Introduction to Mass Spectrometry

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Mass spectrometry

Mass spectrometry is a physico-chemical method, which uses electric and magnetic fields to separate charged particles with the aim to determine their weights (the *m/z* ratio)

Qualitative MS:

- characterization (identification) of organic compounds based on molecular weight of ions, adducts and fragments
- studying the reactions of ions in the gas phase

Quantitative MS:

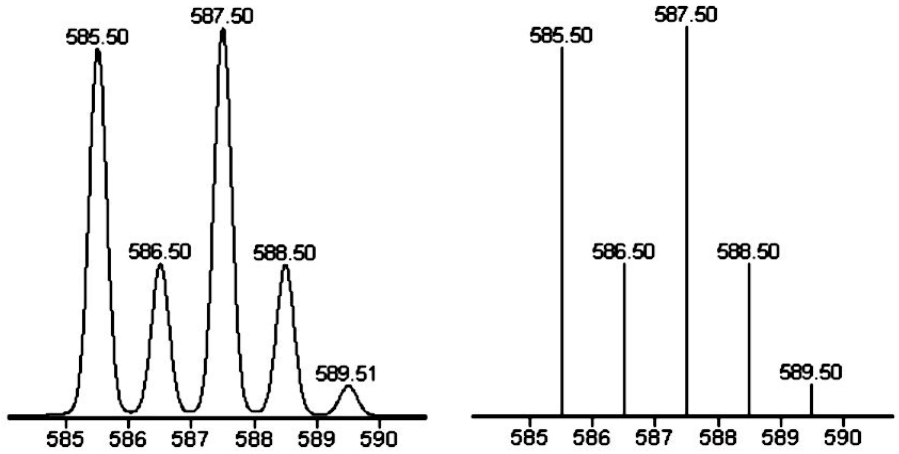
- quantification of organic compounds in the samples based on the intensity of the detector response for the selected ion or group of ions

Mass spectrum

Mass spectrum: A 2D graphical representation of signal intensity versus *m/z* values (the intensity scale is usually normalized 0-100%).

