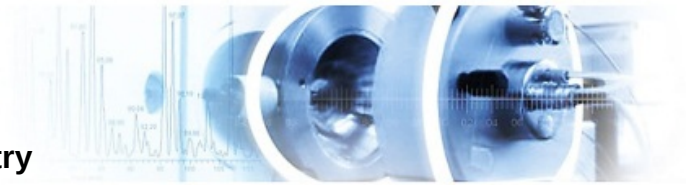
IOCB AS CR, v.v.i.

Research – Service Team Mass Spectrometry



Fourth Short Mass Spectrometry Courses

MINIŠKOLA HMOTNOSTNÍ SPEKTROMETRIE

November 19 – 20, 2013

Why “Miniškola”?

Information for our colleagues – the MS service users

- services provided by the MS group
- organization of the service work
- news
- basic interpretation of data

Demonstration of MS instruments

User training for open-access GC/MS

Discussion, user feedback

Agenda

Tuesday, November 19

Mass spectrometry basics (IOCB Club)

9:00 - 9:15	Opening (J. Cvačka)
9:15 - 9:30	Introduction to mass spectrometry (J. Cvačka)
9:30 - 9:55	MS instrumentation I. (V. Vrkoslav)
	break
10:25 - 10:45	MS instrumentation II. (V. Vrkoslav)

Services of the MS group (IOCB Club)

10:45 - 11:15	Small molecules (A. Březinová)
11:15 - 11:35	Biomolecules (M. Hubálek)

Training for open access instruments (lab. 58c)

14:00 - 16:00	Training for open-access GC/MS (A, ground floor NW, 58c; V. Vrkoslav)

Agenda

Wednesday, November 20

Acquiring & interpreting MS data (IOCB Club)

9:00 - 9:30	Ion mobility spectrometry (J. Jaklová-Dytrtová)
9:30 - 10:10	Experimental strategies in proteomics (J. Horáková)
	break
10:40 - 12:00	Interpretation of small molecule spectra (J. Cvačka)

Demonstration of the instruments (lab. 24 and 63)

14:00 - 16:00	Orbitrap – High resolution and tandem MS (A, ground floor NW, 63; J. Cvačka)
	MALDI – Large and small molecules (A, basement SW, 24; V. Vrkoslav)
	Proteomics data interpretation (A, basement SW, 24; M. Hubálek)

Research-Service Team Mass Spectrometry

& PhD and MSc students

Research

- Identification of organic compounds by common MS methods
- New procedures for analysis of organic compounds based on MS and chromatography
- Electrochemistry/MS, Ion mobility MS

Services

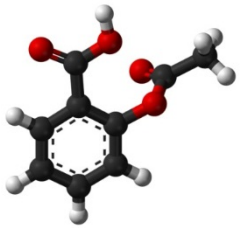
- Services towards characterization and structure elucidation of organic compounds

http://www.uochb.cz/web/structure/200.html

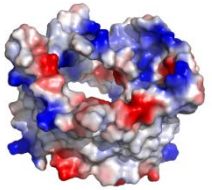
Services

Analysis of Small Molecules

Analysis of Bio-molecules



Open Access Instruments



Services: Small molecules

Routine services:

- Low resolution MS spectra of small molecules
  EI/CI, ESI, APCI, MALDI; (+/-)

- High resolution MS spectra of small molecules
  EI/CI, ESI, APCI, MALDI; (+/-)

On demand services:

- HPLC/MS, GC/MS
- Fragmentation spectra (MS/MS)

A. Březinová
Tue 10:45

Anna Březinová (tel. 117)

Services: Biomolecules

Routine services:

- Determination of molecular weight of biomolecules

  MALDI or ESI-MS analysis

- Identification of proteins

  peptide mass fingerprinting (MALDI-TOF)

  identification using ESI-MS/MS

M.Hubálek
Tue 11:15

On demand services:

- *Protein quantification (label free, SILAC, iTRAQ)*
- *Post-translation modifications*

Martin Hubálek (tel. 117)

Services: Open access instruments

Room 58c (SV)
7/24

Room 53 (LS)
working hours

The open access instruments:

- 2 **GC/MS** (LR EI, nonpolar and polar column)
- **LC/MS** (LR ESI, APCI, MS^n)

Authorized users:

IOCB employees and students *trained by the MS staff*

Google Calendar
on-line reservations

Notes and rules:

- the instruments can be reserved

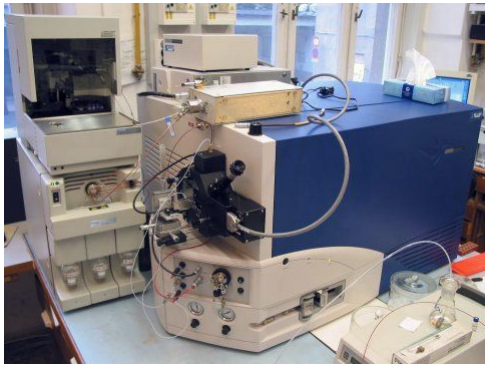
- each measurement must be registered in a logbook

- the users are responsible for damages cased by misuse of the instruments

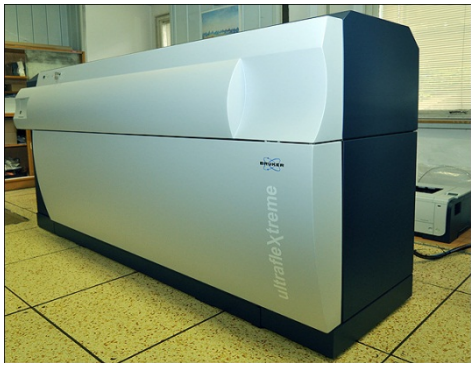
- priority of the MS staff for routine services, maintenance etc.

