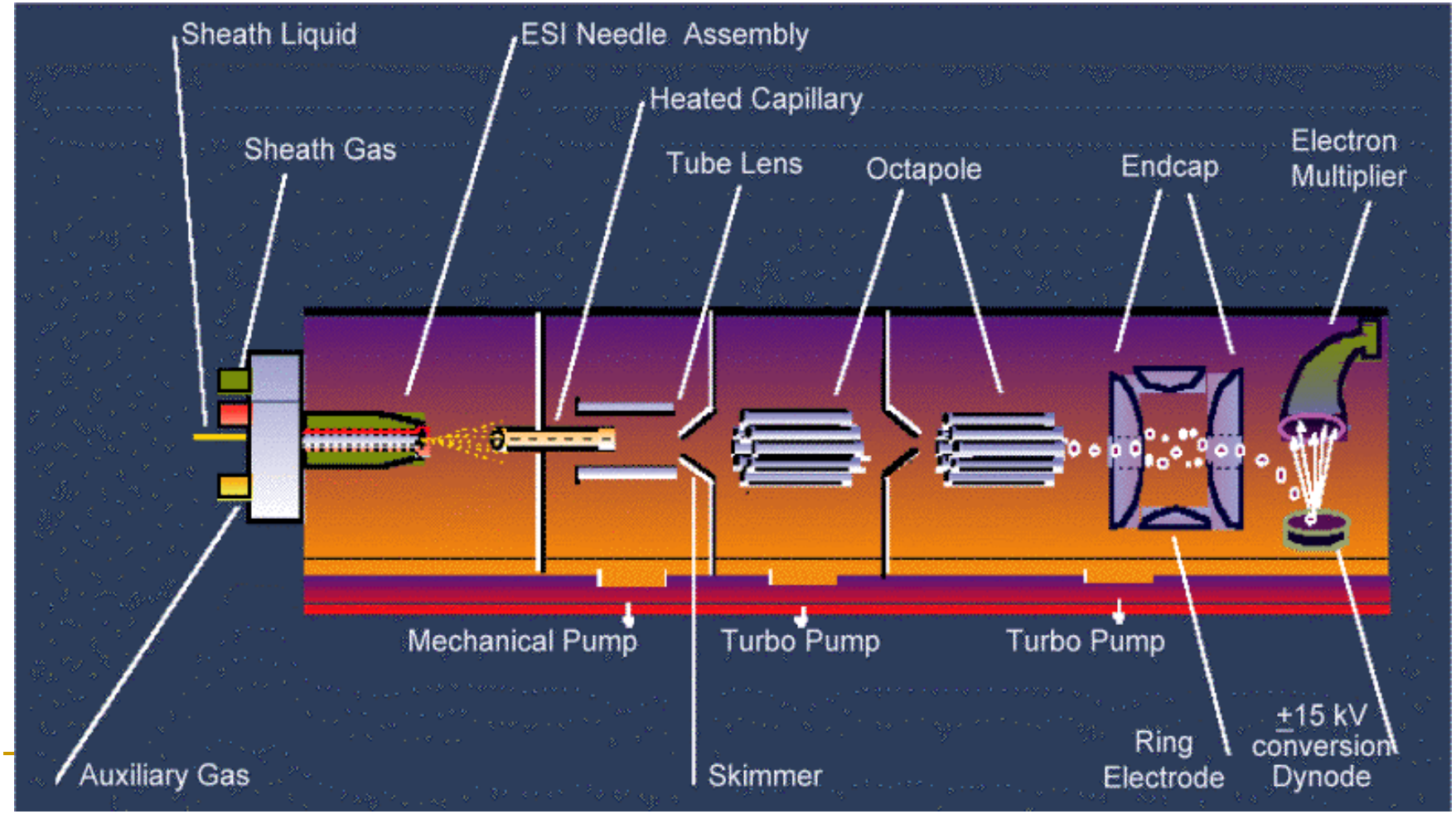
Limitações:

- interpretação dos dados de espécies com múltiplas cargas
- ruim para substâncias neutras e pouco polares
- muito sensível a contaminantes
- corrente iônica baixa

Intervalo de massa: tipicamente até 150.000 Da.