Spectrum → *profile*

Spectrum → *bar graph*

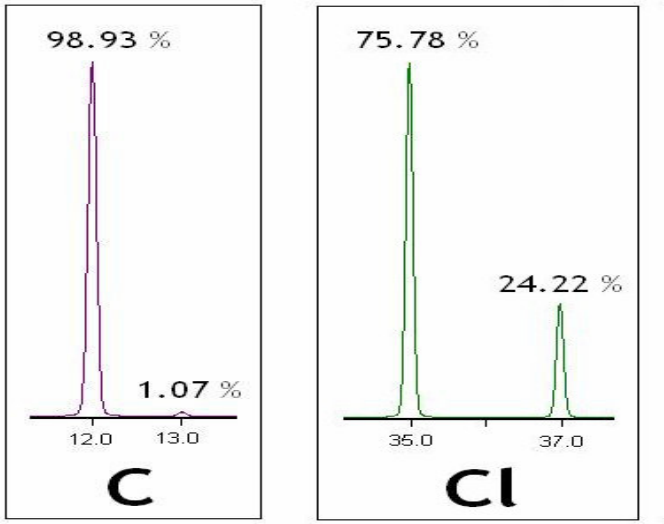


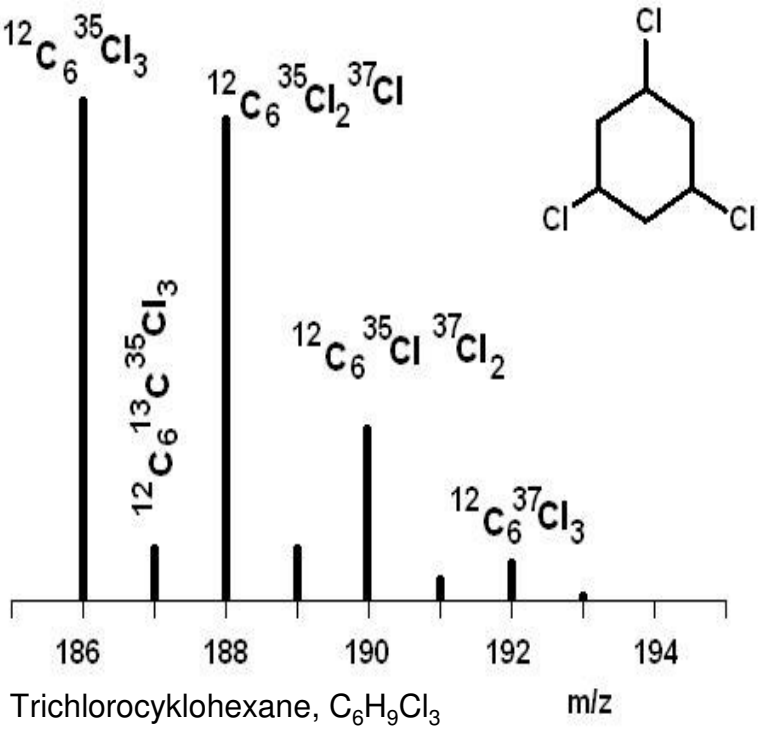
Profile (continuum): record of MS detector, allows determination of peak width (resolution)

Bar graph (centroides): transformed spectrum for easier reading (position = peak center of gravity, intensity = peak height or area)

Isotopes

Isotopes: atoms of chemical element that have the same number of protons but different numbers of neutrons (different weights)





Trichlorocyklohexane, $C_6H_9Cl_3$

Natural mixtures of isotopes: Relative proportions of isotopes in the elements is constant.

The isotopic composition of a polyatomic ion is given by combination of the isotopes in the individual atoms that form it.

Units of mass

Base unit of mass: **kilogram kg**

Kilogram is equal to the mass of the *International Prototype Kilogram* (IPK) stored in a vault at the International Bureau of Weights and Measures in Sèvres, France.

Non-SI unit: **atomic mass unit u**

It is defined as 1/12 of the rest mass of an unbound neutral atom of carbon-12 in its nuclear and electronic ground state. It has a value of $1.660538921 \times 10^{-27}$ kg.

Non-SI unit: **dalton Da**

Dalton is used instead of atomic mass units in biological MS for higher weight. It is not an SI unit.

quantity: ***m/z***

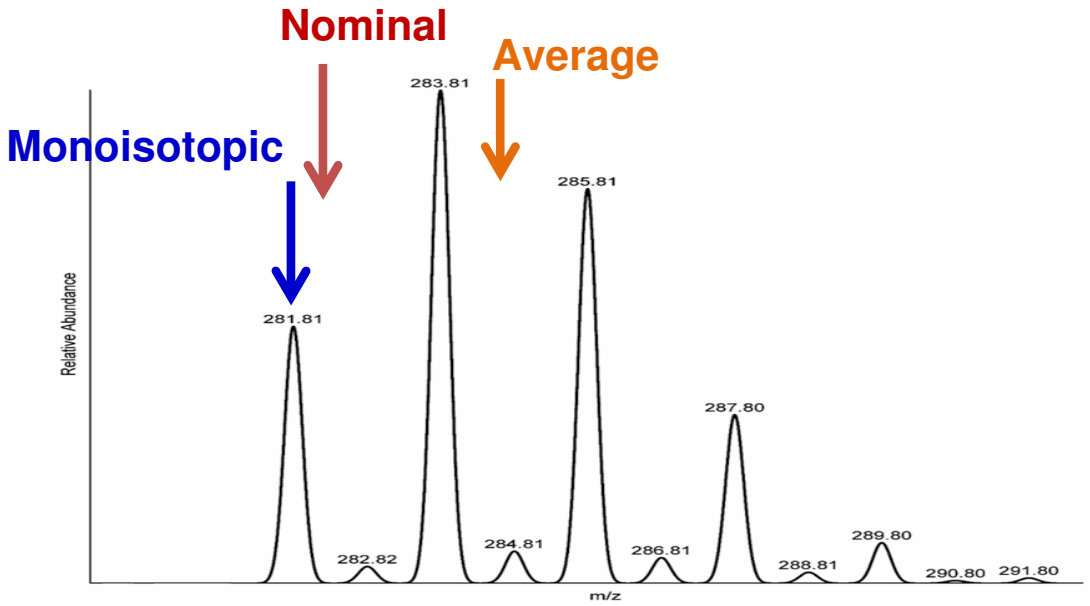
m/z is a dimensionless quantity used to describe ions in the spectrum. The unit **thomson (Th)** is sometimes used.

