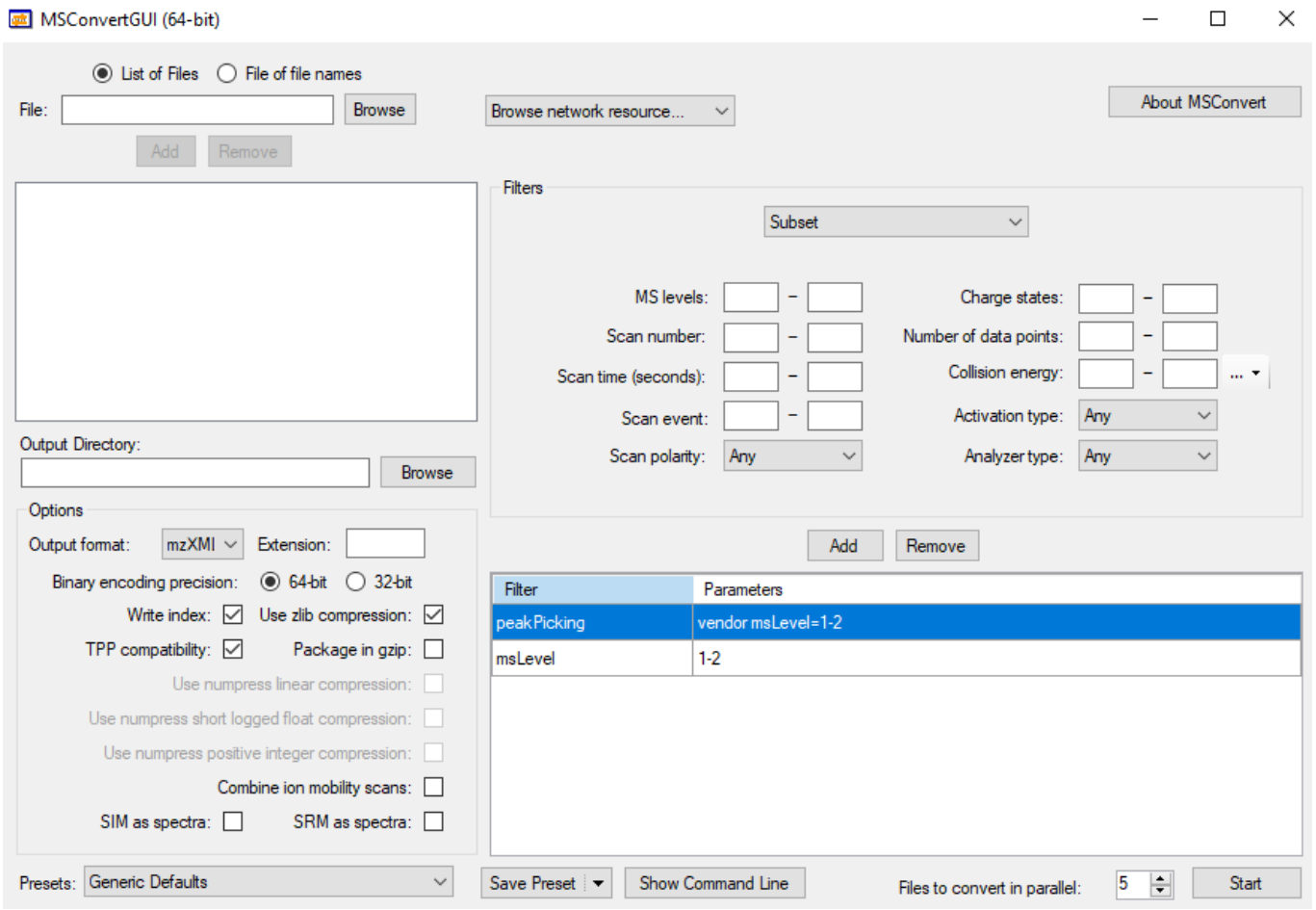
LC-MS-Cheet-Sheet

https://github.com/plyush1993/LC-MS-Cheet-Sheet/tree/main

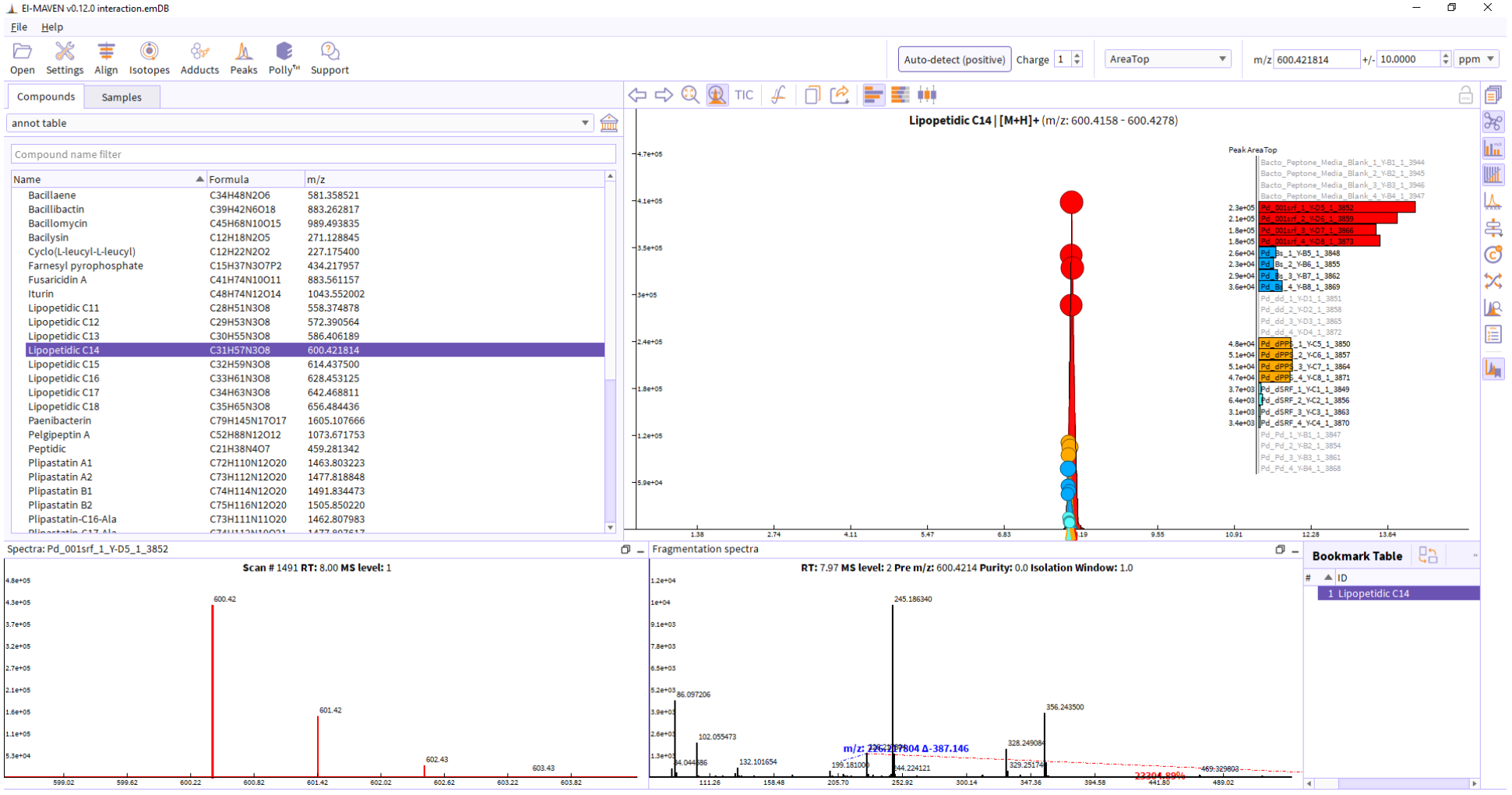
MSConvert

Convert raw LC-MS to mzXML, mzML



