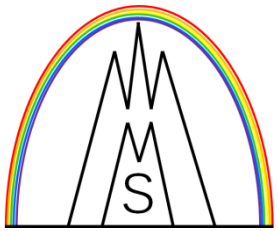


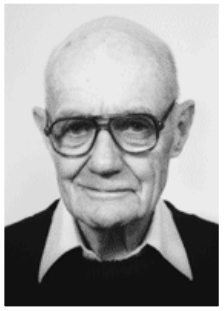

# What is Mass Spectrometry?

Martin Hubálek

**Mass Spectrometry** (**MS**) is an analytical technique that sorts ions based on their mass to charge ratio

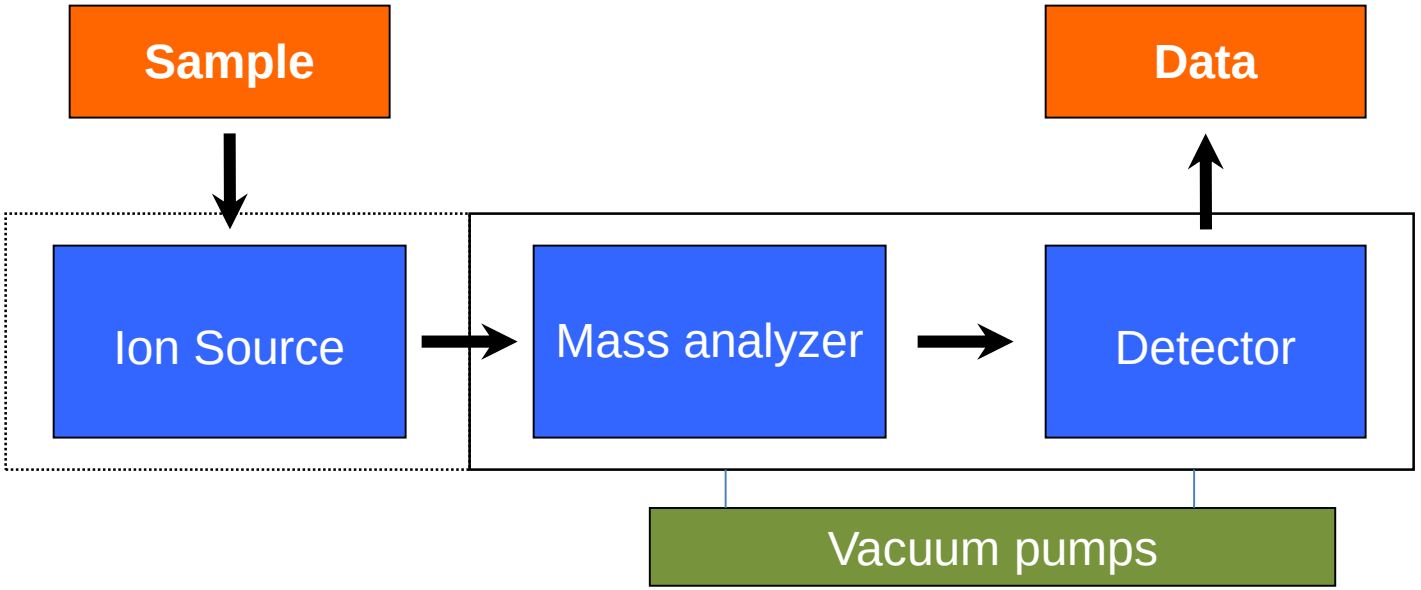
***Mass spectrometry*** *is the* ***art*** *of measuring atoms and molecules to determine their molecular weight. Such mass or weight information is sometimes sufficient, frequently necessary, and always useful in determining the identity of a species. To practice this art one puts charge on the molecules of interest, i.e., the analyte, then measures how the trajectories of the resulting ions respond in vacuum to various combinations of electric and magnetic fields.*

*Clearly, the sine qua non of such a method is the conversion of neutral analyte molecules into ions. For small and simple species the ionization is readily carried by gas-phase encounters between the neutral molecules and electrons, photons, or other ions. In recent years, the efforts of many investigators have led to new techniques for producing ions of species too large and complex to be vaporized without substantial, even catastrophic, decomposition.*