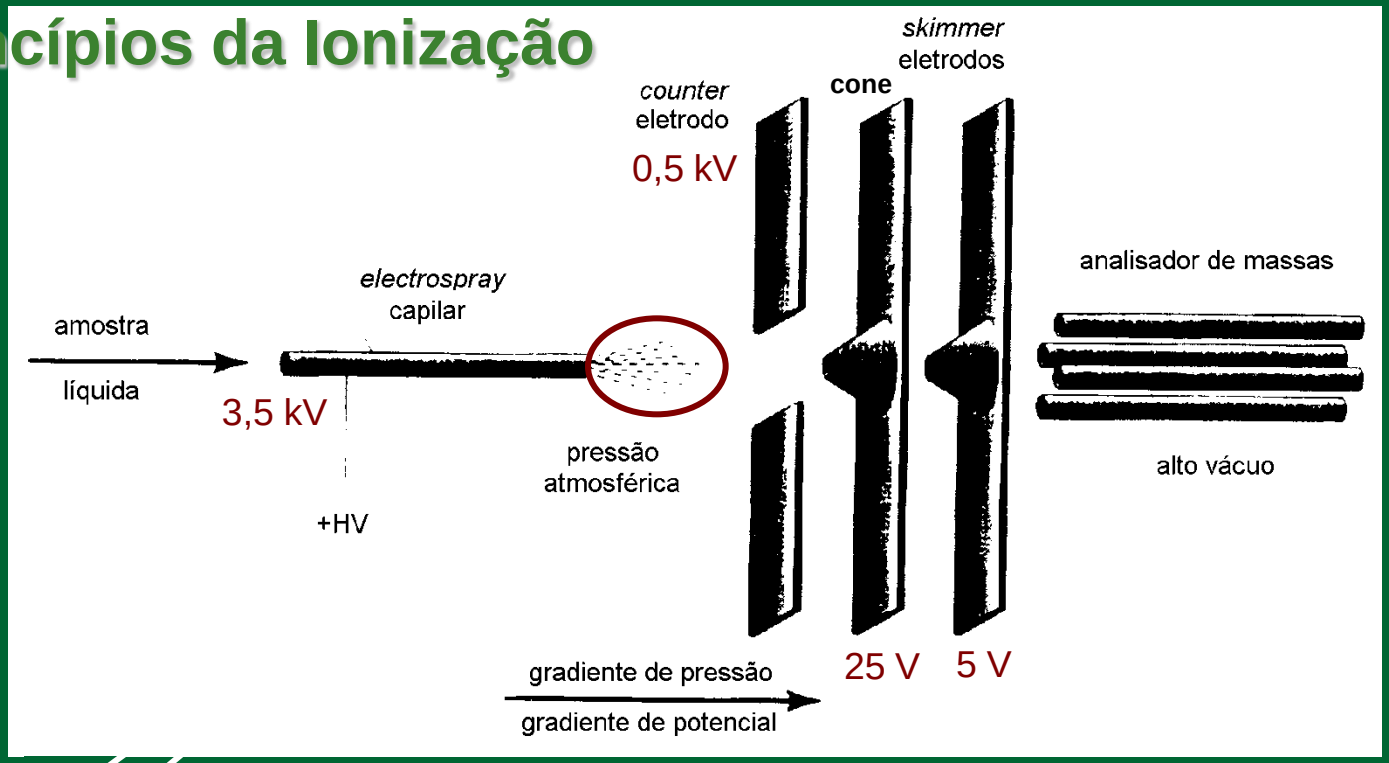
Electrospray

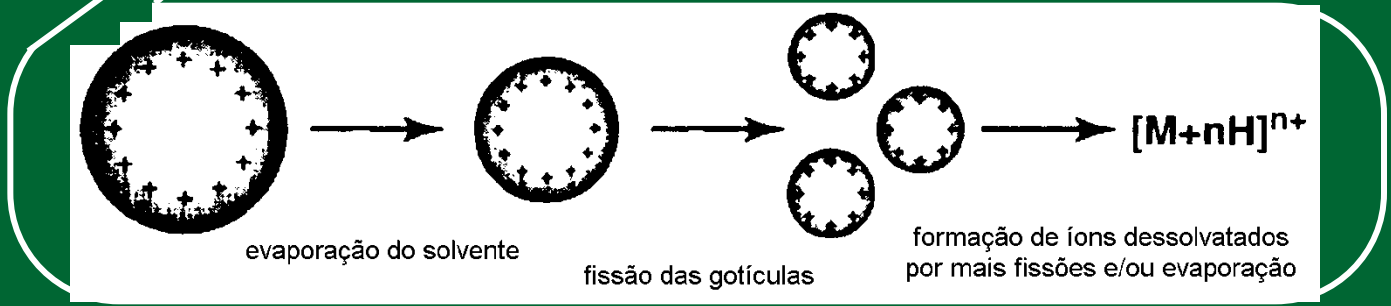
Esquema Geral



Electrospray

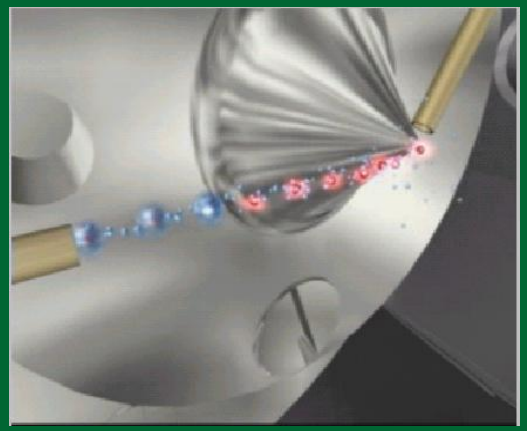
Princípios da Ionização

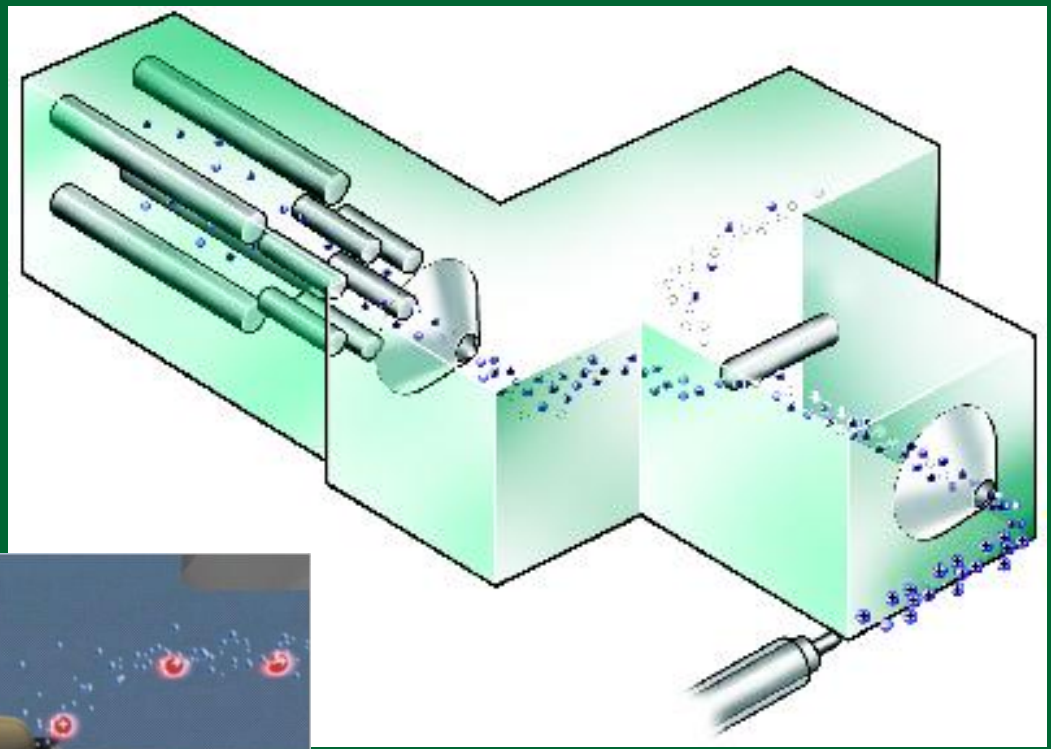


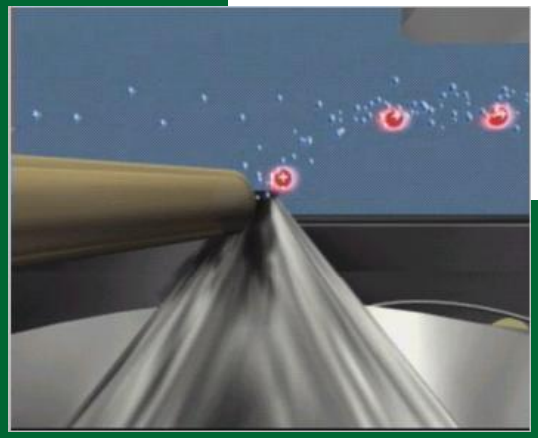


Electrospray

Fluxo dos Íons

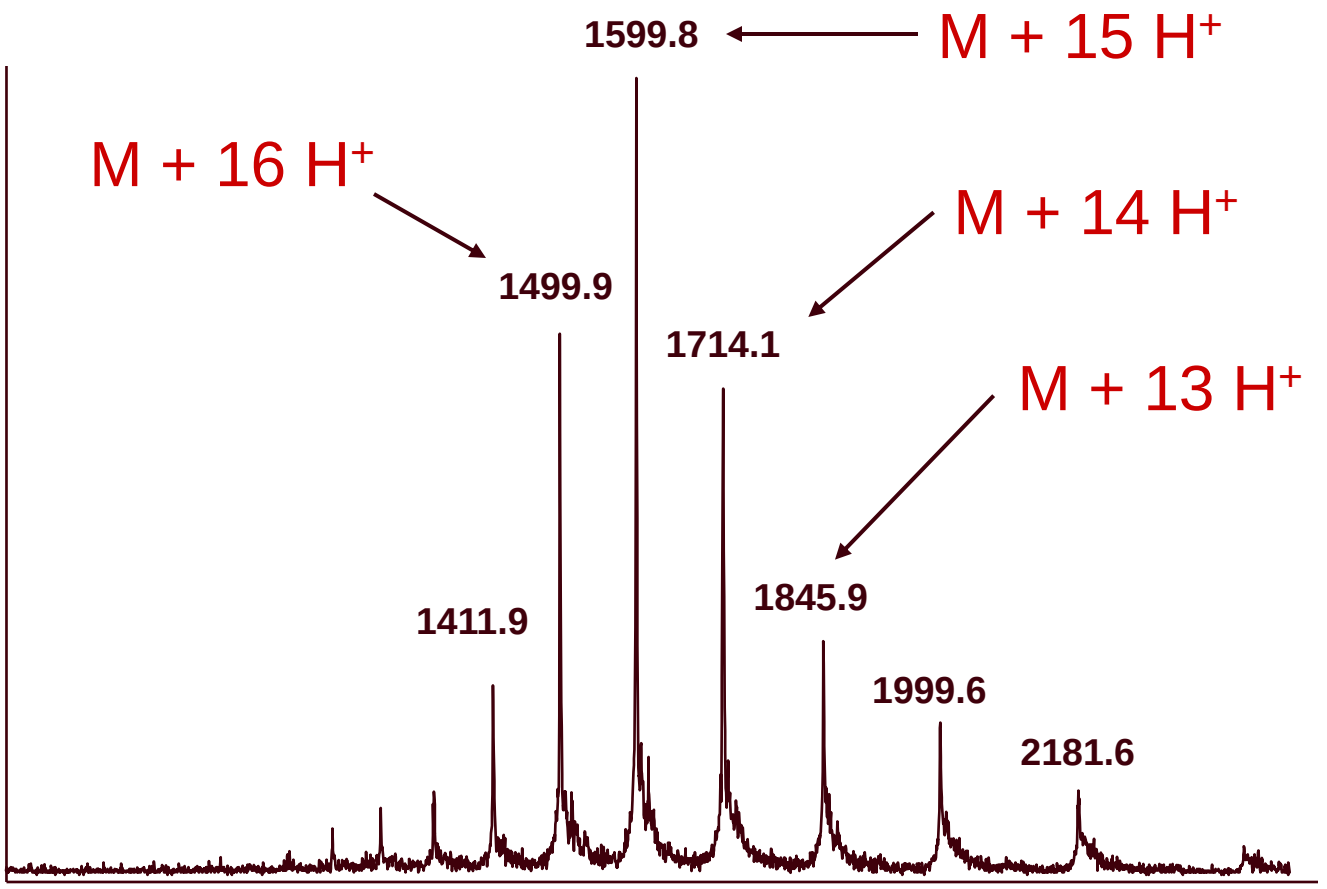






ThermoQuest

ESI Spectrum of Trypsinogen (MW 23983)



ESI

(low femtomole to zeptomole)

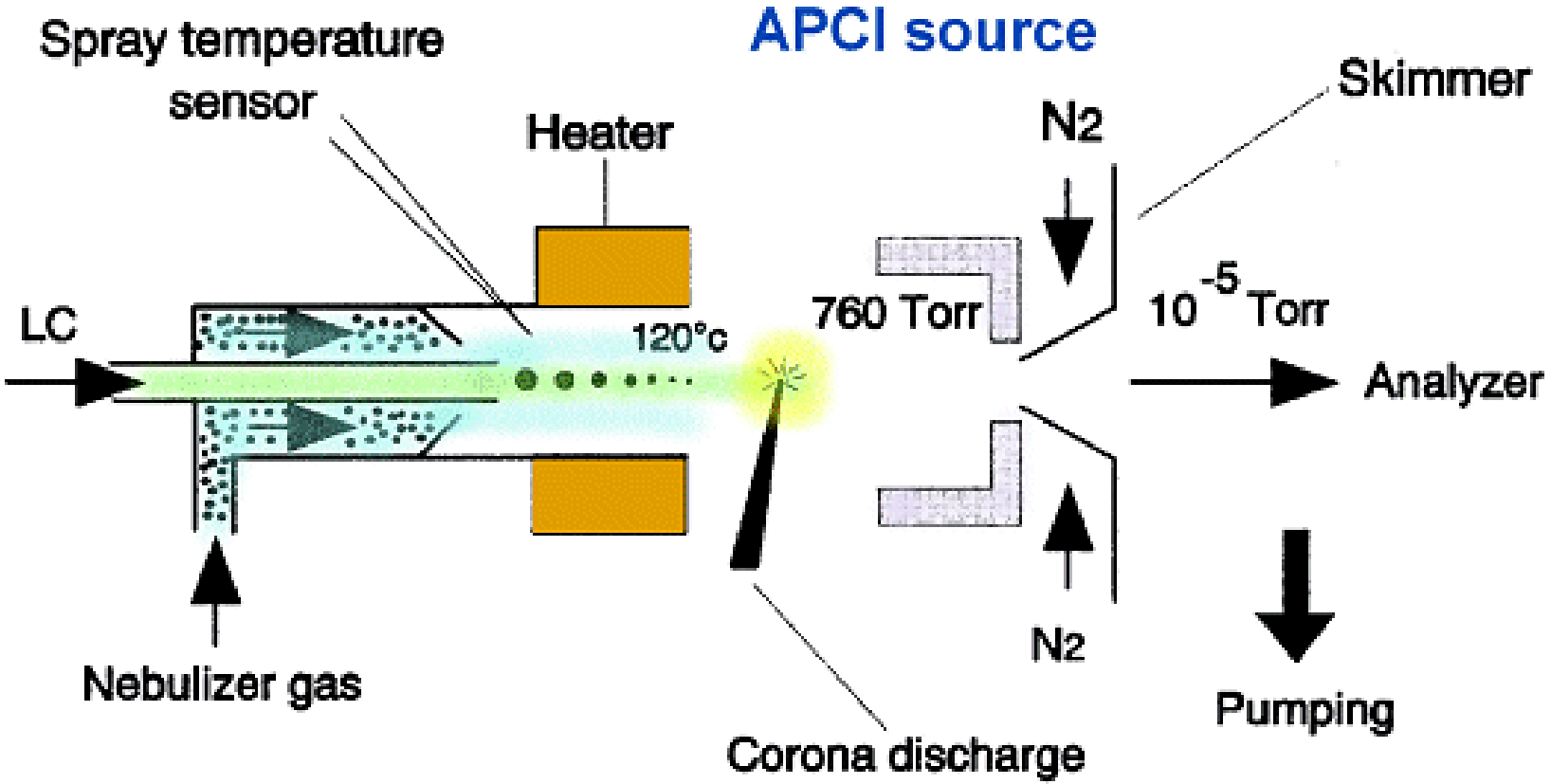
Advantages

- Parent Ion
- High Mass Compounds (>100,000 amu)
- Thermally Labile Compounds (<0º C)
- Easy to Operate
- Interface to HPLC
- Zeptomole sensitivity with nanospray

Disadvantages

- No Fragmentation
- Need Polar Sample
- Need Solubility in Polar Solvent (MeOH, ACN, H_2O, Acetone are best)
- Sensitive to Salts
- Supression

APCI



http://www.rzuser.uni-heidelberg.de/~bl5/ency/ency.html

APCI

(high femtomole)

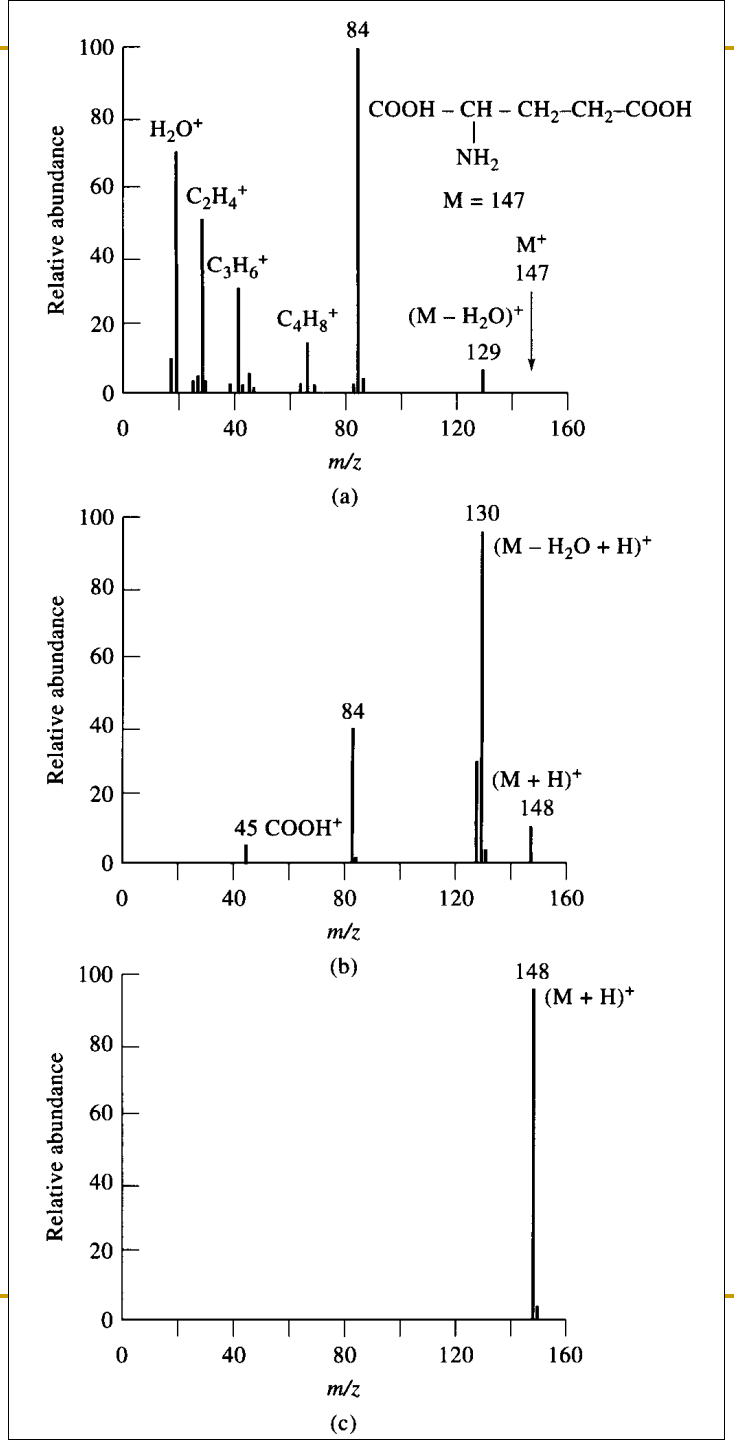
Advantages

- Parent Ion
- Insensitive to Salts
- Interface to HPLC
- Can use Normal Phase Solvents
- Handles High Flow Rates

Disadvantages

- Need Volatile Sample
- Need Thermal Stability

Glutamic Acid



Electron Impact (EI)

