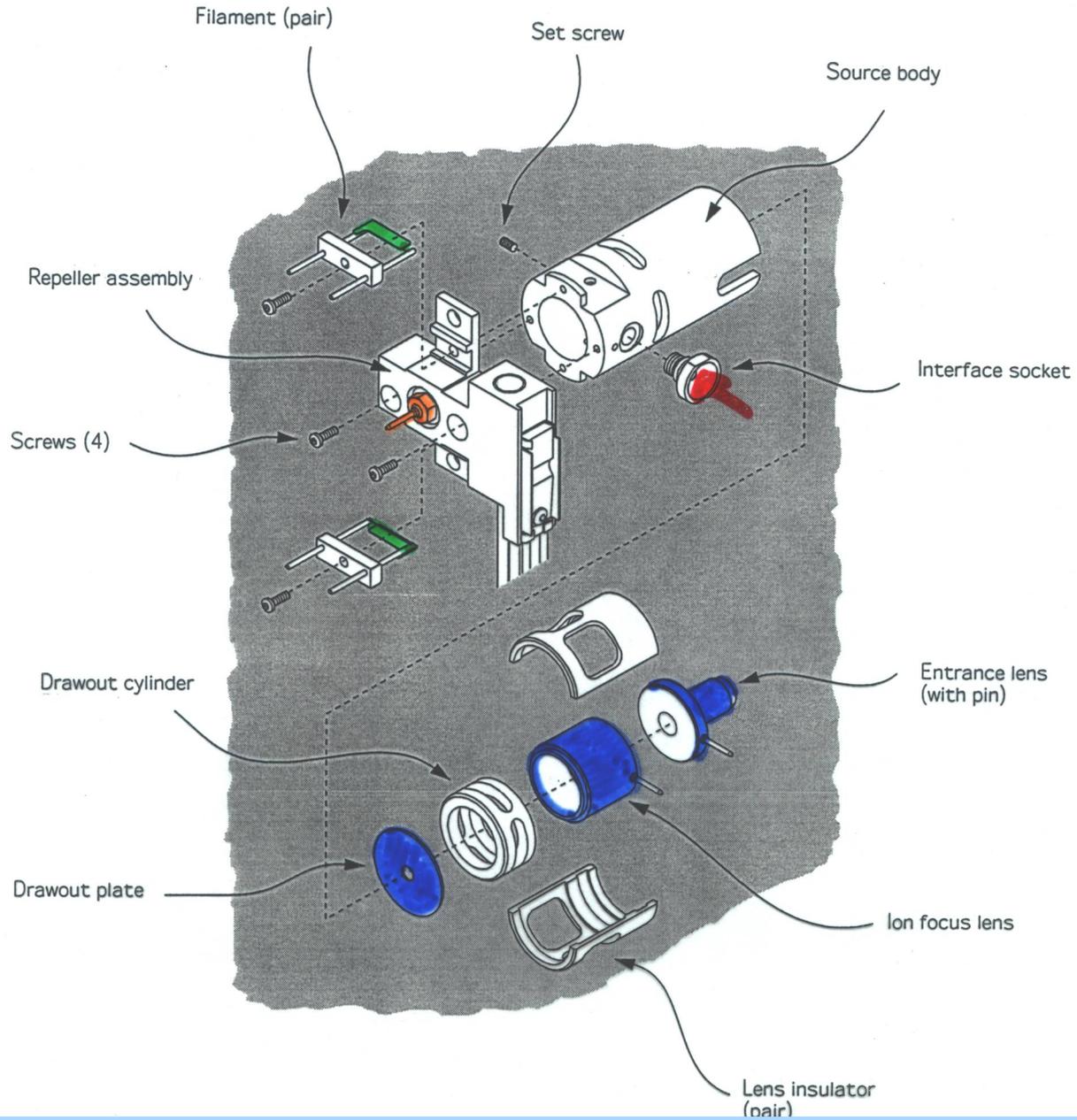
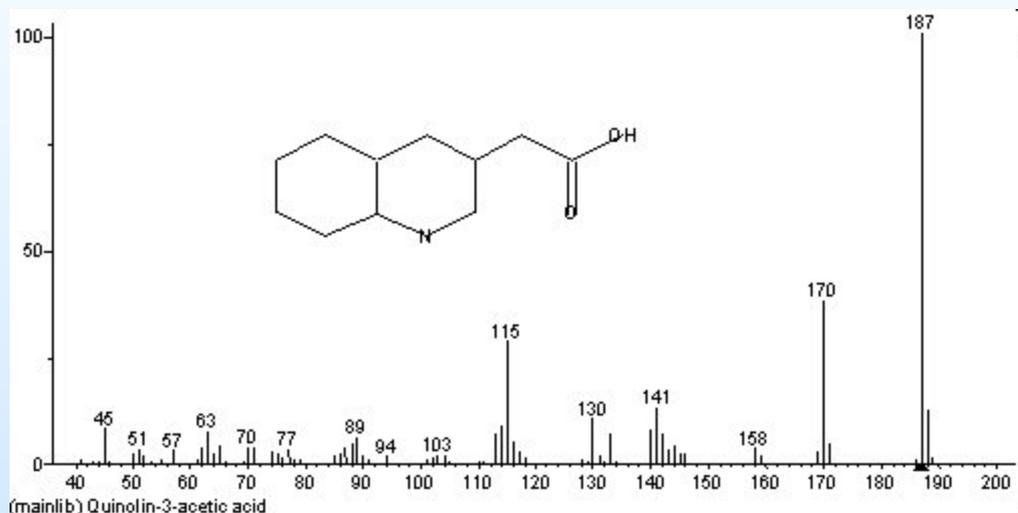


MSD Ion Source



What do I get from the instrument?



Name: Quinolin-3-acetic acid
Formula: C₁₁HgNO₂
MW: 187 CAS#: N/A NIST#: 128384 ID#: 79578 DB: mainlib
Other DBs: None
Contributor: LAC, NIDDK, NIH, Bethesda, MD 20892

10 largest peaks:

187 999	170 376	115 282	141 128	188 123
130 102	114 86	45 80	140 79	63 72

70 m/z Values and Intensities:

41 6	43 4	44 4	45 80	46 1
50 24	51 32	52 14	53 5	55 7
57 29	61 8	62 35	63 72	64 21
65 40	66 4	69 3	70 37	71 33
74 25	75 22	76 13	77 31	78 10
79 10	85 18	86 23	87 33	88 41
89 59	90 16	91 7	94 14	101 6
102 12	103 17	104 15	105 4	110 1
111 1	113 68	114 86	115 282	116 49
117 26	118 13	128 7	129 2	130 102
131 17	132 1	133 66	134 5	140 79
141 128	142 67	143 29	144 40	145 20
146 20	158 33	159 14	169 26	170 376
171 45	186 8	187 999	188 123	189 11

ABUNDANCE
↑
%RA %TIC?

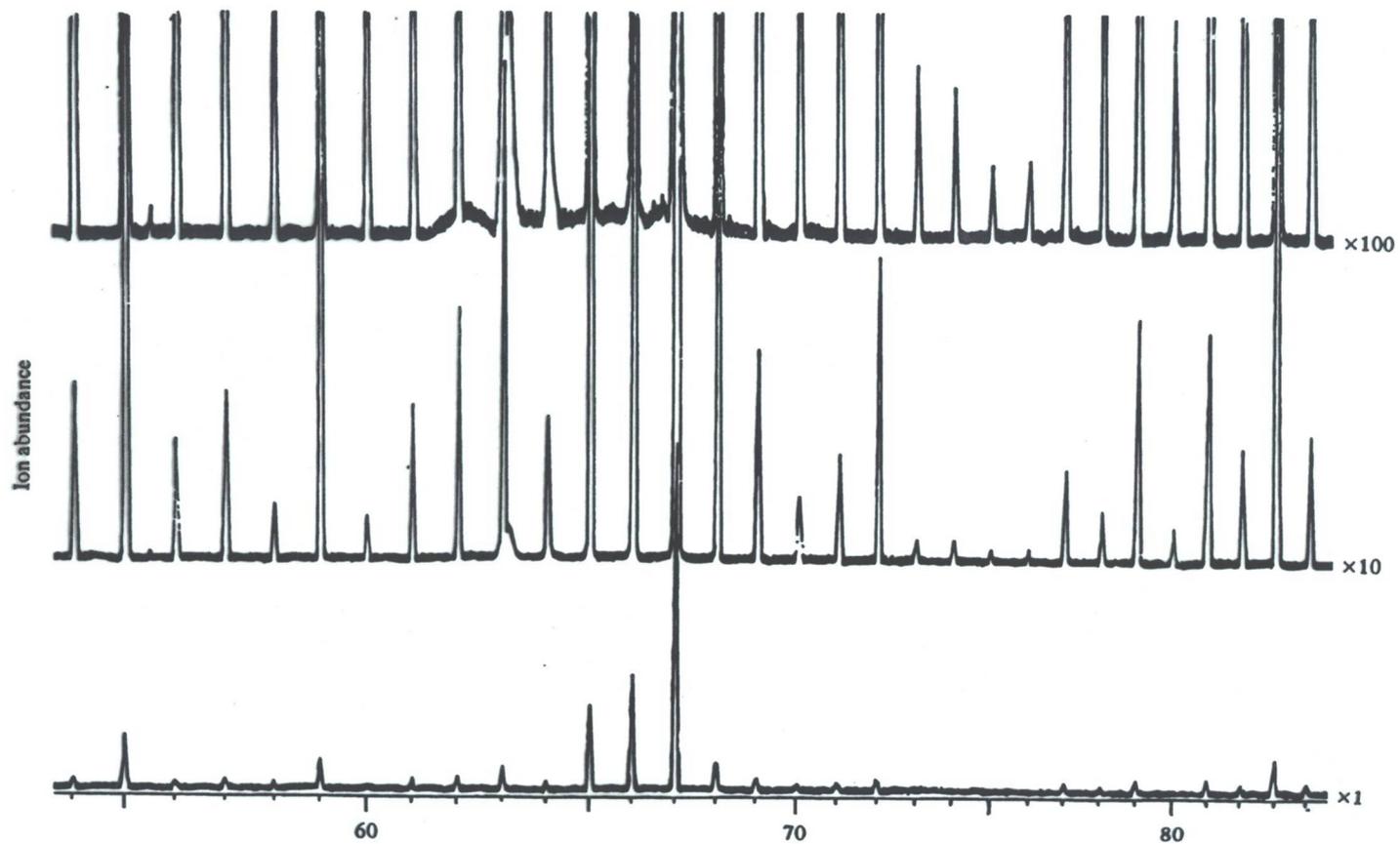


Figure 1.2. Part of a mass spectrum recorded on photographic paper (the three traces record the spectrum at increasing sensitivities from the lower to the upper trace).

