ENVIRONMENTAL SCIENCE AND ENGINEERING PROGRAM

Environmental Analytical Chemistry

I. Principles of Mass Spectrometry

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History

Modern mass spectrometer:
- J. J. Thomson (1906 Nobel Prize in Physics)

Higher accuracy mass spectrometers:
- F. W. Aston (1920 Nobel Prize in Chemistry), A. J. Dempster

Advances in vacuum technology and electronics:
- A. Neir

Time-of-flight analyzers:
- Wiley and McLaren 1955

Quadrupole analyzer:
- W. Paul (1989 Nobel Prize in Physics)

Electrospray ionization
- J. Fenn (2001 Nobel Prize in Chemistry), M. Dole

Matrix assisted laser desorption/ionization (MALDI)
- Tanaka (2001 Nobel Prize in Chemistry)

Mass spectrometry can be defined as an instrumental approach that allows for the mass measurement of molecules in very low quantities (as low as 100 * 10^{-18} moles).

The five basic parts of any mass spectrometer are:

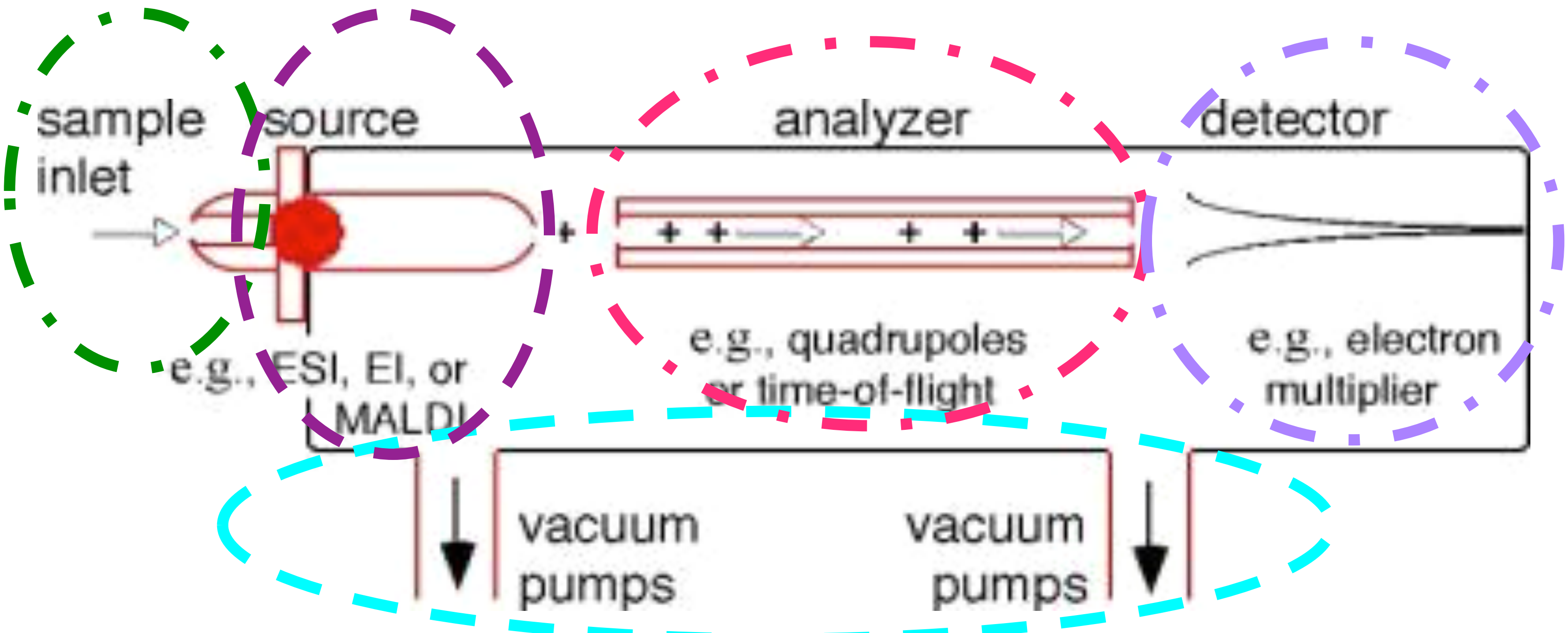
1) A vacuum system.

2) A sample introduction device.

3) An ionization system ($A \Rightarrow A^{+}$).

4) A mass analyzer (m/z).

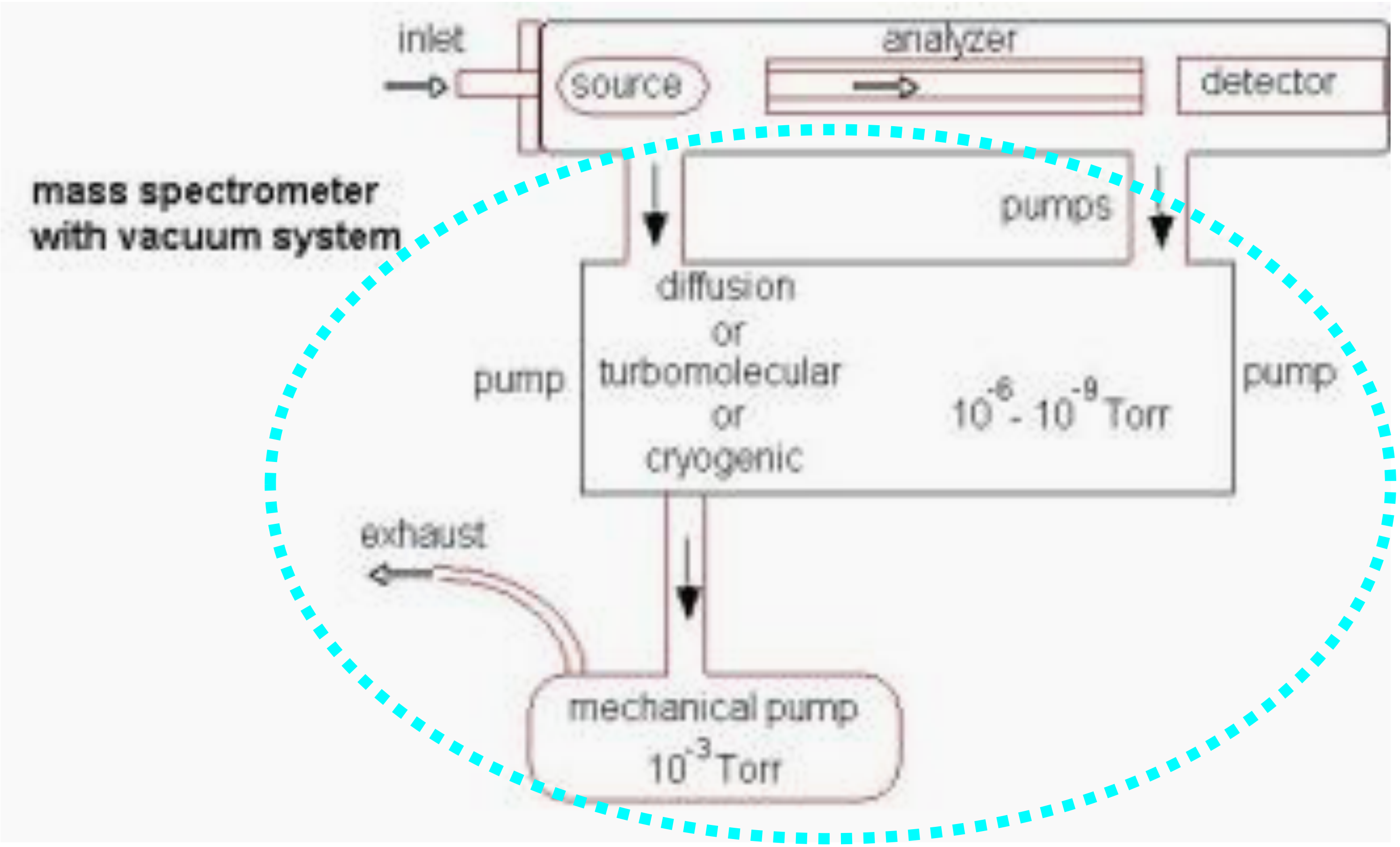
5) An ion detector.



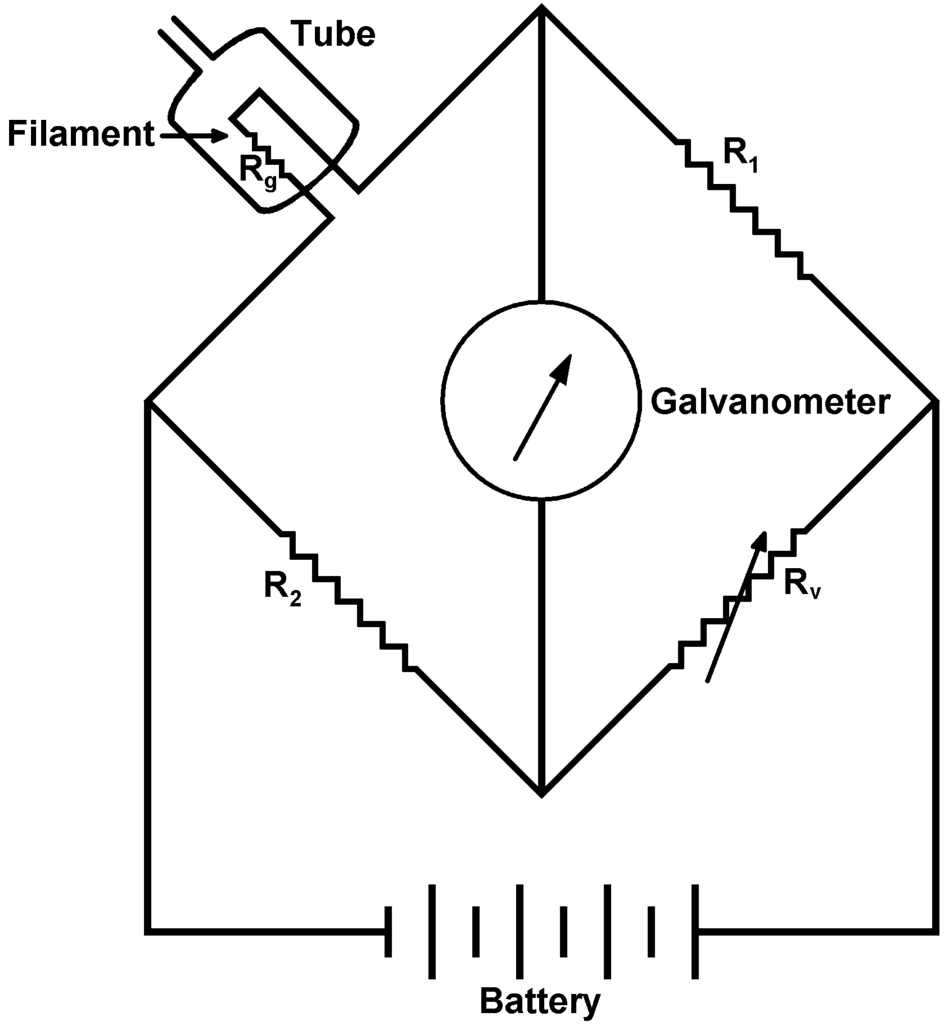
Theory and Practice of Mass Spectrometry

1. Vacuum System

Mass spectrometry requires a low pressure to operate.

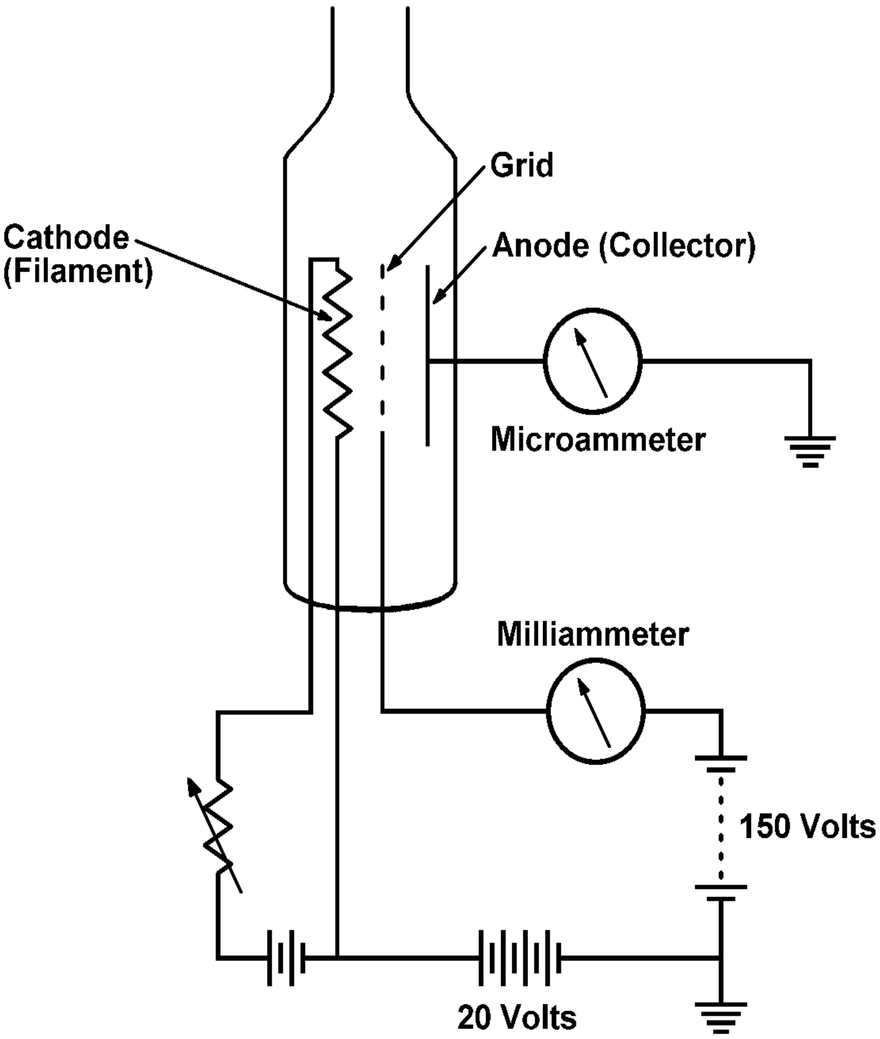


Pirani or Convectron™ Gauge



- Low vacuum measurement
- Operates on principle of the Wheatstone bridge (R_g is a thermistor)
- It uses the thermal conductivity of gases to measure pressure.
- The system is pumped down: there are less molecules and therefore less collisions. Fewer collisions mean that less heat is removed from the wire and so it heats up. As it heats up its electrical resistance increases. A simple circuit utilising the wire detects the change in resistance and once calibrated can directly correlate the relationship between pressure and resistance.

Ion Gauge



- High vacuum measurement
- Outgasing used to dispel contaminants
- Ions are formed at the filament and attracted toward collector
- Ions striking grid generate current

Common pressure gauges

Gauge	Pressure Range	Typical Use
Manometer	760 - 1 torr	systems near atmospheric pressure
Thermocouple gauge	1 - 10^{-3} torr	monitoring mechanical pumps
Ionization gauge	10^{-3} - 10^{-9} torr	high-vacuum systems

Common vacuum pumps

Pump	Lowest Attainable Pressure	Typical Use
Mechanical pump	10^{-3} - 10^{-4} torr	roughing or backing pump
Diffusion pump	10^{-6} torr	vacuum lines
Turbomolecular pump	10^{-9} torr	high-vacuum systems

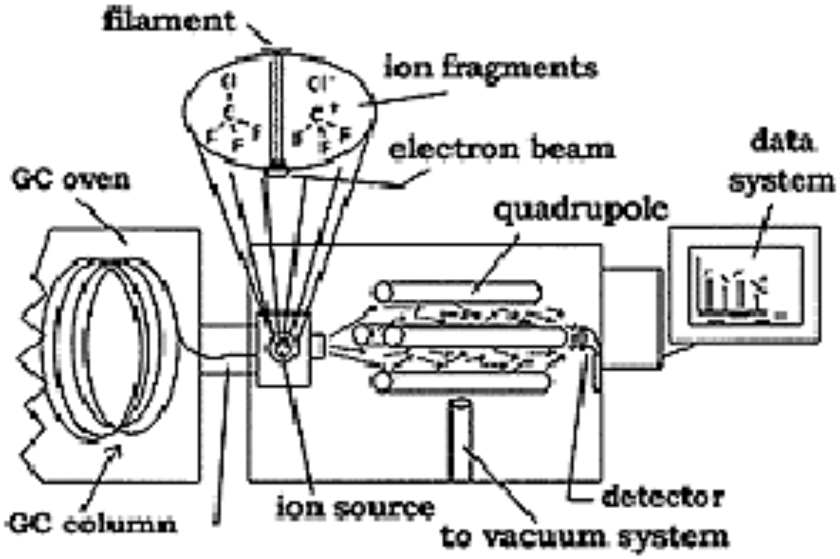
Sample Introduction Device

The sample inlet is the interface between the sample and the mass spectrometer

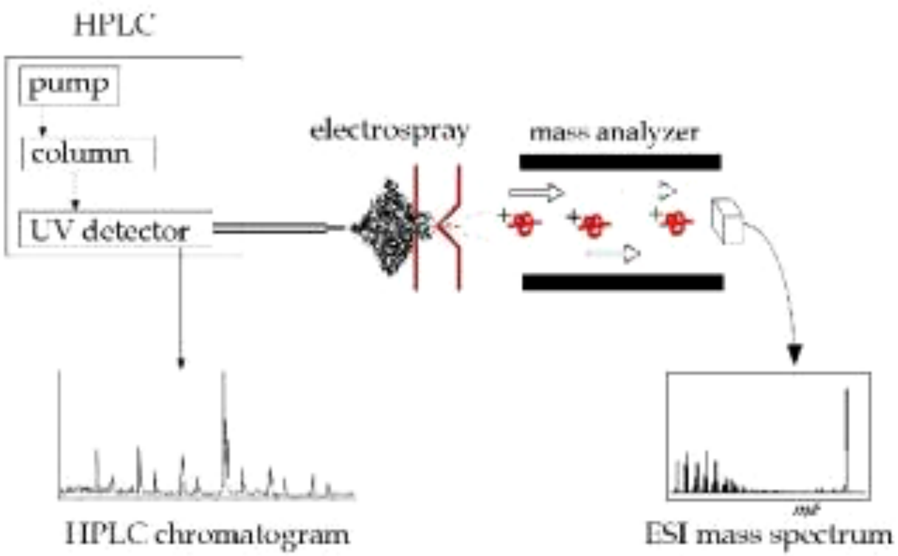
To introduce <u>pure compounds</u>:
samples are placed on a probe which is then inserted through a vacuum lock into the ionization source