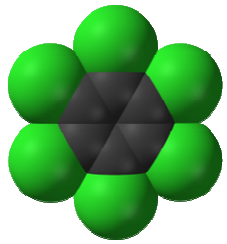
Masses in MS

Nominal Mass: mass calculated from integer masses of the most abundant naturally occurring isotopes (e.g., CO_2: 12u + 2 x 16u = 44 u)

Monoisotopic Mass: mass calculated from exact masses of the most abundant naturally occurring isotopes (e.g., CO_2: 12.0000 + 2 x 15.9949 = 43.9898)

Average Mass: mass calculated from weighted average masses of the isotopes based on their natural abundances (e.g., CO_2: 12.01 + 2 x 16.00 = 44.01)



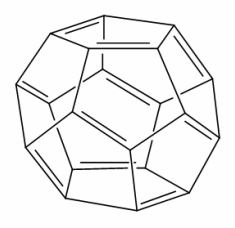


hexachlorobenzene
C_6Cl_6

Deviations from nominal mass – Mass defect

The exact masses differ from the nominal masses. Depending on the elements present the masses are either higher (positive mass defect, mass sufficiency) or lower (negative mass defect, mass deficiency).

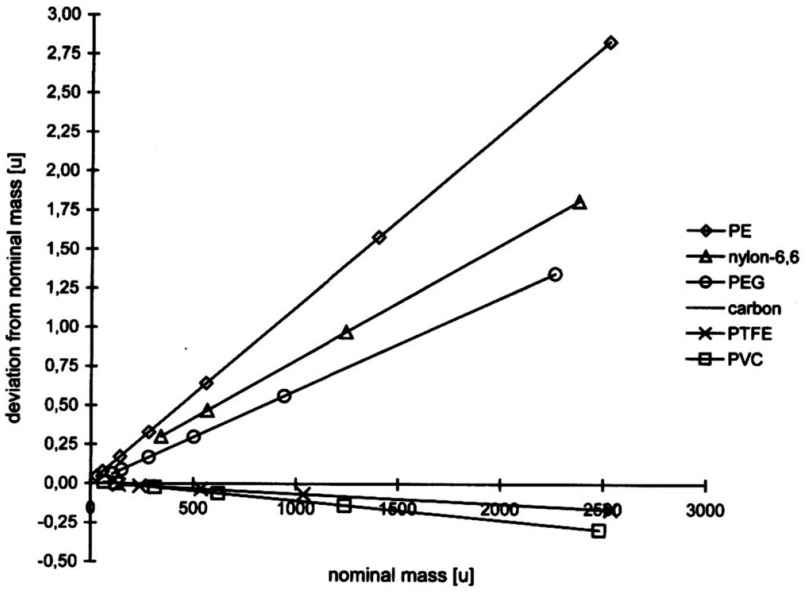
Example: fullerene C20



[5]fullerene-C20 C_{20}
M=240.00000u (0 mmu)

