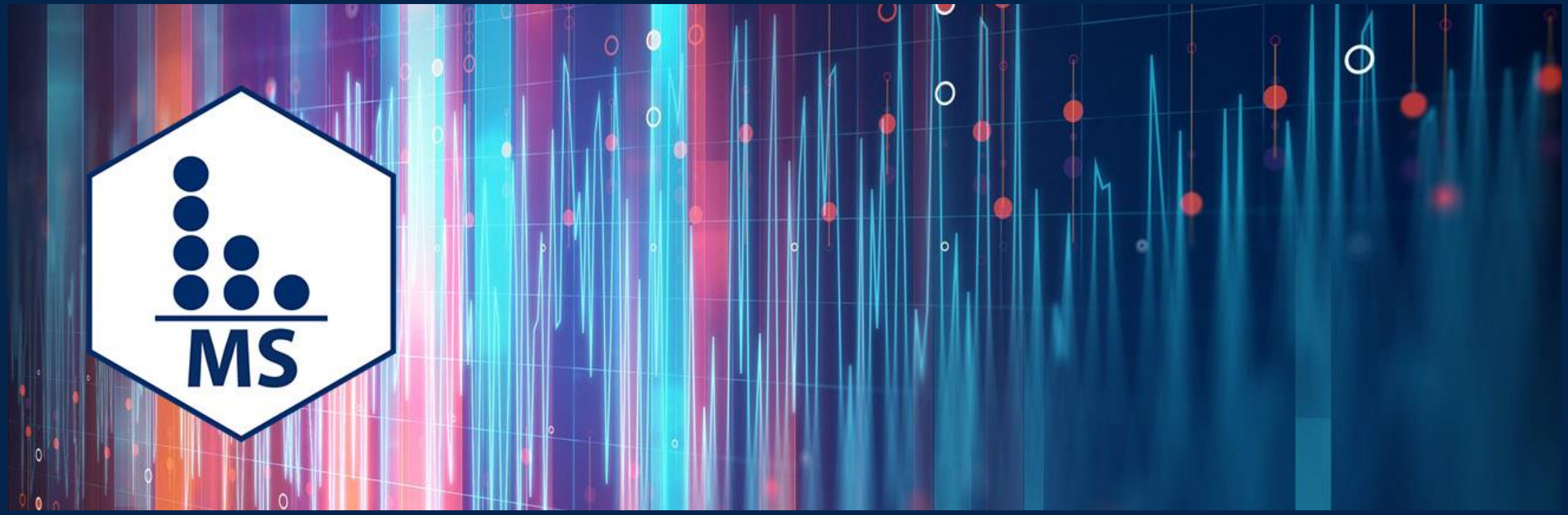
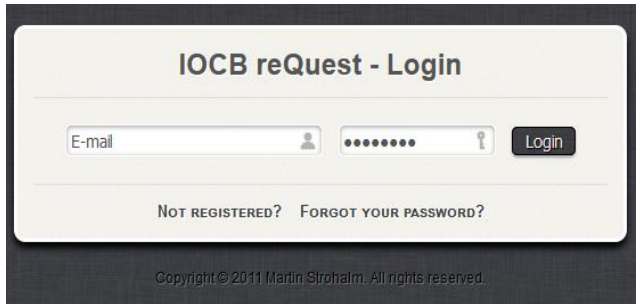
ÚOCHB AV ČR
IOCB PRAGUE

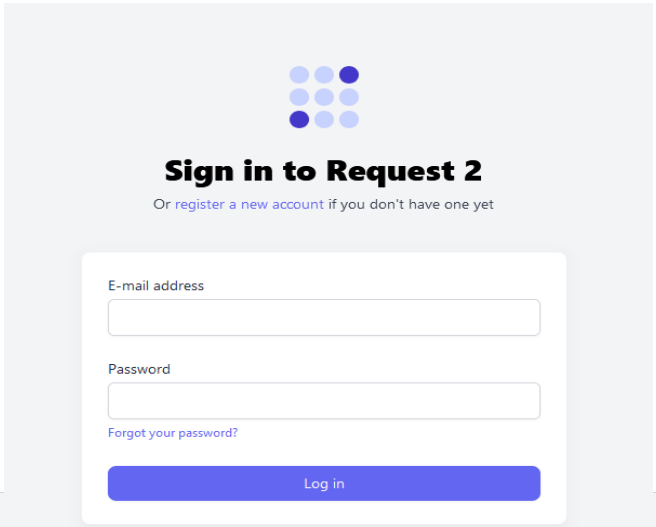


Sample analysis, tips and tricks

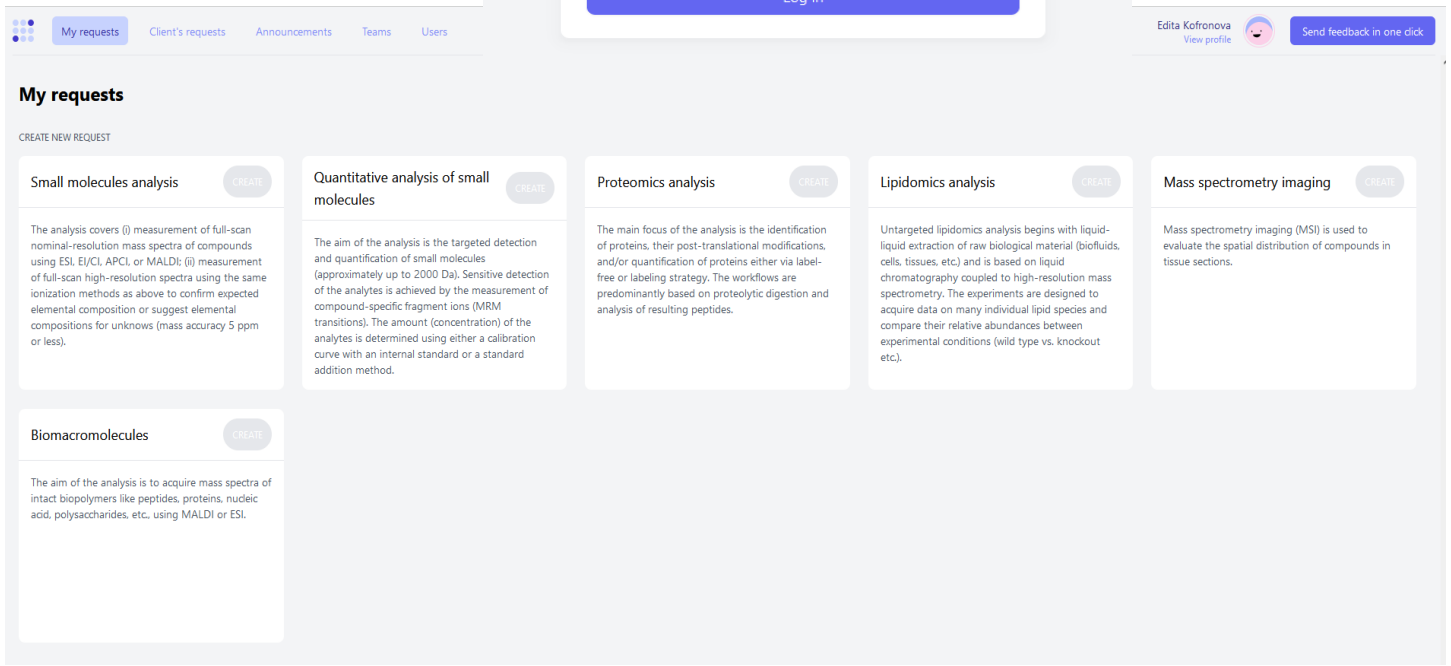
Edita Kofroňová | 25.10. 2024

ReQuest - Future





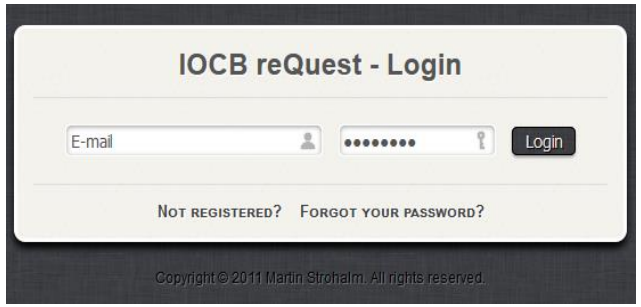
https://request.uochb.cas.cz:8443/users/login.php



Request 2

- After last MS group Days, we were in testing phase
- Thank you all users, who helped us with testing this system

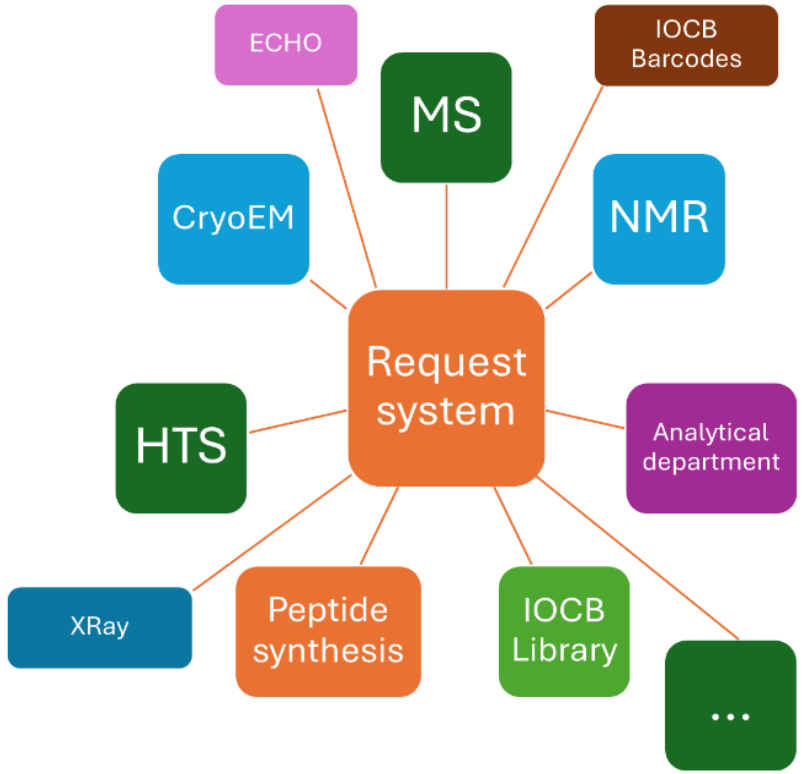
ReQuest - Future



https://request.uochb.cas.cz:8443/users/login.php

New REQUEST

- Based on Request 2, NEW system will be prepared
- All Core Facilities (09/2025)
- Connected with Intraweb login
- Many features