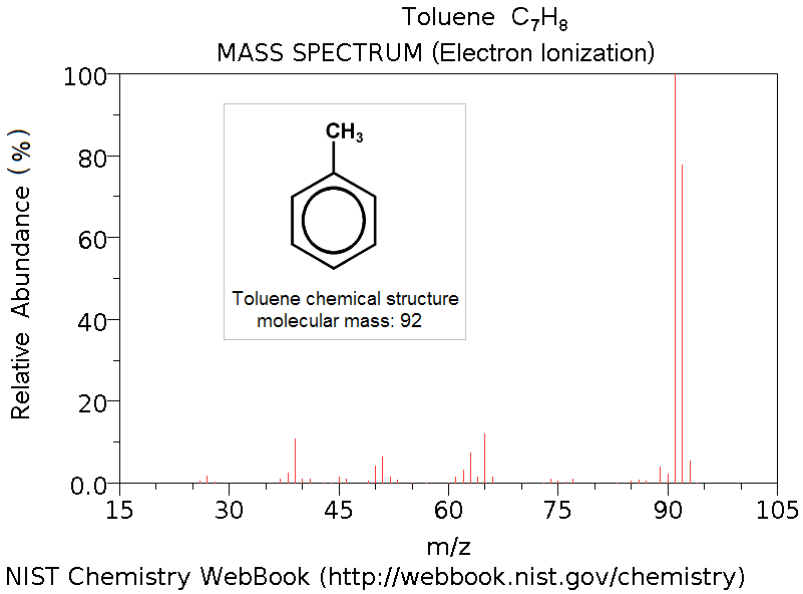
John B. Fenn

# General components of Mass Spectrometer



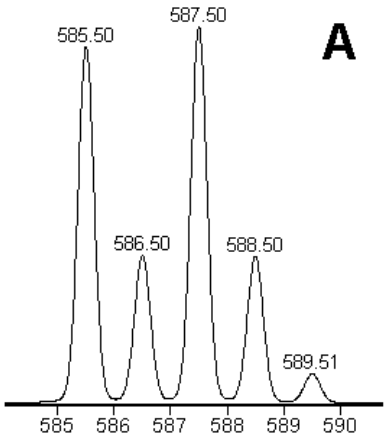
# Mass spectrum

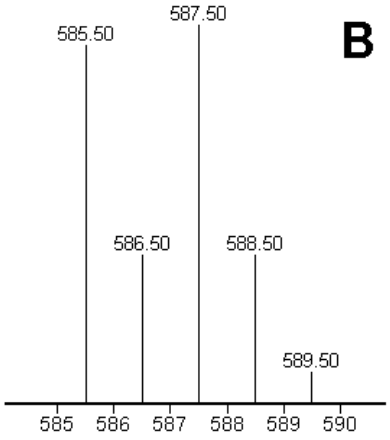
Grafical interpretation of ion intensity on mass to charge ratio (*m/z*).