Vladimír Vrkoslav (tel. 237)

Mass spectrometers



Q-TOF micro (Waters)
Small molecules
(LR); ESI, APCI



UltraflexTreme (Bruker)
Small molecules, biomolecules
(LR, HR); MALDI

LTQ Orbitrap XL (Thermo)
Small molecules, biomolecules
(HR); ESI, APCI, nanoESI

TripleTOF (AB Sciex)
Biomolecules
(HR); nanoESI

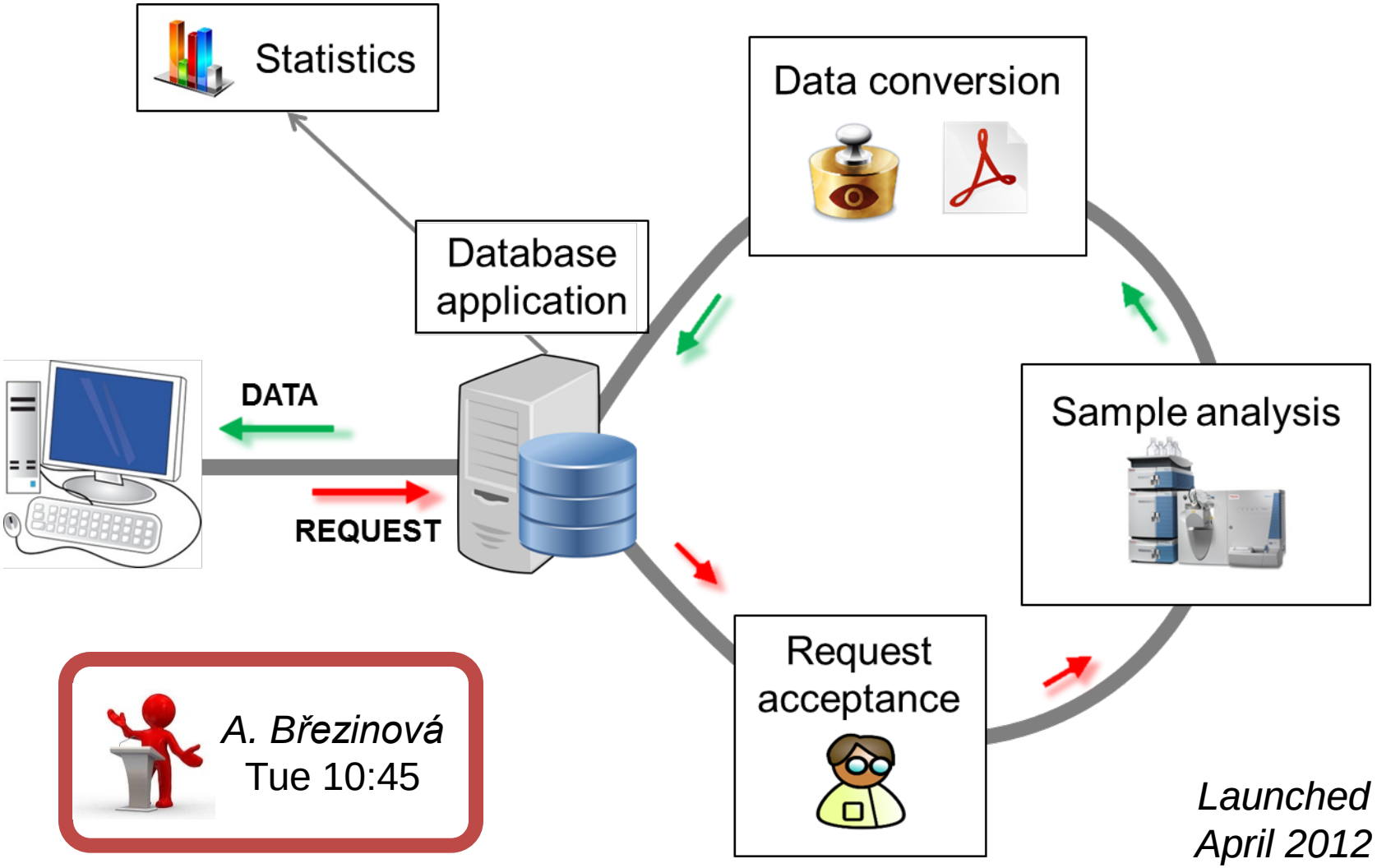
J. Jaklová-Dytrtová
Wed 9:00

Synapt G2 (Waters)
Ion mobility, small & biomolecules
(HR); nanoESI, ESI, APCI

GCT Premier (Waters)
Small molecules
(HR); EI, CI

ReQuest: Management of analysis requests

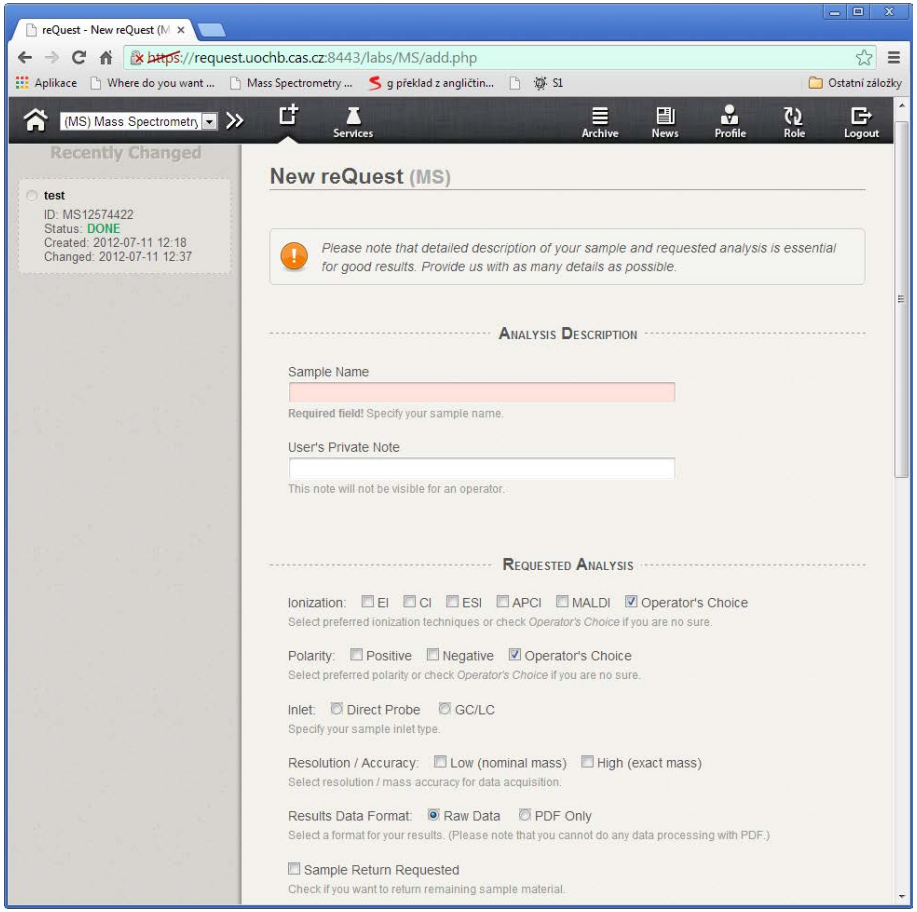
http://request.uochb.cas.cz