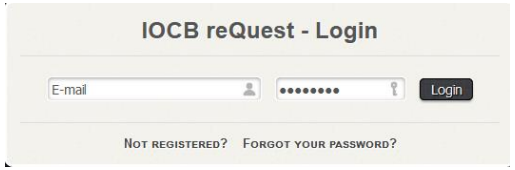
Request systém

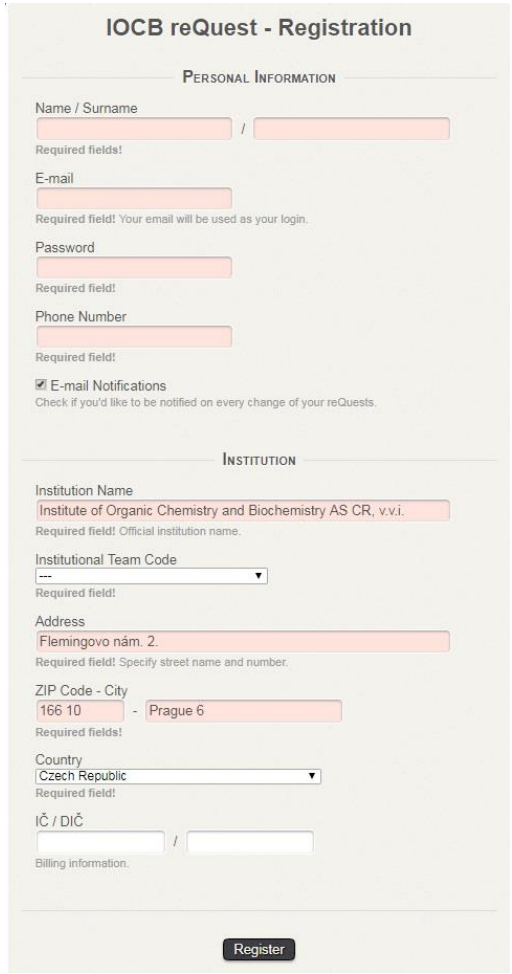
Illustrating picture

Matúš Drexler

IOCB Data Coordinator

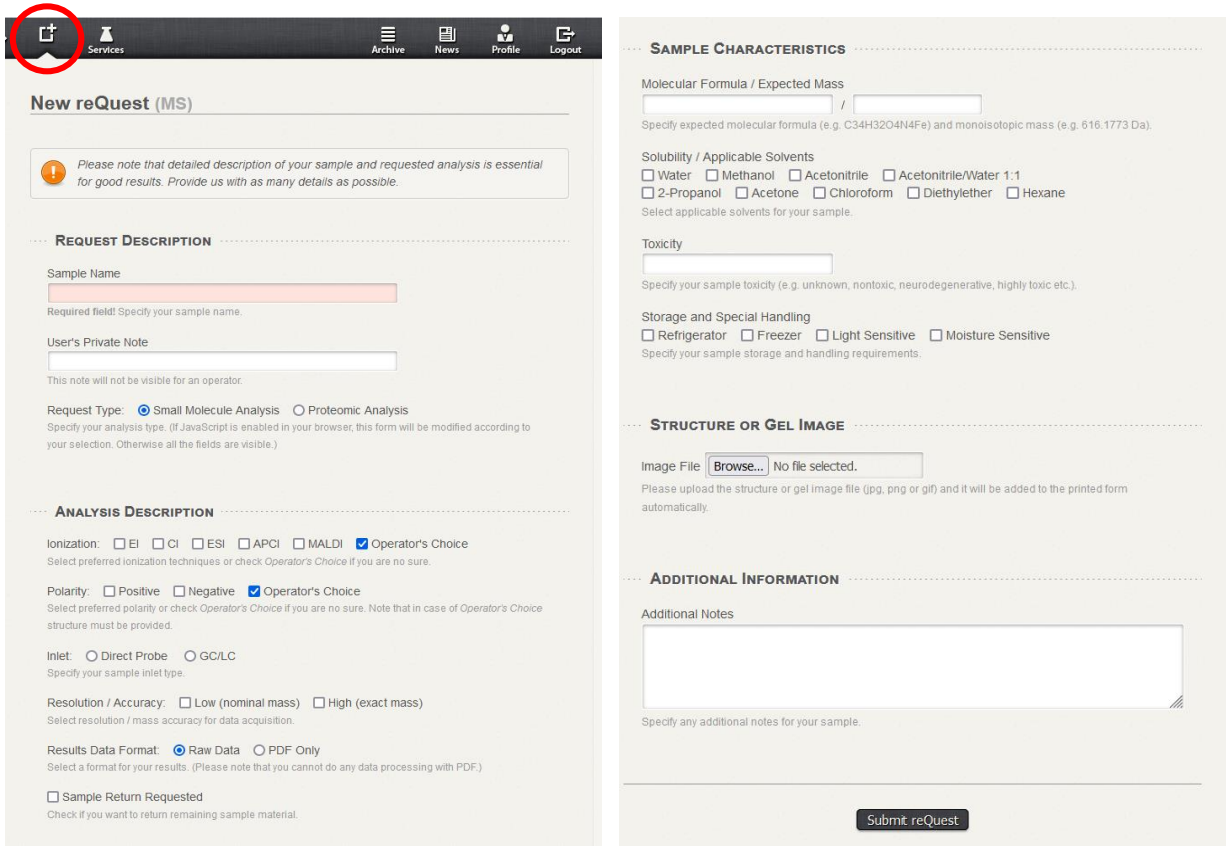
ReQuest – New Users





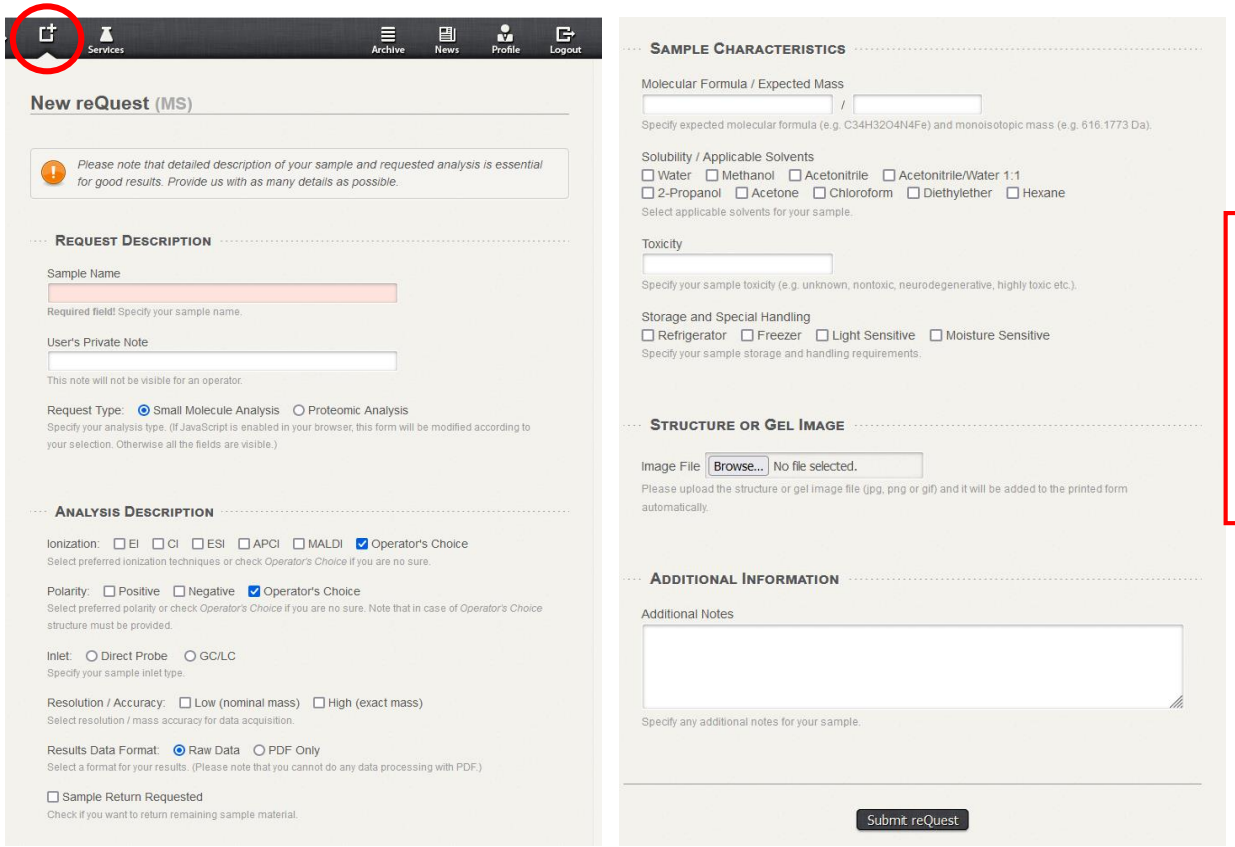
- IOCB phone and email preferred
- **Your account to be handed over to your boss when leaving IOCB (MS stuff cannot retrieve or change password)**
- Except for ReQuest system, no backup of outputs (.pdf or .msd files)
- Backup of measured data on data storage (**true raw files**) (from 2007 on ds.uochb.cas.cz)
  - 1 year – device computer of each mass spectrometer
  - each 3 months – backup to data storage or backup computer
- Be prepared to discuss your wishes when new system of requests will be developed for all Core Facilities (Matúš Drexler – Data Coordinator)

ReQuest – Sample submission



- Sample name must correspond to label on vial (readability, appropriate marker, transparent tape)
- Ionization
  - “operator´s choice” or specify (really need all?)
- Polarity
  - “operator´s choice” or specify (really need both?)
- Inlet
  - ESI/APCI – direct inlet
  - EI/CI – direct inlet or column
- Resolution/Accuracy
  - HIGH resolution – necessary **Formula, Exact mass**
  - LOW resolution – Nominal mass or Range of masses
- Solubility – choose 2 or 3 most suitable solvents
- Data format

ReQuest – Sample submission



- **Structure is highly important for us**
  - upload file in .jpg, .png, .gif, .cdxml format or draw to paper request
- If you don't want disclosed structure, come and discuss

- Sample name must correspond to label on vial (readability, appropriate marker, transparent tape)
- **Ionization**
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- **Polarity**
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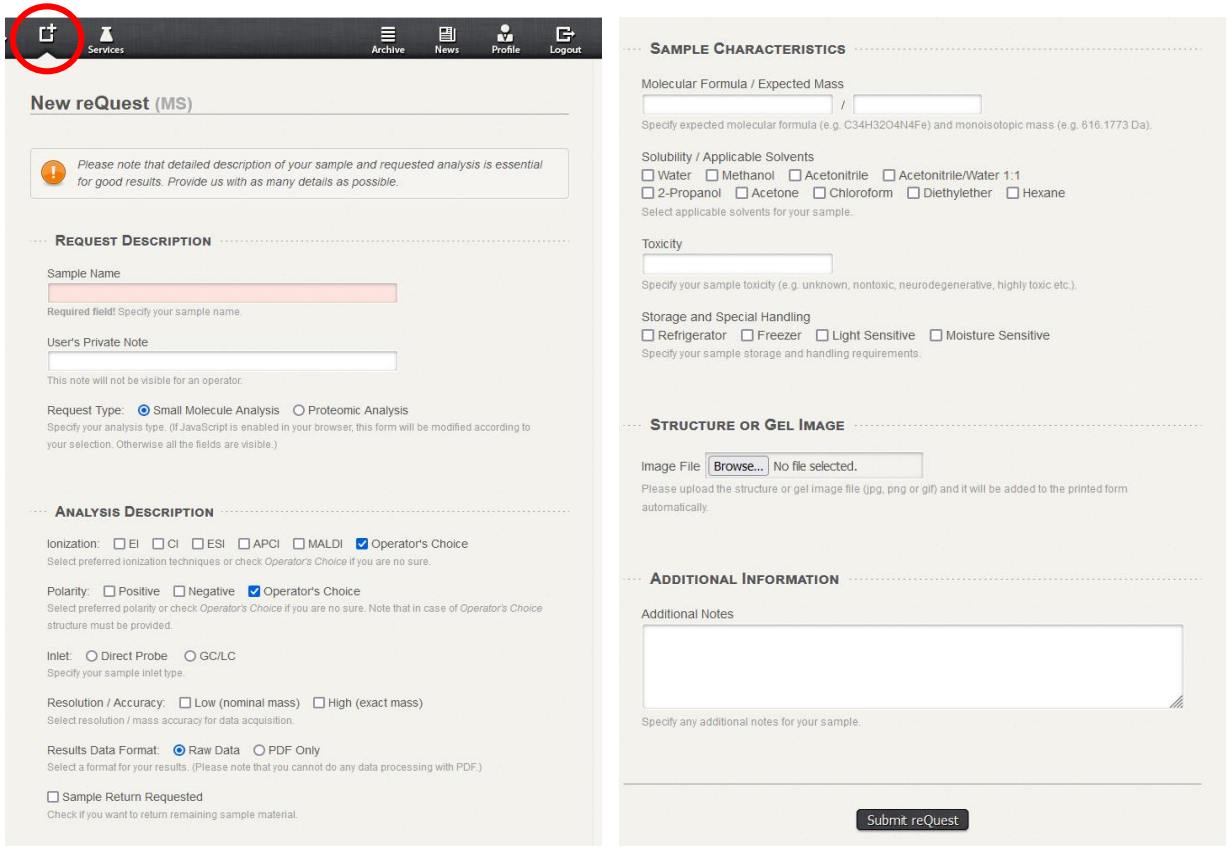

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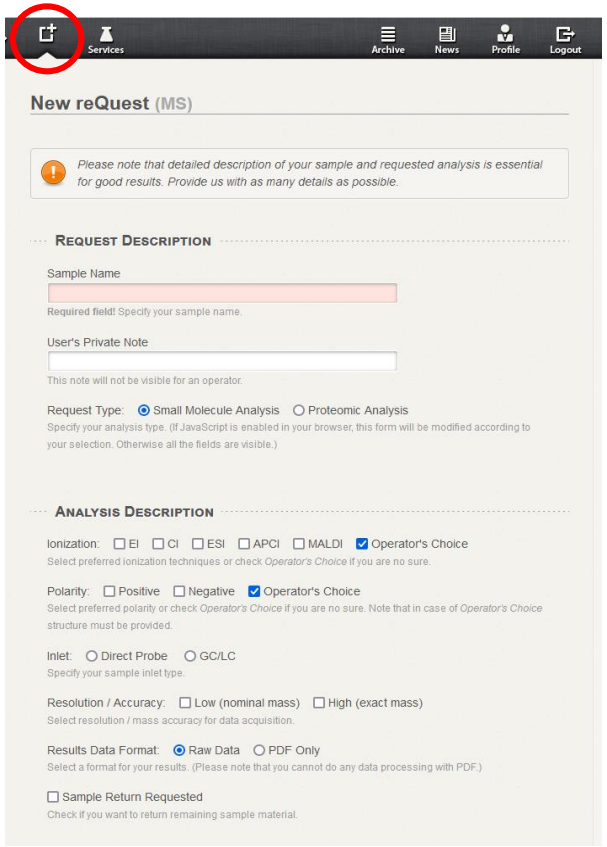

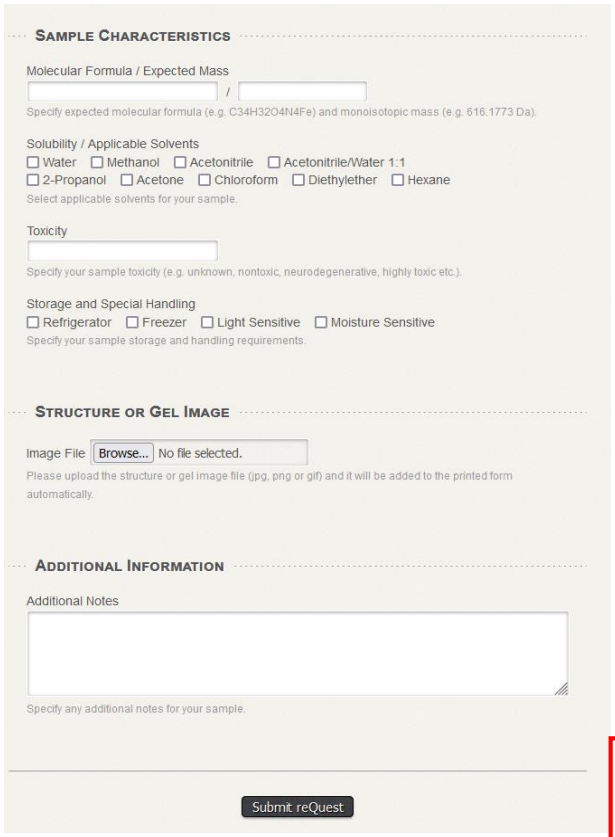

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  - **HIGH resolution – necessary Formula, Exact mass**
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- Solubility – choose 2 or 3 most suitable solvents
- Data format