Table 1.3. Ion abundances in the mass spectrum of decahydronaphthalene.^a

Mass	%RA	%TIC	Mass	%RA	%TIC
41	64.02	5.25	80	5.99	0.49
42	9.79	0.80	81	86.55	7.10
43	7.19	0.59	82	78.53	6.44
50	0.67	0.06	83	15.70	1.29
51	3.02	0.25	84	17.16	1.41
52	1.82	0.15	85	0.82	0.07
53	12.99	1.07	91	2.14	0.18
54	21.23	1.74	93	2.87	0.24
55	46.84	3.84	94	3.50	0.29
56	25.85	2.12	95	60.05	4.93
57	1.94	0.16	96	99.19	8.14
63	0.46	0.04	97	11.35	0.93
65	3.73	0.31	108	0.81	0.07
66	8.24	0.68	109	30.31	2.49
67	100.0	8.20	110	9.07	0.74
68	59.97	4.92	111	0.42	0.03
69	35.17	2.89	123	0.89	0.07
70	3.19	0.26	137	0.91	0.07
71	0.45	0.04	138	99.35	8.15
77	5.02	0.41	139	10.37	0.85
78	1.08	0.09	140	0.31	0.03
79	9.43	0.77			

Notes:

^a All peaks over m/z 40 of greater than 0.3 per cent relative abundance are included.

~ 57.5 m/z ??

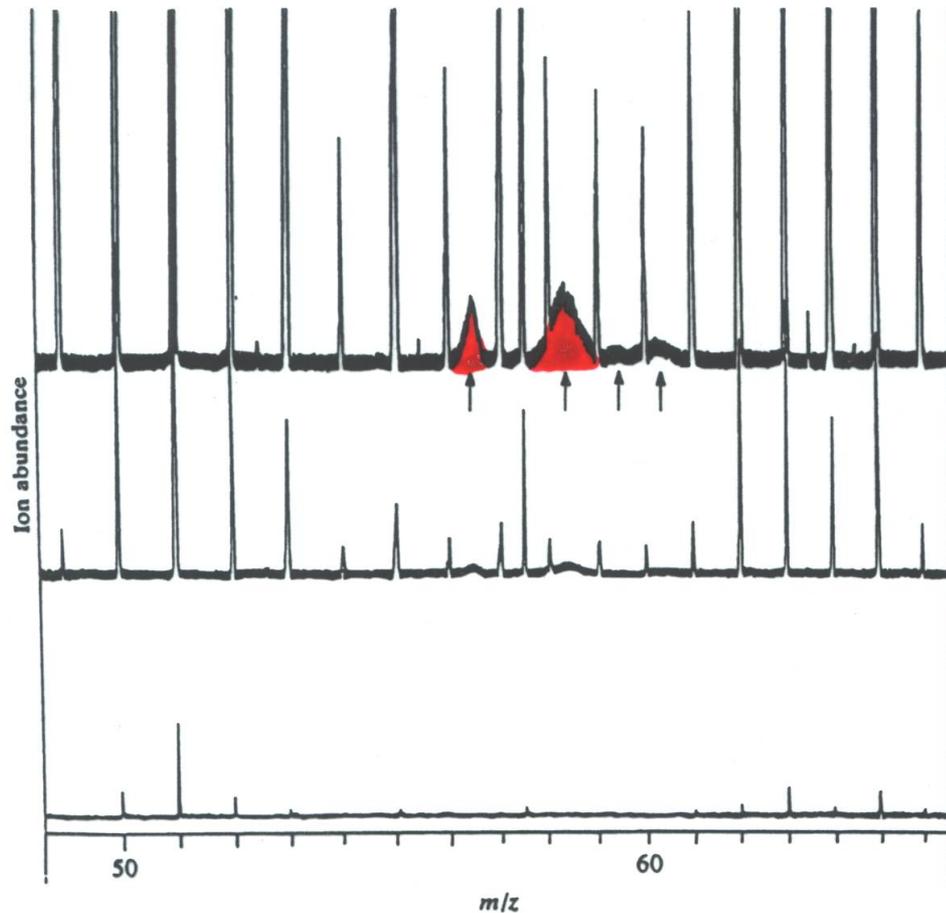


Figure 1.9. Peaks in a mass spectrum (indicated by arrows) representing product ions arising from fragmentation of metastable precursor ions. Normal product ions (produced in the ion source) give rise to narrow peaks at integral mass but product ions from metastable decomposition outside the source are usually seen as broad peaks at non-integral mass. Note also the fairly abundant doubly charged ions at m/z 57.5.

56.3 m/z ??
58.7 m/z . .

~ 57.5 m/z ??

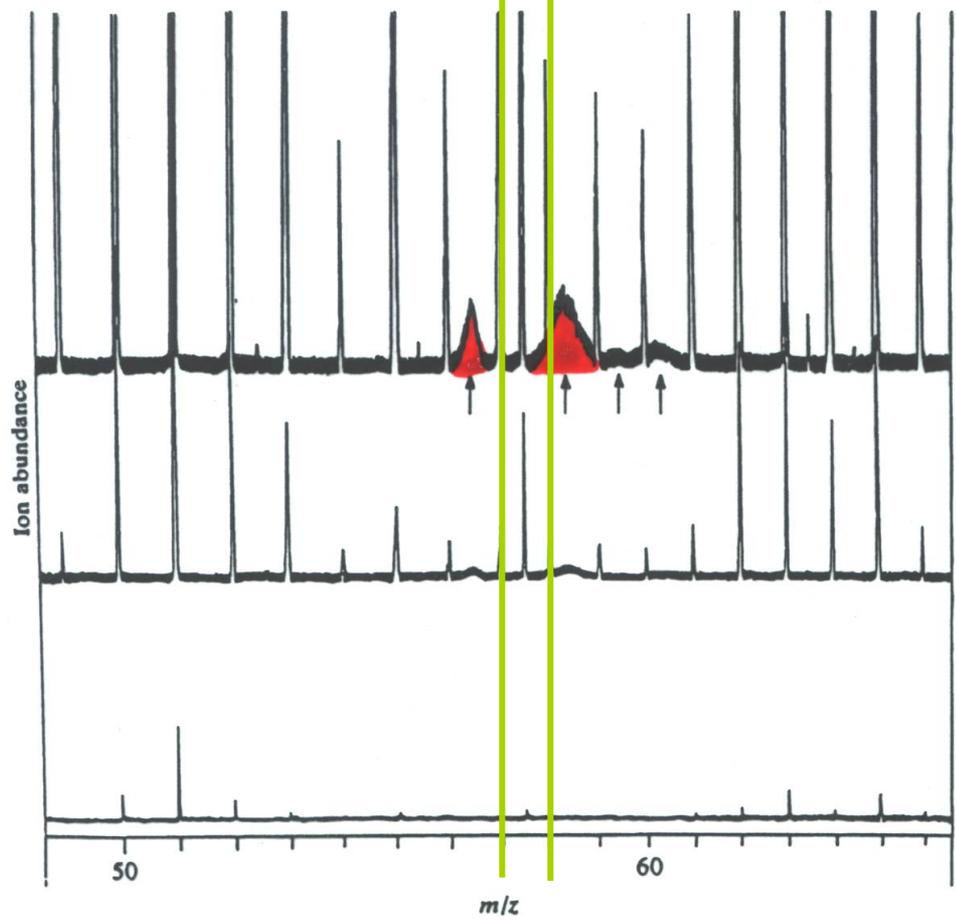


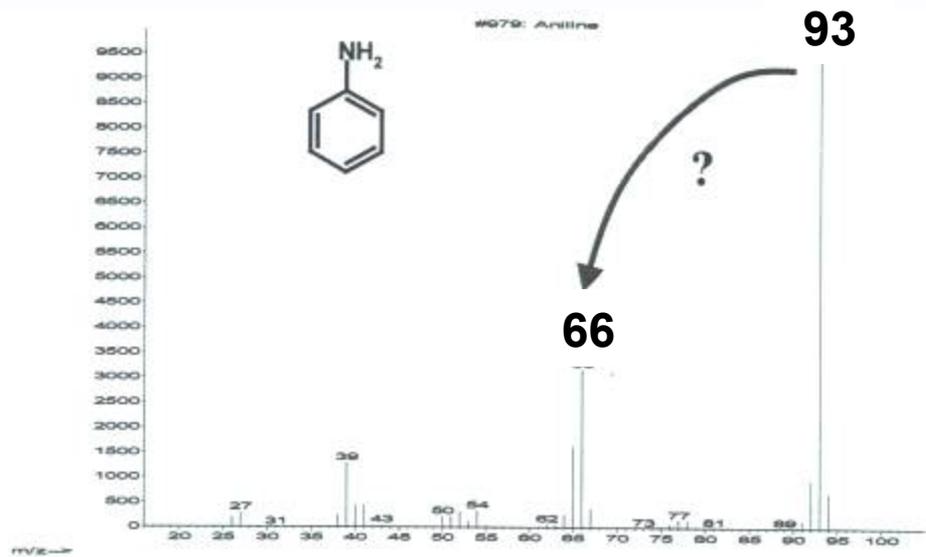
Figure 1.9. Peaks in a mass spectrum (indicated by arrows) representing product ions arising from fragmentation of metastable precursor ions. Normal product ions (produced in the ion source) give rise to narrow peaks at integral mass but product ions from metastable decomposition outside the source are usually seen as broad peaks at non-integral mass. Note also the fairly abundant doubly charged ions at m/z 57.5.

