Mass spectrometry

By

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- Mass spectrometry is routinely used along with IR, NMR and UV for structure determination,

  (elucidation of structure) .
- its basic theory is different from the others.
- In mass spectrometry no characteristic selective absorption of radiation is involved as in the case of the other three methods, also, in the mass spectrometry, the compound undergoes irreversible chemical changes unlike in the others, where the changes are reversible physical changes.

- Mass spectrometry is a method of chemical analysis that measures the mass-to-charge ratio (m/z) of atoms or molecules in a sample.
- By measuring the m/z, scientists can often determine the exact molecular weight of the components in the sample and thereby identify unknown compounds.

- Mass spectrometry is often paired with chromatography—gas (GC/MS) or liquid (LC/MS) chromatography.
- While chromatography separates the components of an unknown compound, mass spectrometry analyzes the components for accurate identification.

How a Mass Spectrometer Works

- While there are many different types of mass spectrometry, mass spectrometers all have universal features.
- Every mass spectrometer features a way to ionize a sample's atoms or molecules, a **mass analyzer**, and a **means of detecting** or counting the number of ions of a specific m/z value.
- The mass analyzer separates the ionized masses based on the m/z and outputs them to the detector for detection and quantification.

- There's much variety in the **mass analyzer** component, and there are many different ways to measure the m/z ratio of the ions in a sample, each with strengths and limitations. There are also many different forms of **detectors** to detect or count the number of ions of a specific m/z value, such as electron multipliers, Faraday cups, and microchannel plates.

What kind of info can mass spec give you?

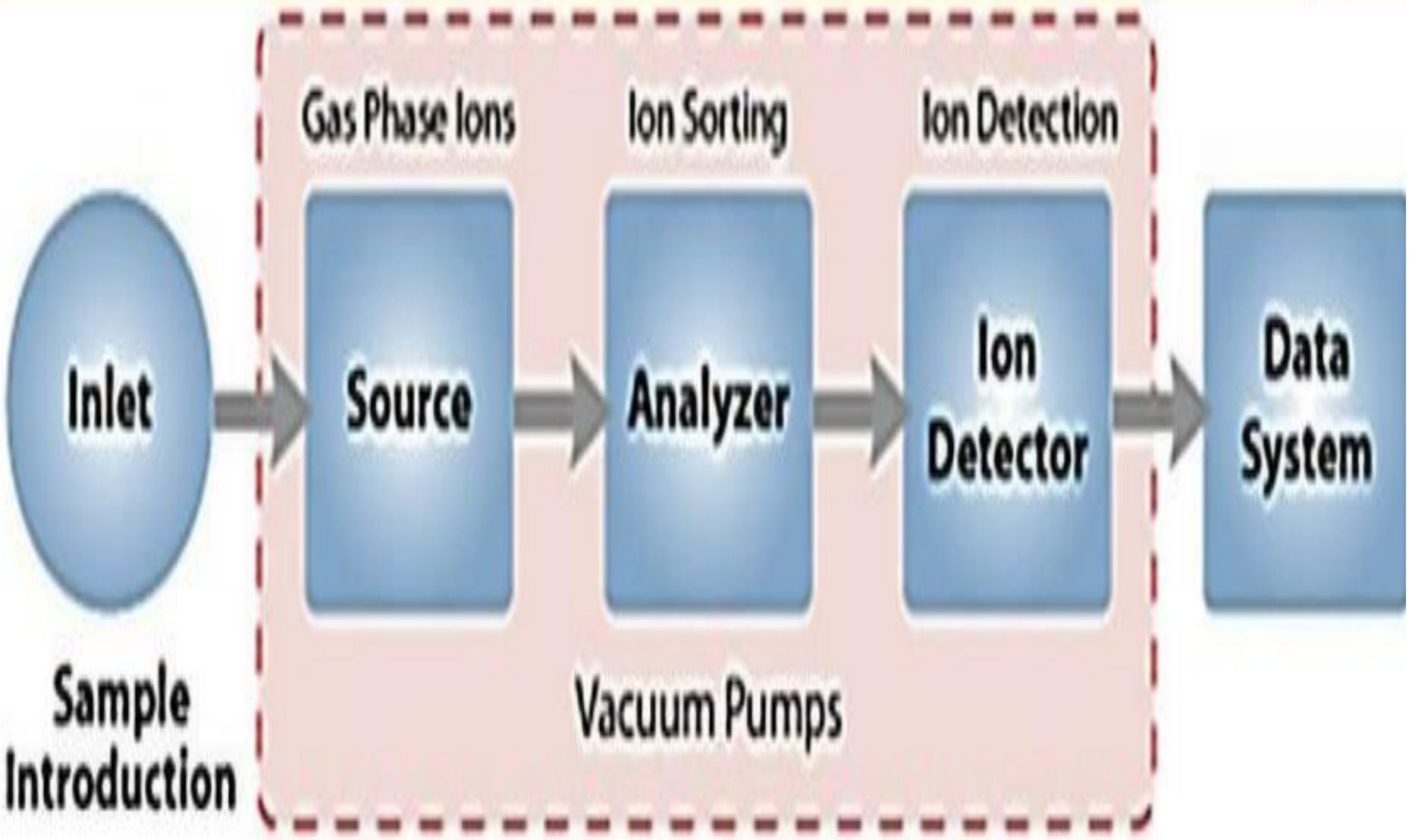
- Molecular weight
- Elemental composition (low MW with high resolution instrument)
- Structural info (hard ionization or CID)
- can provide information about fragmentation patterns.

Types of Mass Spectrometry

- AMS (Accelerator Mass Spectrometry) .
- Gas Chromatography-MS .
- Liquid Chromatography-MS
- ICP-MS (Inductively Coupled Plasma-Mass spectrometry)
- IRMS (Isotope Ratio Mass Spectrometry)
- Ion Mobility Spectrometry-MS

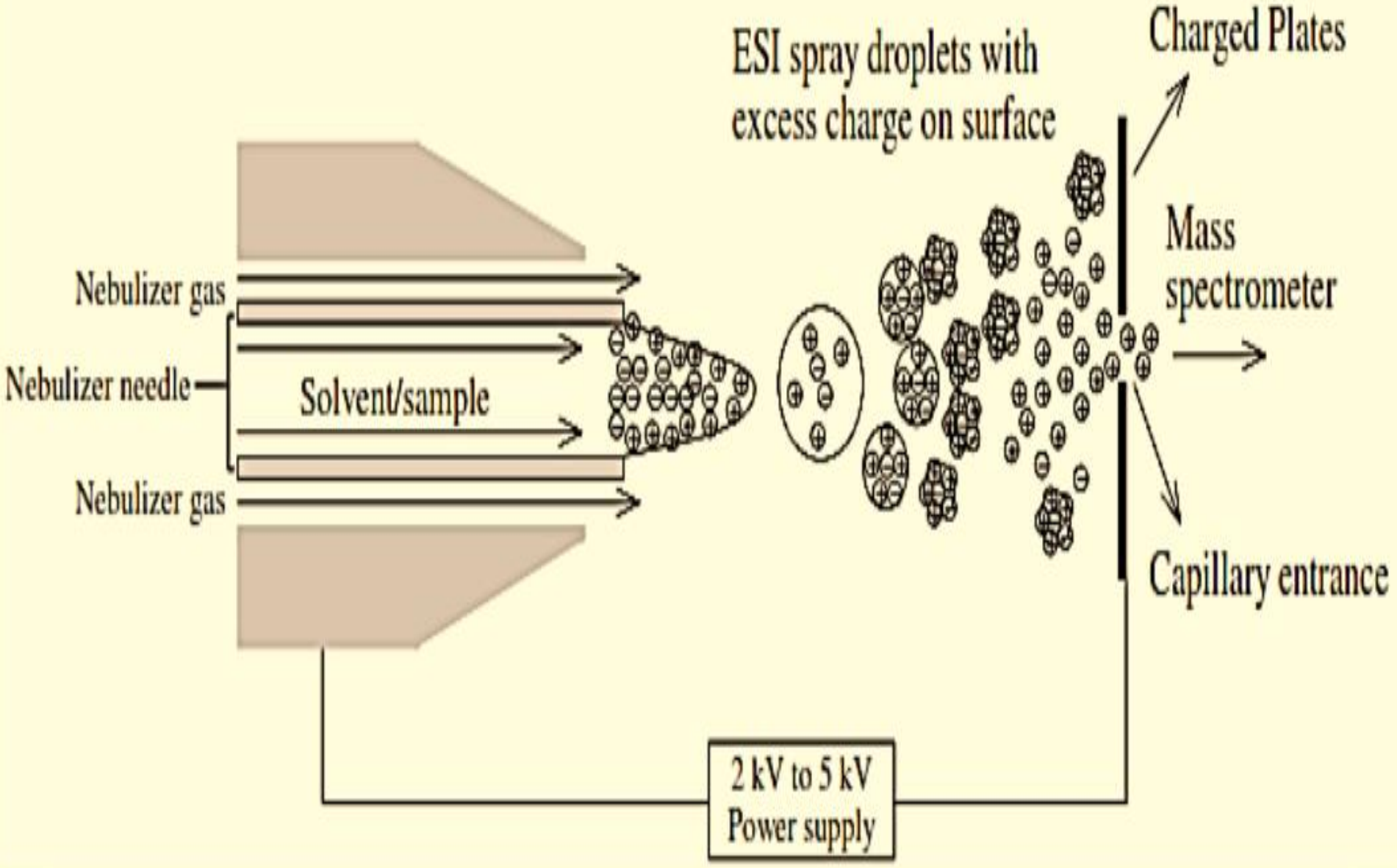
Parts of a Mass Spectrometer

- Sample introduction
- Ion Source (ion formation)
- Mass analyzer (ion separation) high vacuum
- Detector (electron multiplier tube)


Gas Phase Ions
Ion Sorting
Ion Detection
Inlet
Source
Analyzer
Ion Detector
Data System
Sample Introduction
Vacuum Pumps

How does it work?

- Gas-phase ions are separated according to mass/charge ratio and sequentially detected

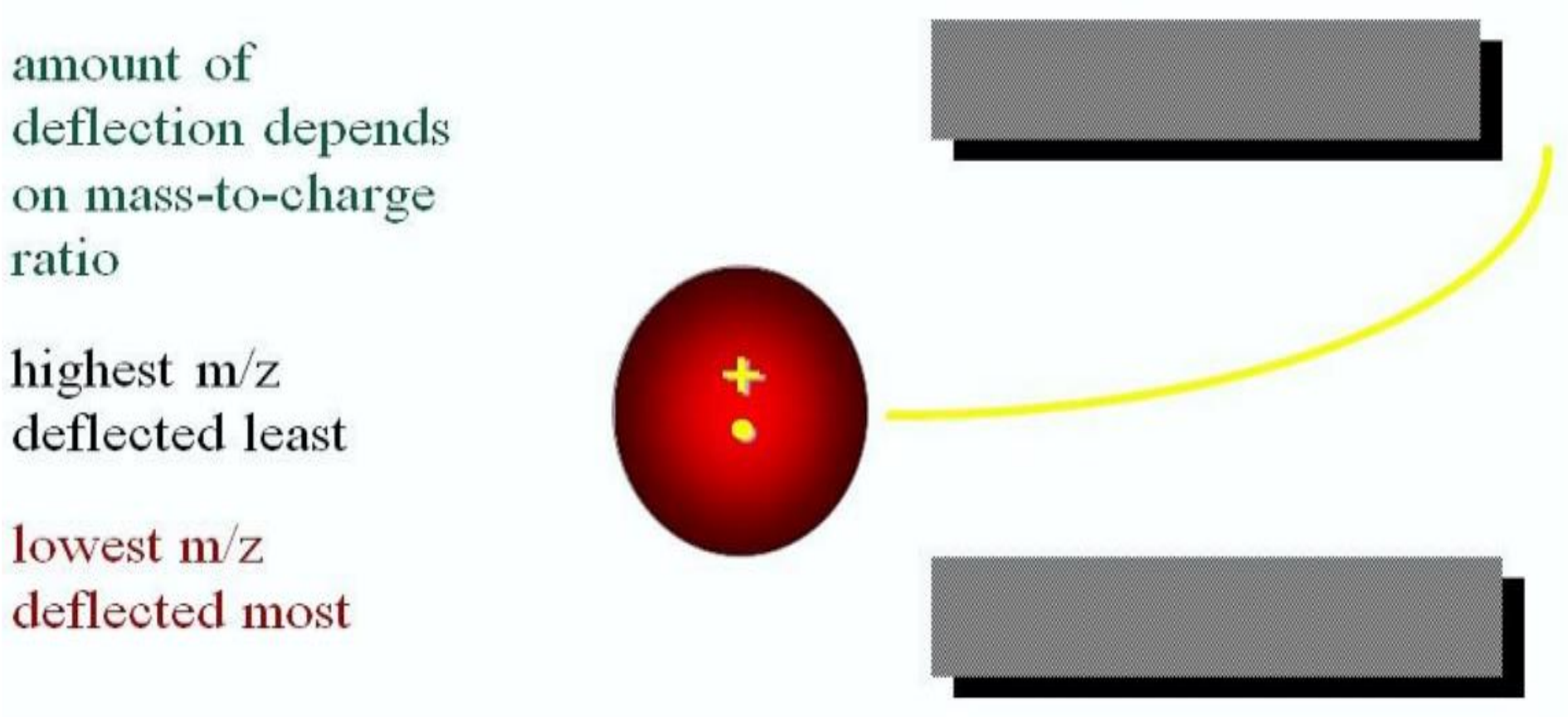


A diagram showing the evaporation of solvent leading to individual ions in an electrospray instrument.

- **Principles of Electron-Impact Mass Spectrometry:**
- Atom or molecule is hit by high-energy electron from an electron beam forming a positively charged, odd-electron species called the molecular ion.

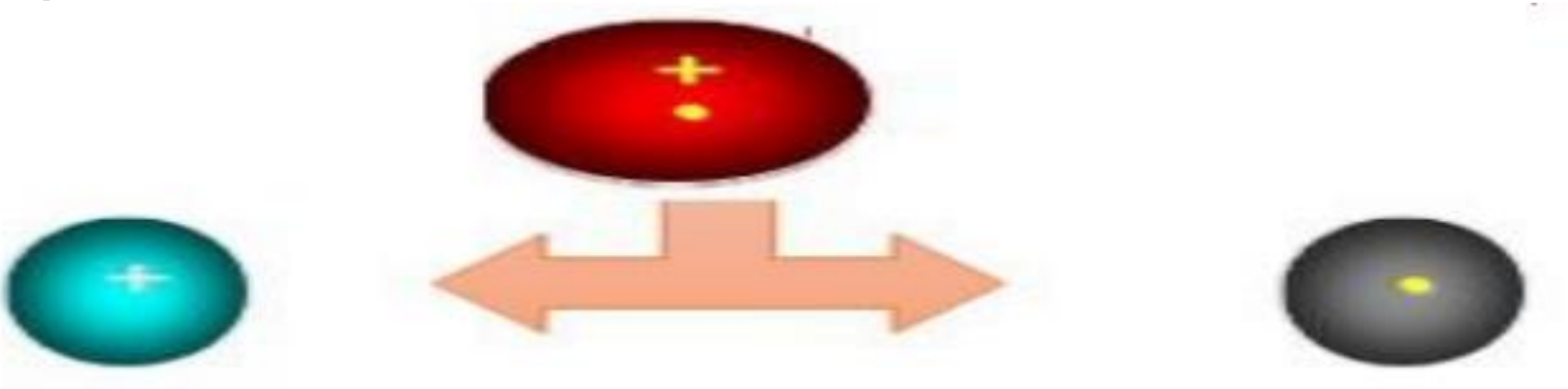
$$M + \text{Beam e 70-ev)} \rightarrow M^{+} + e$$

Molecular ion passes between poles of a magnet and is deflected by magnetic field

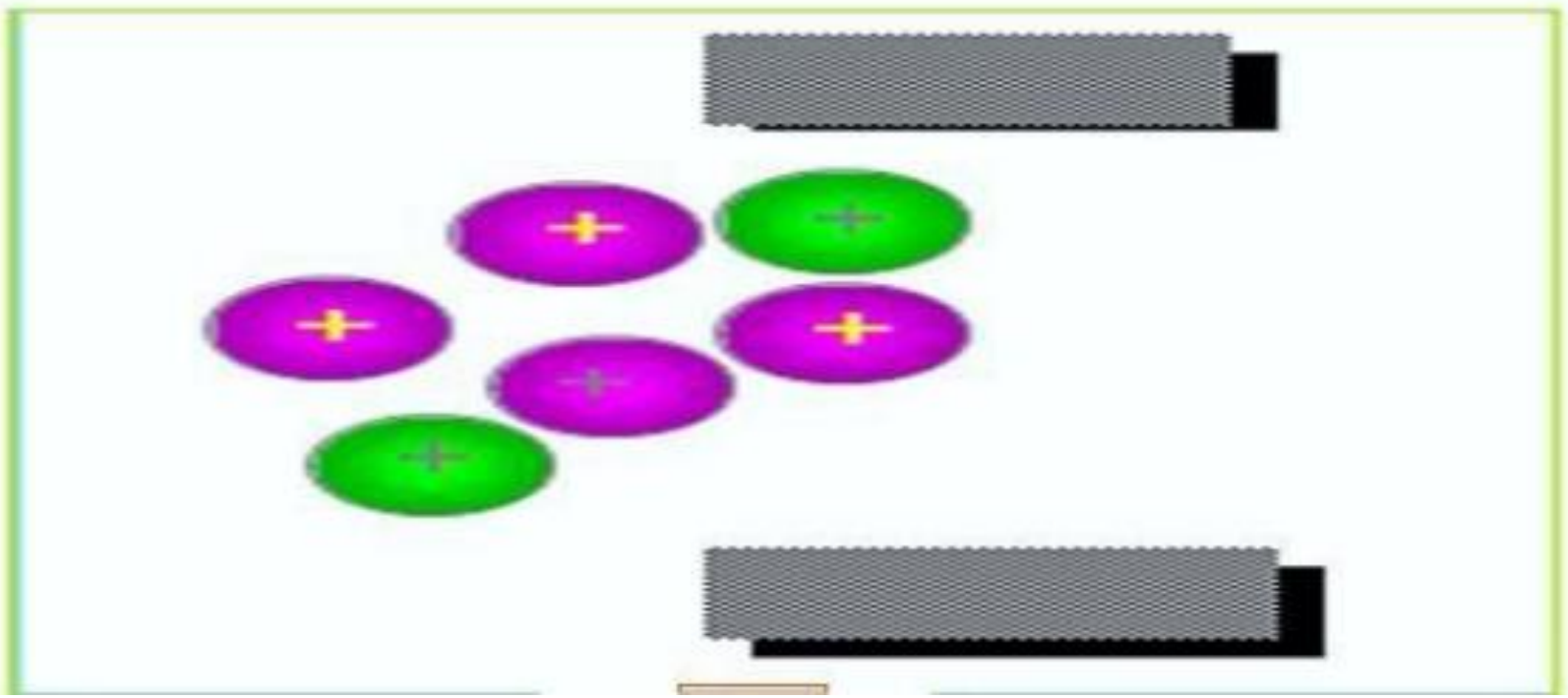


However, the molecular ion often fragments to a mixture of species of lower m/z. The molecular ion dissociates to a cation and a radical