spectrum → continuous

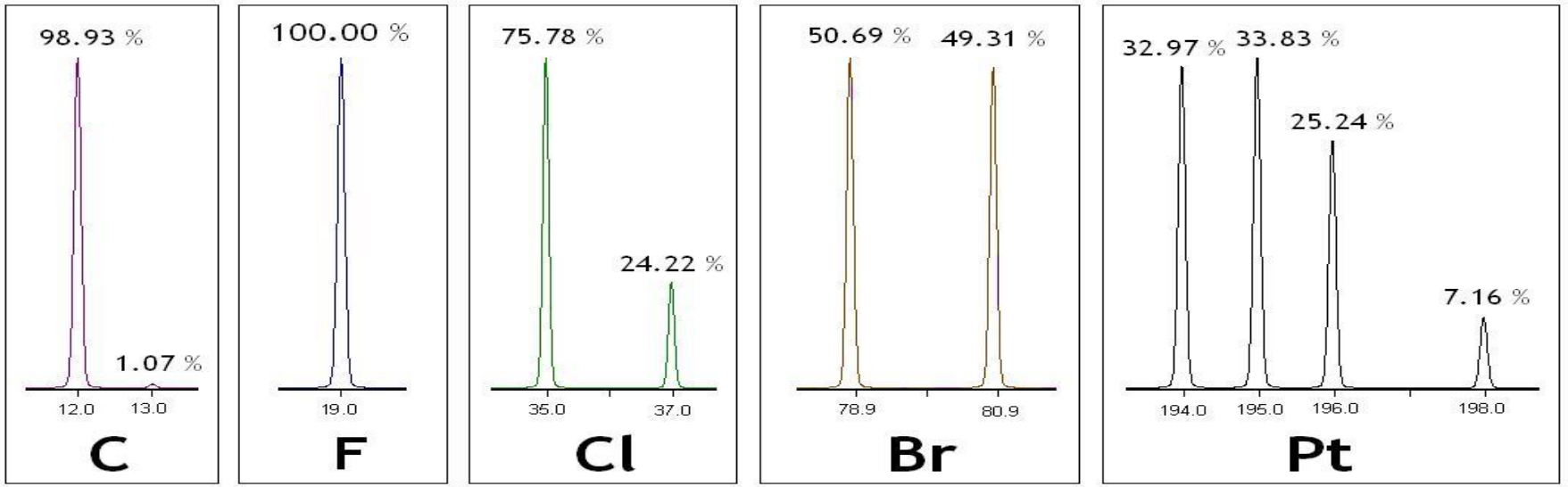
spectrum → centroided



# Isotopes

Natural mix of isotopes – distribution of individual isotopes is constant in nature




**elements**

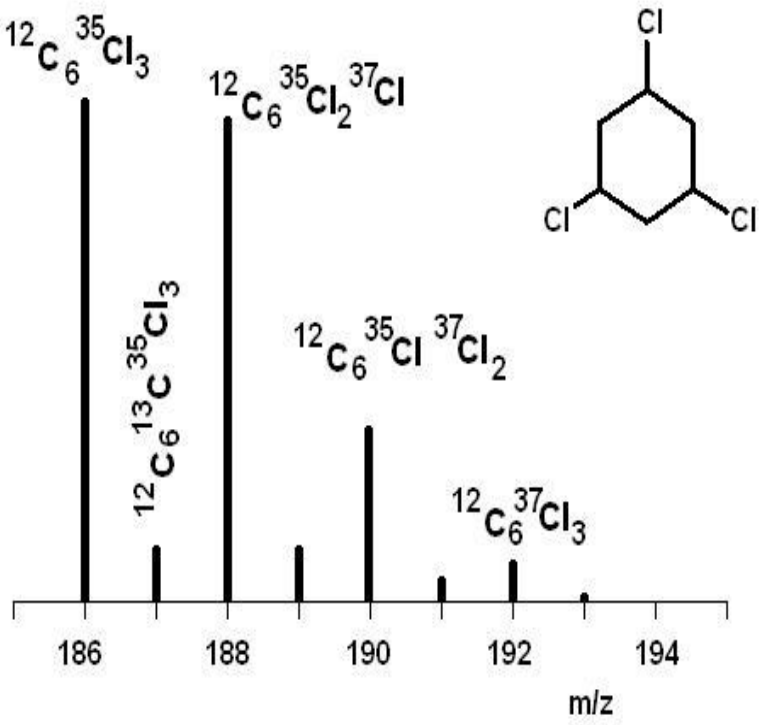
X (monoisotopic): $^{19}F$, $^{23}Na$, $^{31}P$, $^{127}I$

X+1: hydrogen ($^{1}H$, $^{2}H$), carbon ($^{12}C$, $^{13}C$), nitrogen ($^{14}N$, $^{15}N$)