ReQuest: Management of analysis requests

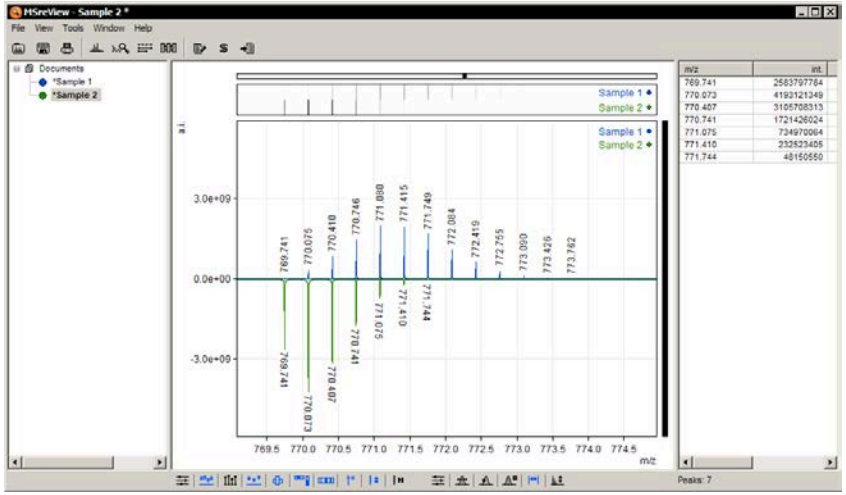
reQuest

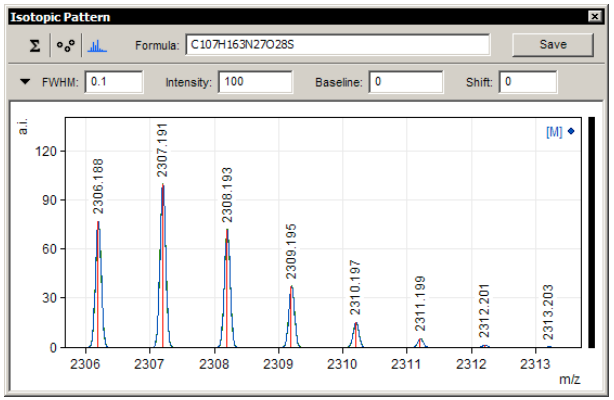
Web-based sample submission



MSreView

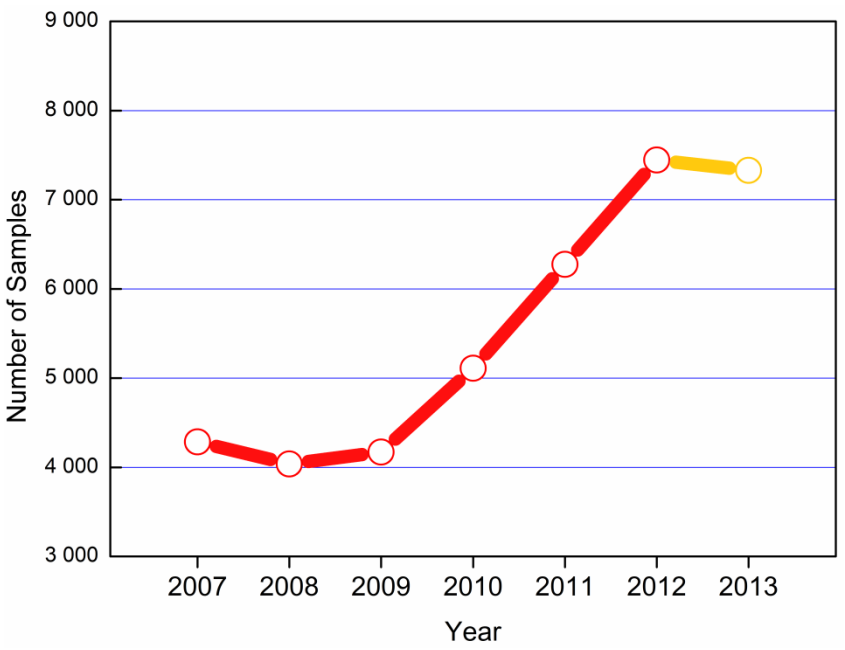
Software for working with mass spectra



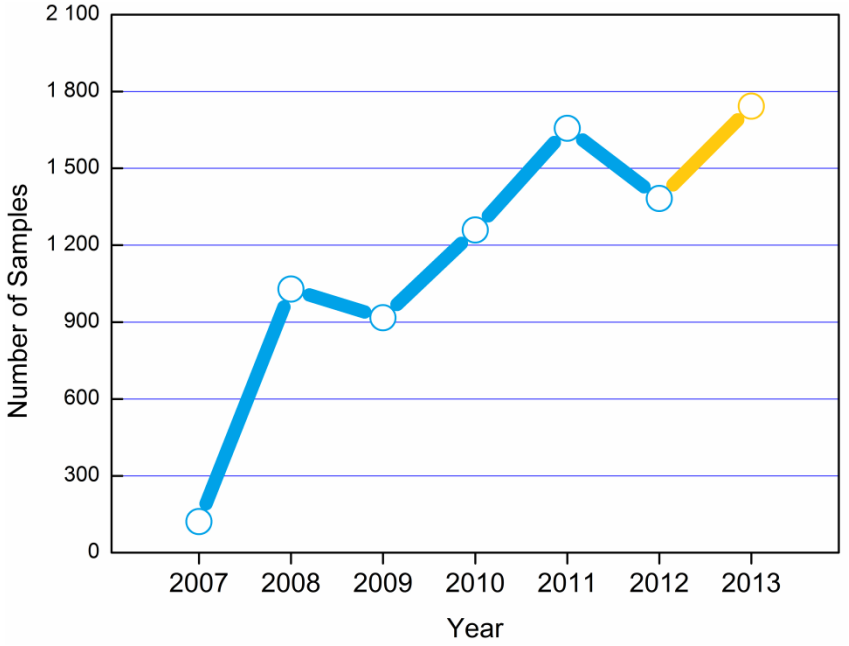


MS services seen by statistics

SMALL MOLECULES: LR & HR spectra



OPEN ACCESS GC/MS



Average duration of MS analysis (small molecules): **3.3** days

Data format requested: **44 %** .msd (raw data), **56 %** .pdf (graphics)

Moving labs and instruments …

I. January 2012

- From **106/107 SV** to **64/64a SZ** (Orbitrap) and **58c SZ** (GC/MS)