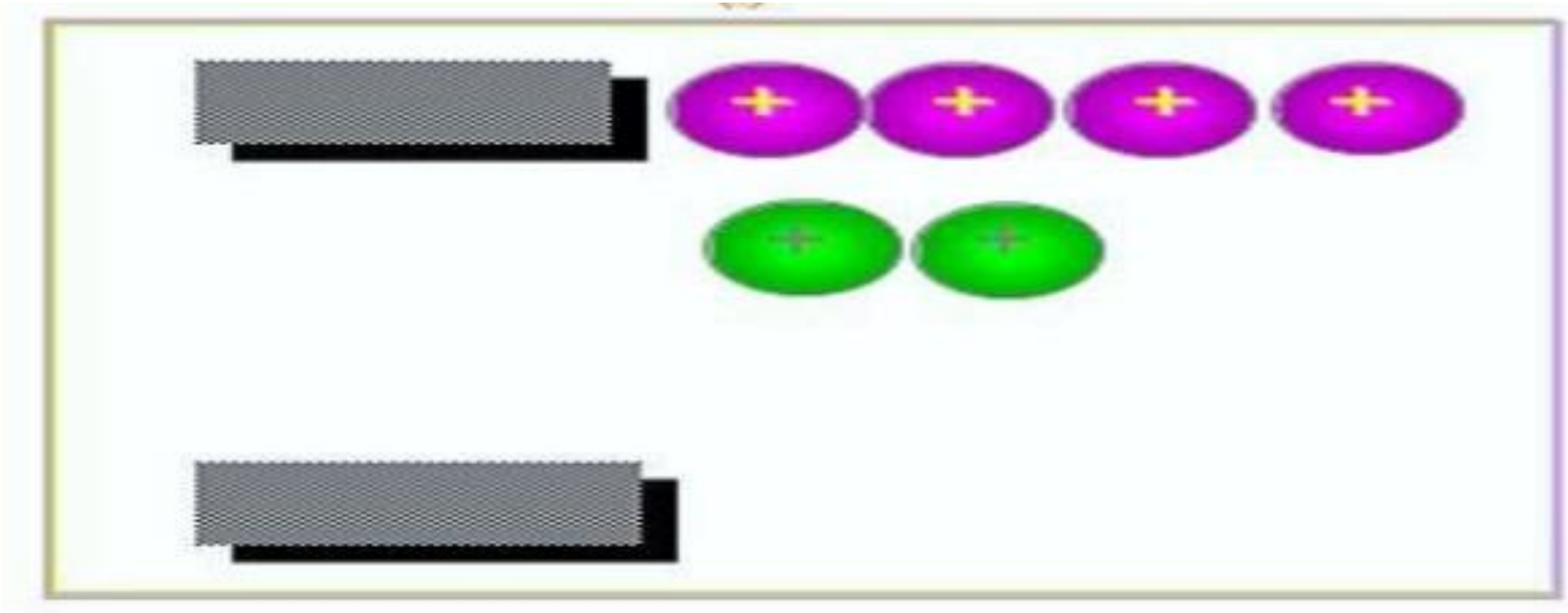
- Usually several fragmentation pathways are available and a mixture of ions is produced.



- mixture of ions of different mass gives separate peak for each m/z



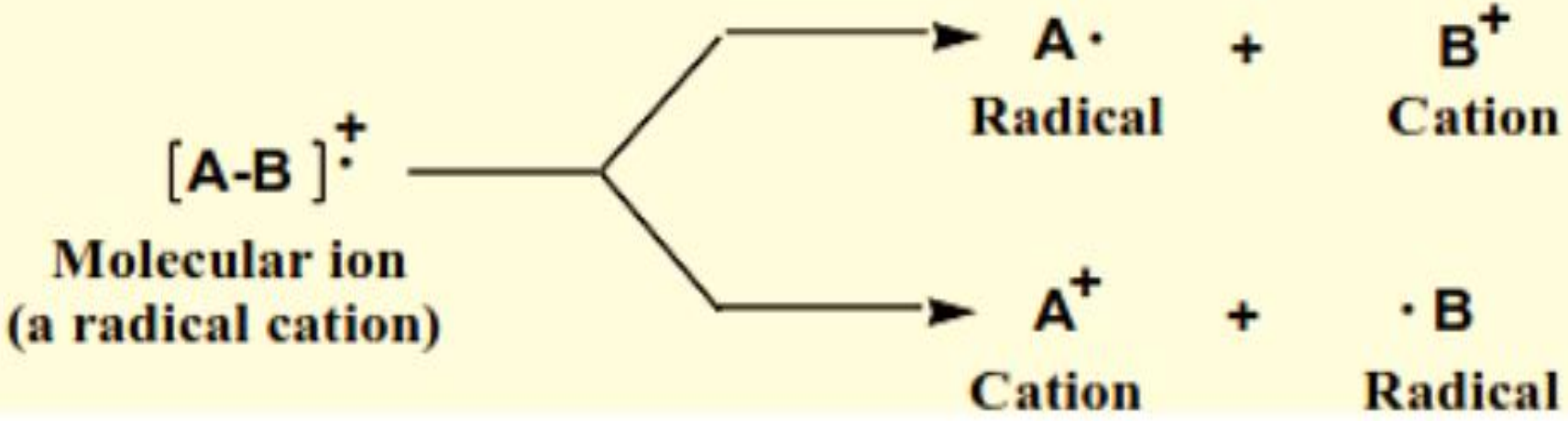
- intensity of peak proportional to percentage of each ion of different mass in mixture separation of peaks depends on relative mass.

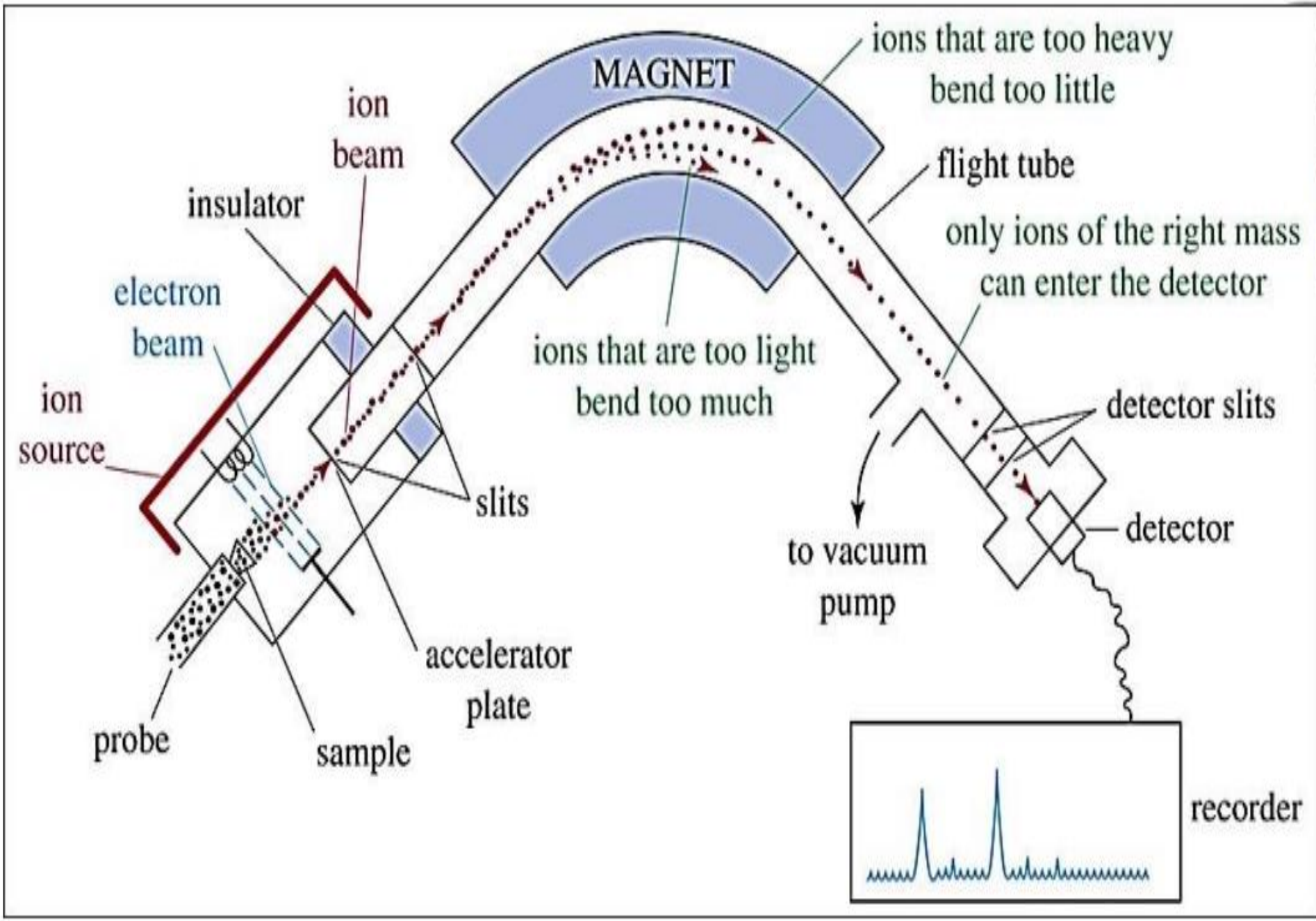


- In mass spectrometry, a substance is bombarded with an electron beam having sufficient energy to fragment the molecule. The positive fragments which are produced (cations and radical cations) are accelerated in a vacuum through a magnetic field and are sorted on the basis of mass-to-charge ratio.
- Since the bulk of the ions produced in the mass spectrometer carry a unit positive charge, the value m/e is equivalent to the molecular weight of the fragment

• The analysis of mass spectroscopy information involves the re-assembling of fragments, working backwards to generate the original molecule. A schematic representation of a mass spectrometer is shown below:

- **Fragmentation of a molecular ion, M, produces a radical and a cation. Only the cation is detected by MS**




MAGNET
ions that are too heavy
bend too little
ion
beam
flight tube
insulator
only ions of the right mass
can enter the detector
electron
beam
ions that are too light
bend too much
ion
source
detector slits
slits
detector
to vacuum
pump
accelerator
plate
probe
sample
recorder

- A very low concentration of sample molecules is allowed to leak into the ionization chamber (which is under a very high vacuum) where they are bombarded by a high-energy electron beam.
- The path of the charged molecules is bent by an applied magnetic field.
- Ions having low mass (low momentum) will be deflected most by this field and will collide with the walls of the analyzer.
- Likewise, high momentum ions will not be deflected enough and will also collide with the analyzer wall.

- Ions having the proper mass-to-charge ratio, however, will follow the path of the analyzer, exit through the slit and collide with the Collector
- This generates an electric current, which is then amplified and detected.
- By varying the strength of the magnetic field, the mass- to charge ratio which is analyzed can be continuously varied.

High-Resolution Molecular Ion

- A unique molecular formula (or fragment formula) can often be derived from a sufficiently accurate mass measurement alone (high-resolution mass spectrometry).

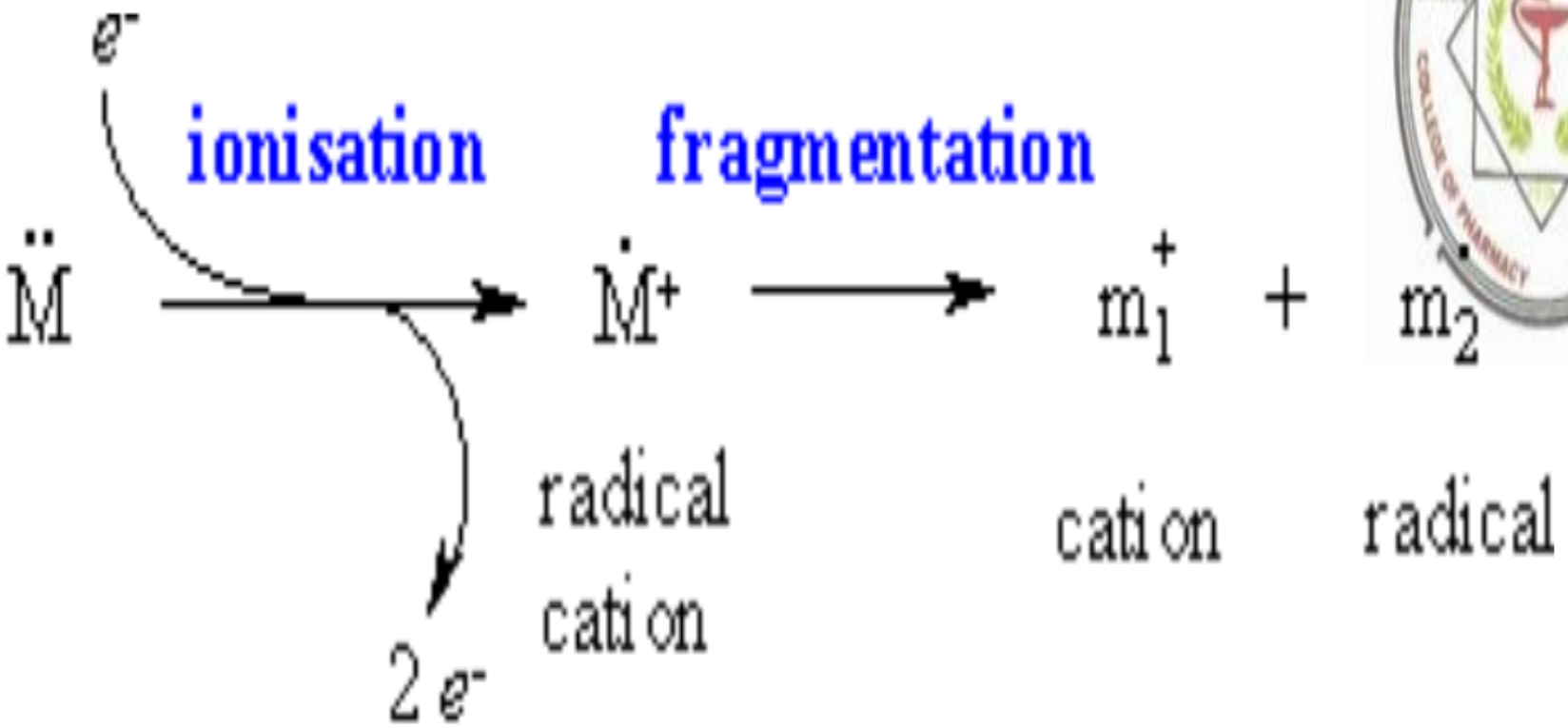
- This is possible because the nuclide masses are not integers (see Table 2.2). For example, we can distinguish at a unit mass of 28 among CO, N_2, CH_2N, and C_2H_4.