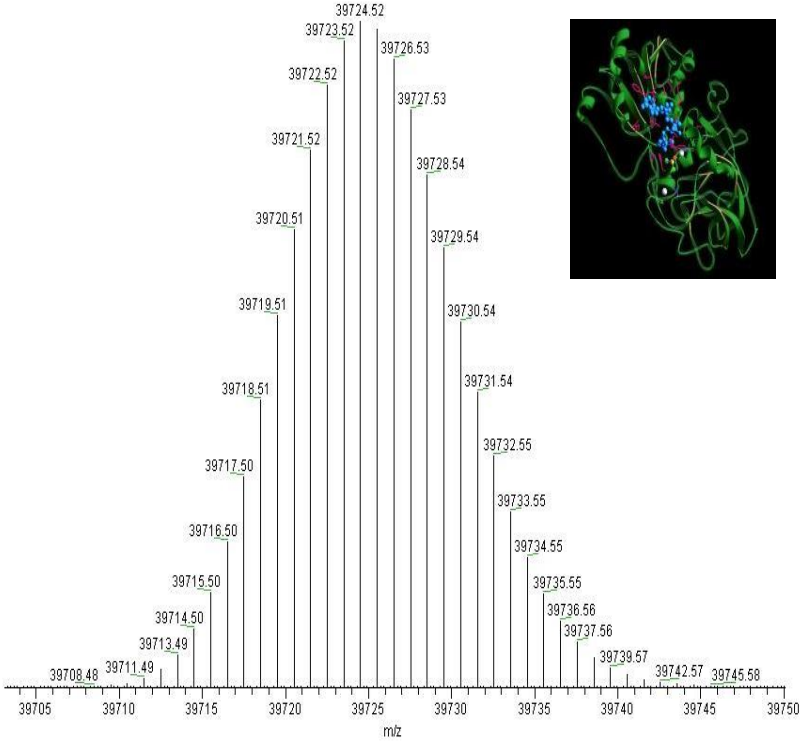
X+2: chlorine ($^{35}Cl$, $^{37}Cl$), brom ($^{79}Br$, $^{81}Br$) oxygen ($^{16}O$, $^{18}O$)

# Isotopic composition of ion

Depends on a combination of isotopes of the atoms within the molecule




trichlorcyklohexan
$C_6H_9Cl_3$




alcoholdehydrogenase
$C_{1764}H_{2859}N_{469}O_{516}S_{26}$

# mass units

**unified atomic mass unit -** non-SI unit

1/12 of the mass of an unbound neutral atom of carbon-12 in its nuclear and electronic ground state

u = 1,660 57.$10^{-27}$ kg

**dalton Da -** non-SI unit

**Da** vs **u -** alternatives for each other

***m/z*** - Mass to charge ration - dimensionless by definition

**thomson Th –** propossed as unit of mass-to-charge ratio

$$1\ \mathrm{Th} = 1\ \frac{\mathrm{u}}{e} = 1\ \frac{\mathrm{Da}}{e} = 1.036426 \times 10^{-8}\,\mathrm{kg\,C^{-1}}$$

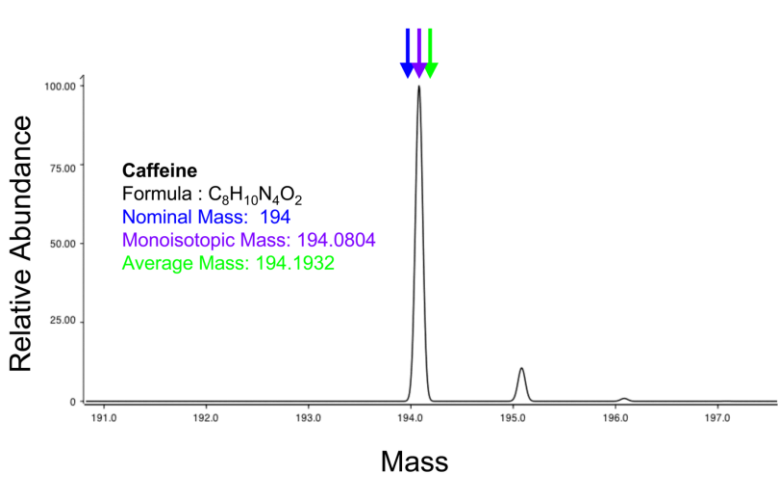
# Mass of ion in MS

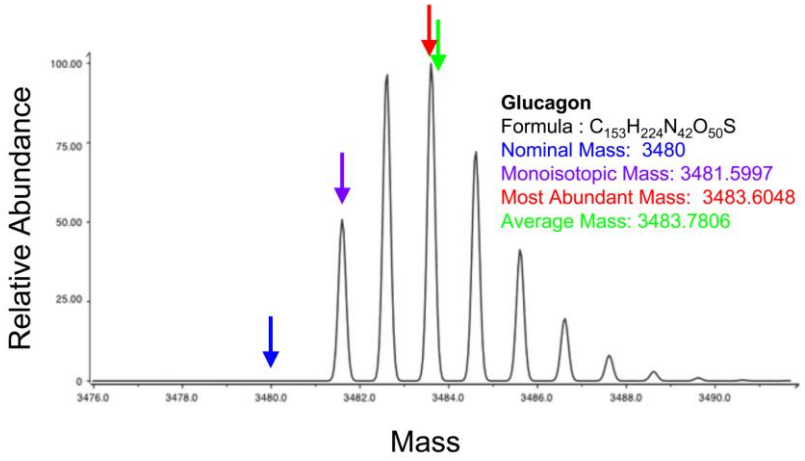
**nominal mass**: is calculated using the integer mass (ignoring the mass defect) of the most abundant isotope of each element. (ex. $CO_2$: 12u + 2 x 16u = 44u)

