CHE680
Chapter 12
Mass Spectrometry

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Chemical Analysis

- Qualitative Analysis

- Quantitative Analysis

Why Mass Spectrometry?

- Spectroscopy (FTIR/UV-Vis/XPS, etc)
- Chromatography (GC/LC)
- Microscopy
- etc

Direct structural analysis is possible by analyzing charged fragments

Mass Spectrometry

- Technique(s) used to measure the masses of ions and their abundances in the gas phase >> molecular weight and structures of compounds

- Consists of following sub-steps
  1. Ionization : Generation of gas phase molecules (and molecular fragments and atoms) and their ionization
  2. Analysis: Separation based upon mass (m) to charge (z) ratio (m/z)
  3. Detection: Detection of separate ions

- Hardware
  1. Ion source
  2. Analyzer (Filter)
  3. Detector

Overview

https://www.youtube.com/watch?v=NuIH9-6Fm6U

Fragmentation

https://www.youtube.com/watch?v=stIwRio9FeM

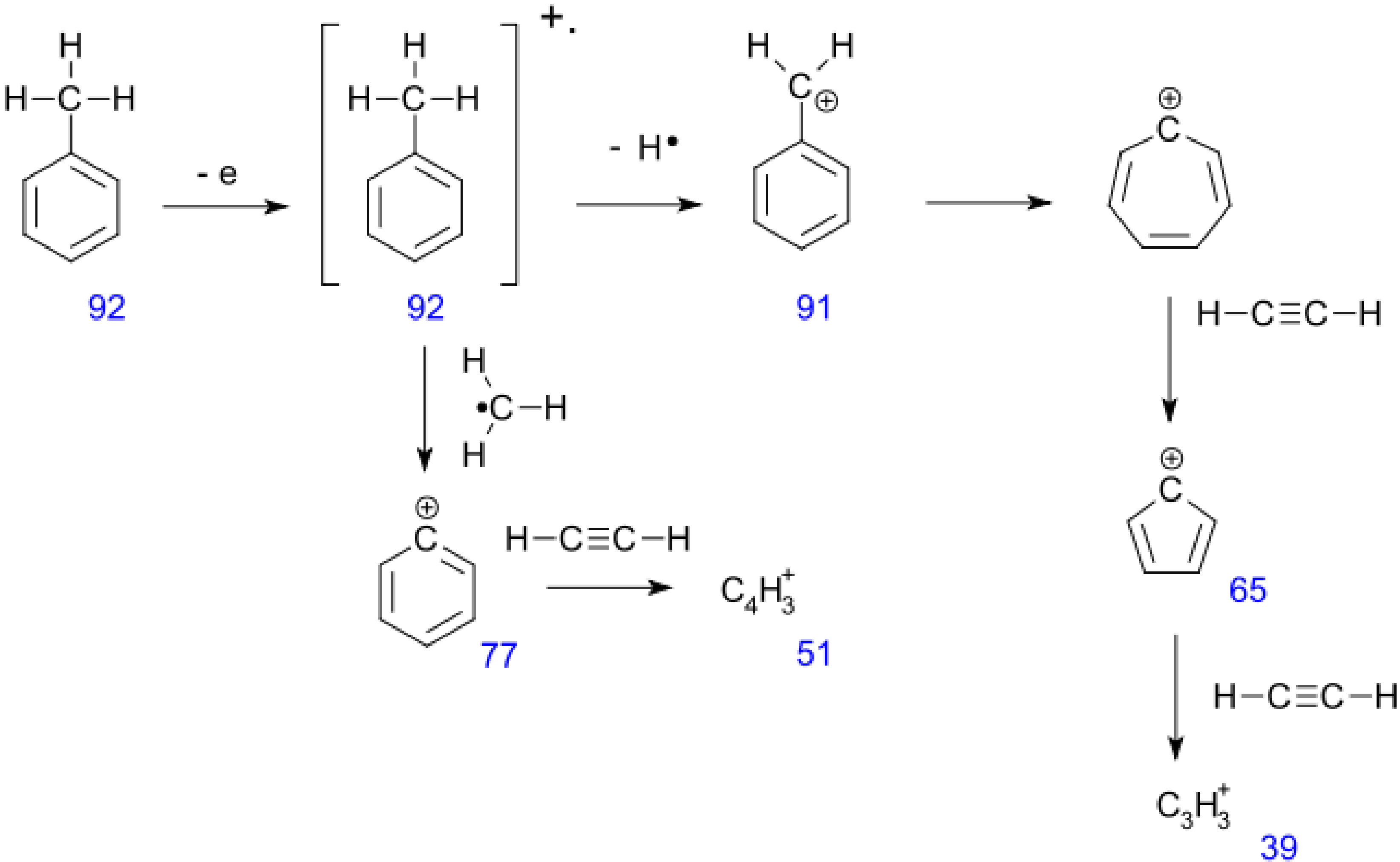
Hardware

- Mass source (ion source):
  1. Molecules are introduced as a gas, liquid, or solid
  2. Vaporization (liquid and solid)
  3. Ionized (several methods, fragmentation or)
     - ➢ Fragmentation
     - ➢ Addition of H^+
  4. Accelerated to analyzer

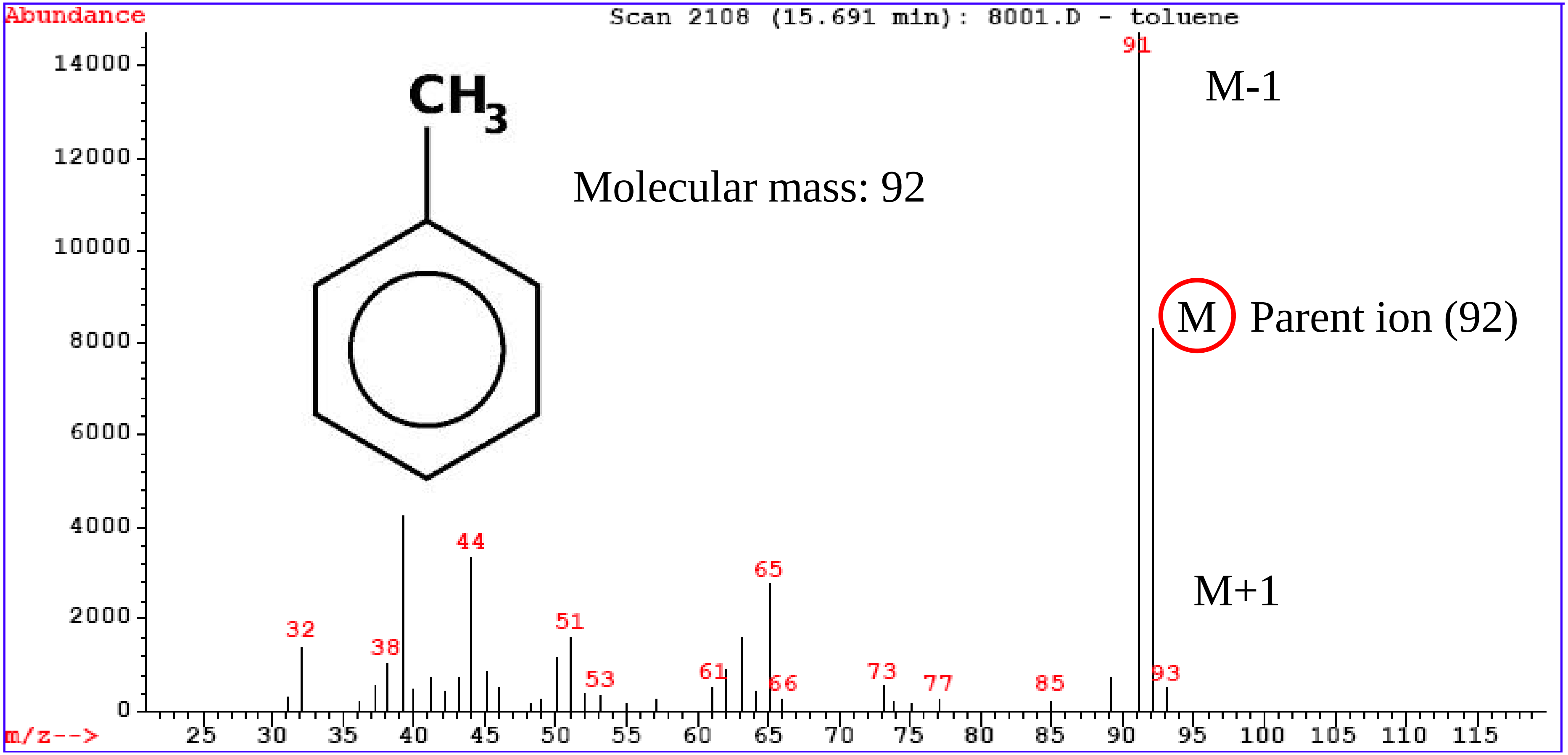
- Analyzer (filter): several methods
  1. Separation by mass (m/z)
  2. Separation by time to detector (from source to detector)

- Detector: count # of ions from analyzer
  Ion multiplier: amplifies current similar to photomultiplier

Production of Ions via Fragmentation



Presentation of Mass Spectrum (Pure Sample)



Sample Introduction

- Direct Introduction:
  1. Syringe: Gas (or liquid) samples
  2. Probe tip: Deposited solid samples

- Introduction via hypernated techniques
  1. GC/MS: GC capillary is directed connected to ionization chamber (protic solvent should be avoided)

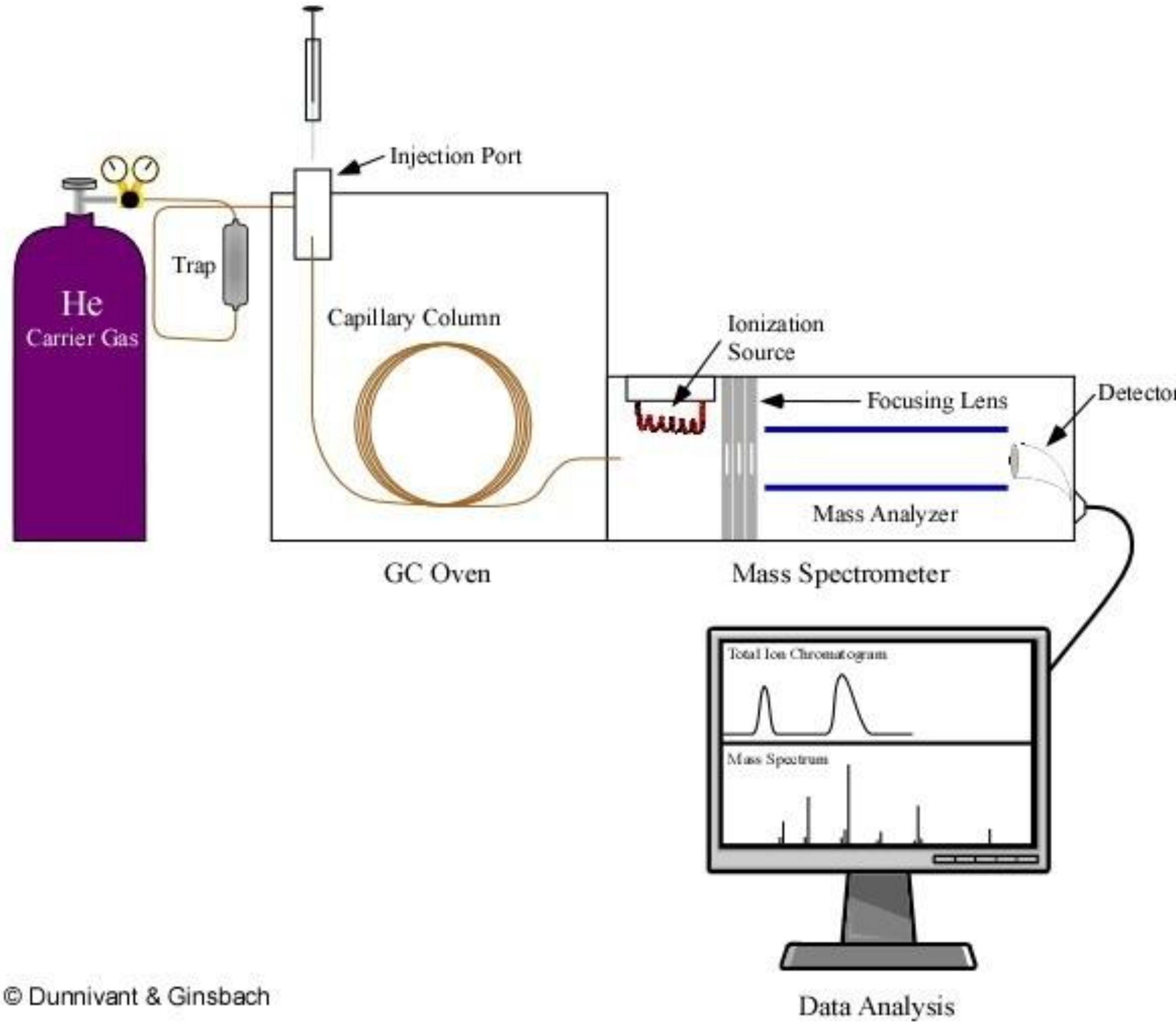
  2. LC/MS: Micro-column (short column) to reduce flow rate (water is poisonous to MS)