CO	N_2	CH_2N	C_2H_4
^{12}C 12.0000	$^{14}N_2$ 28.0062	^{12}C 12.0000	$^{12}C_2$ 24.0000
^{16}O 15.9949		$^{1}H_2$ 2.0156	$^{1}H_4$ 4.0312
27.9949		^{14}N 14.0031	28.0312
		28.0187	

Table 2.2 Exact Masses of Isotopes

Element	Atomic Weight	Nuclide	Mass
Hydrogen	1.00794	^{1}H	1.00783
		D(^{2}H)	2.01410
Carbon	12.01115	^{12}C	12.00000 (std)
		^{13}C	13.00336
Nitrogen	14.0067	^{14}N	14.0031
		^{15}N	15.0001
Oxygen	15.9994	^{16}O	15.9949
		^{17}O	16.9991
		^{18}O	17.9992
Fluorine	18.9984	^{19}F	18.9984
Silicon	28.0855	^{28}Si	27.9769
		^{29}Si	28.9765
		^{30}Si	29.9738
Phosphorus	30.9738	^{31}P	30.9738
Sulfur	32.066	^{32}S	31.9721
		^{33}S	32.9715
		^{34}S	33.9679
Chlorine	35.4527	^{35}Cl	34.9689
		^{37}Cl	36.9659
Bromine	79.9094	^{79}Br	78.9183
		^{81}Br	80.9163
Iodine	126.9045	^{127}I	126.9045

Ionization to Radical Cation Molecular Ion (m^+)

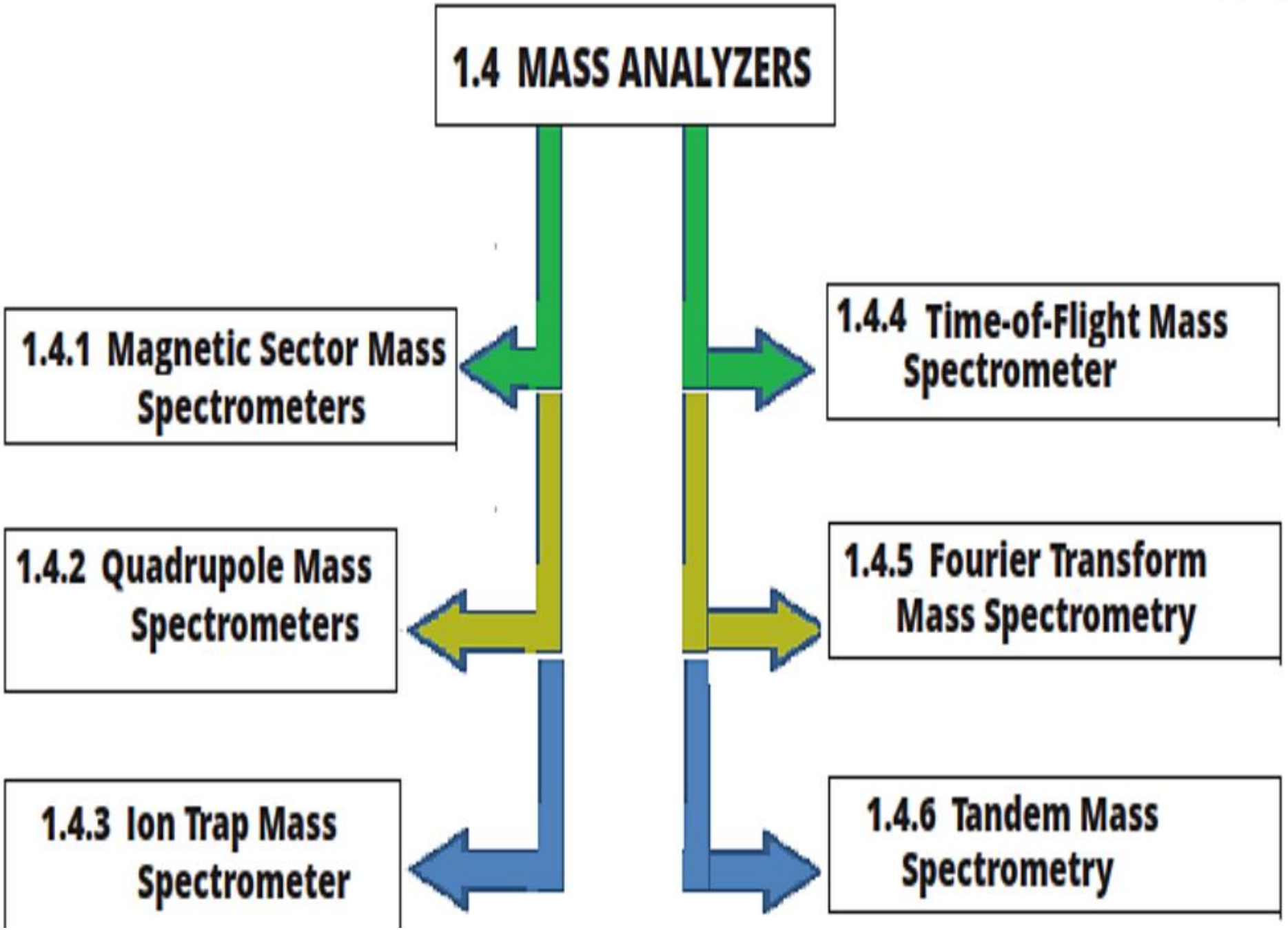


molecule

molecular ion

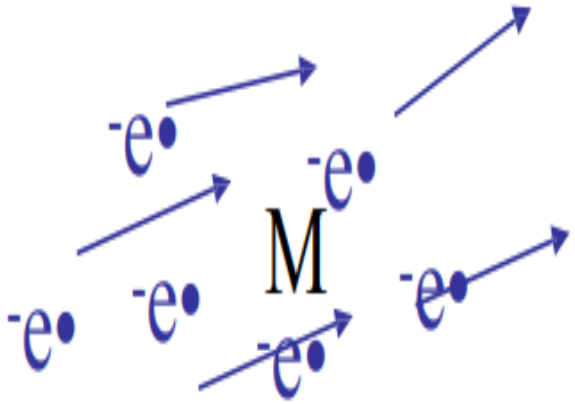
fragment ion

Molecular ion	The ion obtained by the loss of an electron from the molecule also called parent ion
Base peak	The most intense peak in the MS, assigned 100% intensity
M+	Symbol often given to the molecular ion. Mol. With an unpaired e-
Radical cation	+ve charged species with an odd number of electrons
Fragment ions	Lighter cations formed by the decomposition of the molecular ion. also called daughter ion


1.4 MASS ANALYZERS
1.4.1 Magnetic Sector Mass Spectrometers
1.4.2 Quadrupole Mass Spectrometers
1.4.3 Ion Trap Mass Spectrometer
1.4.4 Time-of-Flight Mass Spectrometer
1.4.5 Fourier Transform Mass Spectrometry
1.4.6 Tandem Mass Spectrometry

Molecular Ions give us the molecular mass

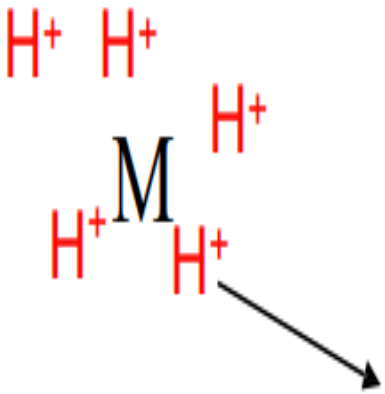
Electron Impact



Dislodges an electron

$M^{+}\bullet$

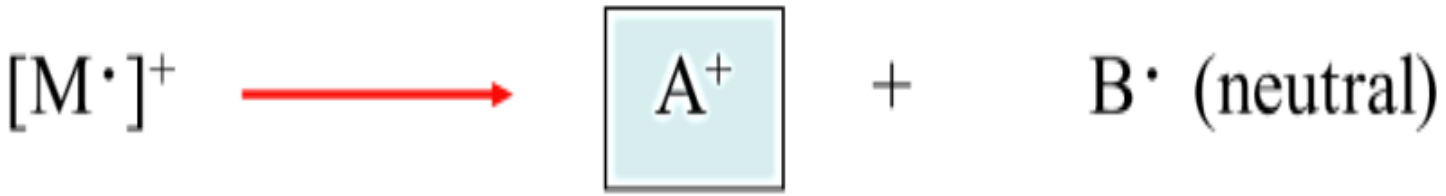
Chemical Ionization



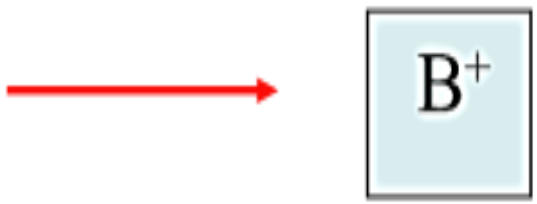
$[M+H]^{+}$

Weighs ***one more*** than MW

EI

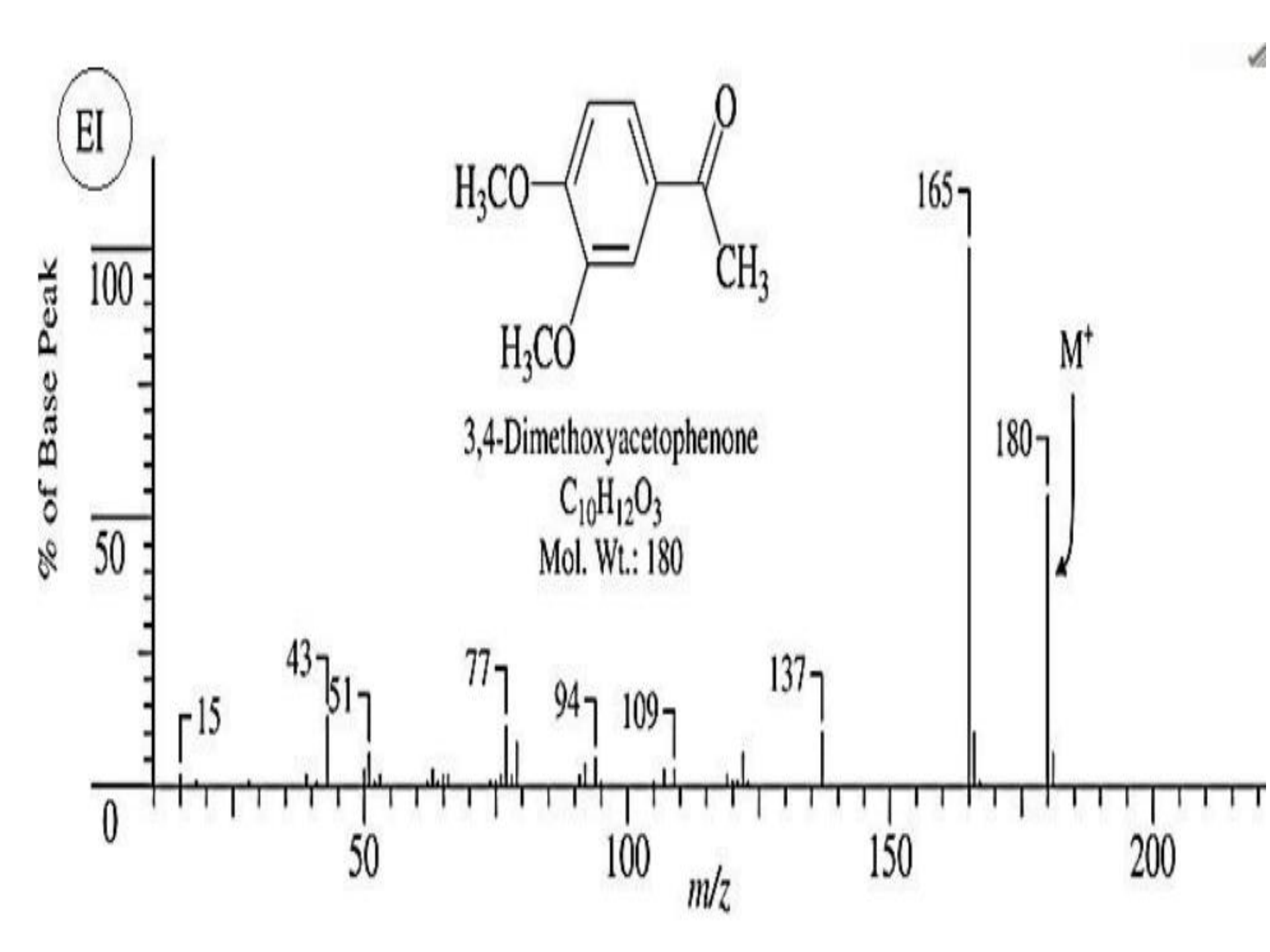


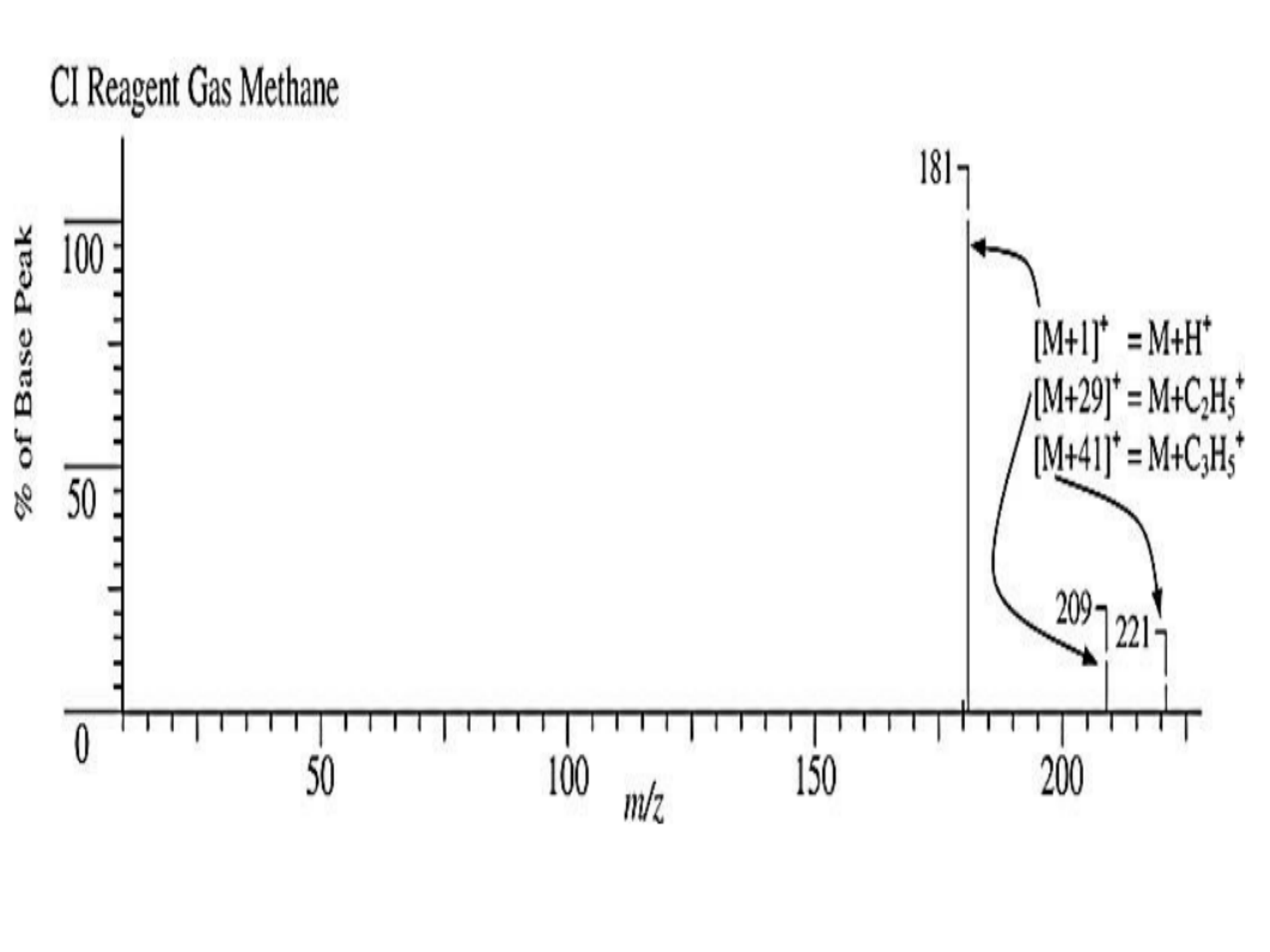
or



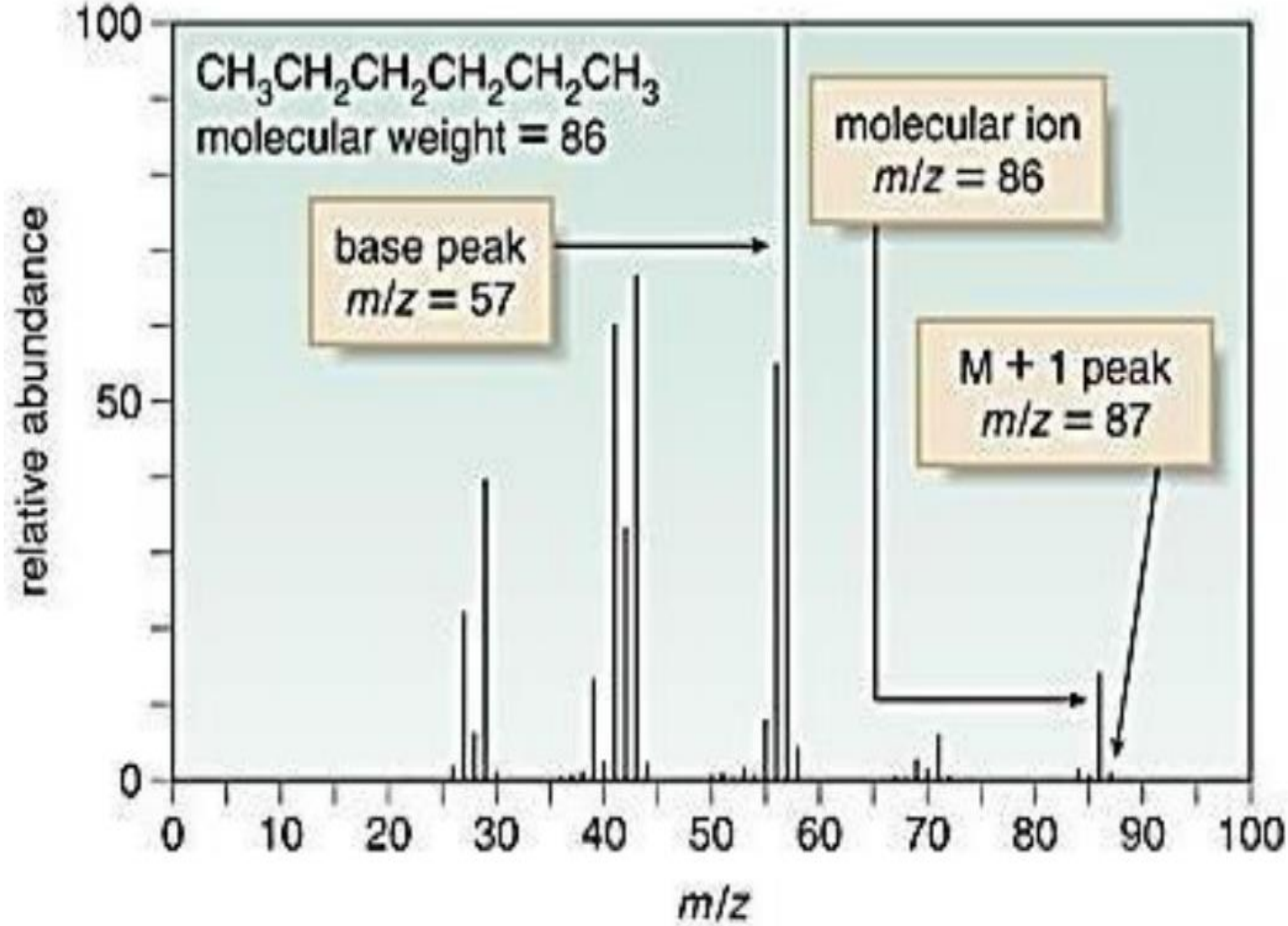
Better carbocation wins and predominates ("Stevenson's Rule")

CI


EI
% of Base Peak
100
50
0
H_3CO
H_3CO
O
CH_3
3,4-Dimethoxyacetophenone
$C_{10}H_{12}O_3$
Mol. Wt.: 180
165
180
M^+
15
43
51
77
94
109
137
50
100
150
200
m/z


CI Reagent Gas Methane
% of Base Peak
100
50
0
50
100
150
200
m/z
181
209
221
[M+1]+ = M+H+
[M+29]+ = M+C2H5+
[M+41]+ = M+C3H5+

- **The mass spectrum** :
- Mass spectra Electron Impact (EI) are routinely obtained at electron beam energy of 70 eV.
- The simplest event that occurs is the removal of a single electron from the molecule in the gas phase by an electron of the electron beam to form the molecular ion, which is a radical cation (M.+) .
- The output of the mass spectrometer shows a plot of relative intensity vs the mass-to-charge ratio (m/e).