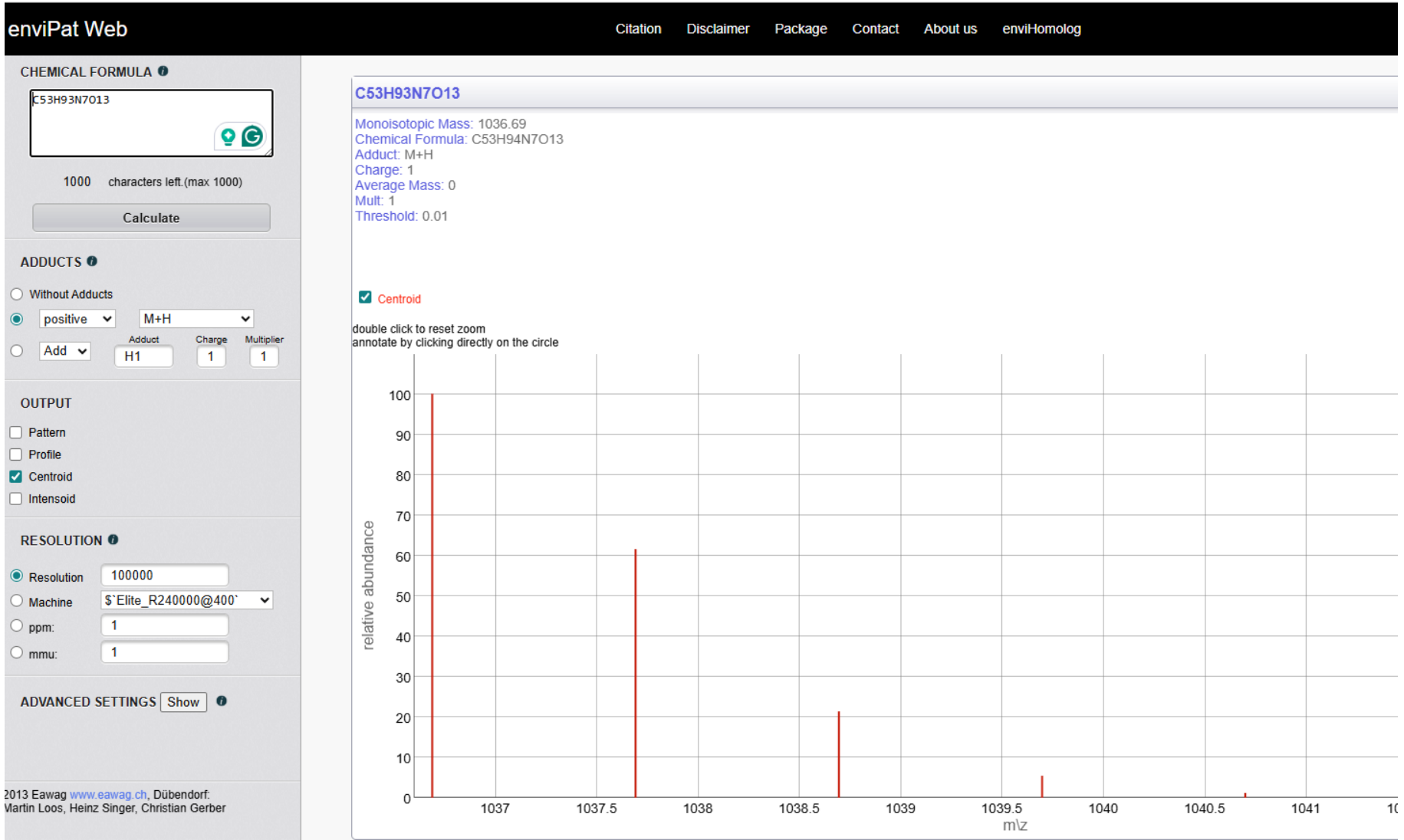
El-Maven

Open LC-MS (mzXML, mzML), get MS1-2 spectra, integrate peaks, search by database



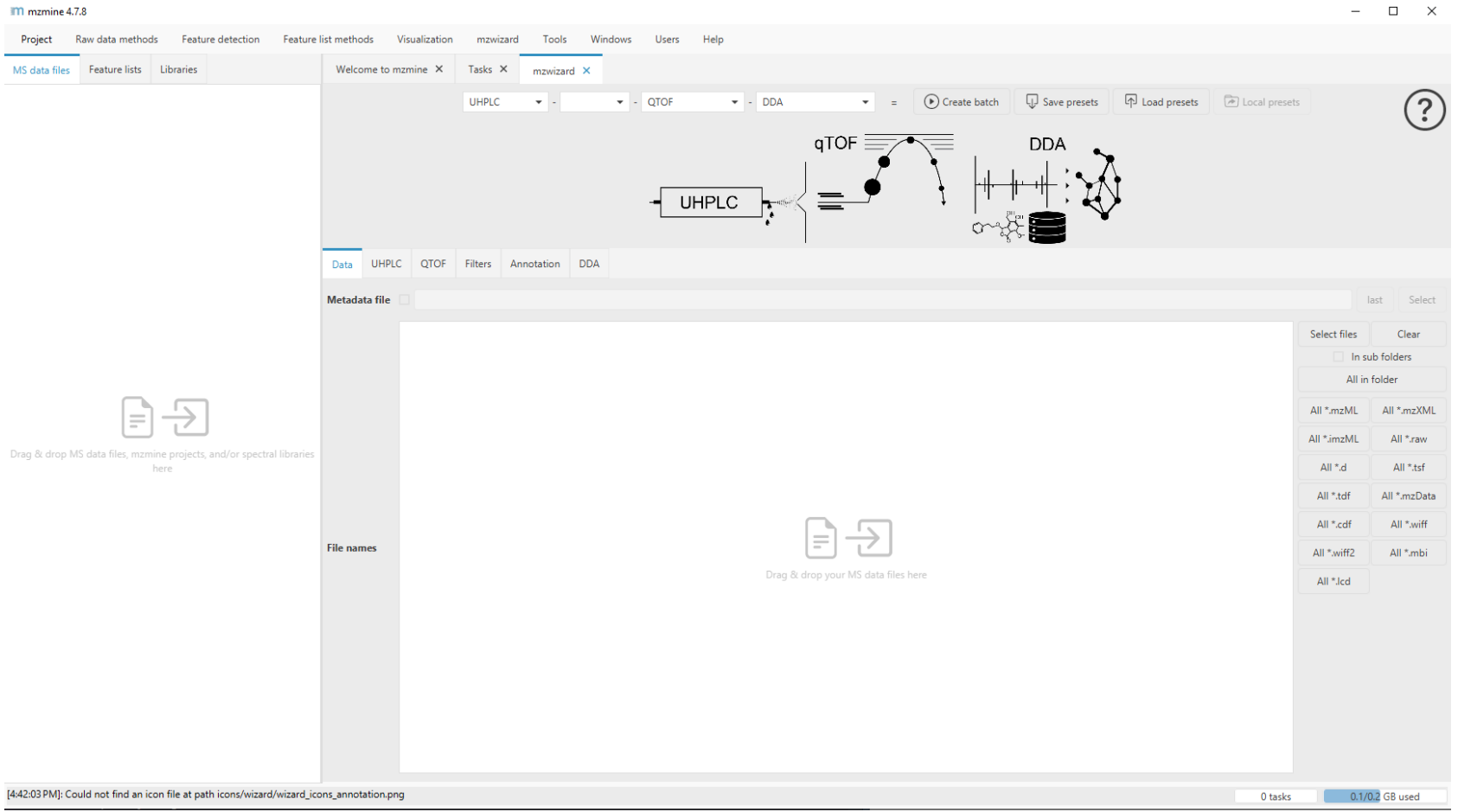