100x

10x

1x

56.3 m/z ??
58.7 m/z . .



Can we use metastable ions to determine fragmentation patterns?

$$m_1 \rightarrow m_2$$

try to find m^*

$$m^* = m_2^2 / m_1$$

Metastable ions are broad peaks with fractionate masses

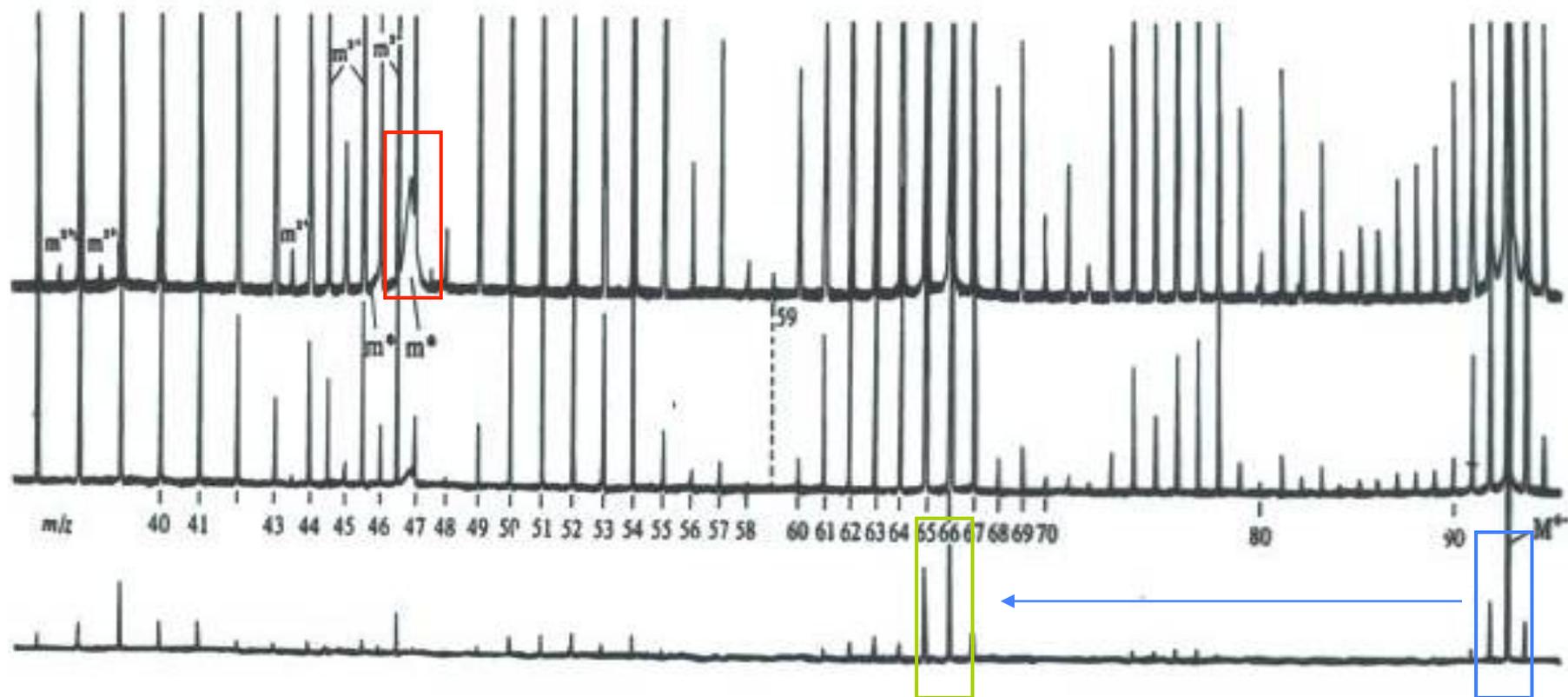


Figure 1.12. The mass spectrum of aniline. The molecular ion (M^+) and the ^{13}C -isotope ion $[M + 1]^+$ are shown. Products of metastable ions (m^*) and doubly charged ions (m^{2+}) are marked. Integral mass-to-charge ratios are given below the middle trace where the spectrum may be counted; where a peak does not appear, as at m/z 59, reference to the top trace will often reveal ions of low abundance.

$$m^* = 46.8 ?$$

$$M^+ = 93 = m_1$$

$$66 = m_2$$

$$m^* = m_2^2 / m_1 = (66)^2 / 93 = 46.8 !$$

Just for clarification

- ***Atomic mass***
- *amu*, atomic mass units (uma??)
- “*Da*” or *Dalton*.
- kD (kiloDalton)
- $1 \text{ amu} = 1.66056 \cdot 10^{-27} \text{ kg}$.
- proton, $m_p = 1.67265 \cdot 10^{-27} \text{ kg}$,
- neutron, $m_n = 1.67495 \cdot 10^{-27} \text{ kg}$.

Atomic masses and isotopes

The atomic weights that we find on the periodic table represent an average for all naturally occurring isotopes.

Example

Chlorine - atomic weight = 35.453

A mixture of ^{35}Cl - 75.77%, ^{37}Cl - 24.23%

We must not only need to account for these isotopes, we can exploit them for determining the number and types of elements that are present.

More on accurate vs. average

● *Nominal mass*

● The *nominal mass* of an ion is calculated by simply adding the *integer masses* of the lightest isotopes of all elements contributing to the molecule

● nominal mass of CH_3Br →

● ^{12}C , ^1H , and ^{79}Br to obtain $M_{\text{nominal}} = 94$ amu.

Accurate or average?

- ***Monoisotopic mass***

- The *monoisotopic mass* is the mass obtained by summing up the *accurate* isotopic masses of the lightest isotopes of all elements contributing to a molecule

- e.g. for CH₃Br

- 12C, 1H, and 79Br → $M_{\text{mono}} = 93.9418 \text{ amu}$

Exact Masses of Some Common Elements and Their Isotopes:

Element	Symbol	Exact Mass (u)	Rel. Abundance %
Hydrogen	¹ H	1.007825037	100.0
Deuterium	² H or D	2.014101787	0.015
Carbon 12	¹² C	12.00000	100.0
Carbon 13	¹³ C	13.003354	1.11223
Nitrogen 14	¹⁴ N	14.003074	100.0
Nitrogen 15	¹⁵ N	15.00011	0.36734
Oxygen 16	¹⁶ O	15.99491464	100.0
Oxygen 17	¹⁷ O	16.9991306	0.03809
Oxygen 18	¹⁸ O	17.99915939	0.20048
Fluorine	¹⁹ F	18.998405	100.0
Sodium	²³ Na	22.9897697	100.0
Silicon 28	²⁸ Si	27.9769284	92.23
Silicon 29	²⁹ Si	28.9764964	5.0634
Silicon 30	³⁰ Si	29.9737717	3.3612
Phosphorus	³¹ P	30.9737634	100.0
Sulfur 32	³² S	31.972074	100.0
Sulfur 33	³³ S	32.9707	0.78931
Sulfur 34	³⁴ S	33.96938	4.43065
Sulfur 36	³⁶ S	35.96676	0.02105
Chlorine 35	³⁵ Cl	34.968854	100.0
Chlorine 37	³⁷ Cl	36.965896	31.97836

High Resolution Accurate Mass Measurement: Elemental Compositions

- Accurate mass determination to several decimal places, using a double-focusing mass spectrometer, enables the determination of molecular formulae and elemental compositions
- For an ion of NH_3 , the measured integer mass would be 17, viz., $m/z = 17/1 = 17$, for $z = 1$.
- In theory, this process can be reversed in that any measured mass leads to an elemental composition.
 - For example, a measured value of 17 would imply the composition, NH_3 .
 - In practice, there are other elemental compositions which could add up to 17. For example, OH (oxygen = 16, hydrogen = 1), CD_2H (carbon = 12, deuterium = 2×2 , hydrogen = 1).