100
50
0
relative abundance
CH3CH2CH2CH2CH2CH3
molecular weight = 86
base peak
m/z = 57
molecular ion
m/z = 86
M + 1 peak
m/z = 87
0
10
20
30
40
50
60
70
80
90
100
m/z

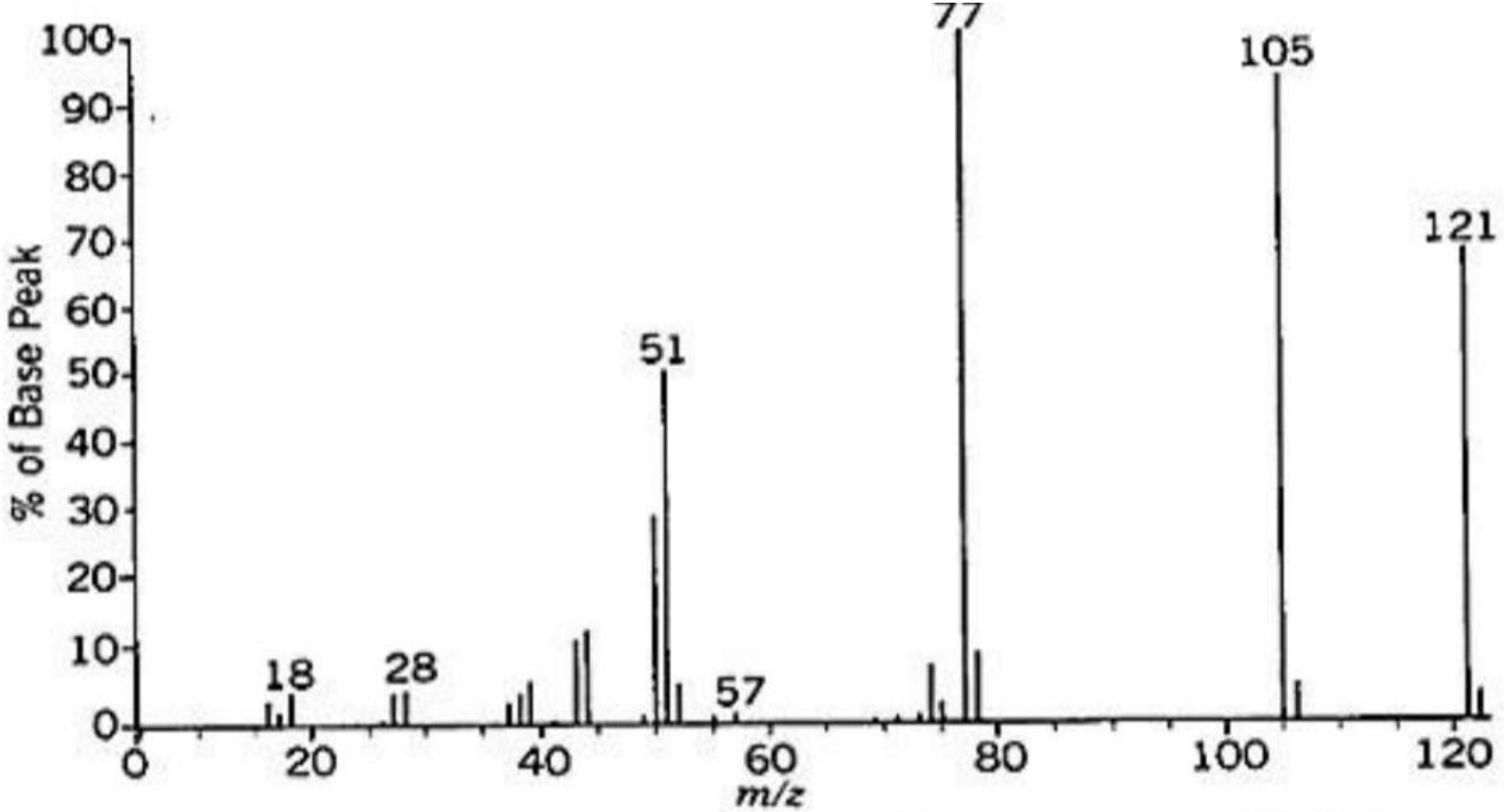


Figure 2.1. Computer-generated, electron-impact (EI) mass spectrum of benzamide $\left(C_6H_5—\overset{\overset{O}{\|}}{C}—NH_2\right)$ in bar graph form.

- The most intense peak in the spectrum is termed the base peak and all others are reported peak in relative to its intensity.
- Because M +• is unstable, some ions decompose to form fragments of radicals and cations that have a lower molecular weight than M+•.
- Electron is first removed from site with lowest ionization potential – non-bonding electrons > pi bond electrons > sigma bond electrons – NB > π > σ • Only CHARGED species are detected.

Recognition of the Molecular IonPeak:

- • The process of fragmentation follows simple and predictable chemical pathways and the ions which are formed will reflect the most stable cations and radical cations which that molecule can form.

molecular ion (M+): is the highest molecular weight peak observed in a spectrum will typically represent the parent molecule, minus an electron.

What information can be determined?

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

Problems in recognition of the molecular ion peak:

- Generally, small peaks are also observed above the calculated molecular weight due to the natural isotopic abundance of 13C, 2H, etc. 2 under electron impact (EI) recognition of the molecular ion peak (M) poses a problem:
- a : the peak may be very weak or may not appear at all
- B: we cannot be sure that is the molecular ion peak and not a fragment peak or an impurity.

- The best solution is to obtain a chemical ionization spectrum. The usual result is an intense peak at M + 1 and little fragmentation.
- C: Many molecules with especially labile protons do not display molecular ions; an example of this is alcohols, where the highest molecular weight peak occurs at m/e one less than the molecular ion (m - 1).

The intensity of the molecular ion peak depends on the stability of the molecular ion. The most stable molecular ions are those of purely aromatic systems.

If substituents that have favorable modes of cleavage arepresent, the molecular ion peak will be less intense, and the fragment peaks relatively more intense

In general:

the following group of compounds will, in order of decreasing ability, give prominent molecular ion peaks:

aromatic compounds > conjugated alkenes > cyclic compounds > organic sulfides > short, normal alkanes

Recognizable molecular ions are usually produced for these compounds in order of decreasing ability:

ketones > amines > esters > ethers > carboxylic acids ~ aldehydes ~ amides ~ halides.

The molecular ion is frequently not detectable in aliphatic alcohols, nitrites, nitrates, nitro compounds, nitriles, and in highly branched compounds.

Fragments can be identified by their mass-to-charge ratio, but it is often more informative to identify them by the mass which has been lost. That is:

The presence of an M − 15 peak (loss of CH_3), or an M − 18 peak (loss of H_2O), or an M − 31 peak (loss of OCH_3 from methyl esters), and so on, is taken as confirmation of a molecular ion peak. An M − 1 peak is common, and occasionally an M − 2 peak (loss of H_2 by either fragmentation or thermolysis), or even a rare M − 3 peak (from alcohols) is reasonable.

- Peaks in the range of M_ 3 to M_14, however, indicate that: • 1. Contaminants may be present. • 2. The presumed molecular ion peak is actually a fragment ion.

Losses of fragments of masses 19 _ 25 are also unlikely (except

for loss of F = 19 or HF = 20 from fluorinated compounds). Loss of 16 (O), 17 (OH), or 18 (H_2O) are likely only if an oxygen atom is in the molecule.

the next step is *chemical ionization* (CI), which usually yields a prominent $[M + H]^+$ peak with little fragmentation.

These CI $[M + H]^+$ ions *(quasimolecular ions)* are often prominent. Chemical ionization spectra sometimes have prominent $[M - H]^+$ ions because of hydride ion abstraction from the $M^{\cdot+}$ ion by CH_5^+. Since the $[M + H]^+$ ions are chemically produced, they do not have the great excess of energy associated with ionization by electron impact, and they undergo less fragmentation. For example, the EI spectrum of 3,4-dimethox-

The "Nitrogen Rule"

- When the number of nitrogen atoms present in the molecule is odd, the molecular mass will be an odd number

  (If m/z is odd, then the number of nitrogens is odd)

- When the number of nitrogen atoms present in the molecule is even or zero, the molecular mass will be an even number.

  (If m/z number is even, the number of nitrogens in the compound is even.)

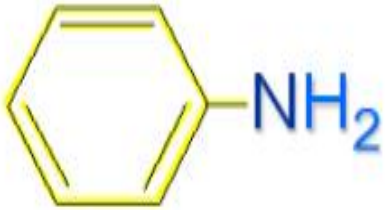
  (Note: 0 is even)

For example, ethylamine, $C_2H_5NH_2$ has one nitrogen atom, and its mass is an odd number (45).

Ethylenediamine, $H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$, has two nitrogen atoms, and its mass is an even number (60)

m / z

93



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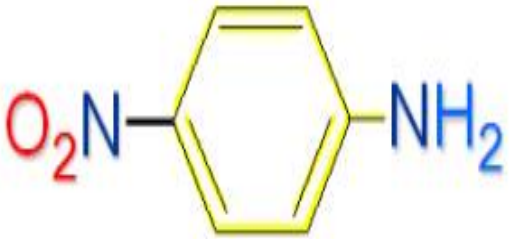

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Sample Introduction/Sources

Volatiles

- Probe/electron impact (EI),Chemical ionization (CI)
- GC/EI,CI

Involatiles

- Direct infusion/electrospray (ESI)
- HPLC/ESI
- Matrix Assisted Laser Adsorption (MALDI)

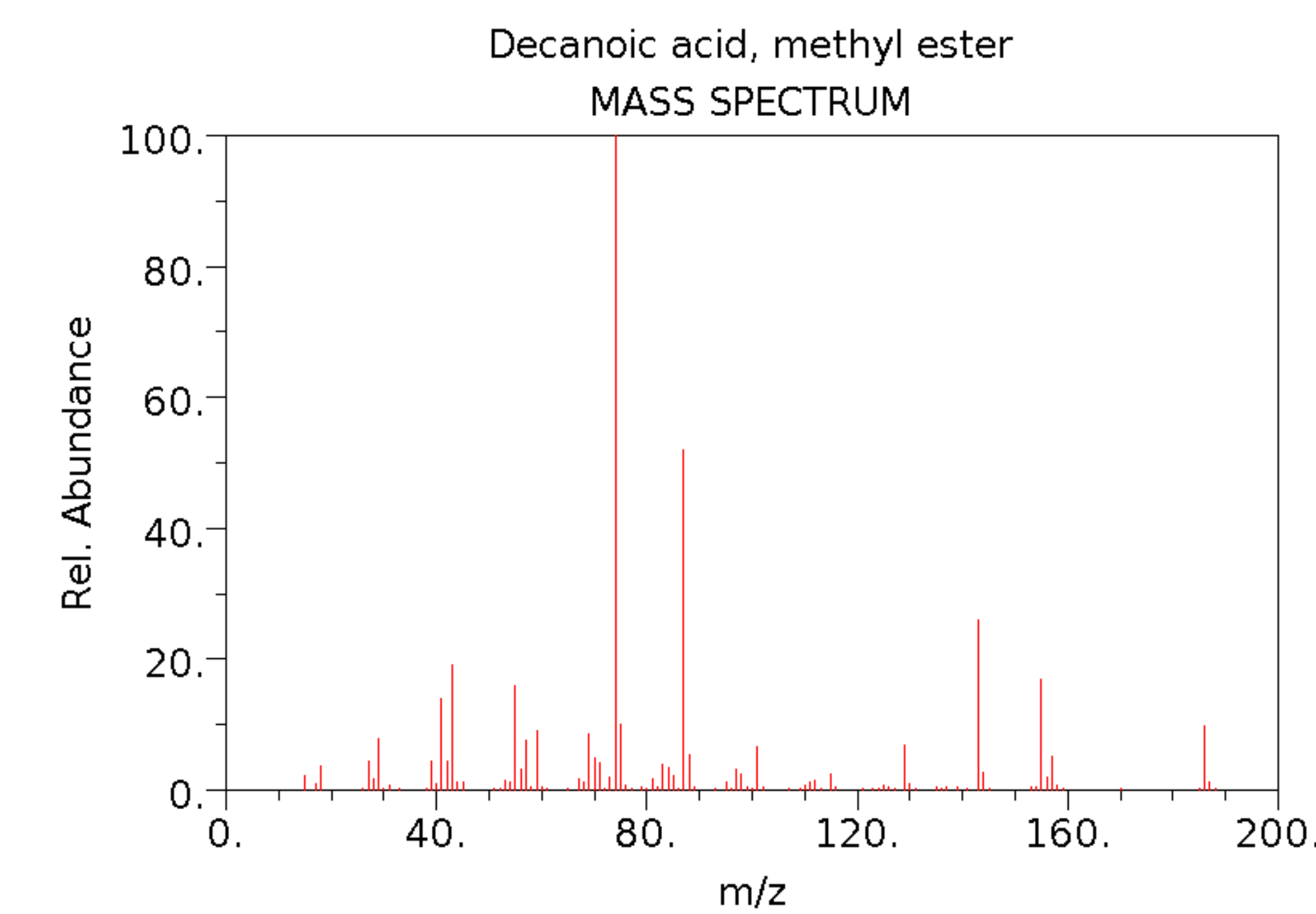
Elemental mass spec

- Inductively coupled plasma (ICP)

EI(Electron Impact), CI(Chemical Ionization)

- **EI (hard ionization)**
  - Gas-phase molecules enter source through heated probe or GC column
  - 70 eV electrons bombard molecules forming M+* ions that fragment in unique reproducible way to form a collection of fragment ions
  - EI spectra can be matched to library stds

  CI (soft ionization)
  - Higher pressure of methane leaked into the source (mtorr)
  - Reagent ions transfer proton to analyte

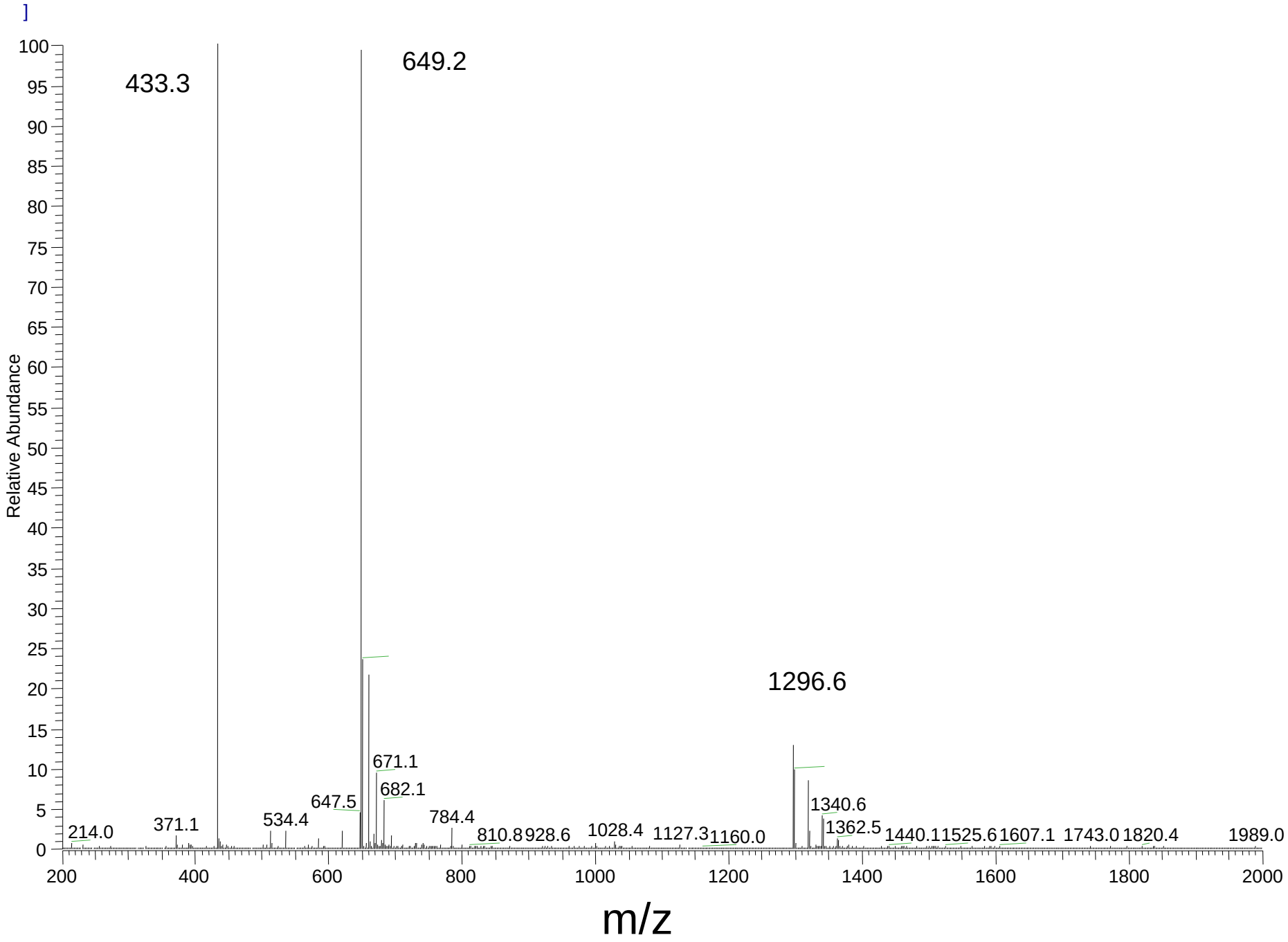

Decanoic acid, methyl ester
MASS SPECTRUM
100.
80.
60.
40.
20.
0.
Rel. Abundance
0.
40.
80.
120.
160.
200.
m/z
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

