



Sample analysis, tips and tricks



ReQuest - Future

IOCB reQuest - Login

E-mail

Login

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<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

Sign in to Request 2
Or register a new account if you don't have one yet

E-mail address

Password

[Forgot your password?](#)

Log in

Edita Kofronova
View profile

Send feedback in one click

My requests Client's requests Announcements Teams Users

CREATE NEW REQUEST

Small molecules analysis CREATE

The analysis covers (i) measurement of full-scan nominal-resolution mass spectra of compounds using ESI, E/CI, APCI, or MALDI; (ii) measurement of full-scan high-resolution spectra using the same ionization methods as above to confirm expected elemental composition or suggest elemental compositions for unknowns (mass accuracy 5 ppm or less).

Quantitative analysis of small molecules CREATE

The aim of the analysis is the targeted detection and quantification of small molecules (approximately up to 2000 Da). Sensitive detection of the analytes is achieved by the measurement of compound-specific fragment ions (MRM transitions). The amount (concentration) of the analytes is determined using either a calibration curve with an internal standard or a standard addition method.

Biomacromolecules CREATE

The aim of the analysis is to acquire mass spectra of intact biopolymers like peptides, proteins, nucleic acid, polysaccharides, etc., using MALDI or ESI.

Proteomics analysis CREATE

The main focus of the analysis is the identification of proteins, their post-translational modifications, and/or quantification of proteins either via label-free or labeling strategy. The workflows are predominantly based on proteolytic digestion and analysis of resulting peptides.

Lipidomics analysis CREATE

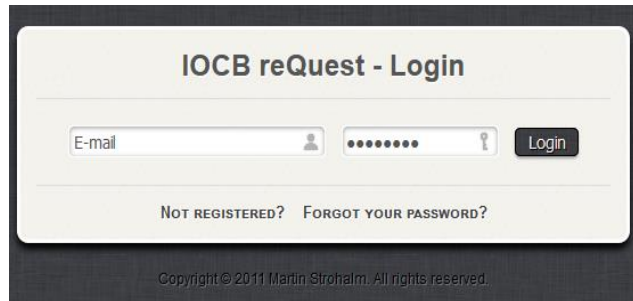
Untargeted lipidomics analysis begins with liquid-liquid extraction of raw biological material (biofluids, cells, tissues, etc.) and is based on liquid chromatography coupled to high-resolution mass spectrometry. The experiments are designed to acquire data on many individual lipid species and compare their relative abundances between experimental conditions (wild type vs. knockout etc.).

Mass spectrometry imaging CREATE

Mass spectrometry imaging (MSI) is used to evaluate the spatial distribution of compounds in tissue sections.



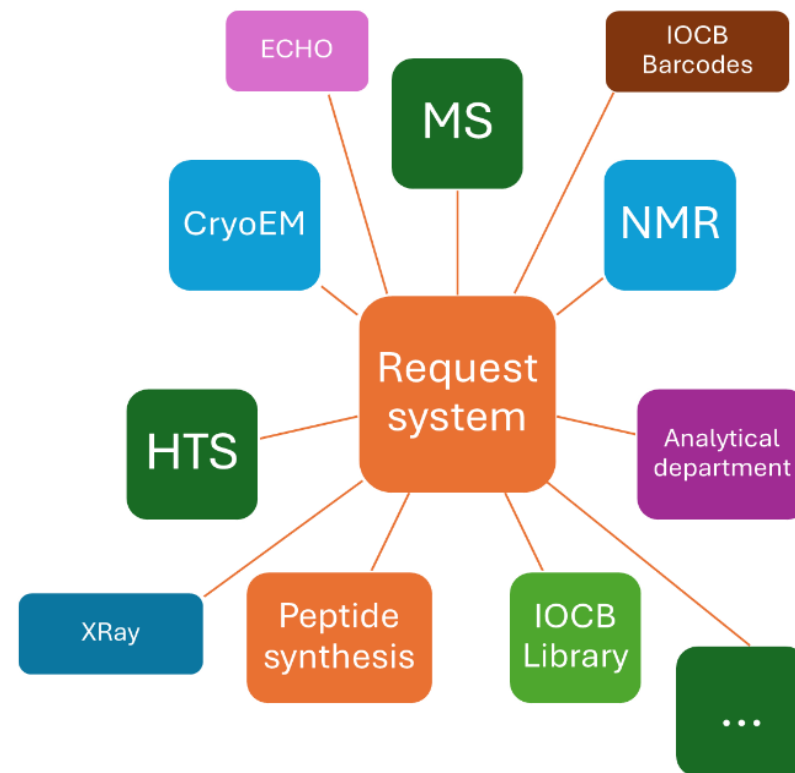
ReQuest - Future



<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 reQuest - Login

E-mail

[NOT REGISTERED?](#) [FORGOT YOUR PASSWORD?](#)

IOCB reQuest - Registration

PERSONAL INFORMATION

Name / Surname /
Required field!

E-mail
Required field! Your email will be used as your login.

Password
Required field!

Phone Number
Required field!

☒ E-mail Notifications
Check if you'd like to be notified on every change of your reQuests.

INSTITUTION

Institution Name
Required field! Official institution name.

Institutional Team Code
Required field!

Address
Required field! Specify street name and number.

ZIP Code - City -
Required field!


Country
Required field!

IČ / DIČ /
Billing information.


- 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

 **Services** **Archive** **News** **Profile** **Logout**

New reQuest (MS)

 Please note that detailed description of your sample and requested analysis is essential for good results. Provide us with as many details as possible.

REQUEST DESCRIPTION

Sample Name

Required field! Specify your sample name.

User's Private Note

This note will not be visible for an operator.

Request Type: ☒ Small Molecule Analysis ☐ Proteomic Analysis

Specify your analysis type. (If JavaScript is enabled in your browser, this form will be modified according to your selection. Otherwise all the fields are visible.)

ANALYSIS DESCRIPTION

Ionization: ☐ EI ☐ CI ☐ ESI ☐ APCI ☐ MALDI ☒ Operator's Choice

Select preferred ionization techniques or check Operator's Choice if you are no sure.

Polarity: ☐ Positive ☐ Negative ☒ Operator's Choice

Select preferred polarity or check Operator's Choice if you are no sure. Note that in case of Operator's Choice structure must be provided.

Inlet: ☐ Direct Probe ☐ GC/LC

Specify your sample inlet type.

Resolution / Accuracy: ☐ Low (nominal mass) ☐ High (exact mass)

Select resolution / mass accuracy for data acquisition.

Results Data Format: ☒ Raw Data ☐ PDF Only

Select a format for your results. (Please note that you cannot do any data processing with PDF.)

☐ Sample Return Requested

Check if you want to return remaining sample material.

SAMPLE CHARACTERISTICS

Molecular Formula / Expected Mass /

Specify expected molecular formula (e.g. C₃₄H₃₂O₄N₄Fe) and monoisotopic mass (e.g. 616.1773 Da).

Solubility / Applicable Solvents

☐ Water ☐ Methanol ☐ Acetonitrile ☐ Acetonitrile/Water 1:1
☐ 2-Propanol ☐ Acetone ☐ Chloroform ☐ Diethylether ☐ Hexane

Select applicable solvents for your sample.

Toxicity

Specify your sample toxicity (e.g. unknown, nontoxic, neurodegenerative, highly toxic etc.).

Storage and Special Handling

☐ Refrigerator ☐ Freezer ☐ Light Sensitive ☐ Moisture Sensitive

Specify your sample storage and handling requirements.

STRUCTURE OR GEL IMAGE

Image File No file selected.

Please upload the structure or gel image file (jpg, png or gif) and it will be added to the printed form automatically.

ADDITIONAL INFORMATION

Additional Notes

Specify any additional notes for your sample.

- 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



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- **Data format**

- **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



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Mass Spectra of Molecules

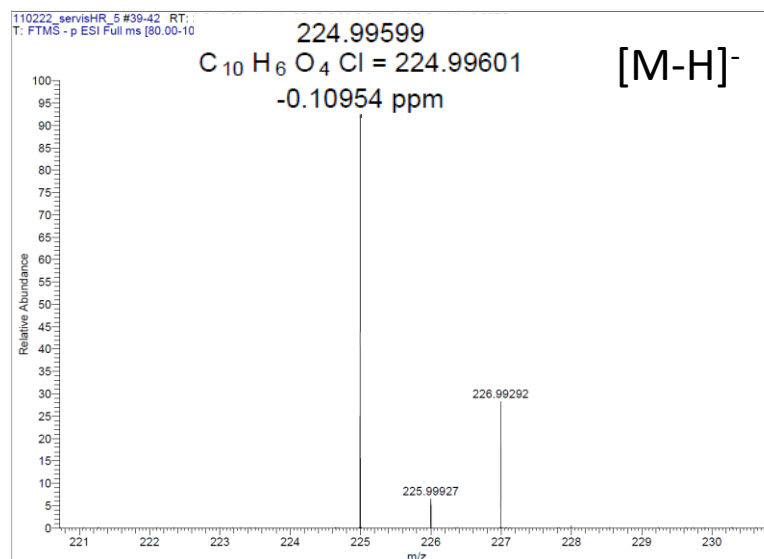
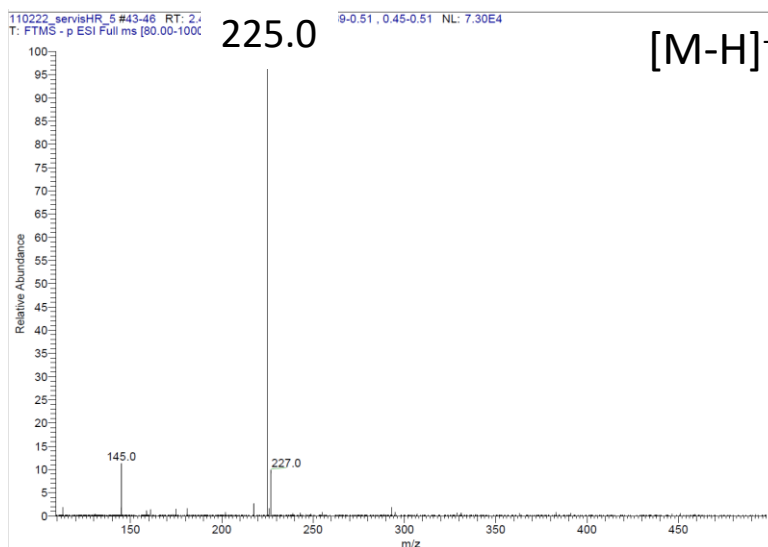
Up to 2000 Da