Capillaries are used to interface the ionization source with other separation techniques:

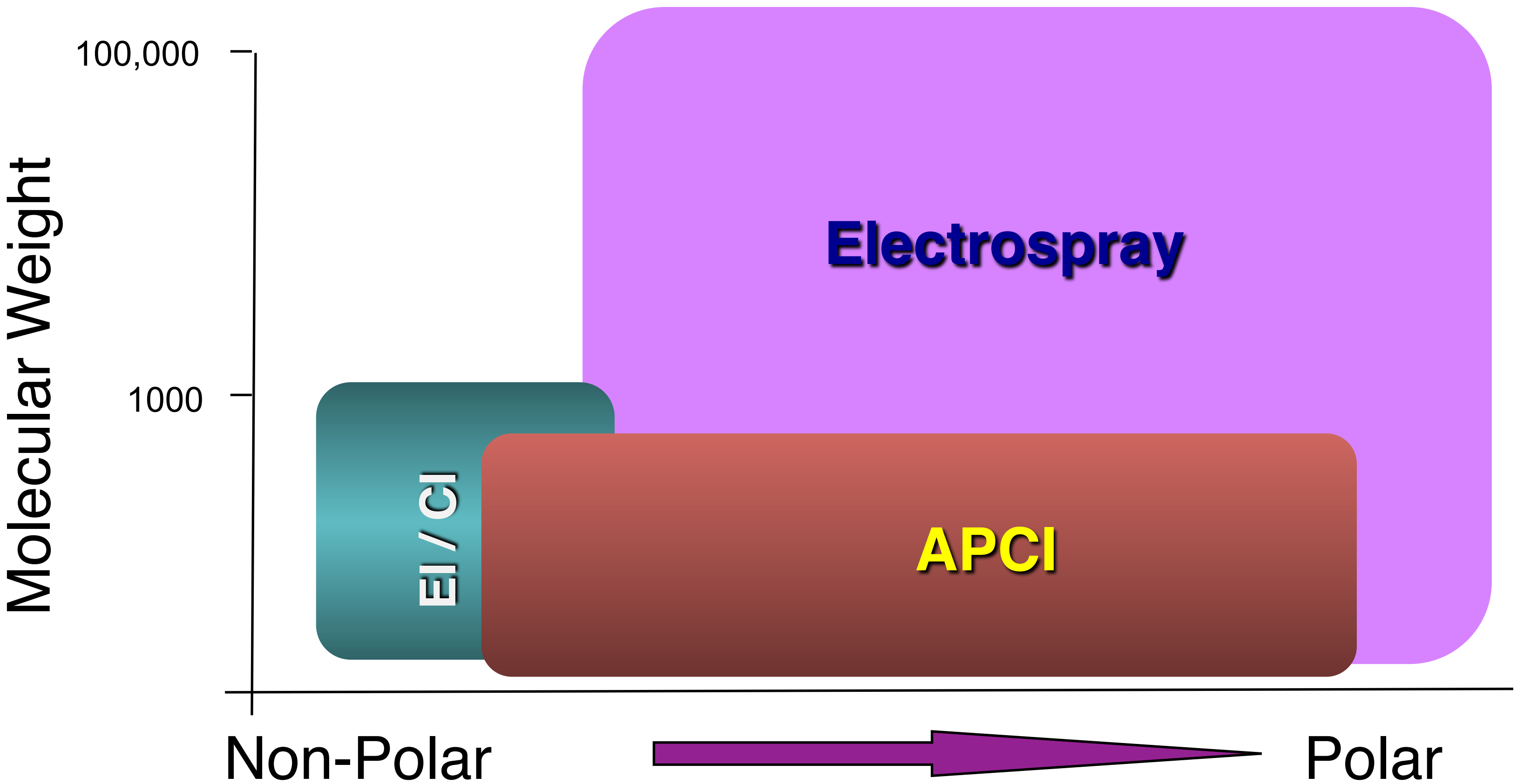
Gas chromatography (GC)



Liquid chromatography (LC)



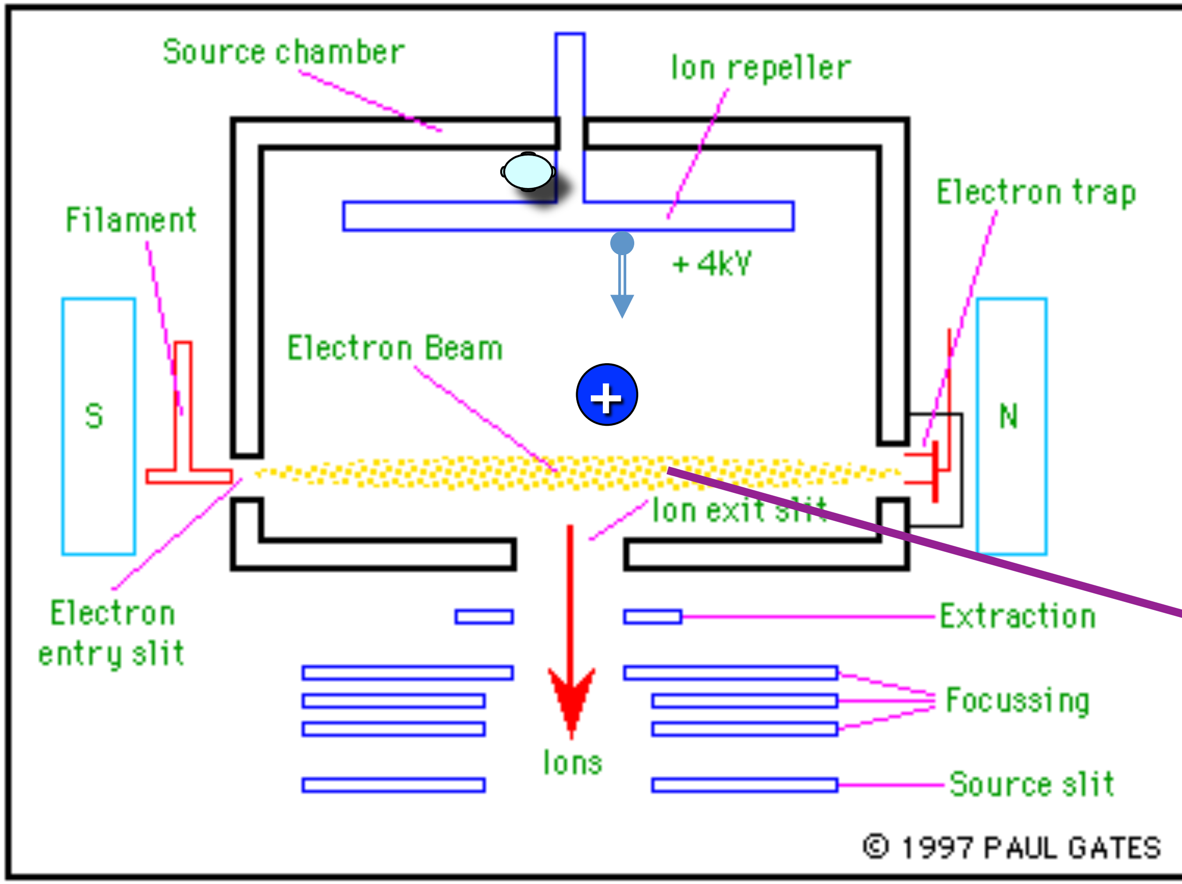
Which Ionisation Mode ?



Ionization Techniques

Electron Ionization – Electron Impact (EI)

Electrons are produced by <u>thermionic emission</u> from a tungsten or rhenium filament (filament current ca. $1*10^{-4}$ Amps).



e^- energy: 70 eV

Mechanisms of ion formation in EI sources

High abundance of ions

$$AB + e^{-*} \text{-----}> A^{+} + B^{-} + e^{-}$$

$$AB + e^{-*} \text{-----}> A^{+} + B^{\circ} + 2e^{-}$$

Low abundance: Molecular ion Formation

$$AB + e^{-*} \text{-----}> [AB^{+\circ*}] + 2e^{-}$$

and $$[AB^{+\circ*}] \text{-----}> AB^{+\circ}$$

Very low abundance but possible

$$AB + e^{-*} \text{-----}> [AB^{2+*}]'' + 3e^{-}$$

and

$$[AB^{2+*}]'' \text{-----}> A^{+} + B^{+}$$

'Self chemical ionization'

$$AH + e^{-*} \text{-----}> AH^{*} + e^{-}$$

and

$$AH^{*} + AH \text{-----}> [AH+H]^{+} + A^{-}$$

Chemical ionization (CI)

A reagent gas is ionized at a pressure of 0.3-1 torr to produce a high yield of reagent ions which may be positively or negatively charged and react with the molecules to form ions which constitute the CI spectrum of the compound.

I) Acid-base reaction type:

M (molecule of sample) + XH^+ (reagent ions) --> MH^+ ("molecular ions")+ X (reagent gas)

II) Complex formation reaction type:

M (molecule of sample) + XH^+ (reagent ions) --> MXH^+ ("molecular ions")

III) Charge transfer reaction type (redox):

M (molecule of sample) + $G^{+\bullet}$ (reagent ions) --> $M^{+\bullet}$ ("molecular ions")+ G (reagent gas)

The reagent gases in positive CI

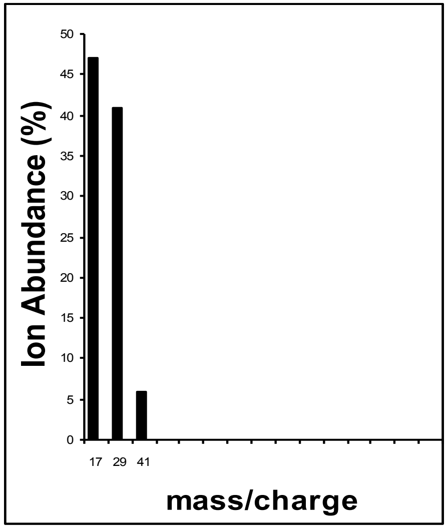
The most utilized reagent gases are methane (CH_4), ammonia (NH_3), isobutane ($i\text{-}C_4H_{10}$) and noble gases for the charge transfer reactions.

CH_4 (EI ionization) --> CH_4^+, CH_3^+, CH_2^+, CH^+, C^+

$CH_4^+ + CH_4^{+\bullet}$ --> CH_5^+ (47%) + CH_3

$CH_3^+ + CH_4$ --> $C_2H_5^+$ (41%) + H_2

$CH_4 + C_2H_3^+$ --> $C_3H_5^+$ (6%) + H_2



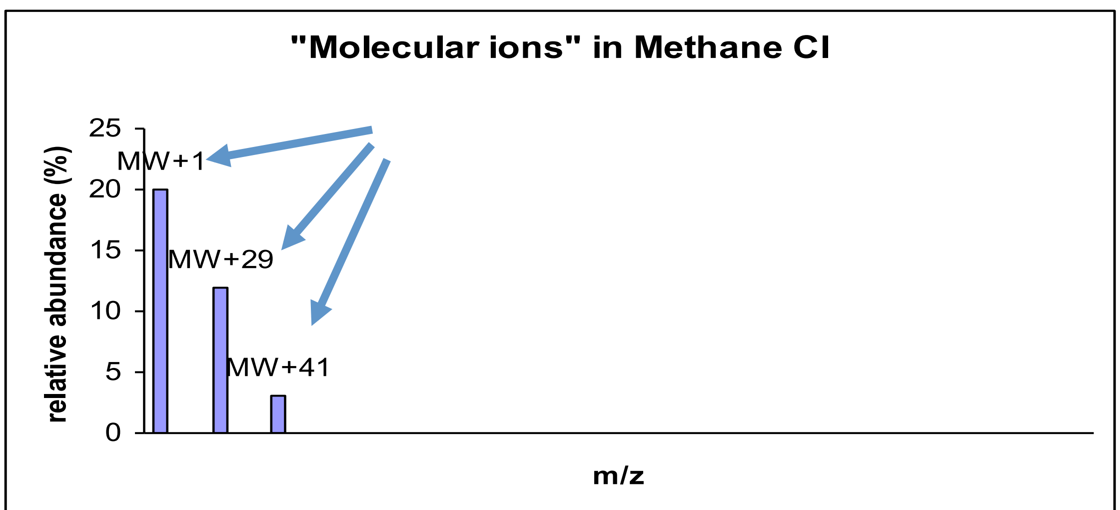
Methane chemical ionization

$CH_5^+ + M \dashrightarrow MH^+ + CH_4\uparrow$ ([m/z]+1)

$C_2H_5^+ + M \dashrightarrow MH^+ + C_2H_4\uparrow$ ([m/z]+1)

$C_2H_5^+ + M \dashrightarrow [M\ C_2H_5]^+$ ([m/z]+29)

$C_3H_5^+ + M \dashrightarrow [M\ C_3H_5]^+$ ([m/z]+41)



$M_2 - M_1 = 28$ amu
$M_3 - M_1 = 40$ amu
$M_3 - M_2 = 12$ amu
$\Downarrow$
$M_1 - 1 = MW$

Ammonia chemical ionization

NH_3 (EI ionization) --> $NH_3^{+\bullet}$

$NH_3 + NH_3^{+\bullet}$ --> $NH_4^+ + NH_2^\bullet$

$NH_3 + NH_4^+$ --> $(NH_3)_2H^+$

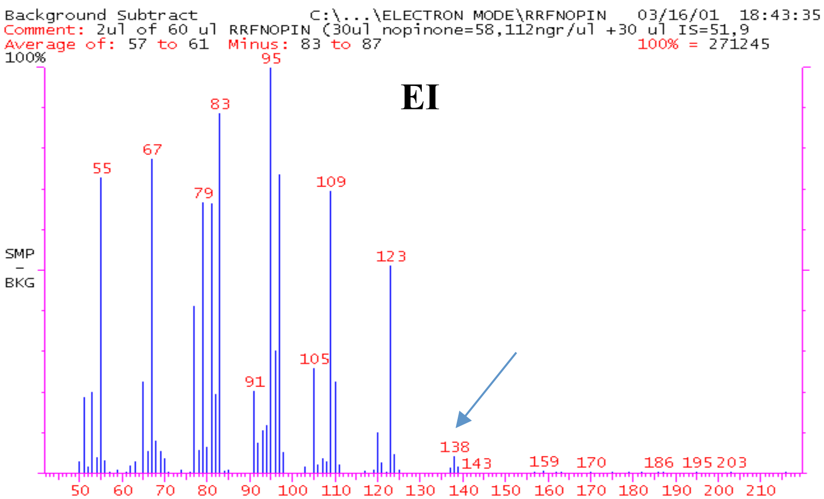
The reagent ions react with the molecules like Brønsted or like Lewis acids:

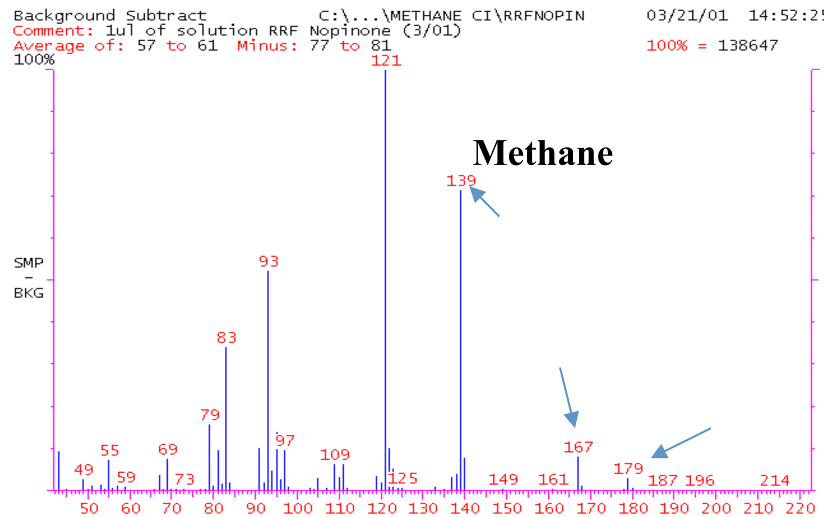
$NH_4^+ + M$ --> $MH^+ + NH_3\uparrow$ ([m/z]+1)

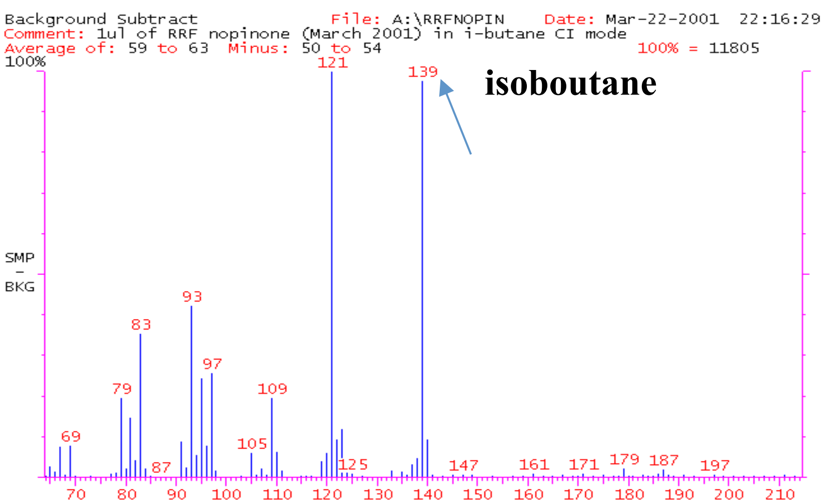
$NH_4^+ + M$ --> $[M\ NH_4]^+$ ([m/z]+18)