CI/ ion-molecule reaction

- $2CH_4 + e^- \rightarrow CH_5^+$ and $C_2H_5^+$
- $CH_5^+ + M \rightarrow MH^+ + CH_4$
- The excess energy in MH^+ is the difference in proton affinities between methane and M, usually not enough to give extensive fragmentation

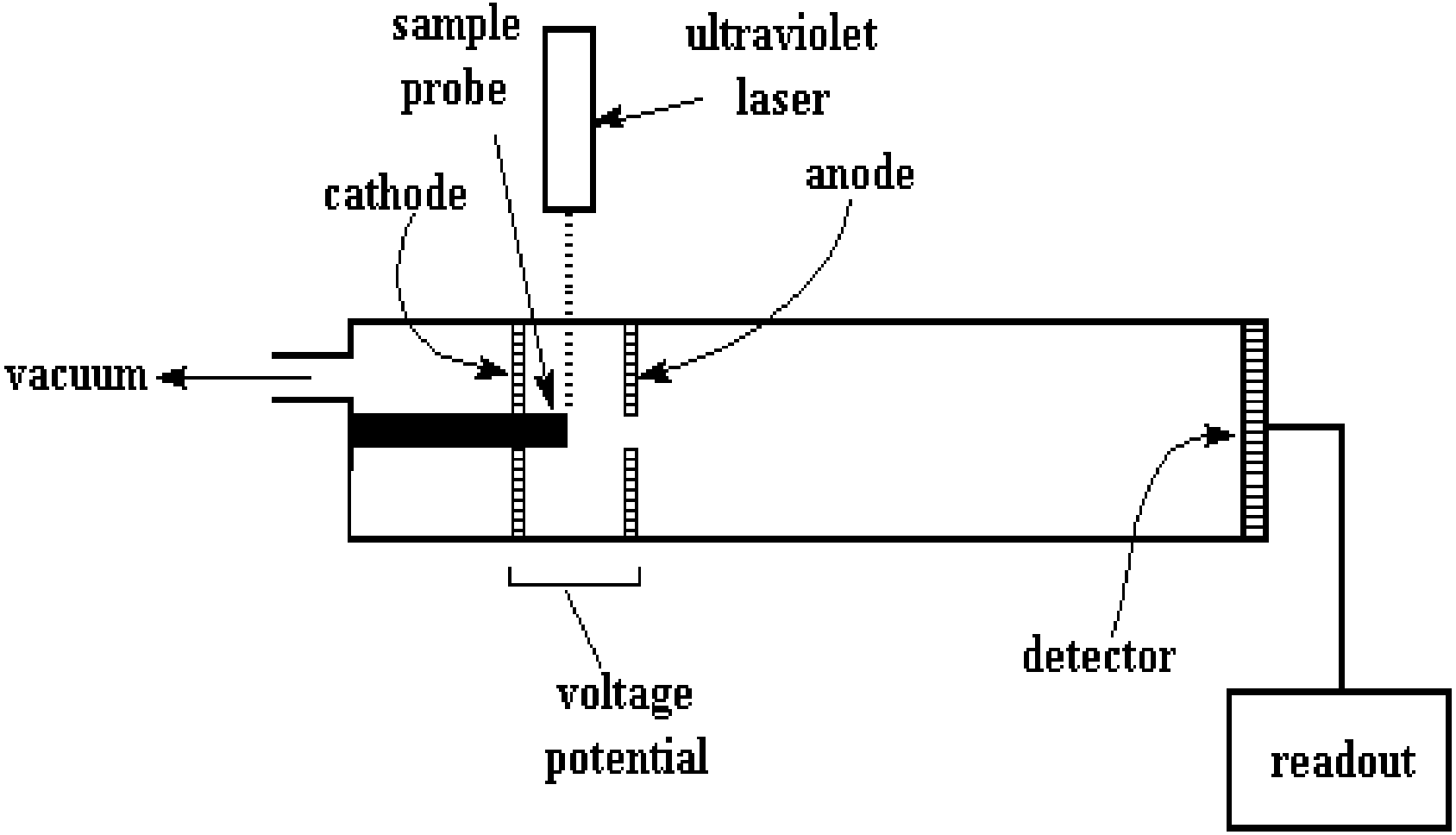
Electrospray

- 5 kV voltage on a needle
- Nebulization gas
- Produces gas-phase protonated analytes
- Little to no fragmentation
- Multiple charging
- 10 μM angiotensin at 5 μl/min direct infusion, MW 1269


]
433.3
649.2
1296.6
671.1
682.1
647.5
534.4
784.4
371.1
214.0
810.8
928.6
1028.4
1127.3
1160.0
1340.6
1362.5
1440.1
1525.6
1607.1
1743.0
1820.4
1989.0
Relative Abundance
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
200
400
600
800
1000
1200
1400
1600
1800
2000

MALDI

- Matrix -UV absorber, ex. picolinic acid, cinnimic acid
- Singlely charged ions
- Need mass analyzer with a large m/z range – TOF
- Laser pulse as opposed to continuous source



A simplified diagram of a MADLI apparatus

(After Creel, H., *Trends in Polym. Sci.*, 1993, *1(11)*, 336-342.)

Mass Analyzers

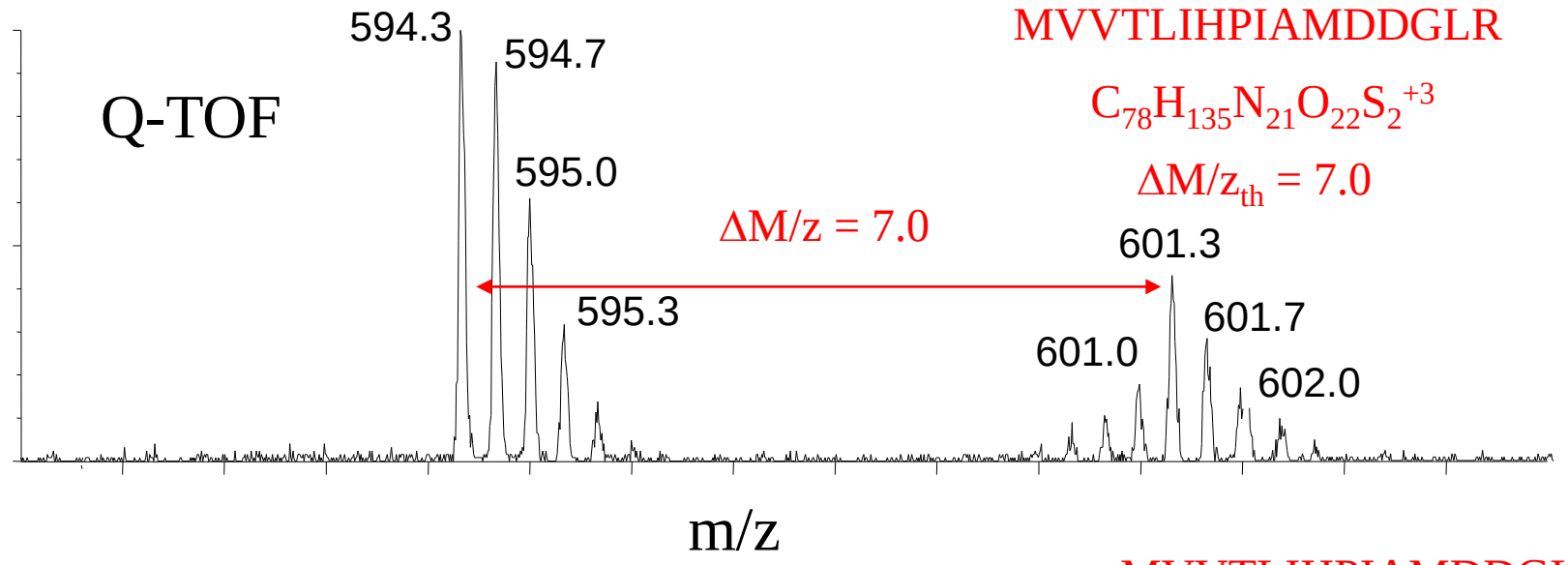
- Low resolution
  - Quadrupole
  - Ion trap
- High resolution
  - TOF time of flight
  - Sector instruments (magnet)
- Ultra high resolution
  - ICR ion cyclotron resonance

Resolution

- $R = m/z/\Delta m/z$
- Unit resolution for quad and trap
- TOF up to 15000
- FT-ICR over 30000
  - MALDI, Resolve ^{13}C isotope for a protein that weighs 30000
  - Resolve charge states 29 and 30 for a protein that weighs 30000

High vs low Res ESI

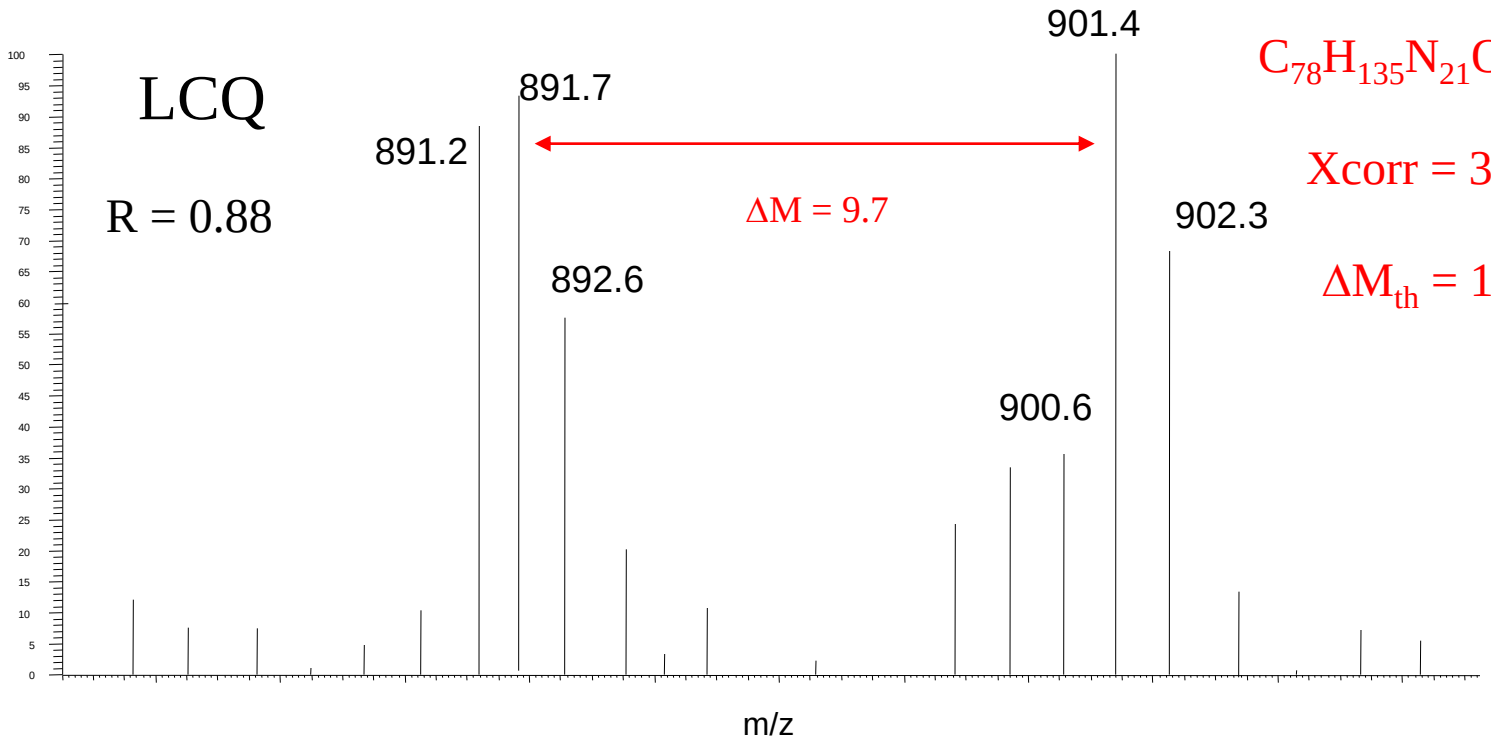
- Q-TOF, ICR
  - complete separation of the isotope peaks of a +3 charge state peptide
  - Ion abundances are predictable
  - Interferences can be recognized and sometimes eliminated
- Ion trap, Quad
  - Unit resolution



MVVTLIHPIAMDDGLR

$C_{78}H_{135}N_{21}O_{22}S_2^{+3}$

$\Delta M/z_{th} = 7.0$



MVVTLIHPIAMDDGLR

$C_{78}H_{135}N_{21}O_{22}S_2^{+2}$

Xcorr = 3.09

$\Delta M_{th} = 10.5$

Exact Mass Determination

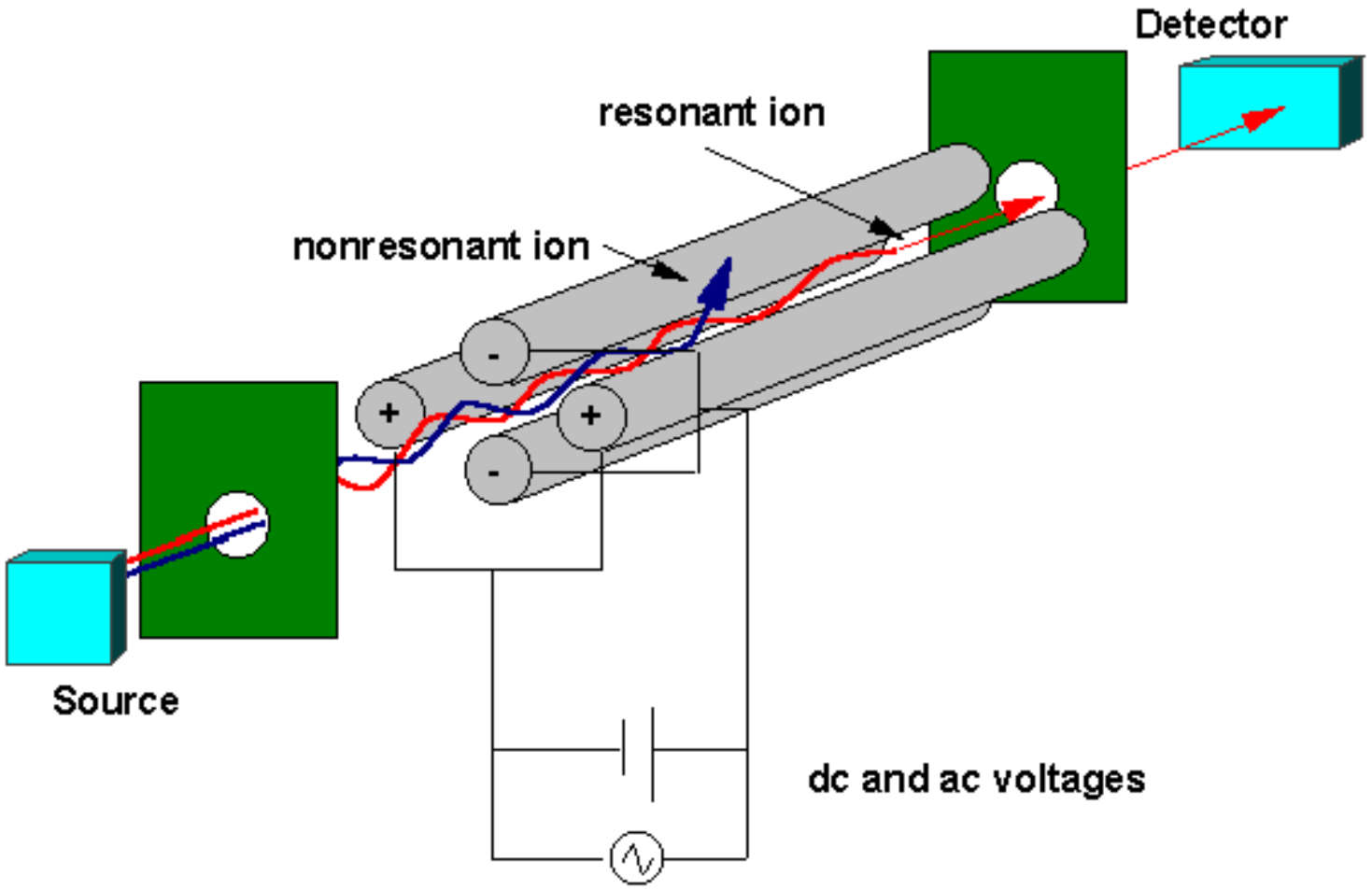
- Need Mass Spectrometer with a high mass accuracy – 5 ppm (sector or TOF)
- $C_9H_{15}NO_4$, FM 201.1001 (mono-isotopic)
- Mass accuracy = {(Mass Error)/FM}*10^6
- Mass Error = (5 ppm)(201.1001)/10^6 =

  $\pm$ 0.0010 amu

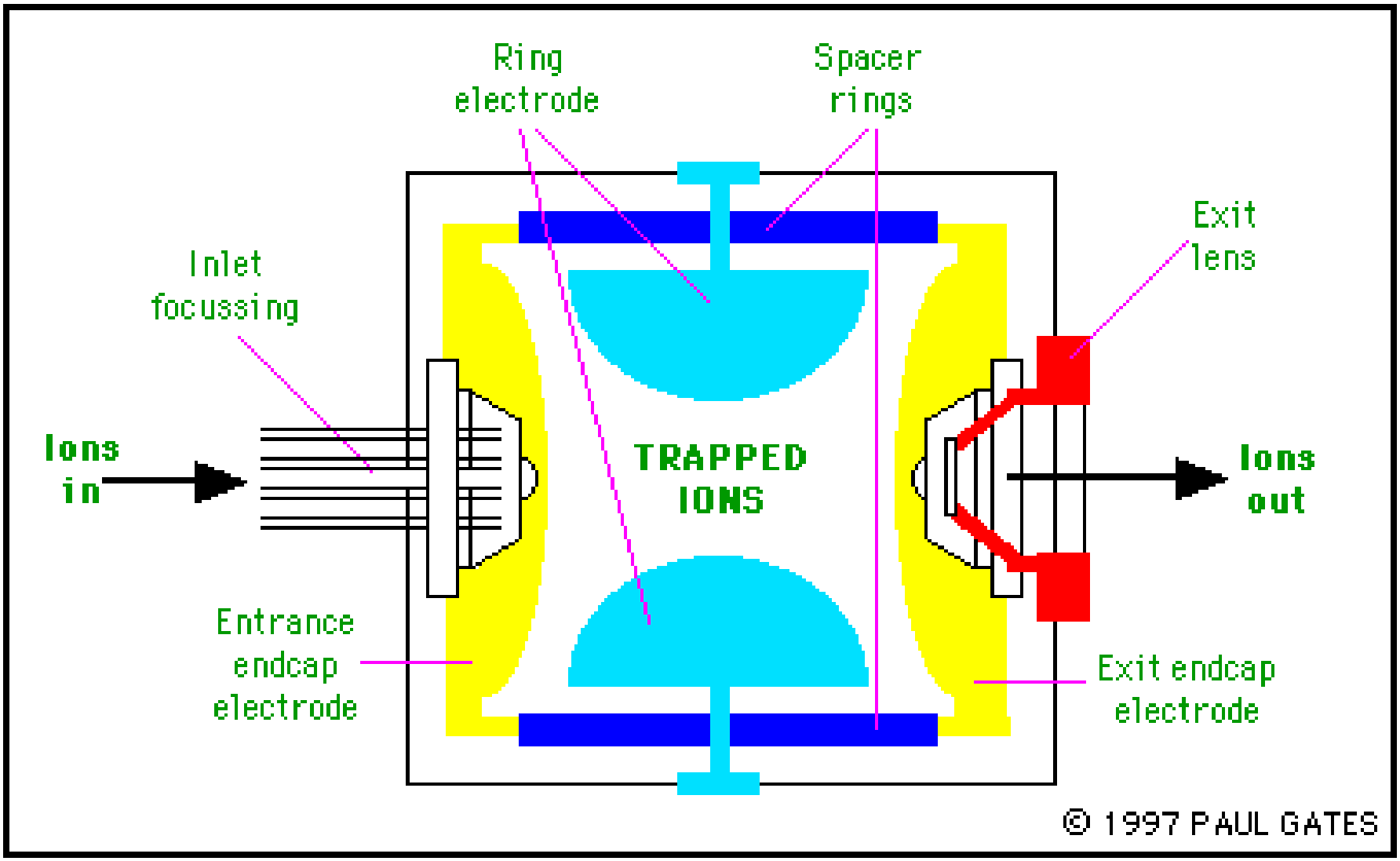
Mass accuracy

- Mass Error = (5 ppm)(201.1001)/10^6 =
  $\pm$ 0.0010 amu
- 201.0991 to 201.1011 (only 1 possibility)
- Sector instruments, TOF mass analyzers
- How many possibilities with MA = 50 ppm?
  with 100 ppm?