**Monoisotopic mass**: is the sum of the masses of the atoms in a molecule using the unbound, ground-state, rest mass of the principal (most abundant) isotope for each element instead of the isotopic average mass. (ex. $CO_2$: 12.0000u + 2 x 15.9949u = 43.9898u)

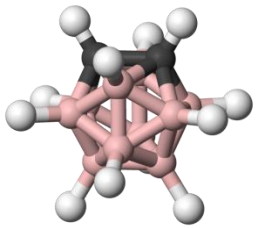
**Average mass**: obtained by summing the average atomic masses of the constituent elements (ex. $CO_2$: 12.01u + 2 x 16.00u = 44.01u)

**Most abundant mass:** mass of the molecule with the most highly represented isotope distribution, based on the natural abundance of the isotopes.



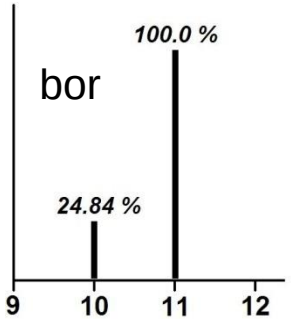



# Mass of ion in MS



karboran

$C_2B_{10}H_{12}$, $[M-H]^-$

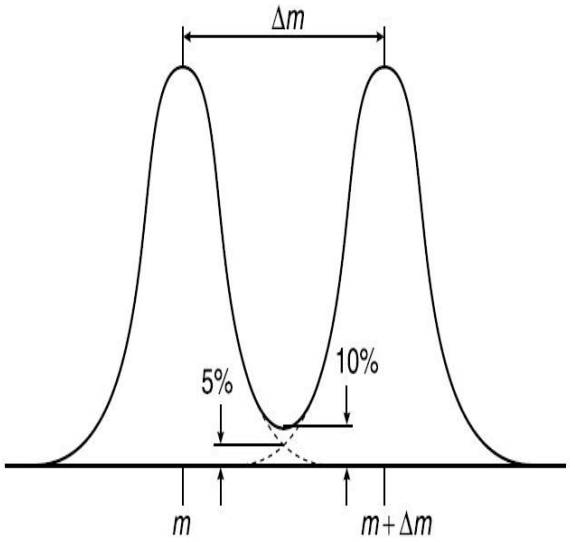


# Resolution in MS

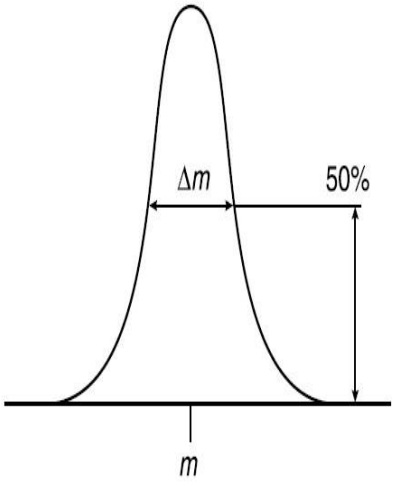
measures of the ability to distinguish two peaks of slightly different mass-to-charge ratios *ΔM*, in a mass spectrum

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

**10% valley**




**FWHM**



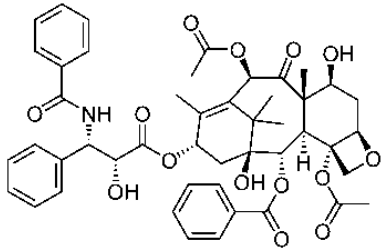

*Two definitions:*

**Resolution – 10% valley**:
the closest spacing of two peaks of equal intensity with the valley (lowest value of signal) between them less than a specified fraction of the peak height.