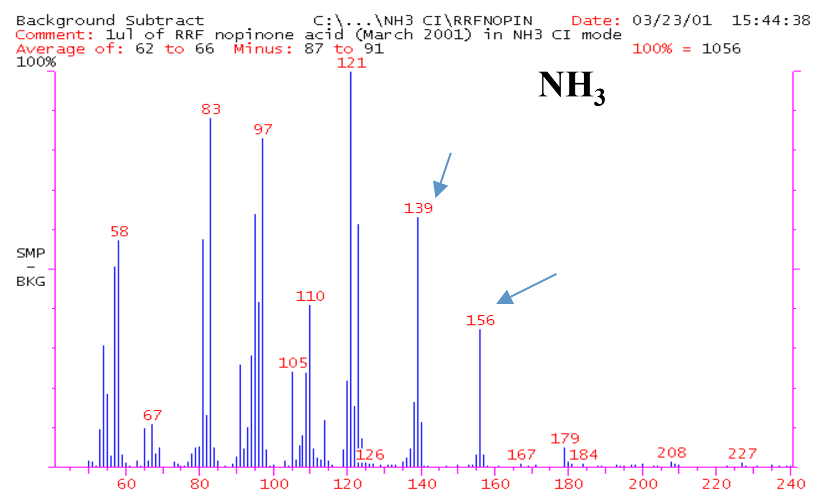
Mass Spectrometric Study in Chemical Ionisation

Nopinone (Ret. Time 4.05) MW=138

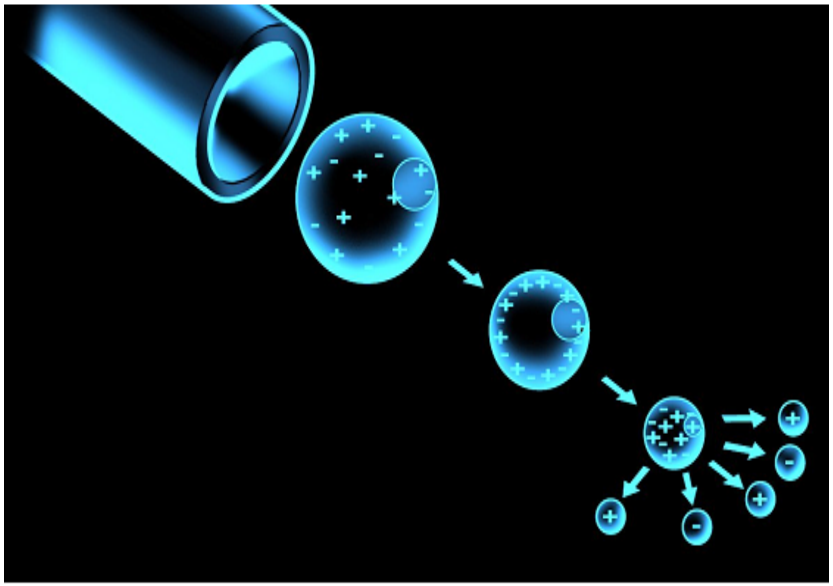




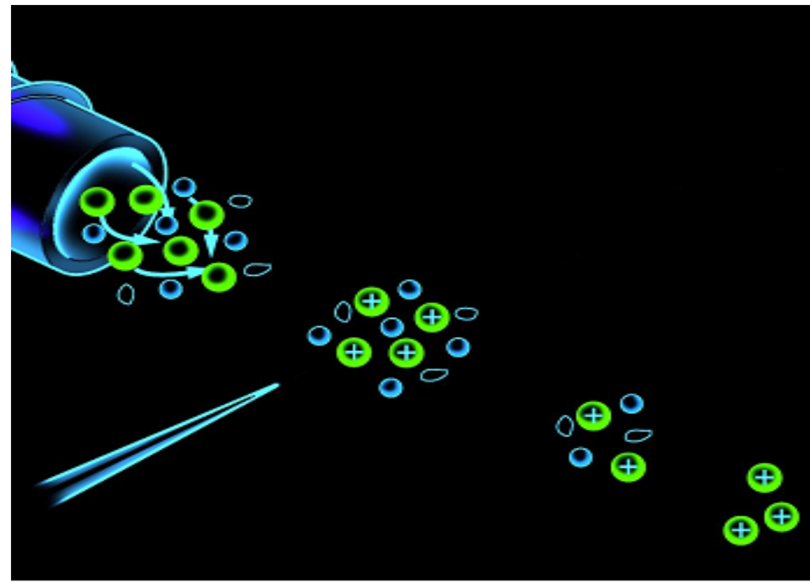




Atmospheric Pressure (API) Ion Generation



Electrospray, Microspray, Nanospray Ionisation

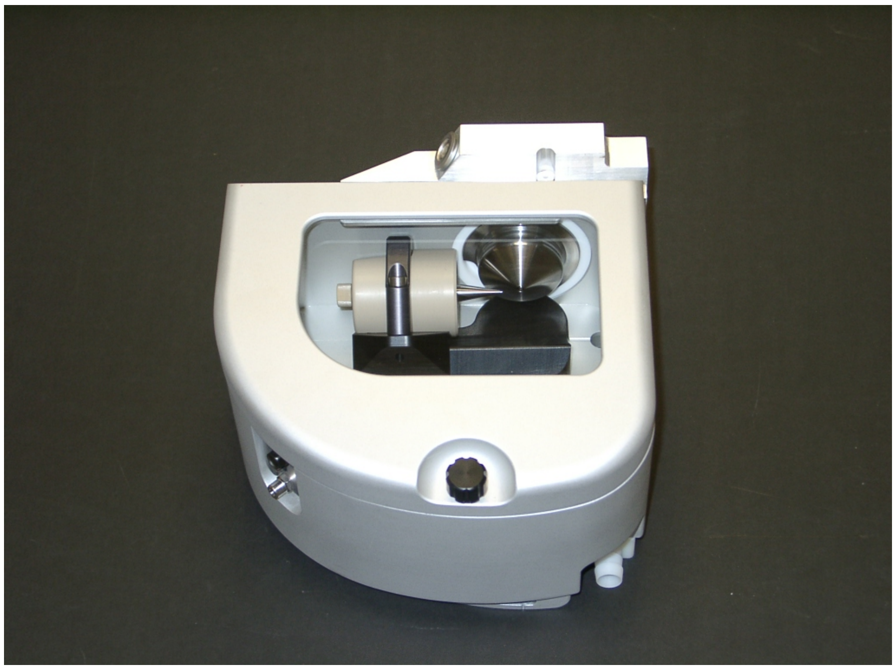


Atmospheric Pressure Chemical Ionisation

TSQ Quantum Discovery API Probes

Electrospray

APCI

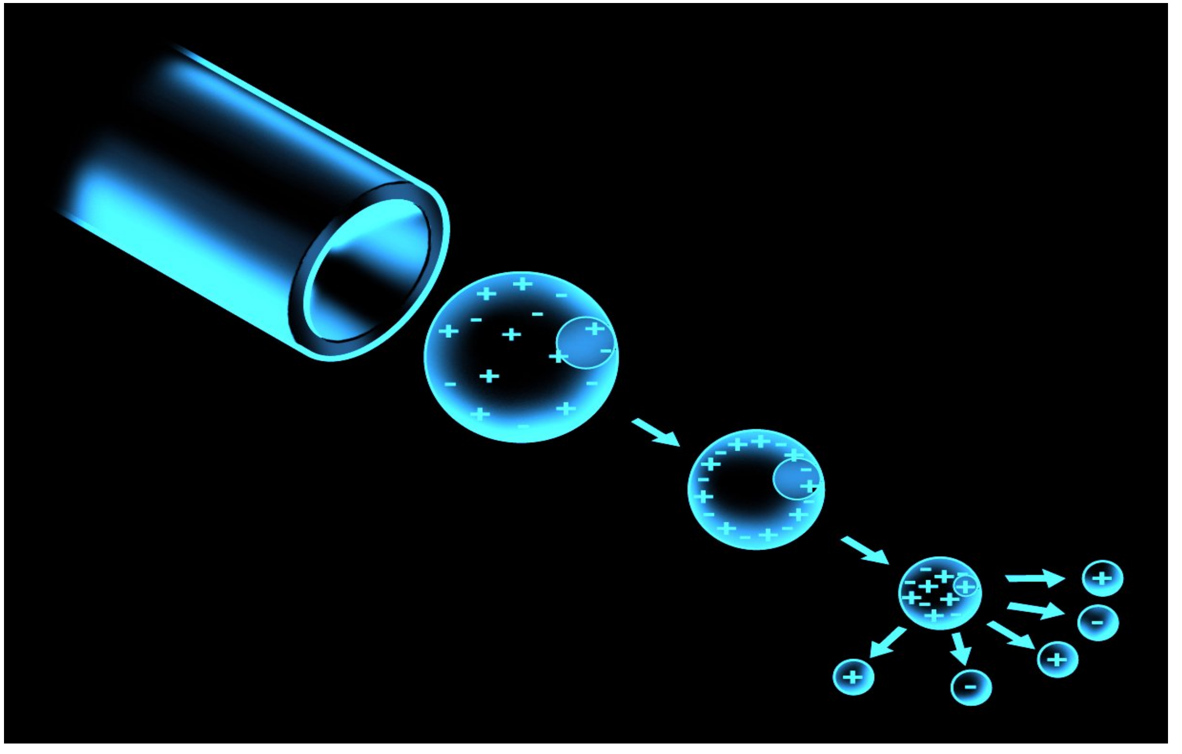


Adjustable Angle ES Source

Fixed orthogonal APCI source

Electrospray Probe

- Adjustable Angle Electrospray Geometry
- Optimal LC flow capability (50 µl/min – 800 µl/min)
- Accepts fused silica or metal needle
- Max voltage 8 kV, 100 µA

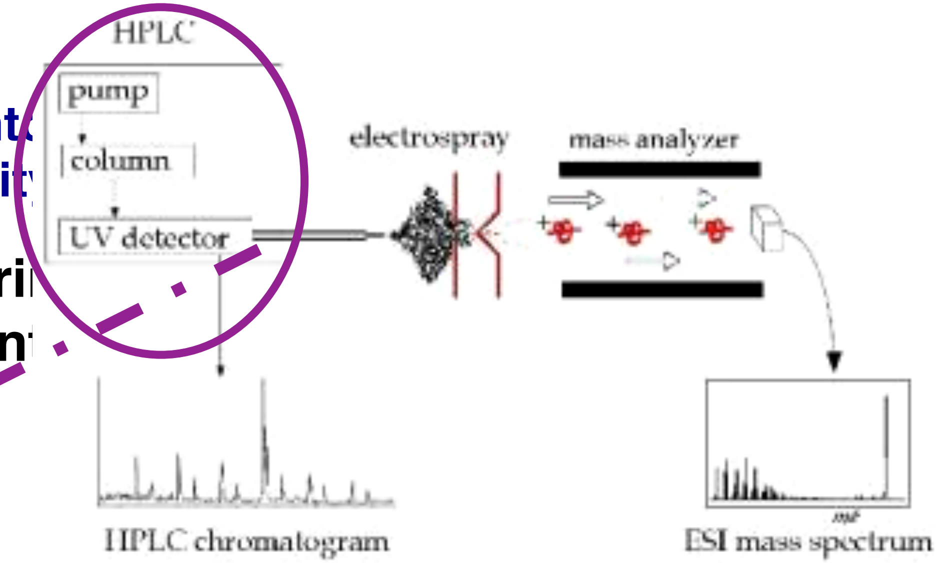


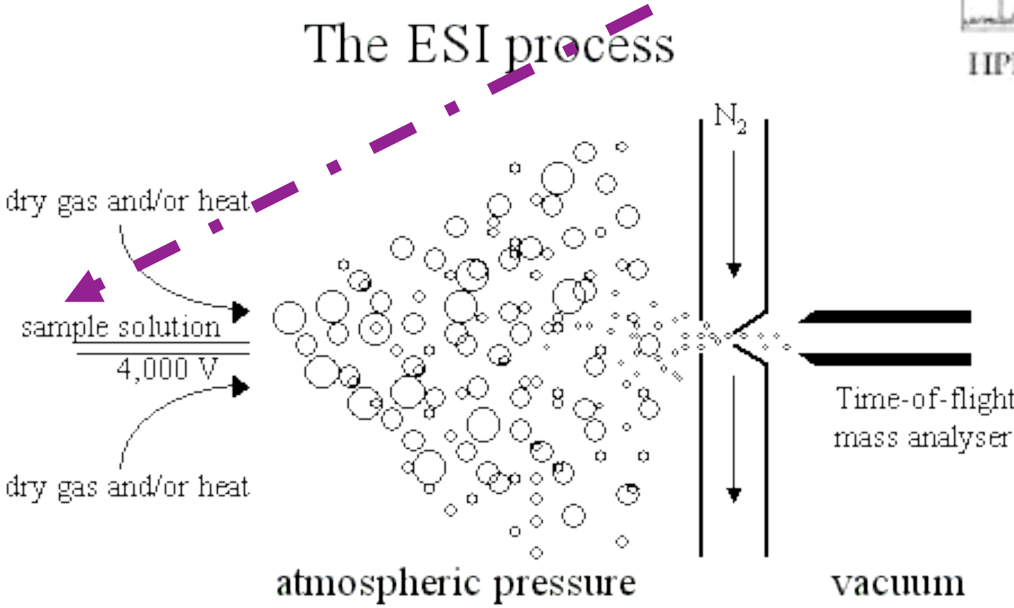
Electrospray Ionization (ESI)

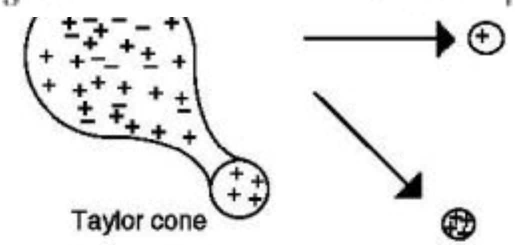
Large charged droplets are produced by forcing of the analyte solution through a needle, at the end of which is applied a potential (e.g. 4,000 V)

The emerging solution is dispersed int of charged droplets of the same polarit

The solvent evaporates away, shri and increasing the charge concent



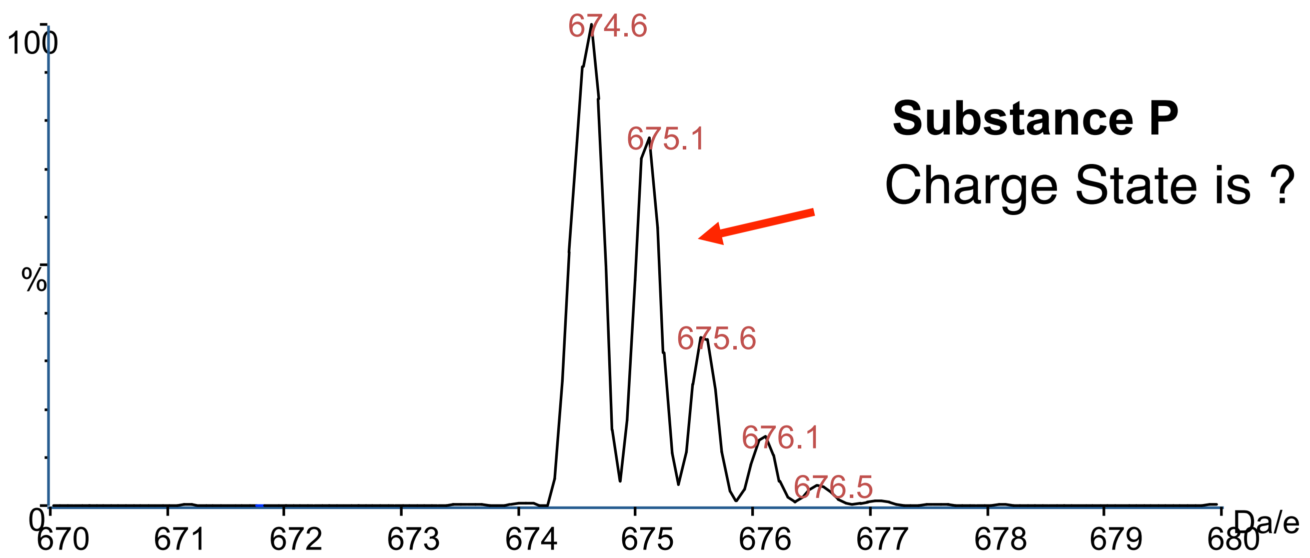




The charges are statistically distributed: formation of multiply charged ions.

