High-Resolution Mass spectrometry (HR-MS, HRAM-MS) (FT mass spectrometry)

MS that enables **identifying elemental compositions** (empirical formulas) from **accurate m/z data**

7.05.2018 1

Atomic masses (atomic weights)

- Aatomic mass unit is 1/12 of the mass of ^{12}C isotope
- **Atomic mass A_r** of an atom (molecular mass M_r of a molecule) is the ratio of the atomic mass of the atom (or isotope, molecule or ion) to the 1/12 part of the ^{12}C isotope mass
  - By definition: $A_r(^{12}C) = 12.00000$
  - Subscript r: it is a relative number, i.e. strictly saying, without a unit
    - For distinguishing from other unitless quantities atomic masses are often expressed as quantities with units: *Dalton* or *amu* (*atomic mass unit*)

7.05.2018 2

Nominal and exact atomic masses

- ... of isotopes
- **Nominal atomic mass**: The sum of numbers of protons and neutrons in the atomic nucleus
  - This is what a low-resolution MS shows for small and medium size molecules
  - The sum of nominal atomic masses of atoms in a molecule is the **nominal molecular mass of the molecule**
- **Exact atomic mass**: The ratio of the atomic mass of the atom to the 1/12 part of the ^{12}C isotope mass

8.05.2018 3

Atomic masses of isotopes

- Atomic masses of isotopes are **more or less** multiples of the number of protons and neutrons, **but not exactly!**
  - $A_r(^{12}C) = 12.000000$ amu
  - $A_r(^{13}C) = 13.003360$ amu
  - $A_r(^{14}N) = 14.003069$ amu
  - $A_r(^{16}O) = 15.994920$ amu
  - $A_r(e^-) = 0.0005486$ amu

7.05.2018 4

Example: CO, N_2 ja C_2H_4

- The nominal molecular mass of CO, N_2 and C_2H_4 is 28
- The exact molecular masses of molecules composed of the main isotopes:
  - CO: 27.994920 amu (isotopes: $^{12}C^{16}O$)
  - N_2: 28.006150 amu (isotopes: $^{14}N_2$)
  - C_2H_4 28.031300 amu (isotopes: $^{12}C_2{}^1H_4$)
- The respective molecular ions:
  - CO^+: 27.994371
  - N_2^+: 28.005601
  - $C_2H_4^+$: 28.030752

These ions are called isobaric ions

8.05.2018 5

Atomic masses of elements

- Natural elemets are mostly **mixtures of isotopes:**
  - Atomic mass of natural C is $A_r(C) = 12.011$
    - Approximately 1.10% of natural carbon is ^{13}C
  - Atomic mass of natural Cl is $A_r(Cl) = 35.453$
    - Approximately 75% of natural Cl is ^{35}Cl and ca 25% is ^{37}Cl
- **Mass spectrometer enables separating these isotopes**
  - **In the case of Cl there will be two peaks**
  - **m/z difference is ca 2 amu**
  - **Intensity ratio is roughly 75 : 25**

7.05.2018 6

Resolving power of mass spectrometer

- Can be expressed as:
  - The lowest Δm value, which is still separable by an MS
    - Can be expressed as Δm/m*1 000 000
      - Unit: ppm
  - Via R: R = m / Δm
- With many MS instruments:
  - Δm ~ m, i.e. Δm is not constant throughout the m/z axis
  - R is in broad terms constant throughout the m/z axis

All „m“-s in this presentation have to be interpreted as „m/z“ values!

High-resolution MS, HRMS
(High-resolution accurate-mass MS, HRAM-MS)

- MS that enables:
  - separating ions with the same **nominal** masses but different **exact** masses and
  - determining **high-accuracy m/z** values
- As a result it enables:
  - **Distinguishing** between different compounds in complex mixtures without separation
  - **Identification** of compounds with very high selectivity

High-resolution mass spectrometry: example

- In order to distinguish between CO^+ and $C_2H_4^+$ very high resolution is not needed, because:
  - the m/z values are low
  - the difference between m/z values is big: 0.0364 amu (ca 1300 ppm)
  - necessary resolution: $R = 28 / 0.0364 = 770$
- What about CO^+ and N_2^+?