- 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

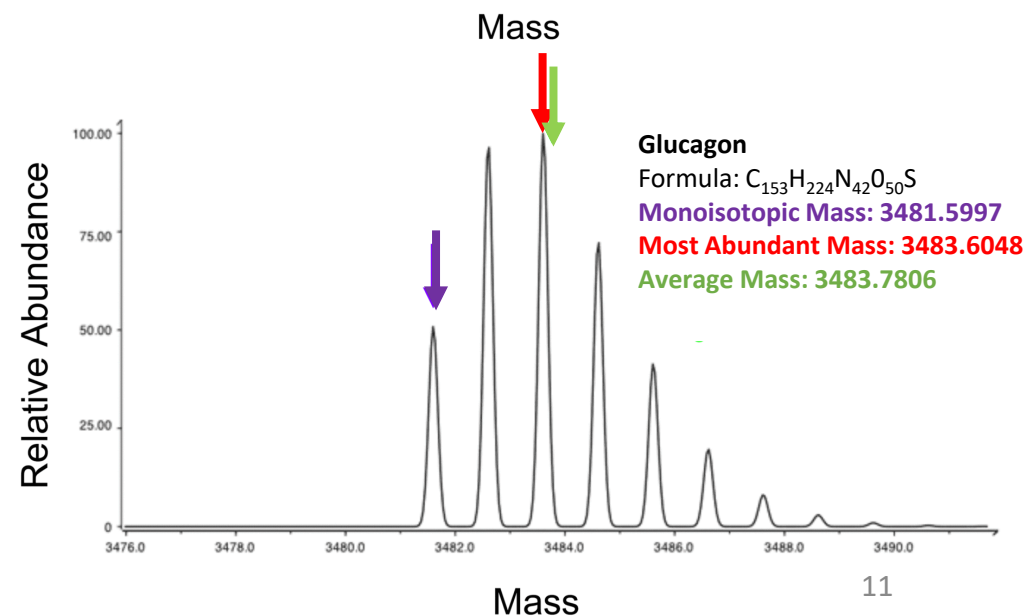
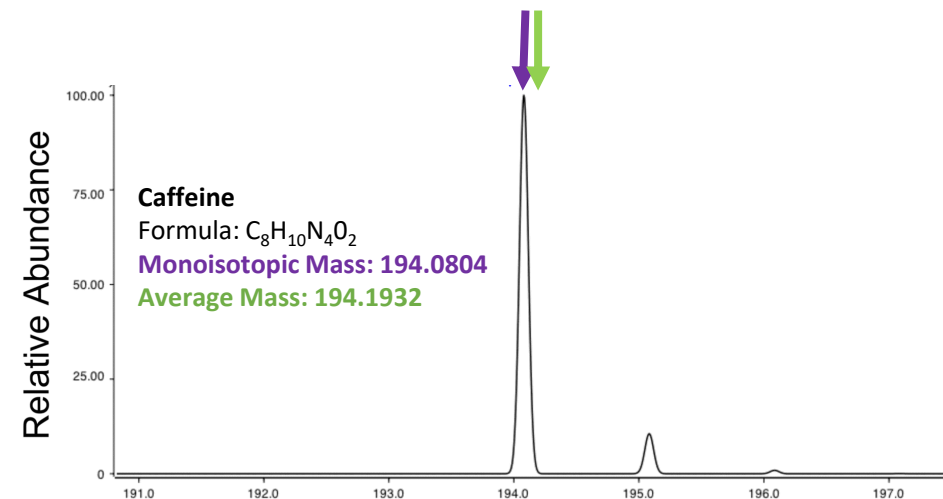
Up to 12 000 Da

- 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





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Results Data Format: ☒ Raw Data ☐ PDF Only

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Molecular Formula / Expected Mass

Solubility / Applicable Solvents

Toxicity

Storage and Special Handling

STRUCTURE OR GEL IMAGE

Image File

ADDITIONAL INFORMATION

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- **Useful information for us – “old ReQuest” – it does not include all information**

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- **Solubility – choose 2 or 3 most suitable solvents**
- **Data format -.pdf, .msd or .mzML (.mzxML)**



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ADDITIONAL INFORMATION

Additional Notes

Specify any additional notes for your sample.

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	

- Additional information

- Useful information for us – “old ReQuest” – it does not include all information



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**

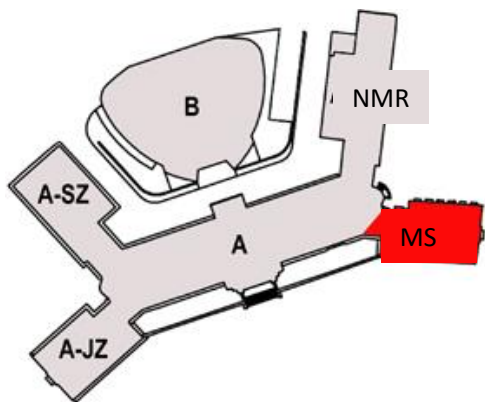
ionizability of sample is < ionizability of plasticizers = **failed**



ReQuest – Sample Submission



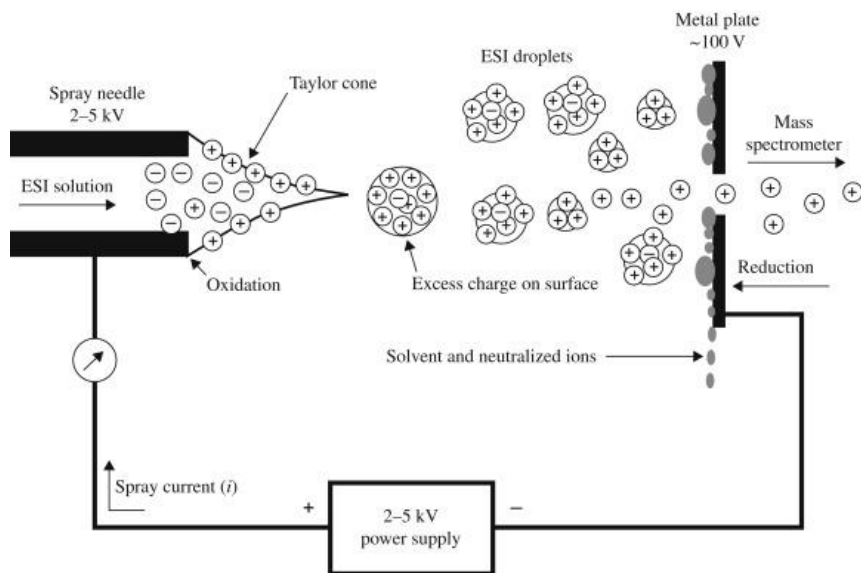
- Printed ReQuest form with the sample
 - if you have one mass and a lot fraction, single paper ReQuest is OK
 - however, each sample must be written under its own ReQuest separately
- Light sensitive sample – cover with aluminum foil or put to refrigerator
- Moisture sensitive sample – cover with parafilm
- 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



Building A
SE-wing
1st floor

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



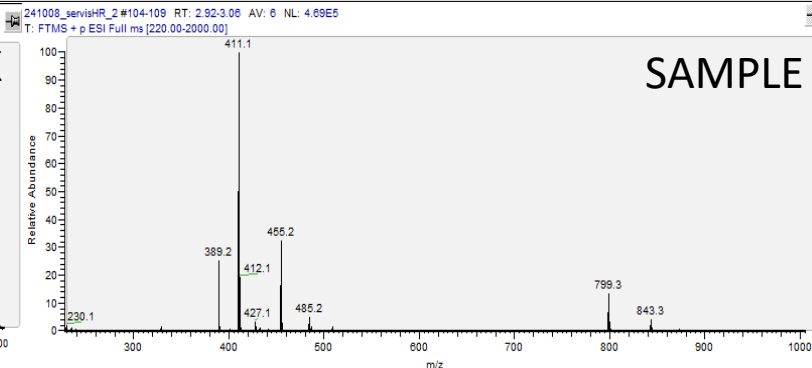
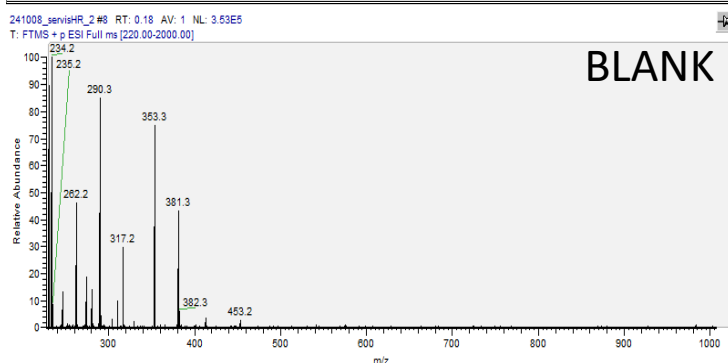
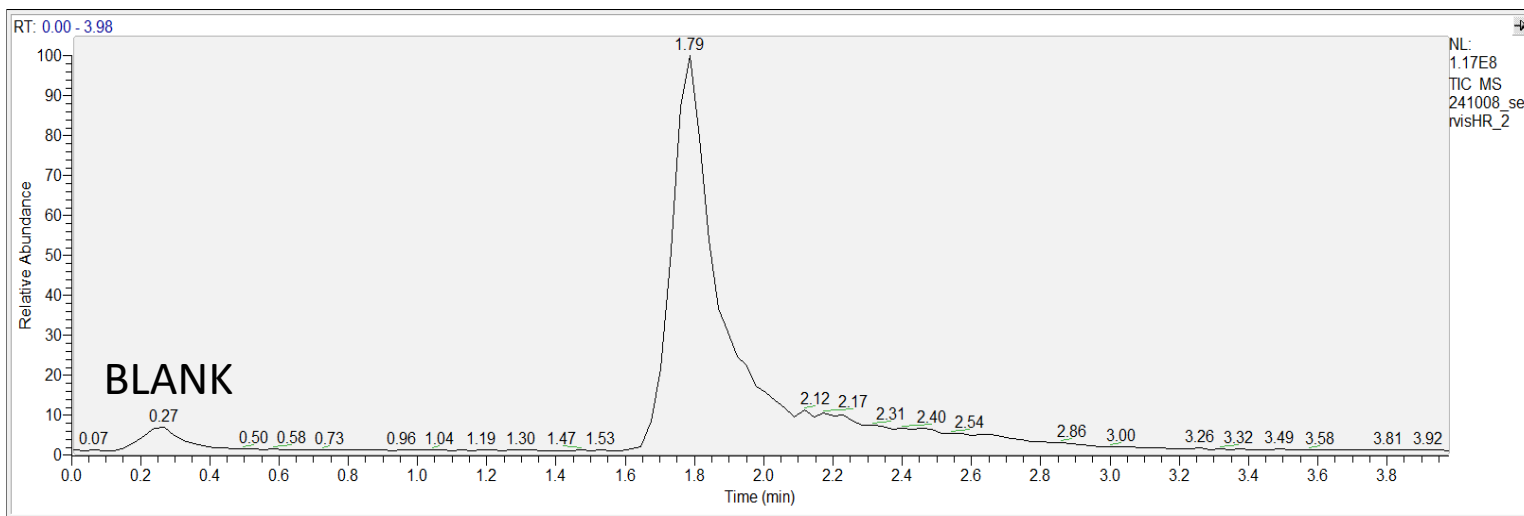
Samples – Salts, Buffers and Other Additives