GC-MS vs LC-MS

- GC-MS: Gas phase sample to MS
  1. Easy to ionize and fragmentation
  2. Limited to low molecular weight (m/z ~ 600)

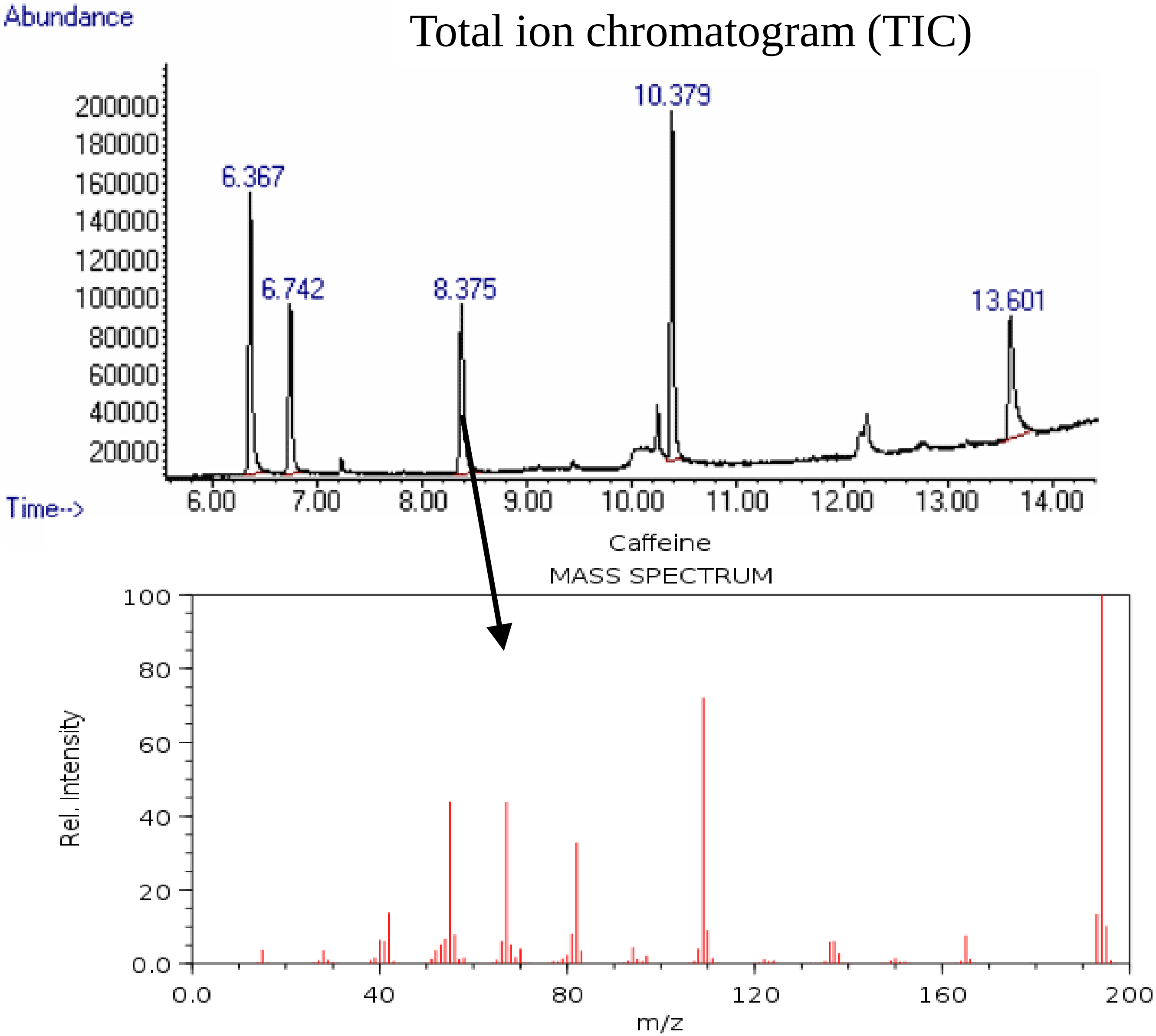
- LC-MS: Liquid phase sample to MS
  1. Difficult to ionize
  2. Hard to fragment
  3. MS-MS is popular option
  4. Wide range of molecular weight (m/z ~4000, 100 kD)

GC/MS


Injection Port
Trap
He
Carrier Gas
Capillary Column
Ionization
Source
Focusing Lens
Detector
Mass Analyzer
GC Oven
Mass Spectrometer
Total Ion Chromatogram
Mass Spectrum
Data Analysis

Typical Data from GC/MS

Total ion chromatogram (TIC)



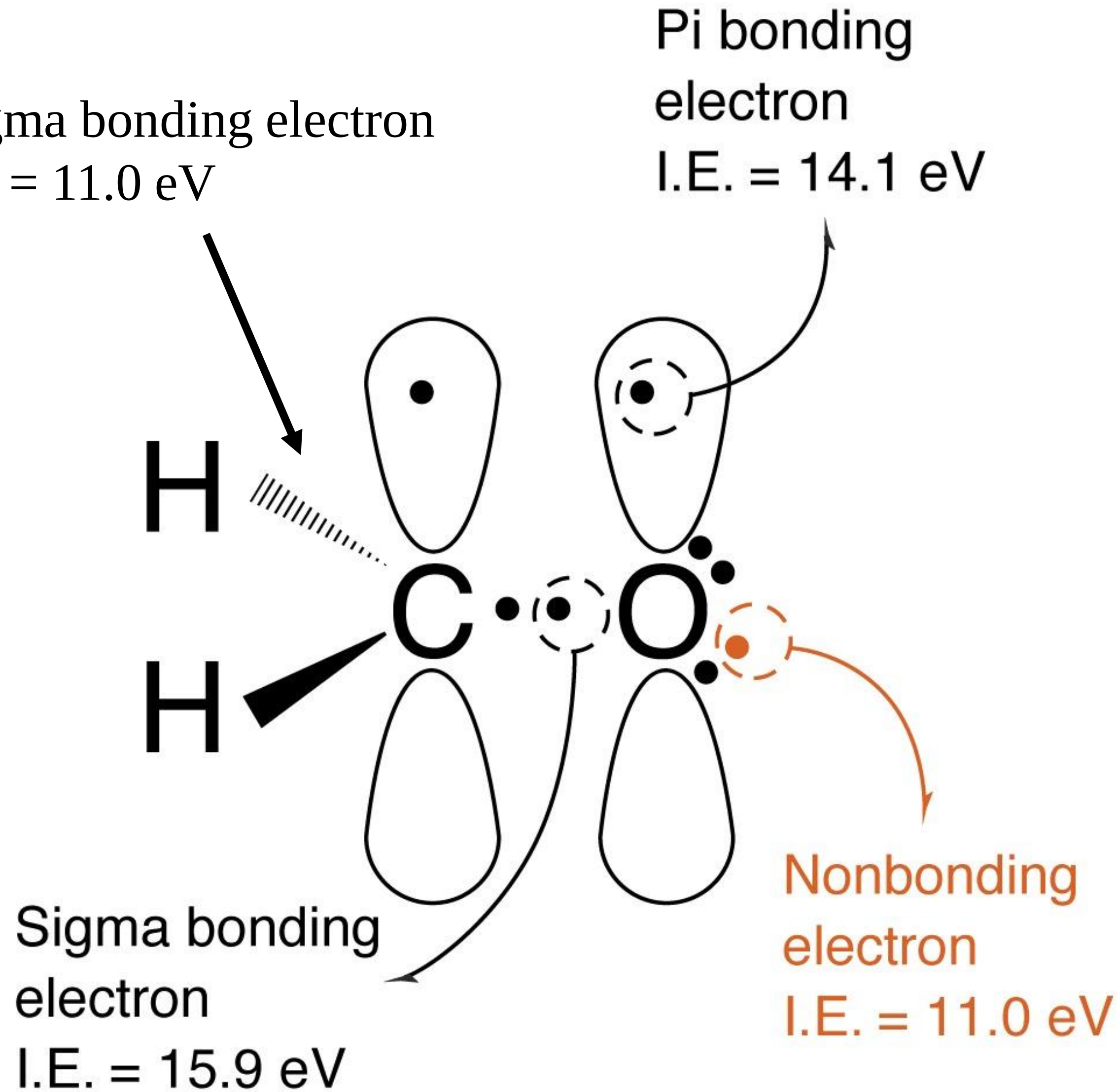
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Similar to chromatograms

Structural information

How to Fragment to Ions

Sigma bonding electron
I.E = 11.0 eV

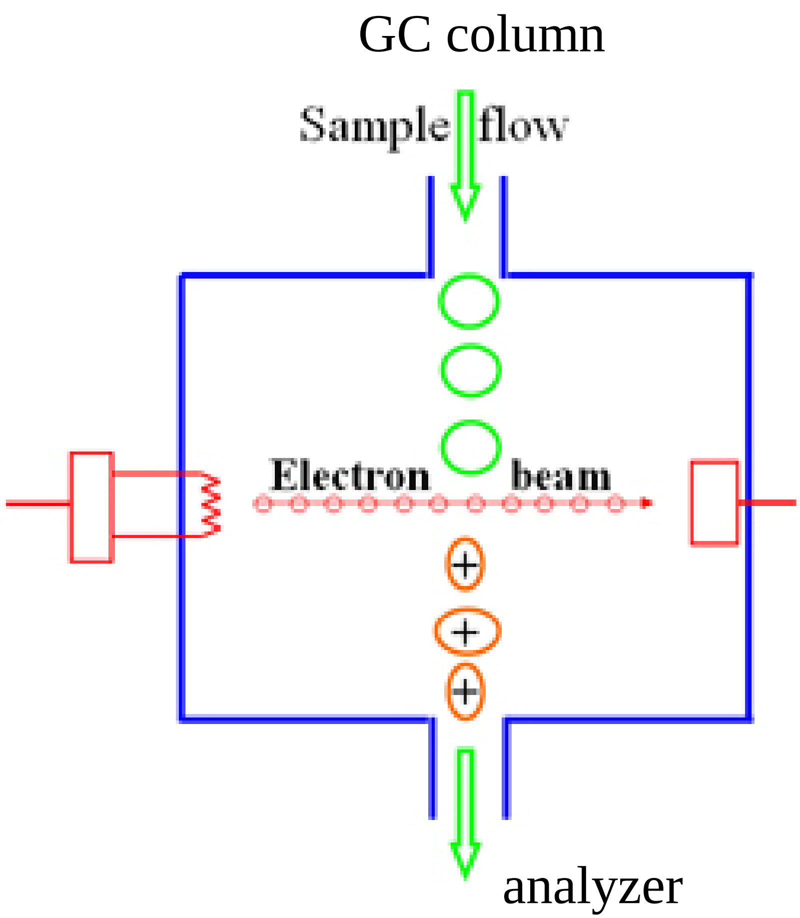


Typical Ionization Methods

- GC-MS
  - EI (electron ionization)
  - CI (chemical ionization)

Electron Ionization (EI)