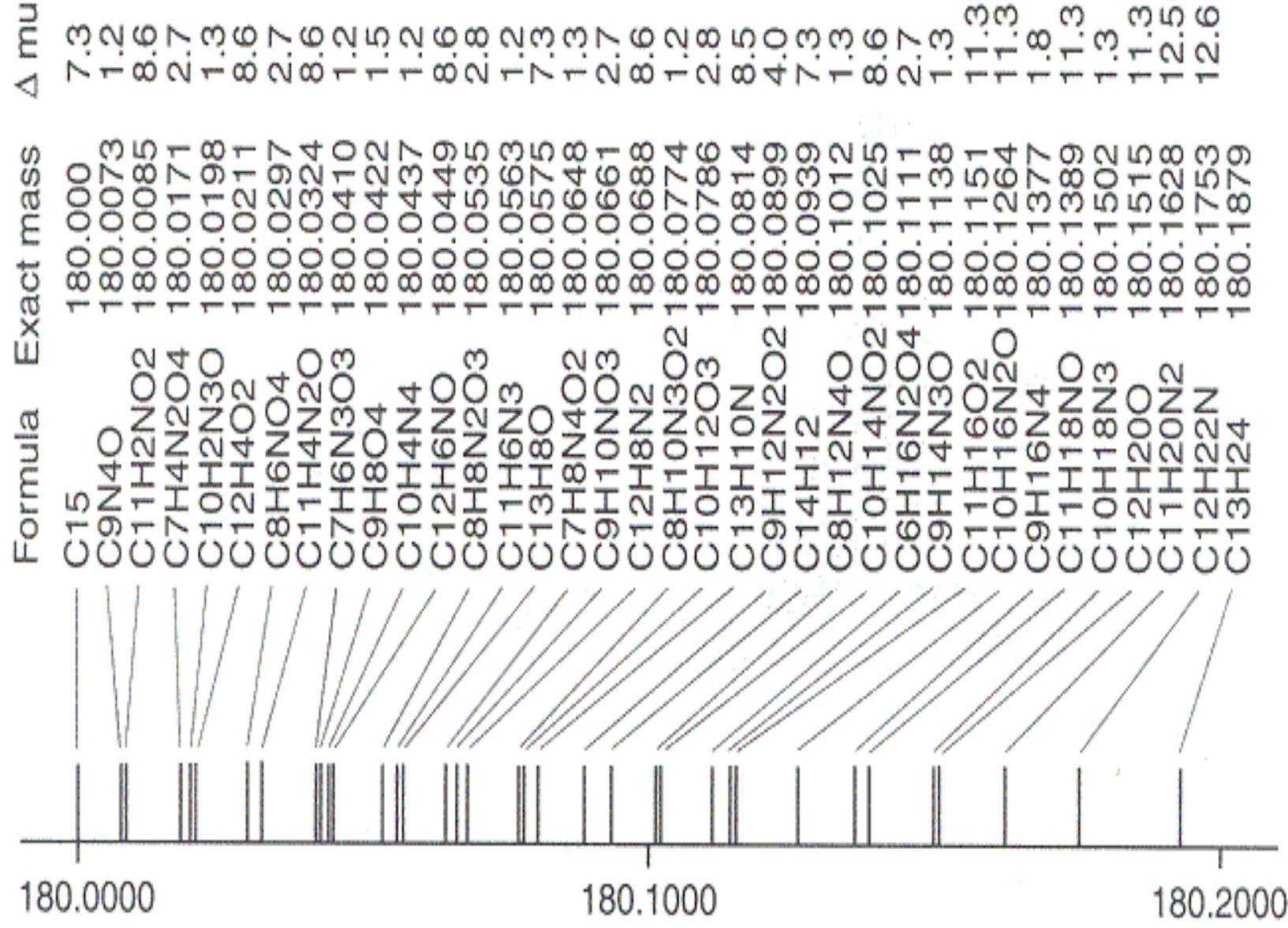
High Resolution Accurate Mass Measurement: Elemental Compositions

- For larger masses, the possibilities increase enormously.
 - At mass 100, there would be literally thousands of possible elemental compositions → unit mass is not enough!
- In fact atomic masses are not integers!
 - C = 12.0000.
 - O = 15.9949,
 - N = 14.0031,
 - H = 1.0078 and so on...
- The accurate mass for ammonia (NH₃) is:
 $14.0031 + 3 \times 1.0078 = 17.0265$
- The accurate mass of OH is: $15.9949 + 1.0078 = 17.0027$,
 - giving a mass difference of 0.0238 units between NH₃ and OH, which are potentially separable.
- A mass spectrometer which can measure mass correct to several decimal places rather than just integer mass can be used to measure such differences
- → RESOLUTION IS IMPORTANT → ELEMENTAL ANALYSIS

Accurate Mass Determination

Integer Mass = 58				Accurate Mass	
<u>*C</u>	<u>H</u>	<u>N</u>	<u>O</u>		m/z measured 58.04189
1	-	1	2	57.992902	
1	2	2	1	58.016711	
1	4	3	-	58.040520	
2	2	-	2	58.005478	
2	4	1	1	58.053096	
2	6	2	-	58.053096	
3	6	-	1	58.041862	C₃H₆O
3	8	1	-	58.065671	
4	10	-	-	58.078247	

* Compositions are read from left to right. Thus the fifth entry would be C₂H₄NO, of accurate mass 58.053096.



What is mass resolution?

$$R = \frac{m}{\Delta m}$$

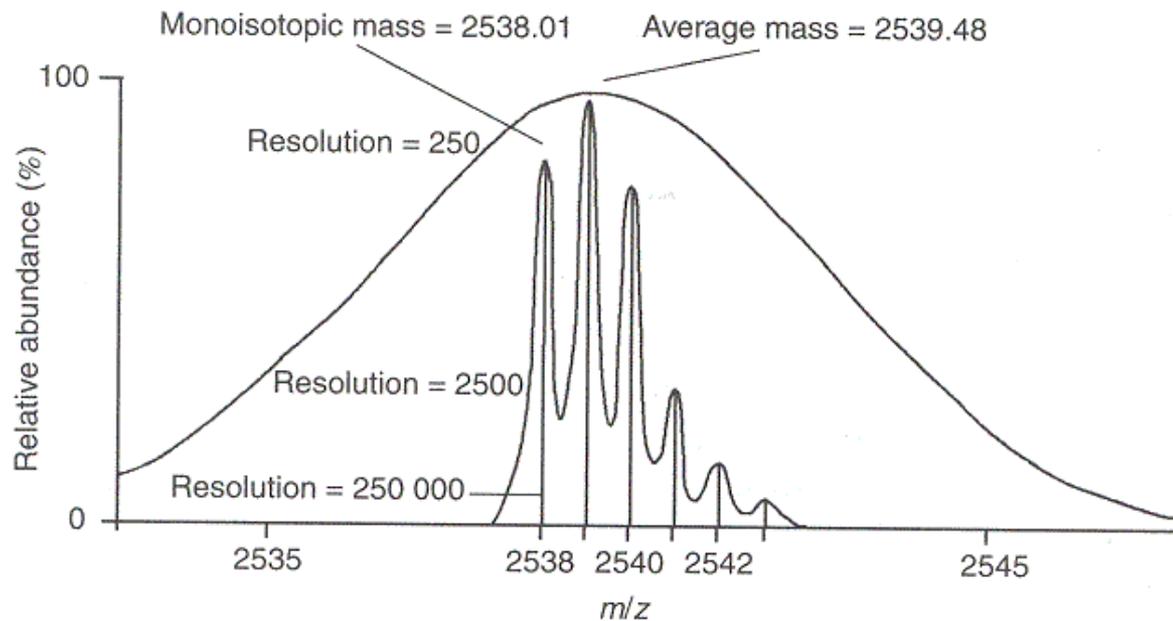


Figure 7.1

Mass spectrum of isotopic cluster of a singly protonated peptide ($C_{101}H_{145}N_{34}O_{44}$) with monoisotopic mass = 2 538 015 u and average mass = 2 539 483 Da at resolutions of 250 (resolution typically obtained for linear TOF), 2500 (quadrupole) and 250 000 (FTICR). (Reproduced (modified) from Ref. 16 permission)

Lets go back to resolution: what do I need to separate them?

● The accurate mass for ammonia (NH_3) is:
 $14.0031 + 3 \times 1.0078 = 17.0265$

● The accurate mass of OH is:
 $15.9949 + 1.0078 = 17.0027,$

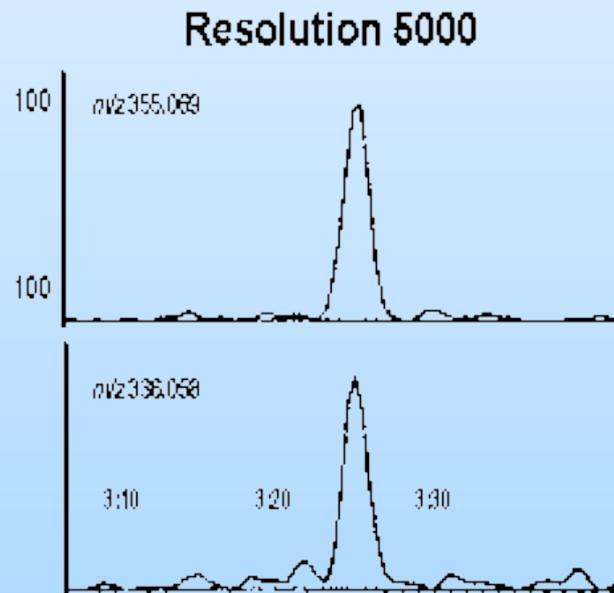
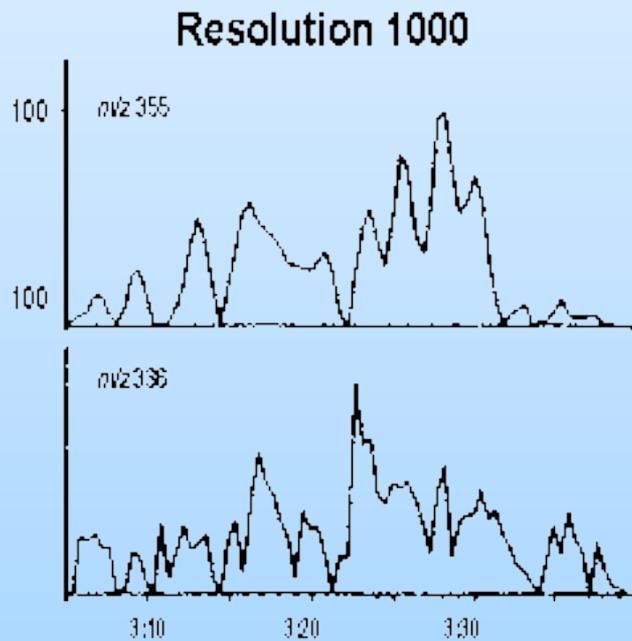
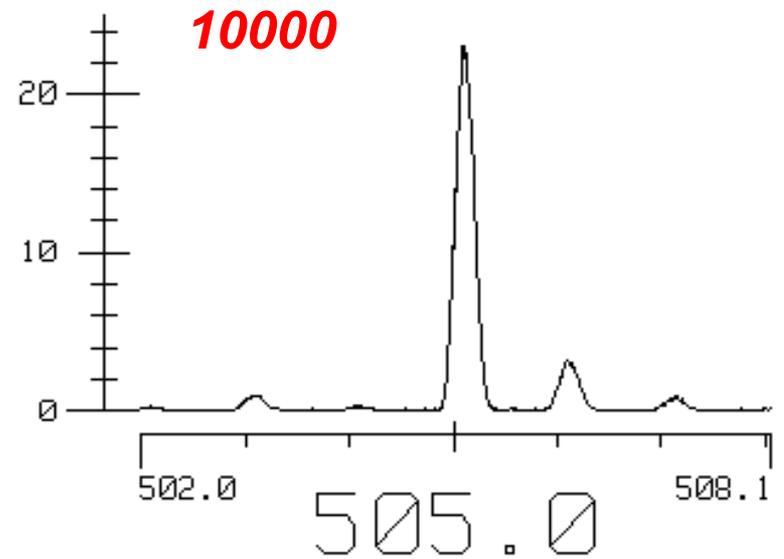
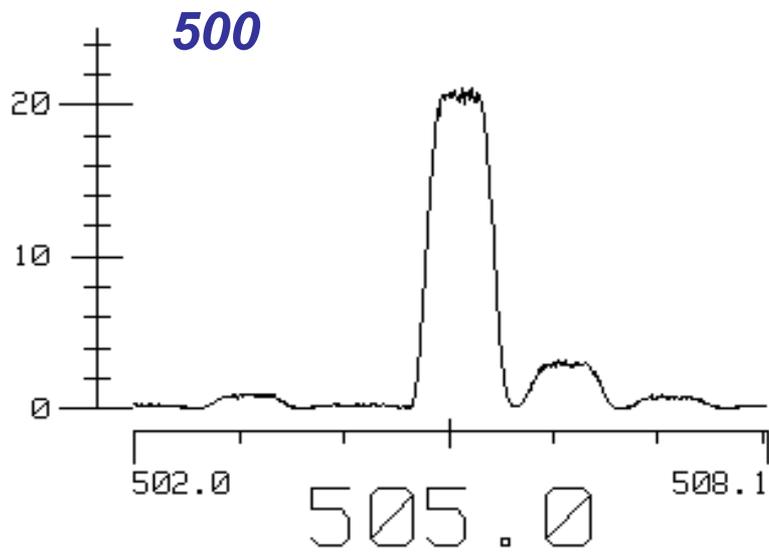
● giving a mass difference of 0.0238 units between NH_3 and OH, which are potentially separable.