II. September 2013 (to former D. Schroeder's labs)

- From **33 LS** to **23 JZ** (MALDI, TripleTOF) and **25 JZ** (Q-tof micro)

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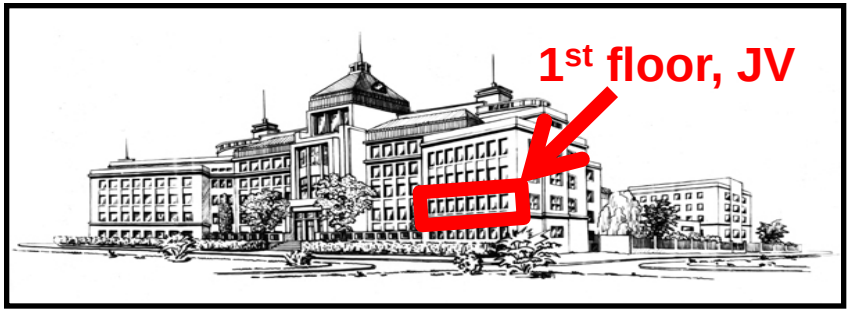
III. end of 2013 (to former I. Valterová's labs)

- From **274 PS** to **72 JZ** (Synapt), low-res. instruments to **75a JZ** and **78 JZ**

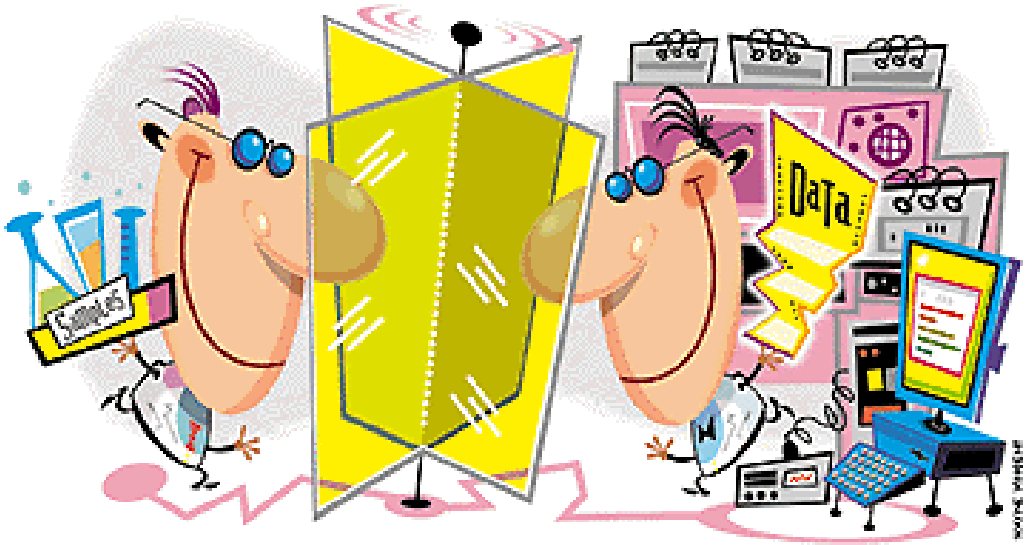
IV. 2014/2015 (to final labs)

- all instruments to **1NP JV**



MS Service is here for you…

We value your thoughts and opinions! Please feel free to contact us with your comments, questions or special service needs.