Field Ionization

Field Desorption

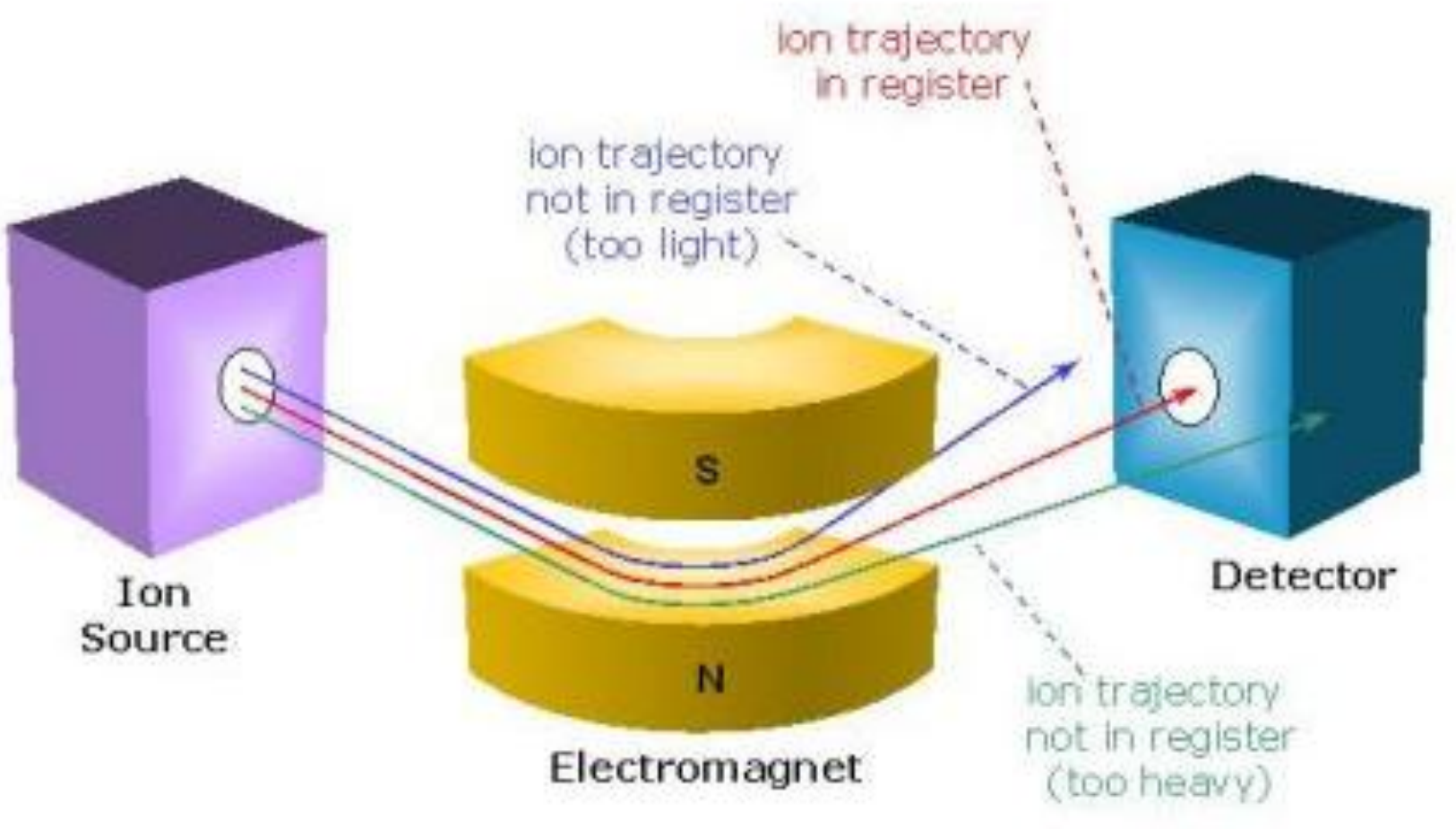
Mass Analyzers

- 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)

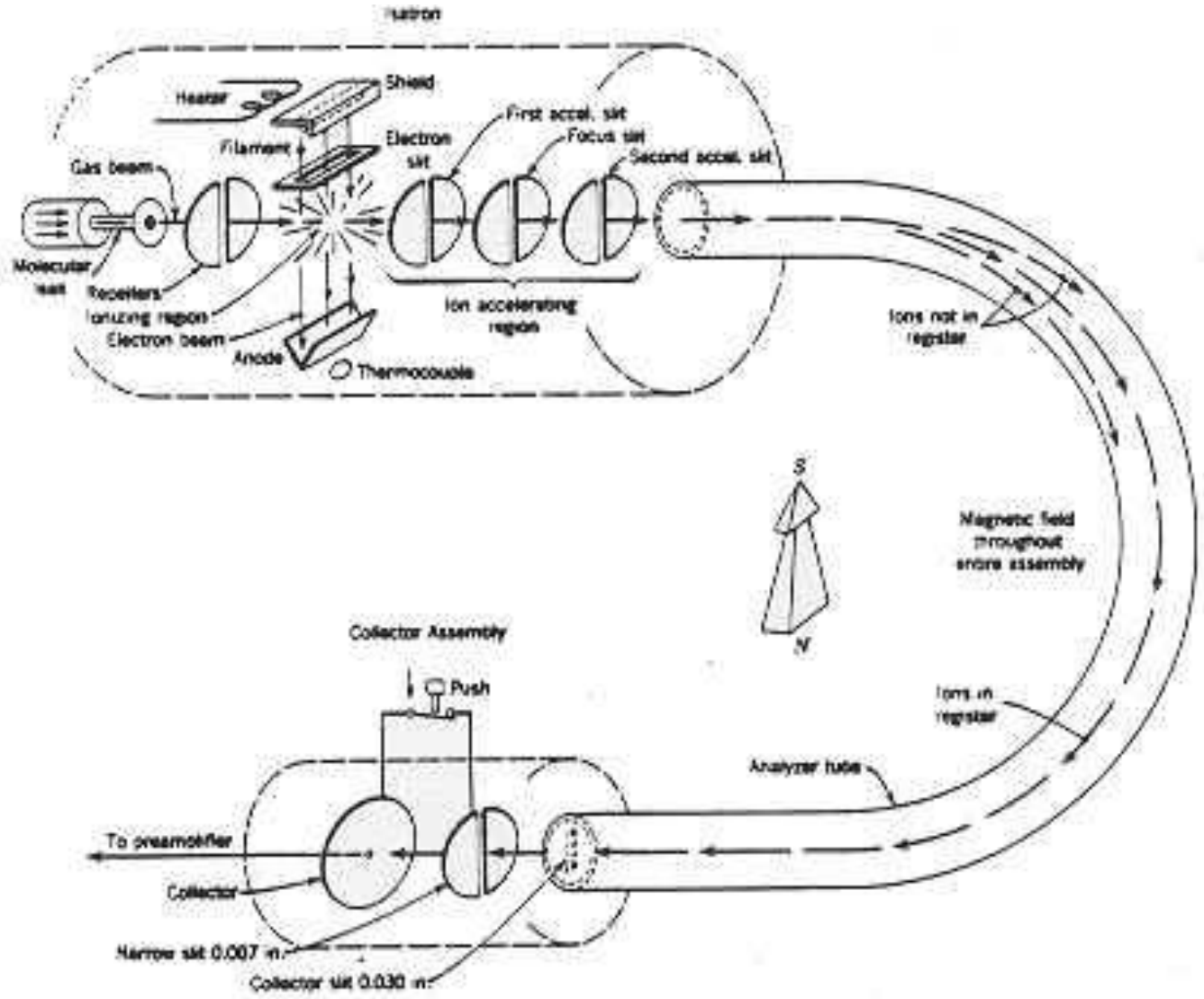
Different Types of Mass Analyzers

- Magnetic Sector Analyzer (MSA)
  - High resolution, exact mass, original MA
- Quadrupole Analyzer (Q or Q*)
  - Low (1 amu) resolution, fast, cheap
- Time-of-Flight Analyzer (TOF)
  - No upper m/z limit, high throughput
- Ion Cyclotron Resonance (FT-ICR)
  - Highest resolution, exact mass, costly

Magnetic Sector Analyzer



Magnetic Sector Analyzer



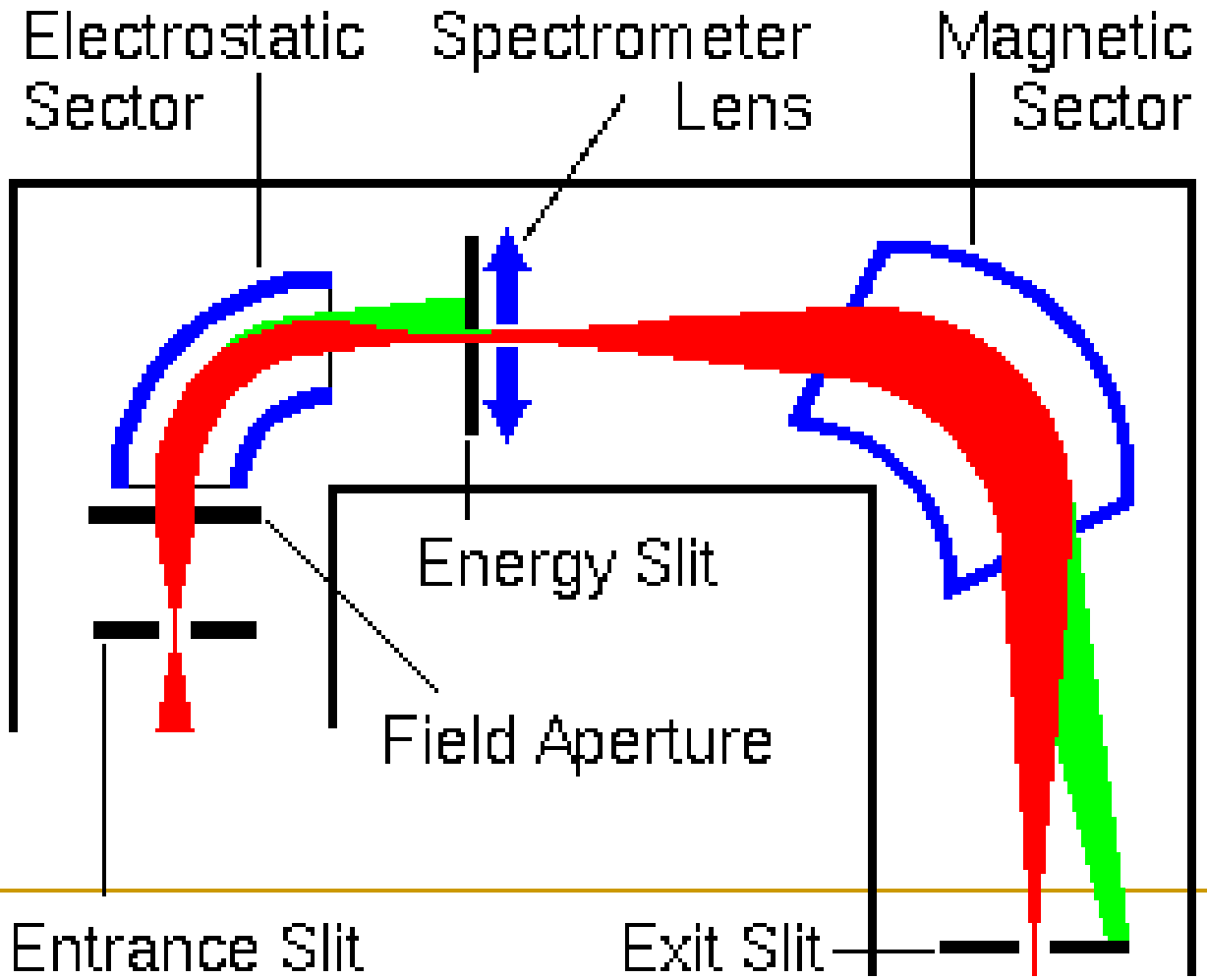
The Mass Spec Equation

$$\frac{m}{z} = \frac{B^2 r^2}{2V}$$

M = mass of ion
z = charge of ion
V = voltage

B = magnetic field
r = radius of circle

Double-Focusing Magnetic Sector



Double-Focusing Magnetic Sector

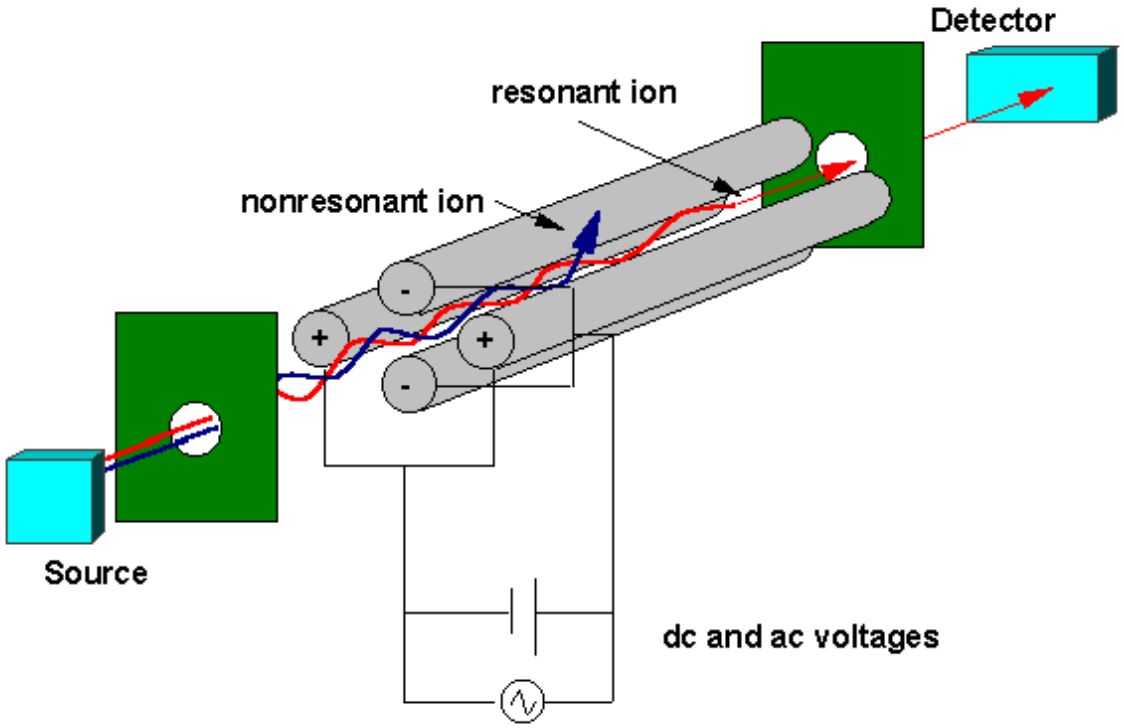
Advantages

- Very High Resolution (60,000)
- High Accuracy (<5 ppm)
- 10,000 Mass Range

Disadvantages

- Very Expensive
- Requires Skilled Operator
- Difficult to Interface to ESI
- Low resolution MS/MS without multiple analyzers