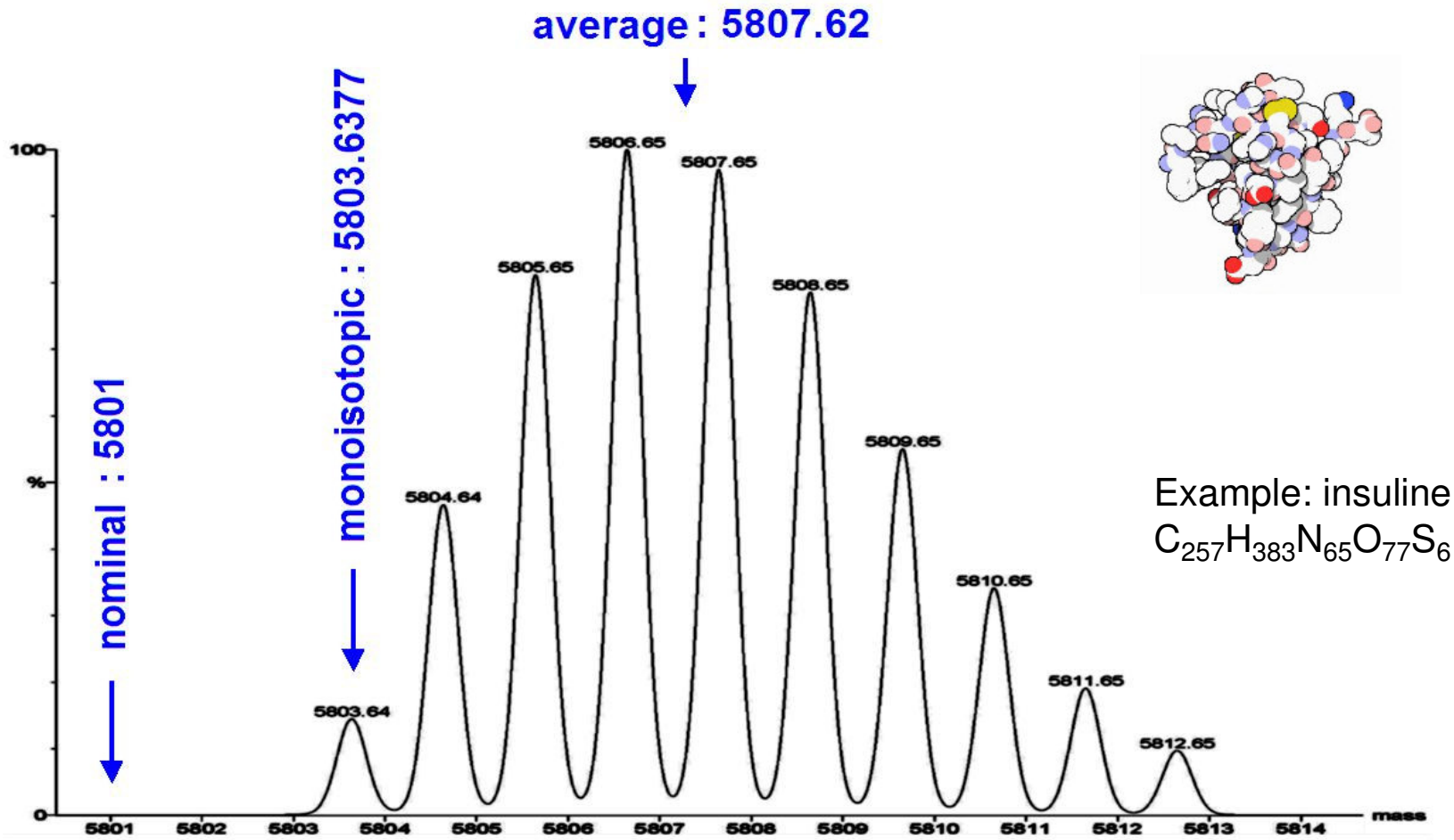
dodecahedrane $C_{20}H_{20}$
M= 260.15650u (+156.90 mmu)

perfluorododecahedrane $C_{20}F_{20}$
M=619.96807u (-31.93 mmu)



The use of nominal masses is limited to the low mass range (Mass defect above ~500 u is higher than 0.5 u and thus rounded to higher integer value).