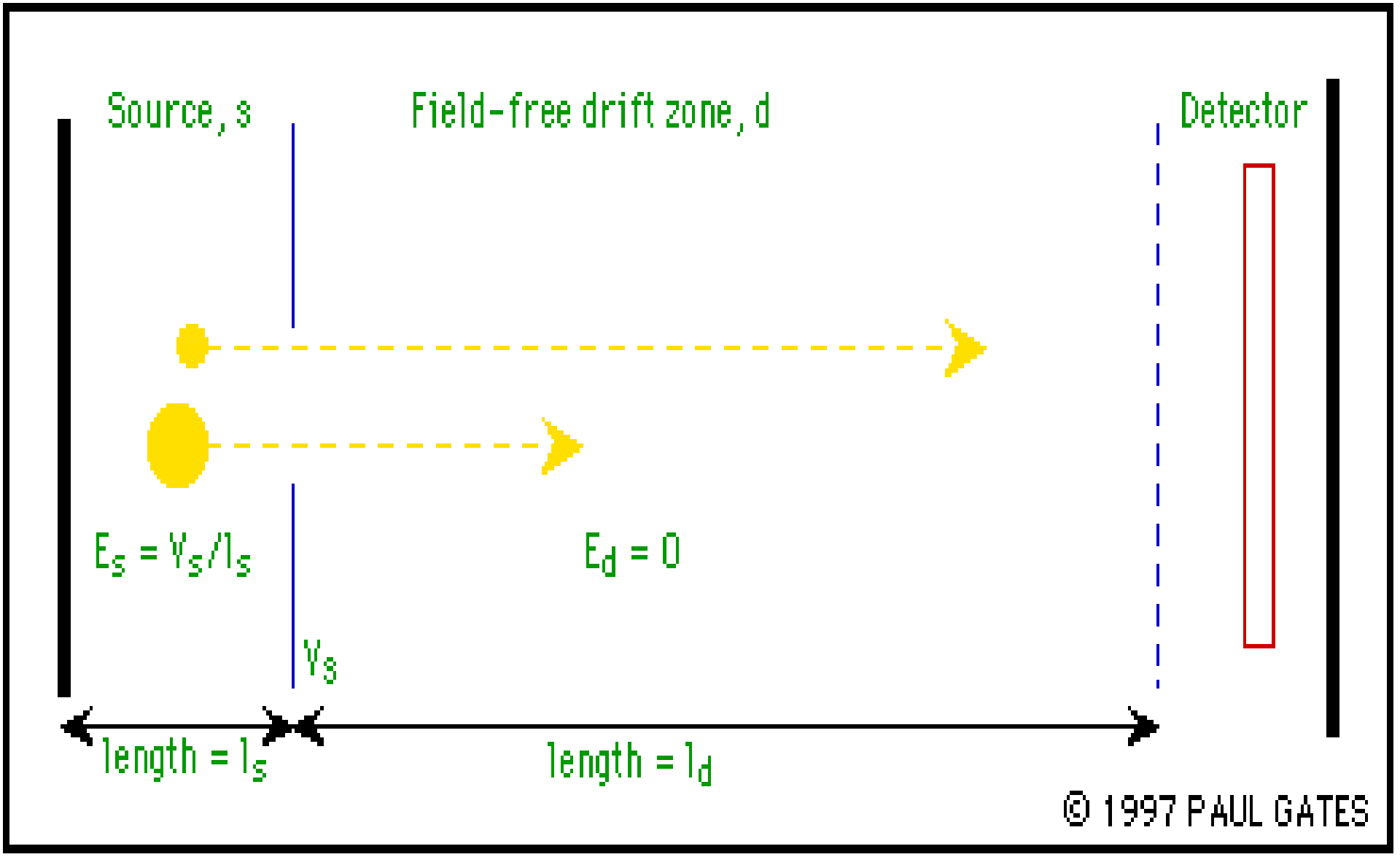
Quadrupole Mass Ion Filter



Ion Trap


Ring electrode
Spacer rings
Exit lens
Inlet focussing
Ions in
TRAPPED IONS
Ions out
Entrance endcap electrode
Exit endcap electrode
© 1997 PAUL GATES

Time of Flight -TOF



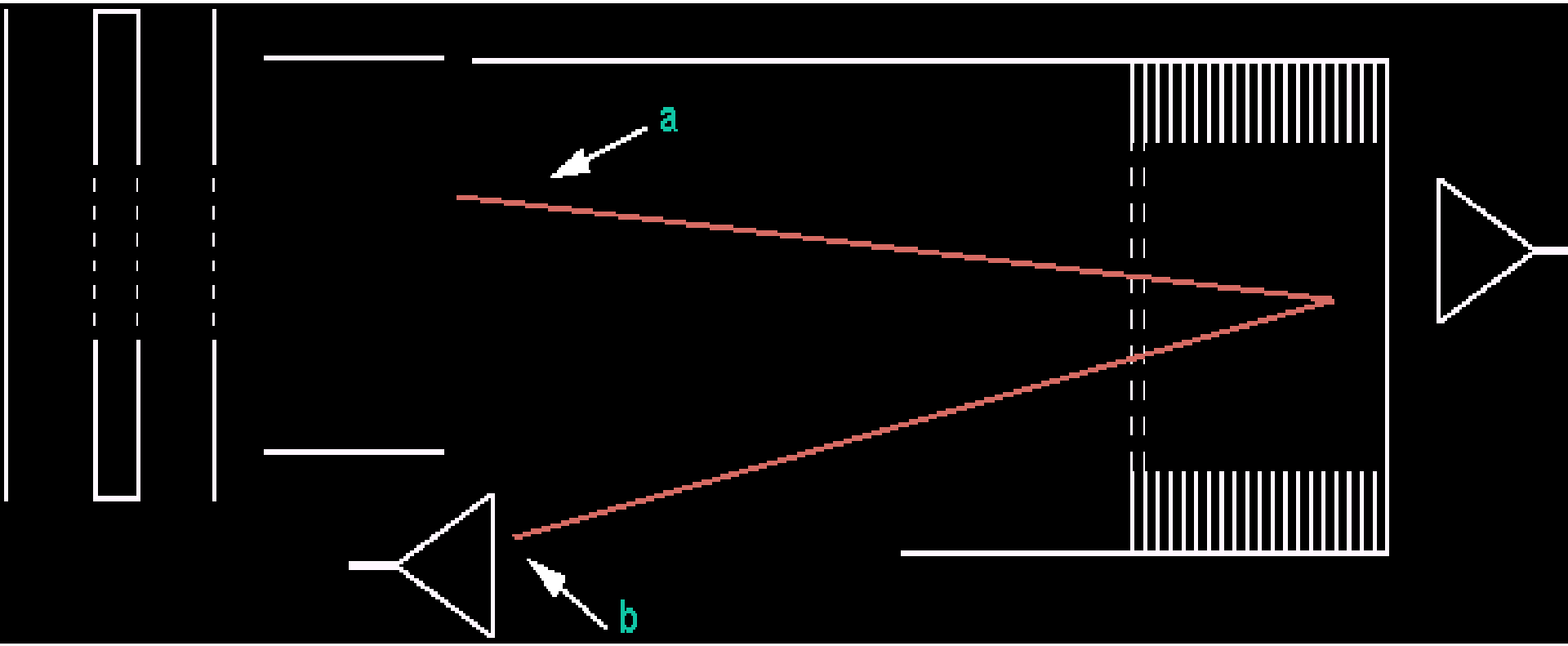
$$\frac{m_i}{z_i} = 2eE\, l_s \left(\frac{t_i}{l_d} \right)^2$$

Where:

- m_i = mass of analyte ion
- z_i = charge on analyte ion
- E = extraction field
- 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 (1.6022x10-19 C)

TOF with reflectron

http://www.rmjordan.com/tt1.html



Sector instruments

http://www.chem.harvard.edu/mass/tutorials/magnetmovie.html

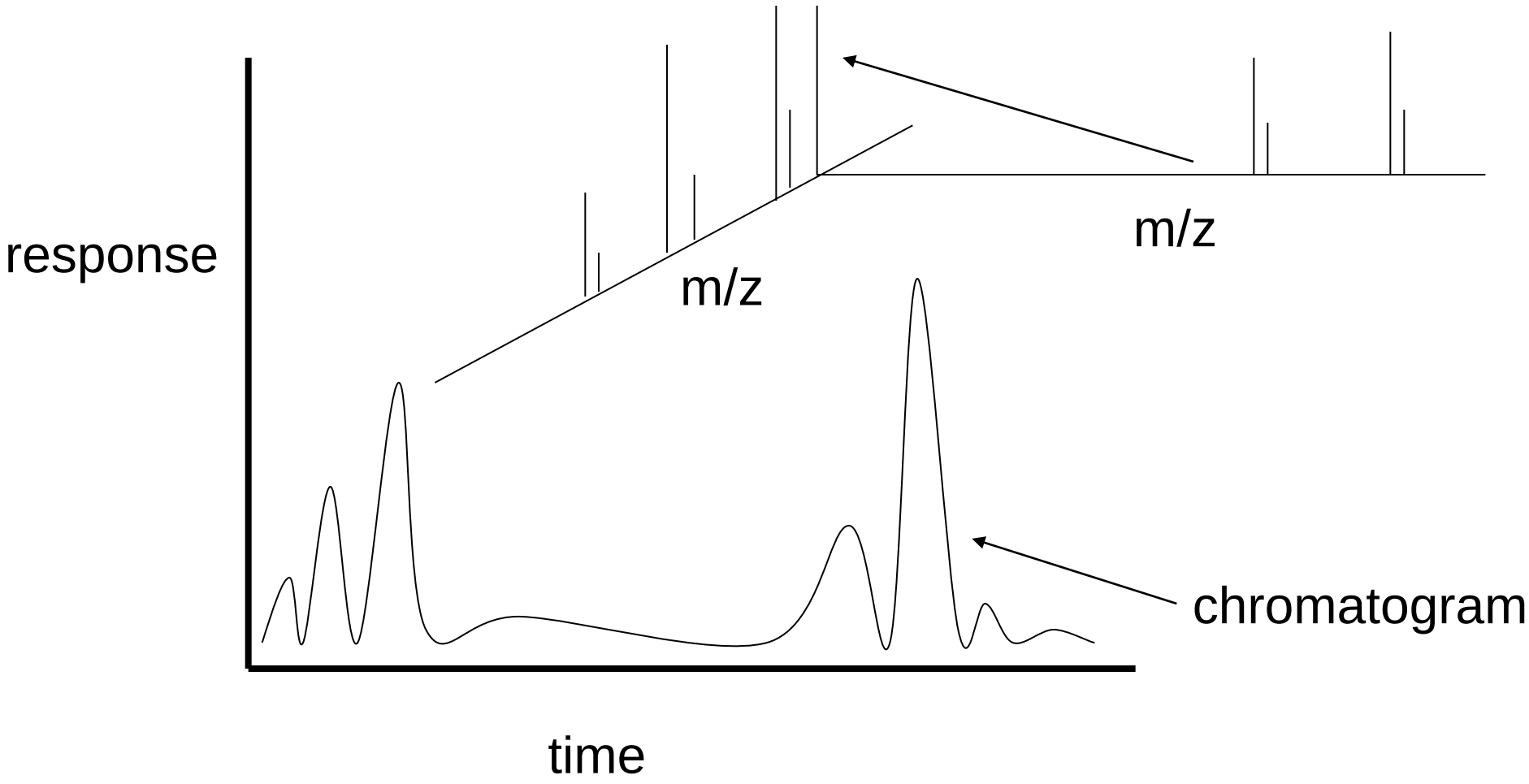
FT-ICRMS

- http://www.colorado.edu/chemistry/chem5181/MS_FT-ICR_Huffman_Abraham.pdf

CID or MS-MS

- MS-MS
  - sequencing the peptides or oligonucleotides
  - structural characterization of drugs and metabolites
  - Assay development, sensitivity enhancement

HPLC-MS-MS



hybrids

- Ex. Q-TOF
  - Trap has excellent sensitivity (can store essentially all ions), mass selectivity (can store ions of a particular m/z ratio
  - TOF is a high resolution mass analyzer
- Triple quadruple
  - Neutral loss scan

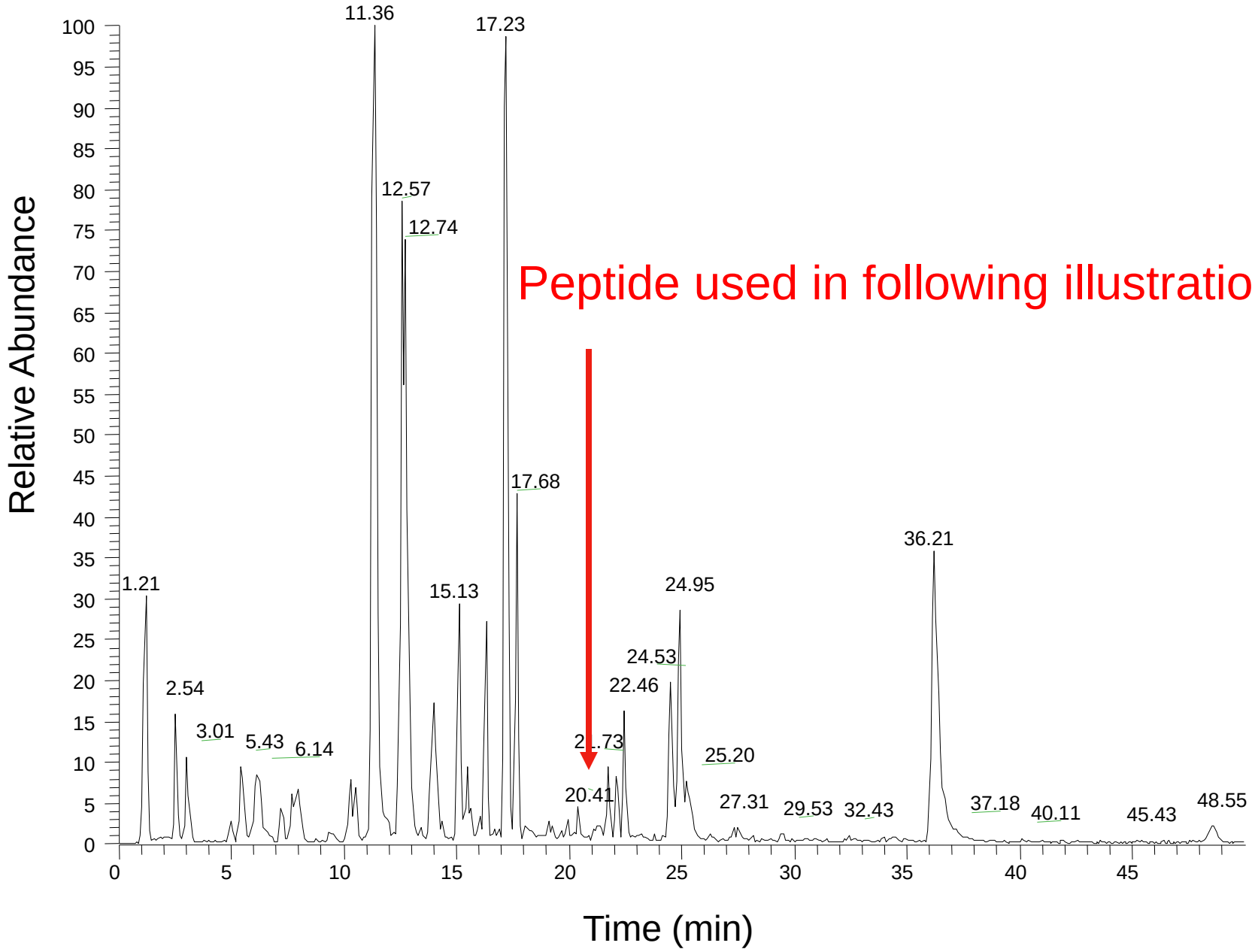
HPLC-MS

- Reverse-phase HPLC
  - Separation of involatiles (peptides)
  - The lower the flow the greater the sensitivity
  - Column ID (300 μm – 50 μm)