Quadrupole Mass Analyzer



Quadrupole Mass Filter

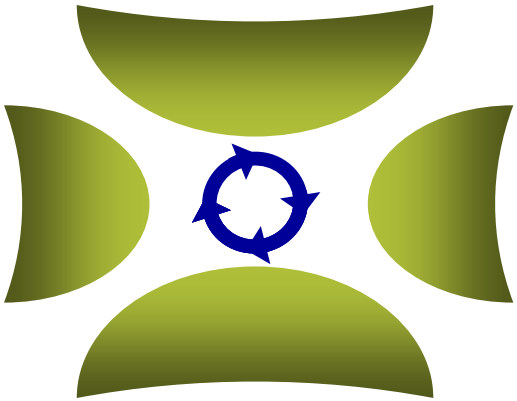
Advantages

- Inexpensive
- Easily Interfaced to Many Ionization Methods

Disadvantages

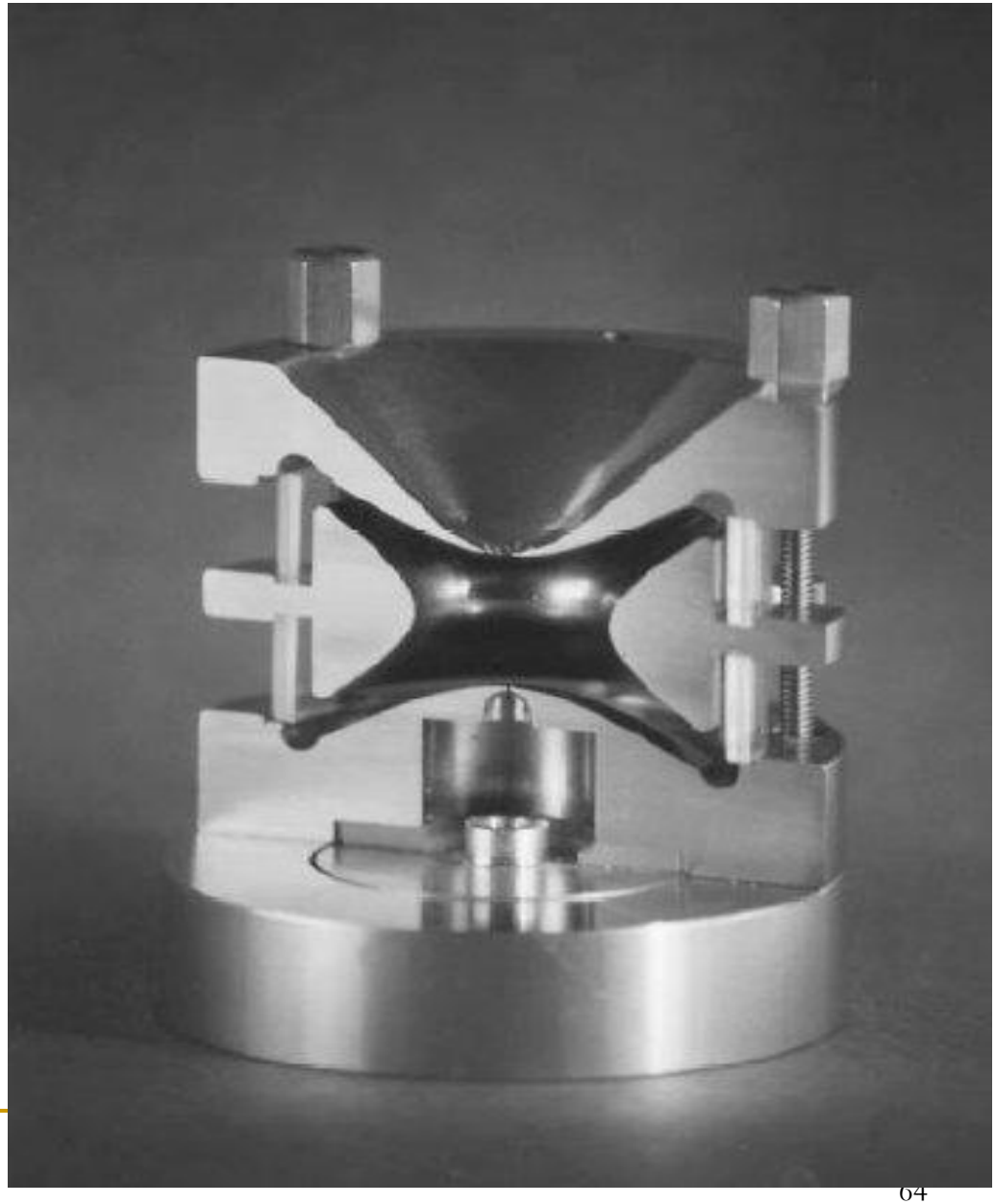
- Low Resolution (<4000)
- Low Accuracy (>100ppm)
- MS/MS requires multiple analyzers
- Low Mass Range (<4000)
- Slow Scanning

Ion Trap Mass Analyzer



Top View

Cut away side view



Quadrupole Ion Trap

Advantages

- Inexpensive
- Easily Interfaced to Many Ionization Methods
- MS/MS in one analyzer

Disadvantages

- Low Resolution (<4000)
- Low Accuracy (>100ppm)
- Space Charging Causes Mass Shifts
- Low Mass Range (<4000)
- Slow Scanning

Linear Time-of-Flight (TOF)

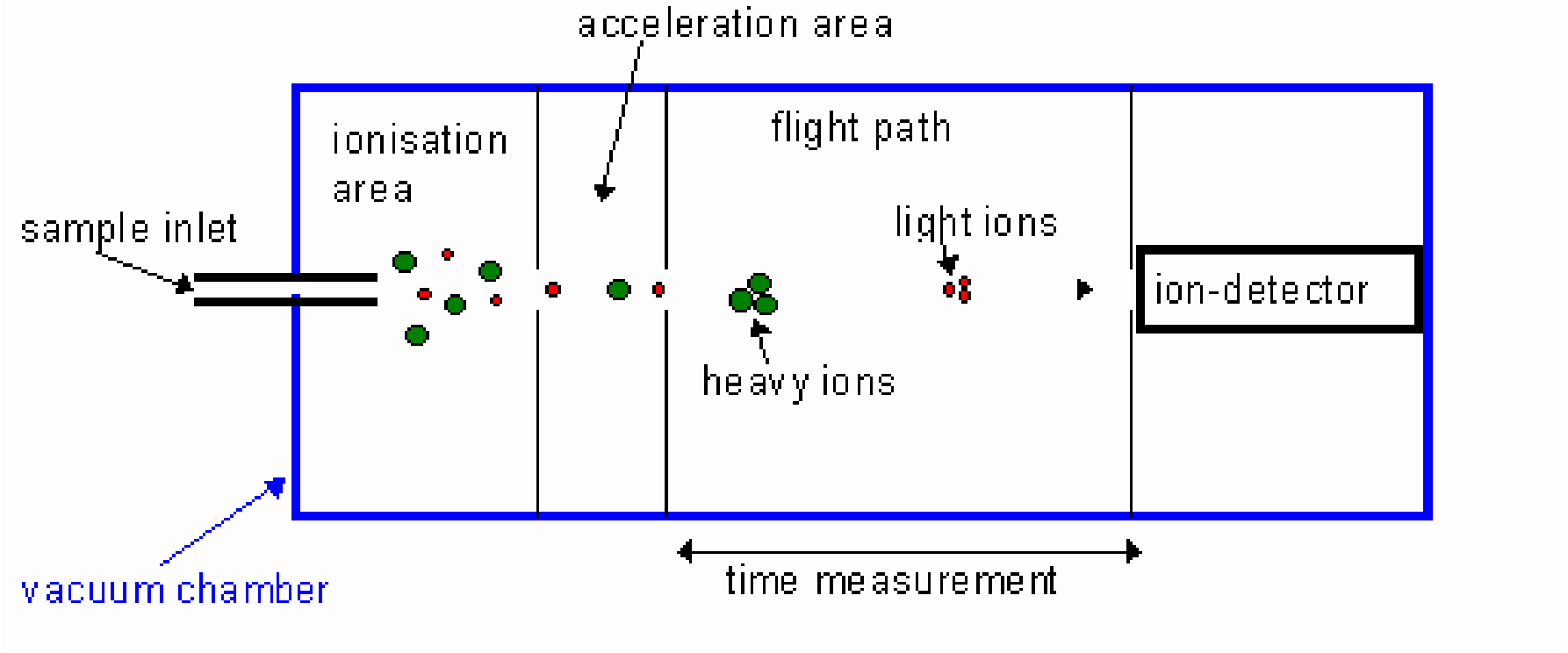
Advantages

- Extremely High Mass Range (>1 MDa)
- Fast Scanning

Disadvantages

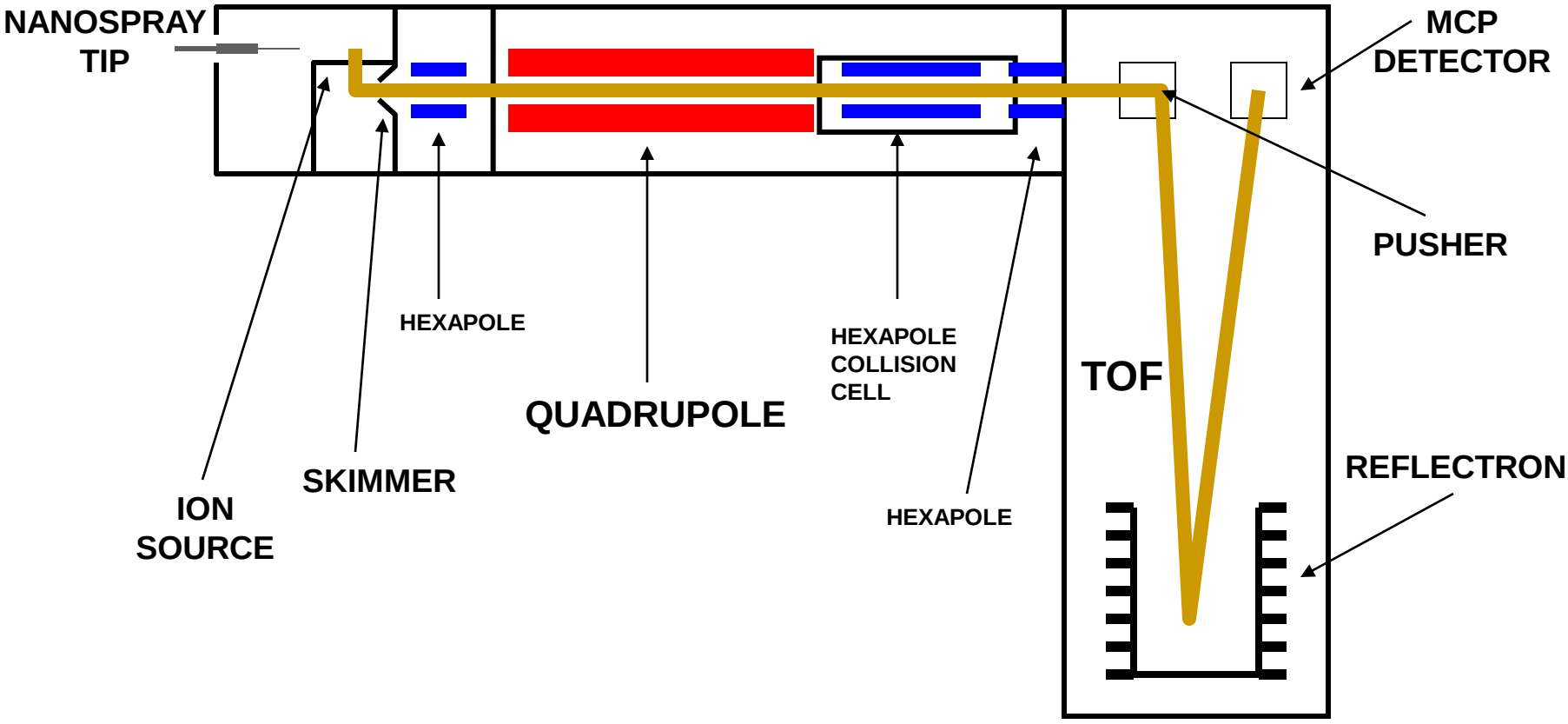
- Low Resolution (4000)
- Low Accuracy (>200ppm)
- MS/MS not possible