Multiply Charged Ions- Mass Assignments

- Single Charge - apparent mass = (M+H)/1
- Double Charge - apparent mass = (M+2H)/2
- Triple Charge - apparent mass = (M+3H)/3
- n charges - apparent mass = (M+nH)/n



The isotopes of doubly charged ions are separated by 0.5 amu
The isotopes of triply charged ions are separated by 0.33 amu

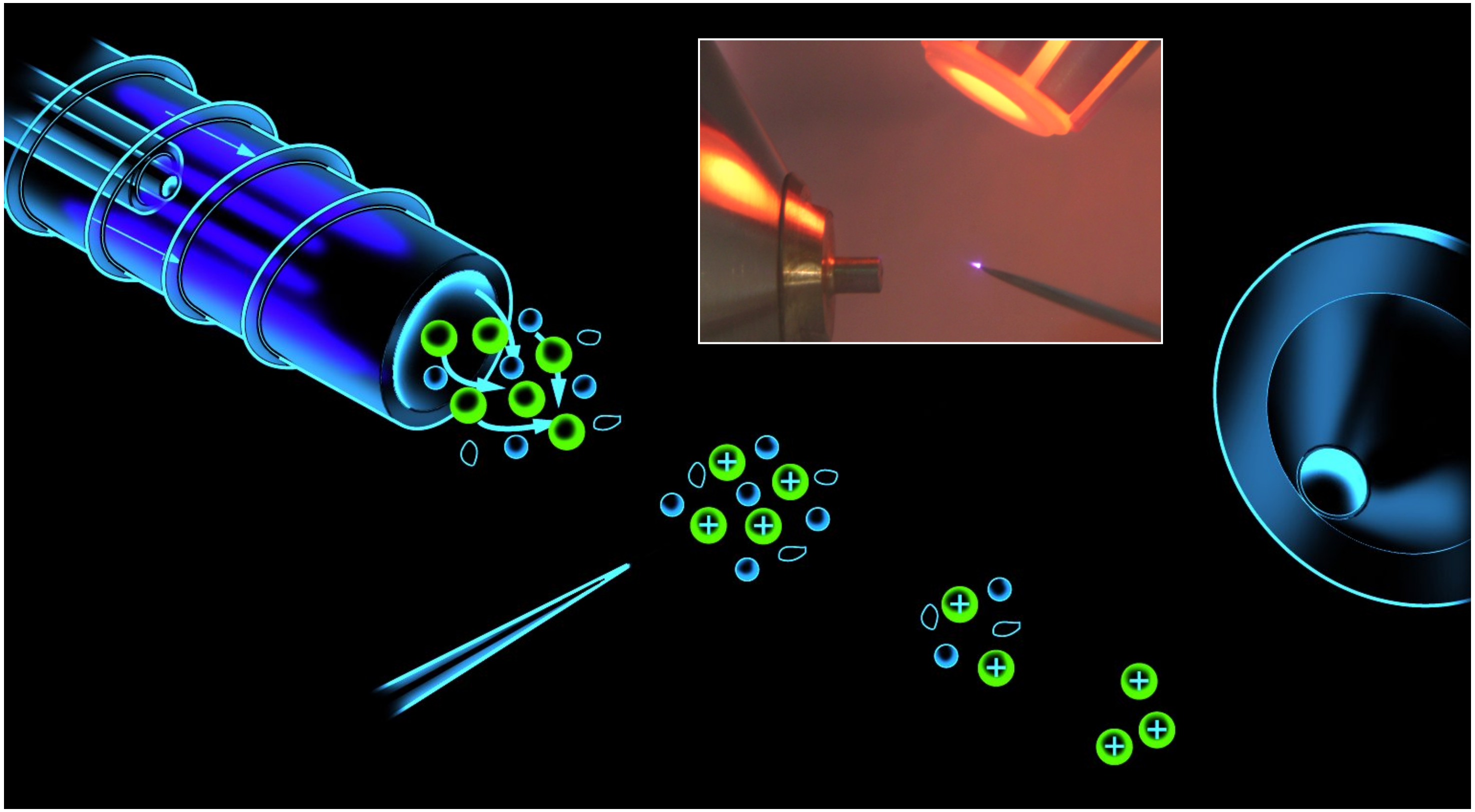
APCI Probe: *Atmospheric Pressure Chemical Ionization*

The liquid effluent is introduced directly into the APCI source

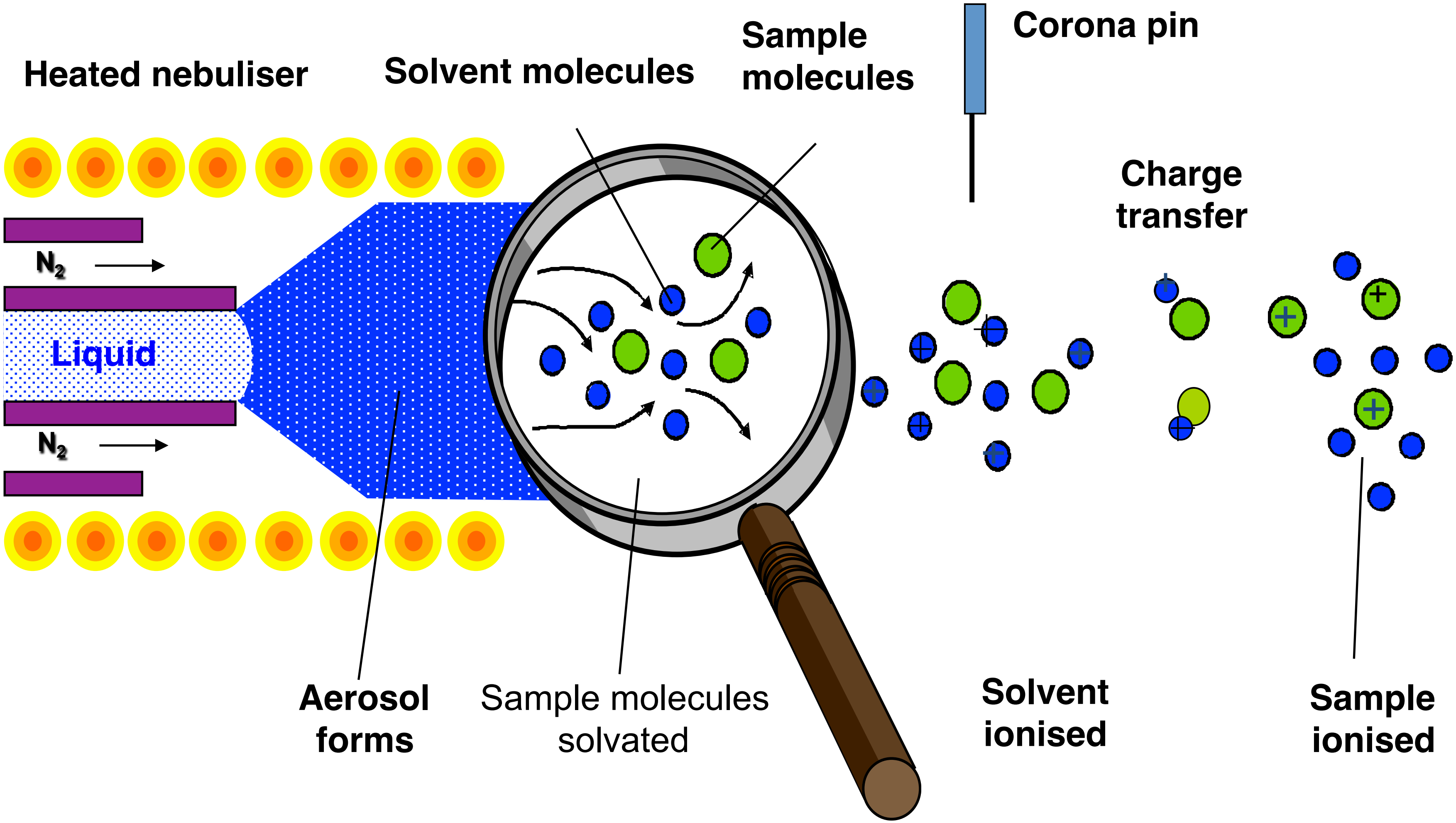
APCI source contains a heated vaporizer: rapid vaporization

Ionization occurs through a corona discharge: reagent ions from the solvent vapor

Vaporized molecules carried through ion-molecule reaction at atmospheric pressure.



Mechanism of ion generation in APCI



Chemical ionization of sample molecules is very efficient at atmospheric pressure (high collision frequency)

Protonation (MH^+) occurs in the positive mode, and e^- transfer or H^+ loss ($[M-H]^-$) in the negative mode.

APCI Reaction Mechanism

Primary ion formation:

$$H_2O + e^- \longrightarrow H_2O^{+\cdot} + 2e^-$$

Secondary ion formation:

$$H_2O^{+\cdot} + H_2O \longrightarrow H_3O^+ + {}^{\cdot}OH$$

Positive analyte ion formation:

$$H_3O^+ + \textit{Analyte} \longrightarrow [\textit{Analyte+H}]^+ + H_2O$$

Negative analyte ion formation:

$${}^{\cdot}OH + \textit{Analyte} \longrightarrow [\textit{Analyte-H}]^- + H_2O^{+\cdot}$$

Chemistry Considerations

ESI:

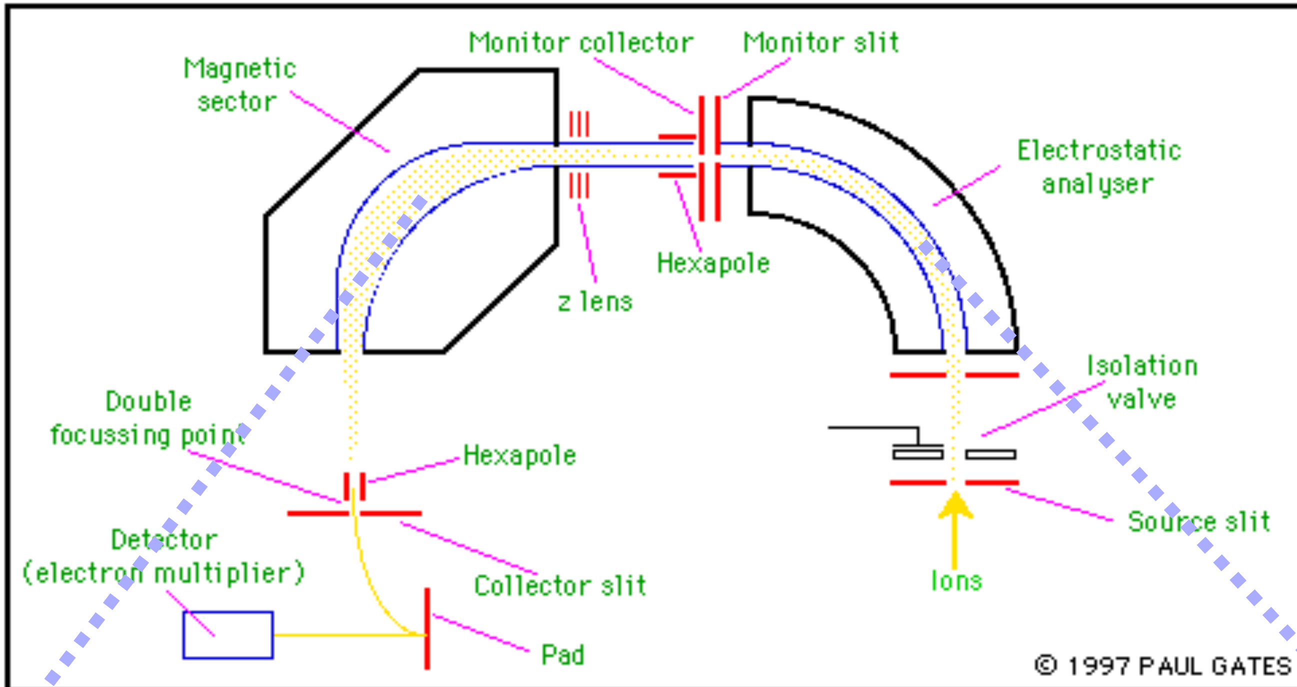
- Ions are predominantly pre-formed in solution
- Technique works well with Polar analytes
- Good for Thermally labile analytes
- Good for Large Molecules (Proteins/Peptides)

APCI:

- Ions are formed by gas phase chemistry
- Technique works well with Non-Polar analytes
- Good for Volatile / Thermally Stable analytes
- Good for Small Molecules (Steroids)

Ionization	Analytes	Introduction	Max mass	Capability
EI	Relatively small, volatile	GC or probe	1,000 Daltons	Provides structure info
CI	Relatively small, volatile	GC or probe	1,000 Daltons	Molecular ion $[M+H]^+$
ESI	Peptides, Proteins, Nonvolatile	LC or syringe	200,000 Daltons	Ions often multiply charged

1) Double Focusing (Sector) Analysis



$$\frac{m_i v^2}{r} = B z_i v$$

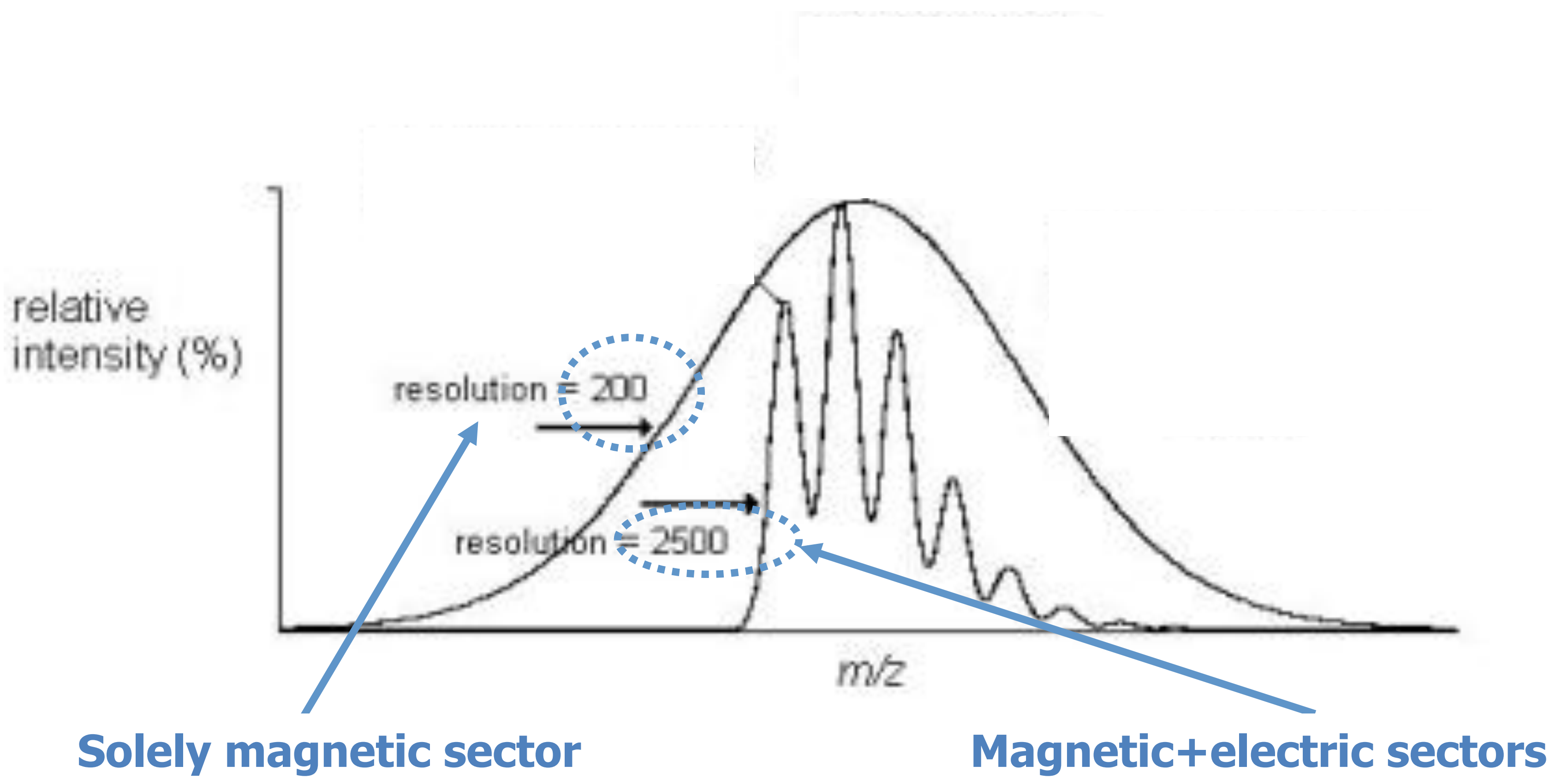
$$\text{kinetic energy} = \frac{m_i v^2}{2} = z_i V_a$$

$$\frac{m_i}{z_i} = \frac{B^2 r^2}{2V_a}$$

scanned

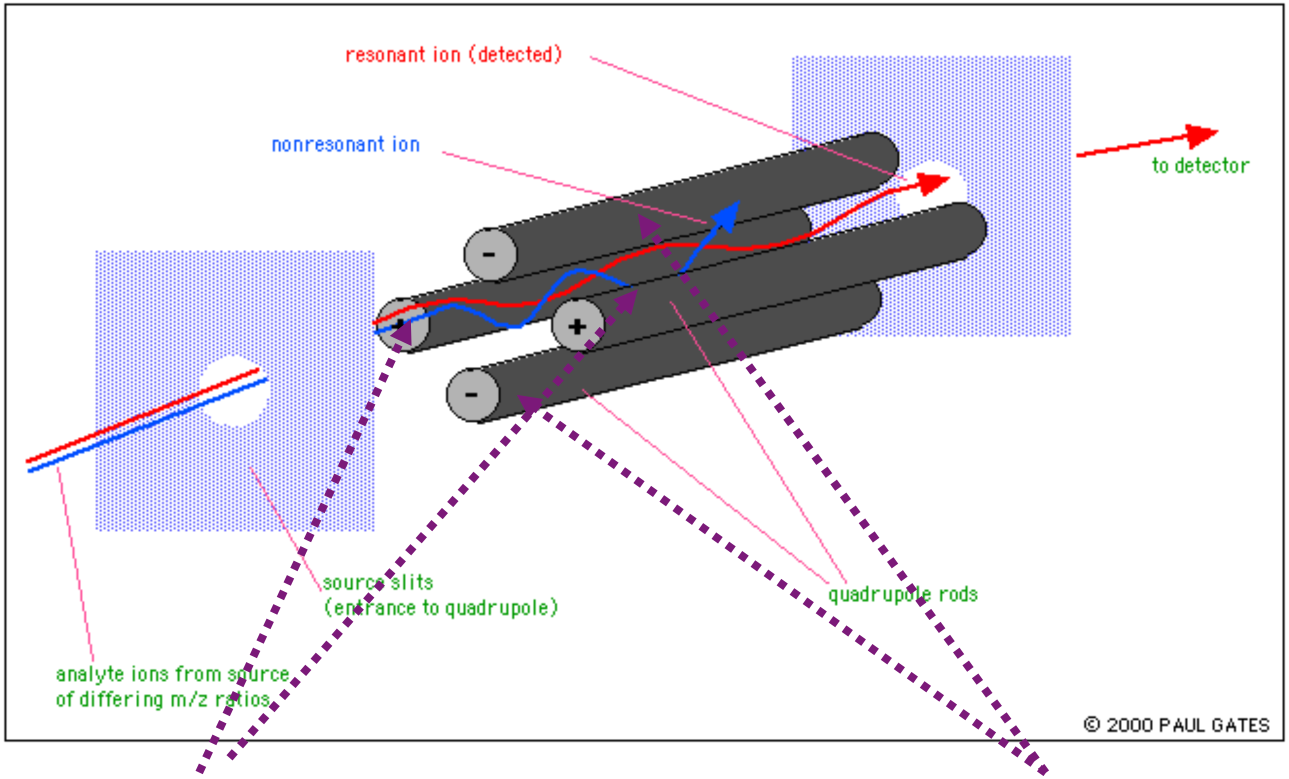
fixed

Increase in resolution allows for better peak distinction within a spectrum



3) Quadrupole Mass Analyzer

Electric fields are used to separate ions according to their m/z values



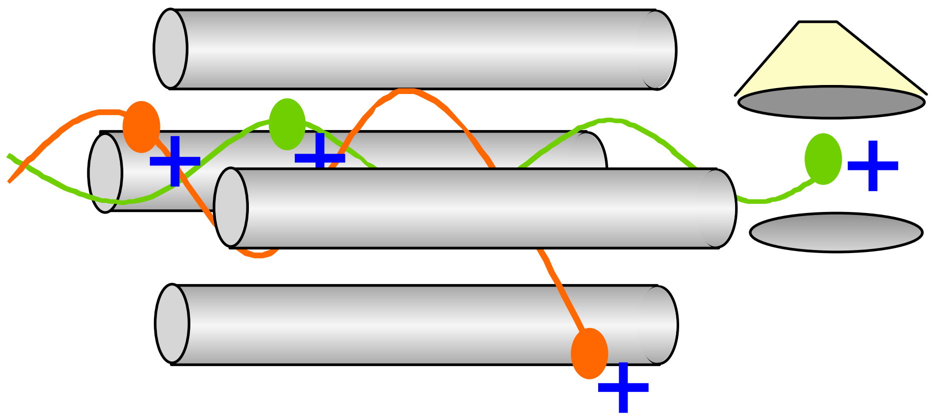
+(U+Vcos(ωt)) **-(U+Vcos(ωt))**

$$m/z = f\,(U,V,\omega)$$

Choice of U, V and ω (1-2 MHz): only one m/z will oscillate stably through the quadrupole mass analyser to the detector