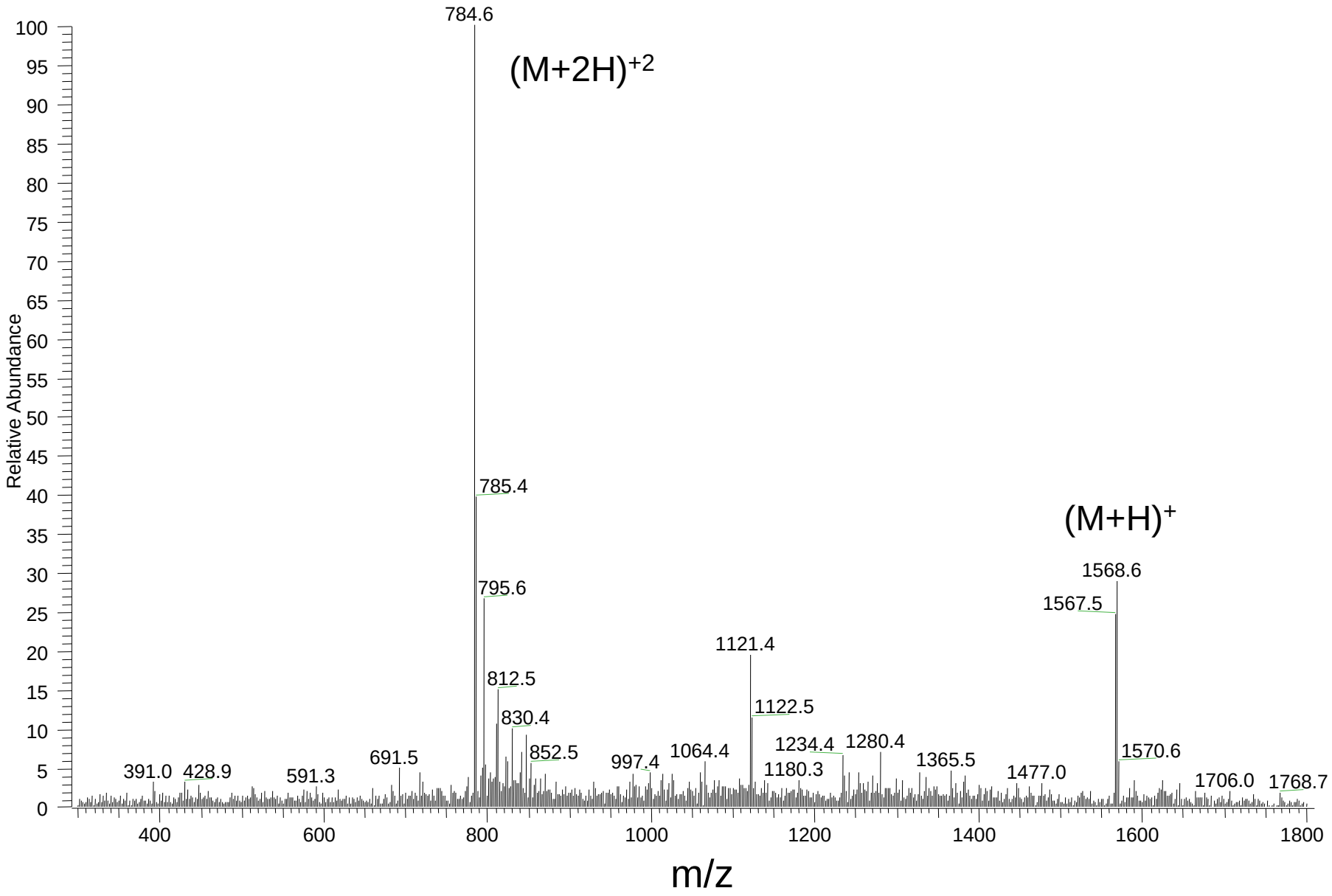
Proteomics using LC-MS

- Protein identification, characterization, quantification
- Extract proteins, fractionate proteins (typically using 2D-gel electrophoresis)
- Digest protein(s) with a protease to produce peptide mixture (lysine, arginine)
- LC-MS-MS analysis
- Database searching identifies proteins
  - Mascot, Expasy (tools)

RP-HPLC Separation of a Tryptic Digest of BSA

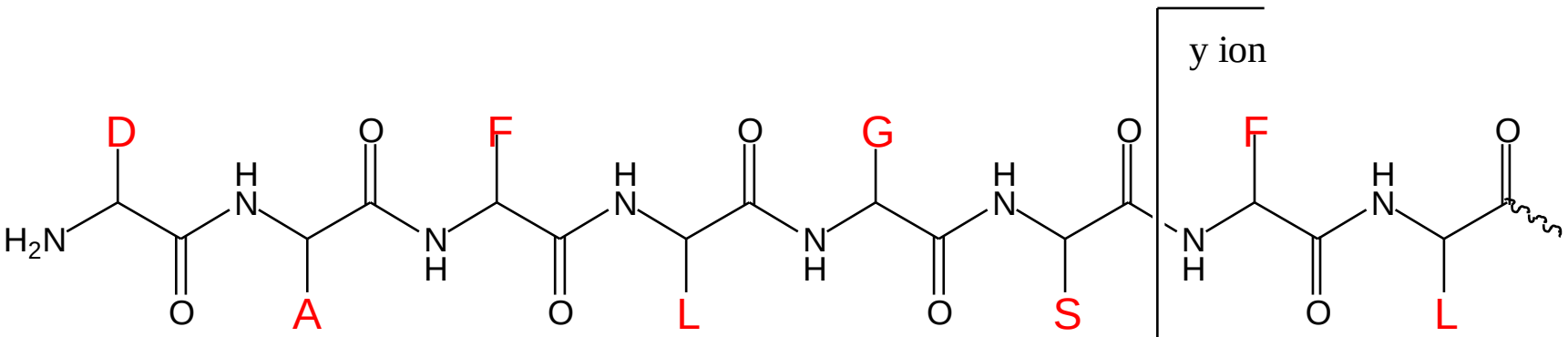


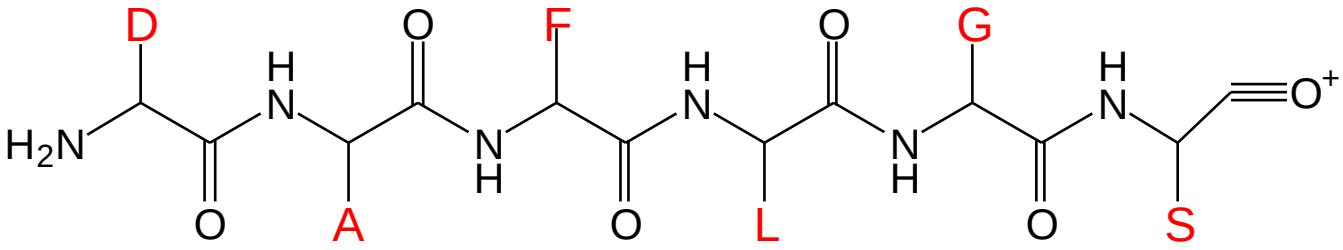
Mass Spectrum of a Tryptic Peptide from BSA



N term ⟶ C term

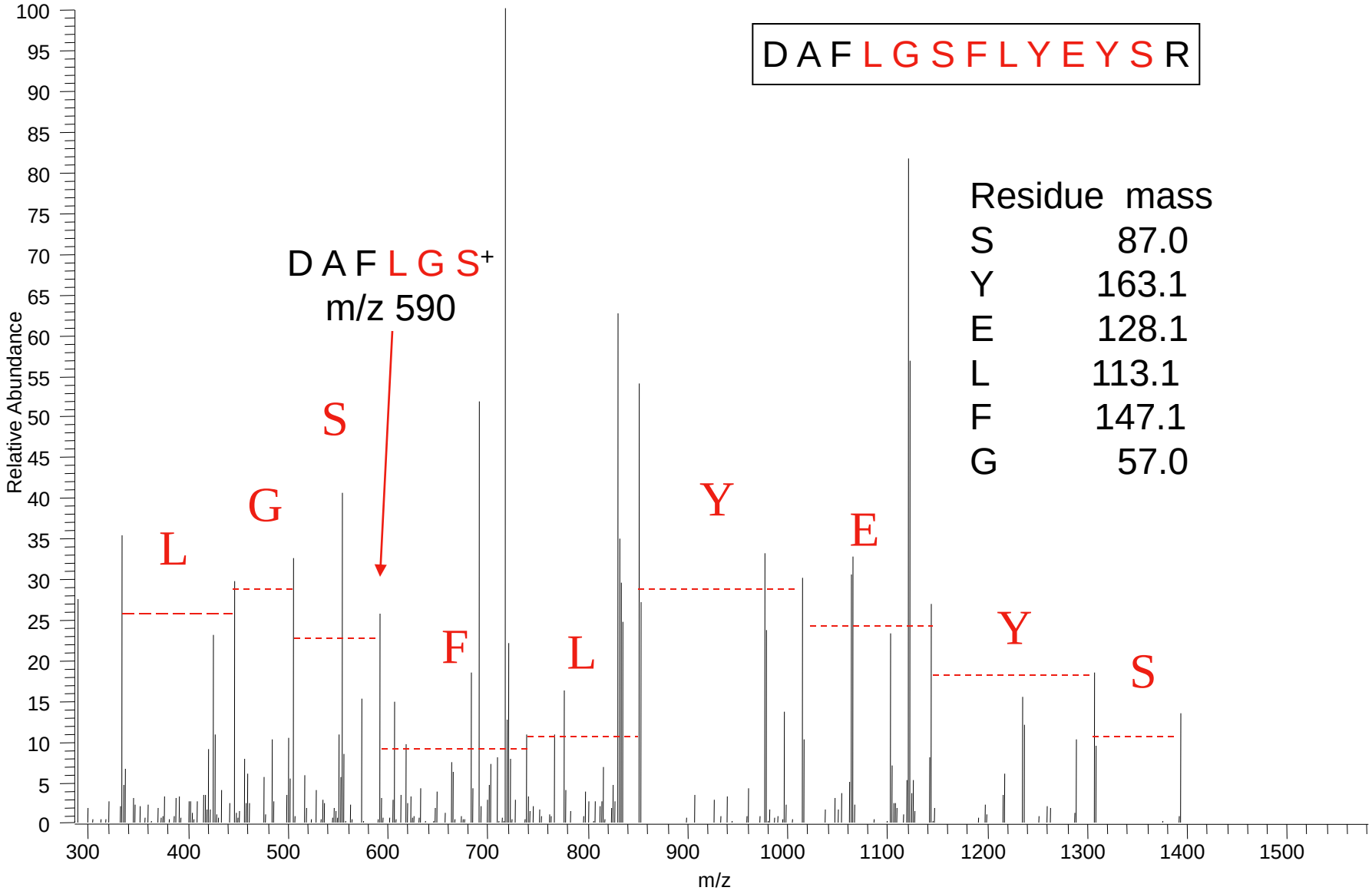
D A F L G S F L Y E Y S R



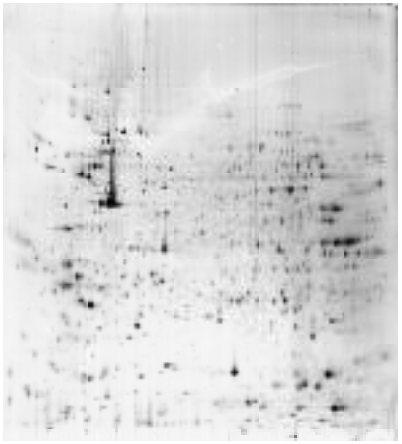


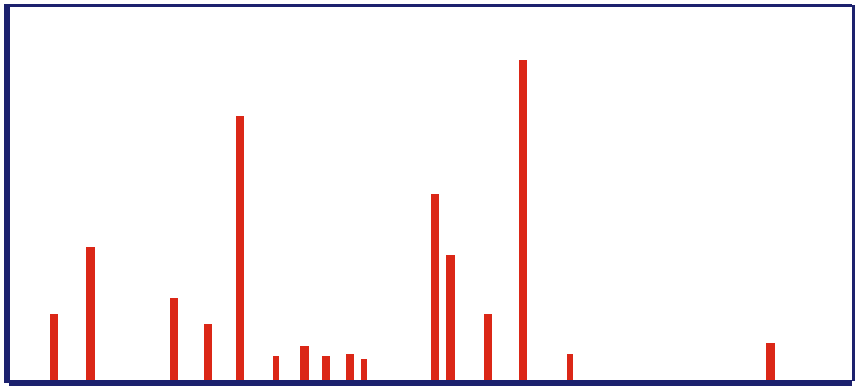
b ion
(m/z 590)