Linear Time-of-Flight (TOF)



http://www.kore.co.uk/graphics/MS-200_tof.gif

Q-TOF Mass Analyzer



Reflectron Time-of-Flight (TOF)

Advantages

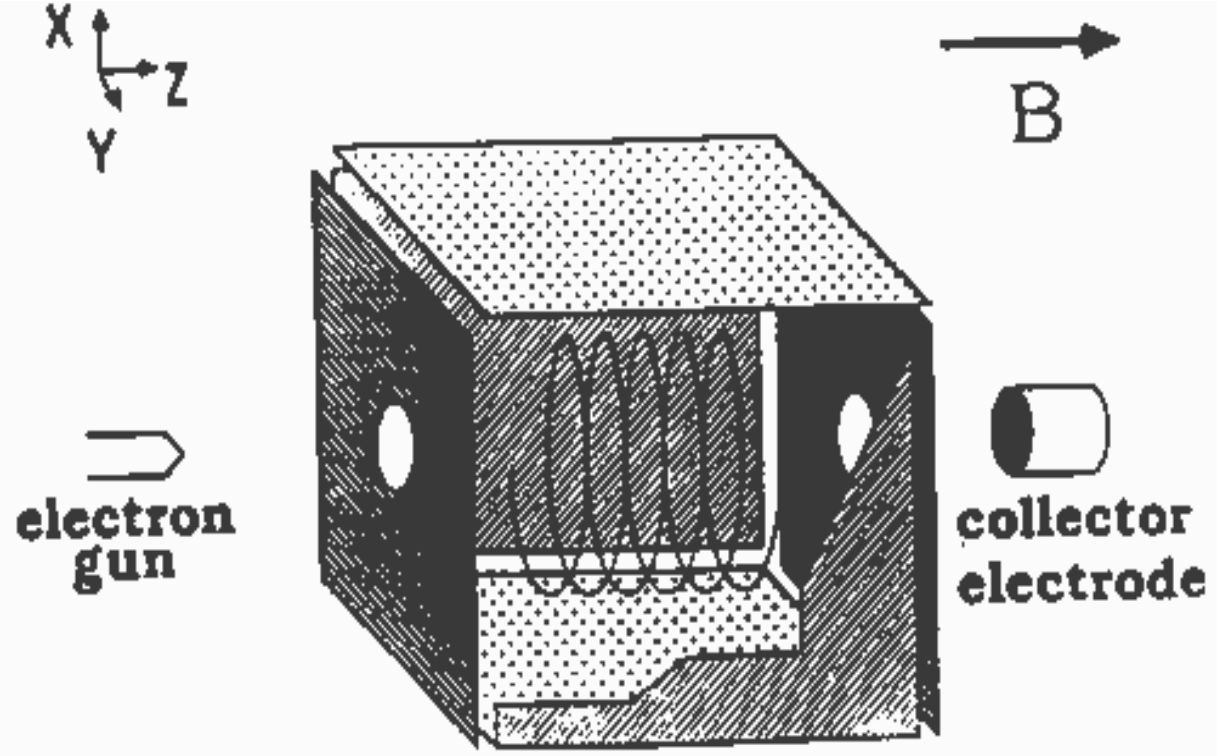
- High Resolution (>20,000 in some models)
- High Accuracy (<5ppm)
- 10,000 Mass Range
- Fast Scanning

Disadvantages

- Low Resolution for MS/MS (PSD)

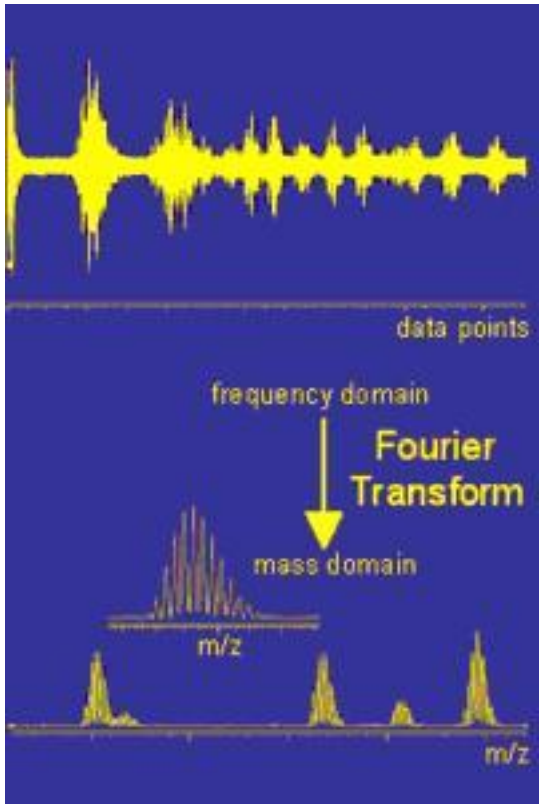
FT-ICR-MS
Analyzer Cell

- Trapping plates left and right
- Excitation plates front and back
- Detection plates top and bottom



Reproduced from Asamoto, B. *FT-ICR/MS;* 1991.

FT-Ion Cyclotron Analzyer



FT-ICR-MS

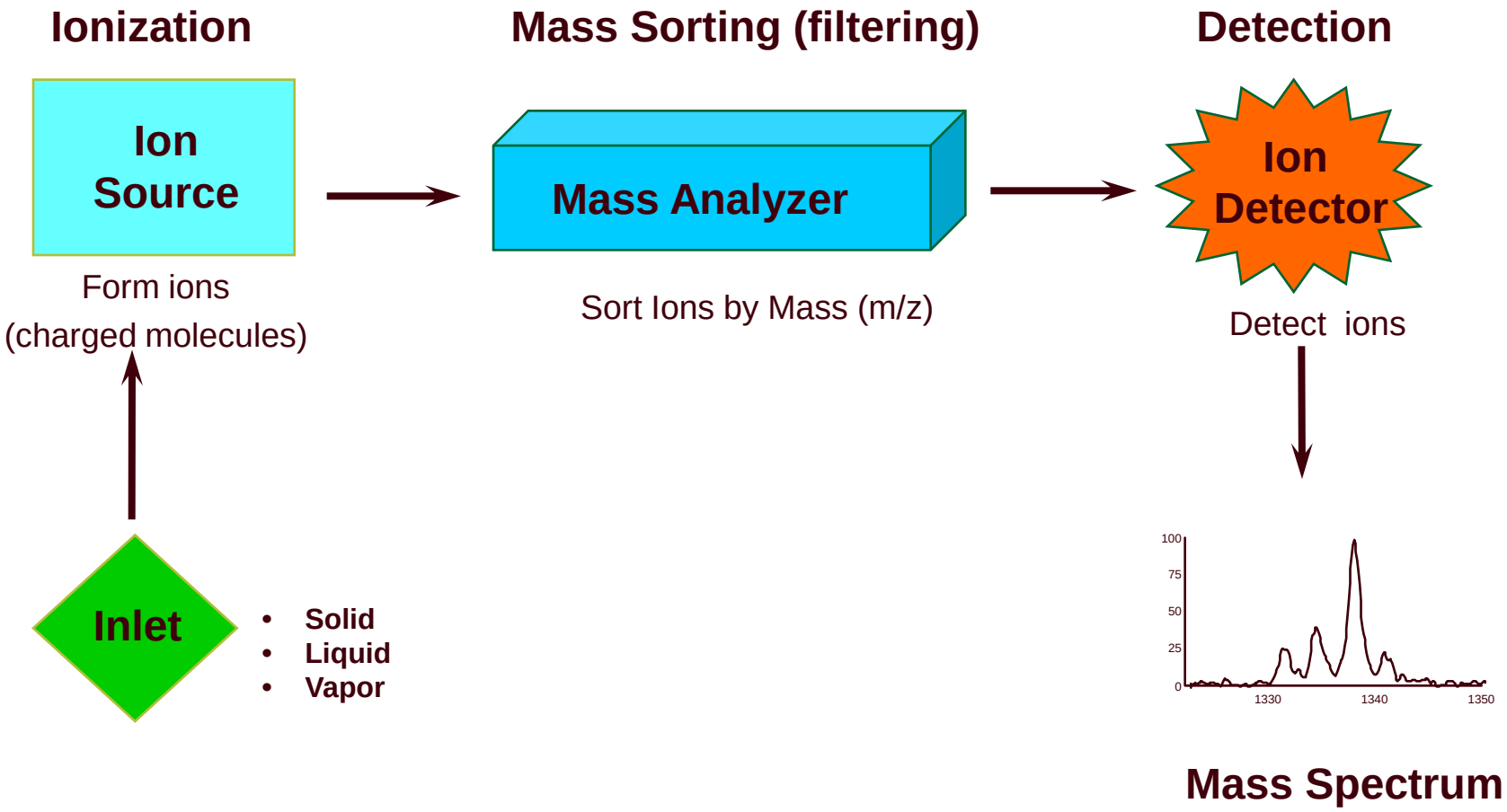
Advantages

- Extremely High Resolution (>500,000)
- Very Good Accuracy (<1 ppm)
- MS/MS in one analyzer

Disadvantages

- Expensive
- Requires Superconducting Magnet
- Slow MS/MS

Summary: acquiring a mass spectrum



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 ??

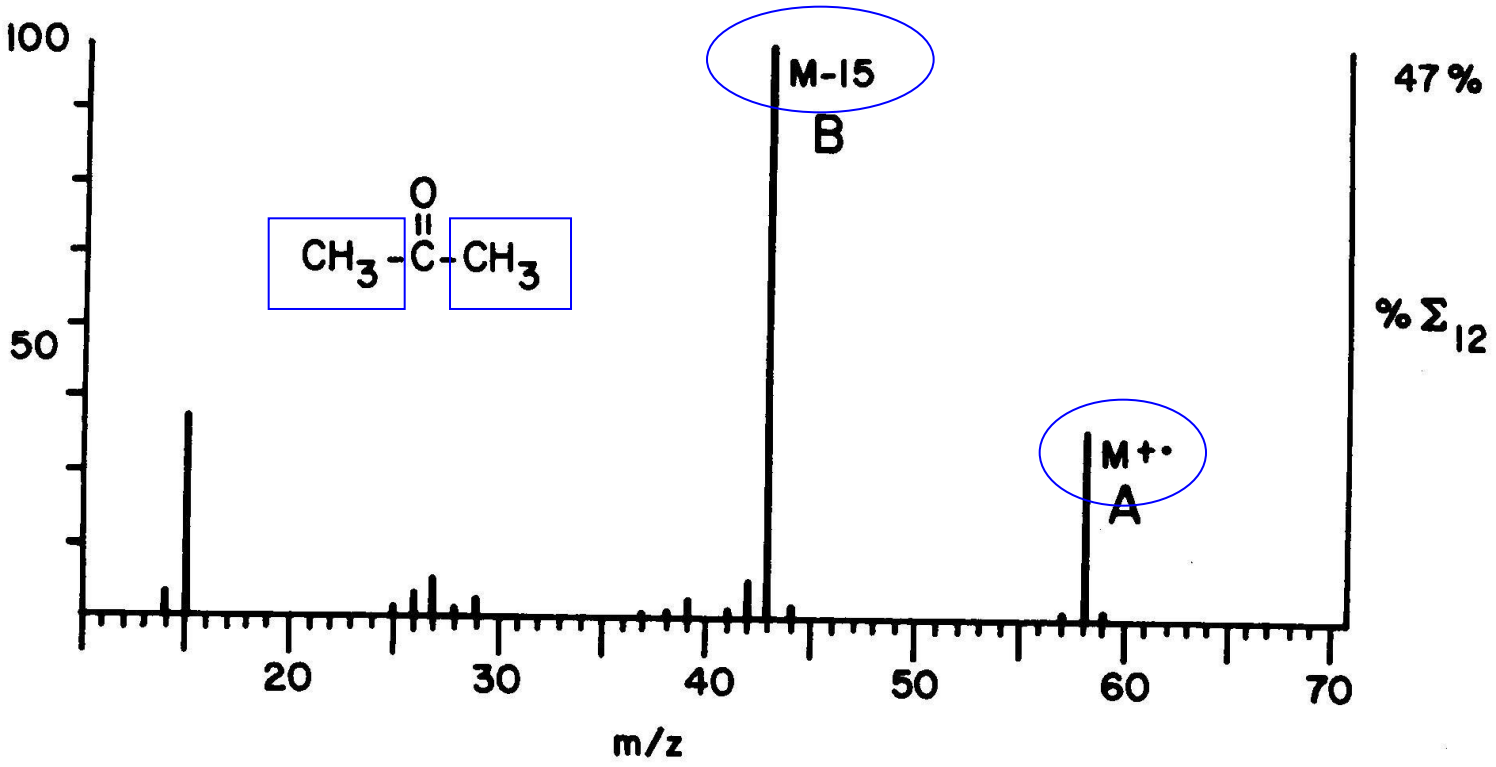


FIG. 1.6. Bar-graph format for the mass spectrum of acetone.