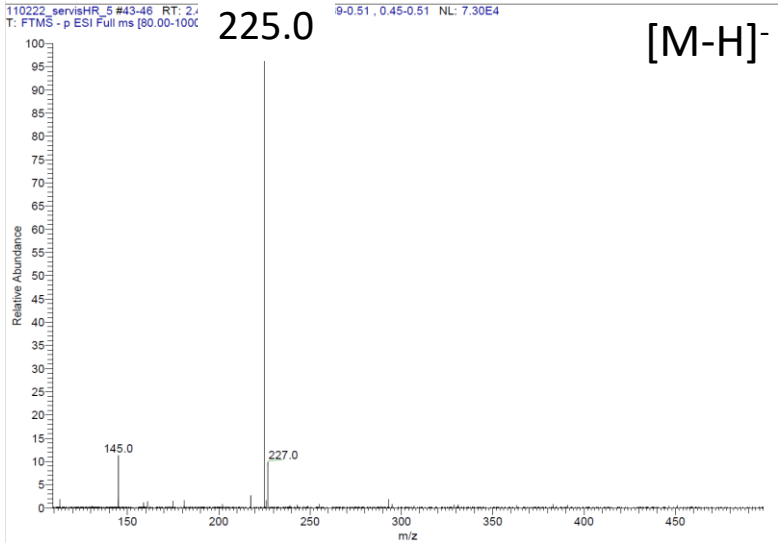
Mass Spectra of Molecules

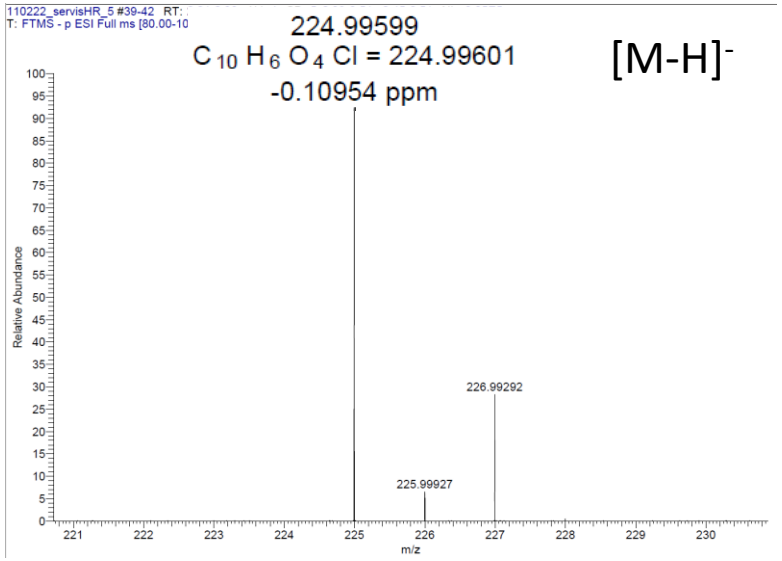
Up to 2000 Da

- Low-resolution mass spectra (ESI, EI/CI, MALDI)
  - identification of product in reaction mix or fractions
  - quick verification of products mass during routine synthesis (QC)

- High-resolution mass spectra (ESI, EI/CI, APCI, APPI, MALDI)
  - to confirm expected elemental composition
  - suggest elemental composition(s) for unknown compounds or impurities

Resolution
EI/CI up to 30 000
ESI/APCI/APPCI up to 100 000





Mass Spectra of Molecules

Up to 50 000 Da (150 000 Da)

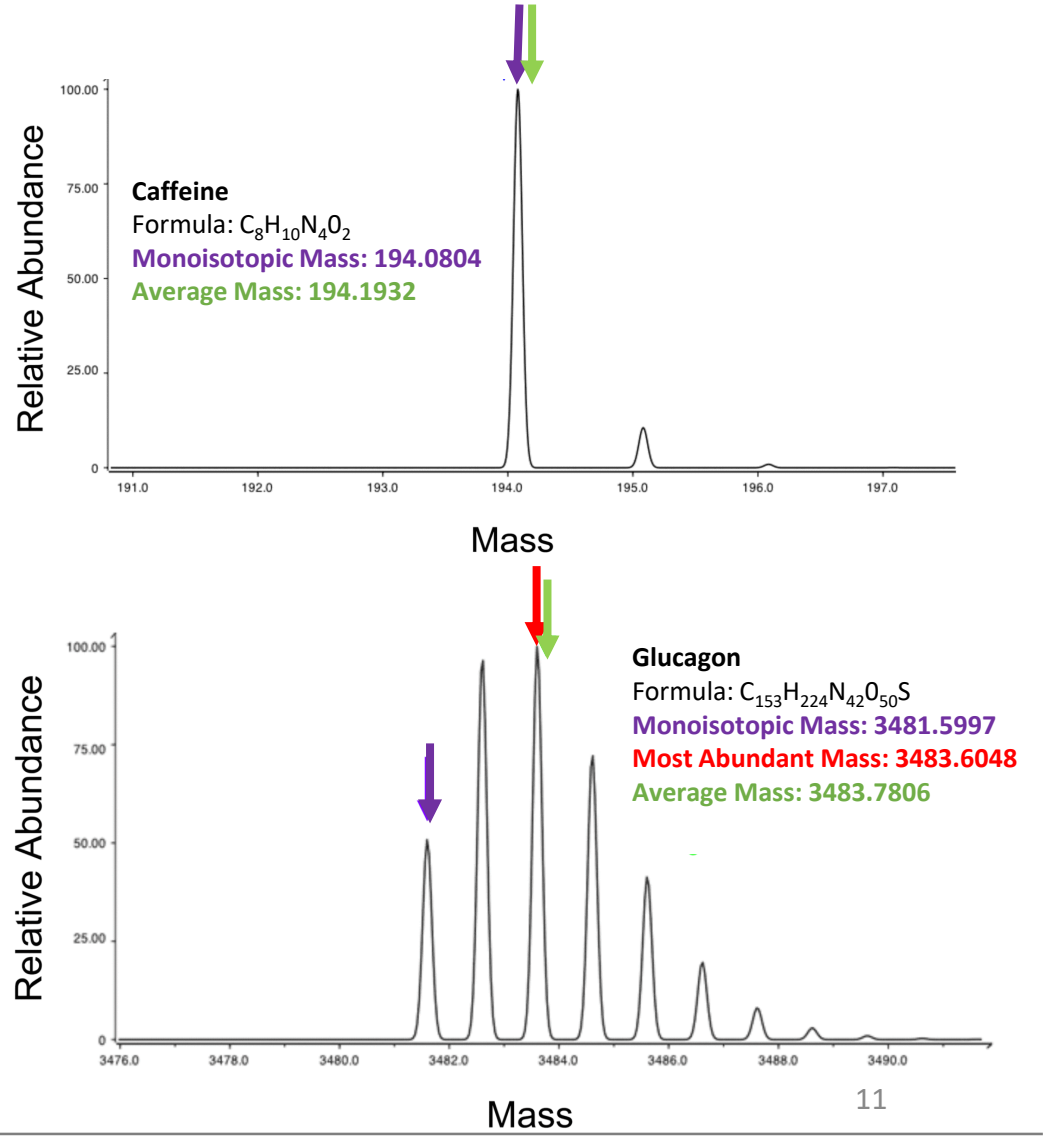
- Low-resolution mass spectra (MALDI)
  - synthetic peptides (QC for Core Facility Peptide Synthesis)
  - offline detection of peptides or proteins
  - oligonucleotides (DNA and RNA)
  - organic compounds – Polymers, Molecular Devices, Helicenes
  - polysaccharides
  - antibody

Up to 12 000 Da

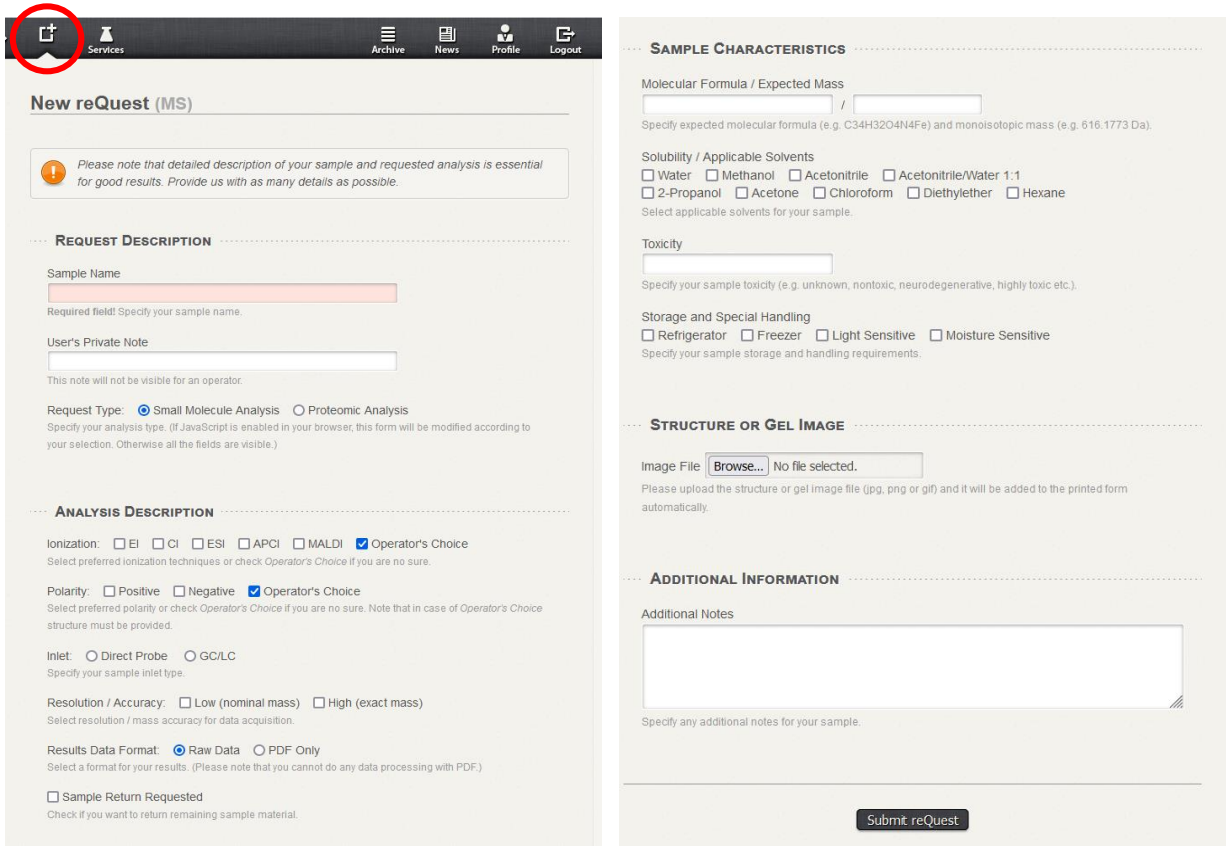
- High-resolution mass spectra (ESI, MALDI)
  - Deconvolution to determine the molecular weight of compounds producing ions in multiple-charged states (ESI)
  - organic compounds – Polymers, Molecular Devices, Helicenes (MALDI)

ReQuest – Sample Submission: Mass

- Monoisotopic mass
  - sum of masses of atoms in molecule using unbound, ground-state, rest mass of principal (most abundant) isotope for each element of the isotopic average mass, instead
    (ex. CO2: 12.0000u + 2 x 15.9949u = 43.9898u)
- Average mass
  - obtained by summing average atomic masses of constituent elements
    (ex. CO2: 12.01u + 2 x 16.00u = 44.01u)
- Most abundant mass
  - mass of molecule with most highly represented isotope distribution, based on natural abundance of isotopes



ReQuest – Sample submission



- **Additional information**
  - **Useful information for us – "old ReQuest" – it does not include all information**

- Sample name must correspond to label on vial (readability, appropriate marker, transparent tape)
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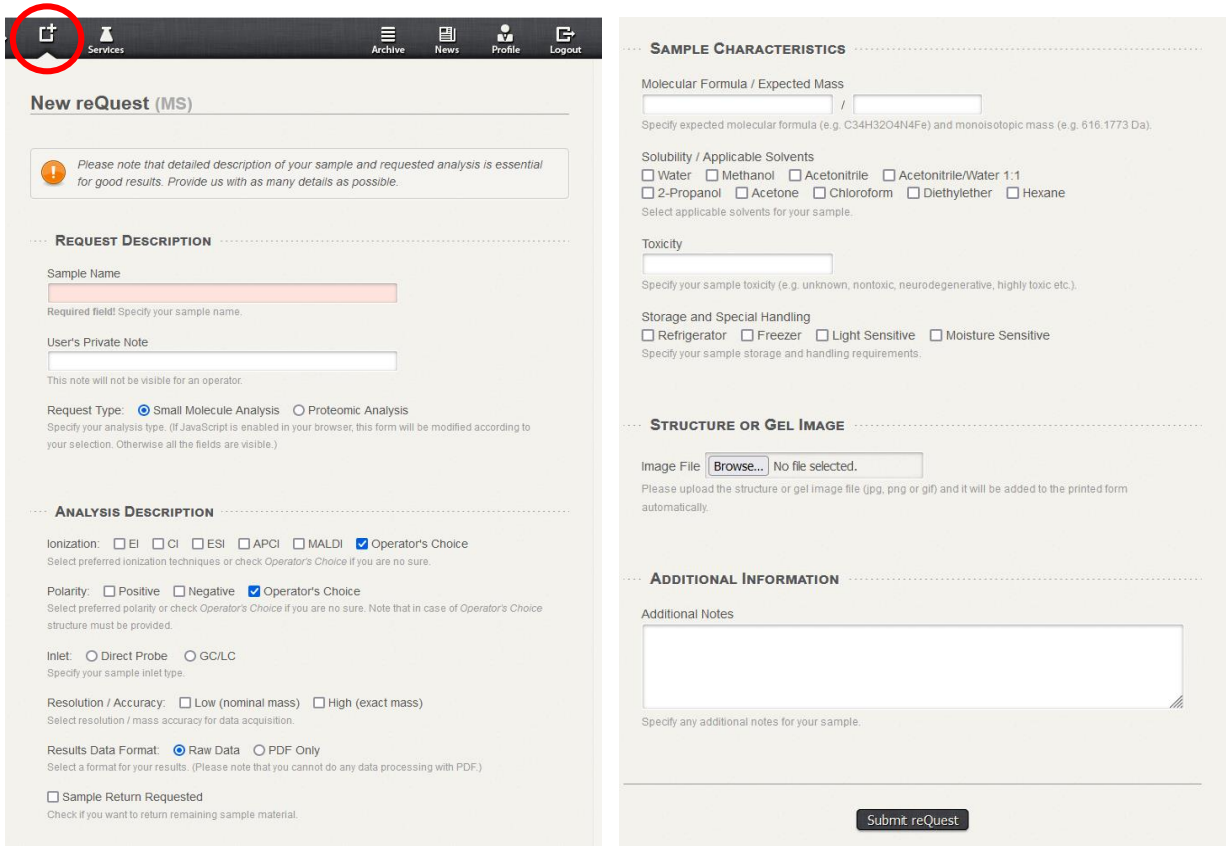
  - HIGH resolution – necessary Formula, Exact mass
  - LOW resolution – Nominal mass or Range of masses
- **Solubility – choose 2 or 3 most suitable solvents**
- **Data format -.pdf, .msd or .mzML (.mzxML)**

ReQuest – Sample submission



- **Additional information**
  - **Useful information for us – "old ReQuest" – it does not include all information**