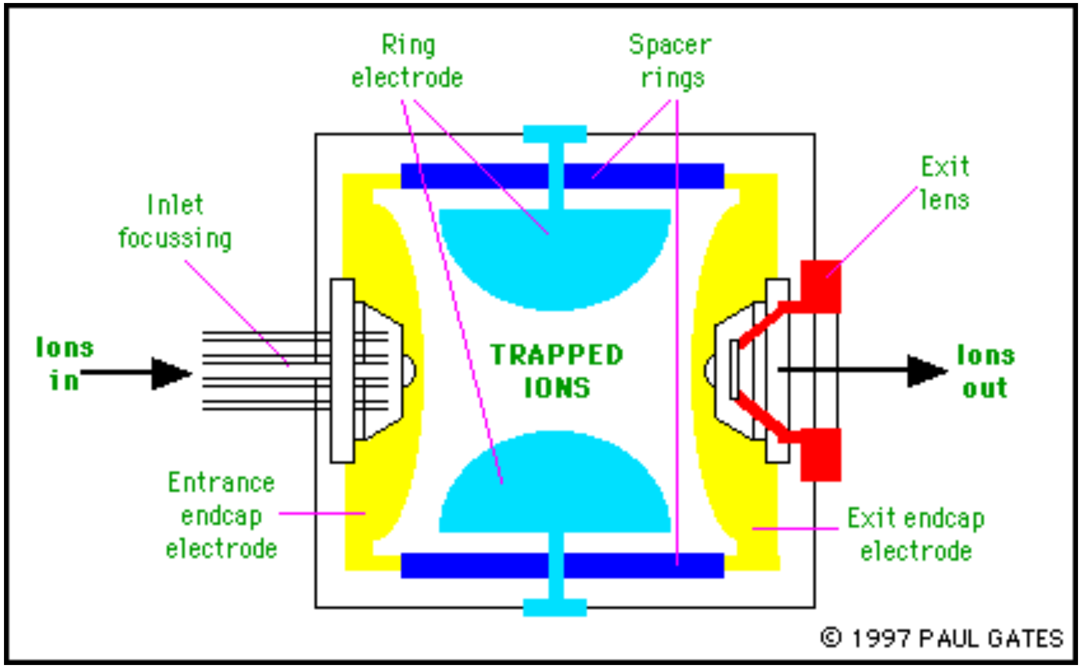
Full Scan v's SIM

Full Scan: **As RF and DC voltages are ramped upward (i.e.the mass analyzer is scanned), ions of successively higher mass-to-charge ratios and having stable trajectories are allowed to pass through the analyzer. If one MS scan between m/z 0 and m/z 500 is completed in one second, then each m/z will be allowed to pass for only 1/500 s.**



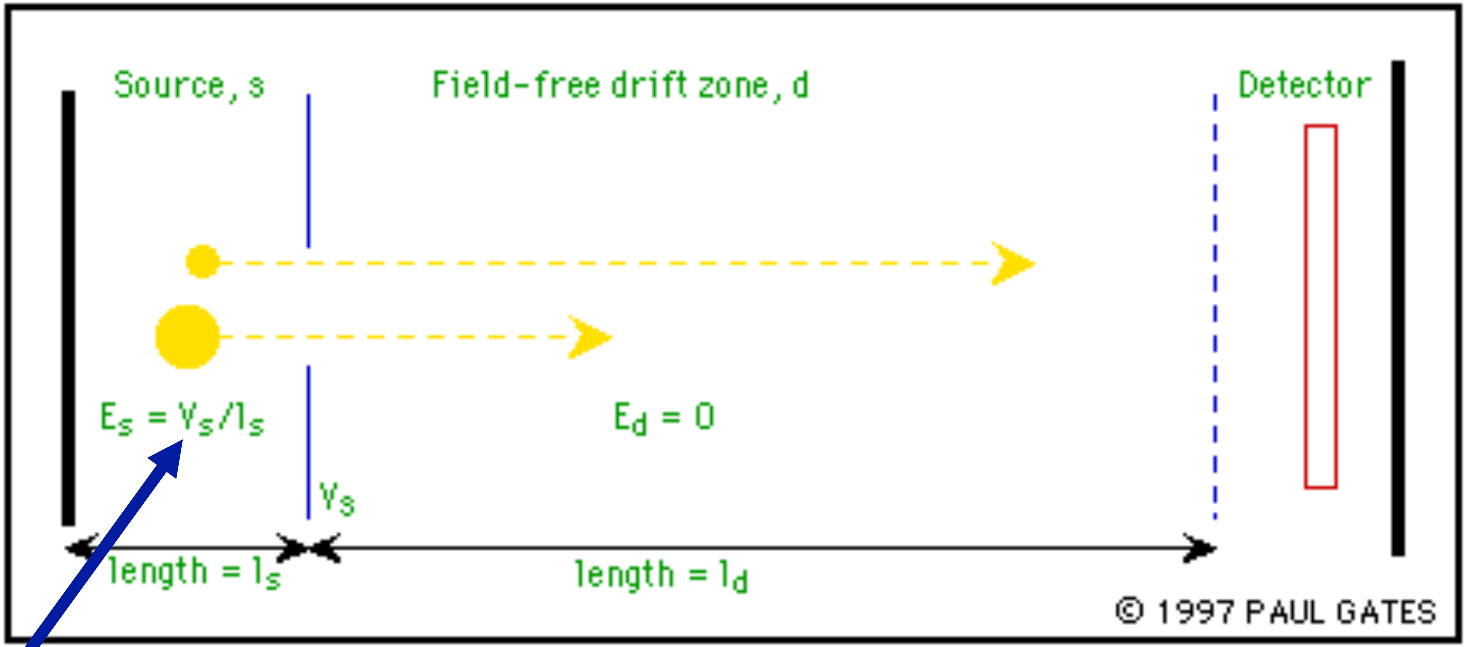
***SIM*:** **If the RF and DC voltages are held constant, ions of a single m/z ratio and having a stable trajectories are transmitted. Data is collected on the ion for a much longer time resulting in improved signal to noise, better peak definition and lower RSD's. SIM can give 15 to 25 times improvement in sensitivity compared to full scan.**

5) Ion Trap Analysis



•Voltages are applied to the 3 <u>electrodes</u> to trap and eject ions according to m/z.
•The ring electrode RF potential, is producing a 3D quadrupolar potential field within the trapping cavity.
•Ions are trapped in a stable oscillating trajectory.
•The trajectory depends on the trapping potential and the m/z.
• Alteration of potentials: Instabilities in trajectory:
Ions are ejected in increasing m/z and focused by the exit lens and detected by the ion detector system.

2) Time-of-Flight (TOF)



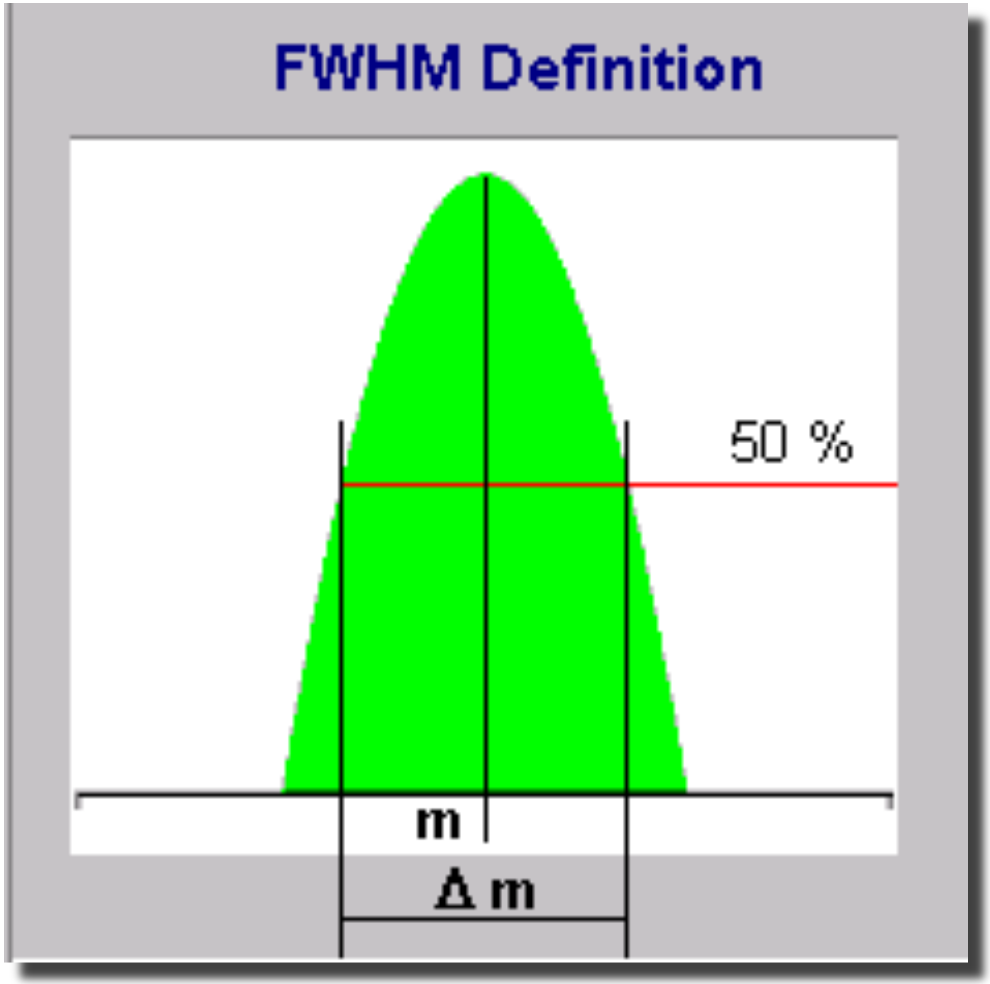
Source extraction potential

$$\frac{m_i}{z_i} = 2eE\,l_s\left(\frac{t_i}{l_d}\right)^2$$

E = extraction field=V_s/l_s
t_i = time-of-flight of ion
l_s = length of the source
l_d = length of the field-free drift region
***e* = electronic charge**