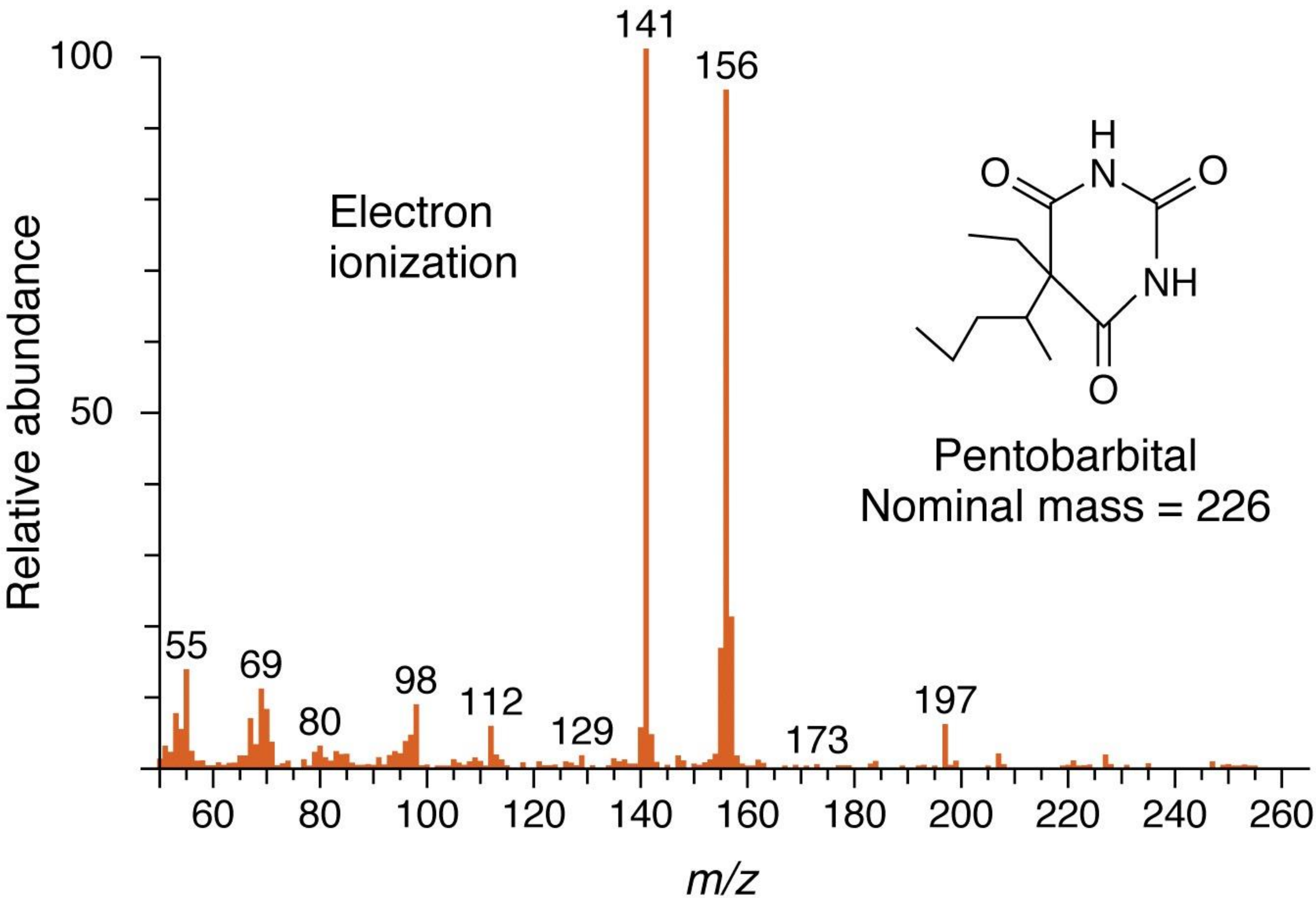
- Ionization via collision of sample molecules with electrons
- Electrons are produced by thermo ionic process (~70 eV)
- Ionization efficiency: ~1/10000 (~0.01%)



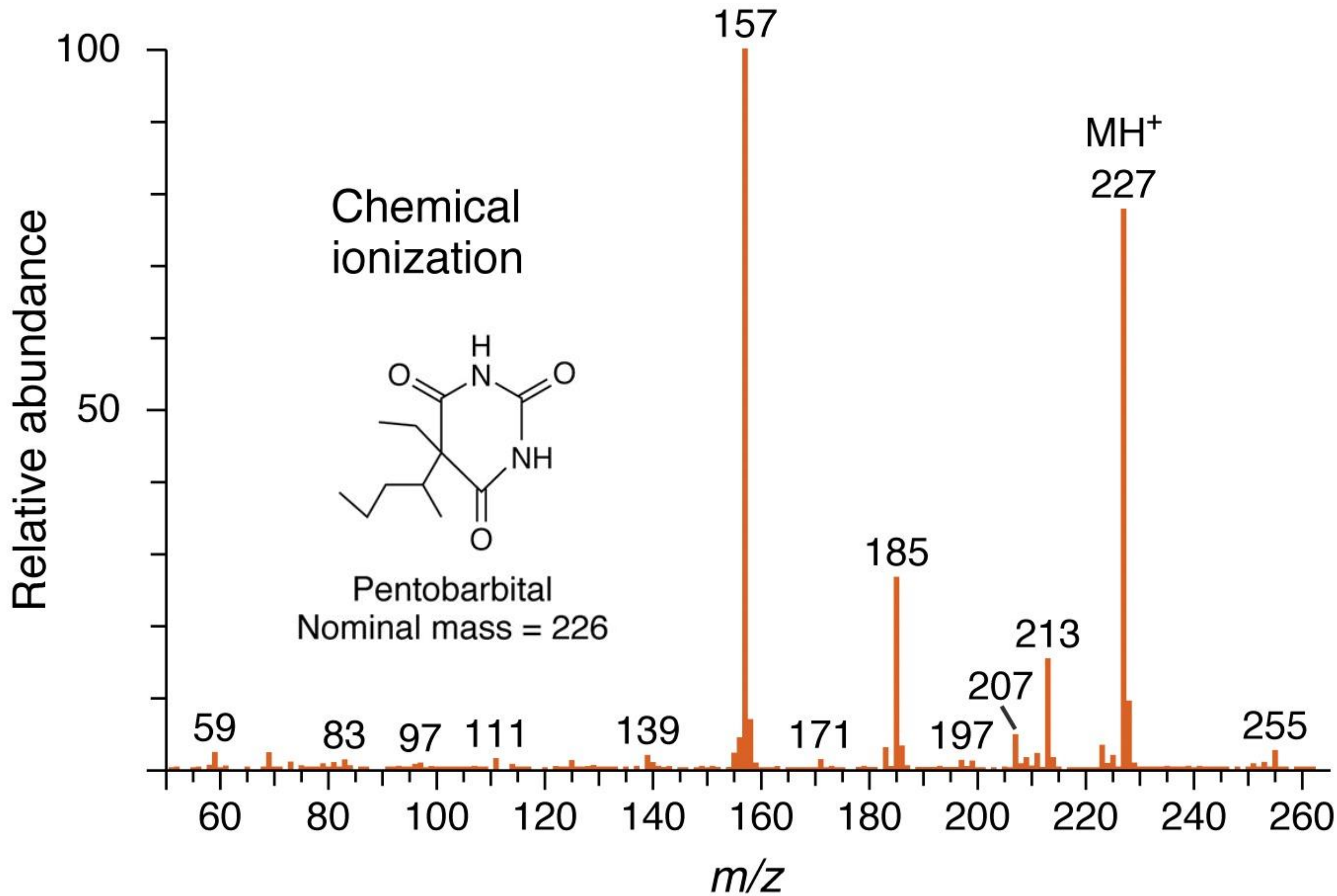
Chemical Ionization (CI)

- Ionization via collision of sample molecules ionized reagent gases with hydrogen (CH_4, NH_3, etc)
- Soft ionization (high concentration of M+1)

Primary reactions :	$CH_4 + e^- \longrightarrow CH4]^{+\bullet} + 2\,e^-$	*(Ionisation)*
Secondary reactions :	$CH_4]^{+\bullet} \longrightarrow CH_3]^+ + H^\bullet$	
	$CH_4]^{+\bullet} + CH_4 \longrightarrow CH_5]^+ + CH_3^\bullet$	*(Autoprotonation)*
	$CH_3]^+ + CH_4 \longrightarrow C_2H_5]^+ + H_2$	
Collisions with M :	$CH_5]^+ + M \longrightarrow MH]^+ + CH_4$	(ion at M+1)
	$C_2H_5]^+ + M \longrightarrow MC_2H_5]^+$	(ion at M+29)
If M is of the type RH :	$CH_5]^+ + RH \longrightarrow R^+ + CH_4 + H_2$	(ion at M-1)
If it is a tert-butyl cation :	$(CH_3)_3C]^+ + M \longrightarrow MH]^+ + CH_2{=}C(CH_3)_2$	



141
156
100
50
Relative abundance
Electron
ionization
55
69
80
98
112
129
173
197
H
O
N
O
NH
O
Pentobarbital
Nominal mass = 226
60
80
100
120
140
160
180
200
220
240
260
m/z



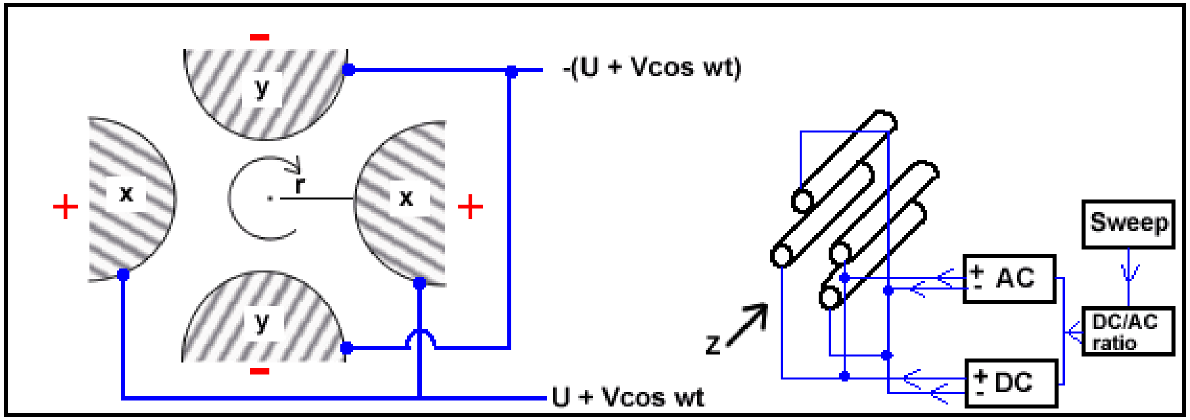
Relative abundance
100
50
157
MH+
227
Chemical
ionization
H
O
N
O
NH
O
Pentobarbital
Nominal mass = 226
185
213
207
59
83
97
111
139
171
197
255
60
80
100
120
140
160
180
200
220
240
260
m/z

Ion Analyzers

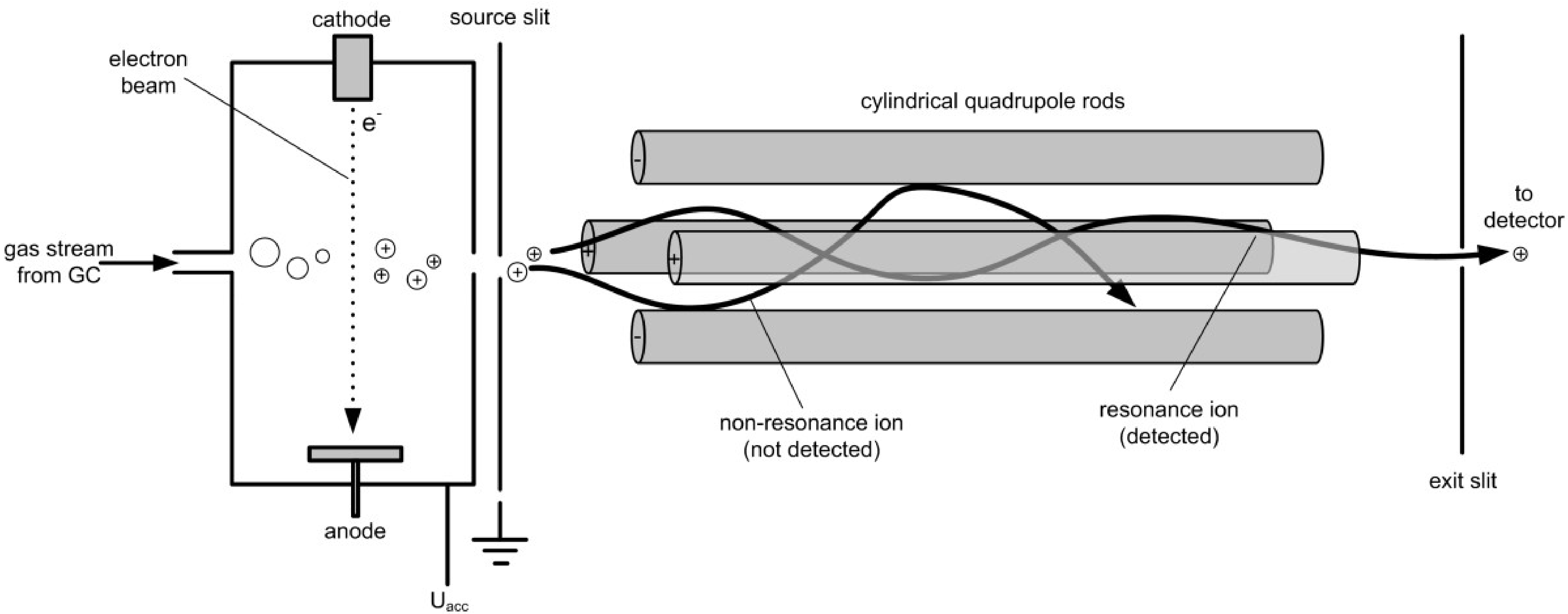
- Make use of m/z (mass/charge ratio) of ions and their speeds
- Magnetic analyzer (EB type): old fashioned
  - Electric sector (E)
  - Magnetic analyzer (B)
- Time of flight (TOF) analyzer
- Quadrupole analyzer
- Ion traps (Orbitrap)