Ionization	Additional information
ESI	LC/MS, assay of purity sample for Martin Svoboda
MALDI	Protein, without buffer
MALDI	Protein, buffer 10mM HEPES, 100mM NaCl
MALDI	ds DNA oligonucleotide, 10 µL of 10 µM solution in water
MALDI	ss RNA oligonucleotide, 10 µL of 20 µM solution in water
MALDI	Polymer, range of masses 400 - 4000Da
ESI	Pure fraction from extract, range of masses 200-2000
MALDI /ESI	MALDI first, if mass is presented, then ESI

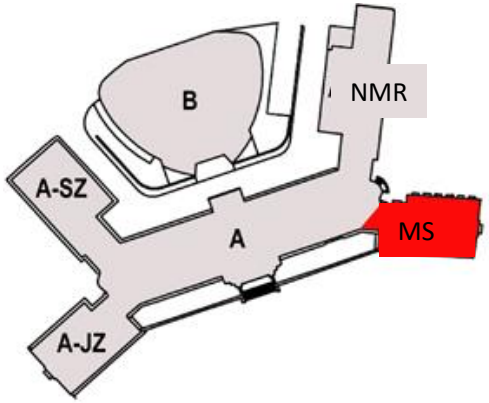
ReQuest – Sample submission: Tubes

- All these sample tubes are suitable for solid samples
- For diluted samples, only glass vials please – especially when samples are diluted in chloroform or dimethyl sulfoxide
- Mass spectrometry is too sensitive to obtain really blank “blank”
- Solvents extract antioxidants and plasticizers from plastic

ionizability of sample is > ionizability of plasticizers = passed

ionizability of sample is < ionizability of plasticizers = failed

ReQuest – Sample Submission

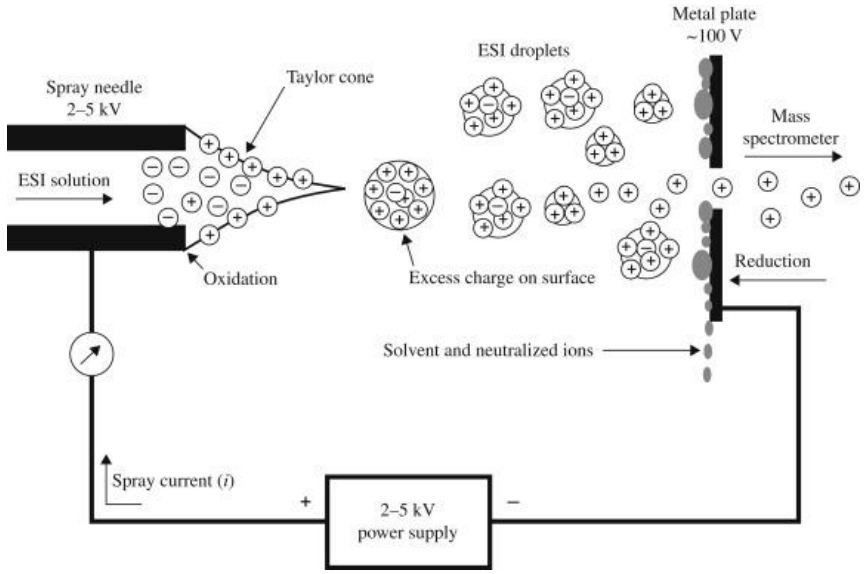


Building A
SE-wing
1st floor

- Printed ReQuest form with the sample
  - if you have one mass and a lot fraction, single paper ReQuest is OK
  - however, each sample must be written under its own ReQuest separately
- Light sensitive sample – cover with aluminum foil or put to refrigerator
- Moisture sensitive sample – cover with parafilm
- Unstable or dilute sample – put to refrigerator or freezer
  - very unstable sample – come and ask for urgent measurement
- Samples are returned to the original place

Samples Requirements – ESI, EI/CI, APCI, APPI

ESI



https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/electrospray-ionization

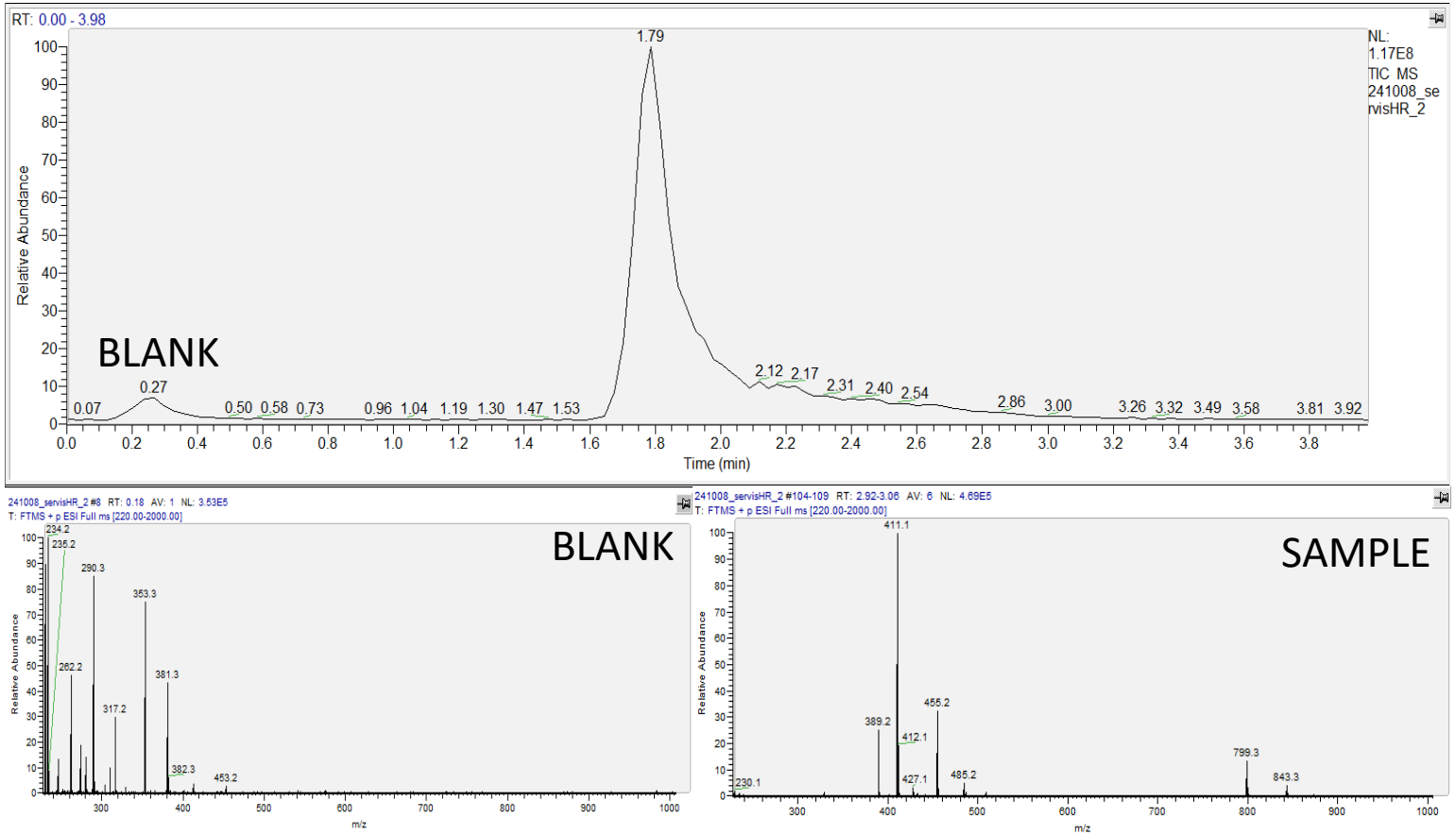
- **Solid samples** – from 0.1 to 1 mg
  - **VISIBLE AMOUNT OF SAMPLE**
  - larger amount of sample when compound contain **nitro group** or azid group, is unstable or light sensitive
- Dilute samples – necessary to enter more information
  - concentration (g/L or mol/L)
  - used solvent – LC/MS purity, avoid strong acid or bases, avoid high boiling points solvent (DMSO, DMF)
    - ESI, APCI – methanol, acetonitrile, water
    - EI/CI – methanol, acetonitrile, hexane, chloroform, dichloromethane
  - used buffers – only volatile
    - avoid phosphates - suppressed signal,and for ESI no TBA and TFA
  - used detergents – only LC/MS compatible type, their concentration

Samples – Salts, Buffers and Other Additives

- Volatile additives can be used in lower concentration (up to 10mM)
  - **formic acid, acetic acid, ammonium acetate, ammonium formate**, TFA (only for MALDI)
- Non-volatile additives – should be avoided because of signal suppression, could create undesired adducts, danger of irreversible machine contamination or could blocked inlet capillary
  - **phosphate, sulfate buffers, SDS, TRIS, TBA,** DMSO, **DMF**

Samples Measurement – ESI/APCI

SAMPLE

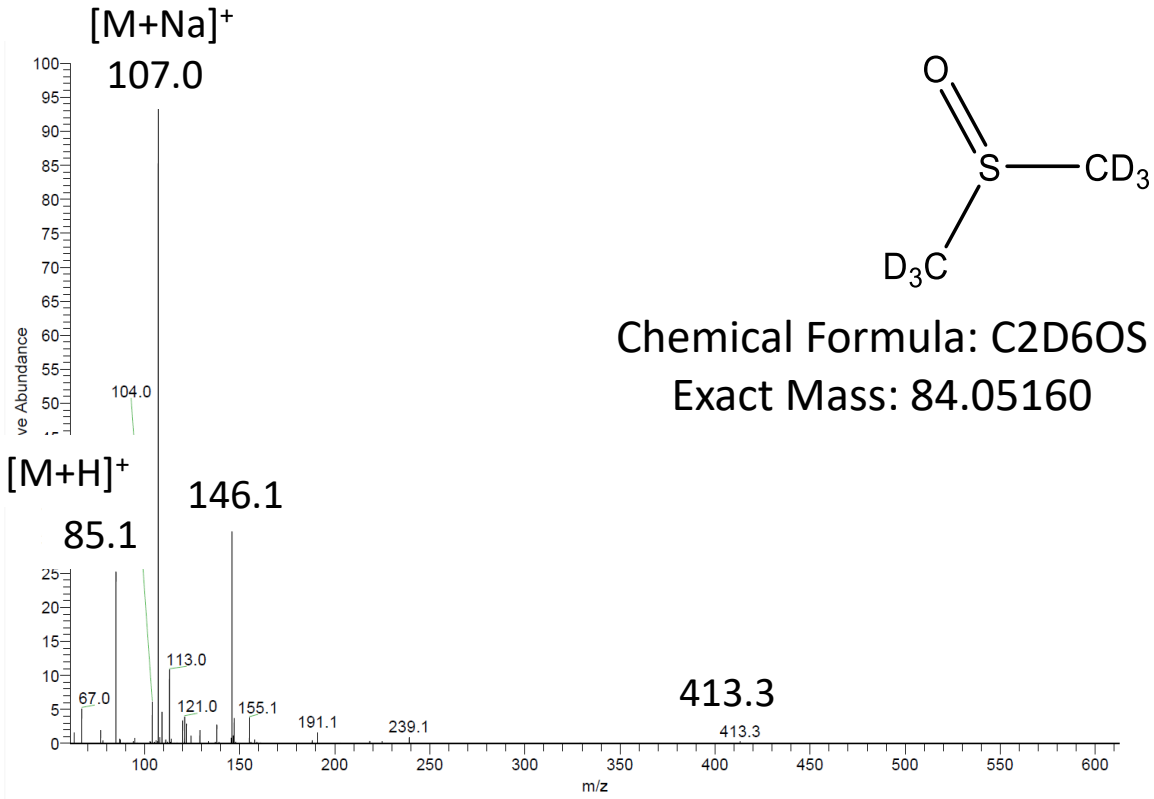


- Direct injection without column
- Injection volume – 10 µL
- Concentration around 0.5 mg/mL
- Isocratic elution
- ESI – mobile phase
  - 80% MeOH, 20%H_2O
  - Problematic for moisture sensitive samples
- APCI – Mobile phase
  - 100% ACN
- Specific analyses

Advantage:

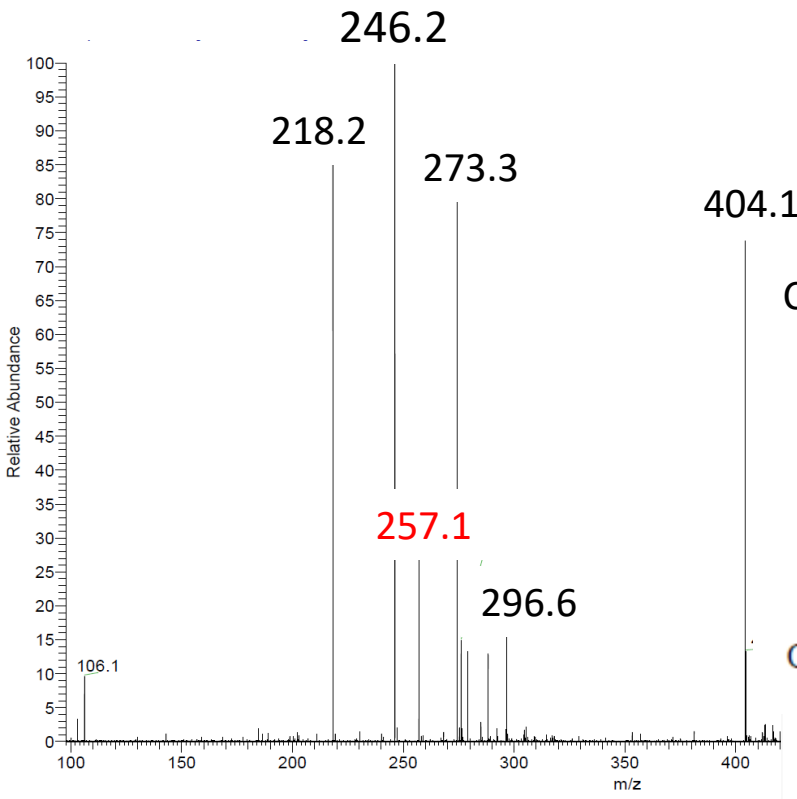
- Subtract spectrum of diluting solvents
- Subtract carry over spectrum of previous over concentrated sample