Deviations from nominal mass – Mass defect



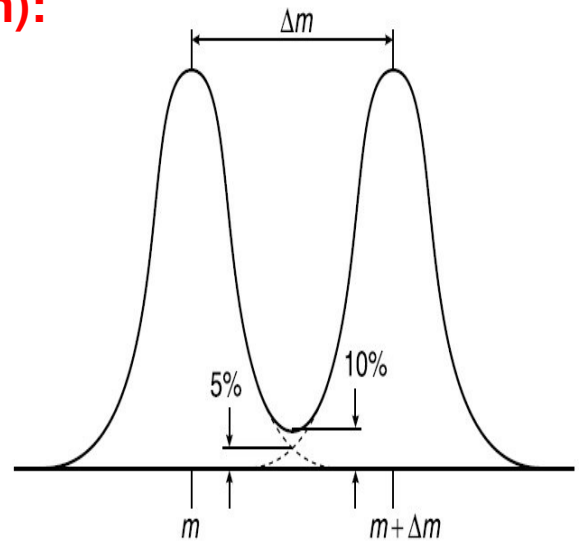
Example: insuline
$C_{257}H_{383}N_{65}O_{77}S_6$

Resolution

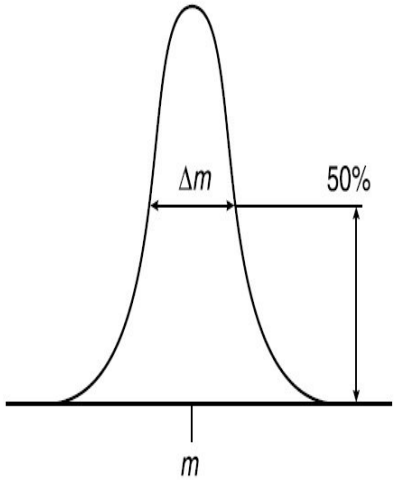
Resolving power (resolution):
the ability of an instrument to separate neighboring peaks

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

10% valley



FWHM

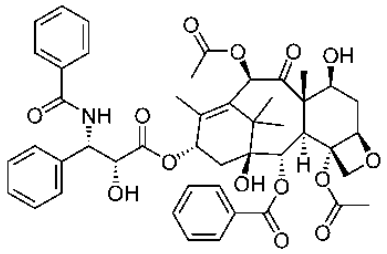


Two definitions of resolution:

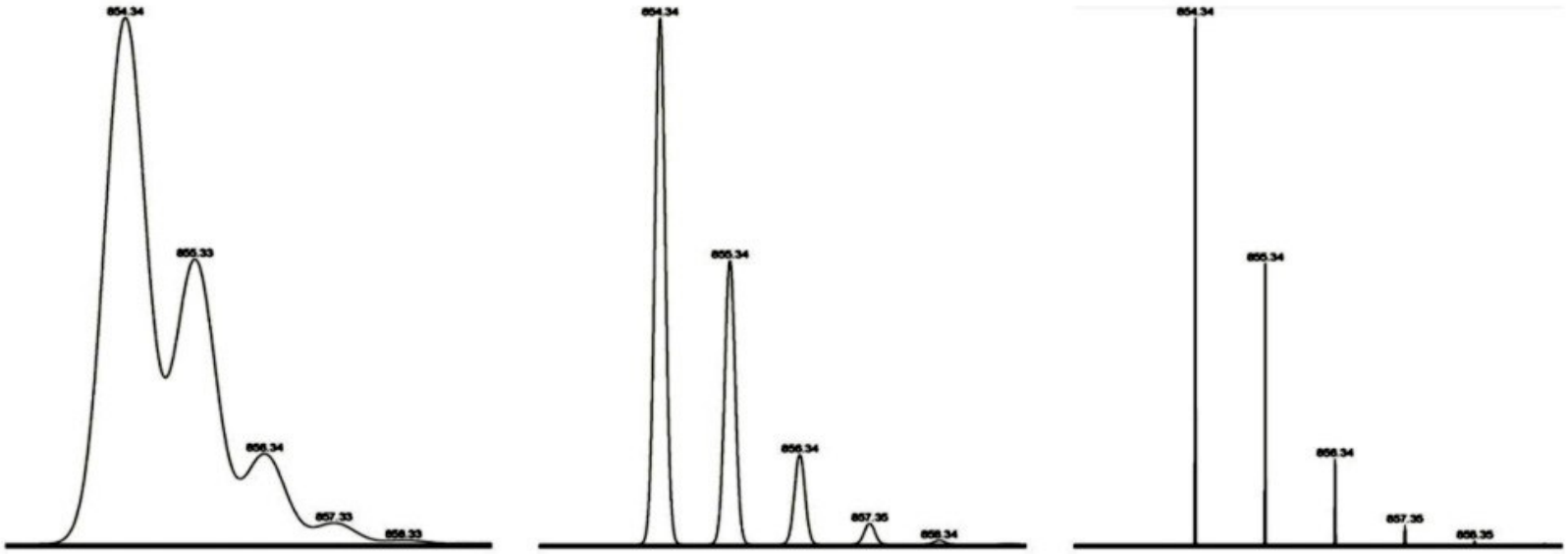
Resolution – 10% valley:
the ratio of an ion mass and the mass difference between equally high peaks when the valley separating their maxima is at 10 % of their intensity. Used for sector instruments (constant resolution in the entire mass range).