Introduction to Mass Spectrometry

Josef Cvačka

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:

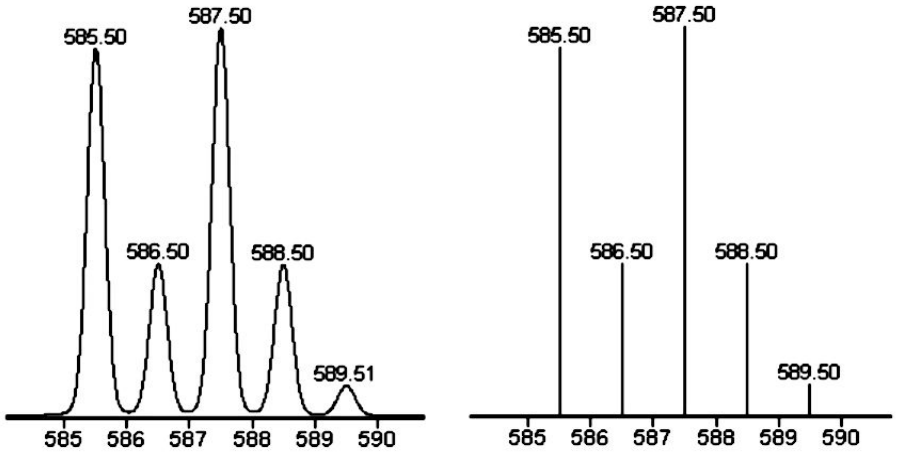
- quantification of organic compounds in the samples based on the intensity of the detector response for the selected ion or group of ions

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*

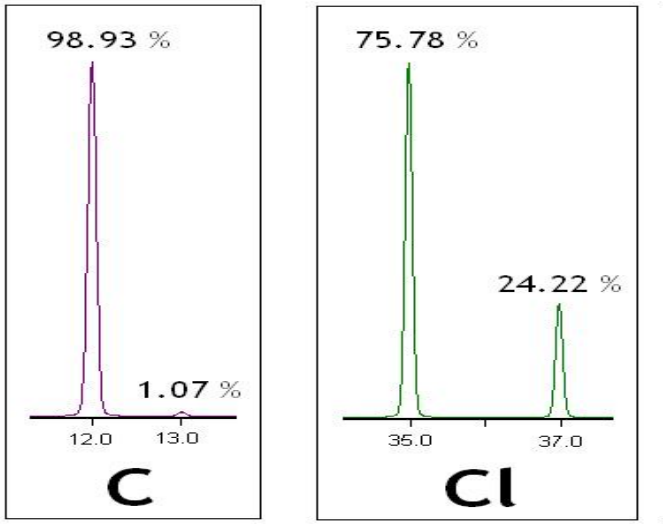


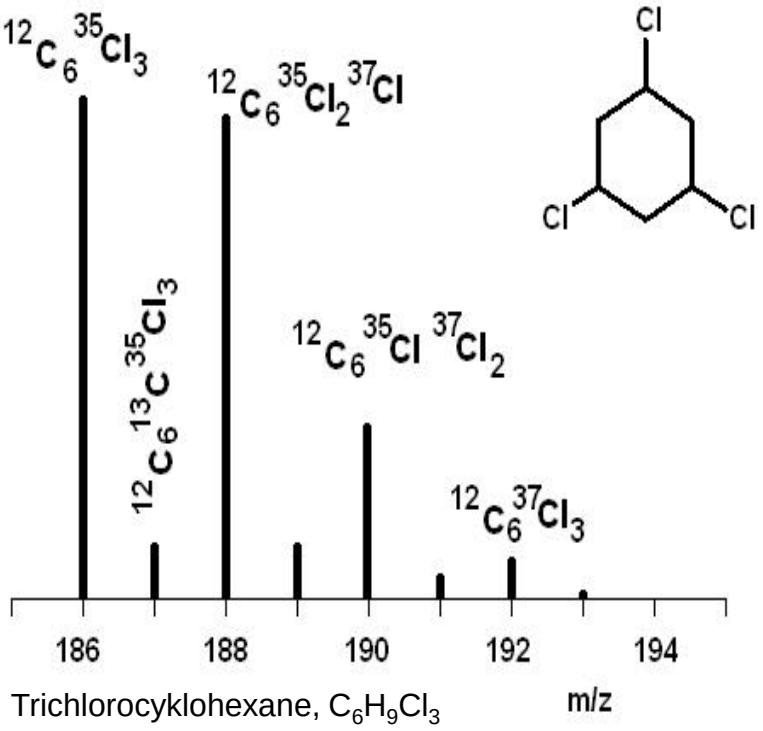
Profile (continuum): record of MS detector, allows determination of peak width (resolution)