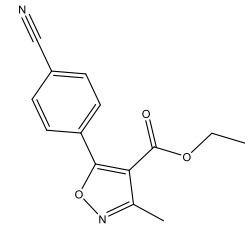
Samples Requirements – DMSO-free Samples



Chemical Formula: C2D6OS
Exact Mass: 84.05160

sample after NMR measurement solvent d_6-DMSO

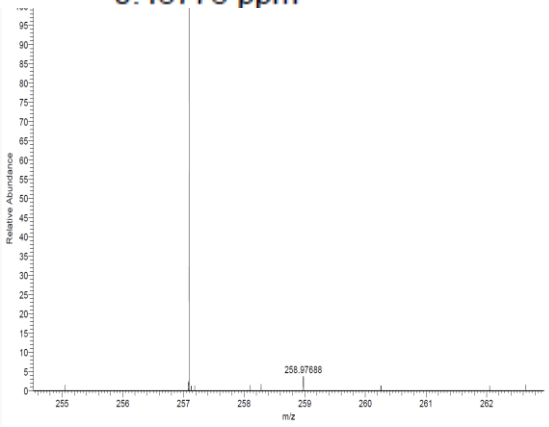




Chemical Formula: $C_{14}H_{12}N_2O_3$
Exact Mass: 256.08479

same sample after lyophilization

257.09219
$C_{14}H_{13}O_3N_2$ = 257.09207
0.48778 ppm

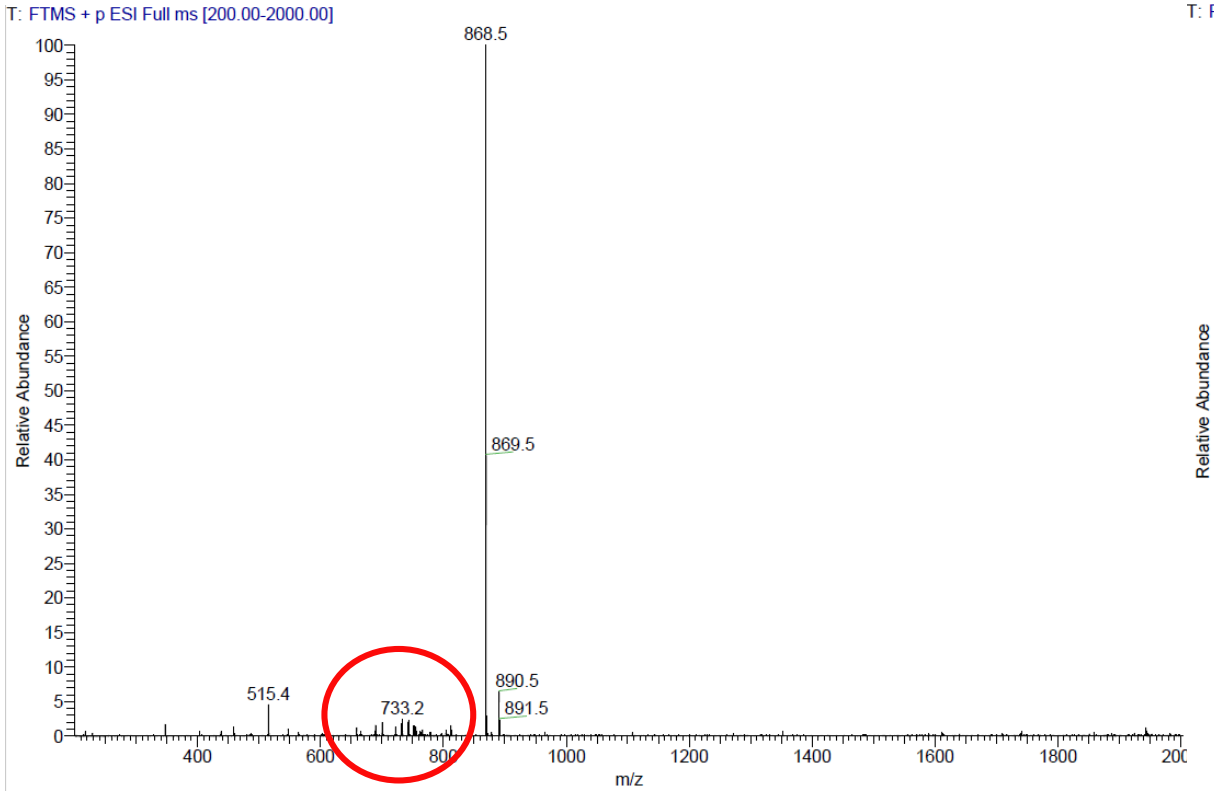


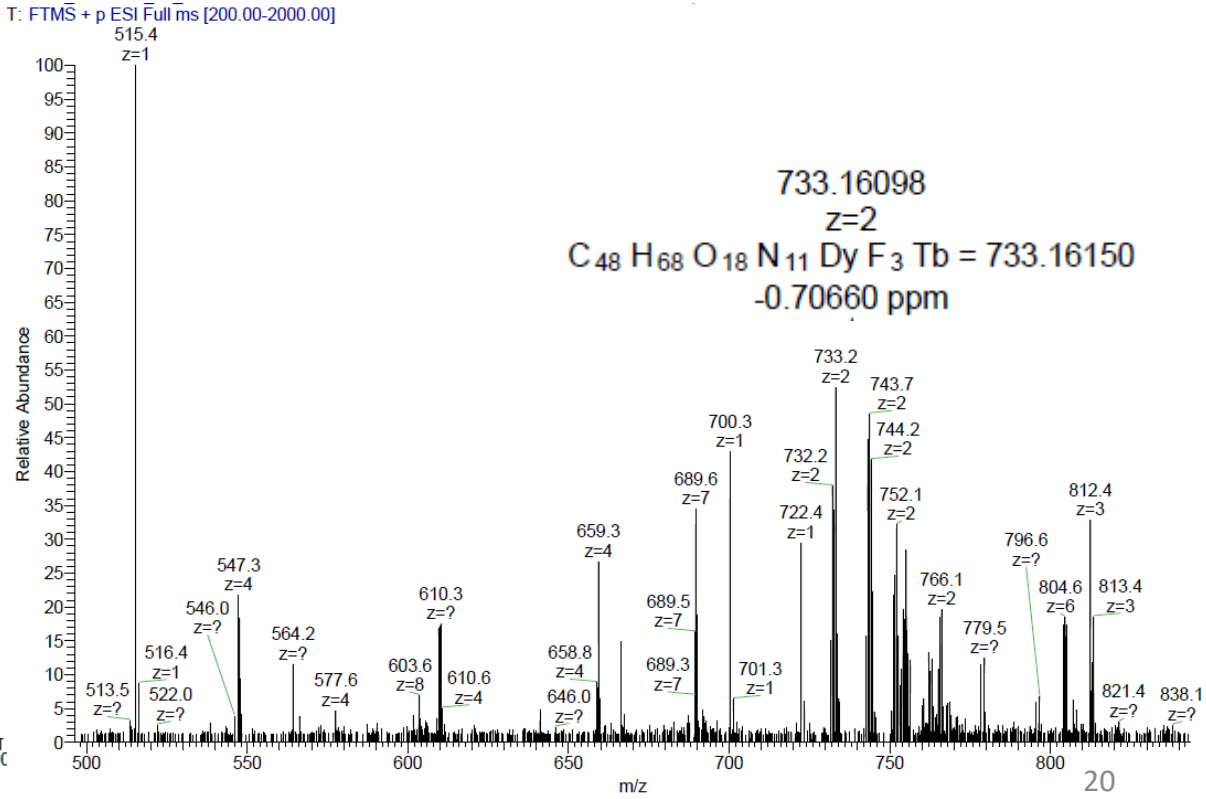
- Supplementary of impacted journal:

MS, *m/z*(%): 258.0 (5, M+H), 257.0 (100, M+H), …- probably not from this analysis
HRMS (ESI+) *m/z* $[M+H]^+$ calcd for $C_{14}H_{13}O_3N_2^+$ 257.09207, found 257.09219

Samples Requirements – Stability in Solution

Formula	$C_{48}H_{66}DyF_3N_{11}O_{18}Tb$
Exact Mass	1464.30845
Additional Notes	Sample (in HPLC vial with insert) in mixture of acetonitrile/water with 0.1% formic acid. 100 µl of sample. concentration approximately 50 µM.





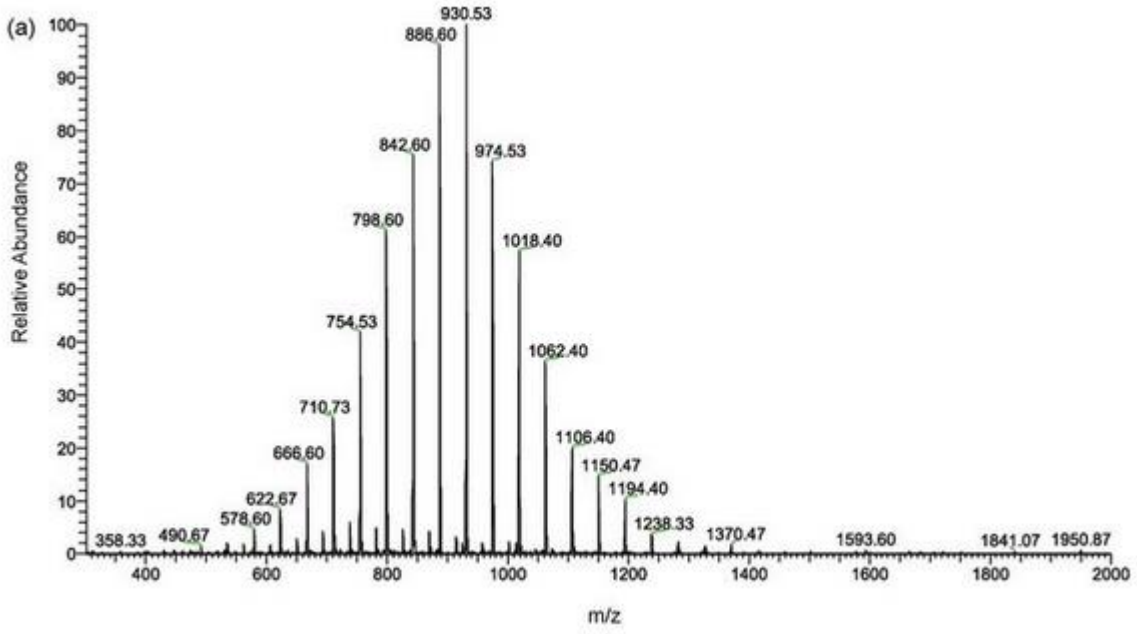
Samples – Common Contaminants

- **Phthalates** – from plastics, contaminated solvents
  - diisobutylphthalate (masses **ESI: 279, 301** , EI: 149, 205, 223, 278)
  - diisooctylphthalate (masses **ESI: 391, 413**, EI: 149, 167, 279, 390)
- **Antioxidants** – from plastics : irganox, irgafos
  - the most common masses **ESI: 663, 685**, EI: 316 ,591, 647, 642
- **Polysiloxans** – from silicone rubber, teflon lined caps from vials
  - the most common masses **ESI: 297, 371, 445, 519**, EI: 73, 147, 221, 295, 355
- **PEGs** – extracted polymer from teflon/silicon septum (+44 series)
- **Amides** – from plastics
  - oleamide **ESI: 282**, stearamide **ESI: 284**, erucamide **ESI: 338, 360**)
- **Detergents** – Triton X-100
- **Fatty acids** – palmitic and oleic acids from skin
  - masses **negESI : 255, 283**

https://www.merckmillipore.com/INTERSHOP/web/WFS/Merck-HK-Site/en_US/-/USD/ShowDocument-Pronet?id=201604.111

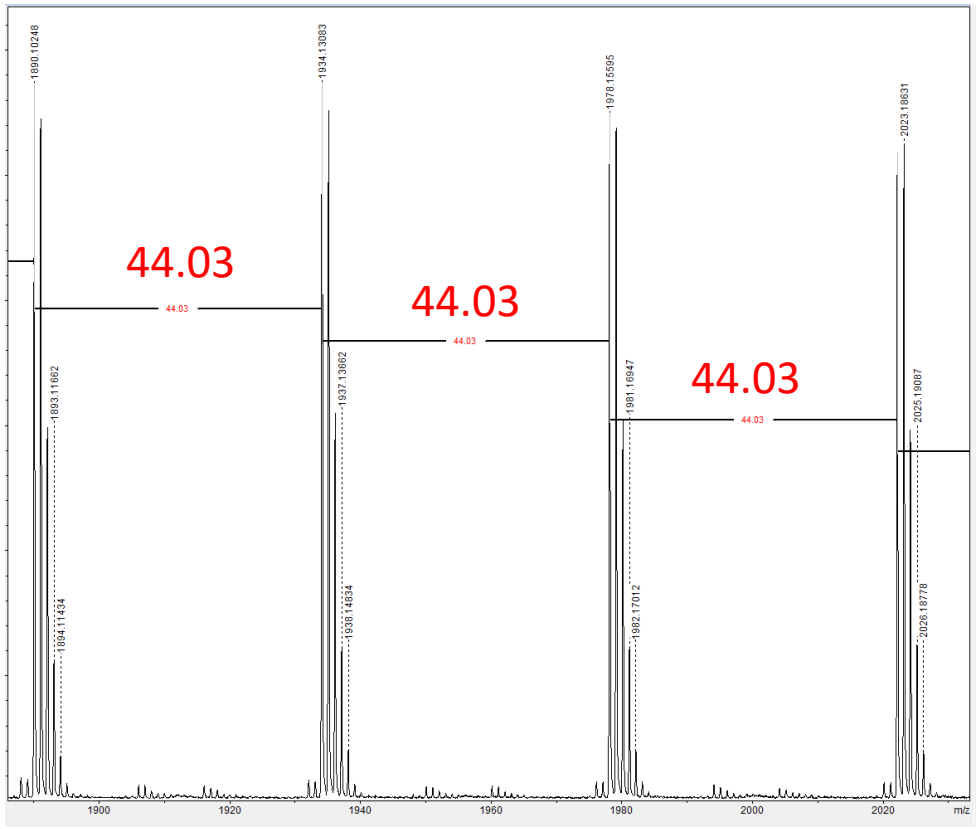
Samples – PEG Contaminants

- **PEGs** – extracted polymer from teflon/silicon septum (+44 series)
- All repeating masses are suspect