Mass resolution vs Mass Accuracy

- **Mass resolution:**
    - The ability of an MS to distinguish beween very close m/z values
    - Does not need that the measured m/z values are very near to the actual m/z values
  - Needed for **resolving mixtures**
- **Mass accuracy:**
    - How close is the measured m/z value to the actual m/z value
    - For achieving high mass accuracy, as a rule, high mass resolution is needed
  - Needed for **identification**

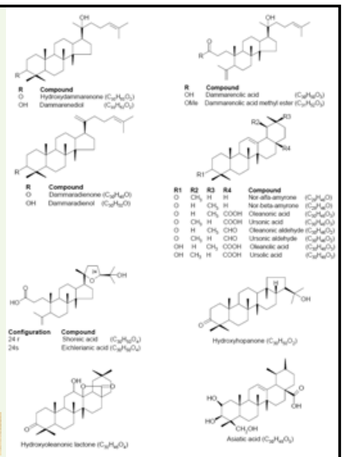
HRMS as a technique can be specific

- If signals with these m/z values are in the spectrum
  - 27.994371
  - 28.005601
  - 28.030752
- Then they cannot correspond to any other molecules than CO, N_2, C_2H_4, respectively
- The specificity only holds for small ions!

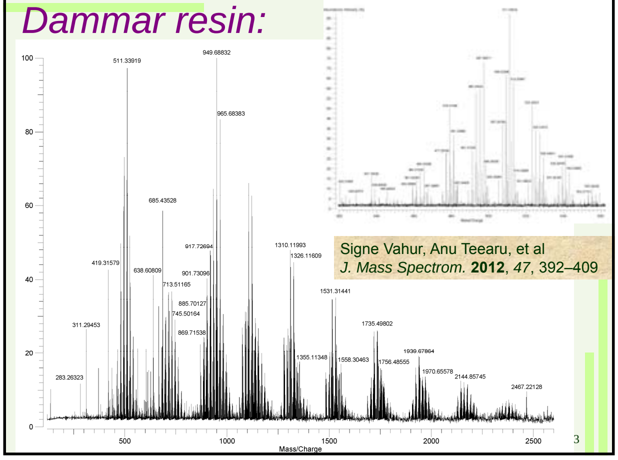
Example: Dammar resin

- A triterpenoid resin:
  - a complex mixture of cyclic isoprenoid compounds with 30 carbon atoms
  - Plus their oxidation and polymerization products

Signe Vahur, Anu Teearu, et al
J. Mass Spectrom. **2012**, *47*, 392–409



Dammar resin:



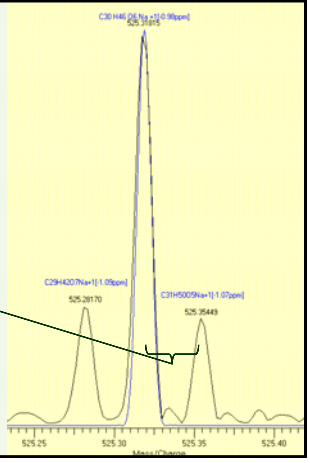
Signe Vahur, Anu Teearu, et al
J. Mass Spectrom. **2012**, *47*, 392–409

3

Identification of components

The FT-ICR spectrometer (7T magnet) easily resolves all three 525 isobars!

Difference 0.0365 amu (69 ppm)
m/z error ca 0.0005 amu (1 ppm)
Resolution achieved higher than
R = 525 / 0.0363 = 14462



Signe Vahur, Anu Teearu, et al
J. Mass Spectrom. **2012**, *47*, 392–409

The higher m/z, the more possible isobars!