EnviPat

MS1 Isotopic Pattern Generating



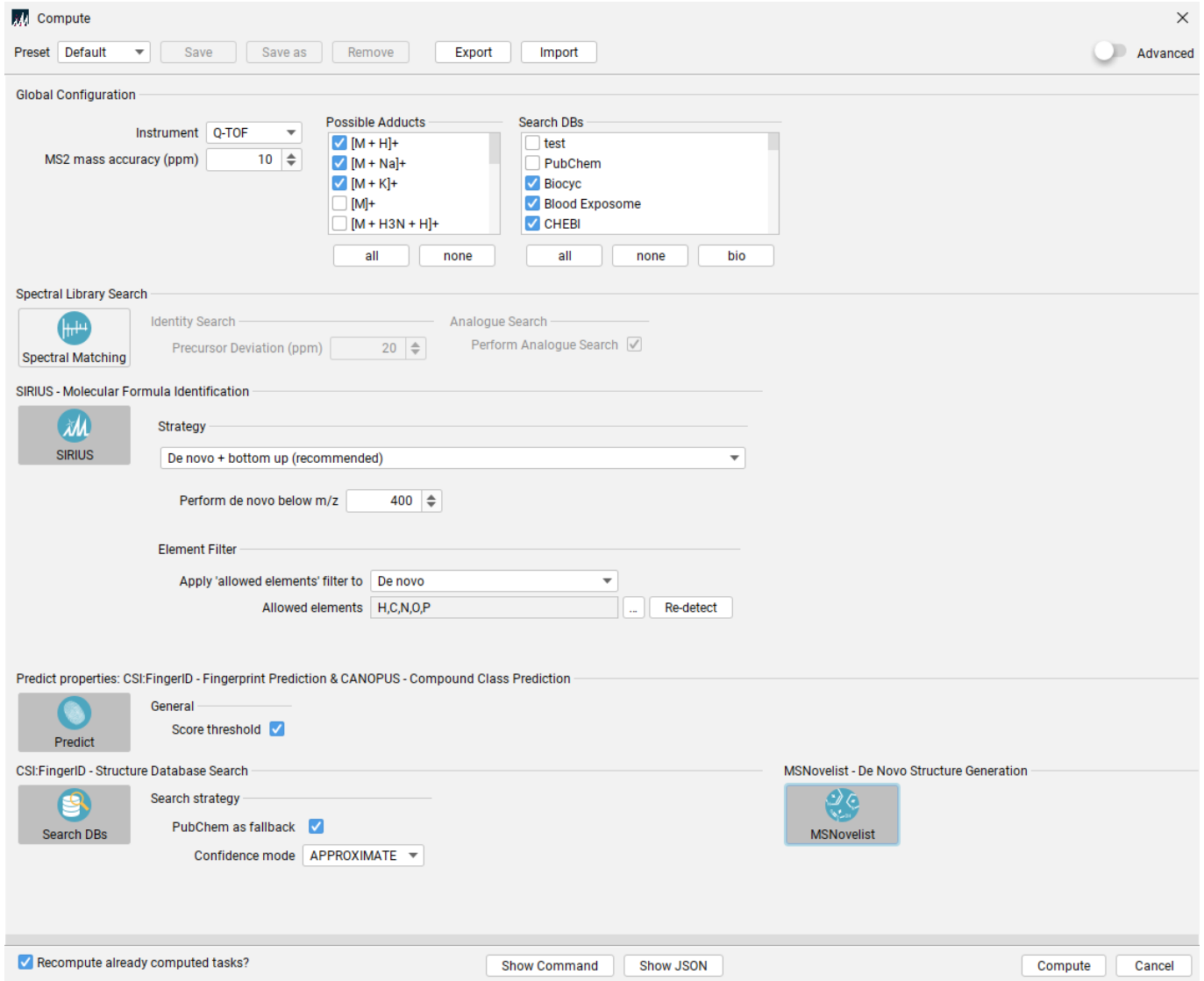
MzMine

Peaks Integration (peak table)
Generating spectra files (mgf)
Annotation / Identification