Resolution

FWHM: Full Width Height Maximum



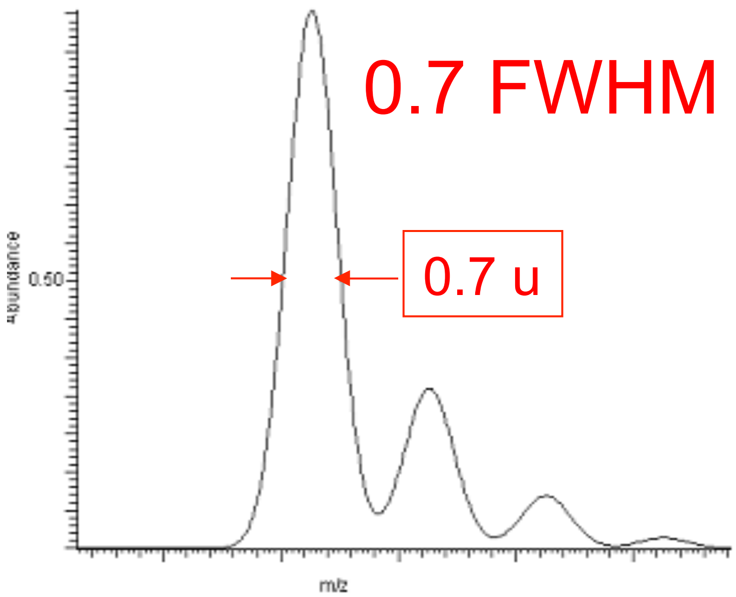
Quadrupoles Ion traps

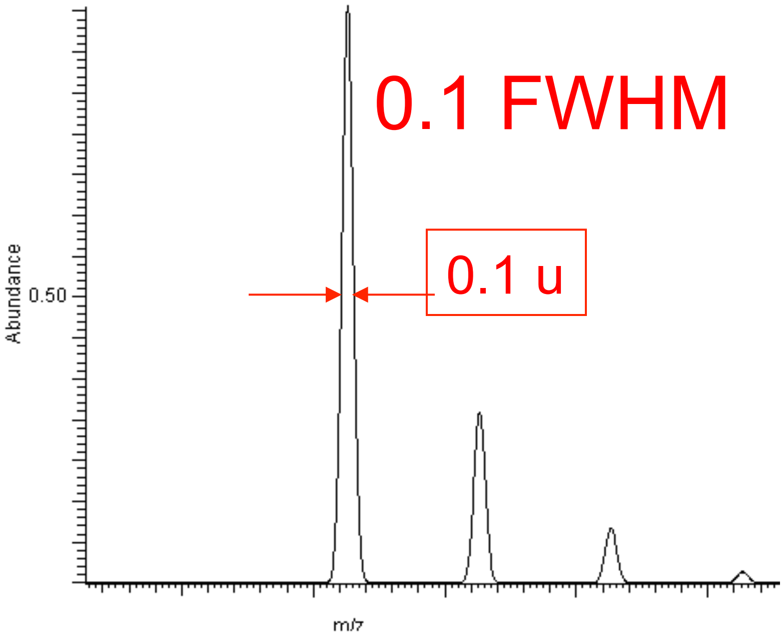
and TOF' s

Constant peak widths

Variable Resolution

“Unit mass Resolution” v’s 0.1 FWHM





m/z 300/ 0.7 = R 428

m/z 500/ 0.7 = R 714

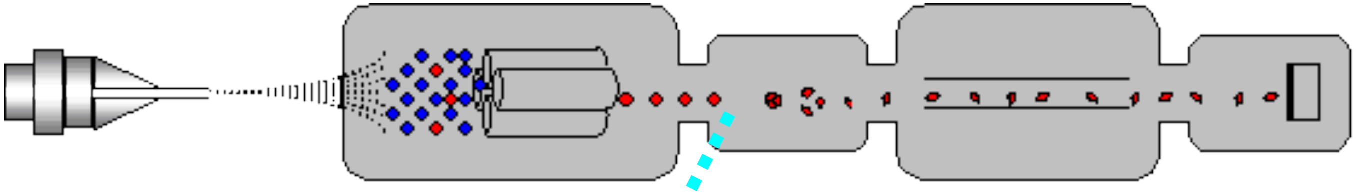
m/z 1000/ 0.7 = R 1428

m/z 300/ 0.1 = R 3000

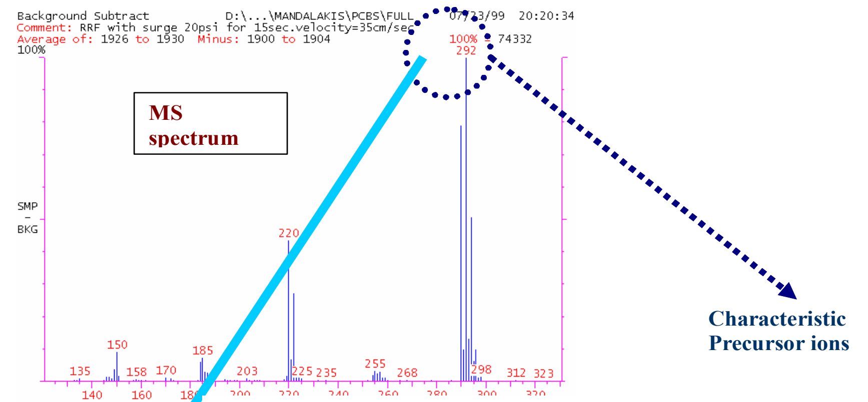
m/z 500/ 0.1 = R 5000

m/z 1000/ 0.1 = R 10,000

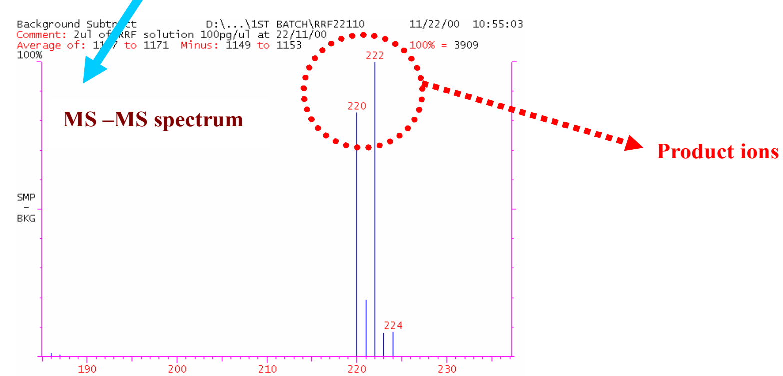
6) Tandem Mass Spectrometry



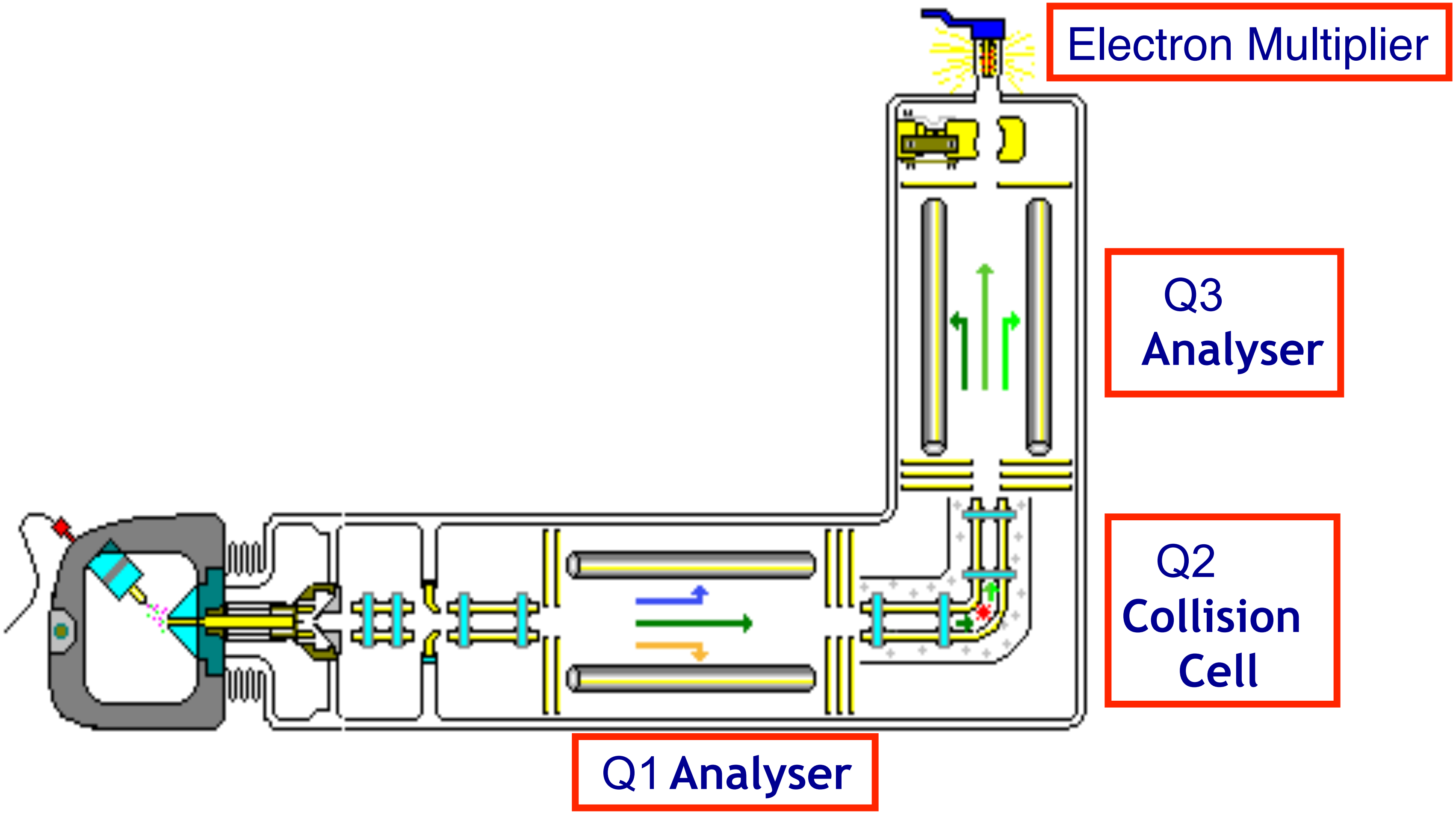
Fragmentation: induction of ion/molecule collisions by collision-induced dissociation (CID).



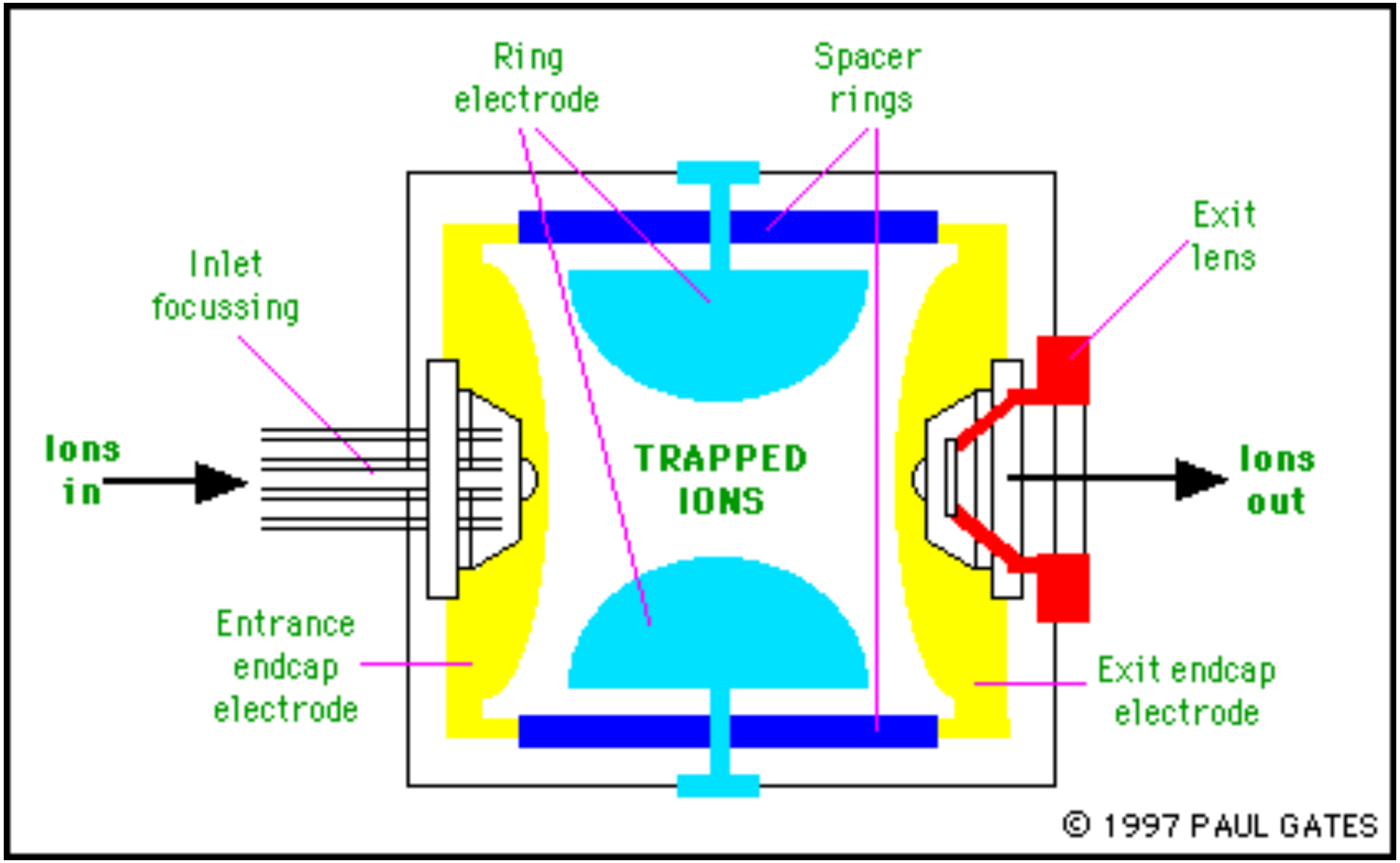
- Isolation of the precursor ion
- Fragmentation of the precursor ion
- Detection of product ions



TSQ Quantum Components



Ion Trap "MS-MS"

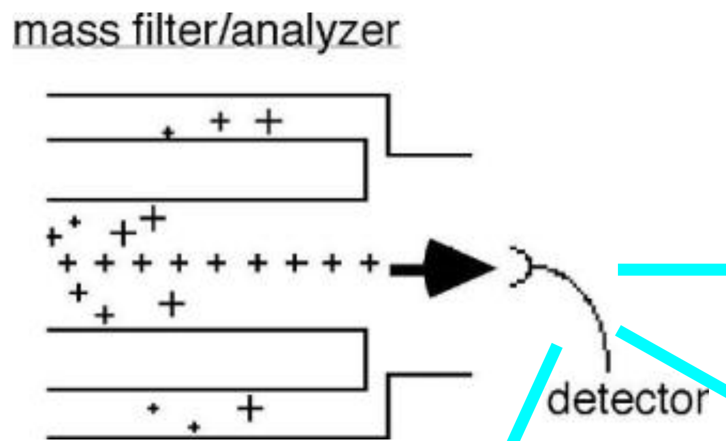


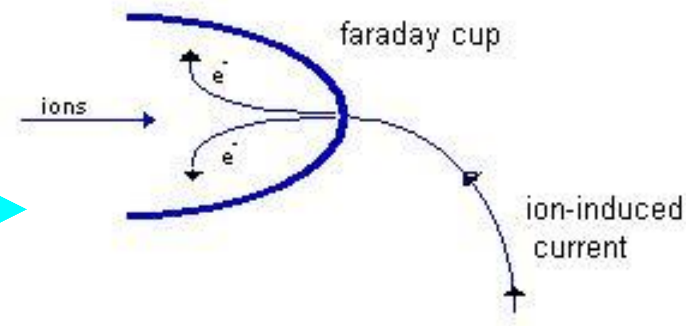
Fragmentation: induction of ion/molecule collisions by collision-induced dissociation (CID) within the ion trap.

Overview of the most used mass analyzers

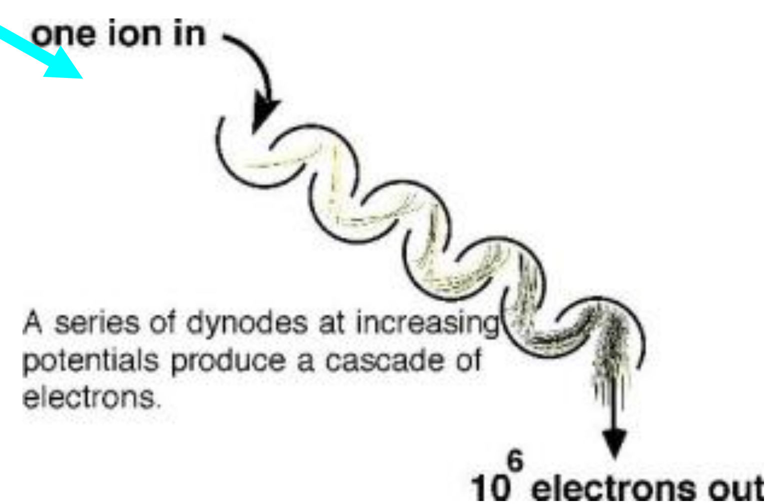
Analyzer	***System capabilities***
Quadrupole	Unit mass resolution, fast scan, low cost
Magnetic and/or Electrostatic	High resolution, exact mass
Time-of-Flight (TOF)	Theoretically, no limitation for m/z maximum, high throughput

5. Ion Detection

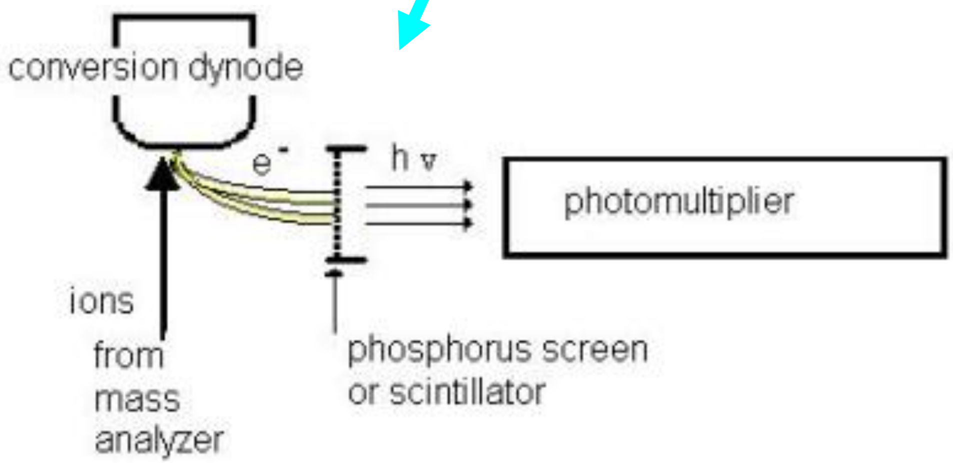




Faraday cup



Electron multiplier



Photomultiplier conversion dynode

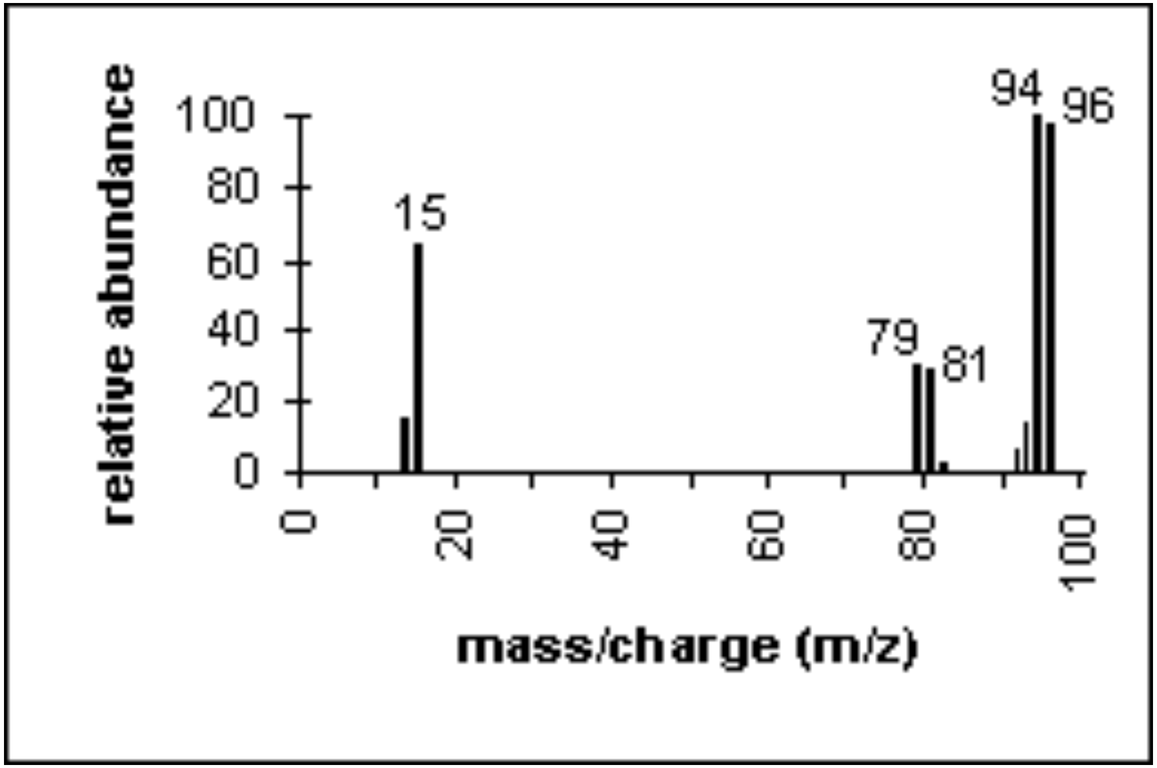
Applications of Mass Spectrometry

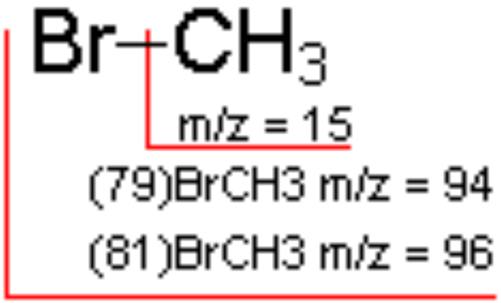
- Atomic Masses
- Geochronology and Geochemistry
- Accelerator MS and dating materials
- Organic Chemistry
- Combinatorial Chemistry
- Biochemistry
- Peptide and DNA sequencing
- Small biomolecule characterization
- Viruses
- Forensics
- Space probes
- Small Mass Spectrometers