$$\rightarrow \Delta m = 0.0238 \text{ amu}$$

$$\rightarrow m = 17.0265 \text{ amu for } \text{NH}_3$$

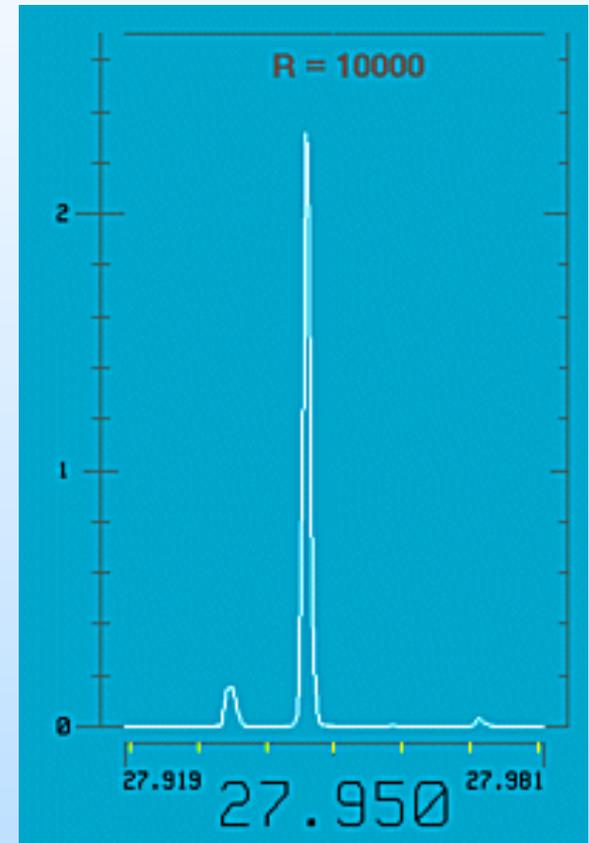
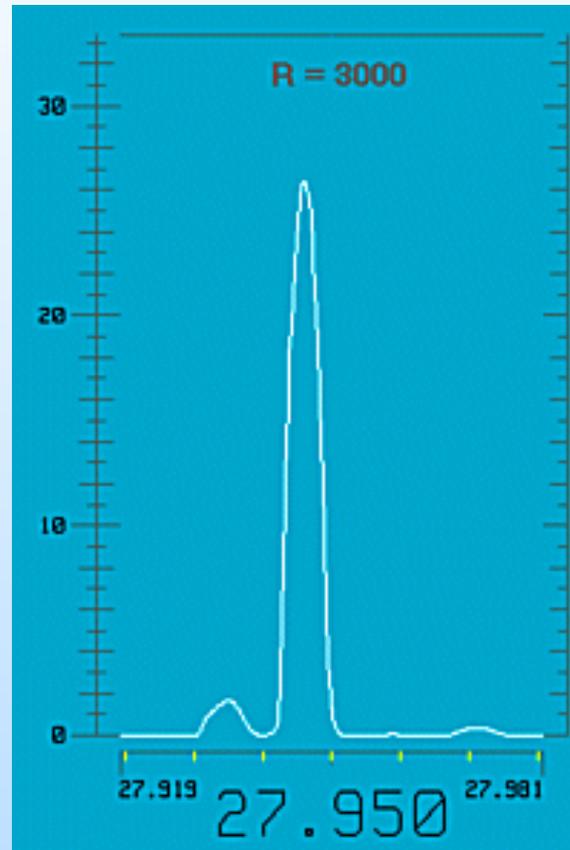
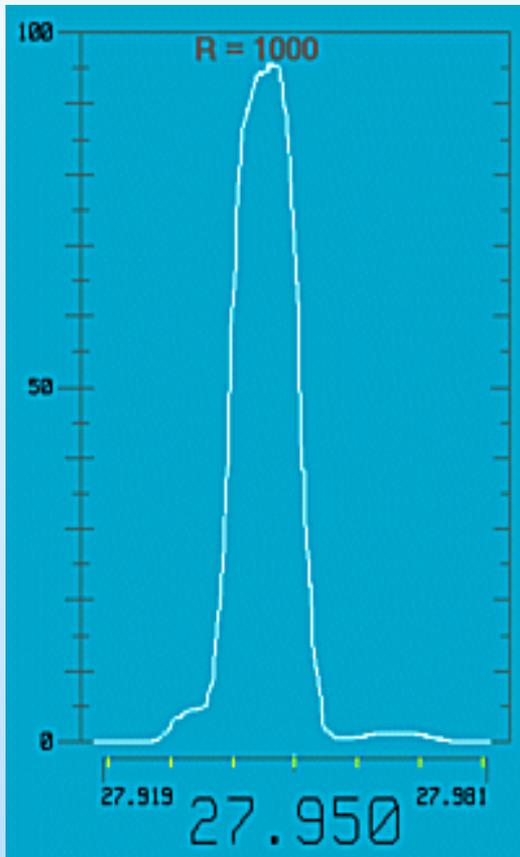
Minimum resolution required:

$$R = 17.0265/0.0238 = 715.4$$



Clenbuterol
 m/z 355

When resolution is not a good thing?



Sensitivity vs. selectivity

ACCURATE MASS, AVERAGE MASS, NOMINAL MASS,... WHAT A MASS!

(a) Average atomic mass for Cl = 35.45 **CLOROETHANE**
Average atomic mass for C = 12.01
Average atomic mass for H = 1.01
∴ Average molecular mass
for C₂H₅Cl = 64.52 (used for weighing)

(b) Isotopic mass for ¹²C = 12
Isotopic mass for ¹³C = 13
Isotopic mass for ³⁵Cl = 35
Isotopic mass for ³⁷Cl = 37
Isotopic mass for ¹H = 1
∴ Isotopic molecular masses
for C₂H₅Cl = 64, 65, 66, 67 (used in mass spectrometry)

Exact Masses of Some Common Elements and Their Isotopes:

Element	Symbol	Exact Mass (u)	Rel. Abundance %
Hydrogen	¹ H	1.007825037	100.0
Deuterium	² H or D	2.014101787	0.015
Carbon 12	¹² C	12.00000	100.0
Carbon 13	¹³ C	13.003354	1.11223
Nitrogen 14	¹⁴ N	14.003074	100.0
Nitrogen 15	¹⁵ N	15.00011	0.36734
Oxygen 16	¹⁶ O	15.99491464	100.0
Oxygen 17	¹⁷ O	16.9991306	0.03809
Oxygen 18	¹⁸ O	17.99915939	0.20048
Fluorine	¹⁹ F	18.998405	100.0
Sodium	²³ Na	22.9897697	100.0
Silicon 28	²⁸ Si	27.9769284	92.23
Silicon 29	²⁹ Si	28.9764964	5.0634
Silicon 30	³⁰ Si	29.9737717	3.3612
Phosphorus	³¹ P	30.9737634	100.0
Sulfur 32	³² S	31.972074	100.0
Sulfur 33	³³ S	32.9707	0.78931
Sulfur 34	³⁴ S	33.96938	4.43065
Sulfur 36	³⁶ S	35.96676	0.02105
Chlorine 35	³⁵ Cl	34.968854	100.0
Chlorine 37	³⁷ Cl	36.965896	31.97836

Atomic masses and isotope

	Mass	RB	Mass	RB	Mass	RB	Class
H	1.0078	100					A
C	12.0000	100	13.0034	1.1			A+1
N	14.0031	100	15.0001	0.37			A+1
O	15.9949	100			17.9992	0.2	A+2
F	18.9984	100					A
S	31.9720	100	32.9715	0.8	33.9679	4.4	A+2
Cl	34.9989	100			36.9659	32.5	A+2
Br	78.9183	100	80.9163	98			A+2

RB = relative abundance

Isotope classes

The A, A+1, A+2 class system is used to indicate the types of isotopes present.

A - Only a single isotope.

This may mean that the element only exists as a single isotope (like fluorine)

The abundance of all but one isotope is too small to use.