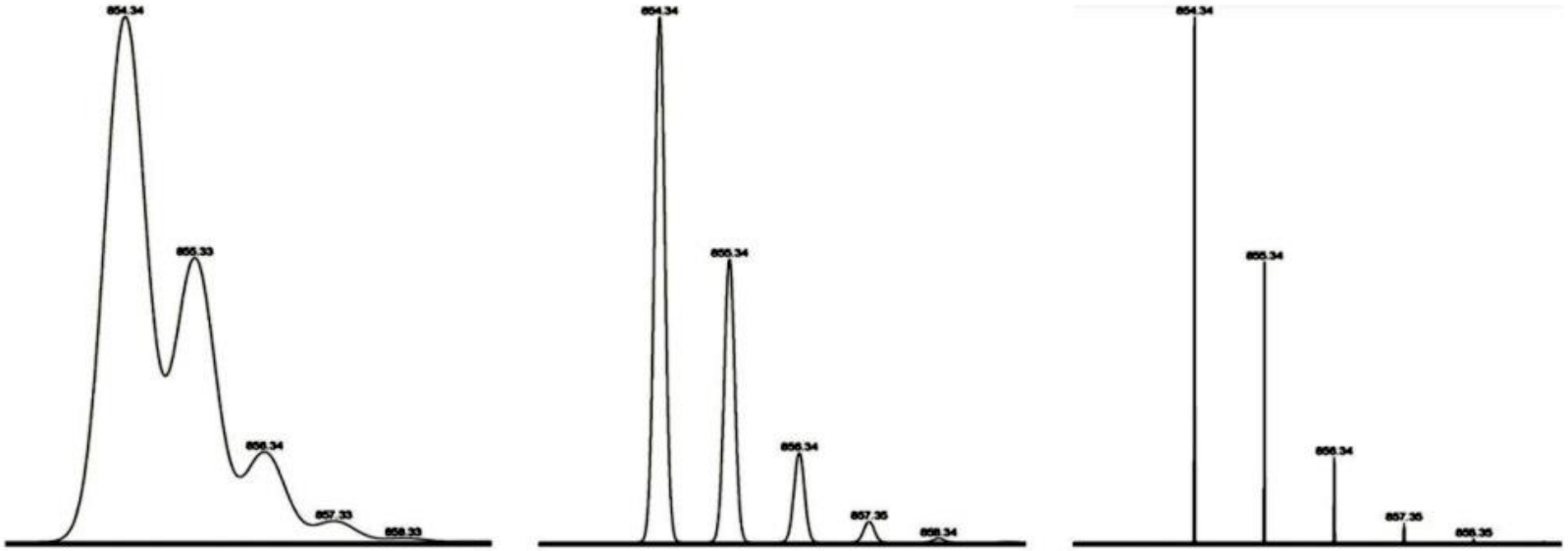
**Resolution – FWHM**:
the width of the peak measured at a specified fraction of the peak height – 50% is called full width at half maximum (FWHM).

*Numerically - FWHM approx. 2times bigger than 10% valley.*

# Resolution in MS




paclitaxel $C_{47}H_{51}NO_{14}$ (Mw 853.3)




**Low resolution** ←→ **high resolution**

# Resolving power of analyzer

Maximum resolving power of different types of analyzers

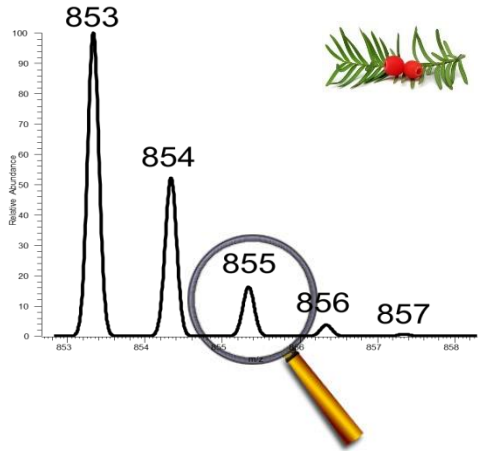
Time-of-flight (oa TOF):
**R = 60 000** FWHM (Maxis 4G, Bruker)

Sector analzer:
**R = 80 000** 10% údolí (AutoSpec Premier, Waters)

High-Field Orbitrap
**R = 240 000** FWHM (*m/z* 400, Orbitrap Elite, Thermo)