MS-MS Spectrum



Peptide Mass Mapping





```
MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE
EQFKGLVLIA FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD
ESHAGCEKSL HTLFGDELCK VASLRETYGD MADCCEKQEP
ERNECFLSHK DDSPDLPKLK PDPNTLCDEF KADEKKFWGK
YLYEIARRHP YFYAPELLYY ANKYNGVFQD CCQAEDKGAC
LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA
RLSQKFPKAE FVEVTKLVTD LTKVHKECCH GDLLECADDR
ADLAKYICDN QDTISSKLKE CCDKPLLEKS HCIAEVEKDA
IPENLPPLTA DFAEDKDVCK NYQEAKDAFL GSFLYEYSRR
HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ
VSTPTLVEVS RSLGKVGTRC CTKPESERMP CTEDYLSLIL
NRLCVLHEKT PVSEKVTKCC TESLVNRRPC FSALTPDETY
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KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
STQTALA
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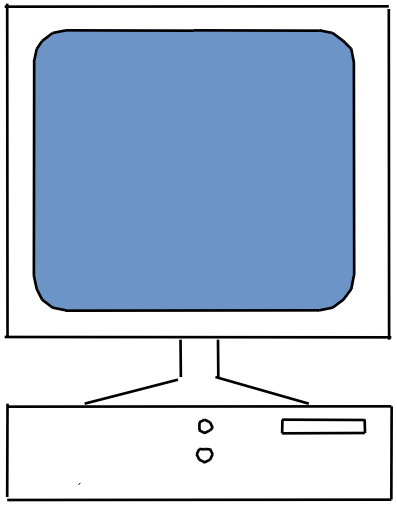


567.95	1249.17
655.84	1305.29
842.19	1439.32
927.16	1480.26
1001.22	1567.23
1083.21	1639.40
1142.29	1750.43
1193.16	2211.37

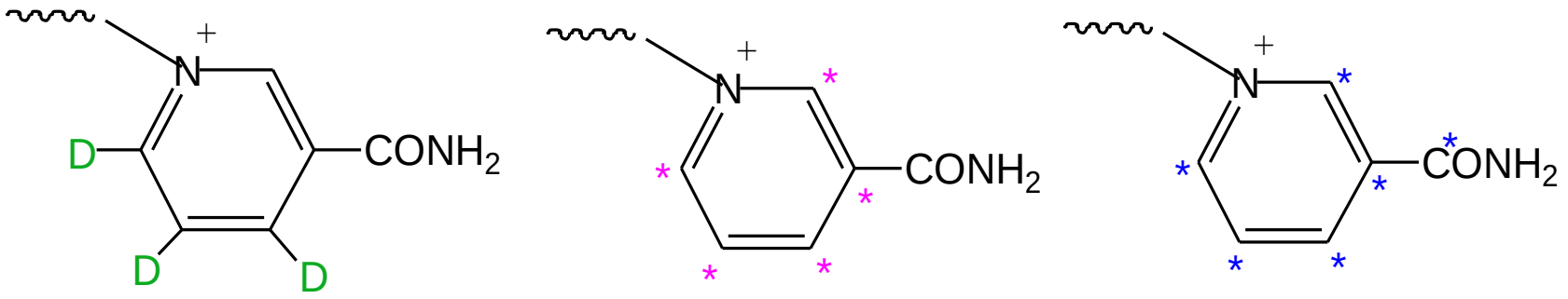
LC-MS-MS analysis

- Characterization of synthetic processes
- Drug metabolism studies – structural elucidation of metabolites
- Quantification of polar molecules in biological samples – NAD

NAD Assay

- Goal to determine the relative importance of the different biosynthetic pathways.
- Stable isotopic incorporation

NAD Synthesized from Labeled Precursors



NAD synthesized from

a) $^{2}H_{4}$ labeled nicotinic acid and nicotinamide (m/z 667)

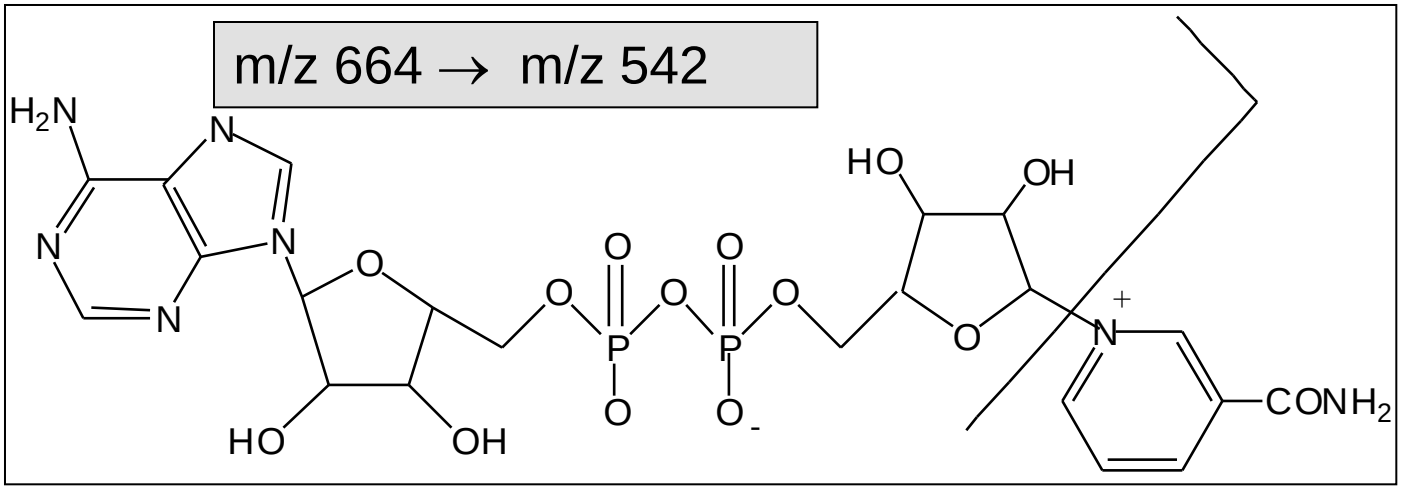
b) $^{13}C_{5}$ labeled tryptophan (m/z 669)

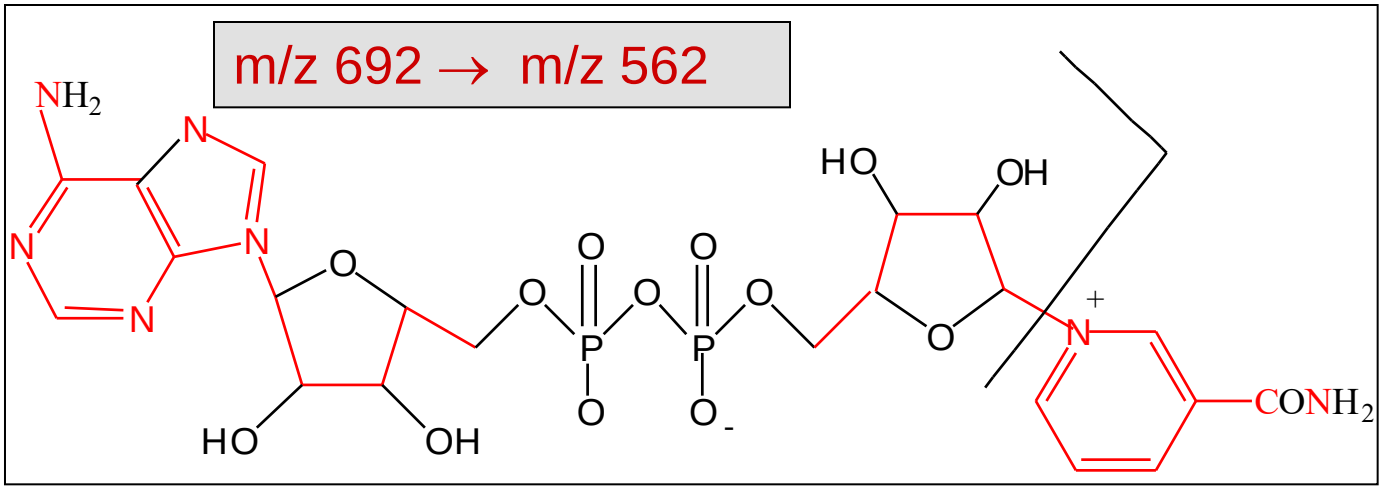
c) $^{13}C_{6}$ labeled quinolinic acid (m/z 670)

The Experimental Strategy

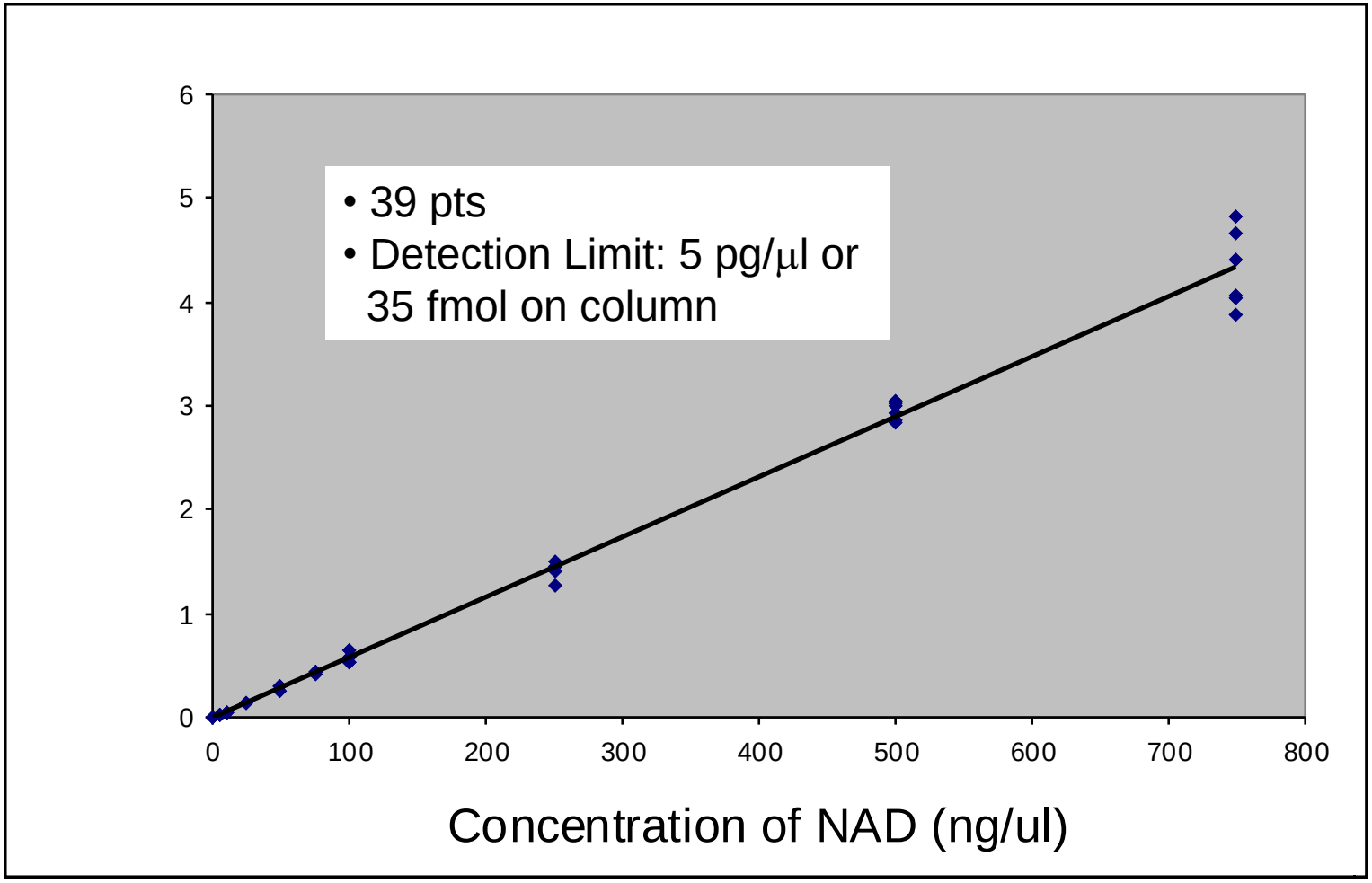
- Culture cells in media containing isotopically labeled precursors for fixed time intervals
- Harvest and lyse the cells, extract the NAD, and quantitate the unlabeled and labeled NAD using reverse-phase LC-MS-MS

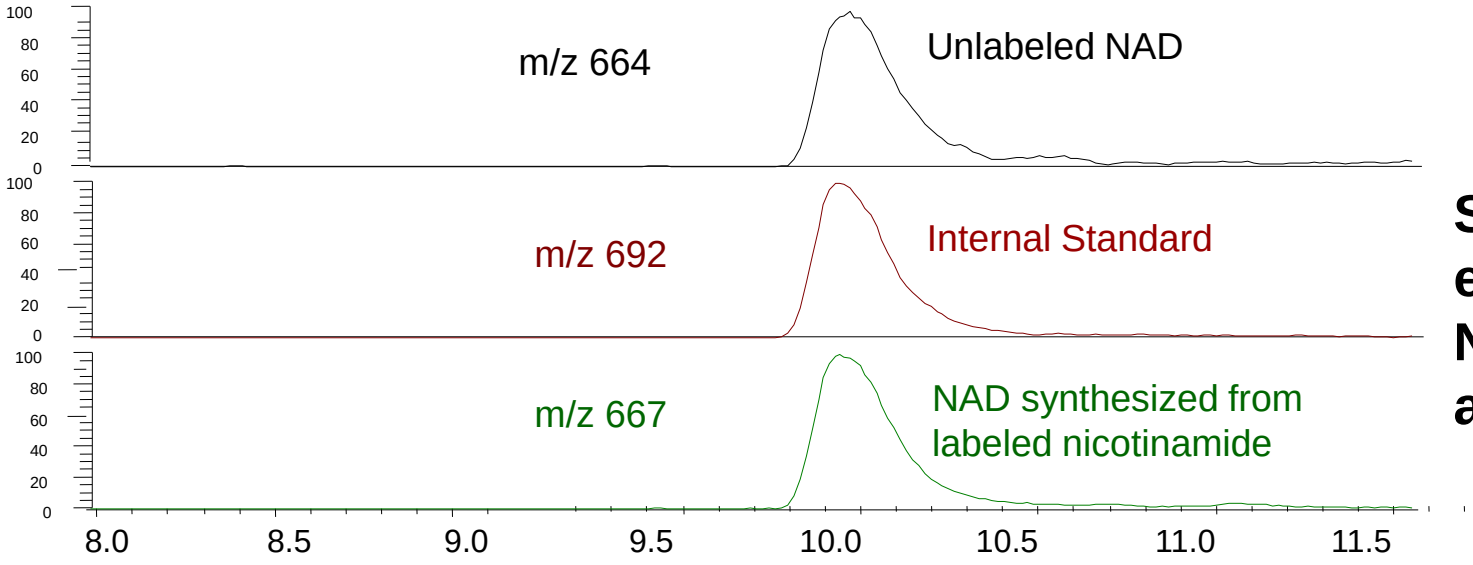
Sensitivity advantage



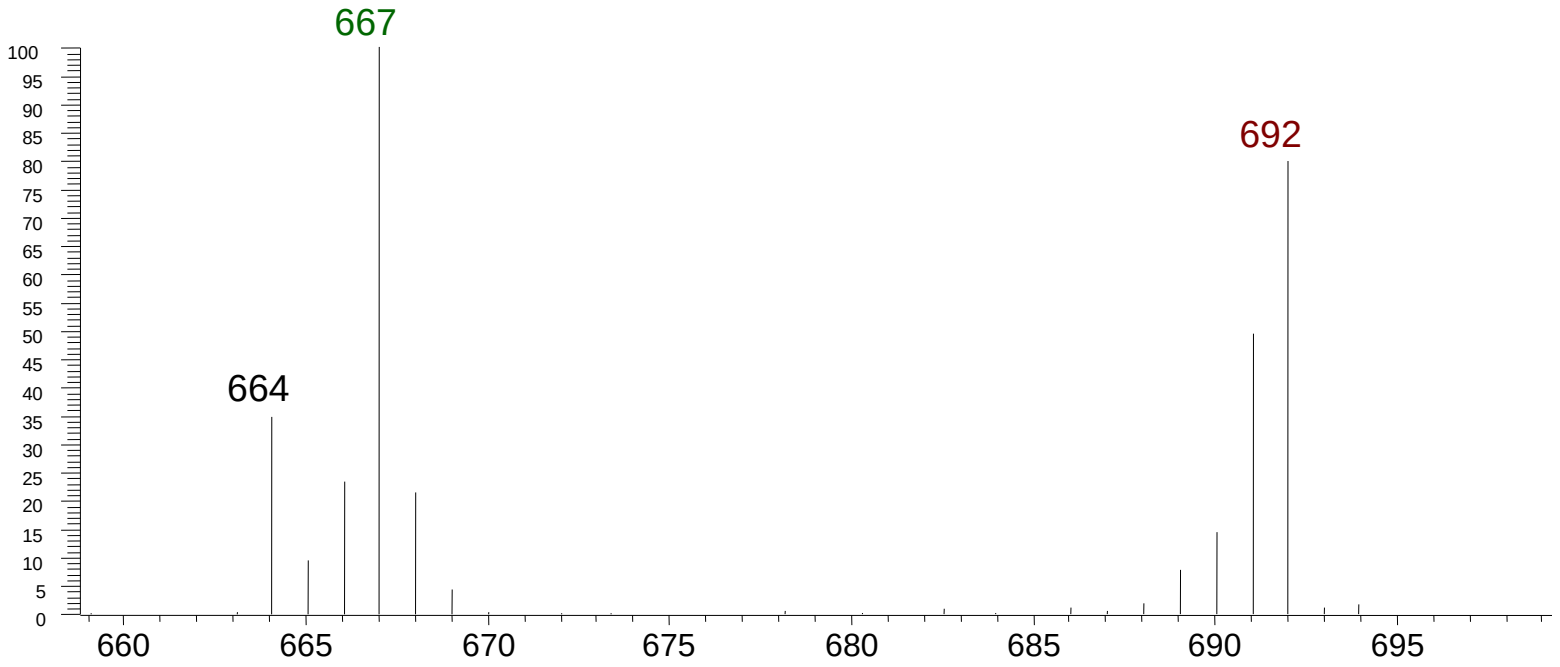


Standard Curve





Sample from experiment 1: Nicotinamide at 72 hrs



Elemental Mass Spectrometry

- ICP-MS (inductively coupled plasma)
- SIMS-TOF (secondary ion mass spectrometry
- CRIMS

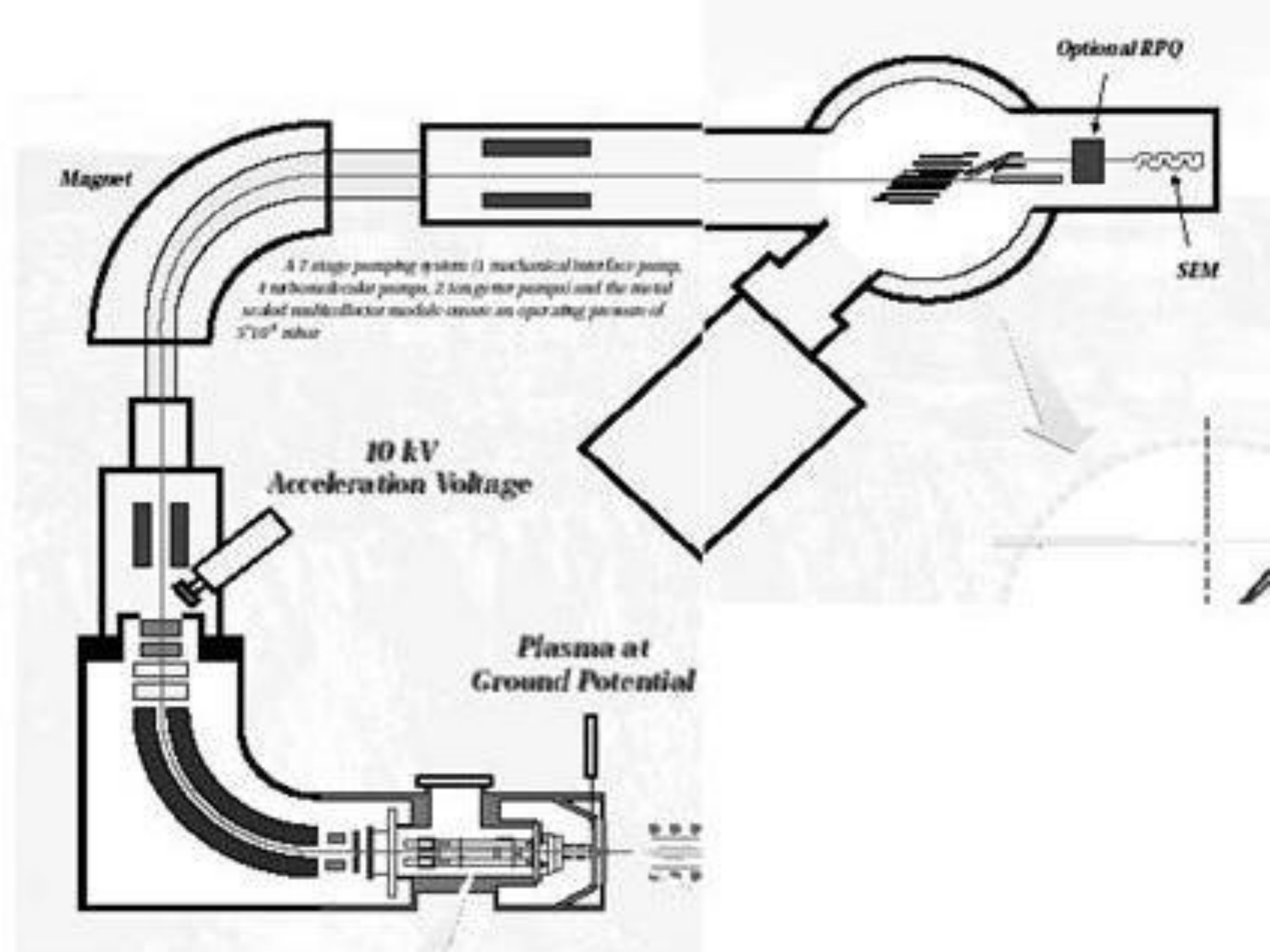
ICP-MS (vs. ICP-UV/vis)

- ICP (ch 10, pg 231-232) ICP-MS (ch 11)
- A spark ignites flowing argon forming a self-sustaining plasma ($T \approx 10000$ K)
- Sample is aspirated/pumped into plasma forming elemental cations and some simple polyatomic ions
- Ions are pushed into mass analyzer by high voltage

Isotope ratio mass spectrometry

- Elemental analysis (geologists, archeologist, isotope tracer studies)
- High resolution sector mass analyzers
- Faraday cups
- ThermoFinnigan Neptune

  http://www.thermo-optek.it/GetBrochure.php?ID=43
- CRI-MS (chemical reaction interface)
  - Converts all carbon to CO_2


Optional RPQ
Magnet
SEM
10 kV
Acceleration Voltage
Plasma at
Ground Potential