Quadrupole Analyzer

- Commonly account in GC/MS or LC/MS
- Positive ions enter quadrupole,
  - it will move along O-z direction
  - Two positive electrode: focusing by repulsion
  - Two negative electrode: defocusing by attraction



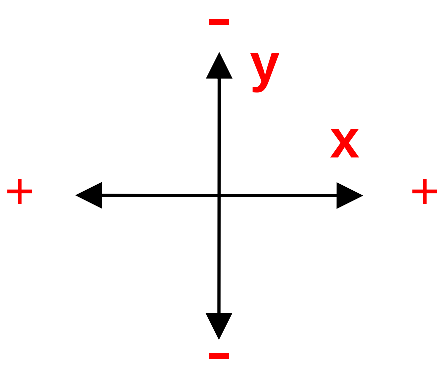
Mass Selection by Changing Field Strength

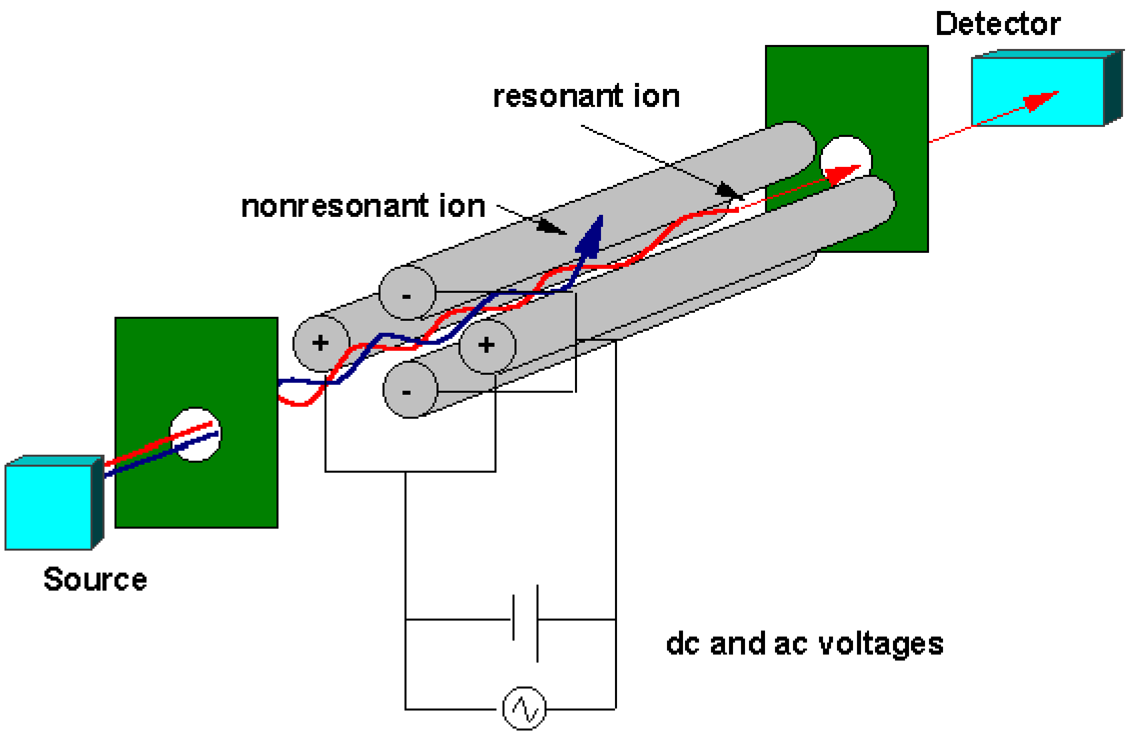


- For a give +/- potential, ions with specific mass can go to detectors and other can't
- Applied +/- potential to quadrupole can be changed
- This is why it is called mass selected detector (MSD) rather than mass spectrometer

Quadrupole Analyzer: Mass Selective Detector (MSD)

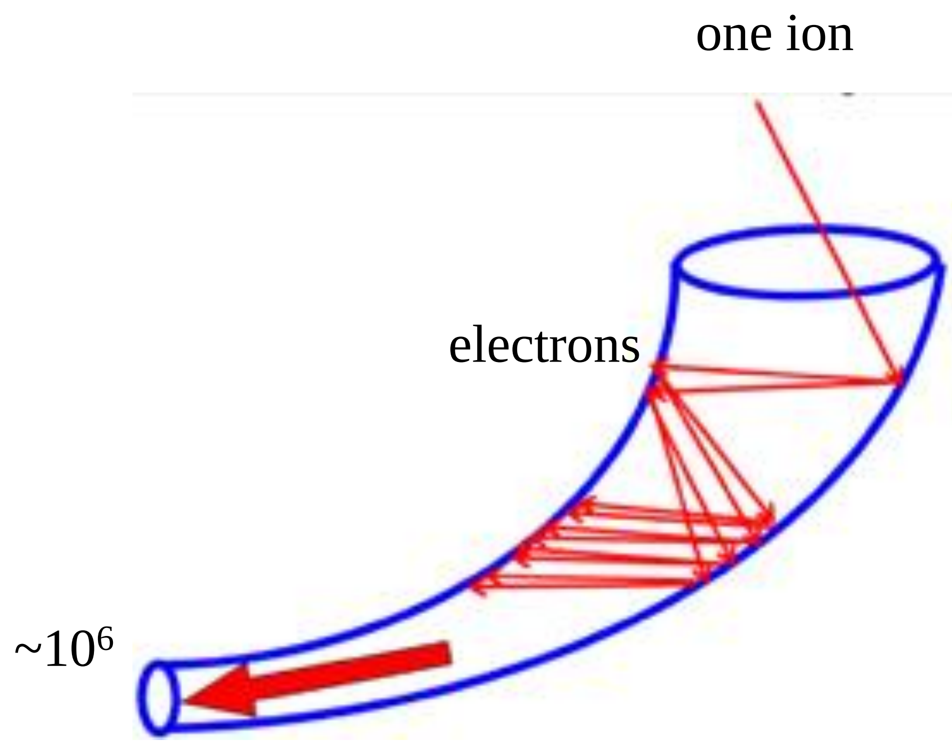
- Ions with zero velocity in the O-y direction: x-O-z plan
  1. Heavy ions: won’t respond to variation of field (resonant)
  2. Light ions: will respond to variation of field and be lost (nonresonant)
- Ions with zero velocity in the O-x direction: y-O-z plan
  1. Heavy ions: will respond to variation of field and be lost (nonresonant)
  2. Light ions: won’t respond to variation of field (resonant)





Ion Detector

- Measurements of electrical charge (current) carried by ions
- Dynode



Calibration of GC MSr

PFTBA (Perfluorotributylamine)