- 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



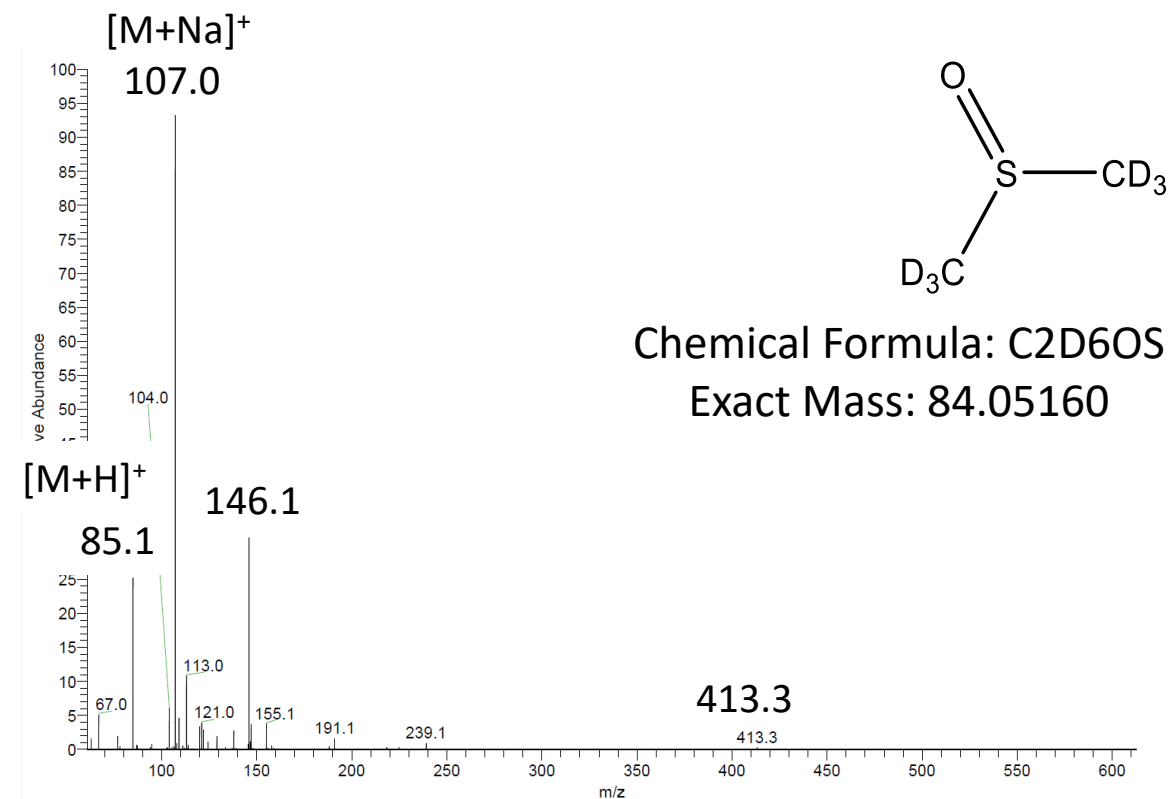
- Direct injection without column
- Injection volume – 10 μL
- Concentration around 0.5 mg/mL
- Isocratic elution
- ESI – mobile phase
 - 80% MeOH, 20% H_2O
 - Problematic for moisture sensitive samples
- APCI – Mobile phase
 - 100% ACN
- Specific analyses

Advantage:

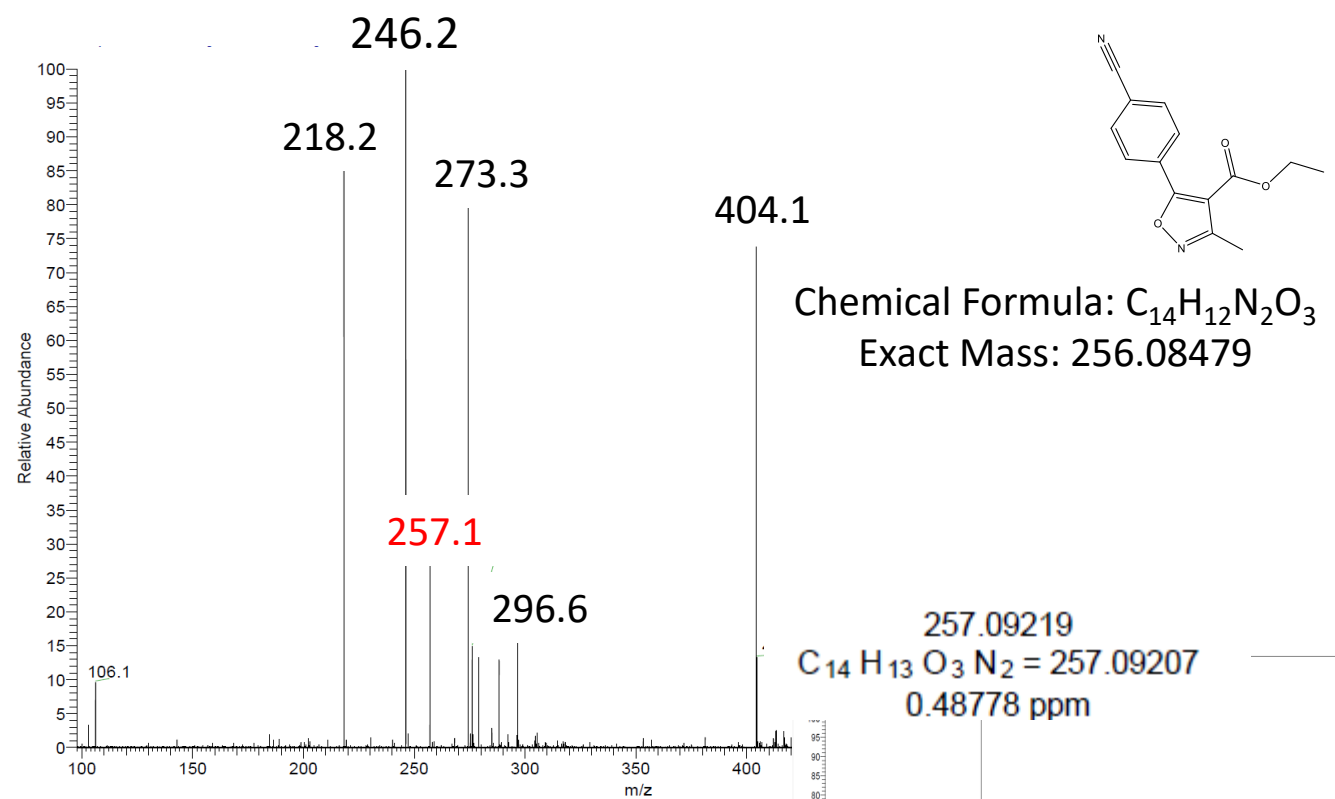
- Subtract spectrum of diluting solvents
- Subtract carry over spectrum of previous over concentrated sample