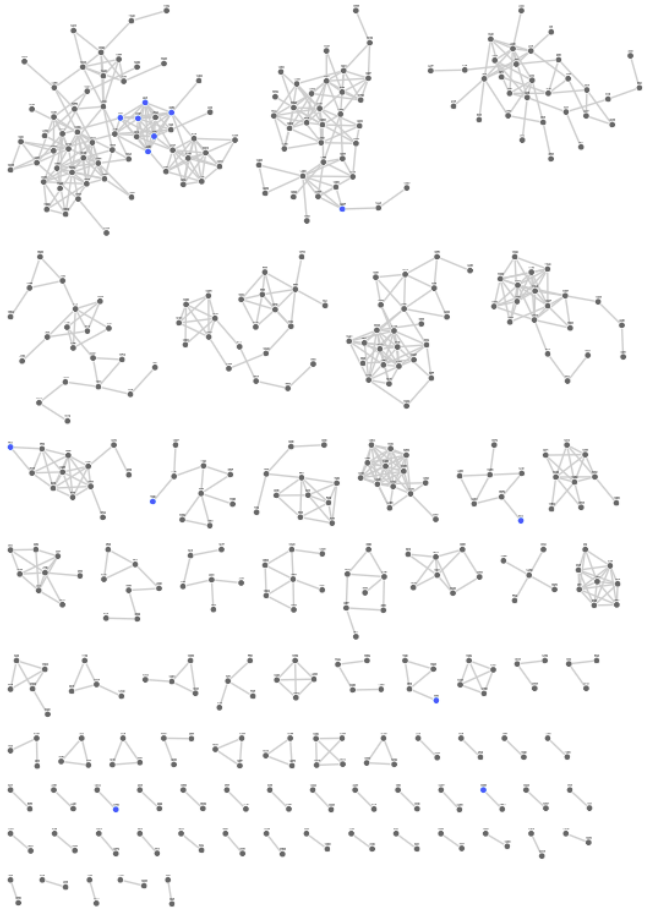
SIRIUS

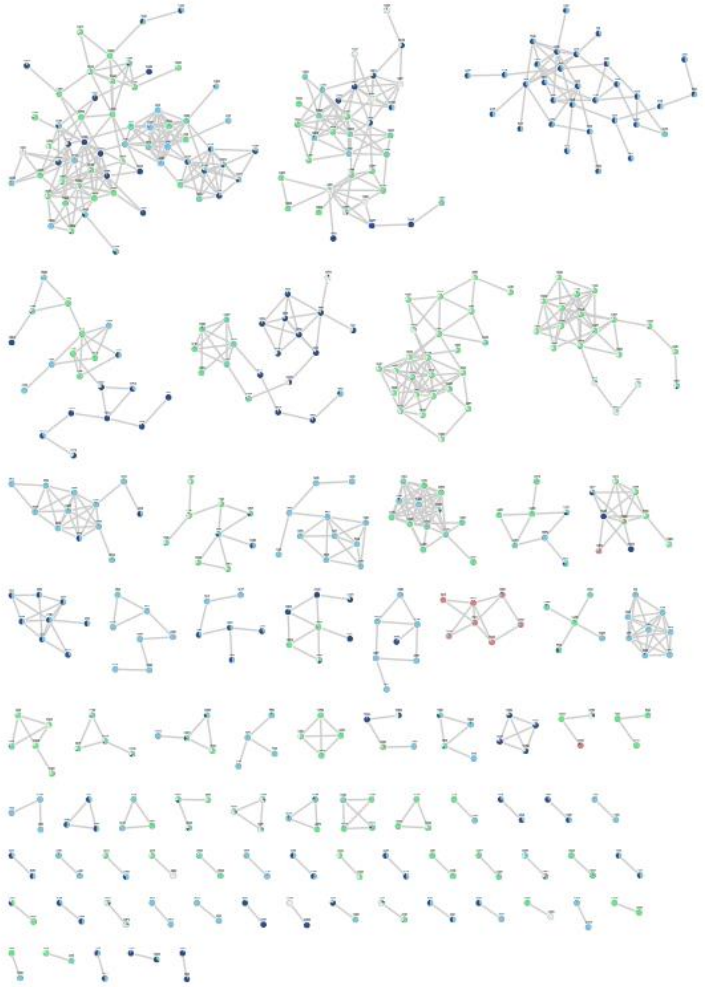
Annotation / Identification (by mgf from mzmine)



GNPS

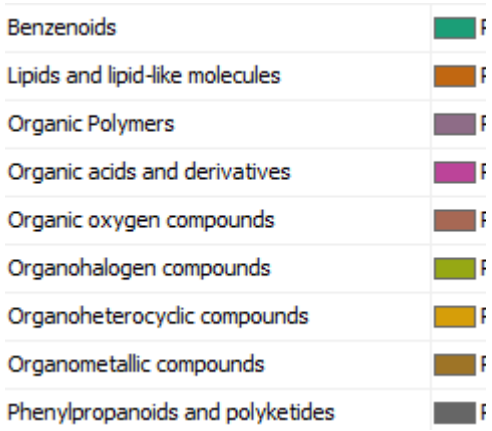
Molecular Networking
(by mgf, peak table from mzmine)