Charge: +2

Measured m/z	Theoretical m/z	[ppm]	Delta [mmu]	Delta	RDB Composition
516.76671	516.76671	0.0	0.0	21.0	$C_{49} H_{71} O_{12} N_{13}$
	516.76647	0.5	0.2	15.0	$C_{49} H_{79} O_{11} N_{9} S_{2}$
	516.76638	0.6	0.3	12.0	$C_{41} H_{75} O_{14} N_{15} S_{1}$
	516.76705	-0.7	-0.3	11.5	$C_{43} H_{77} O_{15} N_{12} S_{1}$
	516.76604	1.3	0.7	16.0	$C_{48} H_{75} O_{16} N_{9}$
	516.76738	-1.3	-0.7	20.5	$C_{51} H_{73} O_{13} N_{10}$
	516.76604	1.3	0.7	21.5	$C_{47} H_{69} O_{11} N_{16}$
	516.76580	1.8	0.9	15.5	$C_{47} H_{77} O_{10} N_{12} S_{2}$
	516.76772	-2.0	-1.0	16.5	$C_{44} H_{73} O_{11} N_{16} S_{1}$
	516.76773	-2.0	-1.0	11.0	$C_{45} H_{79} O_{16} N_{9} S_{1}$
	516.76805	-2.6	-1.3	25.5	$C_{52} H_{69} O_{9} N_{14}$
	516.76537	2.6	1.3	16.5	$C_{46} H_{73} O_{15} N_{12}$
	516.76807	-2.6	-1.4	7.0	$C_{38} H_{79} O_{14} N_{15} S_{2}$
	516.76513	3.0	1.6	10.5	$C_{46} H_{81} O_{14} N_{8} S_{2}$
	516.76513	3.1	1.6	16.0	$C_{45} H_{75} O_{9} N_{15} S_{2}$
	516.76839	-3.3	-1.7	16.0	$C_{46} H_{75} O_{12} N_{13} S_{1}$
	516.76479	3.7	1.9	20.0	$C_{52} H_{75} O_{11} N_{9} S_{1}$
	516.76872	-3.9	-2.0	25.0	$C_{54} H_{71} O_{10} N_{11}$
	516.76470	3.9	2.0	17.0	$C_{44} H_{71} O_{14} N_{15}$
	516.76874	-3.9	-2.0	6.5	$C_{40} H_{81} O_{15} N_{12} S_{2}$
	516.76446	4.3	2.2	11.0	$C_{44} H_{79} O_{13} N_{11} S_{2}$
	516.76897	-4.4	-2.3	12.5	$C_{40} H_{73} O_{16} N_{16}$
	516.76907	-4.6	-2.4	15.5	$C_{48} H_{77} O_{13} N_{10} S_{1}$

Instrument		QqTOF
Mass Error		5 ppm
# of Pro-posals m/z 516.76671		23

http://www.thermo.com/

Mass accuracy ±5 ppm

- The previous slide corresponds to the abilities of the „ordinary“ TOF mass spectrometers
- **It is difficult to pick from the 23 candidates, which is correct**
- **Difficult to identify!**

Mass accuracy ±1 ppm

- This corresponds to FT-ICR

Charge: +2

Measured m/z	Theoretical m/z	[ppm]	Delta [mmu]	Delta	RDB Composition
516.76671	516.76671	0.0	0.0	21.0	$C_{49} H_{71} O_{12} N_{13}$
	516.76647	0.5	0.2	15.0	$C_{49} H_{79} O_{11} N_{9} S_{2}$
	516.76638	0.6	0.3	12.0	$C_{41} H_{75} O_{14} N_{15} S_{1}$
	516.76705	-0.7	-0.3	11.5	$C_{43} H_{77} O_{15} N_{12} S_{1}$

- **It is a lot simpler to pick the correct structure if there are only four candidates!**

Instrument		LTQ-FT 7 T magnet
Mass Error		1 ppm
# of Pro-posals m/z 516.76671		4

The higher the m/z the better mass accuracy is needed for identification!

http://www.thermo.com/

Bruttoformula (empirical formula) vs structure

- Mass spectrum only gives the bruttoformula
- For learning about structure:
  - LC separation
  - MS^n
  - Ion mobility spectroscopy
  - Independent information

FT Mass Spectrometry

- Application of FT (Fourier' transform) in mass spectrometry enables:
  - High **mass resolution**
    - Usual: tens or hundreds of thousands
    - Best: up to 1.N millions
  - High **mass accuracy**
    - Usual: 2-4 ppm
    - Best: 1 ppm and below
  - In some cases also very good limits of detection
    - In principle signal can be obtained from as little as some hundreds of ions
  - Studying gas-phase ion-molecule reactions (ICR)