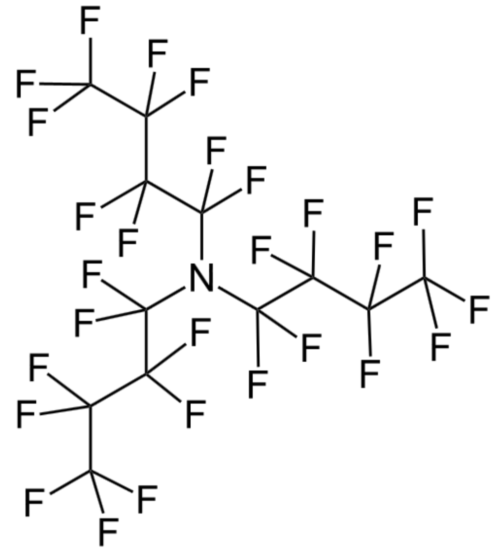
CF_3: 69

CF_3CF_2: 119

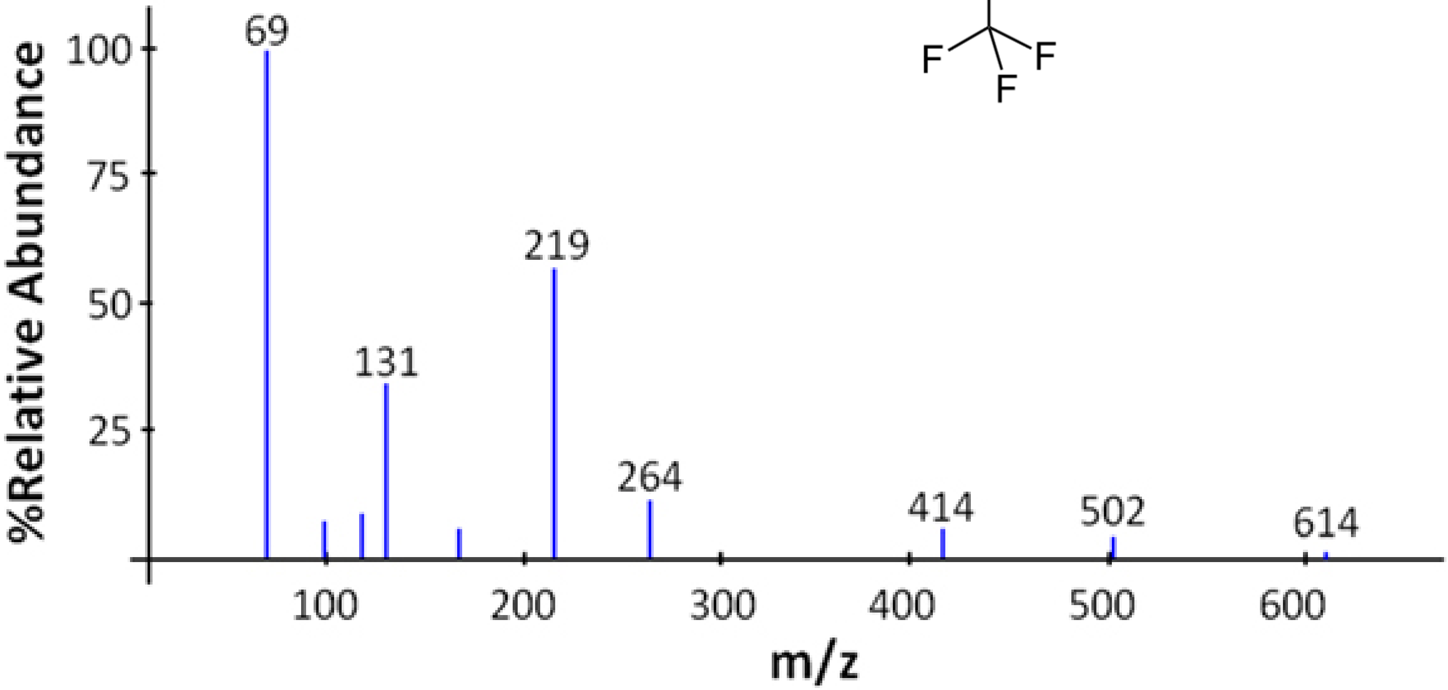
$CF_3CF_2CF_2$: 169

$CF_3CF_2CF_2CF_2$: 219

671-169: 502

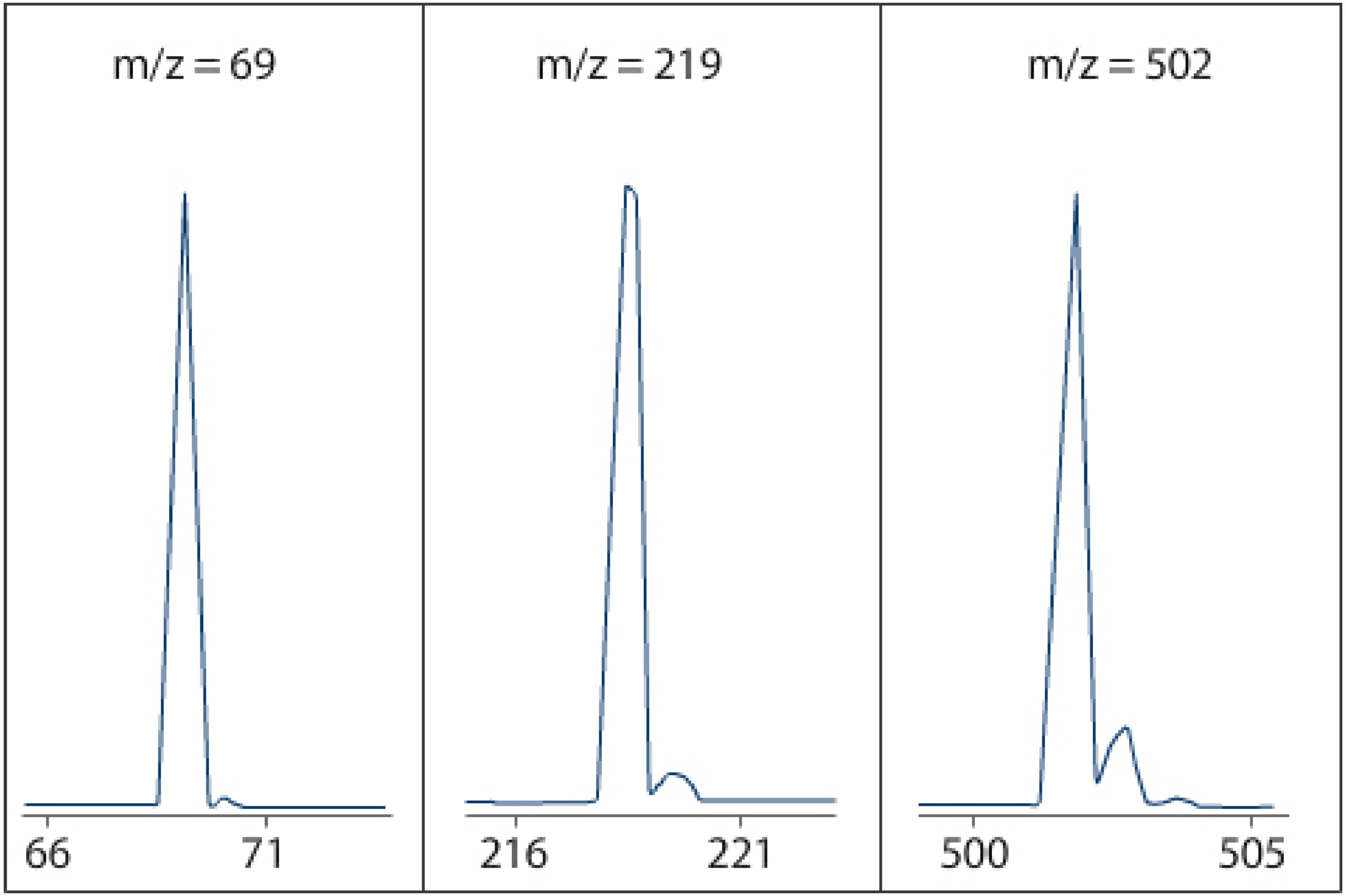


MW: 671

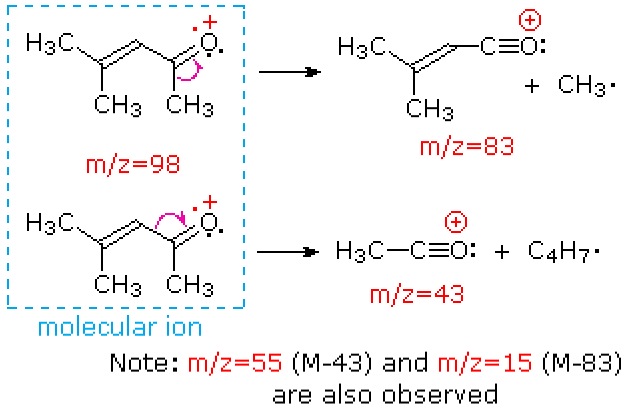


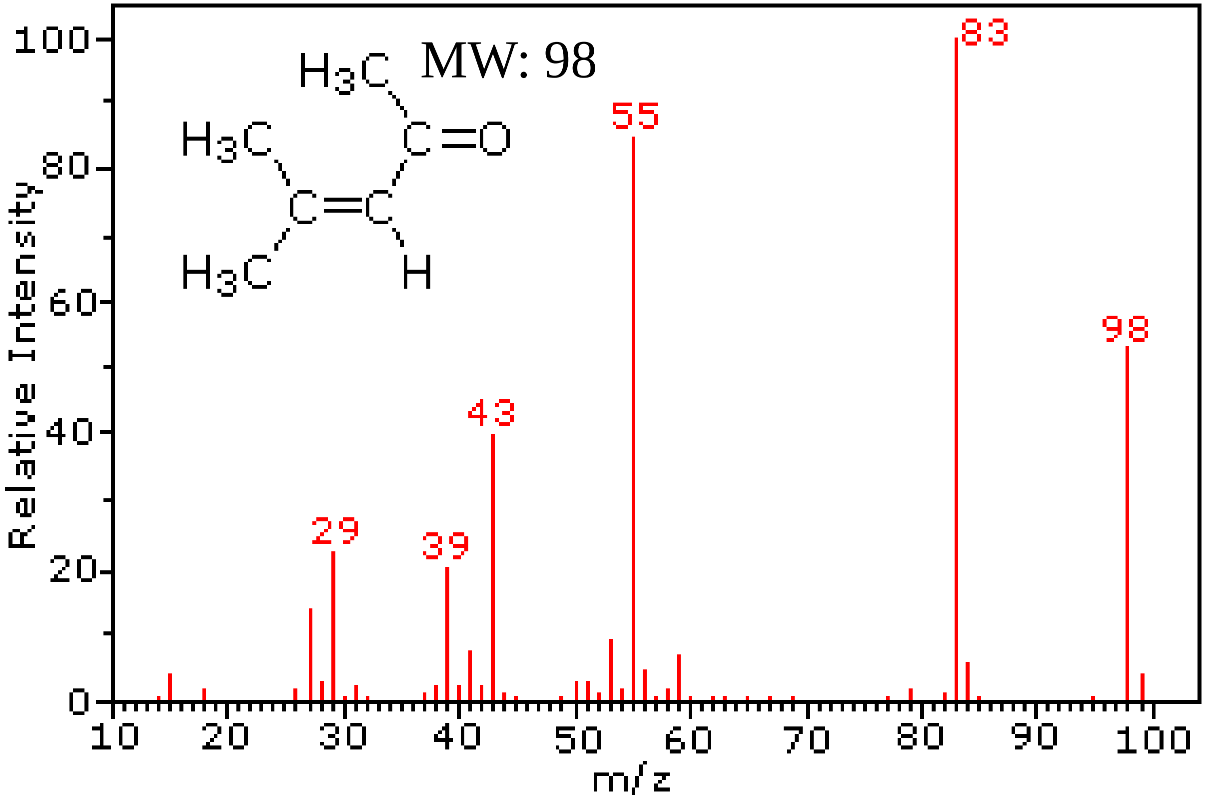
Tuning of Mass Spectrometer

Optimization of MS conditions (EI voltage, etc) using peak shapes, intensity of thee ions from PFTBA



Database is required to confirm the structure: NIST MS Library

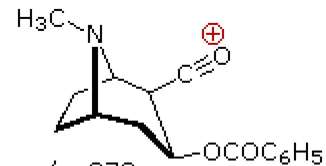




[M –105]