- 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

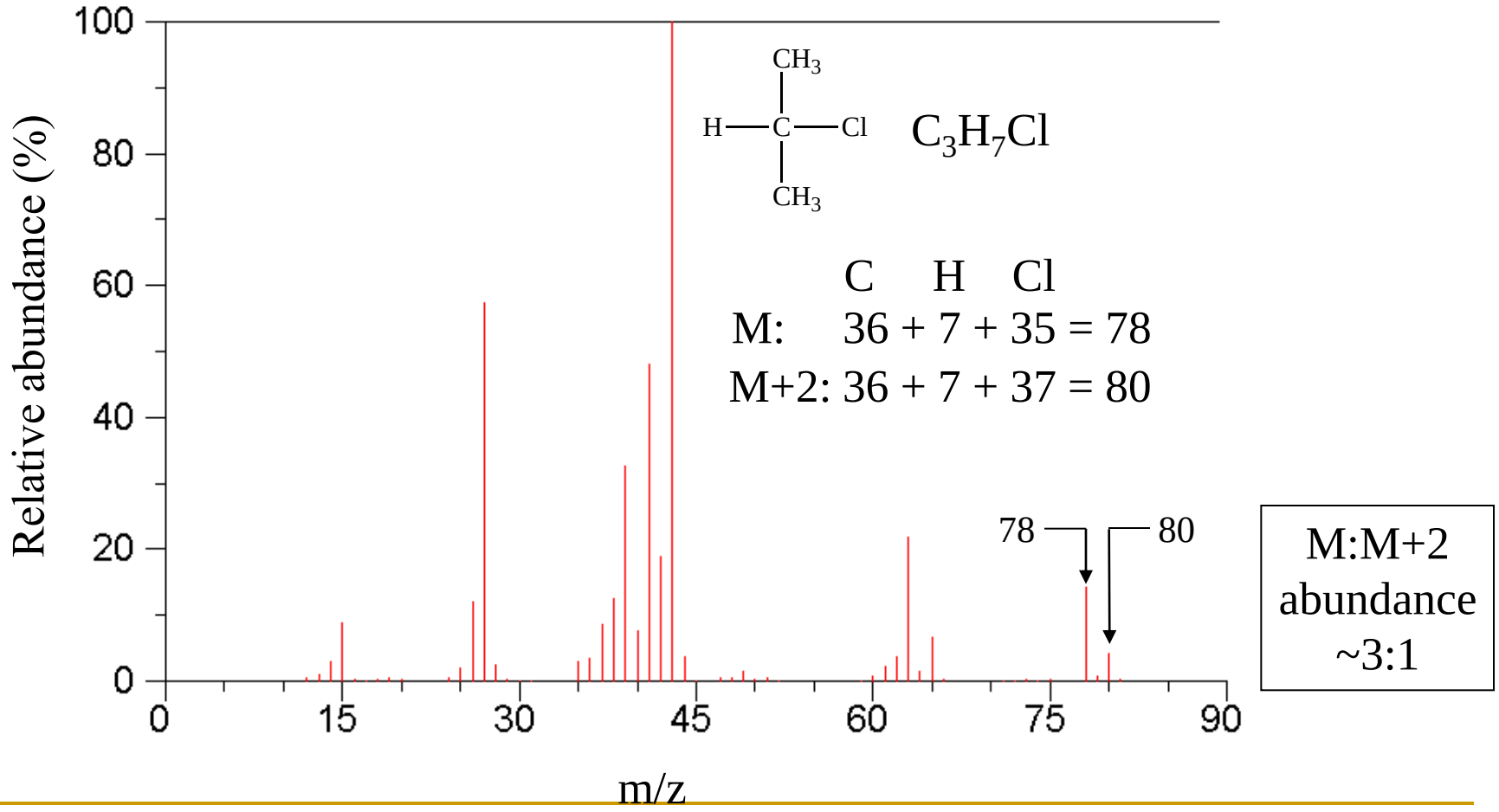
Anything useful from intensity of M+2?