Samples Requirements – DMSO-free Samples

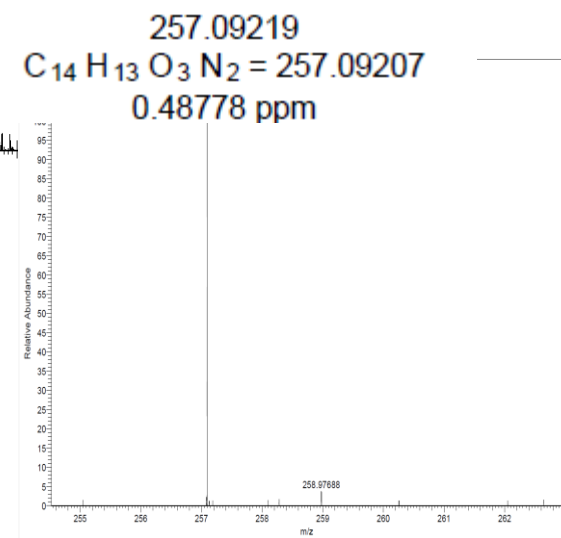


sample after NMR measurement solvent d_6 -DMSO



same sample after lyophilization

- 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





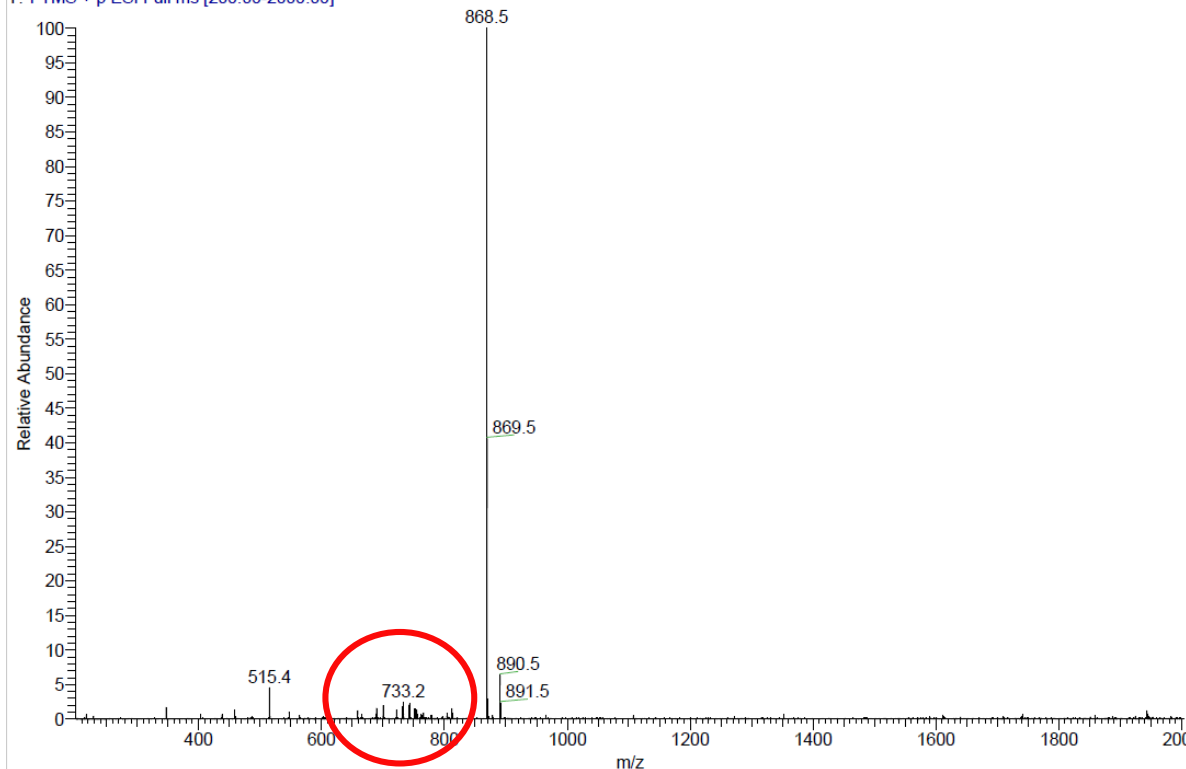
Samples Requirements – Stability in Solution

Formula $\text{C}_{48}\text{H}_{66}\text{DyF}_3\text{N}_{11}\text{O}_{18}\text{Tb}$

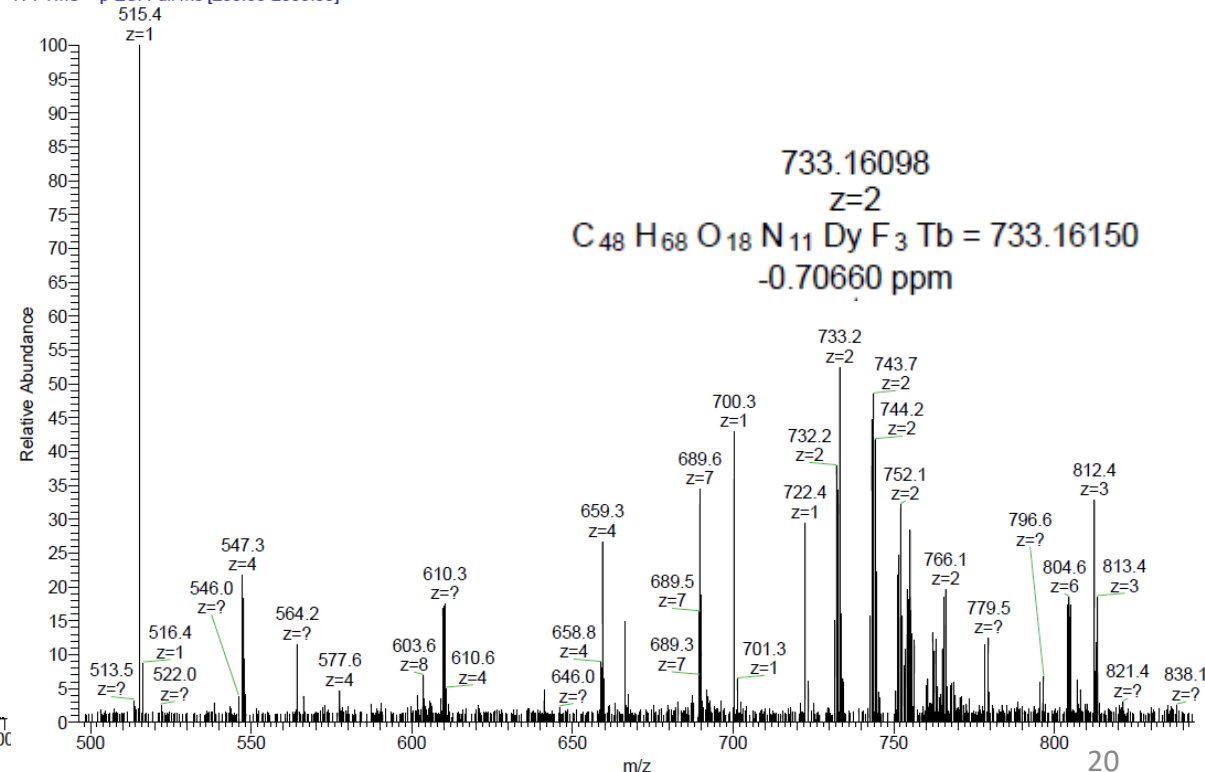
Exact Mass 1464.30845

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 .

T: FTMS + p ESI Full ms [200.00-2000.00]



T: FTMS + p ESI Full ms [200.00-2000.00]





Samples – Common Contaminants

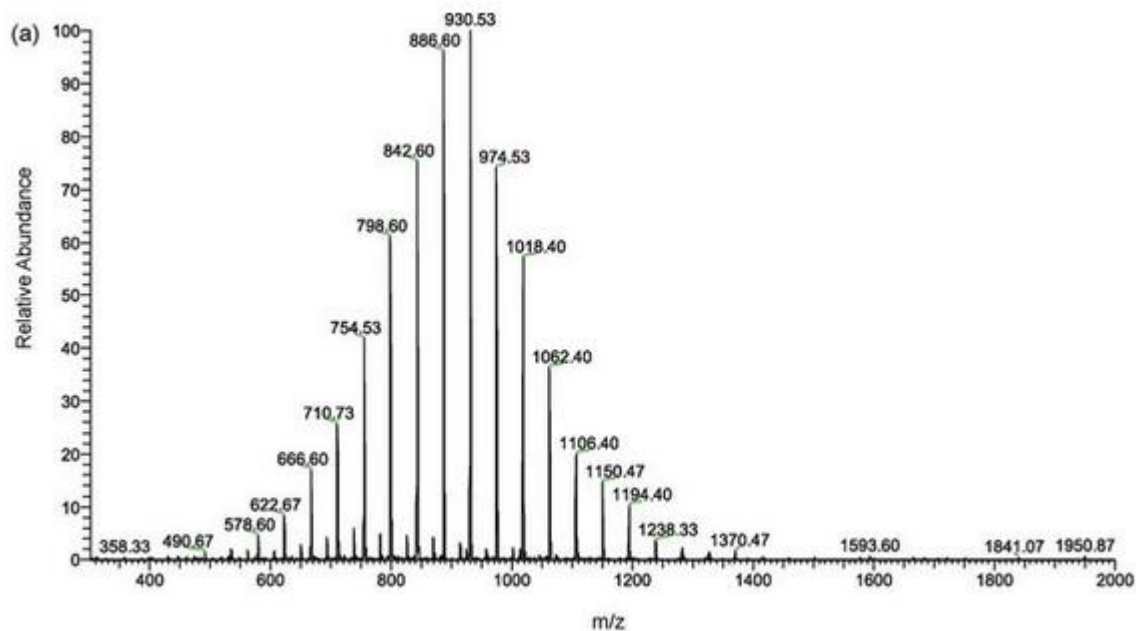
- **Phthalates** – from plastics, contaminated solvents
 - diisobutylphthalate (masses **ESI: 279, 301** , EI: 149, 205, 223, 278)
 - diisooctylphthalate (masses **ESI: 391, 413**, EI: 149, 167, 279, 390)
- **Antioxidants** – from plastics : irganox, irgafos
 - the most common masses **ESI: 663, 685**, 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**