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***

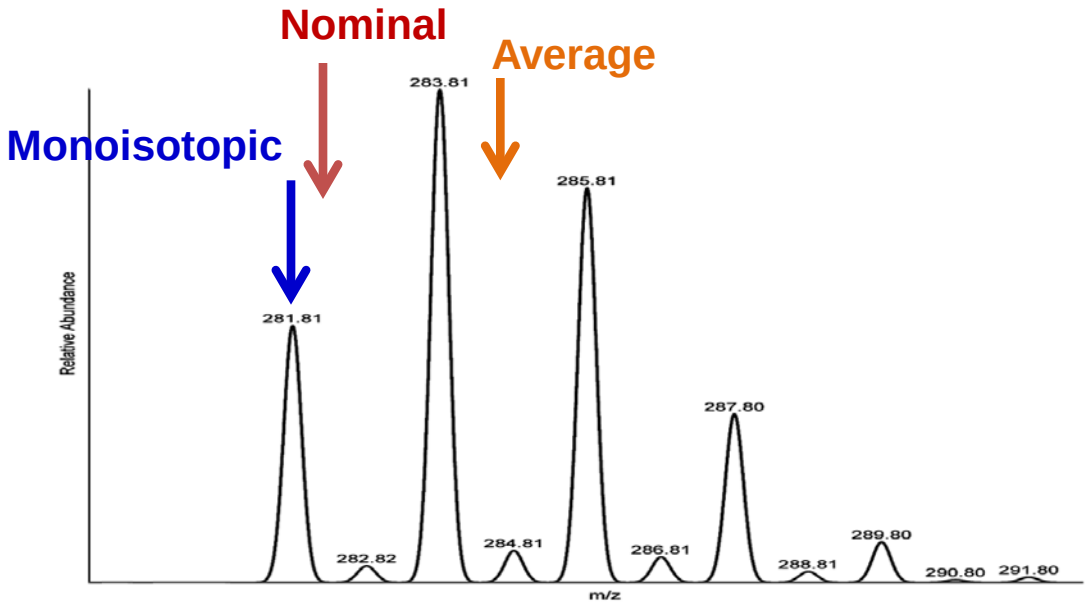
m/z is a dimensionless quantity used to describe ions in the spectrum. The unit **thomson (Th)** is sometimes used.

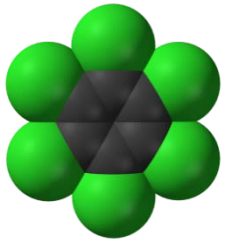
Masses in MS

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)





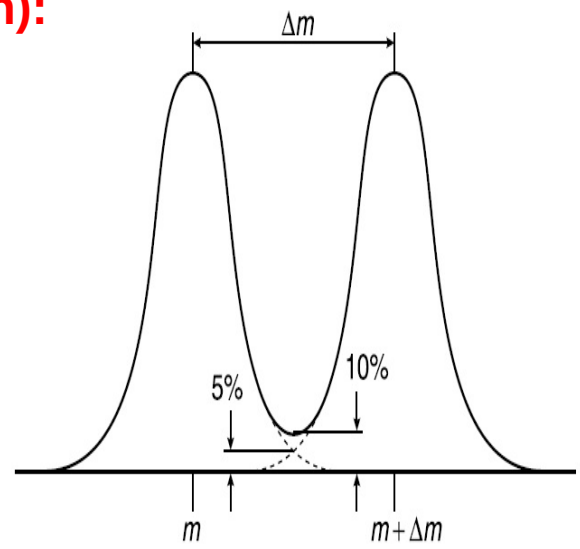
hexachlorobenzene
C_6Cl_6

Resolution

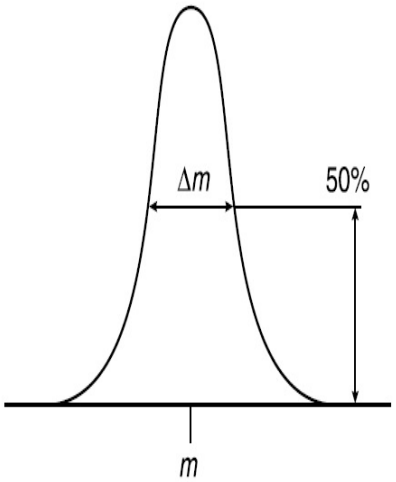
Resolving power (resolution):
the ability of an instrument to separate neighboring peaks

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

10% valley



FWHM

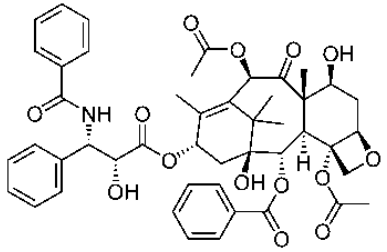


Two definitions of resolution:

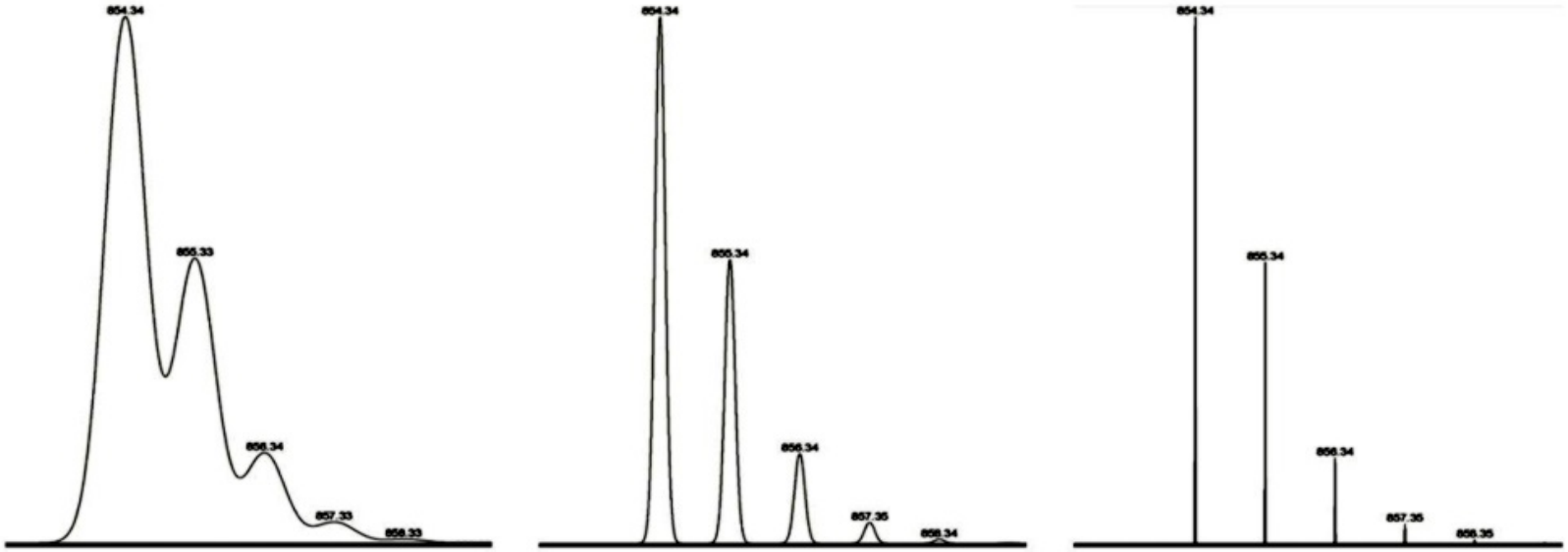
Resolution – 10% valley:
the ratio of an ion mass and the mass difference between equally high peaks when the valley separating their maxima is at 10 % of their intensity. Used for sector instruments (constant resolution in the entire mass range).

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)



Low resolution ←→ **High resolution**

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)