Resolution – FWHM (Full width at half maximum):
The ratio of an ion mass and its peak width at half height. It is used for quadrupole, ion trap and TOF analyzers (constant peak width).

Resolution



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



1 250 5 000 100 000

Low resolution ←→ **High resolution**

Resolving power of mass analyzers

Maximum resolving power of various mass analyzers

Time of flight analyzer (oaTOF):
R = 60 000 FWHM (Maxis 4G, Bruker)

Sector double focusing analyzer:
R = 80 000 10% valley (AutoSpec Premier, Waters)

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

Ion cyclotron resonance (FT-ICR)
R = 600 000 FWHM (calculation for *m/z* 400 a 1s scan, Solarix 18T, Bruker)

Mass accuracy

Mass accuracy – is an error, i.e., the difference between the measured mass and calculated correct value in absolute (mmu) or relative (ppm) mass units

$E_{mmu} = 10^3 (M_{measured} - M_{calculated})$	$E_{ppm} = 10^6 \frac{(M_{measured} - M_{calculated})}{M_{calculated}}$

Calculation of the correct ion mass:

Correct isotope masses

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

Correct charge

Mass of electron (0.5486 mmu) is important !

Example: naphthalene

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

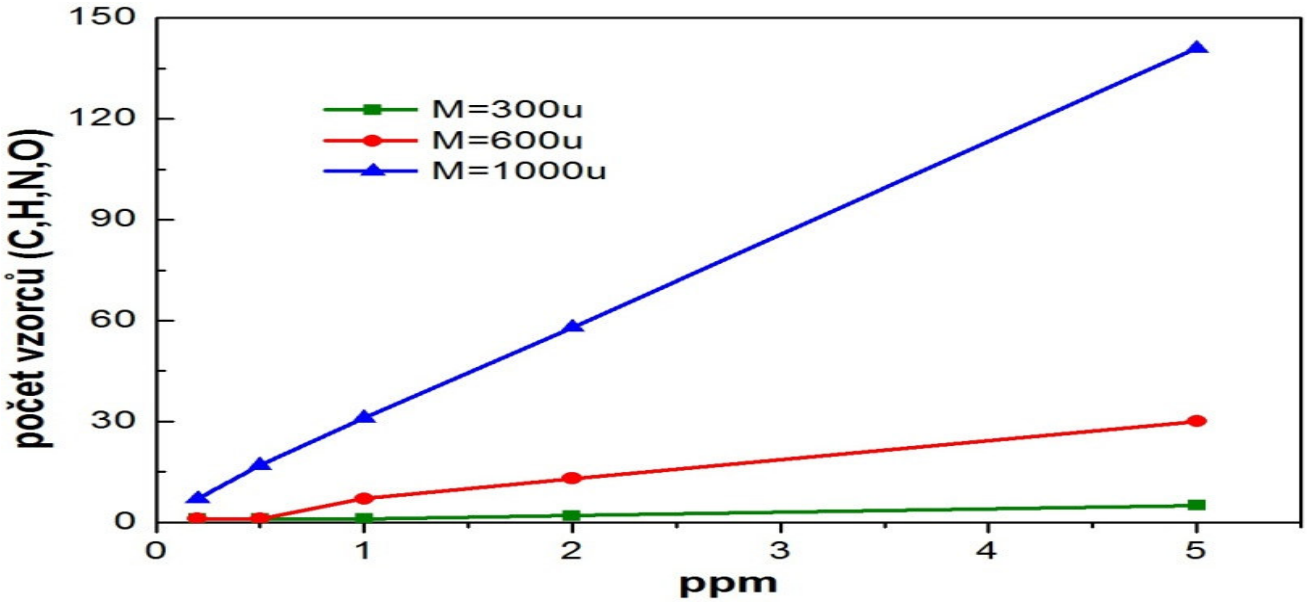
M ($C_{10}H_8$) = 128.0626

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

Calculation of the elemental composition

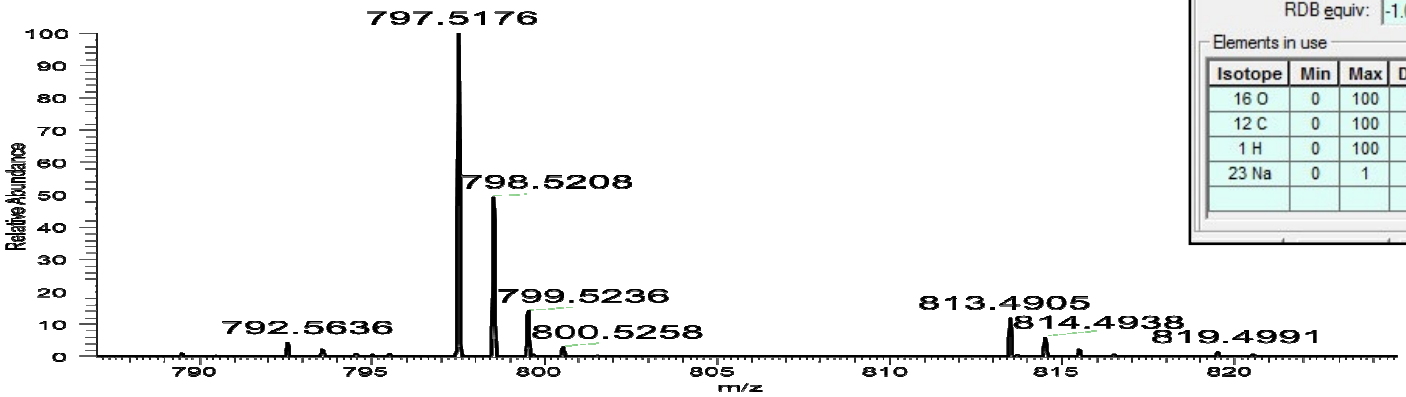
Each elemental composition has a unique mass. At infinitely high mass accuracy we get only the correct composition.

Lower mass accuracy = more possible formulas
Higher weight at the same mass accuracy = more possible formulas



C: 0-100 H: 0-100 N: 0-100 O: 0-100

Calculation of the elemental 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 composition)

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 compositions)

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	$C_{41}H_{74}O_{13}Na$

Mass scale calibration

Mass scale of each mass spectrometer must be calibrated to obtain correct results.

Calibration is performed by measuring spectrum of a calibration substance (mixture) and subsequent correlation of the measured and calculated (i.e. correct) *m/z* values

Types of calibrations :

External calibration

Calibration is carried out before measurement of the sample. Measurements of the calibrant and sample spectra are carried out separately.