https://www.researchgate.net/publication/26785378

-[C2H4O]-; polyethylene glycol, PEG



Exact Mass – Elemental Composition

Mass accuracy: the ratio of the m/z measurement error to true m/z

- Mass accuracy is usually measured in ppm
- For confirmation of expected elemental composition, usually an accuracy of less than 5 ppm is required
  - ESI/EI/CI/APCI – usually less than 2ppm
  - MALDI – less than 5 ppm

$$E_{ppm} = 10^6 \frac{M_{measured} - M_{calculated}}{M_{calculated}}$$

Limits

Charge: 1

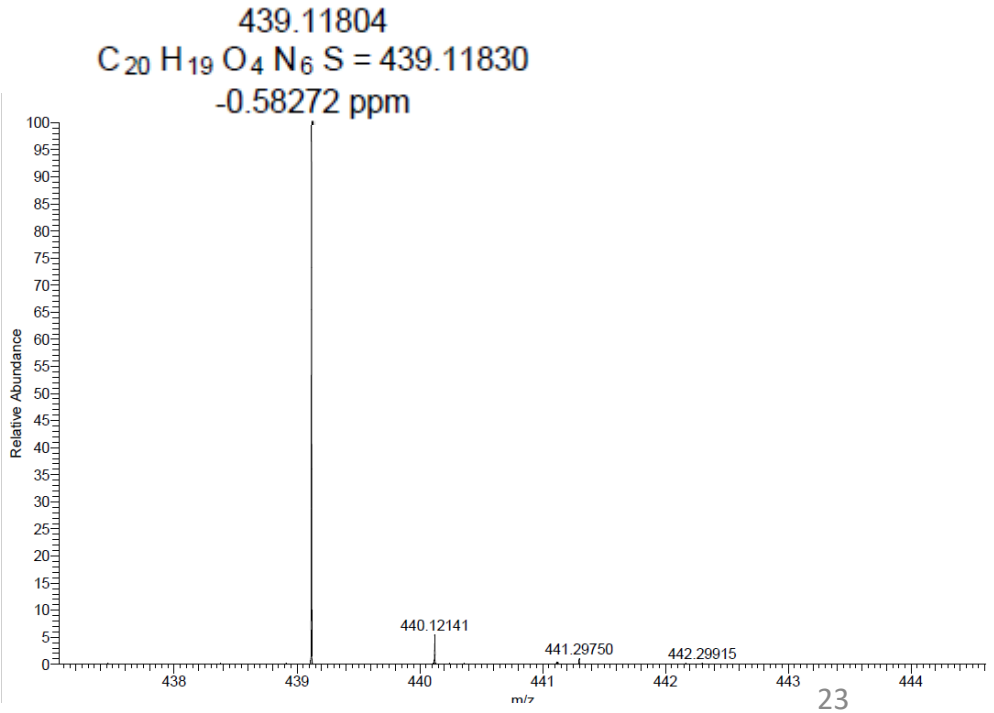
Nitrogen-Rule: Do not use

Mass tolerance: 5.00 ppm

RDB equiv: -1.0-100.0

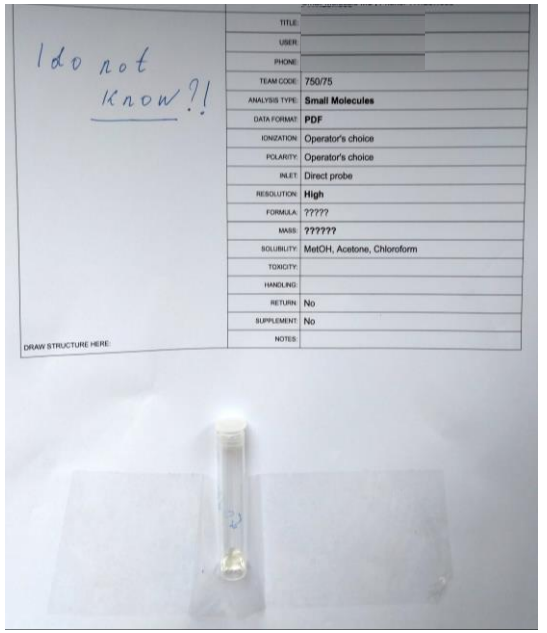
Elements in use

Isotope	Min	Max	DB eq.	Mass
14 N	0	6	0.5	14.003
16 O	0	4	0.0	15.995
12 C	0	20	1.0	12.000
1 H	0	19	-0.5	1.008
32 S	0	1	0.0	31.972

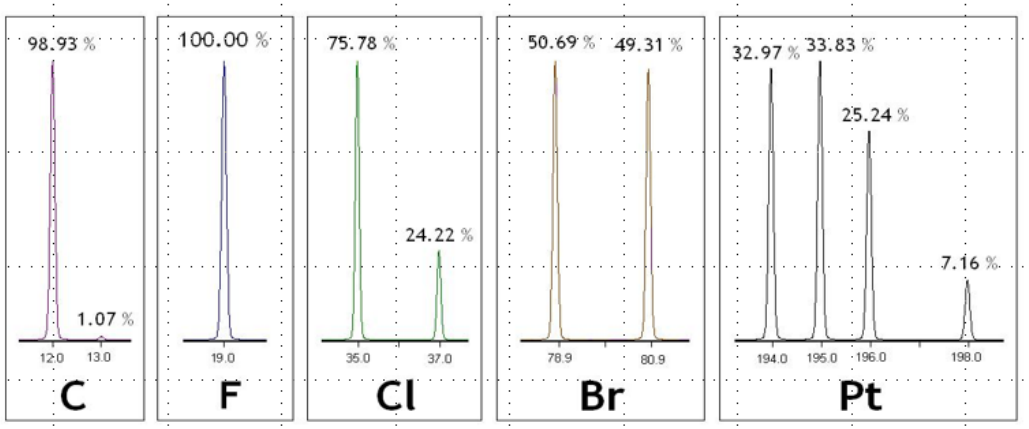


Exact Mass – Elemental Composition

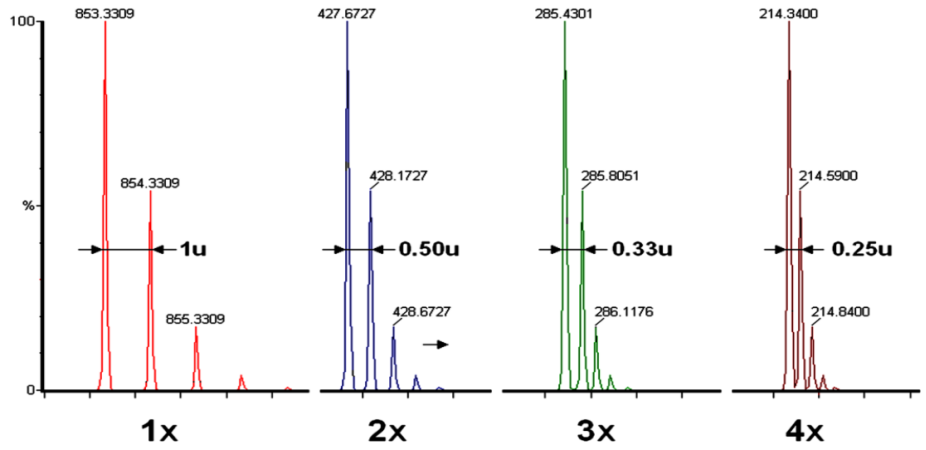
- Unknown sample? We can help!
- Still need as much information as possible
  - which elements could be presented
  - mass range
  - starting material
  - side product - scheme of reaction



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



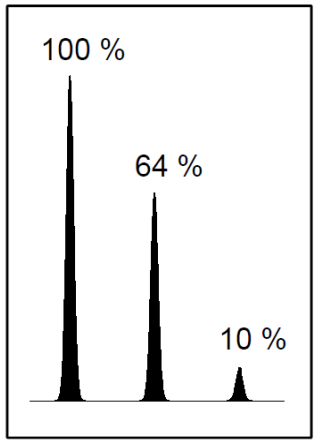
Determining the number of charges

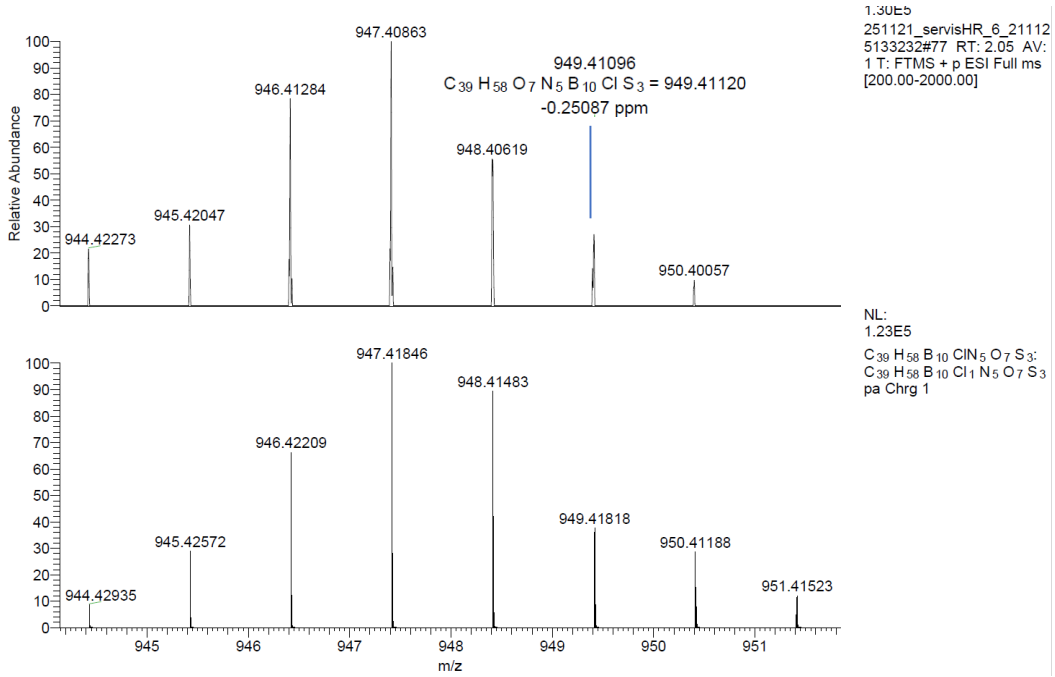


Exact Mass – Elemental Composition

Example: Cl_2

$^{35}Cl^{35}Cl$ (70 Da), P = 1.00 x 1.00 = **1.00**
$^{35}Cl^{37}Cl$ (72 Da), P = 1.00 x 0.32 = 0.32
$^{37}Cl^{35}Cl$ (72 Da), P = 0.32 x 1.00 = 0.32 } **0.64**
$^{37}Cl^{37}Cl$ (74 Da), P = 0.32 x 0.32 = **0.10**





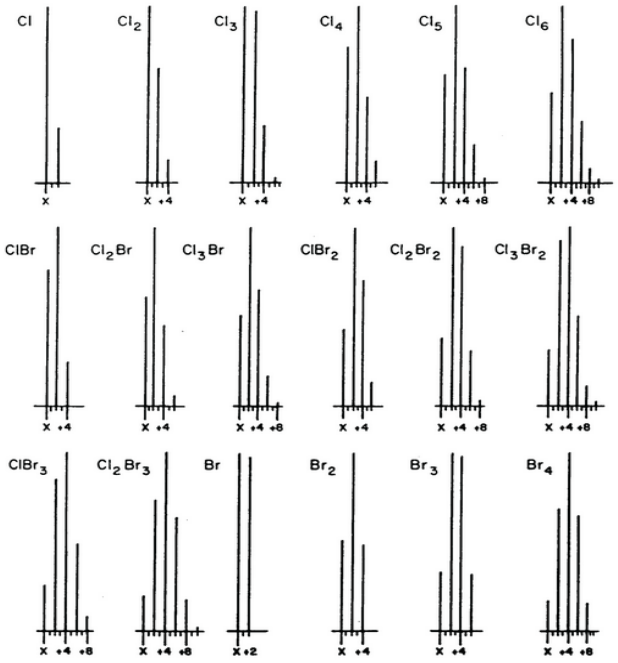
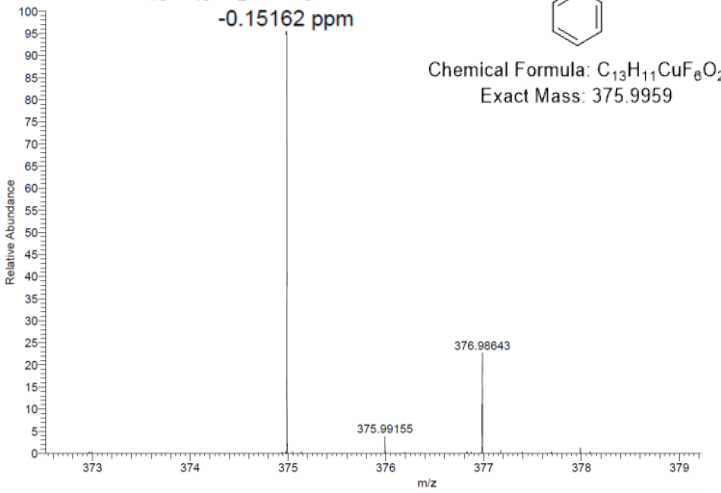
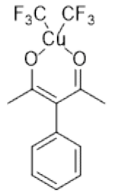