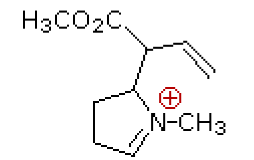
m/z = 198

α-cleavage



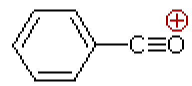
m/z=272

α-cleavage



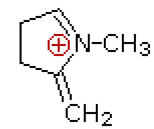
initial m/z=182

C-Y cleavage

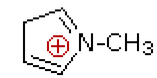


m/z=105

α-cleavage

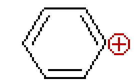


m/z=96



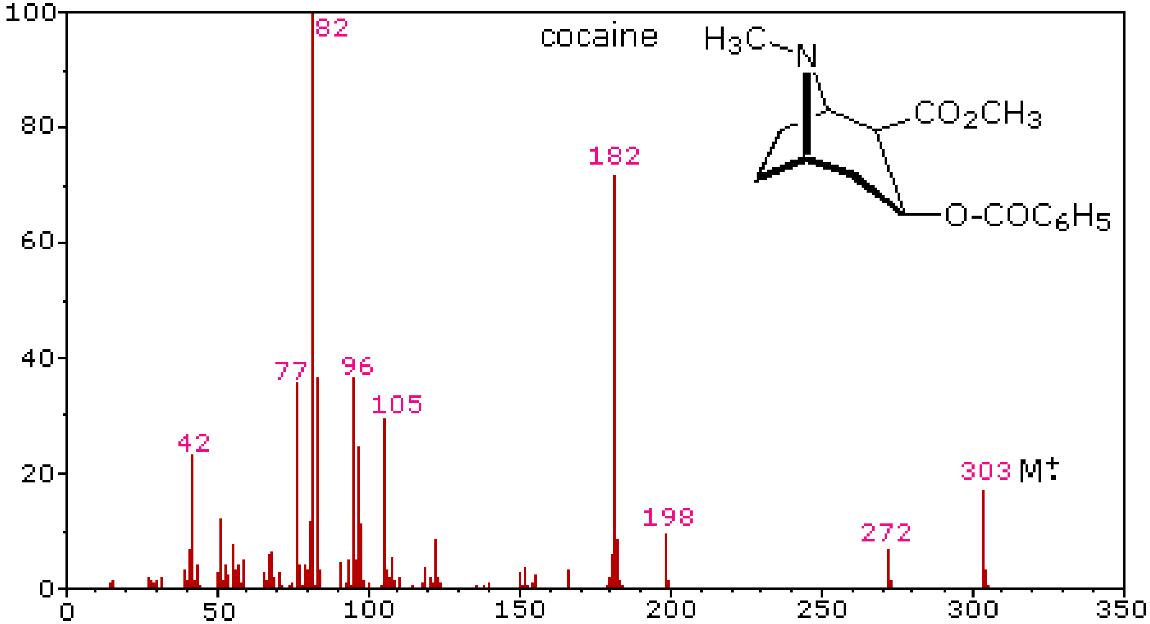
m/z=82

initial α-cleavage



m/z = 77

loss of CO from 105

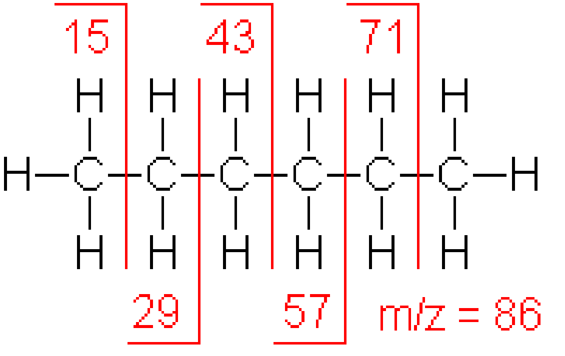


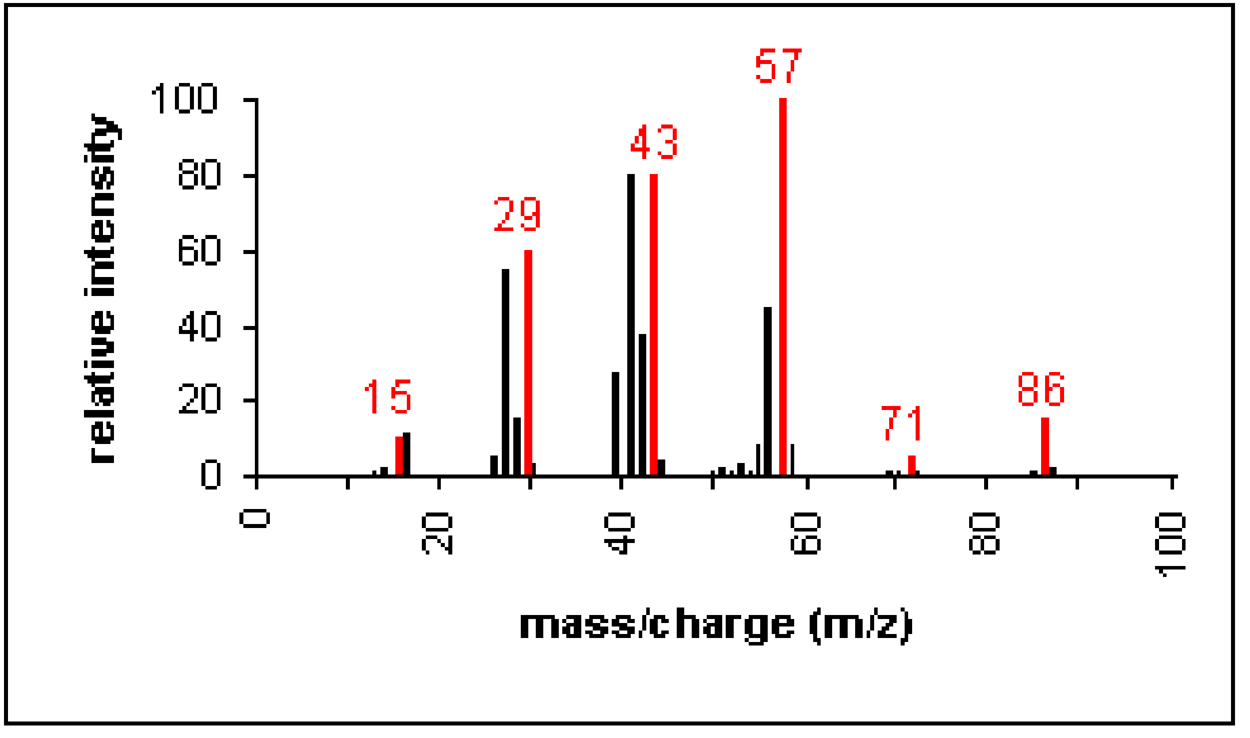
MW: 303

Alkane

- Peak at 15 (CH_3^+), 29 ($CH_3CH_2^+$), 43 ($CH_3CH_2CH_2^+$), 57 ($CH_3CH_2CH_2CH_2^+$), 71 ($CH_3CH_2CH_2CH_2CH_2^+$)
- Clusters of peaks 14 mass units apart (CH_2)

Hexane (C_6H_{14}) with MW = 86.18

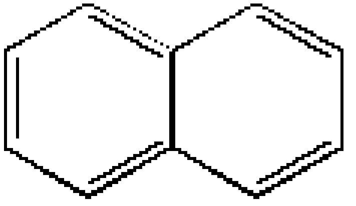




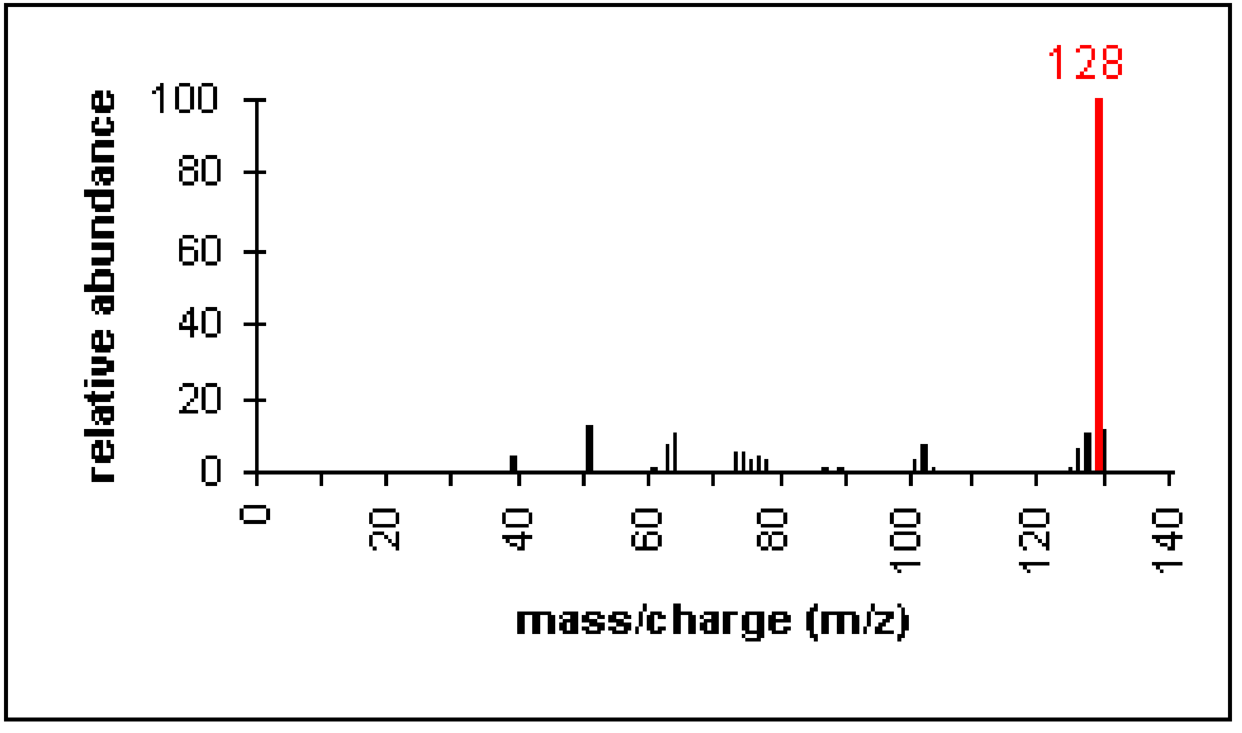
Aromatics

- Molecular ion peaks are base peaks due to the stable structure.

Naphthalene ($C_{10}H_8$)
MW = 128.17



m/z = 128

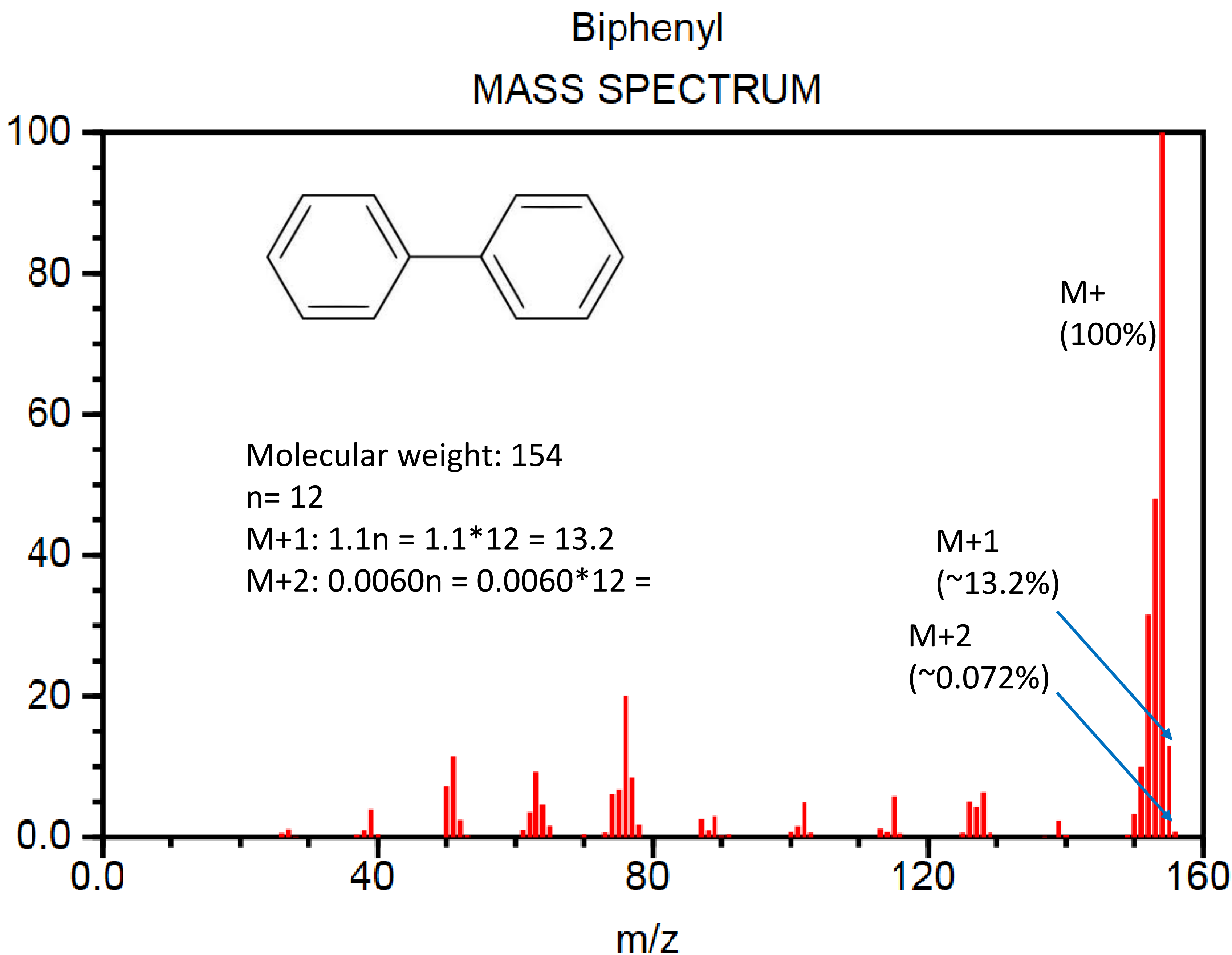


Isotope Effects (M+1, M+2 Peaks)

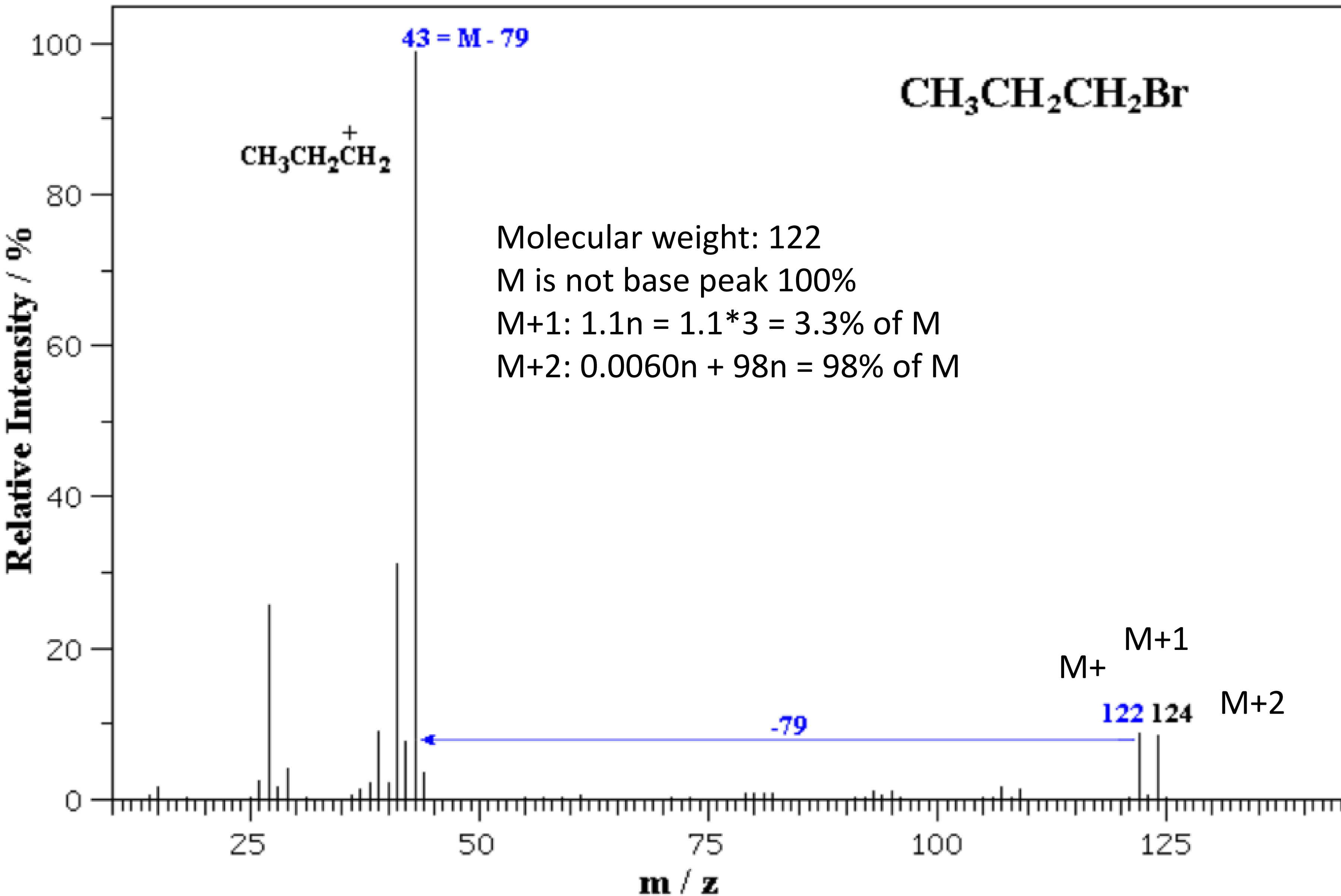
- H, C, N, O, Si, S, Cl, Br, etc
- Atomic weight is average
- MS spec shows