Internal calibration

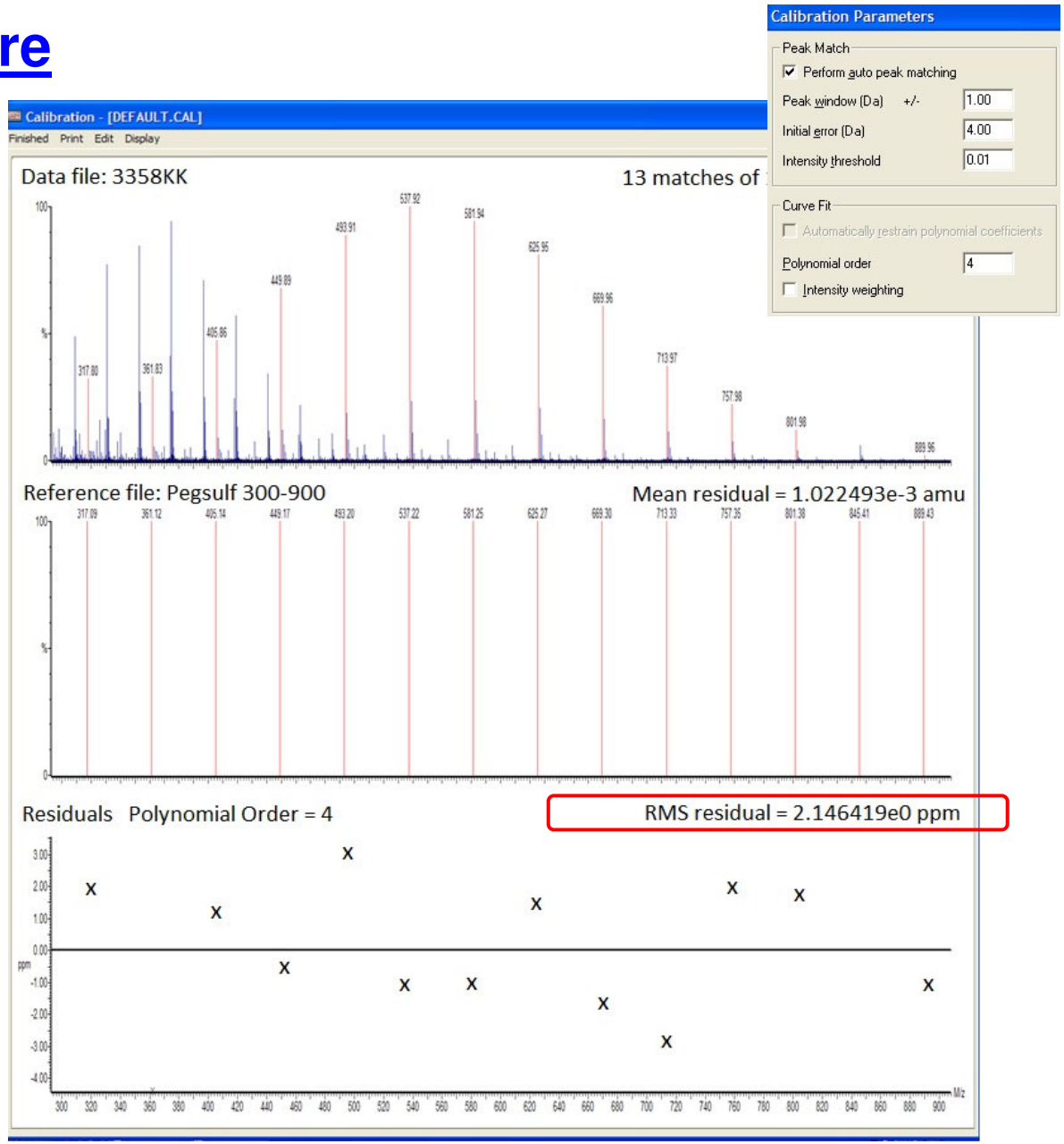
Calibration is carried out from a spectrum containing peaks of both sample and calibrant. Measurements of the calibrant and sample spectra are carried out simultaneously. Internal calibration provides more accurate results.

Calibration procedure

Recorded spectrum

Calibration spectrum (calculated correct *m/z* values)

Mass error for individual peaks



Thank you for your attention !