Figure 2: Peak patterns representing ions with atoms of Cl/B



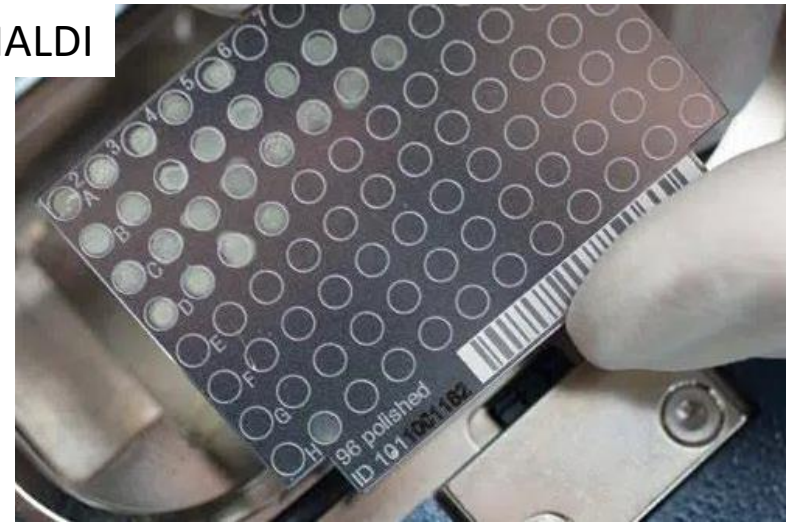


Chemical Formula: $C_{13}H_{11}CuF_6O_2$
Exact Mass: 375.9959

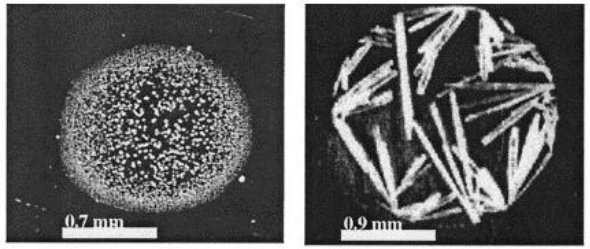
- Isotopic clusters indicate presence of some elements (e.g., Cl, Br, some metals etc.).
- We love isotopic clusters, so we provide prediction, if something unusual appears

Samples Requirements – MALDI

MALDI



Zoom of spots



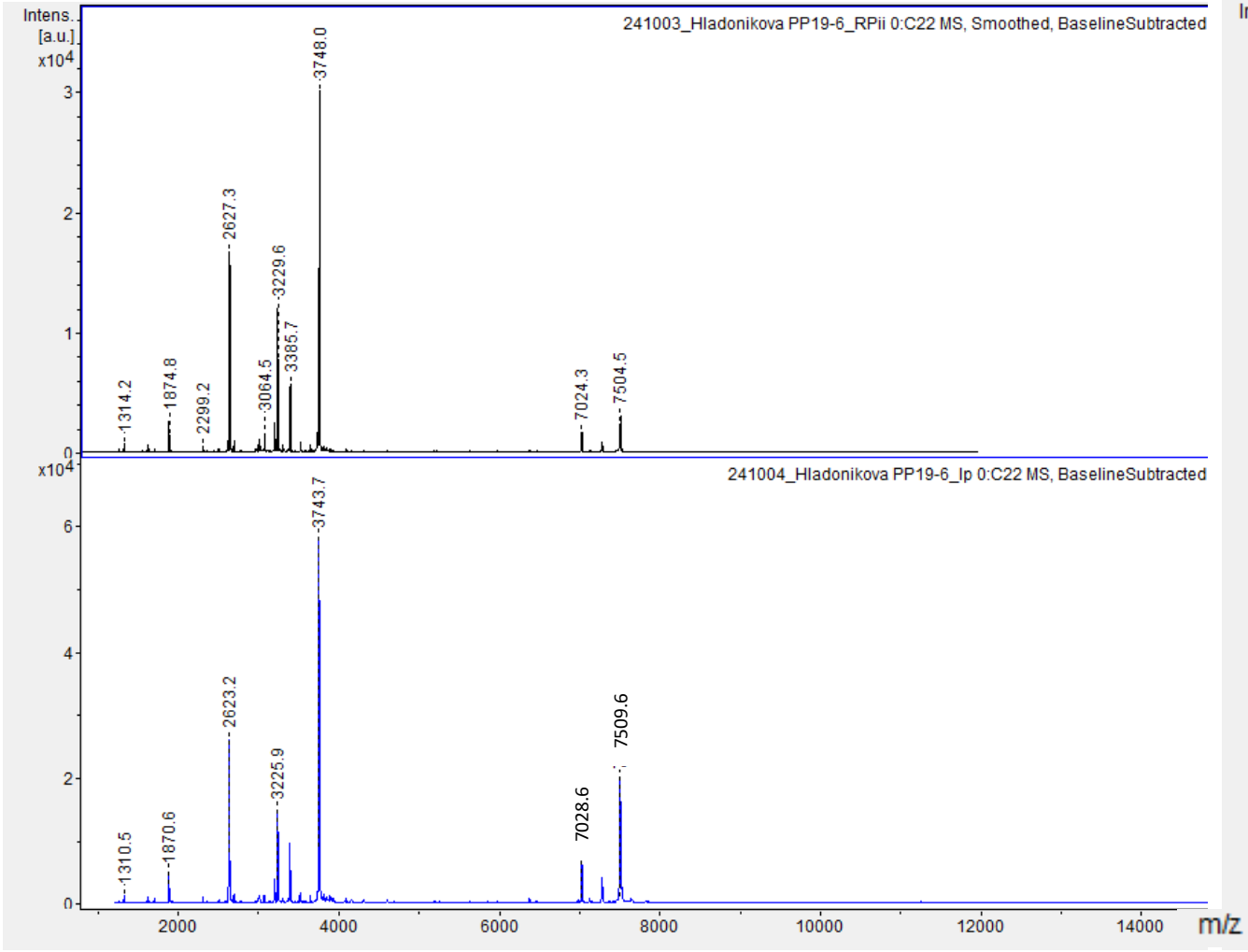
(a) CHCA (b) DHB

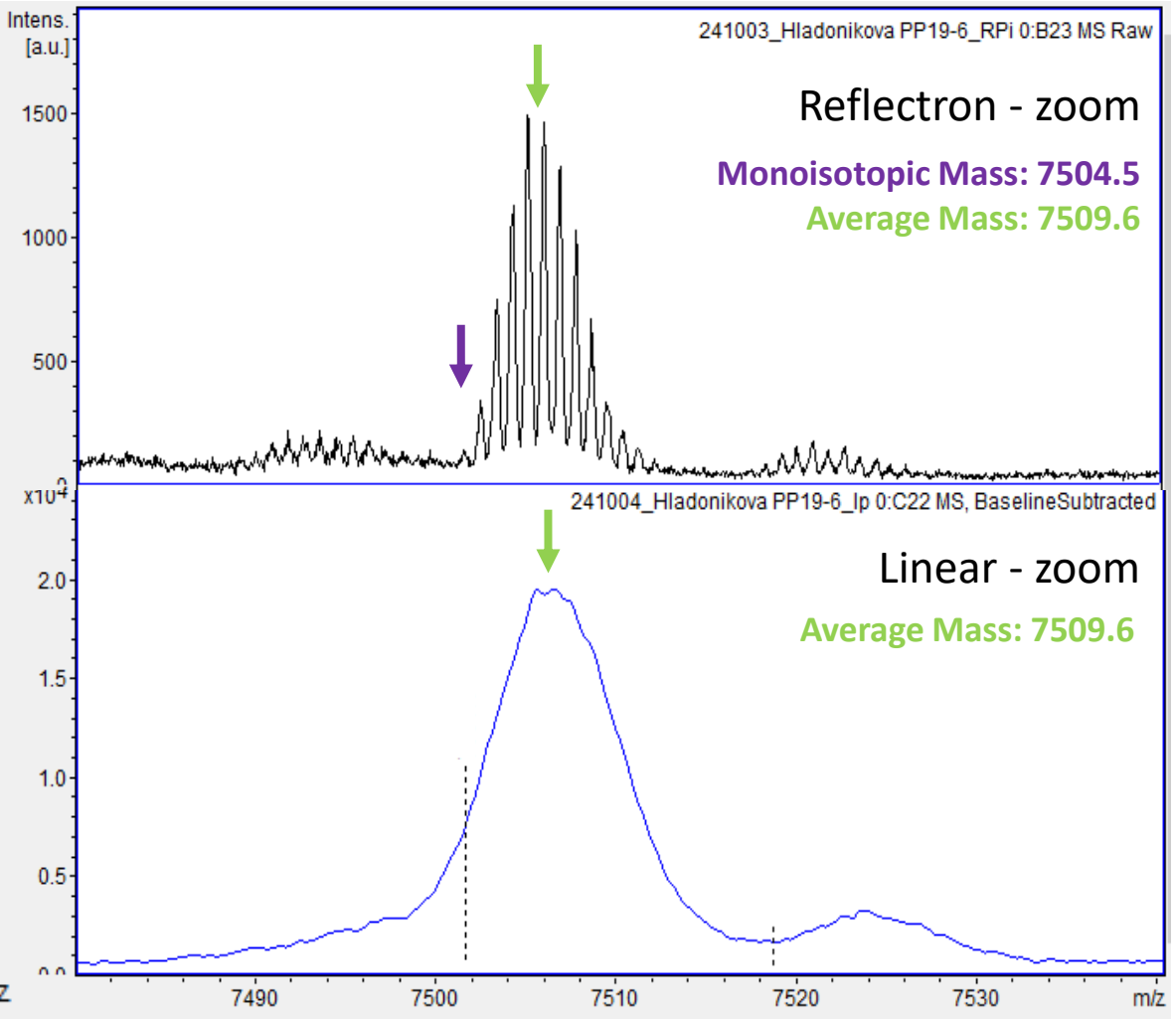
https://www.qlaboratories.com
https://doi.org/10.1016/S1044-0305(03)00262-9

- Solid samples – from 0.1 to 1 mg
- Dilute samples – necessary to enter more information
  - Concentration (g/L or mol/L)
    - Up to 10kDa – concentration 10µM or more
    - Up to 20kDa – concentration 20µM or more
  - Used solvent
    - oligonucleotide only water
    - peptide/protein – 50% acetonitrile with 0.1% FA
    - avoid strong acid or bases
    - avoid high boiling points solvent (DMSO, DMF) – bad crystallization
  - Used buffers (only volatile)
    - phosphate buffers suppress signal
  - Used detergents (type, concentration)

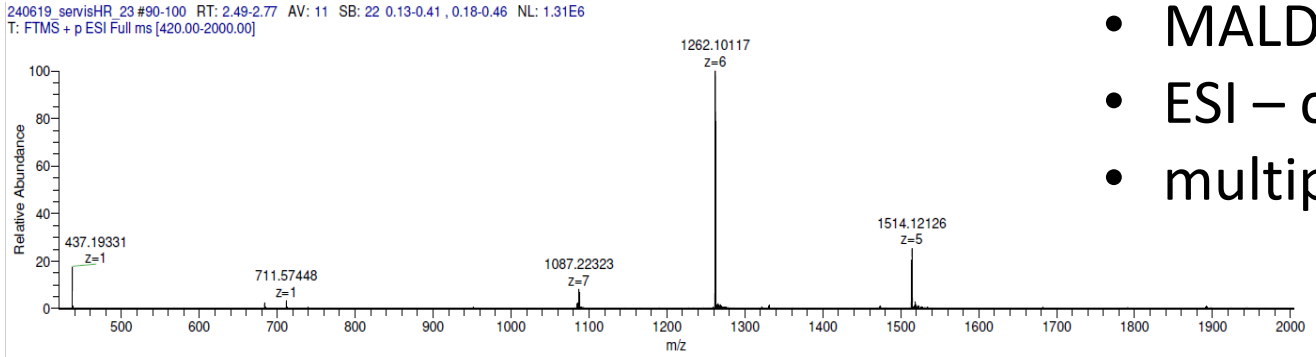
Samples Measurement – MALDI

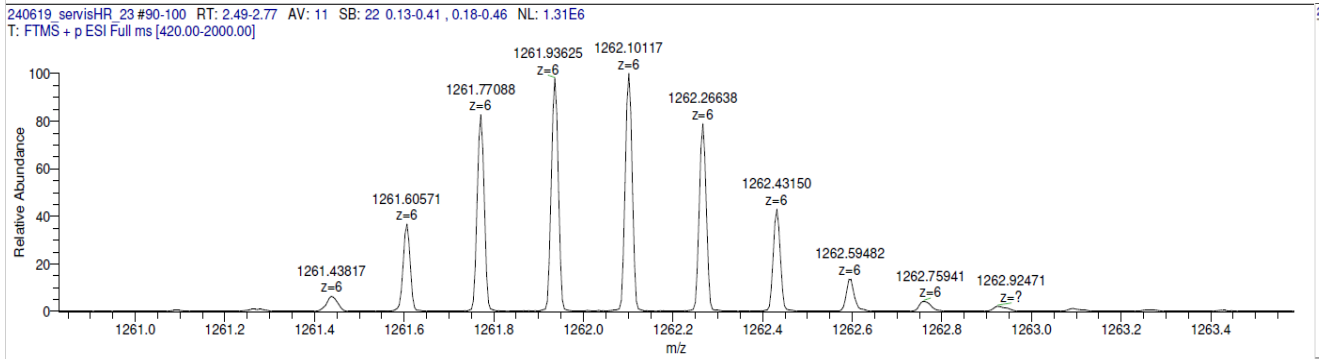
- Insulin – reflectron versus linear mode