(Example. deuterium - 0.0015%)

Isotope classes

A+1

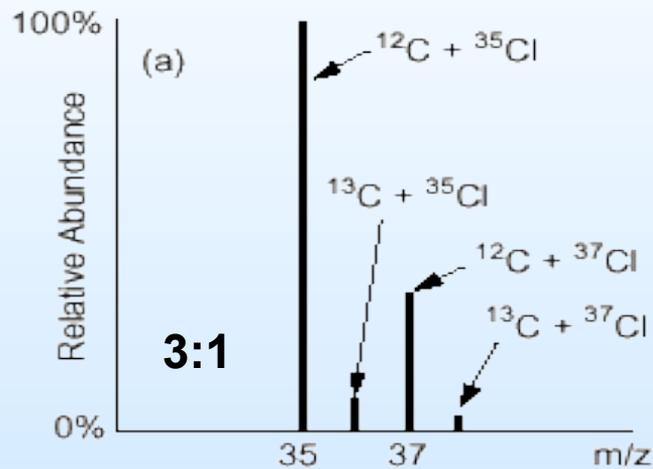
Two isotopes. Examples. C and N

A+2

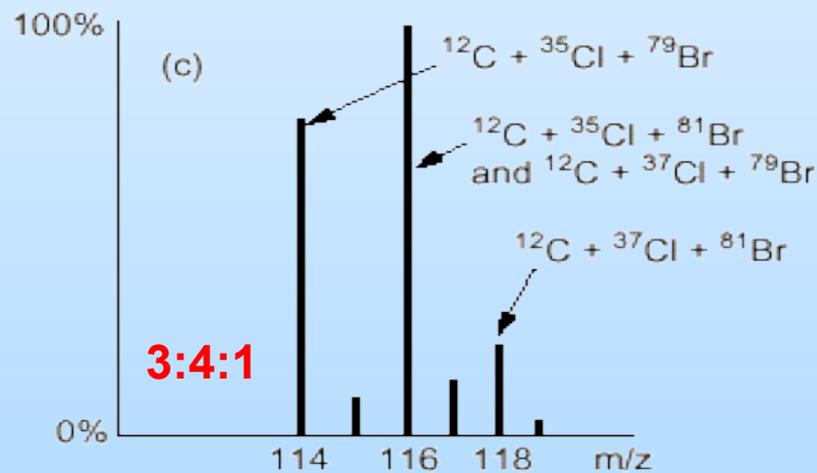
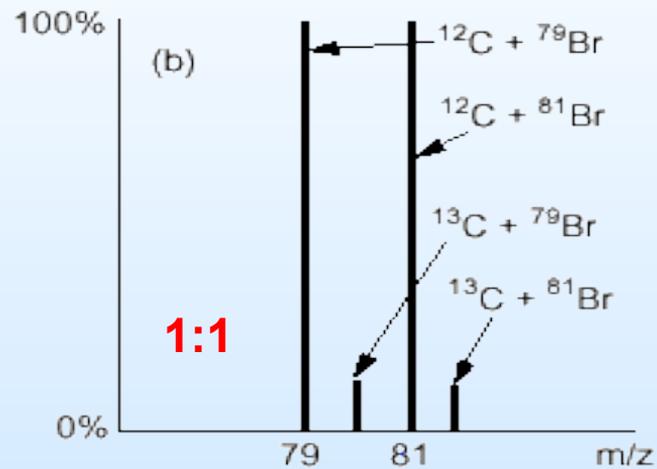
At least two isotopes with the highest mass isotope being +2 from the lowest mass isotope. Examples. O, S, Cl, Br.

In organic MS, the most abundant isotopes are also the lowest mass.

(a) only one chlorine atom

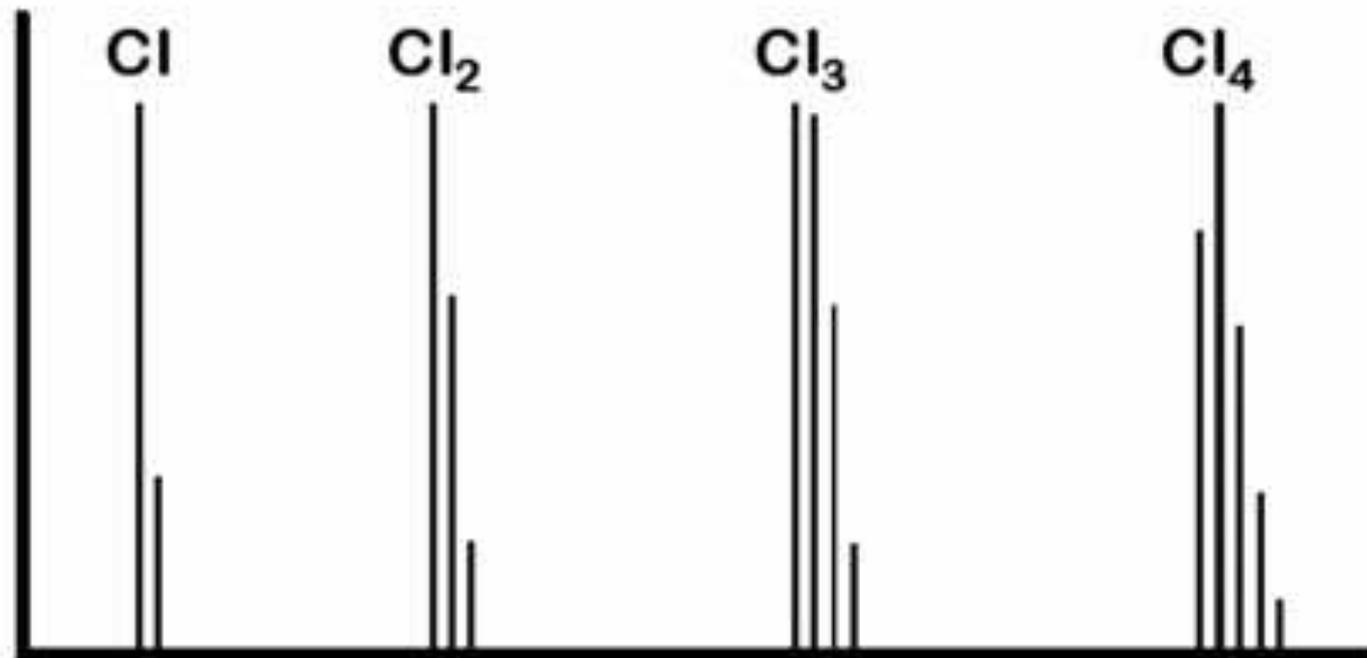


(b) only one bromine atom



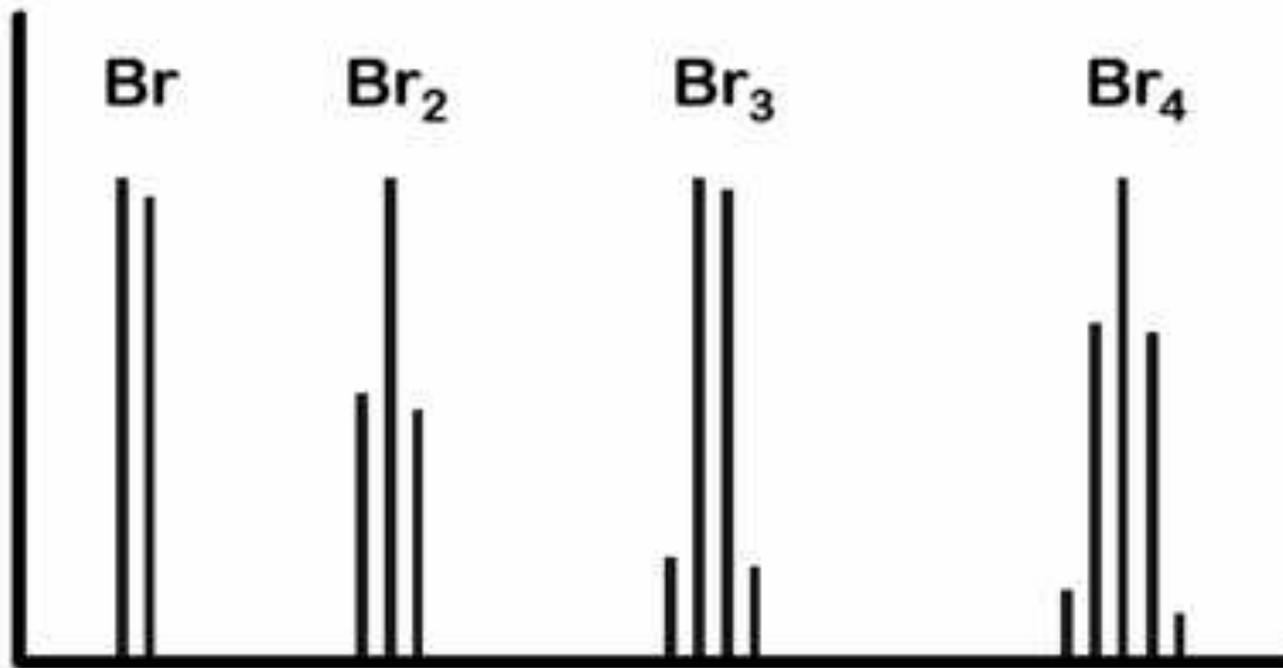
c) one chlorine and one bromine atom

Chlorine



By the time you have 4 chlorine, the A+2 line is significantly larger than the A line.

Bromine



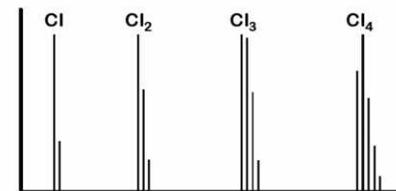
Bromine, which is almost a 1:1 mixture of ⁷⁹Br and ⁸¹Br also has a distinctive pattern.

Chlorine and Bromine

The cluster patterns of polychlorinated and polybrominated species are useful in helping to identify this type of material.