Iontová cyklotronová rezonance (FT-ICR)
**R = 600 000** FWHM (výpočet pro *m/z* 400 a 1s sken, Solarix 18T, Bruker)

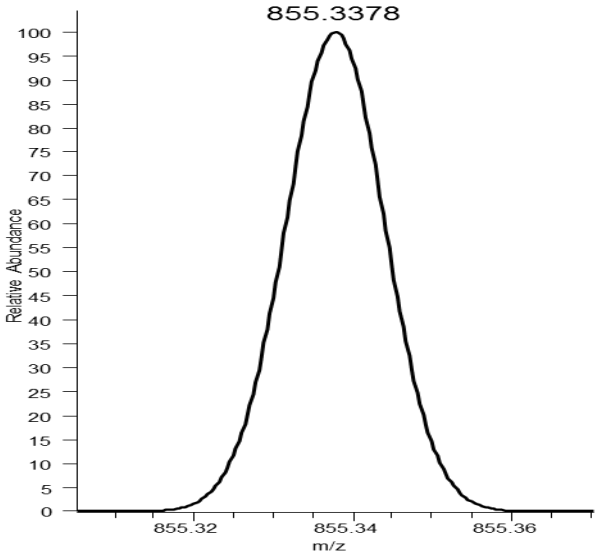
# High resolution spectra



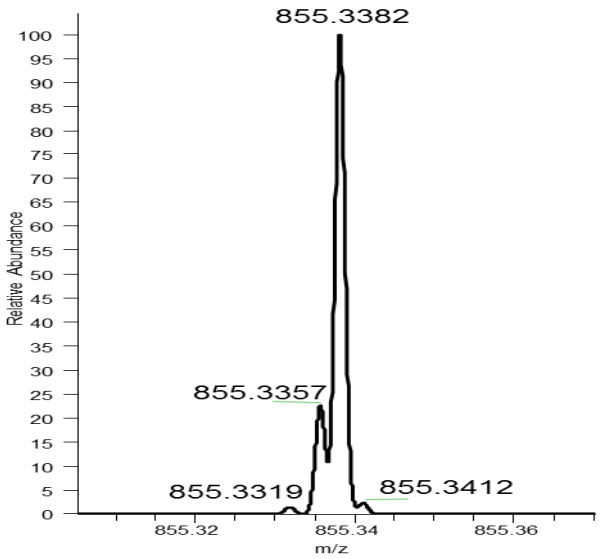

**Separation of isotopes within nominal mass region**

| | |
|---|---|
| 855,3319 | $C_{46}\ ^{13}C\ H_{51}\ O_{14}\ ^{15}N$ |
| 855,3357 | $C_{47}\ H_{51}\ O_{13}\ ^{18}O\ N$ |
| 855,3382 | $C_{45}\ ^{13}C_2\ H_{51}\ O_{14}\ N$ |
| 855,3412 | $C_{46}\ ^{13}C\ H_{50}\ ^{2}H\ O_{14}\ N$ |

**R = 60 000**




**R = 600 000**



# Mass Accuracy

the ratio of the *m/z* measurement error to the true m/z. Mass accuracy is usually measured in ppm or milli mass units.

$$E_{mmu} = 10^3 \left(M_{změřená} - M_{vypočítaná}\right) \qquad E_{ppm} = 10^6 \frac{M_{změřená} - M_{vypočítaná}}{M_{vypočítaná}}$$

**Calculation of exact mass of an ion:**

Correct values of mass of individual isotopes

*G. Audi, A.H. Wapstra, C. Thibault, Nucl. Phys. A 729, 337–676, 2003*

Correct charge

Mass of electron (0.5486 mmu) counts !