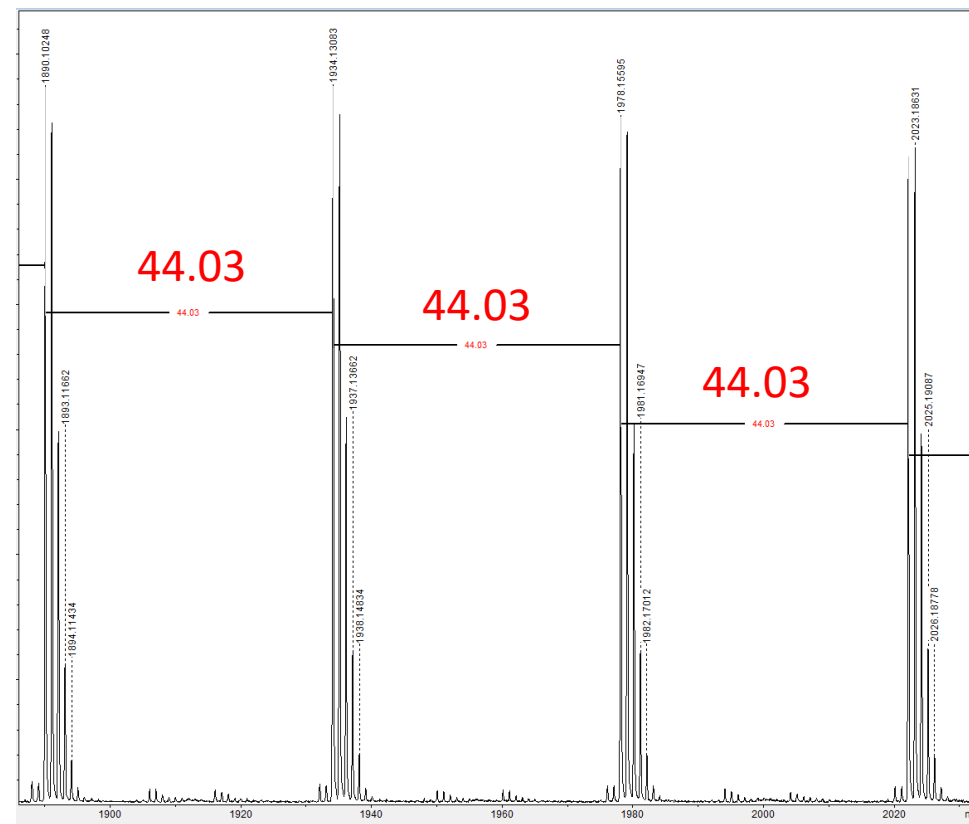
Samples – PEG Contaminants

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

-[C₂H₄O]-; polyethylene glycol, PEG



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





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

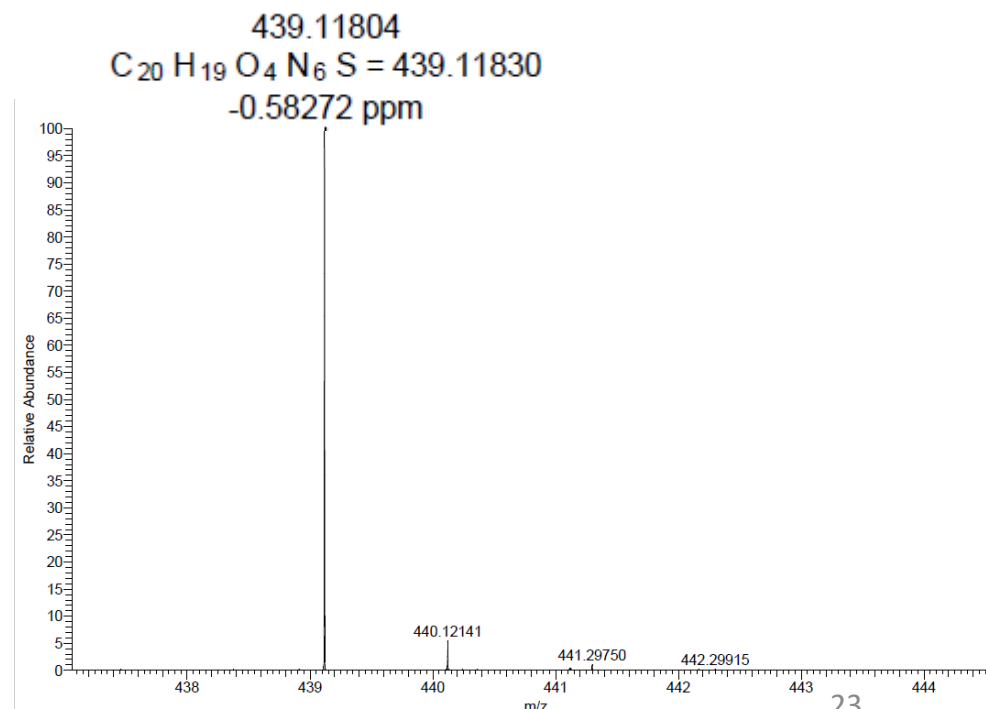
Nitrogen-Rule: Do not use

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

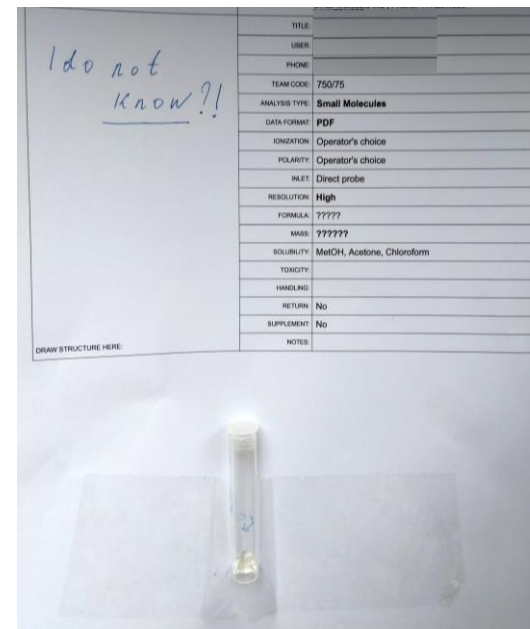
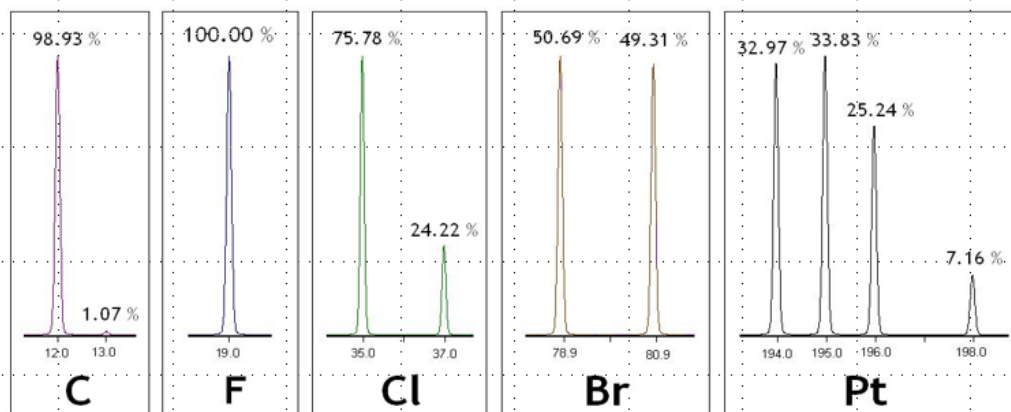




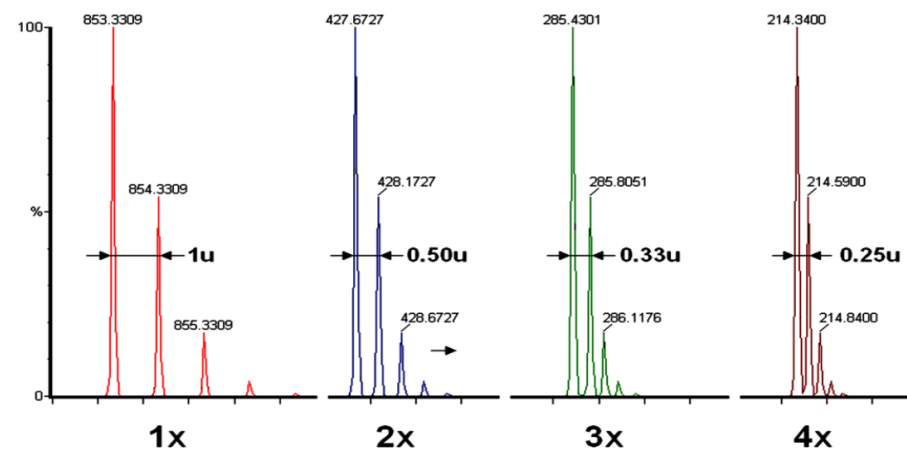
Exact Mass – Elemental Composition

- 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

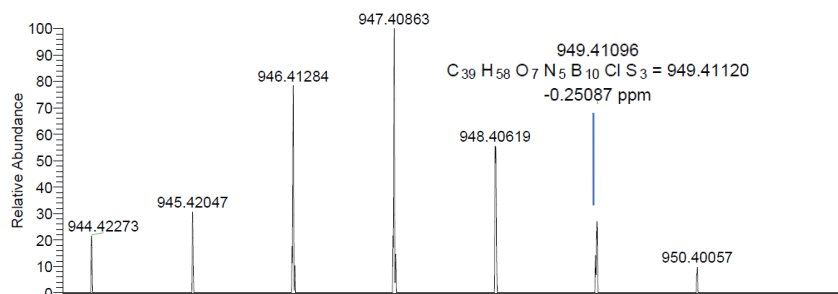
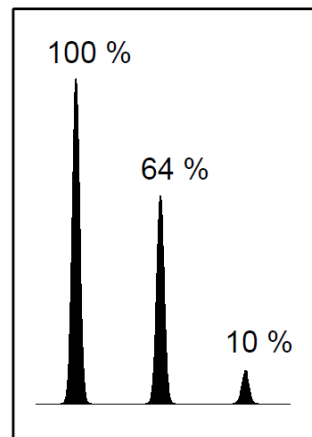


Exact Mass – Elemental Composition

Example: Cl₂

³⁵Cl³⁵Cl (70 Da), P = 1.00 x 1.00 = **1.00**
³⁵Cl³⁷Cl (72 Da), P = 1.00 x 0.32 = 0.32
³⁷Cl³⁵Cl (72 Da), P = 0.32 x 1.00 = 0.32
³⁷Cl³⁷Cl (74 Da), P = 0.32 x 0.32 = **0.10**

0.64



1.30E5
 251121_servisHR_6_21112
 5133232#77 RT: 2.05 AV:
 1 T: FTMS + p ESI Full ms
 [200.00-2000.00]