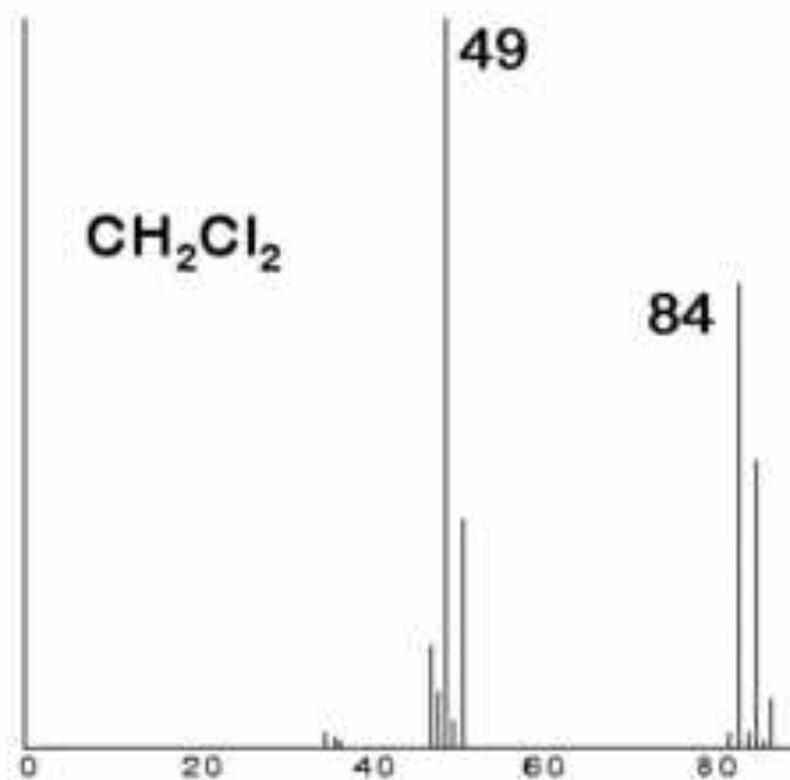
Some elements, like tin can give patterns similar to polyhalogenated species. So another factor to consider is the weight of the proposed molecular ion.

Another example



By the time you have 4 chlorine, the A+2 line is significantly larger than the A line.

Dichloromethane - a common GC/MS solvent



Note the Cl₂ pattern at 84 and the Cl pattern at 49

Table 1.4. *The more important natural isotope abundances for elements commonly occurring in mass spectrometry.**

Element	Isotope (percentage of natural abundance) ^b		
• Hydrogen	¹ H (99.99)		
• Boron	¹⁰ B (19.8)	¹¹ B (80.2)	
• Carbon	¹² C (98.9)	¹³ C (1.1)	
• Nitrogen	¹⁴ N (99.6)	¹⁵ N (0.4)	
• Oxygen	¹⁶ O (99.8)	¹⁸ O (0.2)	
• Fluorine	¹⁹ F (100.0)		
• Silicon	²⁸ Si (92.2)	²⁹ Si (4.7)	³⁰ Si (3.1)
• Phosphorus	³¹ P (100.0)		
• Sulphur	³² S (95.0)	³³ S (0.7)	³⁴ S (4.2)
• Chlorine	³⁵ Cl (75.5)	³⁷ Cl (24.5)	
• Bromine	⁷⁹ Br (50.5)	⁸¹ Br (49.5)	
• Iodine	¹²⁷ I (100.0)		

Notes:

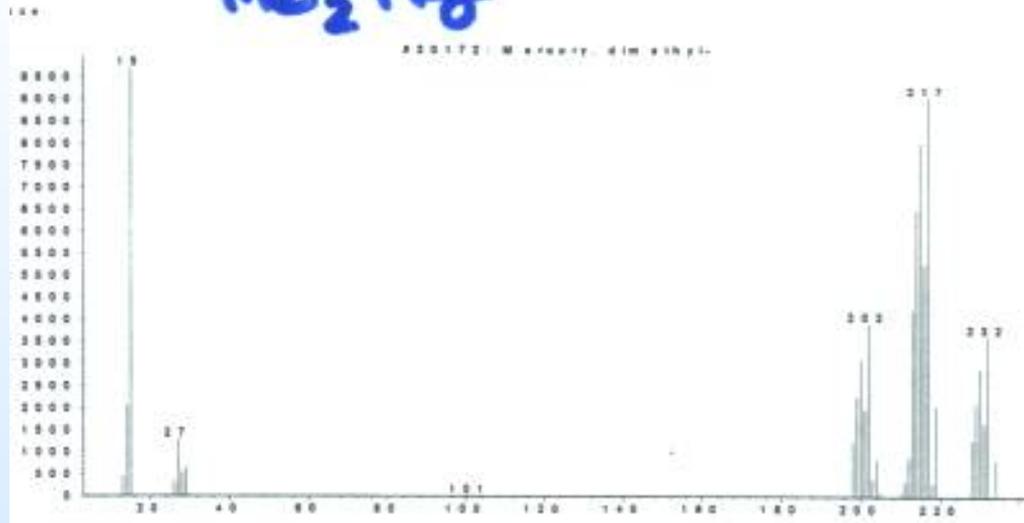
* Metals frequently possess many abundant isotopes and, because of the diversity of organometallic compounds, a listing of metal isotopes has not been included here. Many reference texts contain listings of isotope abundances; see also Beynon (1960) and Kiser (1965).

^b With the exception of hydrogen, percentages are given correct to the first decimal place. Trace isotopes are not included because they have little consequence in mass spectrometry at their natural abundance levels.

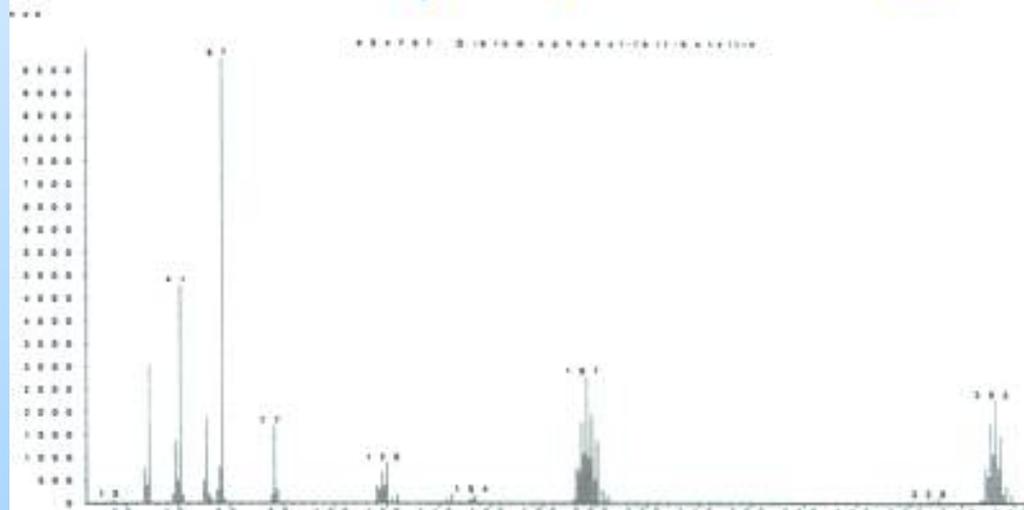
Hg 9 ISOTOPES !

Tin (Sn) 7 ISOTOPES !

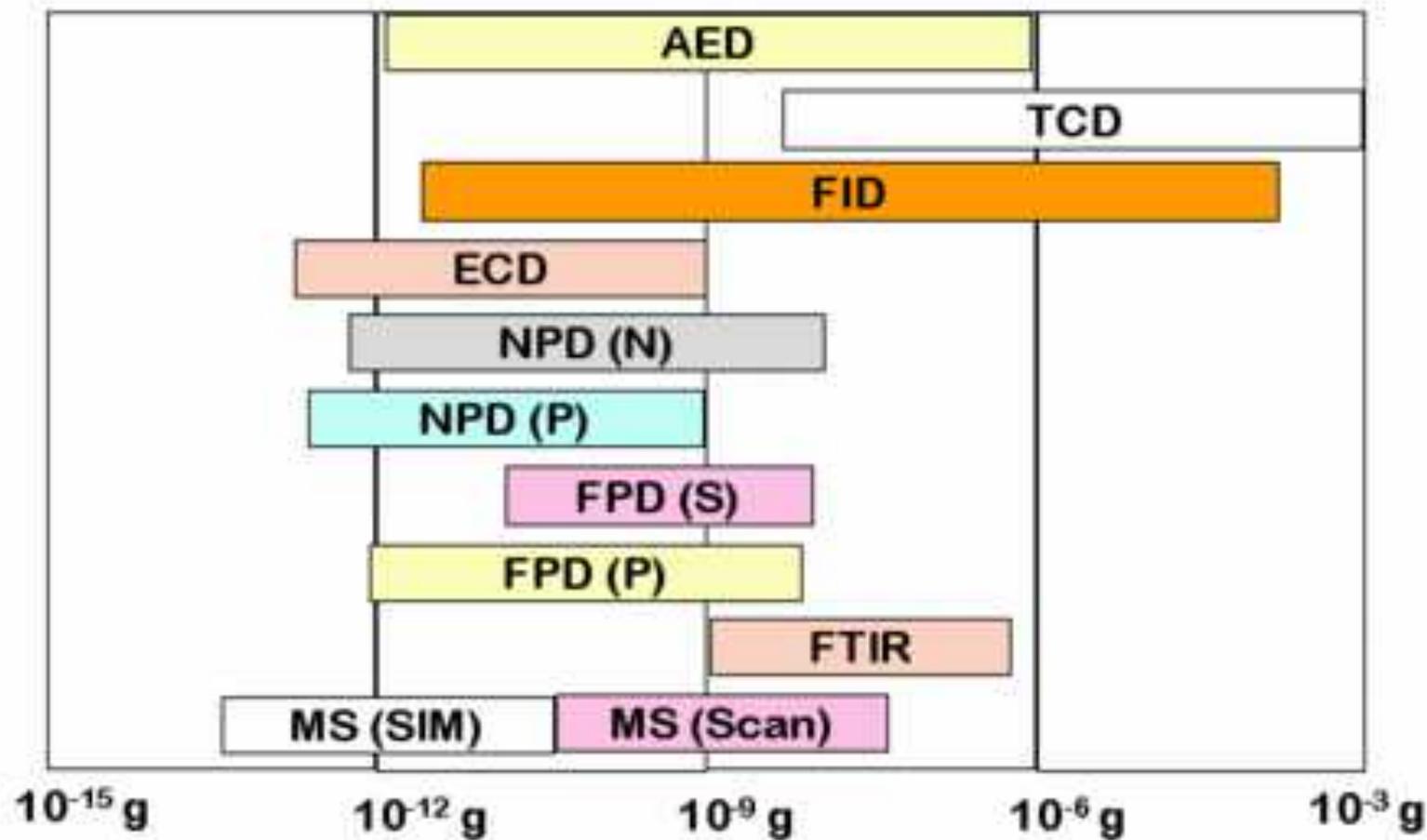
Me₂Hg



Dibromo-phenyl-tert-butyl-Tin



GC detectors sensitivities & ranges



Ionization

A number of ionization techniques exist.