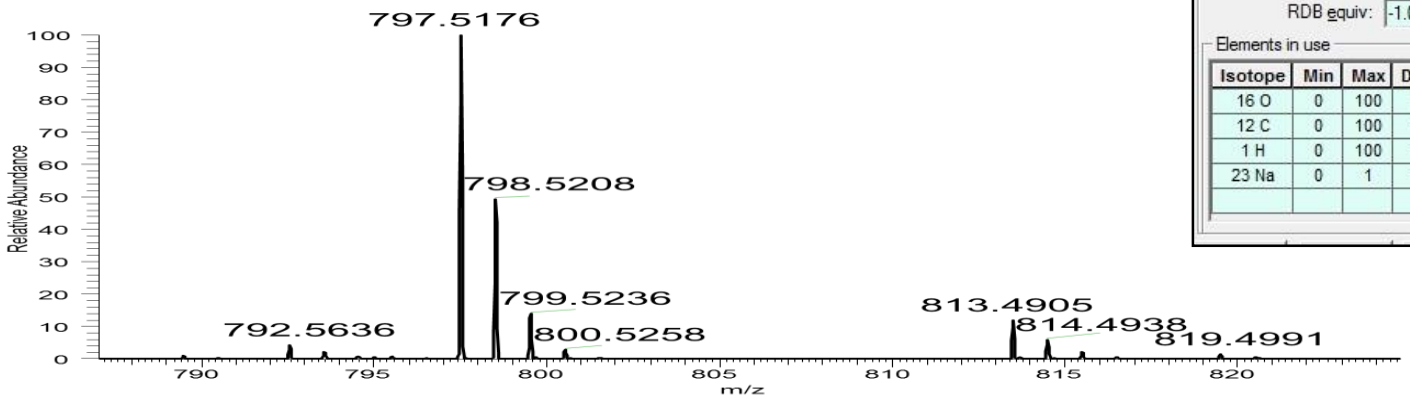
example: naftalen

M ($[C_{10}H_8]^{-\bullet}$) = 128.063149 (+4.3 ppm)

M ($C_{10}H_8$) = 128.0626

M ($[C_{10}H_8]^{+\bullet}$) = 128.062052 (-4.3 ppm)

# Elementar composition




Limits

Charge: 1

Nitrogen-Rule: Do not use

Mass tolerance: 20.00 ppm

RDB equiv: -1.0-100.0

Elements in use

| Isotope | Min | Max | DB eq. | Mass |
|---|---|---|---|---|
| 16 O | 0 | 100 | 0.0 | 15.995 |
| 12 C | 0 | 100 | 1.0 | 12.000 |
| 1 H | 0 | 100 | -0.5 | 1.008 |
| 23 Na | 0 | 1 | -0.5 | 22.990 |

tolerance 5 ppm (3 posibilities)

Elemental composition search on mass 797.52

m/z= 792.52-802.52

| m/z | Theo. Mass | Delta (ppm) | RDB equiv. | Composition |
|---|---|---|---|---|
| 797.5176 | 797.5174 | 0.20 | 8.5 | $C_{45}H_{74}O_{10}Na$ |
| | 797.5198 | -2.81 | 11.5 | $C_{47}H_{73}O_{10}$ |
| | 797.5140 | 4.55 | 20.5 | $C_{54}H_{69}O_5$ |

tolerance 20 ppm (13 possibilities)

Elemental composition search on mass 797.52

m/z= 792.52-802.52

| m/z | Theo. Mass | Delta (mmu) | RDB equiv. | Composition |
|---|---|---|---|---|
| 797.52 | 797.52 | 0.16 | 8.5 | $C_{45}H_{74}O_{10}Na$ |
| | 797.52 | -2.24 | 11.5 | $C_{47}H_{73}O_{10}$ |
| | 797.51 | 3.63 | 20.5 | $C_{54}H_{69}O_5$ |
| | 797.52 | -5.71 | -0.5 | $C_{38}H_{78}O_{15}Na$ |
| | 797.51 | 6.03 | 17.5 | $C_{52}H_{70}O_5Na$ |
| | 797.53 | -8.12 | 2.5 | $C_{40}H_{77}O_{15}$ |
| | 797.53 | -9.22 | 21.5 | $C_{56}H_{70}O_2Na$ |
| | 797.51 | 9.50 | 29.5 | $C_{61}H_{65}$ |
| | 797.53 | -11.63 | 24.5 | $C_{58}H_{69}O_2$ |
| | 797.51 | 11.91 | 26.5 | $C_{59}H_{66}Na$ |
| | 797.50 | 13.01 | 7.5 | $C_{43}H_{73}O_{13}$ |
| | 797.53 | -15.10 | 12.5 | $C_{49}H_{74}O_7Na$ |
| | 797.50 | 15.42 | 4.5 | $C_{41}H_{74}O_{13}Na$ |

*Thank you for your attention*