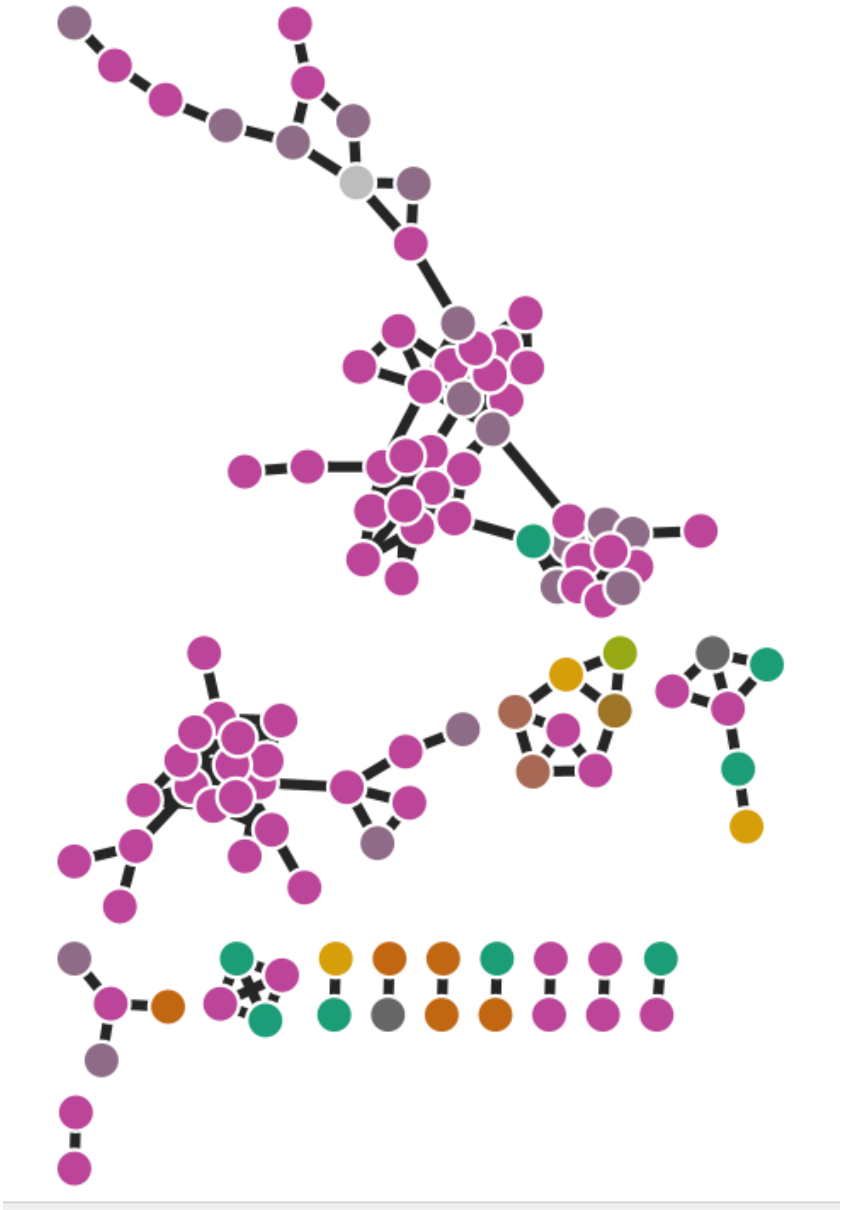




Cytoscape

Combine GNPS + Sirius

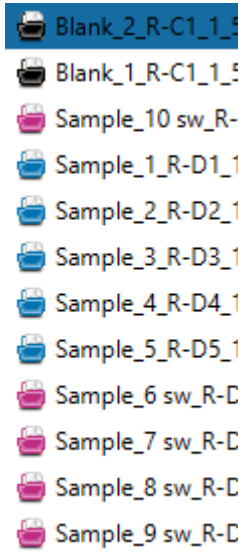


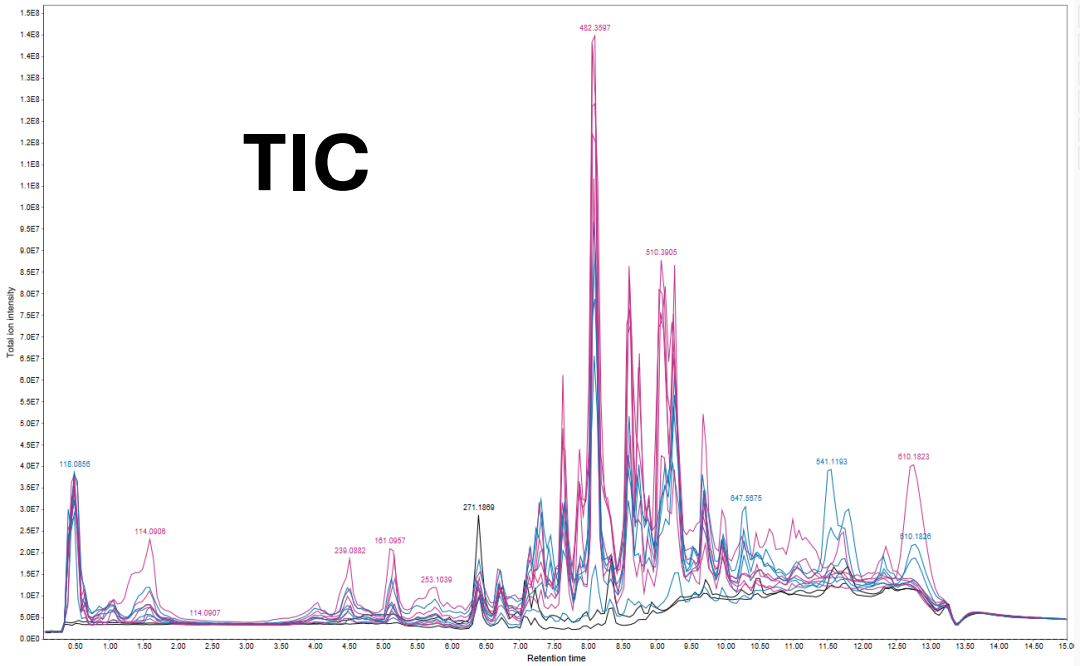


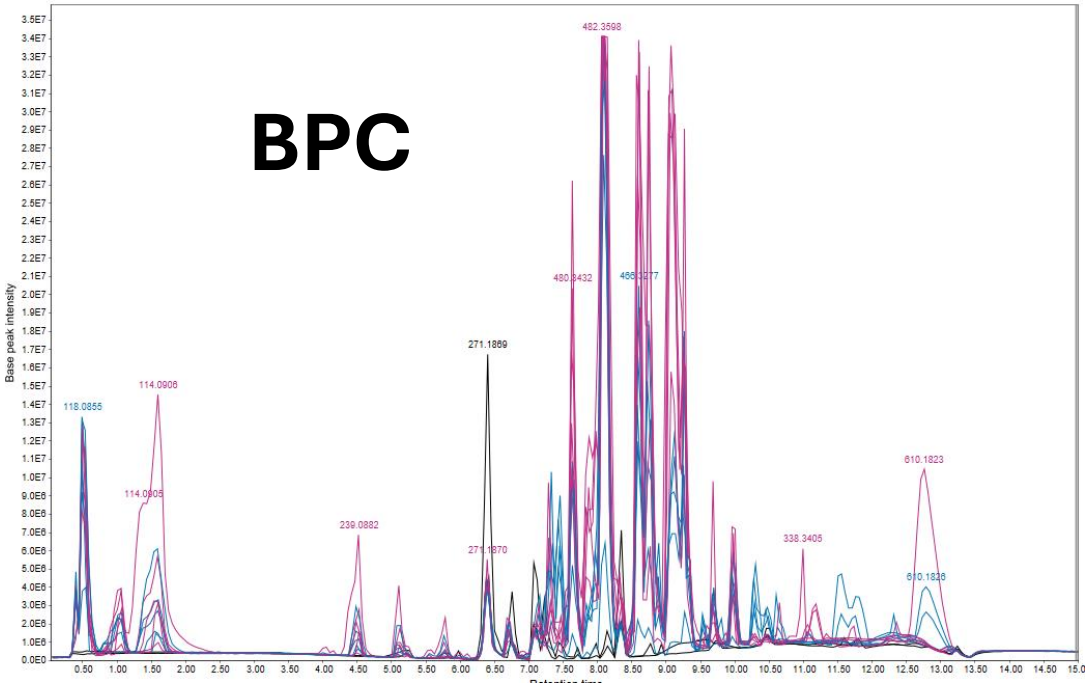
Chromatography (LC-MS)
TIC & BPC

Check:

- Peak shape
- Intensity
- Number of peaks