Types of FT mass spectrometers

There are two types of FT-MS systems:

- **FT-ICR** (*Fourier Transform Ion Cyclotron Resonance*)
  - Classical FT-MS
  - Highest resolution and mass accuracy
  - Enables studying ion-molecule reactions
  - Is losing market share because is too expensive and Orbitrap gives similar performance
- **FT-Orbitrap**
  - Completely new type of MS
  - Developed in the beginning of this century
  - Somewhat less capable but a lot cheaper to buy and to operate

The principle of FT-ICR

- 1. Ions are produced using some usual ionisation method
  - ESI is the most common
- 2. The ions are directed to the ICR cell
  - The cell walls are (in simplified terms) as capacitor plates
  - The cell is in **very strong magnetic field**
    - Superconducting magnet, usually 4 to 12 Tesla
  - The cell is in **high vacuum**
    - Depending on applications $n \cdot 10^{-7}$ mbar (ion-molecule reactions) to $n \cdot 10^{-10}$ mbar (HR applications)
    - At ca $5 \cdot 10^{-9}$ mbar the mean free path of ions is ca 10 km!

FT-ICR Cell

Superconducting magnet

The magnet is cooled by liquid He and liquid N_2

The principle of FT-ICR

- 3. The ions start **cyclotroning** in the cell
  - Ions are kept in the cell by:
    - Magnetic field on the sides
    - Trapping plates at the ends of teh cell
    - The radius of the movement is initially much below 1 mm
    - The ions are not "in phase"

The principle of FT-ICR

- The resonance frequency of an ion:

$$\omega = \frac{B}{m/z}$$

z – ion charge, m – ioon mass, B – magnetic field strength

- As a first approximation the **frequency depends on magnetic field strength and m/z ratio**
  - For accurate m/z ratio measurement it is necessary to measure frequency accurately
  - This equation in theoretical, in practice it is more complex and mass calibration is used

The principle of FT-ICR

- 4. While cyclotroning it is possible to carry out manipulations with the ions:
  - Reactions of ions with molecules
    - Some gas is leaked into the cell so that ions can collide with gas molecules
  - Ion fragmentation
  - Selective ejection of some ions (with predefined m/z values or ranges) while retaining others
- These possibilities enable MS/MS in the ICR cell

The principle of FT-ICR

- 5. For detecting ions they are **excited**
  - On one pair of side plates a complex HF pulse is applied, which contains the frequencies of all detected ions
  - As a result all the ions go to orbits with longer radii
    - Several cm, depending on the cell dimensions
  - Ions with the same m/z ratio are now „in the same phase”

The principle of FT-ICR

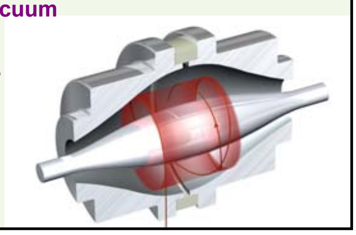
- 6. Cyclotroning of ions that are in the same phase between capacitor plates induces a voltage pulse of complex form
  - It **contains the frequencies of all ions** in the cell
  - Each frequency is represented in the pulse the stronger the more of the respective ion is present in the cell
  - The pulse is collected during several seconds
  - The longer the collection time the
    - Higher the resolution
    - In general, higher the m/z measurement accuracy
  - For long collecting time high vacuum is needed

The principle of FT-ICR

- 7. **Fourier’ transform** is applied to the collected pulse
- A frequency spectrum is obtained, from which mass spectrum is thereafter easily obtained

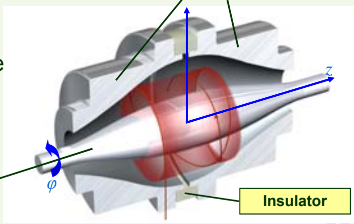
The principle of Orbitap

- 1. Ions are formed using a usual ionization method
  - ESI is the most widespread
- 2. Ions are directed to the Orbitrap cell
  - The cell walls act (in simplified terms) as capacitor plates
  - The cell is in **high vacuum**
    - ca n·10^{-10} mbar
  - **No magnetic field is needed**