Table1

Type	Element	Symbol	Integer Mass[1]	Exact Mass[2]	Percent Abundance	X+1 Factor[3]	X+2 Factor[4]
X	Hydrogen	H	1	1.0078	99.99		
		D or ^{2}H	2	2.0141	0.01		
X+1	Carbon	^{12}C	12	12.0000	98.91		
		^{13}C	13	13.0034	1.1	$1.1n_C$	$0.0060n_{C2}$
X+1	Nitrogen	^{14}N	14	14.0031	99.6		
		^{15}N	15	15.0001	0.4	$0.37n_N$	
X+2	Oxygen	^{16}O	16	15.9949	99.76		
		^{17}O	17	16.9991	0.04	$0.04n_O$	
		^{18}O	18	17.9992	0.20		$0.20n_O$
X	Fluorine	F	19	18.9984	100		
X+2	Silicon	^{28}Si	28	27.9769	92.2		
		^{29}Si	29	28.9765	4.7	$5.1n_{Si}$	
		^{30}Si	30	29.9738	3.1		$3.4n_{Si}$
X	Sodium	Na	23	22.9898	100		
X	Phosphorus	P	31	30.9738	100		
X+2	Sulfur	^{32}S	32	31.9721	95.02		
		^{33}S	33	32.9715	0.76	$0.8n_S$	
		^{34}S	34	33.9679	4.22		$4.4n_S$
X+2	Chlorine	^{35}Cl	35	34.9689	75.77		
		^{37}Cl	37	36.9659	24.23		$32.5n_{Cl}$
X+2	Potassium	^{39}K	39	38.9637	93.26		
		^{40}K	40	39.9640	0.013	$0.012n_K$	
		^{41}K	41	40.9618	6.74		$7.22n_K$
X+2	Bromine	^{79}Br	79	78.9183	50.5		
		^{81}Br	81	80.9163	49.5		$98.0n_{Br}$
X	Iodine	I	127	126.9045	100		



Biphenyl
MASS SPECTRUM
100
80
60
40
20
0.0
0.0
40
80
120
160
m/z
Molecular weight: 154
n= 12
M+1: 1.1n = 1.1*12 = 13.2
M+2: 0.0060n = 0.0060*12 =
M+
(100%)
M+1
(~13.2%)
M+2
(~0.072%)



43 = M - 79
CH3CH2CH2Br
+
CH3CH2CH2
Molecular weight: 122
M is not base peak 100%
M+1: 1.1n = 1.1*3 = 3.3% of M
M+2: 0.0060n + 98n = 98% of M
M+1
M+
M+2
122
124
-79
Relative Intensity / %
m / z
100
80
60
40
20
0
25
50
75
100
125

GC-MS vs LC-MS

- GC-MS: Gas phase sample to MS
  1. Easy to ionize and fragmentation
  2. Limited to nonpolar with low molecular weight (m/z ~ 600)

- LC-MS: Liquid phase sample to MS
  1. Atmospheric ionization
  2. Difficult to ionize
  3. Hard to fragment
  4. MS-MS is popular option
  5. Wide range of polarity and molecular weight (m/z ~4000, 100 kD)

  6. More vigorous ionization techniques are required

Hardware: Same as GC

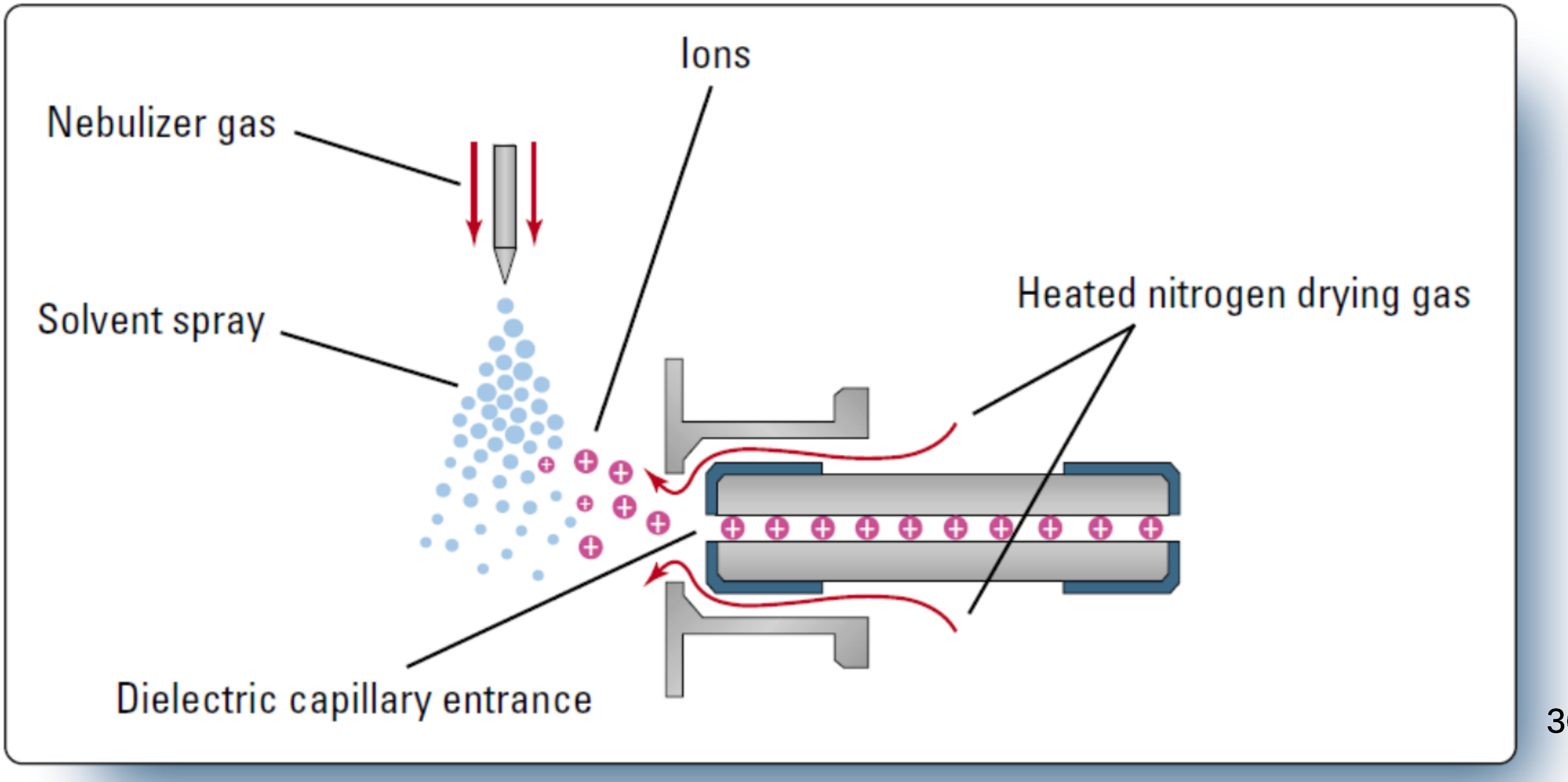
- Mass source (ion source): **ESI**, APCI, APPI, etc
  1. Samples are introduced as liquid
  2. Ionized (several methods)
  3. Accelerated to analyzer

- Analyzer (filter): several methods (quadrupole, ion trap, TOF, etc)

- Detector: dynode, count # of ions from analyzer
  Ion multiplier: amplifies current similar to photomultiplier

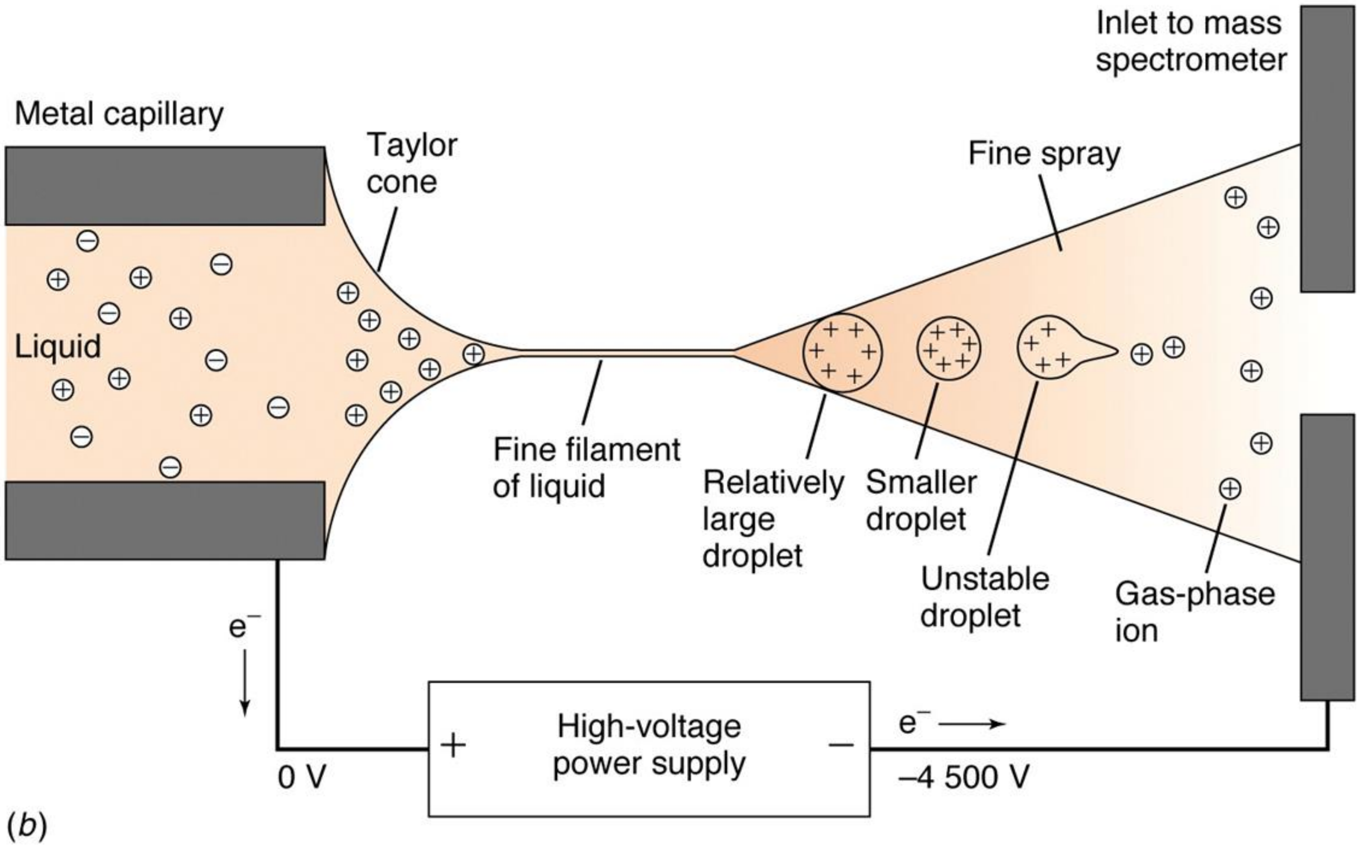
Electronspray Ionization(ESI)

- A small amount of ~0.1% proton source, CH_3COOH (or HCOOH) is added to eluent.
- Hot inert gas (nitrogen) is nebulized with sample (nebulizer gas).
- Liquid sample is under high electric field and drying nitrogen gas.
- Ions are generated (electronspray, ESI).