Electron impact (EI)

Chemical ionization (CI)

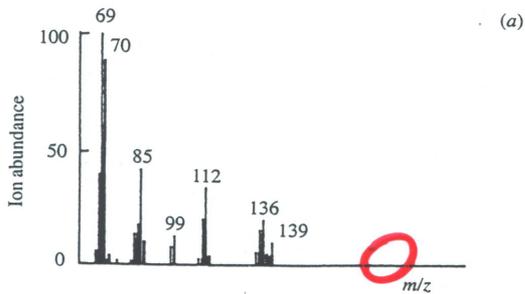
Fast atom bombardment (FAB)

Field ionization

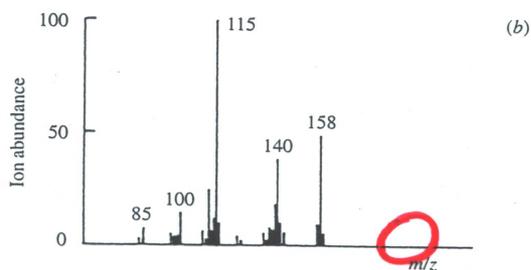
Plasma desorption

SOFT vs HARD

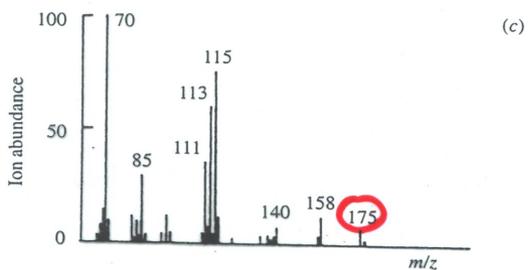
EI



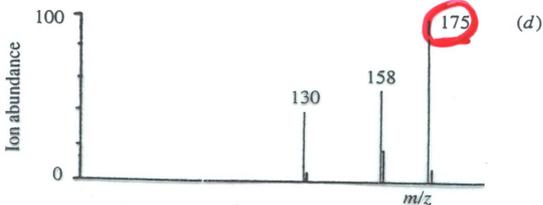
CI
isobutane



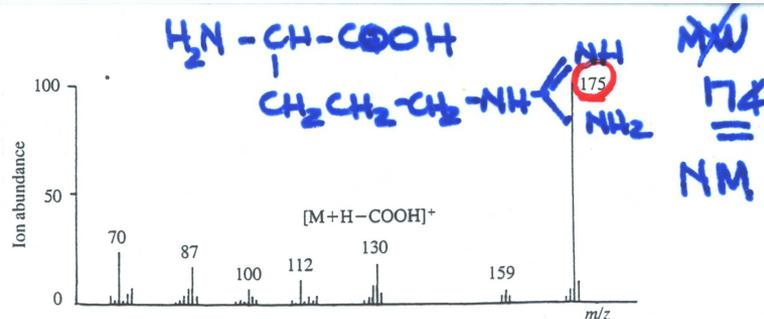
in-beam
CI
isobutane



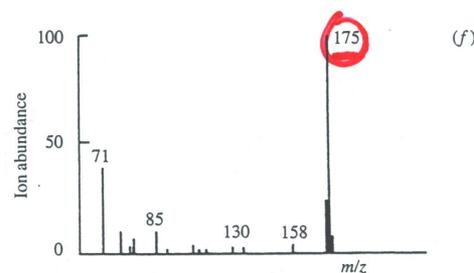
FD



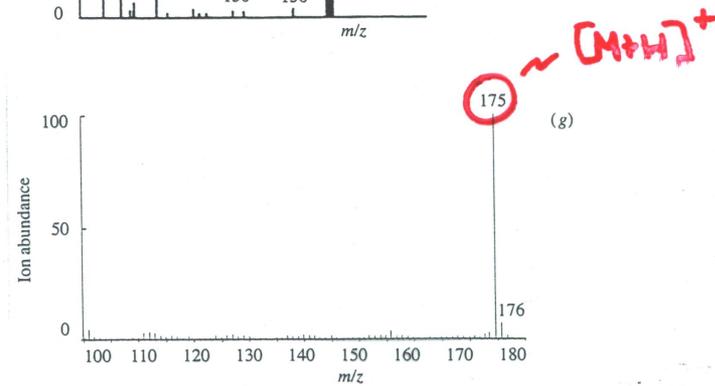
FAB



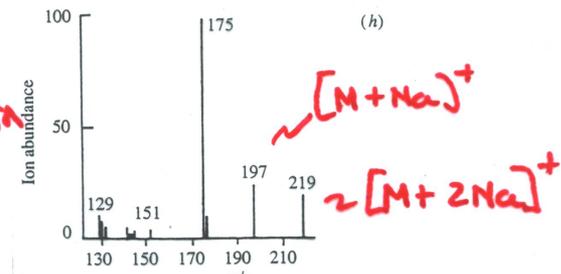
Thermo
spray



ESI



plasma
desorption



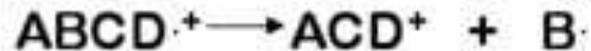
ionization method	typical mass range	polarity of the analyte	positive ions	negative ions	HR-MS	GC-MS	metastable ions	collisionally induced dissociation
EI	1-1000	low	yes	no	yes	yes	yes	yes
CI	60-1200	low-medium	yes	yes	(yes)	yes	yes	yes
FD	1-5000	low-high	yes	no	(yes)	no	no	yes
ESI	100-50000	medium-very high	yes	yes	yes	no	no	yes
FAB	300-5000	low-high	yes	yes	yes	no	yes	yes
LDI	1-1500	low-medium	yes	yes	no	no	no	no
MALDI	500- >100000	low-high	yes	yes	no	no	no	no

MS process

Ionization

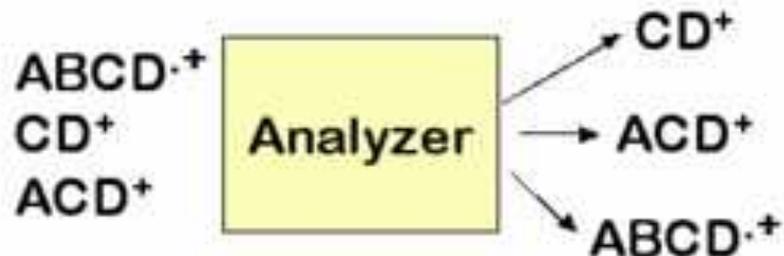


Fragmentation



and so on.

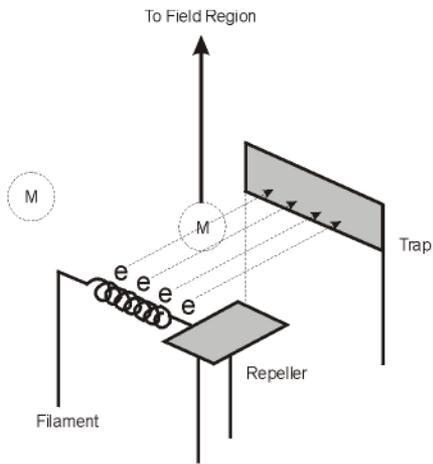
Mass analysis
and detection



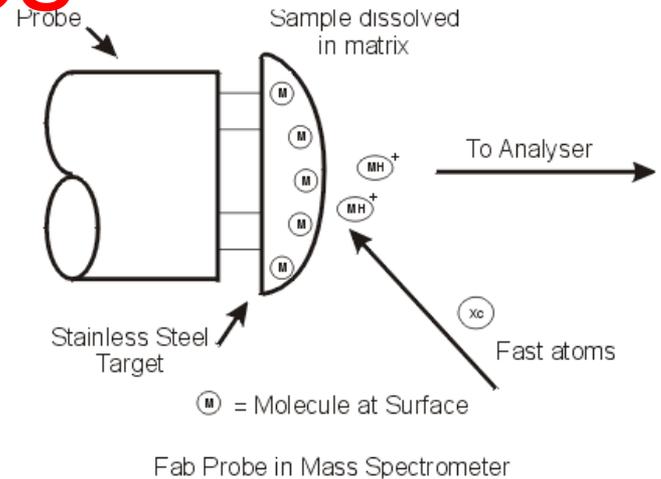
How does a molecule become charged?

- The sample is introduced into the mass spectrometer, which is generally kept under high vacuum ($<10^{-5}$ torr). ???
- Compounds are converted into gas phase molecules either before or during the charging or ionization process, which takes place in the ion source.
- Many types of ionization mode are available: the type of compound to be analyzed and the specific information required determines which ionization mode is the most suitable.
- Once ionized, the molecule ion may fragment, producing ions of lower mass than the original precursor molecule. These fragment ions are dependent on the structure of the original molecule.

Ion Sources



EI Ion Source



Fab Probe in Mass Spectrometer