- Noise level
- Compare with Blanks / Replicate



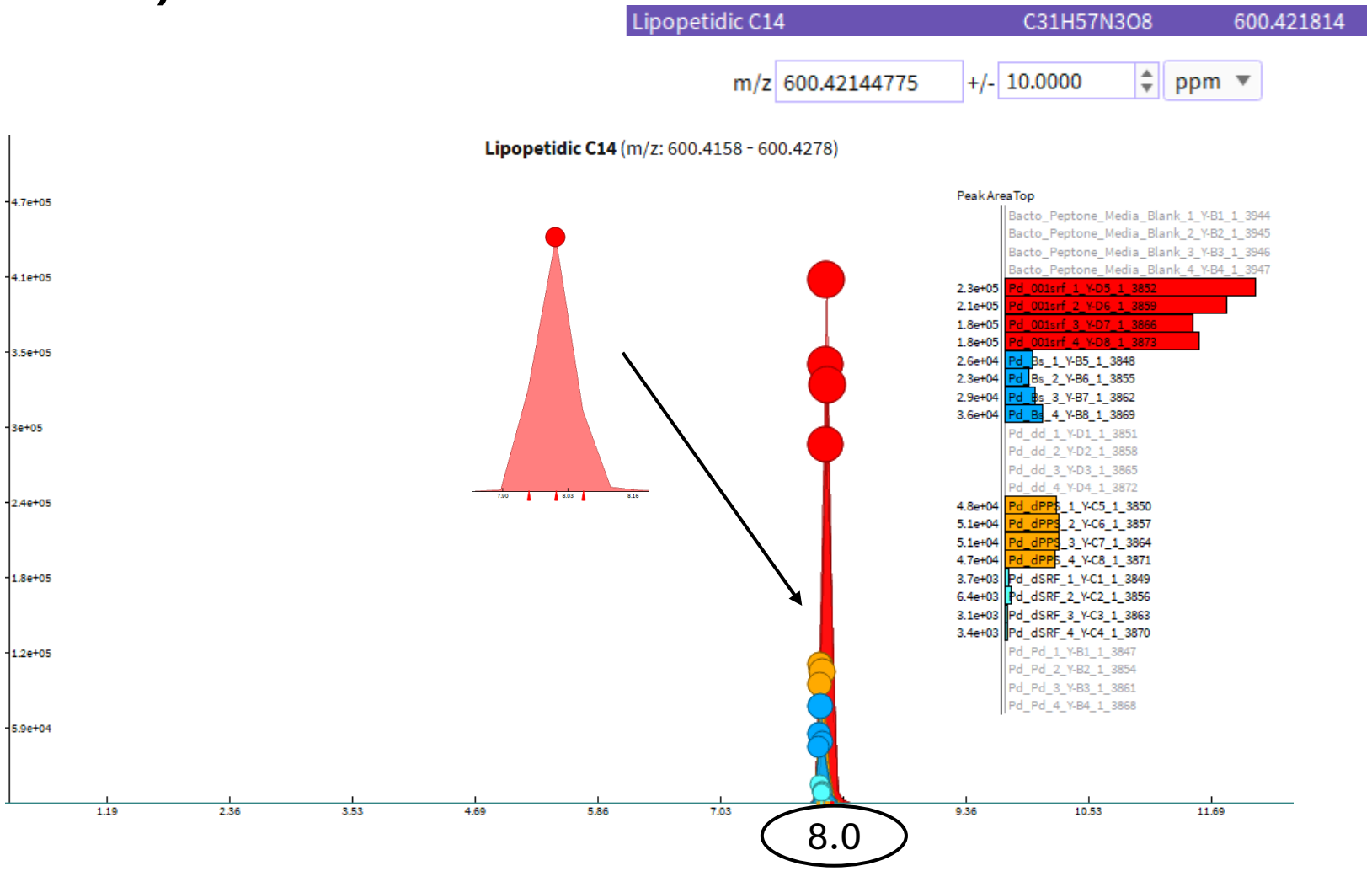




Chromatography (LC-MS)
EIC (MS1)

Check:

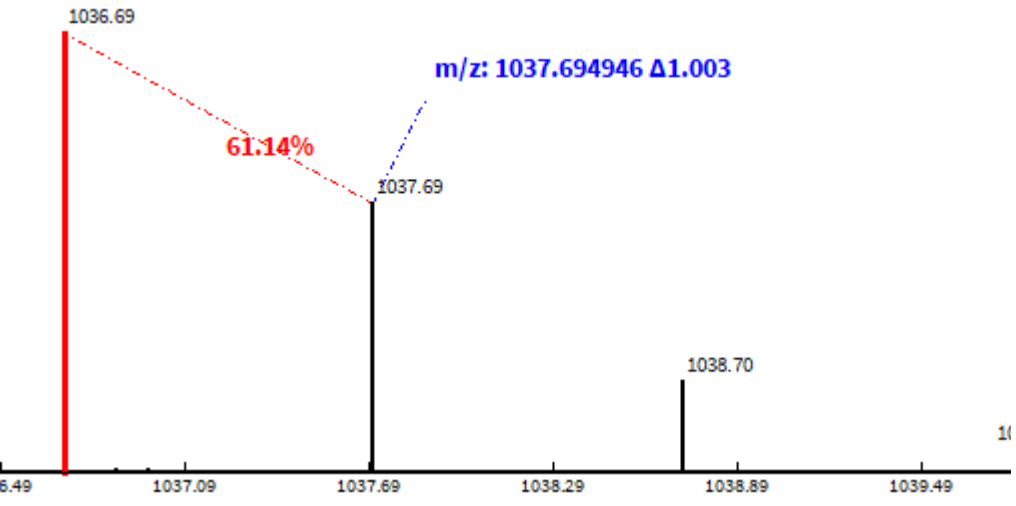
- Peak shape
- EIC in 10 ppm range
- Retention time
- Noise level
- Compare with Blanks / Replicate