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*

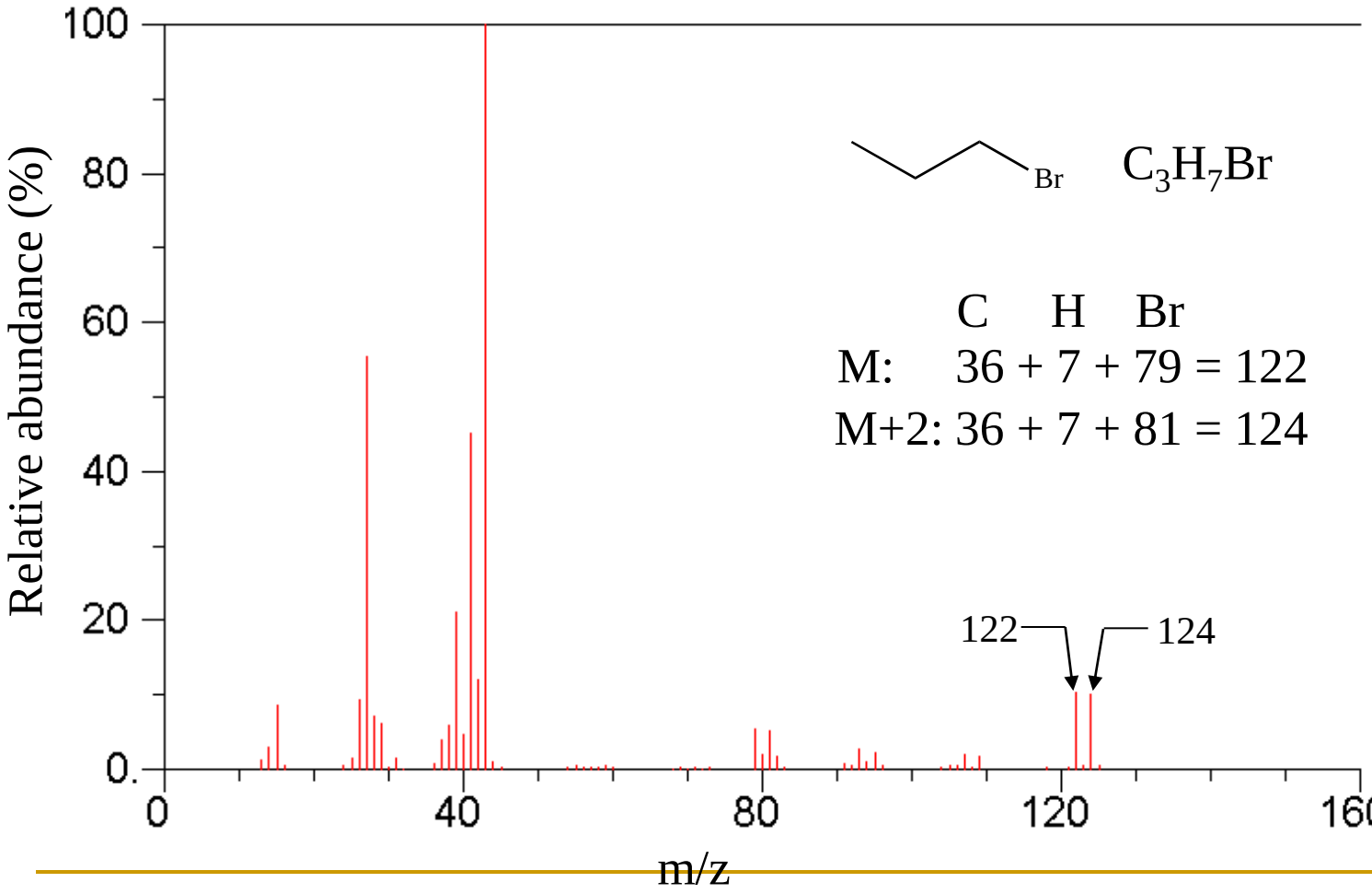
Formula from Mass Spectrum

M+2 Contributors



Formula from Mass Spectrum

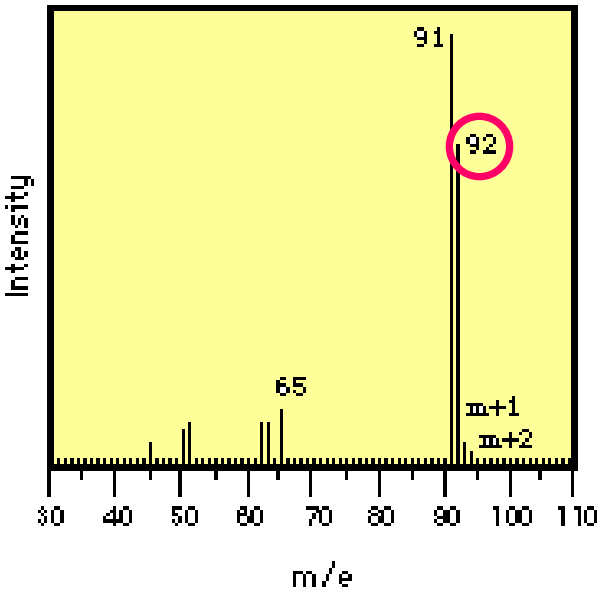
M+2 Contributors

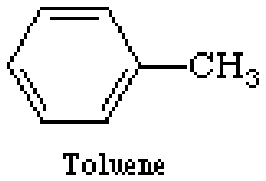


Molecular Ion

- 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 ⟶ $MW=M^{+\cdot}$

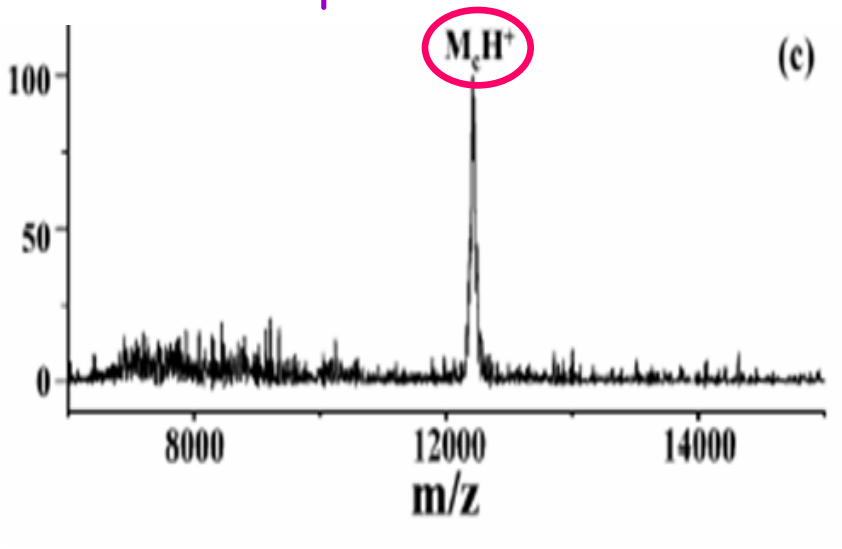




Toluene

$MH^{+}=MW+1$

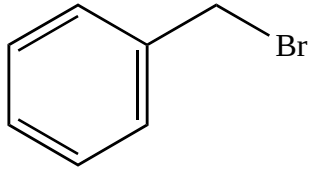
called pseudomolecular ion



Identifying the Molecular Ions

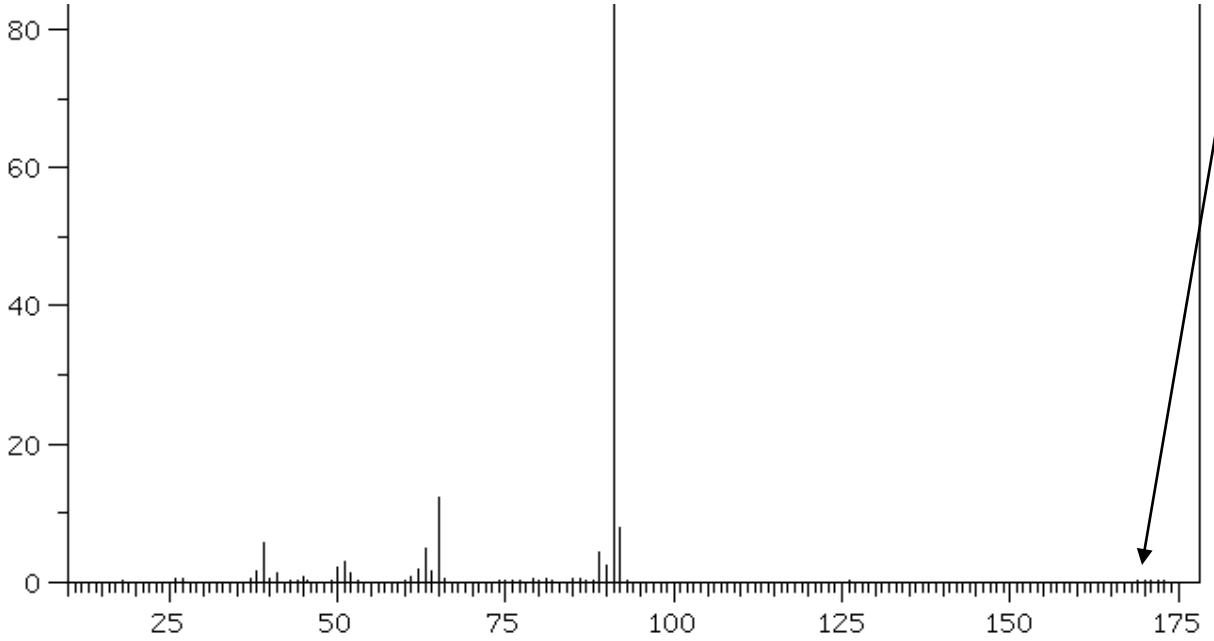
Which peaks are molecular ions?

- Highest m/z not always M
- M+1 has m/z one more than m/z of M



C_7H_7Br

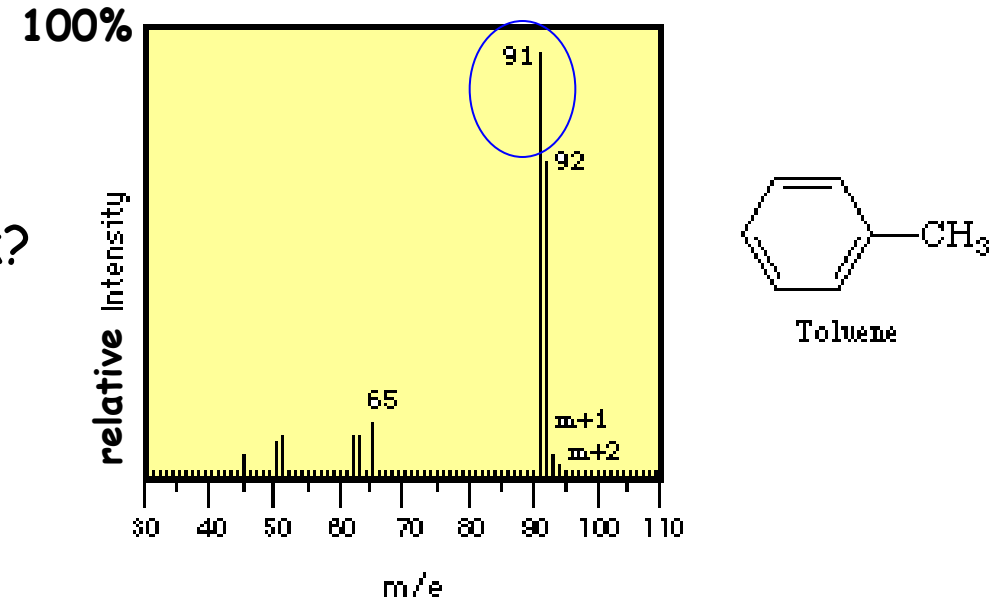
M: m/z = 170



Base peak

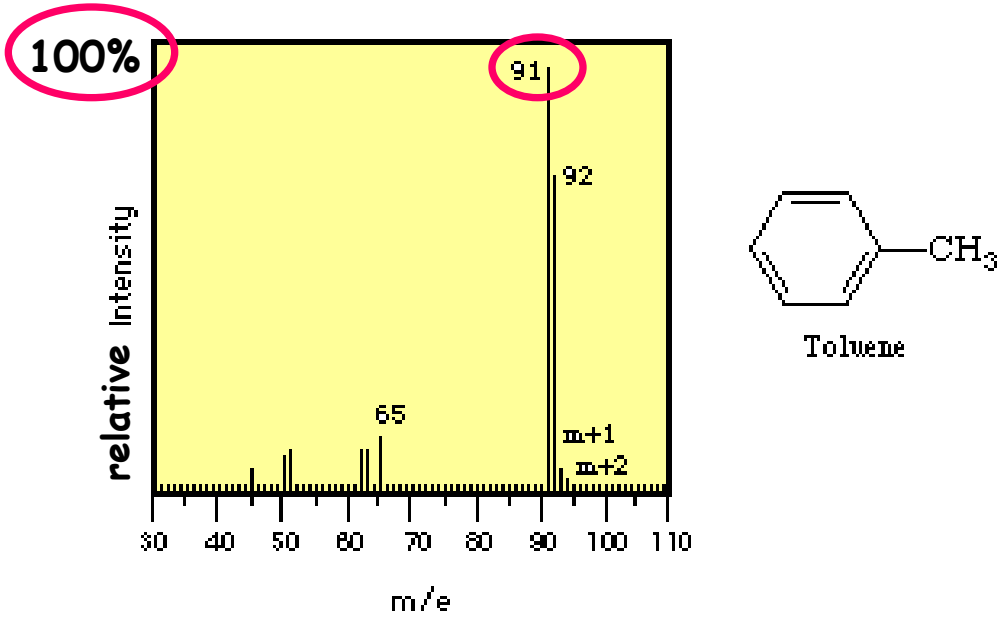
- The base peak is the most intense peak in the mass spectrum.
- It is used as the base against which the intensities of all other peaks are normalized.

Where is the base peak?



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.



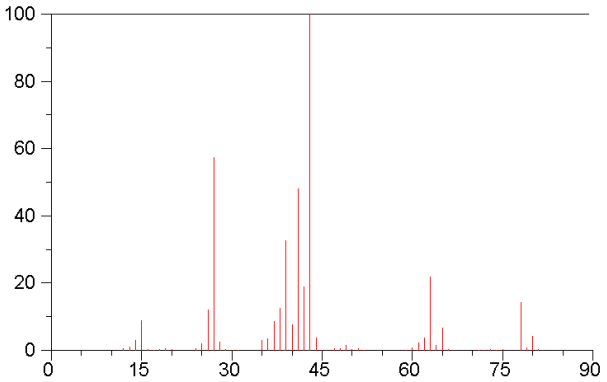
Formula from Mass Spectrum

Summary of Information from Mass Spectrum

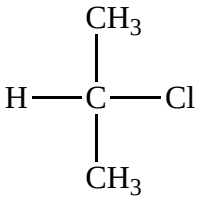
M: Reveals mass of molecule composed of lowest mass isotopes
M+1: Intensity of M+1 / 1.1% = number of carbons
M+2: Intensity reveals presence of sulfur, chlorine, and bromine

Mass Spectrum → Formula → Structure

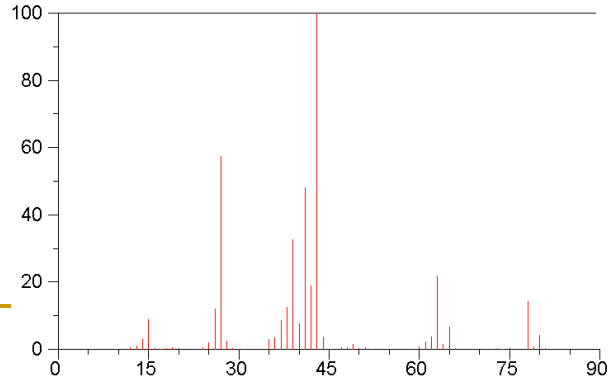
How do we derive structure from the mass spectrum?



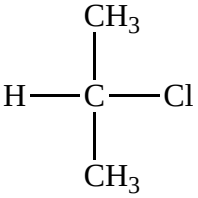
?



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



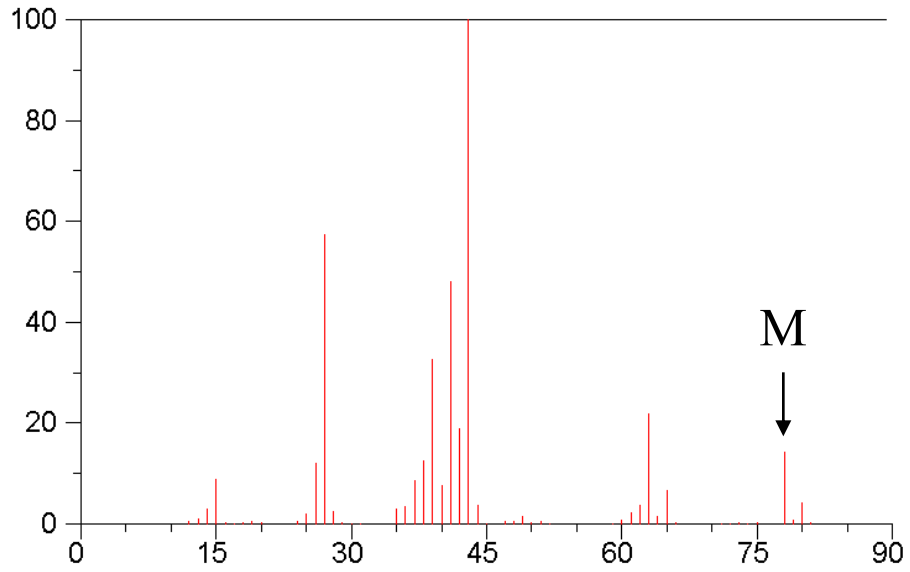
→ C_3H_7Cl →



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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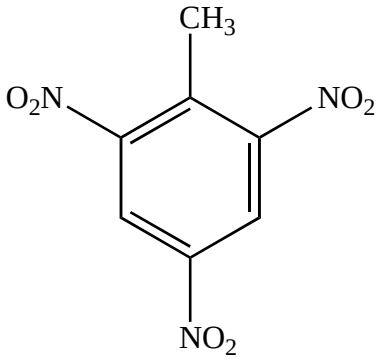
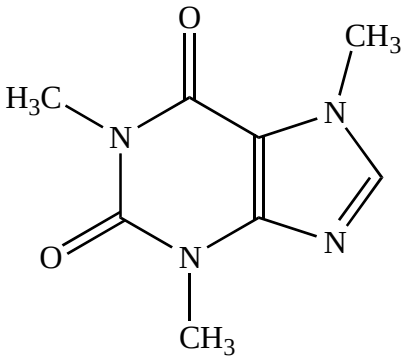
How Many Nitrogen Atoms?

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

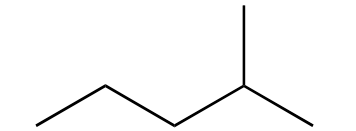
C_3H_7Cl ← even nitrogen count

discarded ~~C_5H_4N~~ ← odd nitrogen count

C_6H_6 ← even nitrogen count

94

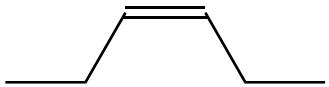
How Many Hydrogen Atoms?



C_6H_{14}

max H for 6 C

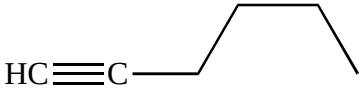
One pi bond



C_6H_{12}

H count = max - 2

Two pi bonds



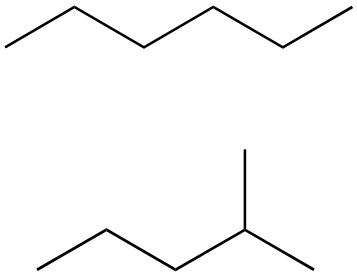
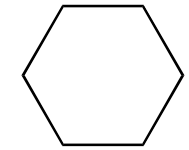
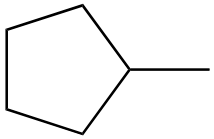
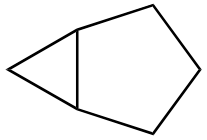
C_6H_{10}

H count = max - 4

Conclusion: Each pi bond reduces max hydrogen count by two

95

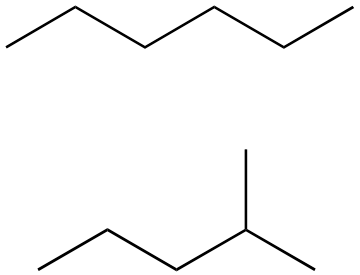
How Many Hydrogen Atoms?