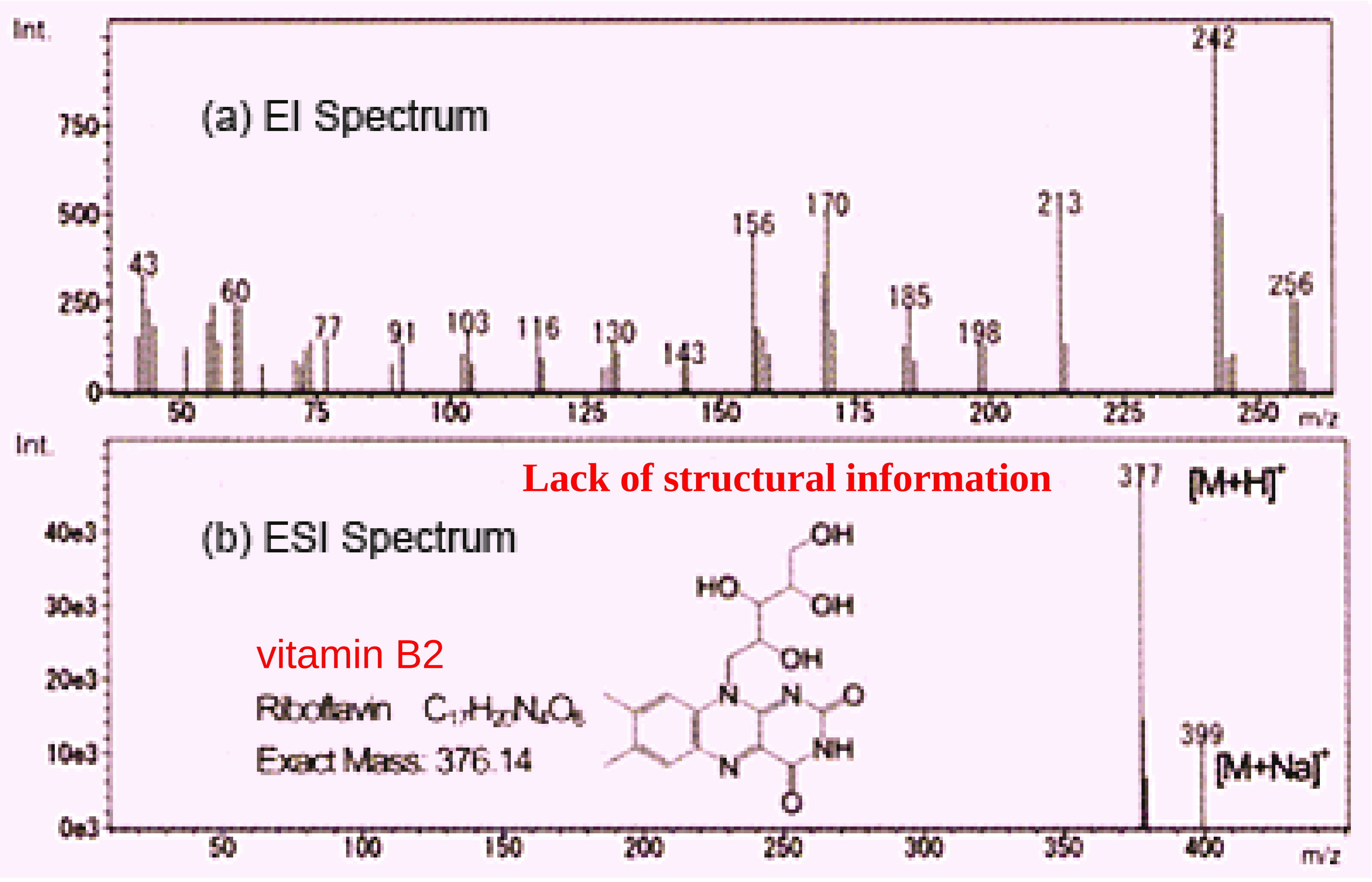


Three Substeps of Ion Formation

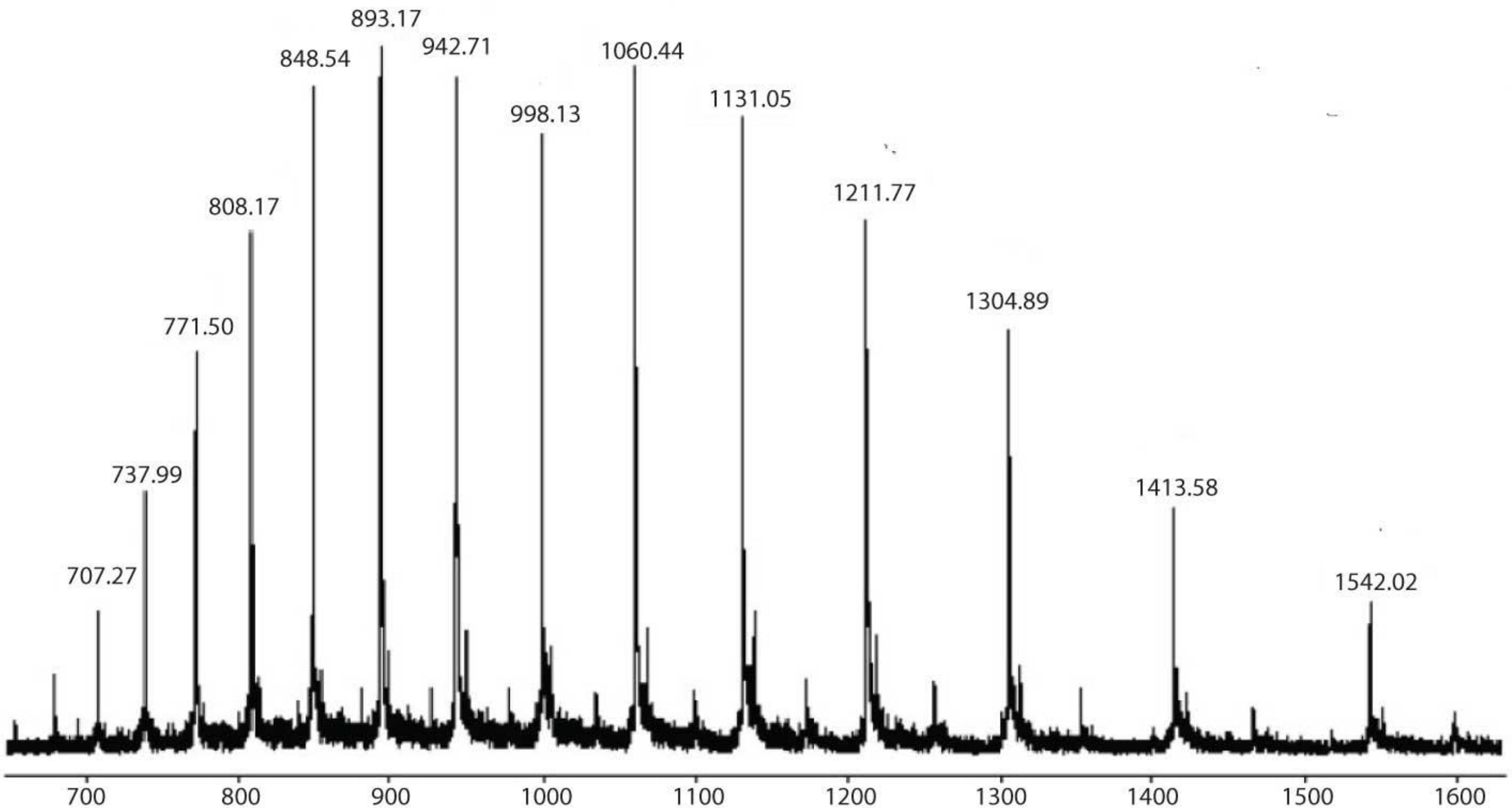
- Droplet formation (Taylor cone & hot nebulizer gas)
- Droplet shrinkage via evaporation of solvent and higher charge density (and/or hot nebulizer gas and fission)
- Gaseous ion formation via complete solvent evaporation



GC vs. LC Mass Spectra



Measuring Multivalent Ions



Molecular Weight Calculation

$m/z = (M + zH)/z$ (Eq.1)

$m = (M + zH)$ (Eq. 2)

$m - zH = M$ (Eq. 3)

$M = z(m/z - H)$ (Eq. 4)

$z_1 = z_2 + 1.$

$z_2 = ((m_1/z_1) - H) / ((m_2/z_2) - (m_1/z_1))$ (Eq. 5)

m/z values 1131 and 1212. We can determine the number of charges on the peak at 1212 using Equation 5:

$z_{1212} = (1131 - 1) / (1212 - 1131) = 14$

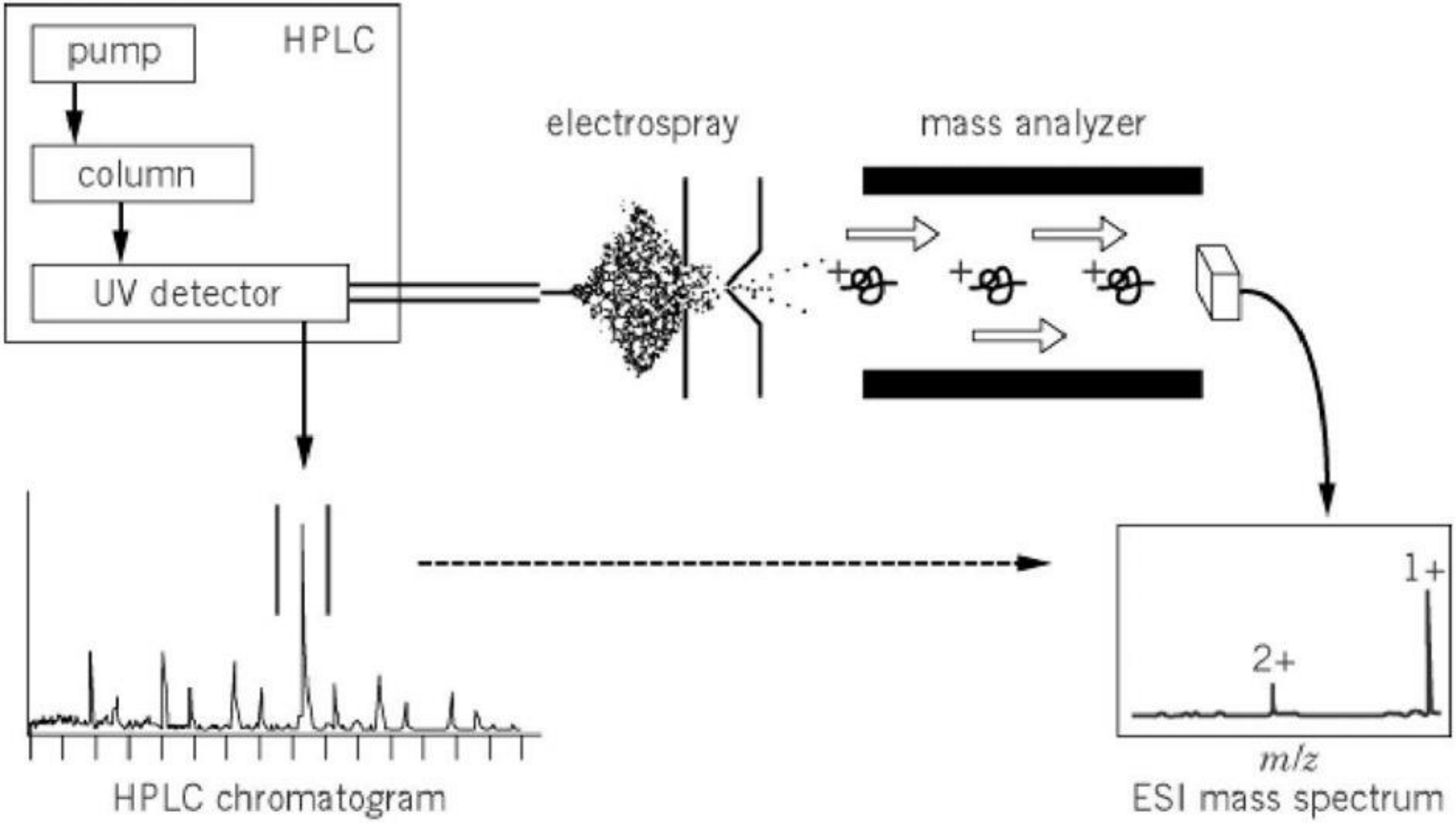
And from Equation 4 the molecular mass is easily determined:

M = 14 (1212-1) = 16,954 Da

Limits of Traditional MS

- $[M+H]^+$ dominates in spectrum
- If m/z of $[A+H]^+$ and $[B+2H]^{2+}/2$ is identical, no resolution in TIC
  - Isomers with same molecular weight
- Structural determination is not feasible/impossible

LC-MS



pump
HPLC
column
UV detector
electrospray
mass analyzer
1+
2+
m/z
HPLC chromatogram
ESI mass spectrum