Basic Spectra Interpretation

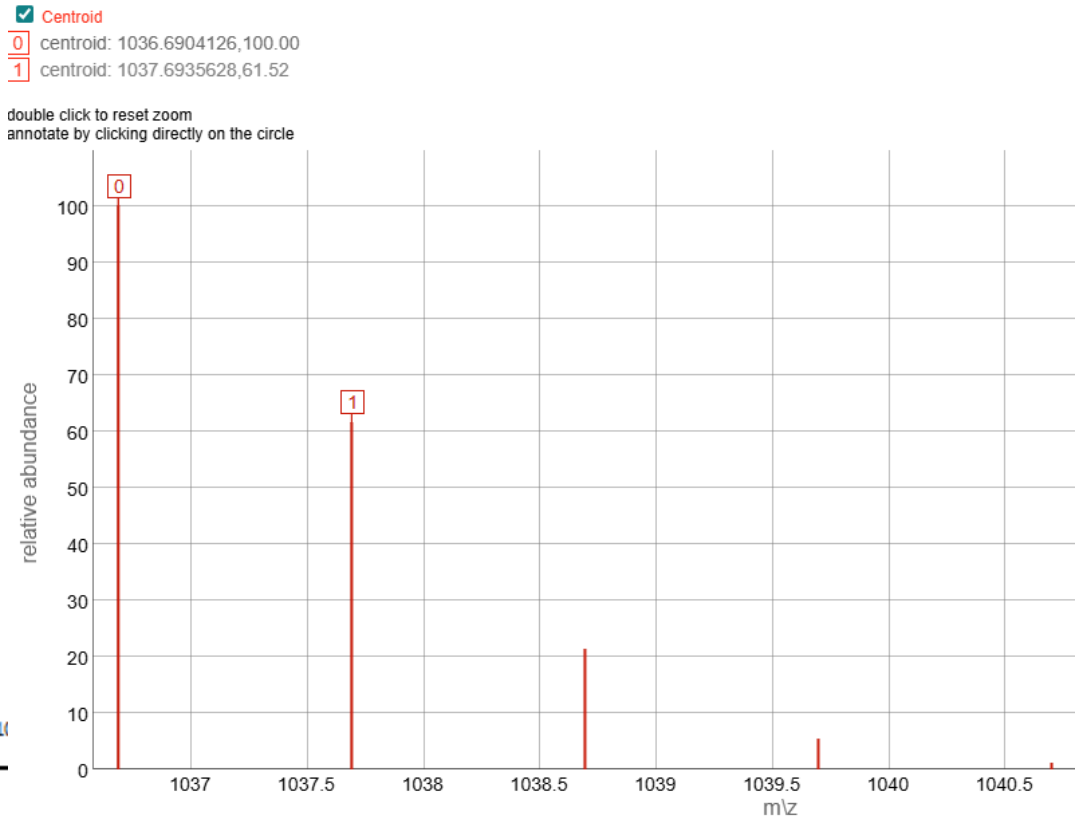
Charge z = +1

- Difference
- Ratio



Monoisotopic Mass: 1036.69
Chemical Formula: C53H94N7O13
Adduct: M+H
Charge: 1
Average Mass: 0
Mult: 1
Threshold: 0.01