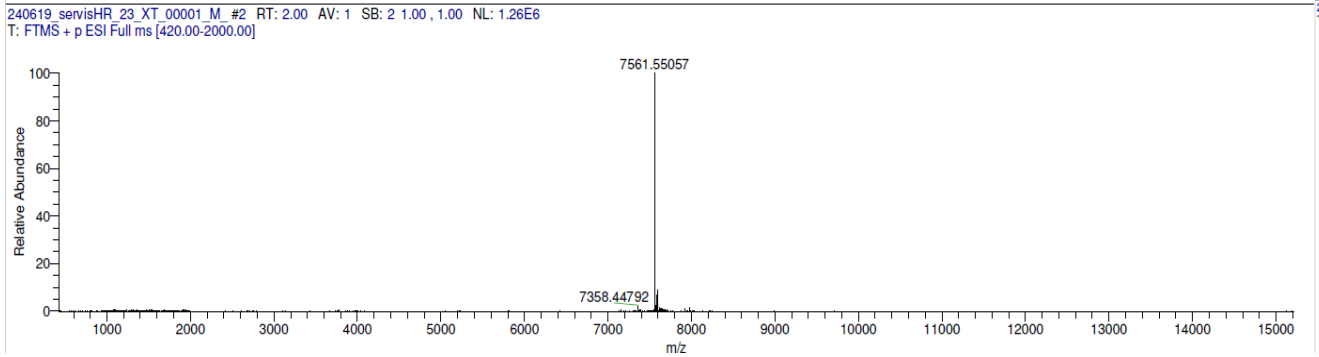


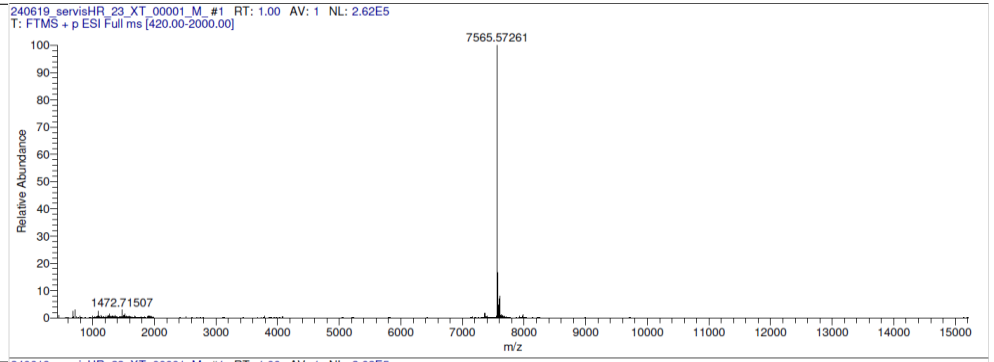


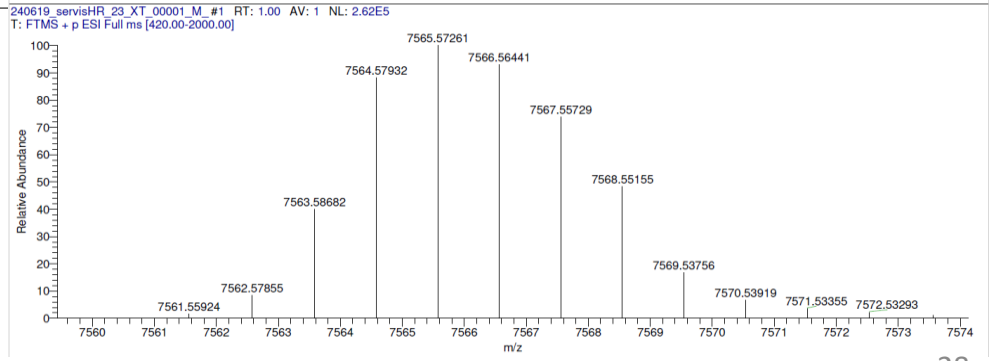
Samples Measurement – ESI – Deconvolution





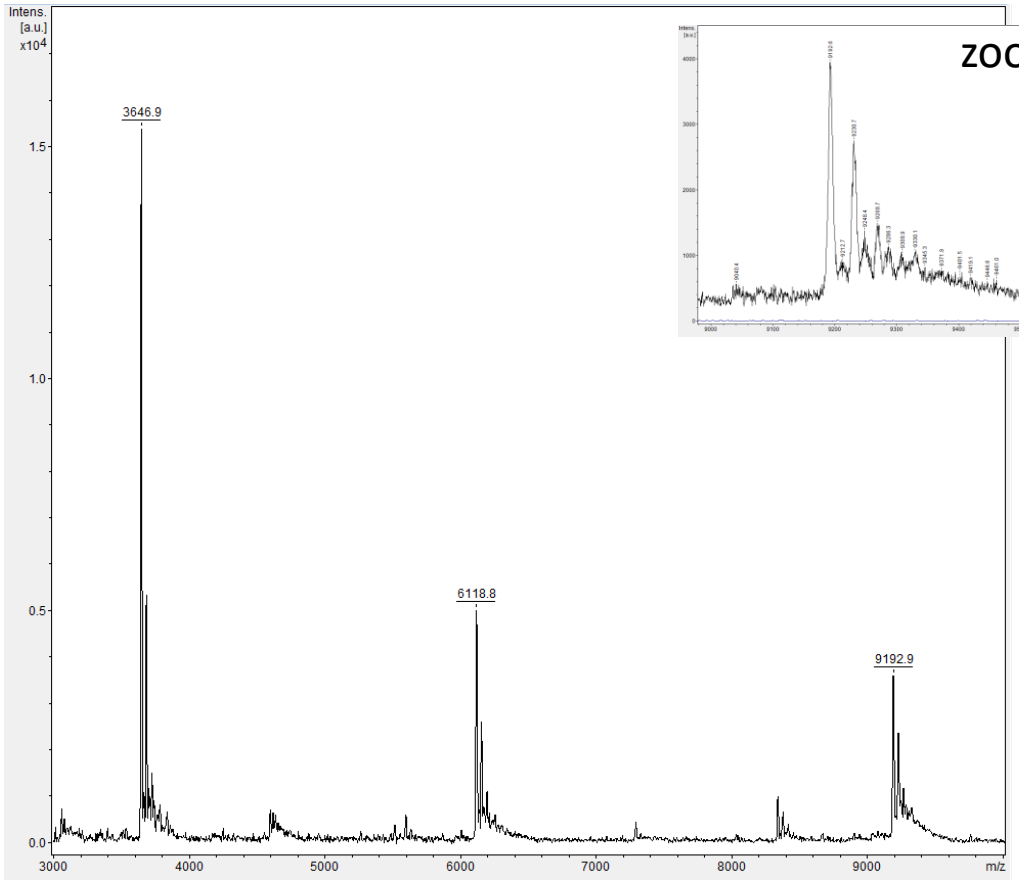






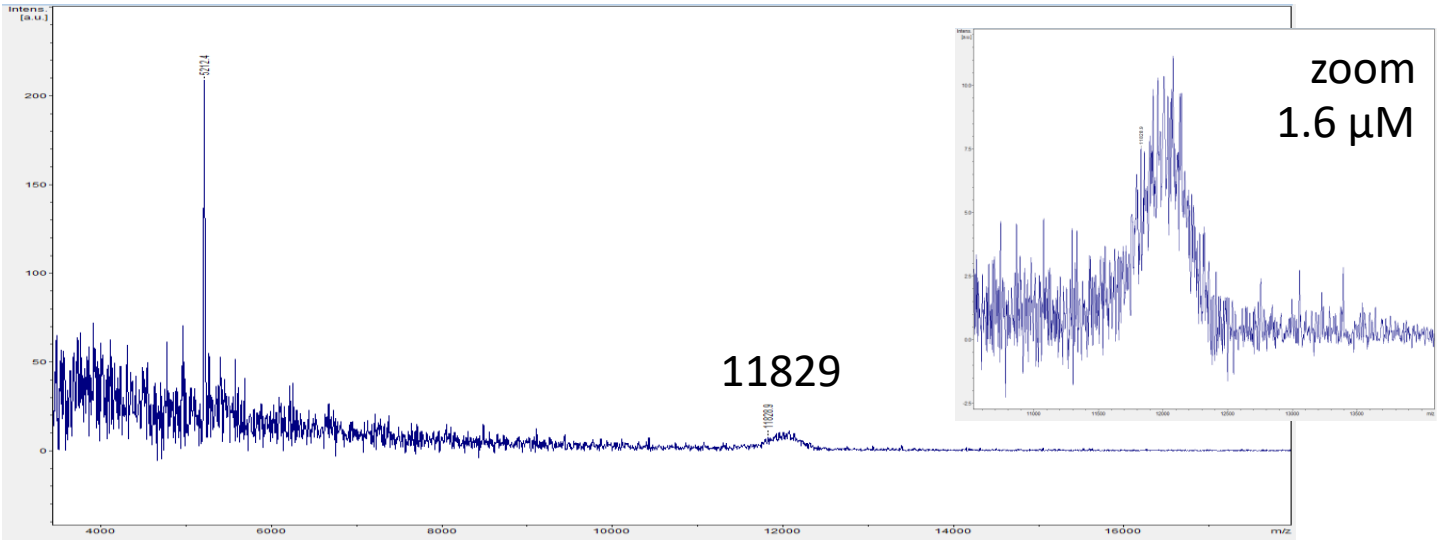
- MALDI – around 8000Da error ± 8Da
- ESI – confirmation of exact mass of insulins
- multiply charged positive ions – deconvolution

MALDI – Oligonucleotides



Oligo Standard

Oligonucleotide	[M+H]+ Average	[M] Average
Oligo 12 (12mer) ACG TAC GTA CGT	3646.4	3645.4
Oligo 20 (20mer) ACG TAC GTA CGT ACG TAC GT	6118.0	6117.0
Oligo 30 (30mer) ACG TAC GTA CGT ACG TAC GTA CGT ACG TAC	9192.0	9191.0



Real sample – low concentrated, buffers

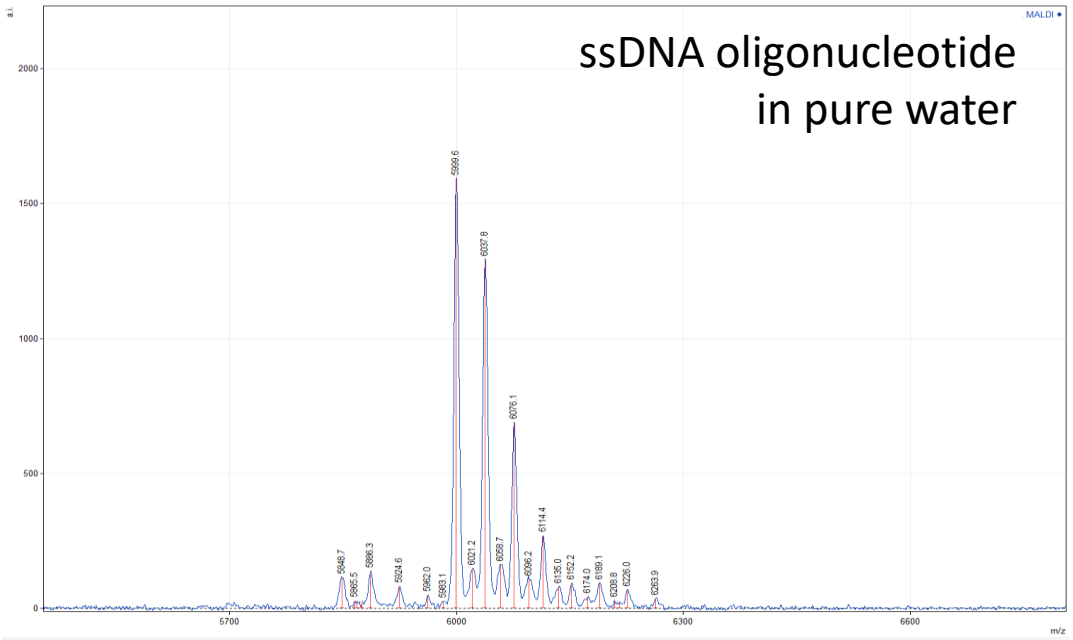
Concentration
Oligo 12 = **0.25** µM, Oligo 20 = **1.25** µM and Oligo 30 = **5** µM
oligo 30 – 20x more than Oligo 12, 1/3 signal

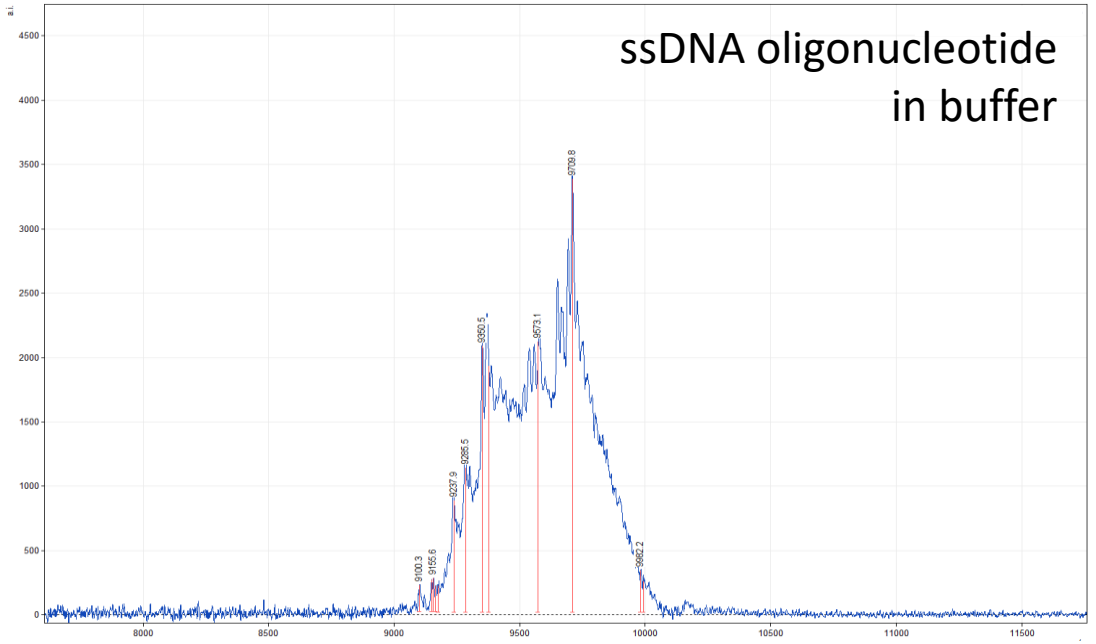
- Requirement

DNA oligo up to 10 000Da – **10 µM**
RNA oligo and DNA oligo up to 20 000Da – **20 µM**

MALDI – Oligonucleotides – Influence of Buffers

- oligonucleotide samples – dried or solution in water
- desalting is crucial in sample preparation
- MALDI in positive – higher response than negative (opposite to ESI)
- DNA oligonucleotides higher intensity than RNA oligonucleotides
- Limits
  - largest oligo seen at our MALDI instrument – 50kDa (rarely), standard up to 20kDa
  - covalent bond of Protein and DNA





How Often Do We Measure?

If instruments work

- **ESI positive** and **EI** – every day
  – time to obtain results: 0-3 days (depending on queue length)
  - capacity of 30 samples per day per instrument
- **ESI negative** – each other day
- **APCI** and **CI** – need exchange probe – less samples – usually once a week
- **MALDI** of organic compounds and peptides – every day
  – time to obtain results: 0-3 days
  - support Peptide Core facilities
  - capacity – approximately 20 samples per day
- **MALDI** of oligonucleotides and polymers – each other day
  - capacity – approximately 10 samples per day

Conclusion

- We really need new system for submitting of samples – ASAP
  - until then – use additional notes
- Sample information is always useful – especially when:
  - analyzing this type of compound for the first time
  - it has not been successful in previous analysis
  - choosing appropriate method for your sample
- Remember the physical principle of this method
  - avoid non-volatile solvents, buffers and additives
- We do our best to provide satisfactory results
- Support Core Facilities
- Feedback make things better

Thank you for your attention!