Common isotopes of the most important elements

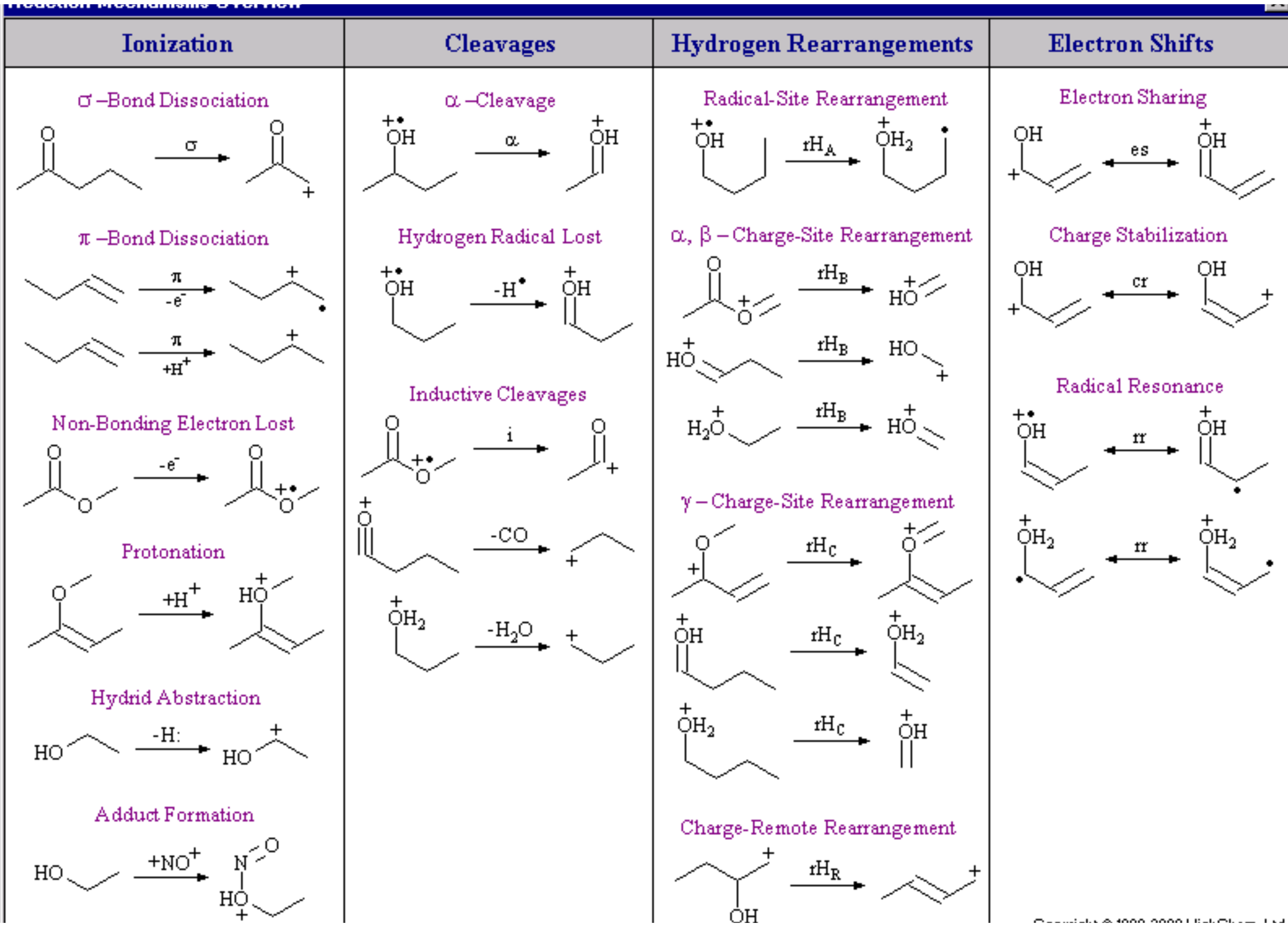
Element	Isotope	Relative Abundance	Isotope	Relative Abundance	Isotope	Relative Abundance
Carbon	^{12}C	100	^{13}C	1.11		
Hydrogen	^{1}H	100	^{2}H	.016		
Nitrogen	^{14}N	100	^{15}N	.38		
Oxygen	^{16}O	100	^{17}O	.04	^{18}O	.20
Sulfur	^{32}S	100	^{33}S	.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0

Methyl Bromide:
An example of how isotopes can aid in peak identification.





The ratio of peaks containing ^{79}Br and its isotope ^{81}Br (100/98) confirms the presence of bromine in the compound


Ionization
Cleavages
Hydrogen Rearrangements
Electron Shifts
σ –Bond Dissociation
α –Cleavage
Radical-Site Rearrangement
Electron Sharing
π –Bond Dissociation
Hydrogen Radical Lost
α, β – Charge-Site Rearrangement
Charge Stabilization
Non-Bonding Electron Lost
Inductive Cleavages
Radical Resonance
Protonation
γ – Charge-Site Rearrangement
Hydrid Abstraction
Adduct Formation
Charge-Remote Rearrangement
-CO
-H:
+NO+

Common Mass Spectrum Fragments

Commonly Lost Fragments

m-15 $\cdot CH_3$

m-17 $\cdot OH$

m-26 $\cdot CN$

m-28 $H_2C{=}CH_2$

m-29 $\cdot CH_2CH_3$ $\cdot CHO$

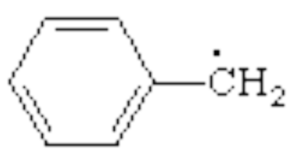
m-31 $\cdot OCH_3$

m-35 $\cdot Cl$

m-43 $CH_3\dot{C}{=}O$

m-45 $\cdot OCH_2CH_3$

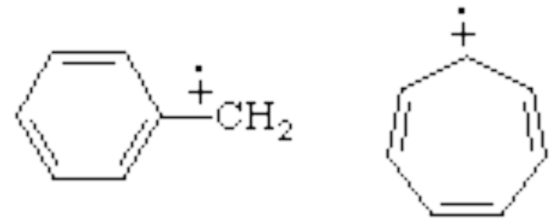
m-91



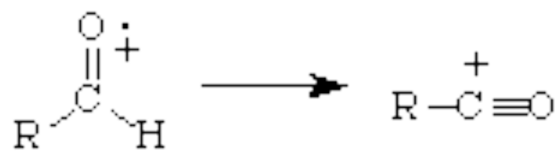
Common Stable Ions

m/e = 43 $CH_3{-}\overset{+}{C}{\equiv}O$

m/e = 91



m/e = m-1



Stages of a mass spectrum interpretation-1:

- ***1. Look for the molecular ion peak:***

- **This peak (if it appears) will be the highest mass peak in the spectrum, except for isotope peaks.**

- **Nominal MW will be an even number for compounds containing only C, H, O, S, Si.**

- **Nominal MW will be an odd number if the compound also contains an odd number of N (1,3,...).**

***Stages of a mass spectrum interpretation-2*:**

- ***2. Calculate the molecular formula:***

The isotope peaks can be very useful, and are best explained with an example.

- **^{12}C has an isotope of ^{13}C. Their abundances are ^{12}C=100%, ^{13}C=1.1%. This means that for every 100 ^{12}C atoms there are 1.1 ^{13}C atoms.**

- ***Example: If a compound contains 6 carbons, then each atom has a 1.1% abundance of ^{13}C.***

If the molecular ion peak is 100%, then the isotope peak (1 mass unit higher) would be 6x1.1%=6.6%.

Stages of a mass spectrum interpretation-3:

- **If the molecular ion peak is not 100% then calculate the relative abundance of the isotope peak to the ion peak.**

- ***Example*: if the molecular ion peak were 34% and the isotope peak 2.3%:**

  (2.3/34)x100 = 6.8%. 6.8% is the relative abundance of the isotope peak to the ion peak.

  Next, divide the relative abundance by the isotope abundance: 6.8/1.1=6 carbons.

- **Look for A+2 elements: O, Si, S, Cl, Br**
- **Look for A+1 elements: C, N**
- **"A" elements: H, F, P, I**

Stages of a mass spectrum interpretation-4:

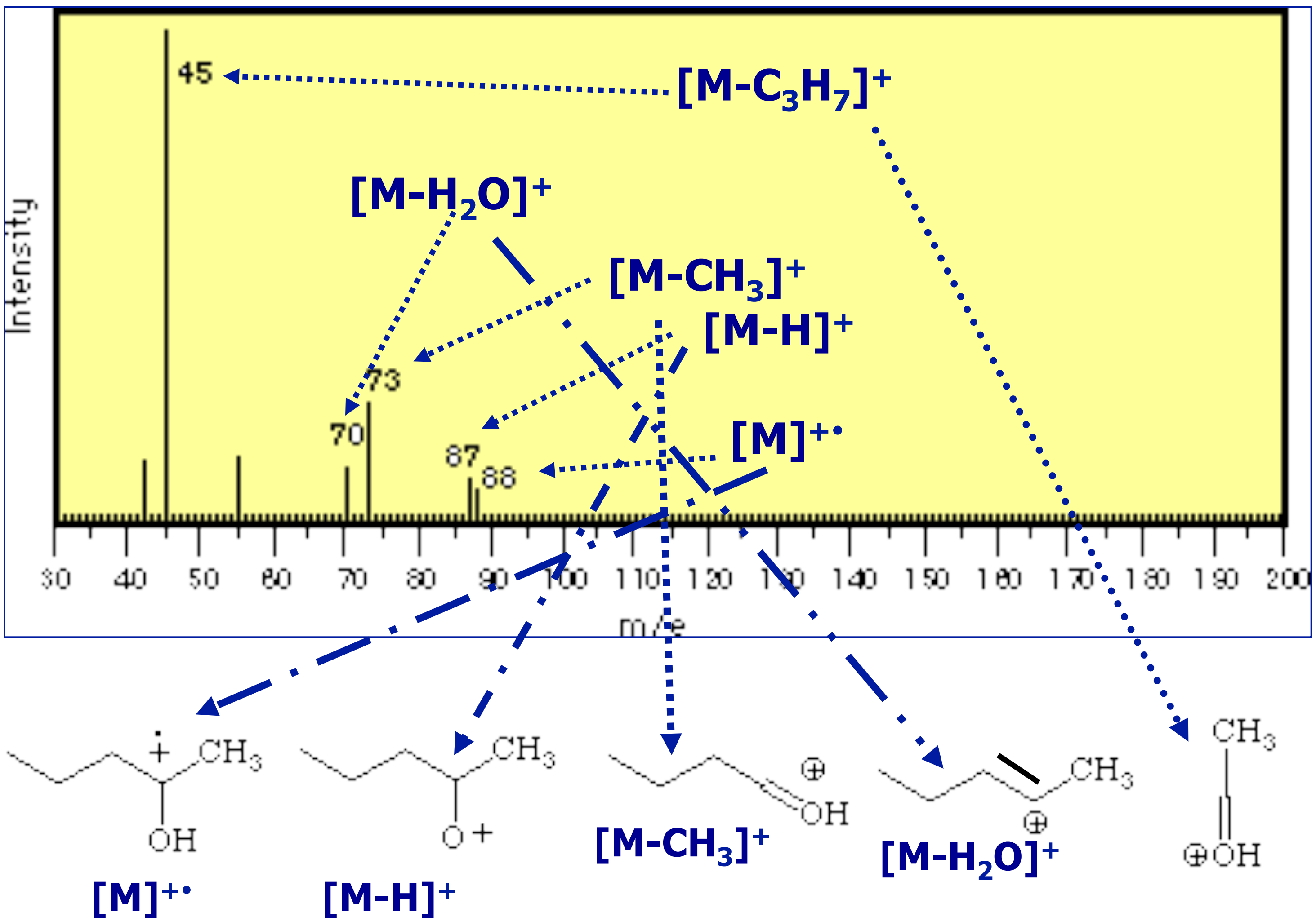
- **3. Calculate the total number of rings plus double bonds:**
- **For the molecular formula: $C_xH_yN_zO_n$**
- **Rings + Double Bonds = $x - (1/2)y + (1/2)z + 1$**

- **4. Postulate the molecular structure consistent with abundance and m/z of fragments.**

  More information on specific fragmentation can be found for each functional group.