NL:
 1.23E5
 C₃₉ H₅₈ B₁₀ Cl₅ O₇ S₃:
 C₃₉ H₅₈ B₁₀ Cl₁ N₅ O₇ S₃:
 pa Chrg 1

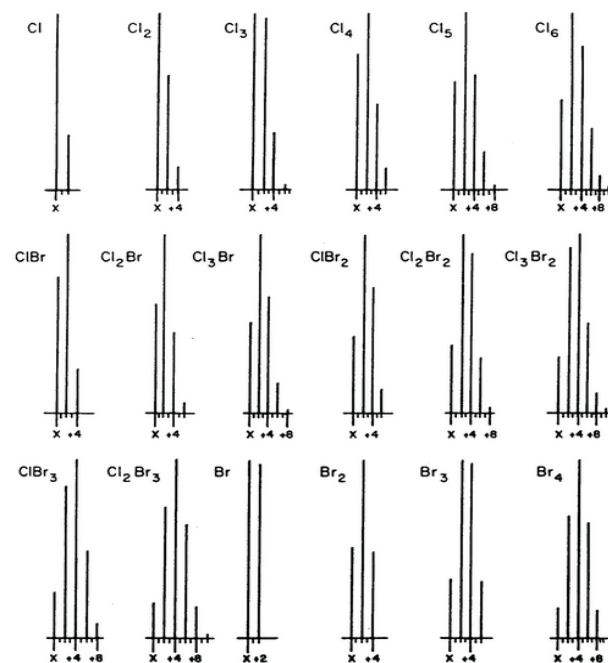
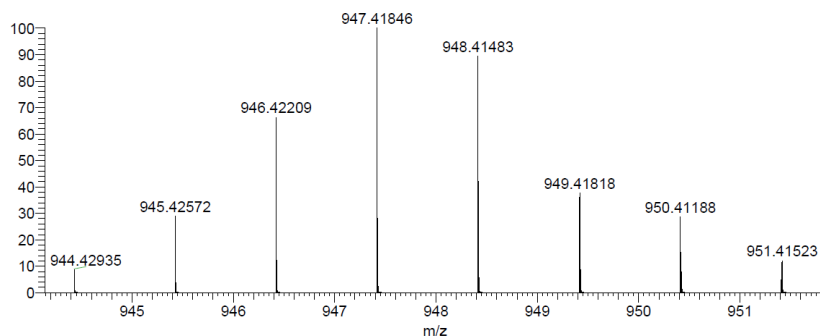
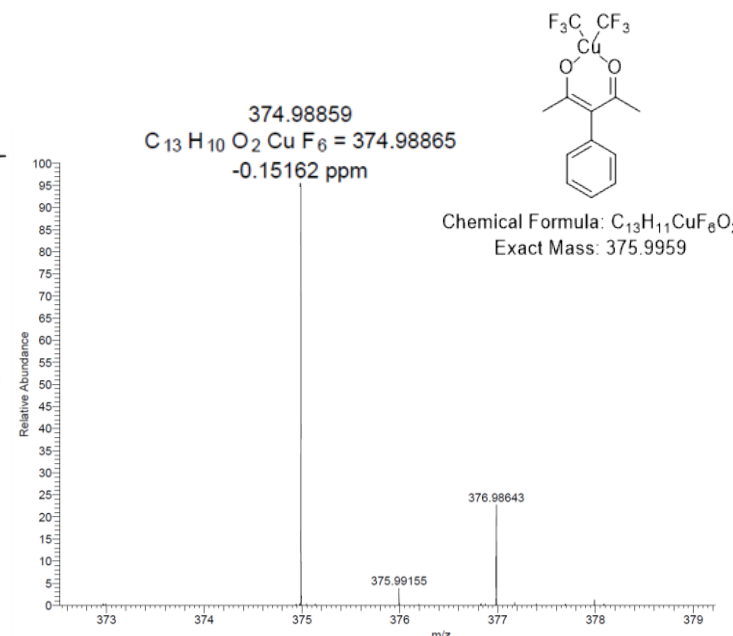


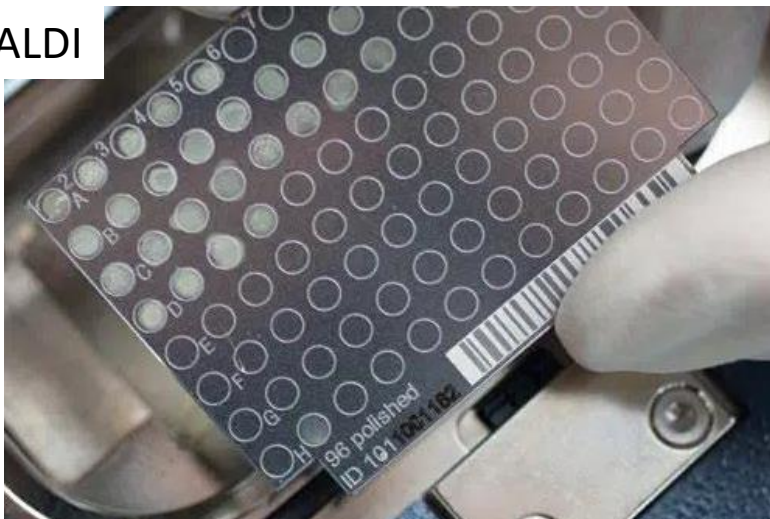
Figure 2: Peak patterns representing ions with atoms of Cl/Br



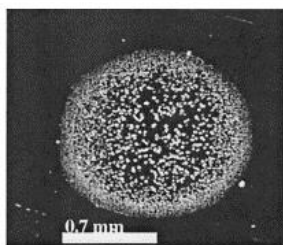
- Isotopic clusters indicate presence of some elements (e.g., Cl, Br, some metals etc.).
- We love isotopic clusters, so we provide prediction, if something unusual appears

Samples Requirements – MALDI

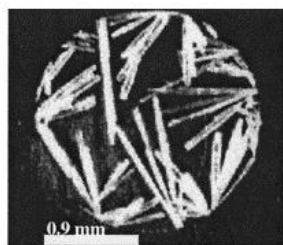
MALDI



Zoom of spots



(a) CHCA



(b) DHB

<https://www.qlaboratories.com>

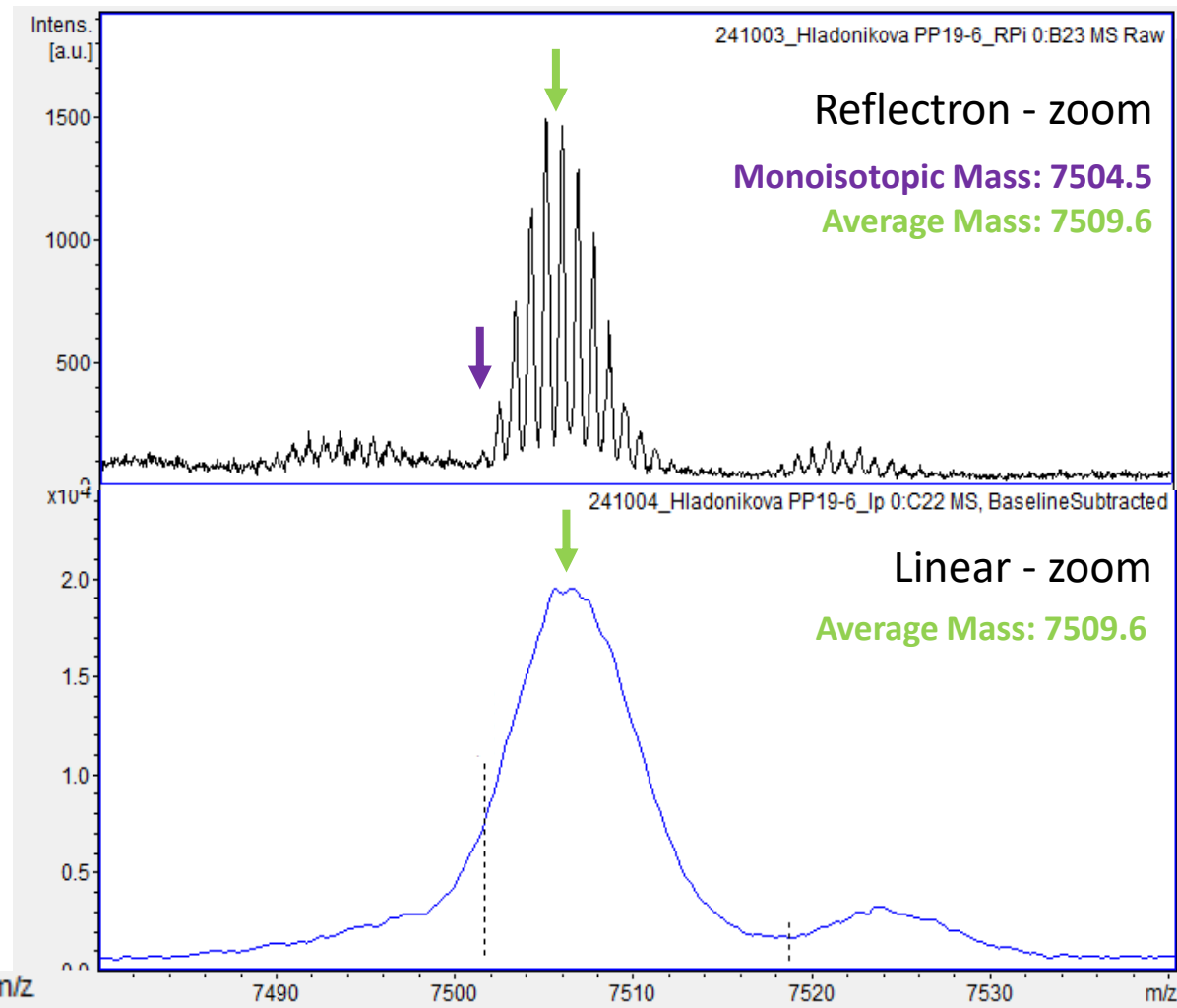
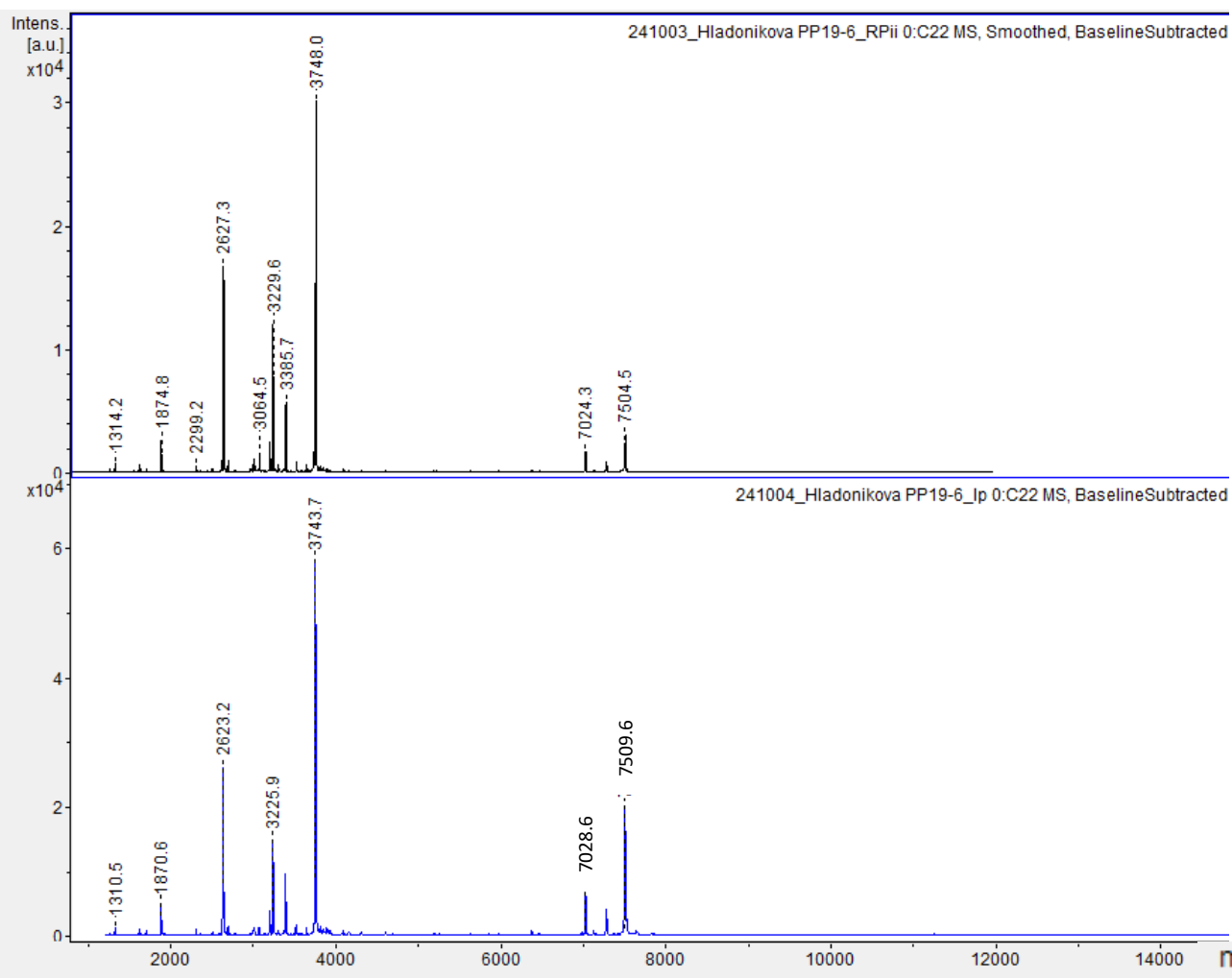
[https://doi.org/10.1016/S1044-0305\(03\)00262-9](https://doi.org/10.1016/S1044-0305(03)00262-9)