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

Conclusion: Each ring reduces max hydrogen count by two

96

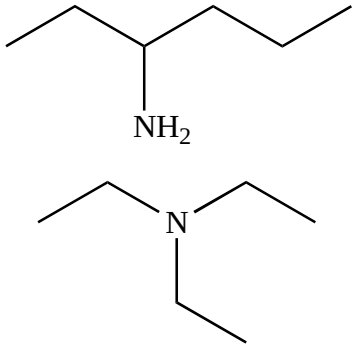
How Many Hydrogen Atoms?



C_6H_{14}

max H for 6 C

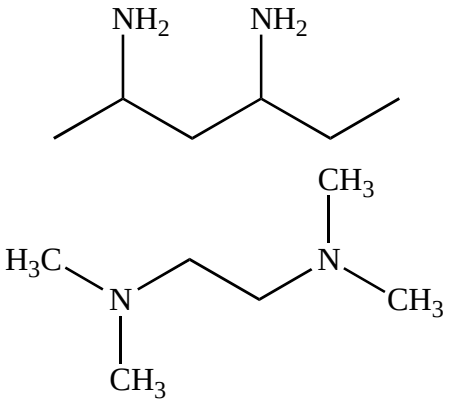
One nitrogen



$C_6H_{15}N$

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

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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

100

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

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Important Performance Factors in Mass Spectrometry

Mass accuracy:

How accurate is the weight measurement?

$$(M_{ave} - M)/M_{ave}, (ppm\ (1/10^6), \%)$$

$$1\ ppm = 10^{-4}\%$$

Resolution: **How well separated are the peaks from each other? M/△M**

Sensitivity: **How small an amount can be analyzed?**

Mass Resolution

- low resolution: unit resolution in the mass range of interest, i.e., a resolution of 100-1000.
  - Unit resolution means that two adjacent peaks in a mass spectrum are resolved sufficiently (with a 10-20% valley).

Unit resolution
(M+1)-M=1
M/1=M

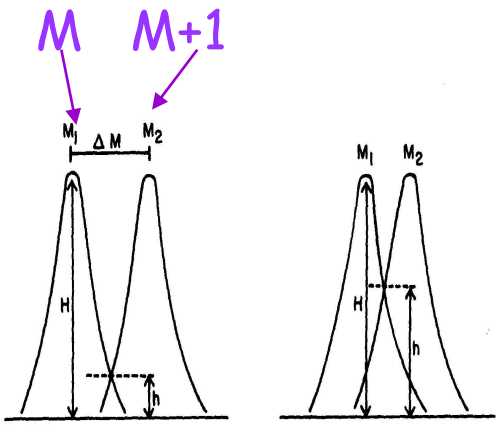


FIG. 1.4. Graphic representation of unit resolution with 15% valley (h/H) definition **(left)** and 50% valley definition **(right)**.

- Medium resolution: 2000-10,000
- High resolution: 10,000 or greater

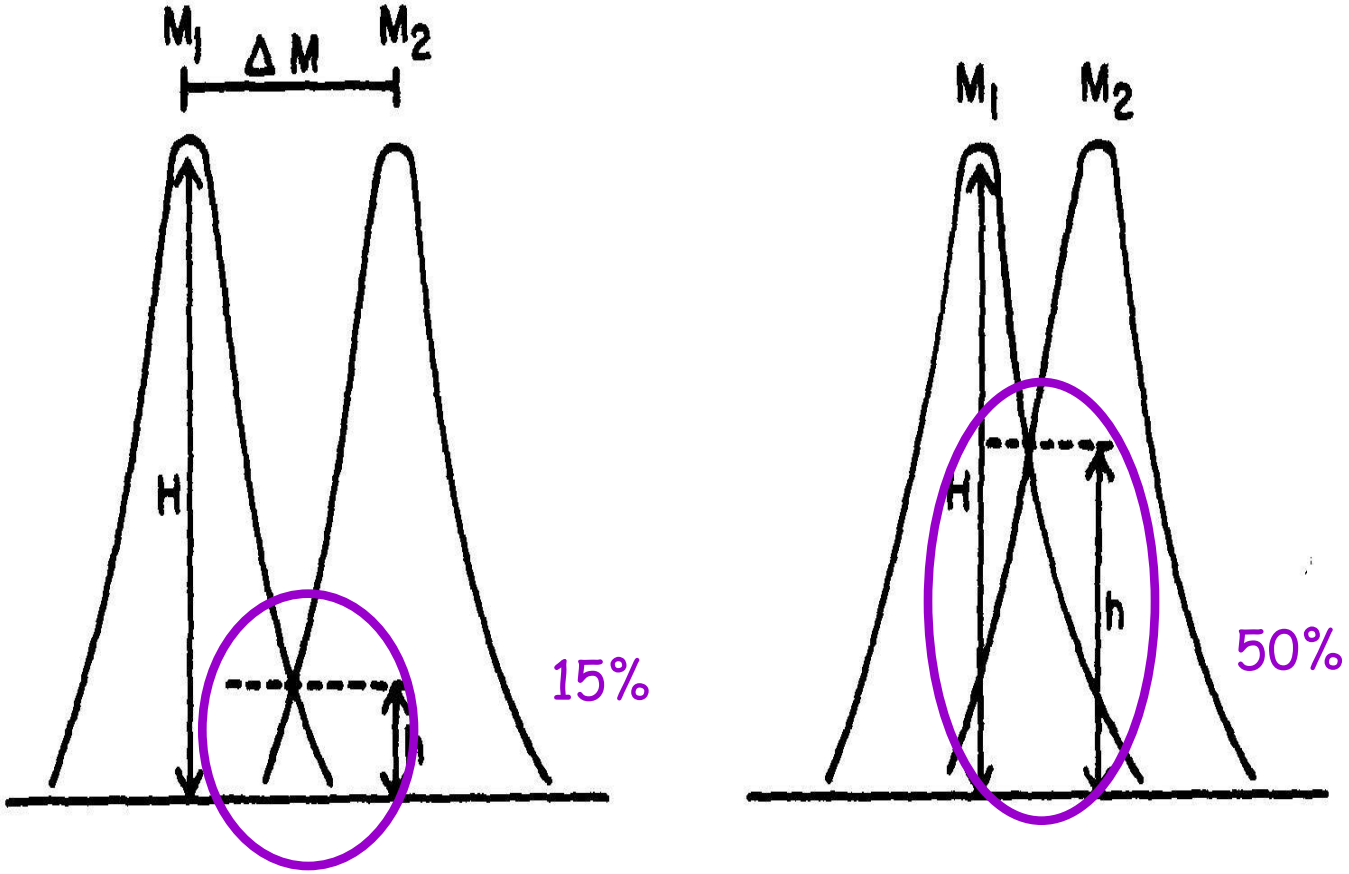


FIG. 1.4. Graphic representation of unit resolution with 15% valley (h/H) definition **(left)** and 50% valley definition **(right)**.

- Numerical expression of resolution can be obtained from the ratio of m/△m
  - m and △m are the m/z values of two adjacent peaks in the mass spectrum
    - Low resolution: △ m=1u
    - High resolution: △ m=0.01u
      - In either case, the numerical value of resolution must be qualified by some indication of the separation of the two peaks.

- Resolution can be evaluated by a peak width definition, **FWHM**

  - m : is the given m/z value of a given peak
  - △ m : the width of the peak measured at a designated peak-height level.
  - FWHM: the peak at half height, also called full width at half maximum

The resolution is 91/0.25 = 364 at m/z 91, where m =91 and △m=10/40=0.25 u

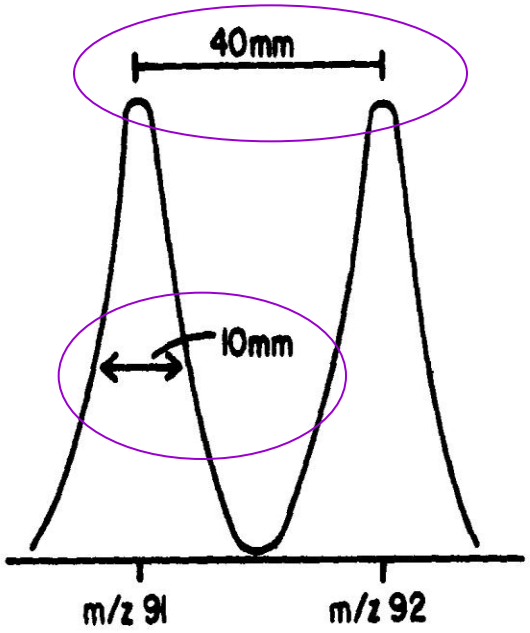
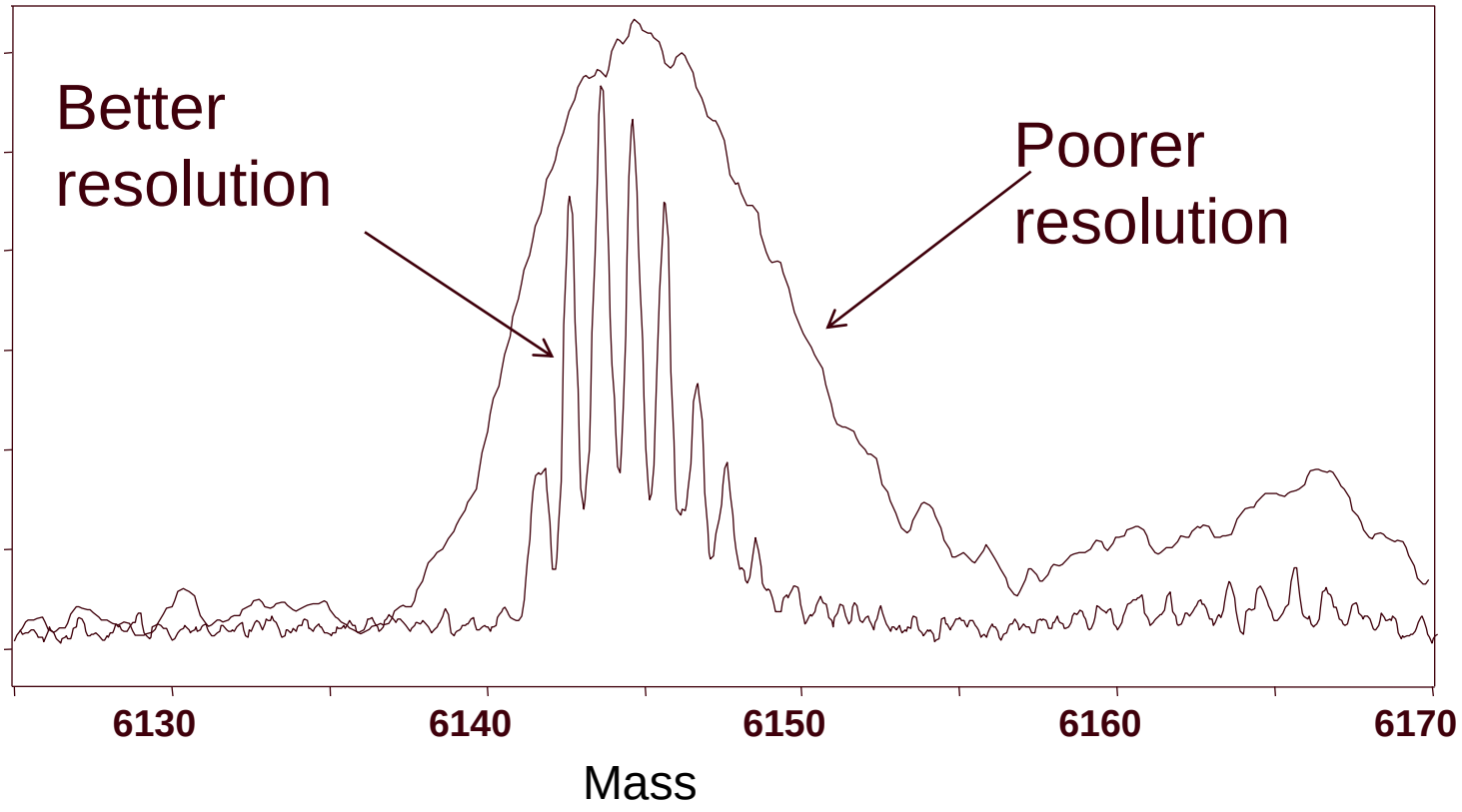


FIG. 1.5. Graphic representation of resolution using peak-width definition: full width at half maximum (FWHM). In this case, the separation of the peak centers is equivalent to four peak widths at half maximum (height).

What if the resolution is not so good?

At lower resolution, the mass measured is the average mass.



Mass measurement accuracy depends on resolution

High resolution means better mass accuracy