The principle of Orbitrap

- We look at positive ions
- Ions:
  - The ions circle around the central electrode
  - Oscillate between the end-electrodes
  - Ions are in the same phase when they enter the cell



The principle of Orbitrap

- The frequency of oscillating along the Z-axis:

$$\omega_z = \sqrt{\frac{k}{m/z}}$$

- *k* is an instrument-specific constant, depends on the instrument and conditions
  - Is determined during mass calibration
- A complex pulse is generated by oscillating ions, which is recorded and FT is applied to get mass spectrum

Using ICR and Orbitrap with LC?

- Intrinsically not very suitable for LC-MS work, but still used extensively
  - The large majority of applications need LC separation
- **Hybrid instruments**
  - Ordinary LC/MS chromatograms are recorded with a LR and fast MS
  - HR-MS is recorded only from the interesting peaks
  - Timings:
    - Either from preliminary chromatograms or
    - via real-time data-dependent analysis (detecting peak start)

TOF

- Mass resolution worse than FT-MS
- Mass accuracy also, but not as much
- Less expensive than Orbitrap
- Speed is a big advantage
  - Real time LC mode is no problem
- MS^2: Q-TOF

Parameter	FT-ICR	Orbitrap	TOF
Mass resolution	Up to 1.N mln	Up to several hundred 1000	Up to several tens of 1000
m/z accuracy	**≤ 1 ppm**	**2 ppm**	2-10 ppm
Magnet needed	**Yes**	No	No
Maximum m/z	Ca 5000	Ca 5000	Ca 100 000
MS^n possibility	Yes	Only hybrid instruments	Only hybrid instruments
Ion-molecule reactions	**Yes**	No	No
Speed	Slow	Slow	**Very fast**
Ion sources	ESI (MALDI)	ESI	MALDI (ESI)

Mass axis calibration

- **The presented theoretical formulas are not sufficiently exact for accurate m/z values**
- For this reason there are additional adjustment equations with empirical correction factors
- These factors are determined using standard compounds, which form ions with well known m/z values
- Two ways:
  - External calibration
  - Internal calibration

Applications

- First of all, cases, where high m/z accuracy is needed
  - **Accurate molecular masses** of compounds
    - Confirming identity of synthesized compounds
      - Standard practice in organic synthesis
  - **Identifying components** in complex mixtures
    - Using m/z values, isotope patterns and MS/MS if needed
      - Can be with or without LC separation
    - Analysis of the type “What are the compounds in my sample?”
  - **Proteomics, metabolomics**, …
- Gas-phase ion-molecule reactions (FT-ICR)

Crude oil analysis

Acc. Chem. Res. **2004,** *37*, 53-59

HR FT-ICR gives the possibility to identify practically all components in crude oil

The overall number is close to 20 000

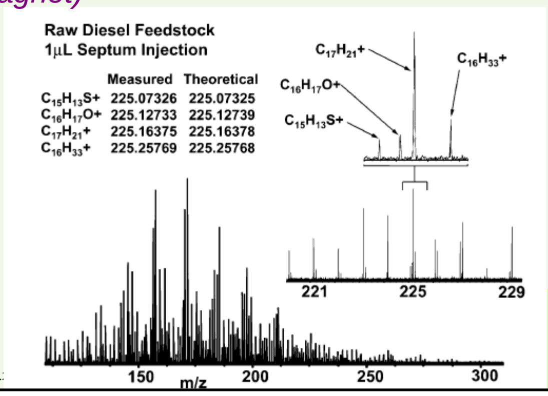
In addition to resolution and mass accuracy it is also important how to ionize

Ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry has recently revealed that petroleum crude oil contains heteroatom-containing (N,O,S) organic components having more than 20 000 distinct elemental compositions (C*c*H*h*N*n*O*o*S*s*).

It is therefore now possible to contemplate the ultimate characterization of all of the chemical constituents of petroleum, along with their interactions and reactivity, a concept we denote as **“petroleomics”**.