- 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 10 μ M or more
 - Up to 20kDa – concentration 20 μ M 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

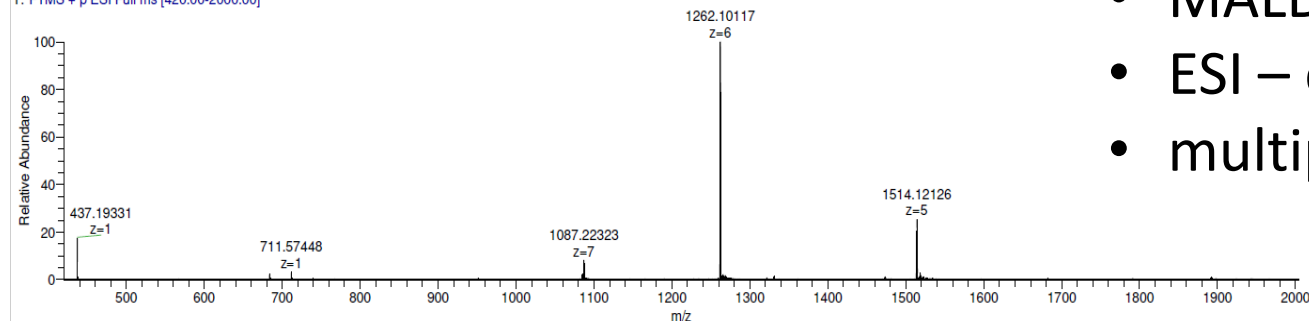




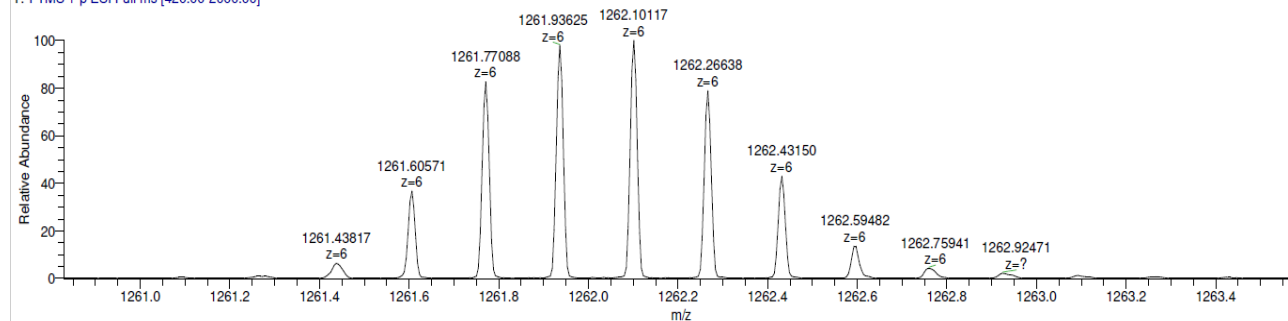
Samples Measurement – ESI – Deconvolution

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

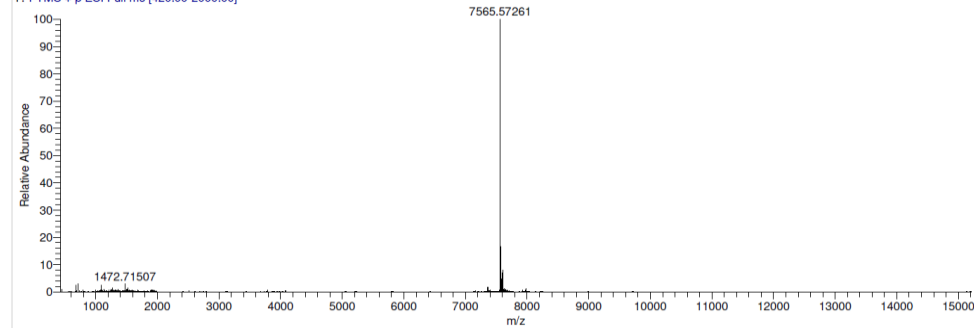
240619_servisHR_23 #90-100 RT: 2.49-2.77 AV: 11 SB: 22 0.13-0.41, 0.18-0.46 NL: 1.31E6
T: FTMS + p ESI Full ms [420.00-2000.00]



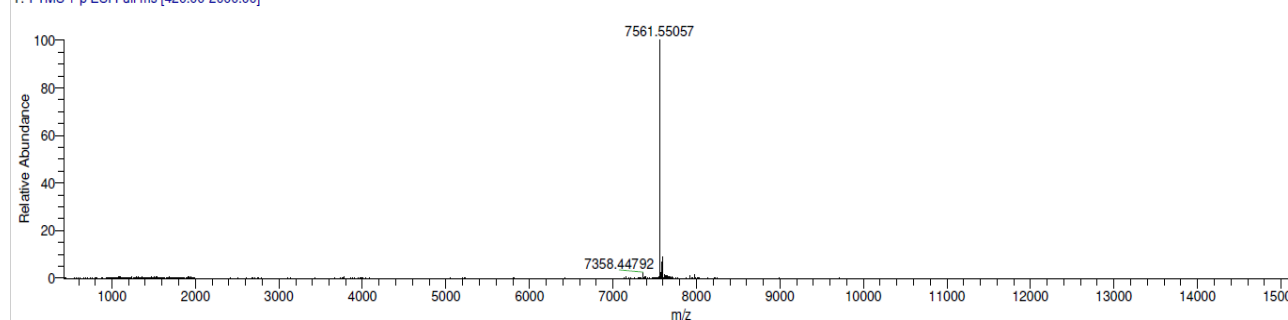
240619_servisHR_23 #90-100 RT: 2.49-2.77 AV: 11 SB: 22 0.13-0.41, 0.18-0.46 NL: 1.31E6
T: FTMS + p ESI Full ms [420.00-2000.00]



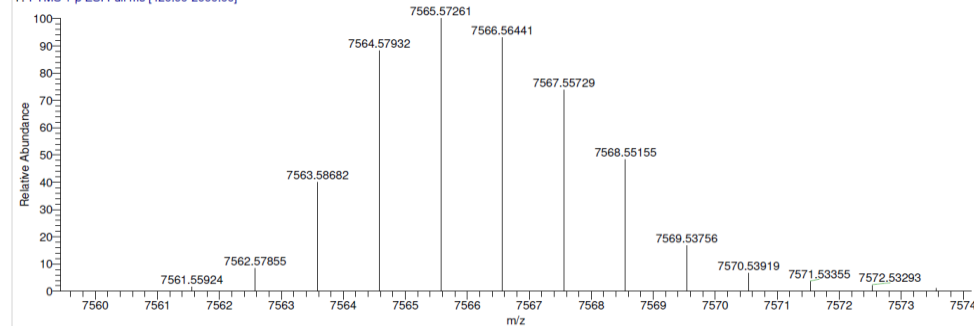
240619_servisHR_23_XT_00001_M_#1 RT: 1.00 AV: 1 NL: 2.62E5
T: FTMS + p ESI Full ms [420.00-2000.00]



240619_servisHR_23_XT_00001_M_#2 RT: 2.00 AV: 1 SB: 2 1.00, 1.00 NL: 1.26E6
T: FTMS + p ESI Full ms [420.00-2000.00]