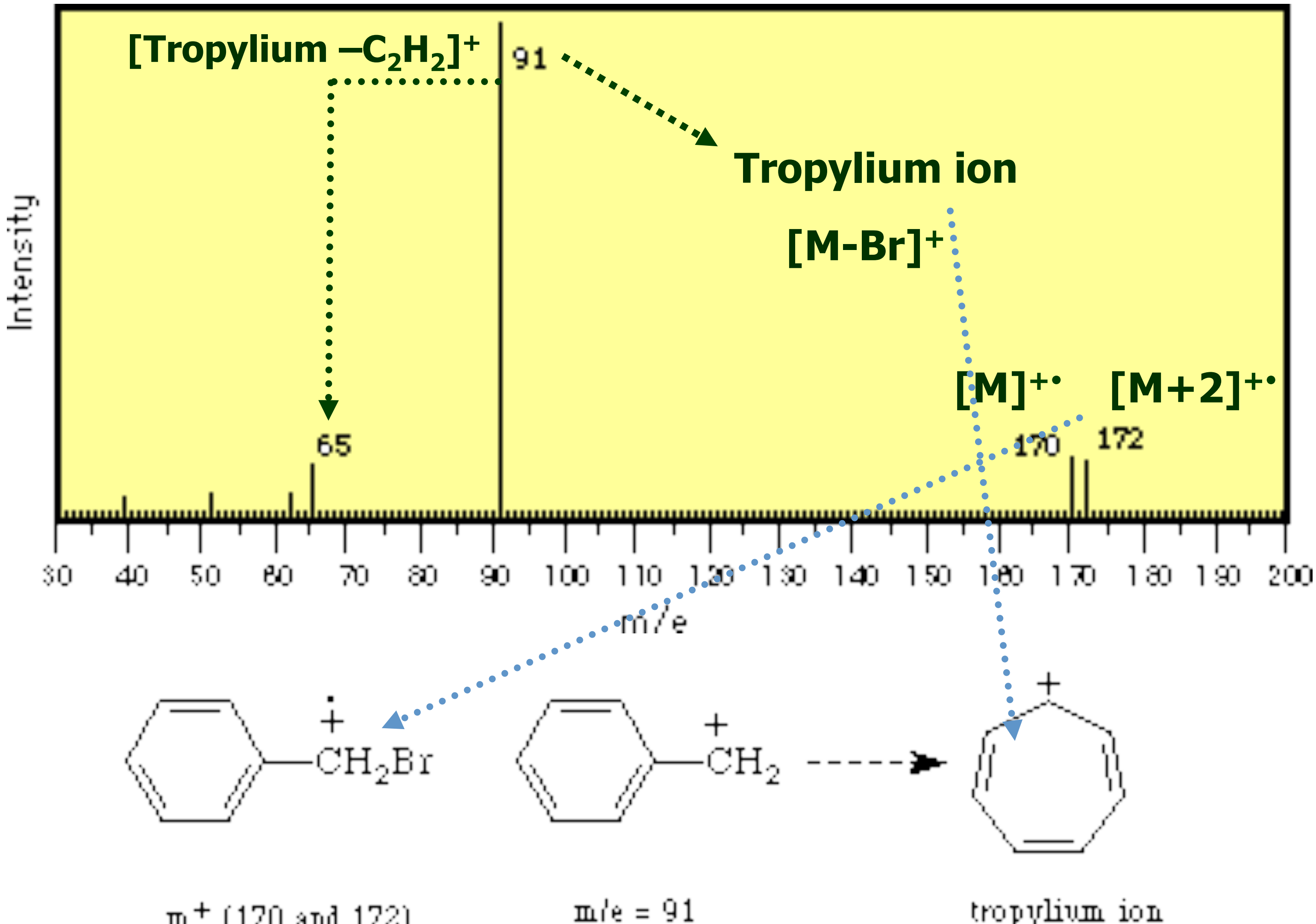
Example #1

Analysis: $C_5H_{12}O$ MW = 88.15



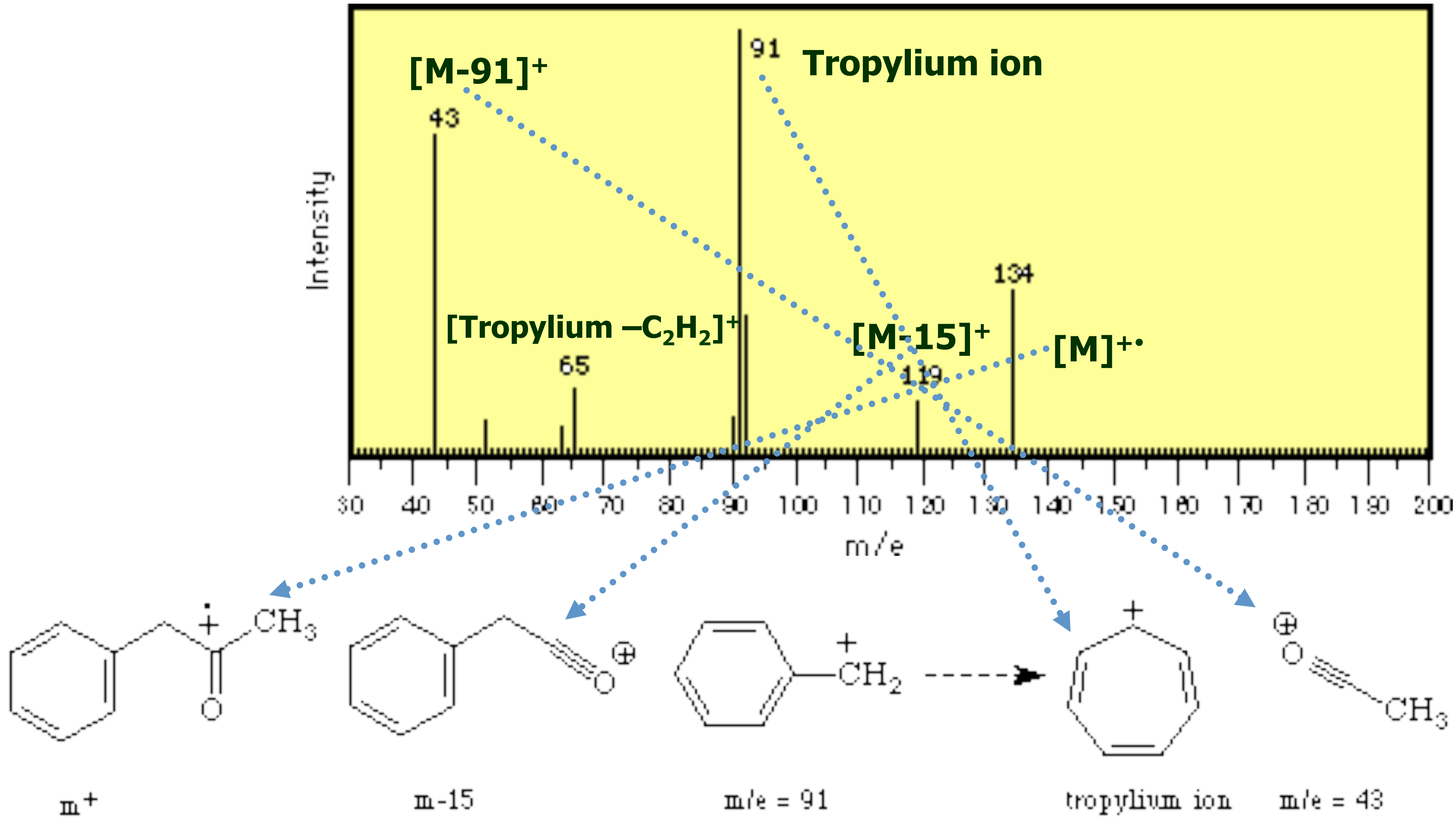
Example #2

Analysis: $C_7H_{12}Br$ MW = 171.04



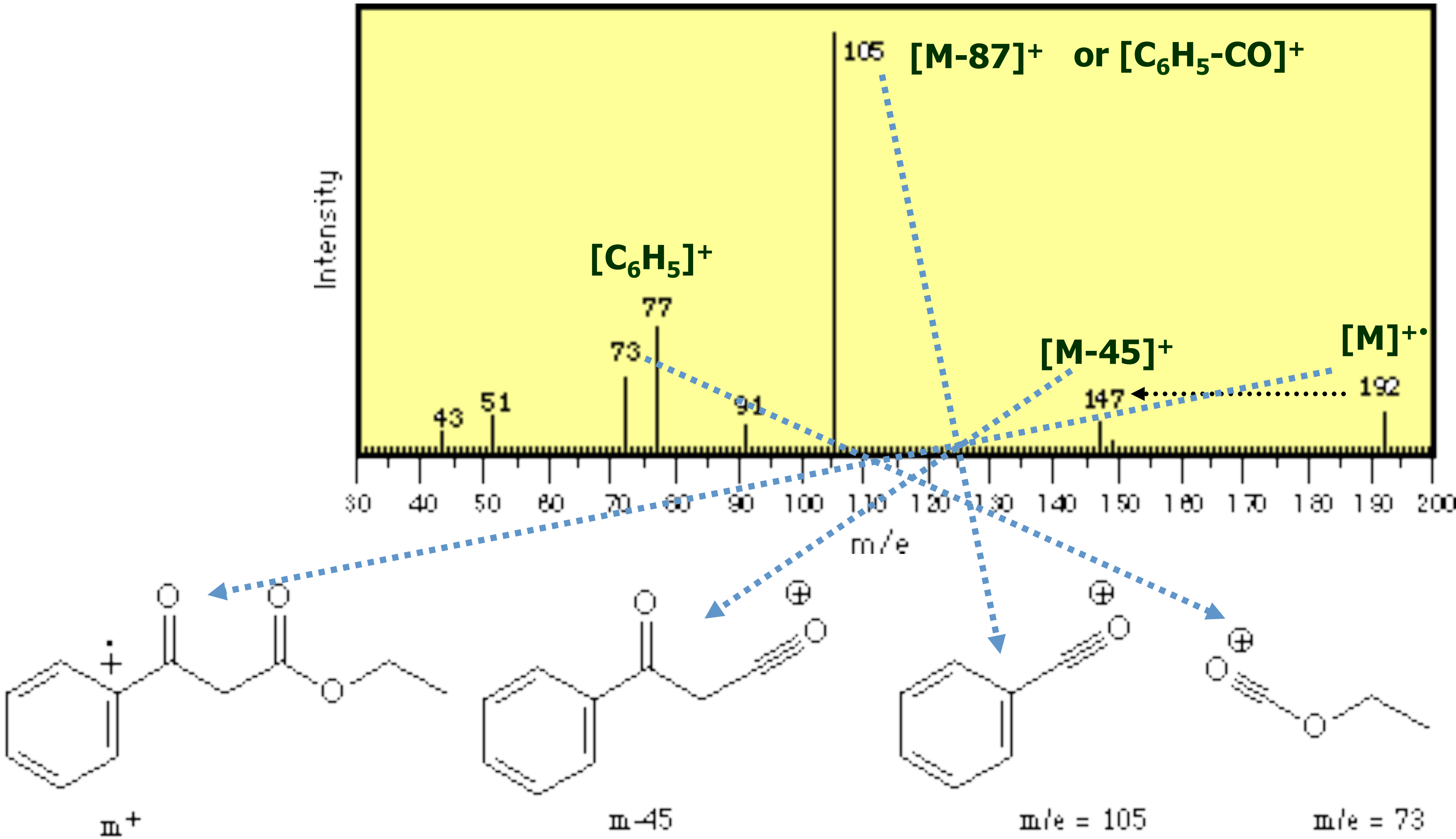
Example #3

Analysis: $C_9H_{10}O$ MW = 134.18



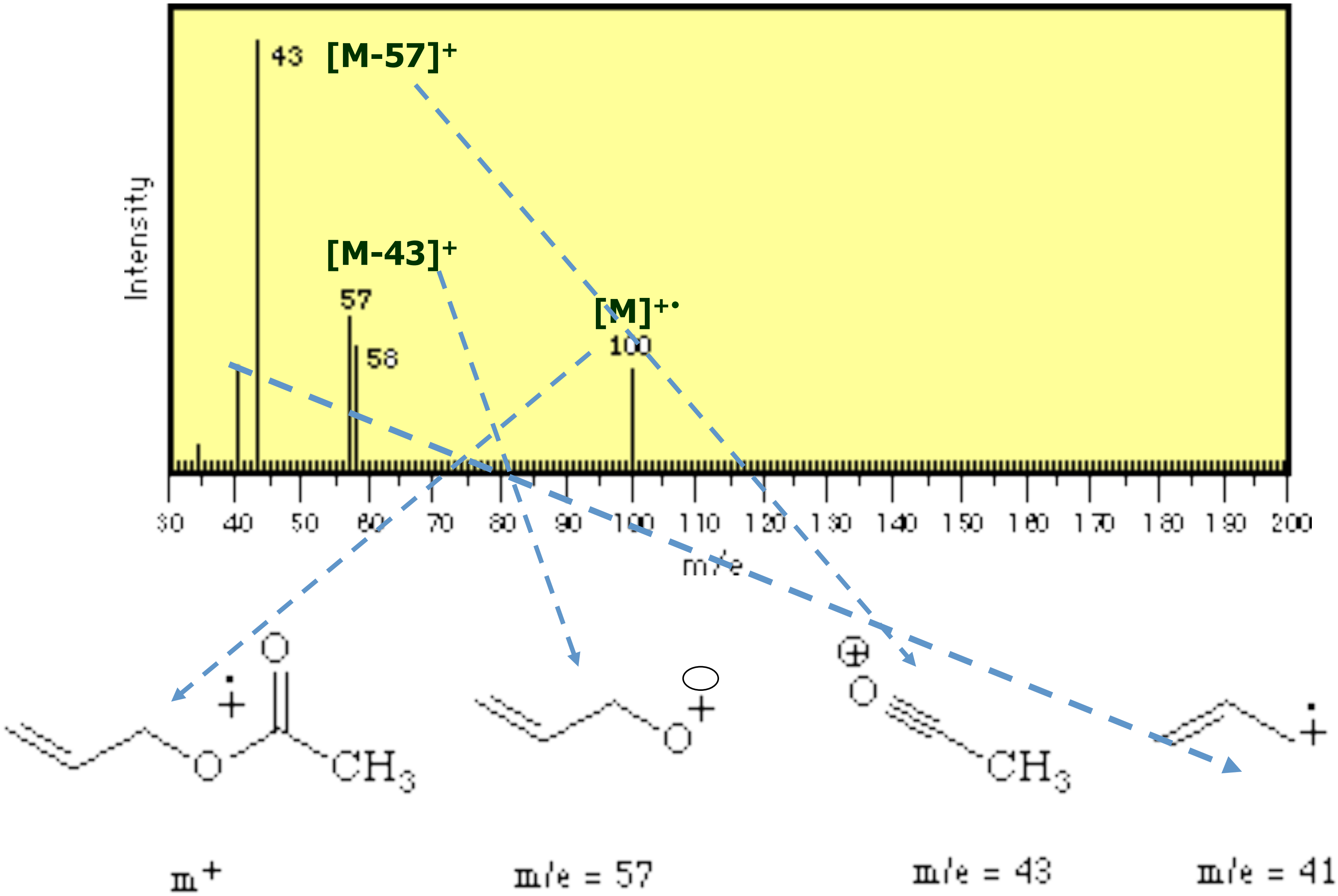
Example #4

Analysis: $C_{11}H_{12}O_3$ MW = 192.21



Example #5

Analysis: $C_5H_8O_2$ MW = 100.12

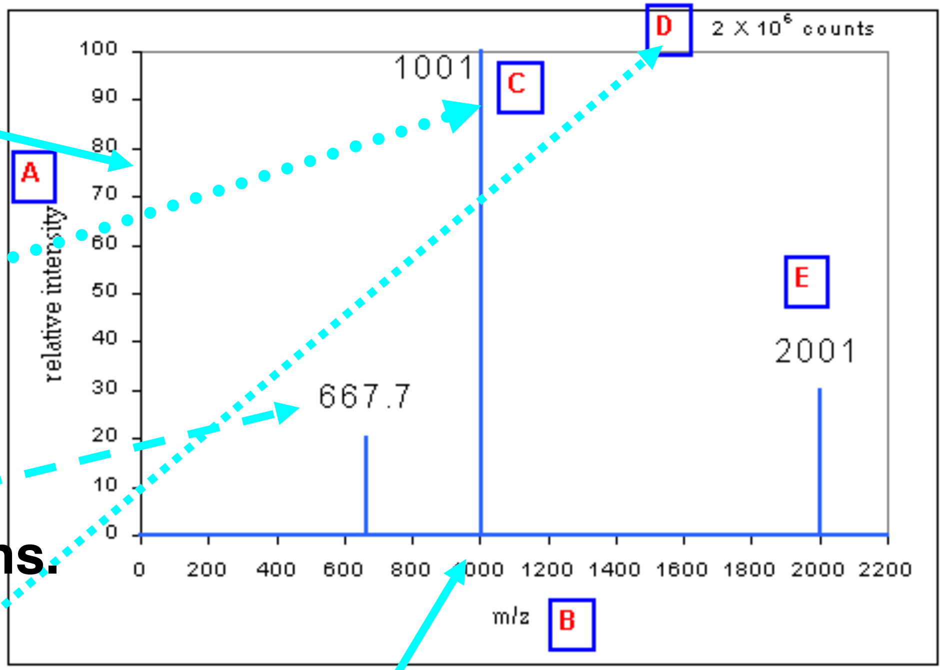


Interpreting Electrospray Mass Spectra

The Y axis is labeled relative intensity.

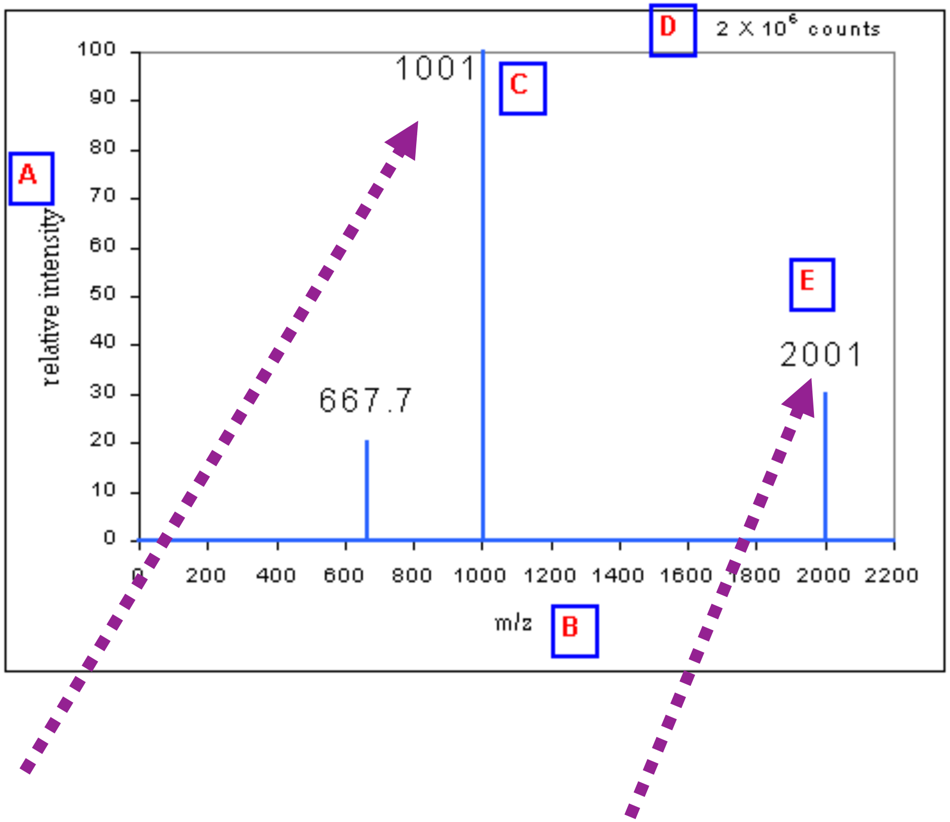
Is the "base peak"

All of the peaks in a spectrum should not be referred to as ions.



The X axis is mass divided by charge, m/z

The spectrum has a certain number of counts associated with the tallest peak in the spectrum.



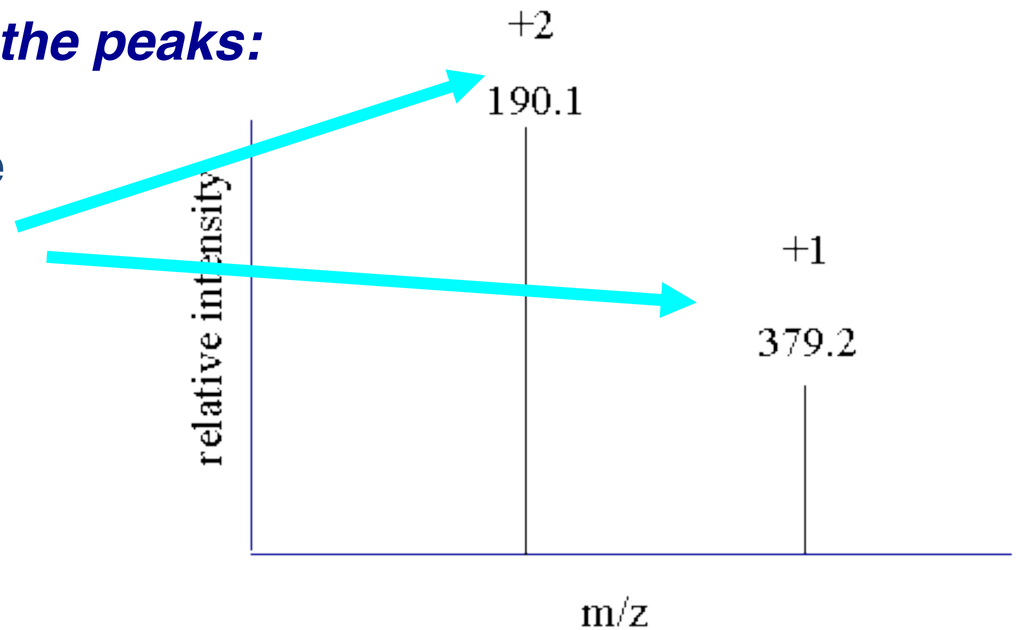
If the mass of a molecule is 2000 and during ESI is charged with 2 $H^+ \Rightarrow$ (2000+2)/2=1001

If the mass is 2000 and is charged with 1 $H^+ \Rightarrow$ (2000+1)/1=2001

Calculating Mass

Determining the charge state of the peaks:

Two adjacent peaks are from the same compound and differ by only one charge



$$190.1 = m_a/z_a$$
$$379.2 = m_b/z_b$$

$$m_a = 190.1\,(z_a)$$
$$m_b = 379.2\,(z_b)$$

If the 2 peaks differ by one charge:

$$m_a = m + 1 = 190.1(z+1)$$
$$m_b = m = 379.2(z)$$

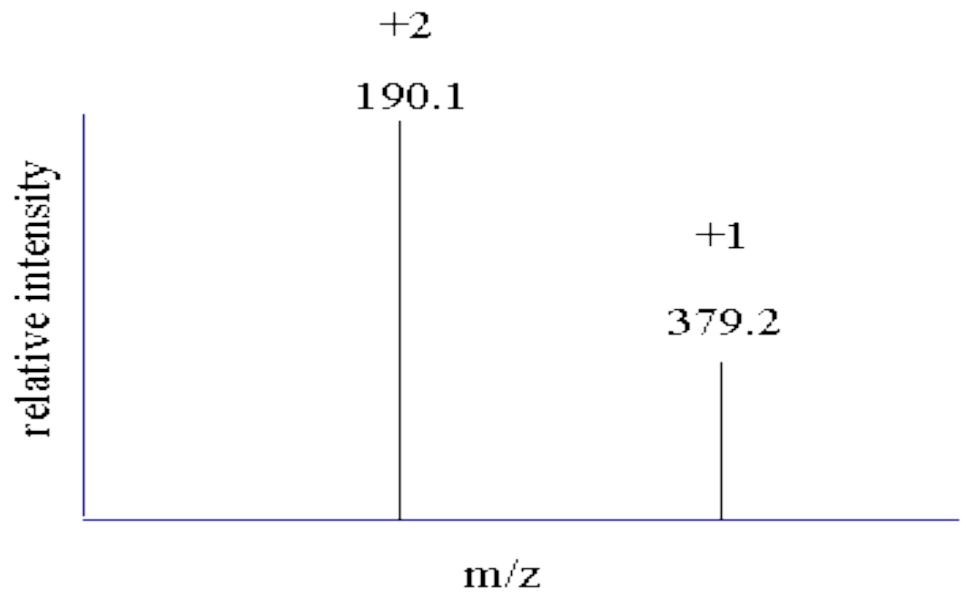
m assumed the same for both peaks

$$m = [190.1(z+1)] - 1$$

$$190.1\,z + 189.1 = 379.2\,z$$
$$z = 1$$

Two peaks in the spectrum are mathematically related :

- **the charge state of the 379.2 peak is +1**
- **the charge state for the 190.1 peak is +2**



Charge		Calculation		Unprotonated Mass	
+1		(379.2 - 1)*1 =		378.2	
+2		(190.1 - 1) *2 =		378.2	
				average	**378.2**