Such knowledge has already proved capable of distinguishing petroleum and its distillates according to their geochemical origin and maturity, distillation cut, extraction method, catalytic processing, etc.

Crude oil analysis: sample spectrum (5.6 T magnet)



Proteomics

- Proteomics is the large-scale study of proteins, particularly their structures and functions:
  - Which proteins are present in which parts of the organism
  - What is their abundance and dynamics
  - What are their functions etc.
- The **proteome** of an organism is the collection of all of its proteins
  - PROTEin complement expressed by a genOME - Marc R. Wilkins (1994)
  - The **proteome** is the entire set of proteins expressed by a genome, cell, tissue, or organism at a certain time.

MS – the key to modern proteomics

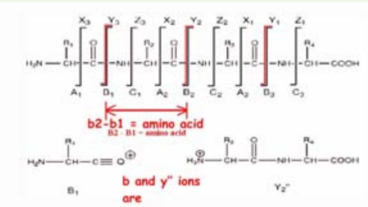
- Proteomics uses different approaches
  - The majority of them are nowadays based on MS
- Mostly one of two:
  - MALDI-TOF or LC-QTOF
    - Classical peptide fingerprinting, not much used currently
  - FT-MS
    - (FT-ICR) or FT-Orbitrap
    - Is gaining popularity thanks to the advances in LC-MS
    - MS/MS experiments

MS Proteomics

- Every amino acid fragment has a unique mass (except for leucine and isoleucine)
  - Proline 97.05276
  - Arginine 156.10111
  - …
- Protein M obtainable from amino acid M-s
  - Protonation and charge type have to be considered also
- The masses of ions formed from any protein or peptide are accurately predictable
  - **Being composed of amino acids is of much help in identifying**

Protein fragments

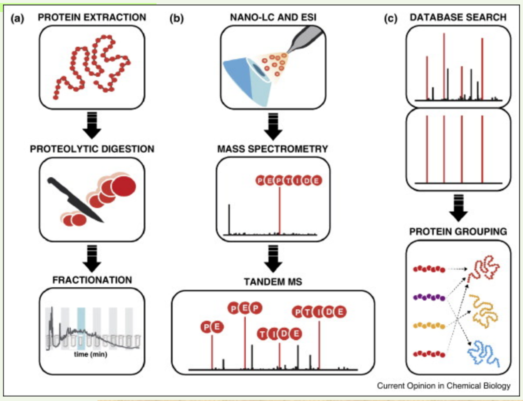
- **Digestion with a protease (most commonly trypsin)**
  – Trypsin cleaves proteins after arginine and lysine
- **MS/MS experiments**
  – In MS fragmentation of protonated proteins or peptides occurs via specific pathways:



  – B, C, Y and Z fragments are the most widespread; type of fragments depends on the fragmentation technique

Bottom-up FT-MS/MS proteomics

- The proteins are digested using a protease (trypsin, LysC, GluC, chymotrypsin etc)
- The formed mixture of peptides
  – is separated by LC and
  – is analysed using FT-MS/MS
- The peptides are identified
  – by m/z (gives accurate peptide mass)
  – Using MS/MS (provides information about the peptide sequence)
- Proteins are identified based on identified peptide sequences



Current Opinion in Chemical Biology, **2015**, *24*, 11–17

Top-down FT-MS proteomics

- Full-length proteins are
  – separated by LC or electrophoresis
    • Sometimes separation is even not needed
  – Ionized by ESI
  – Directed to FT-MS for analysis
- m/z of the entire molecule can be measured
  – Up to 60 kDa is easy, larger proteins are more difficult
  – Usually not accurate enough to identify a protein
- MS/MS experiments in the ICR or HCD cell
    • IRMPD, ECD, ETD, SORI-CAD, HCD
    • The obtained fragments are matched via database search

More on proteomics

- What was explained above was strongly simplified
- Real proteins are often not just sequences of amino acids but contain covalent post-translational modifications (PTM) and co-factors
  – Phosphorylation (serine, threonine, tyrosine)
  – Methylation (lysine, arginine)
  – Glycosyl side chains
  – Metal complexes
    • Often acting as active centres in enzymes