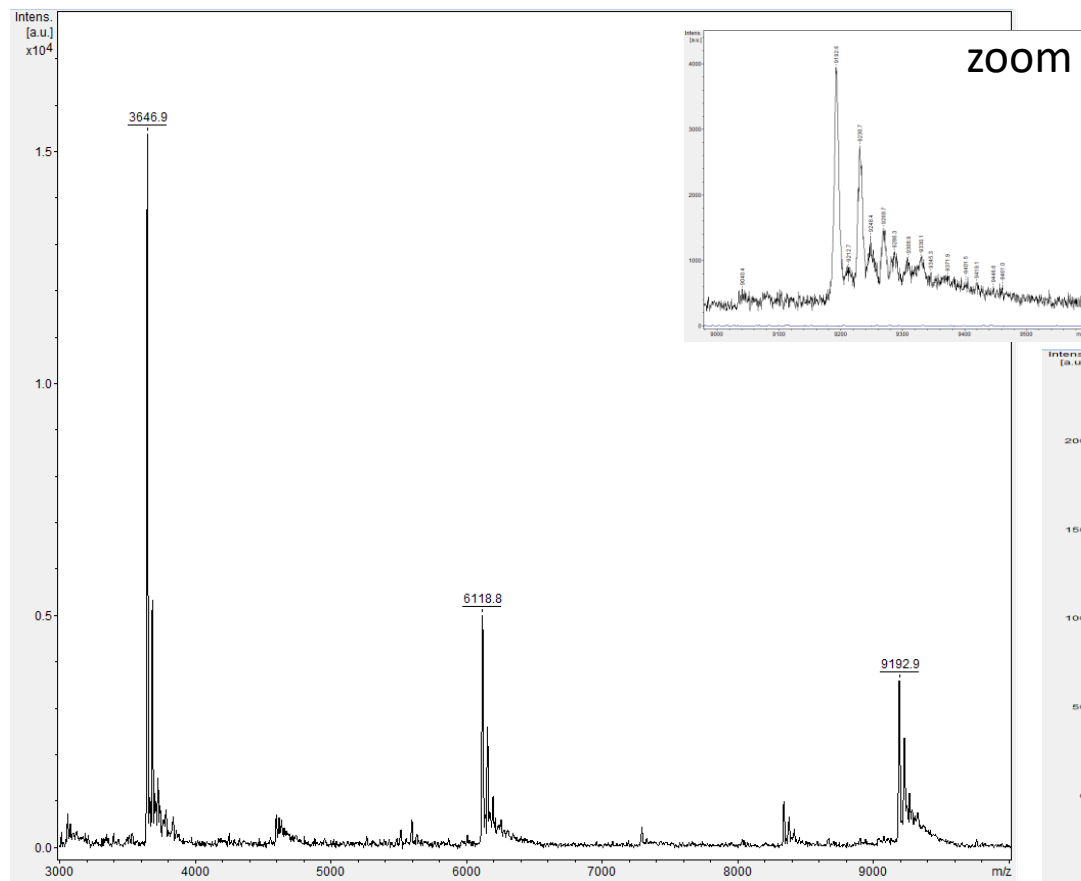


240619_servisHR_23_XT_00001_M_#1 RT: 1.00 AV: 1 NL: 2.62E5
T: FTMS + p ESI Full ms [420.00-2000.00]



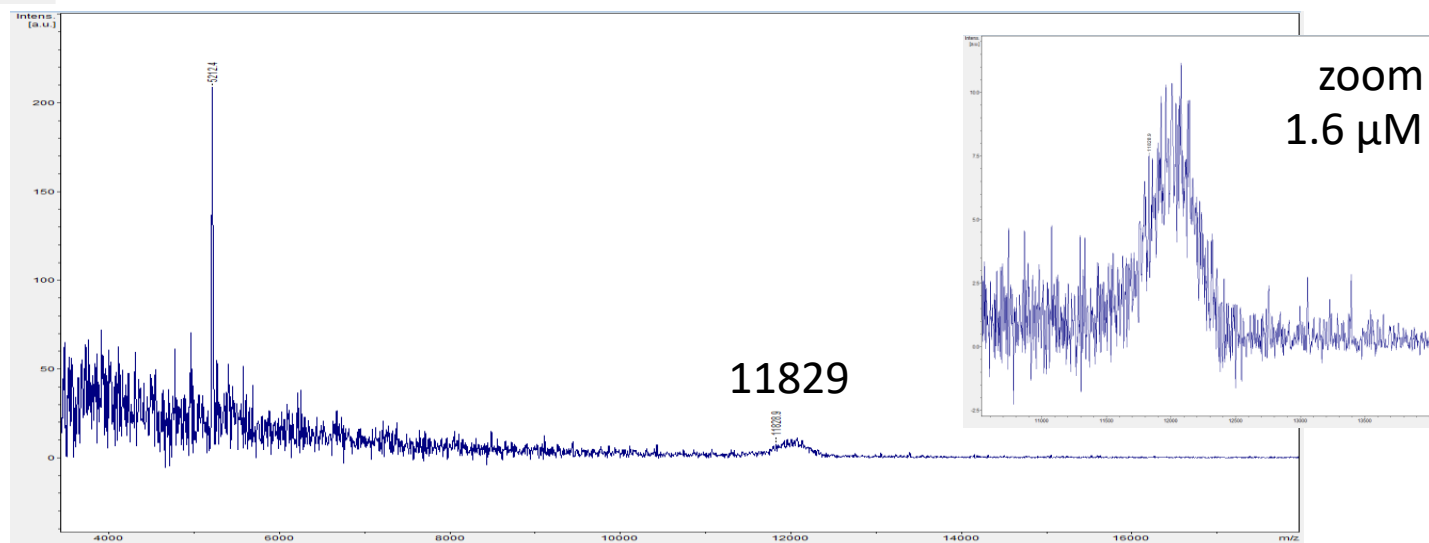


MALDI – Oligonucleotides



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

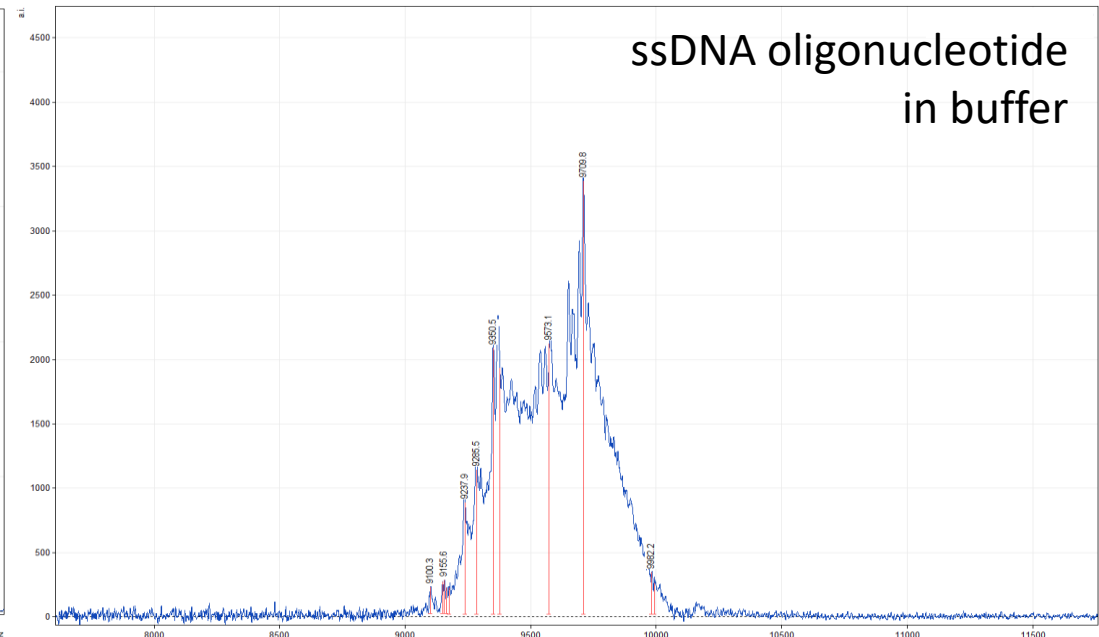
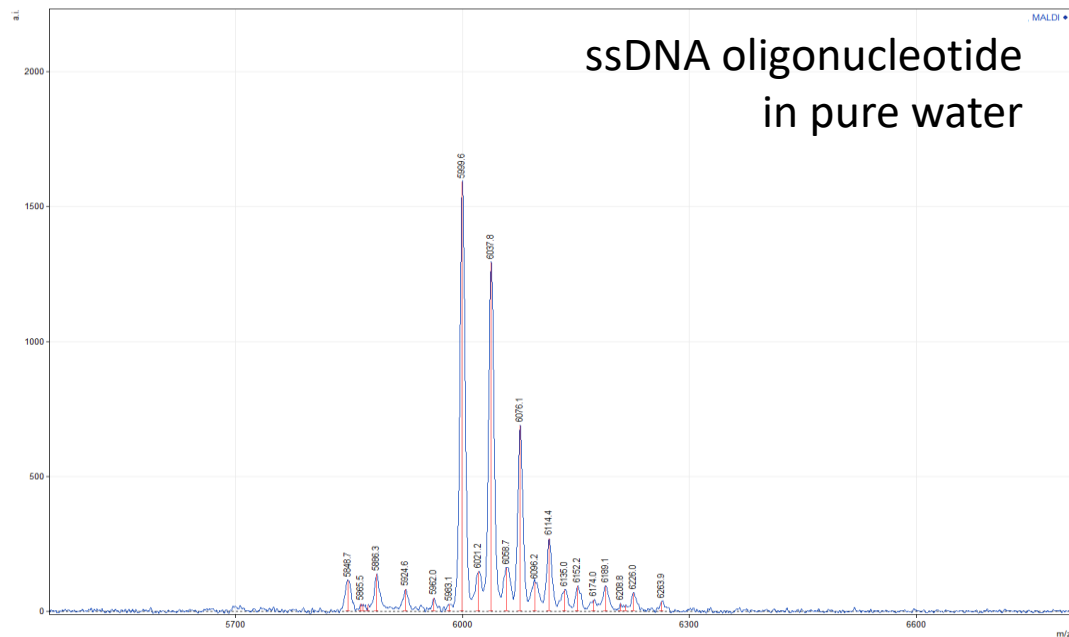
Concentration

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!