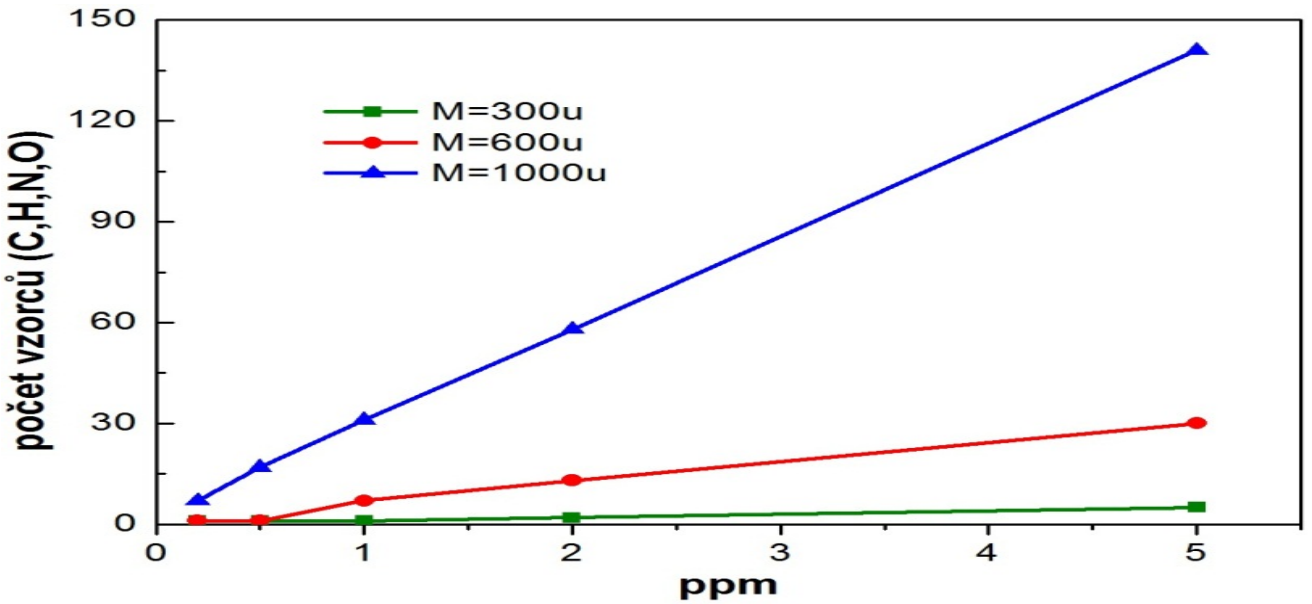
M ($C_{10}H_8$) = 128.0626

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

Calculation of the elemental composition

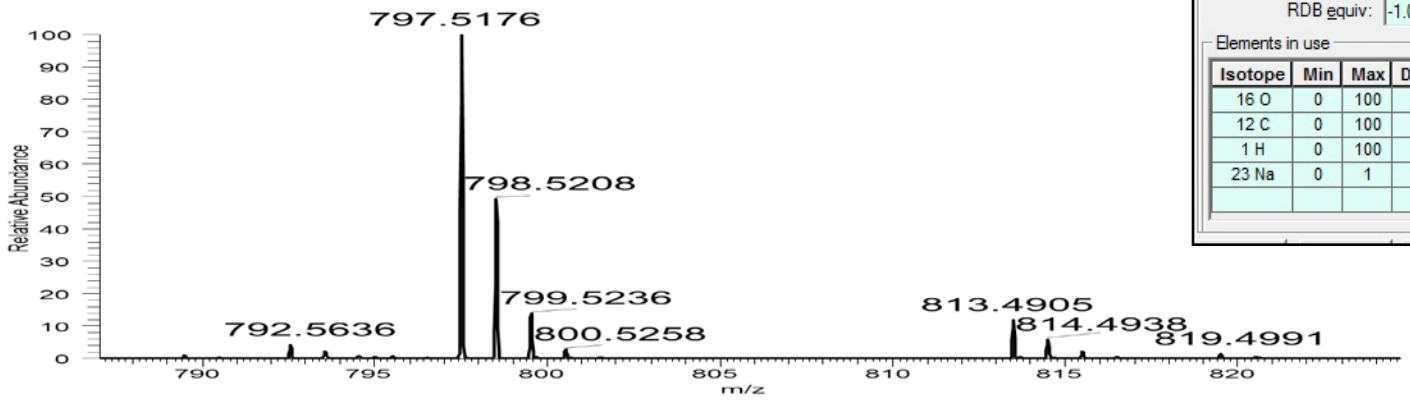
Each elemental composition has a unique mass. At infinitely high mass accuracy we get only the correct composition.

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

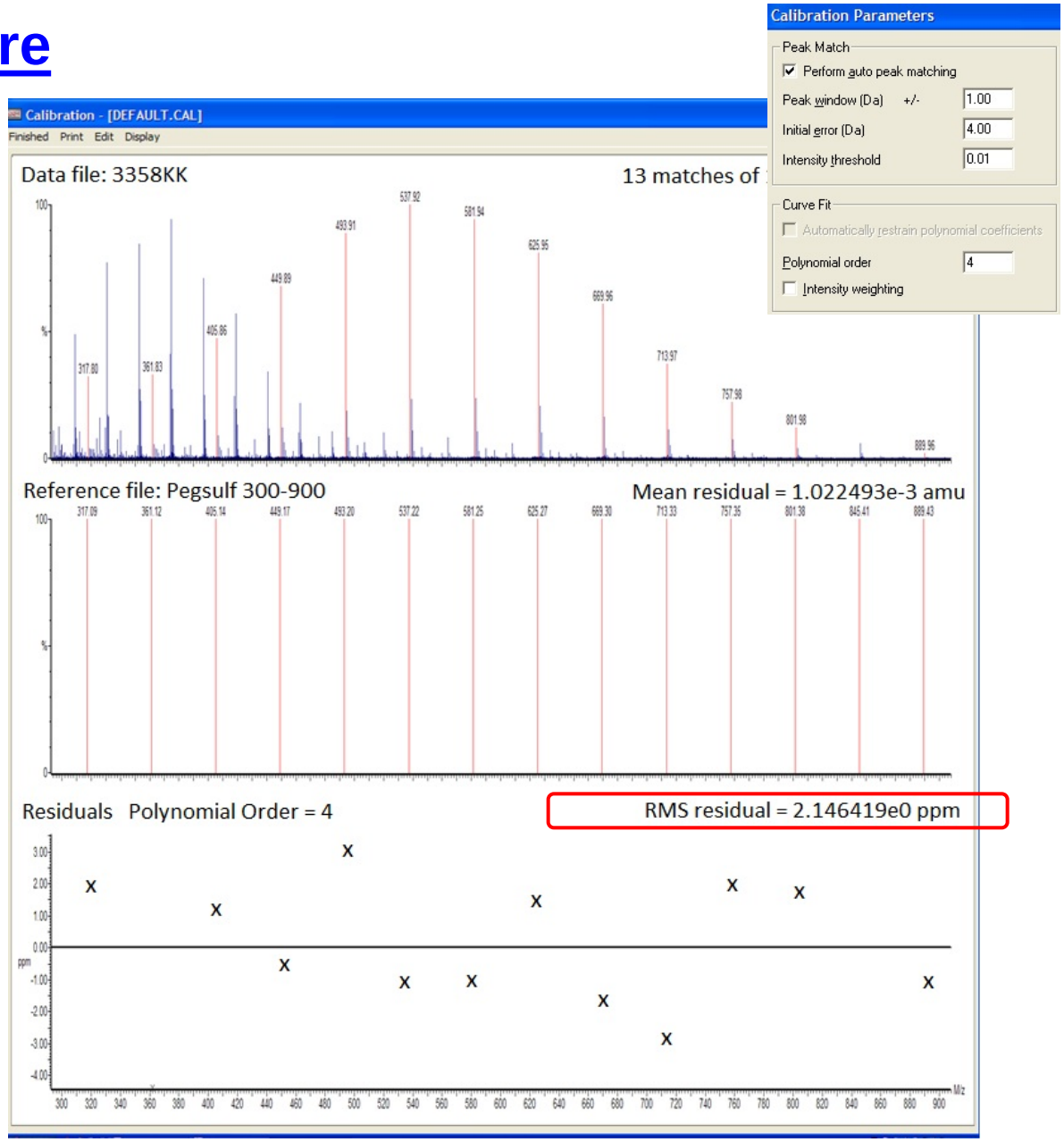
Calibration is carried out from a spectrum containing peaks of both sample and calibrant. Measurements of the calibrant and sample spectra are carried out simultaneously. Internal calibration provides more accurate results.

Calibration procedure

Recorded spectrum

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

Mass error for individual peaks



Thank you for your attention !