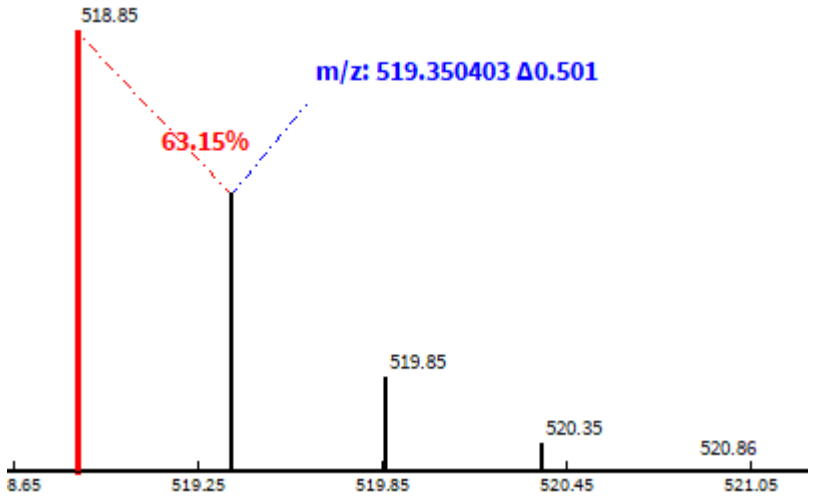
Surfactin C15



Basic Spectra Interpretation

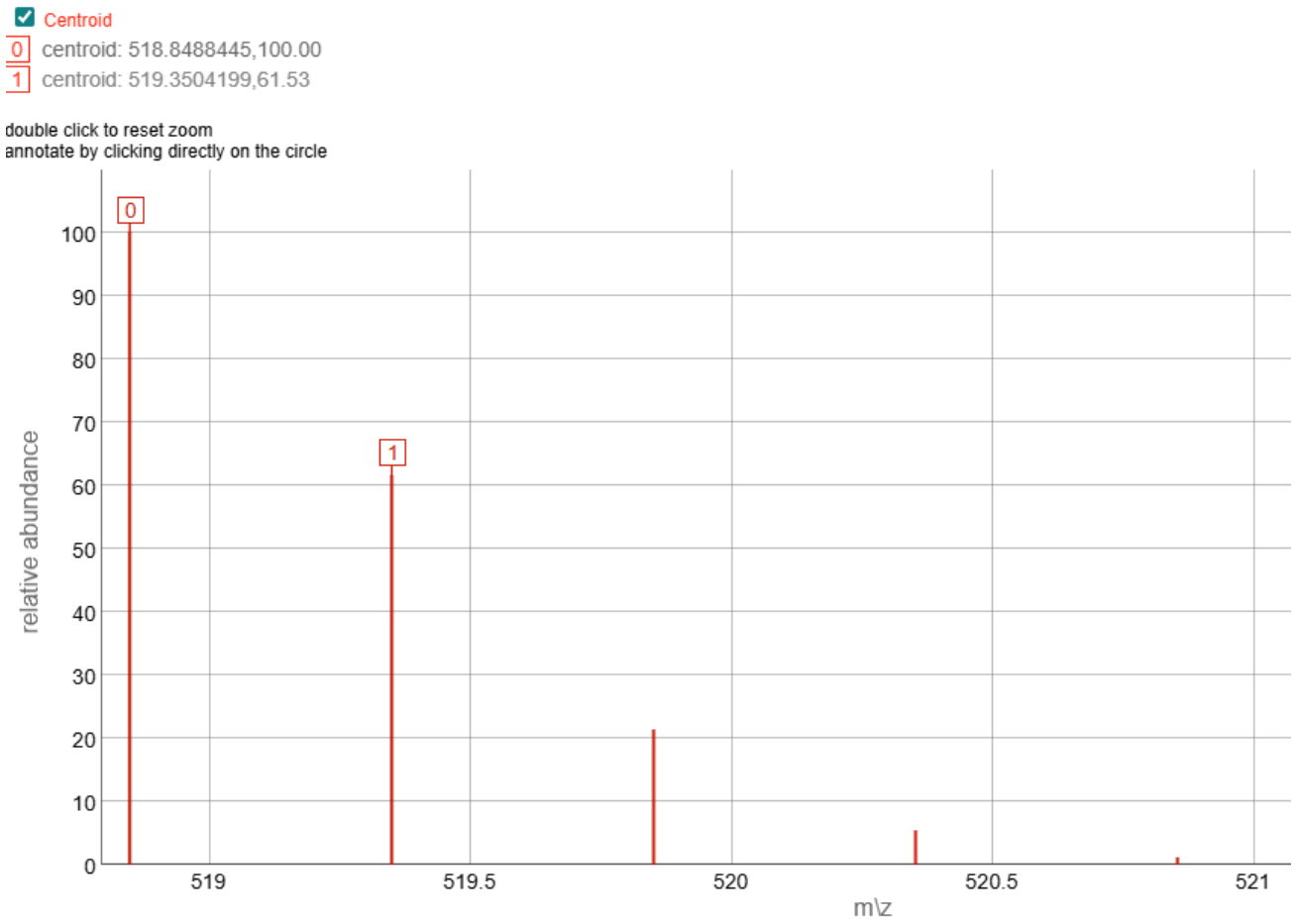
Charge z = +2

- Difference
- Ratio



Monoisotopic Mass: 518.8488
Chemical Formula: C53H95N7O13
Adduct: M+2H
Charge: 2
Average Mass: 0
Mult: 1
Threshold: 0.01

Surfactin C15

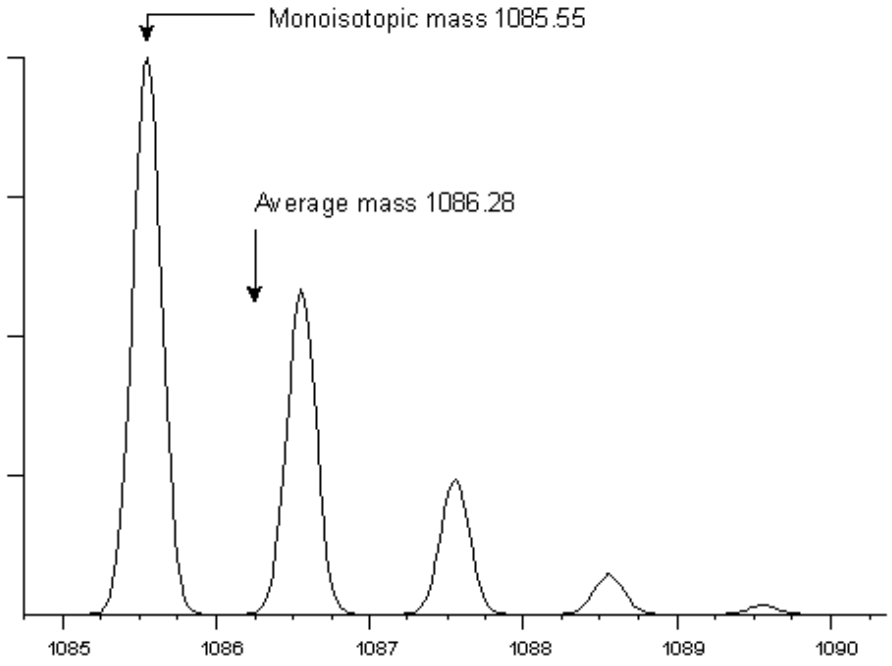


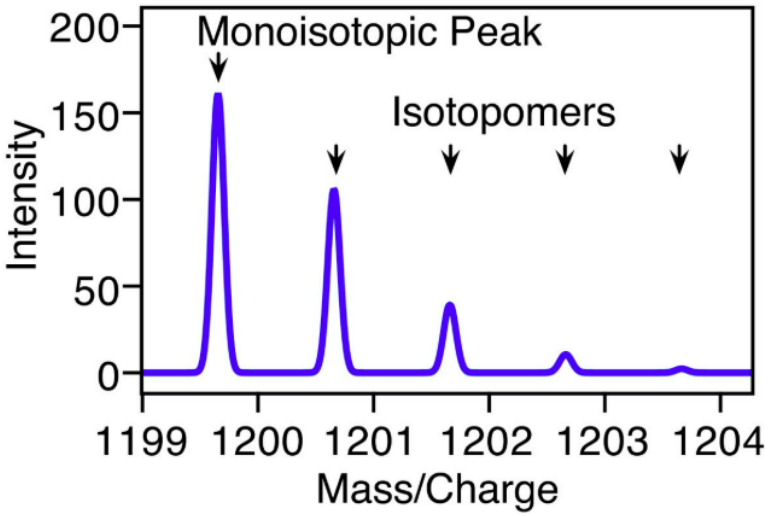
Basic Spectra Interpretation

Monoisotopic Mass

Monoisotopic mass is the exact mass of a molecule or ion, calculated by summing the masses of the most abundant, naturally occurring stable isotope

Peptide HLKTEAEMK

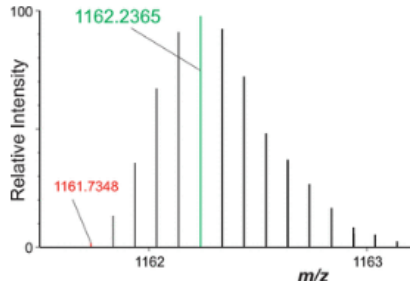




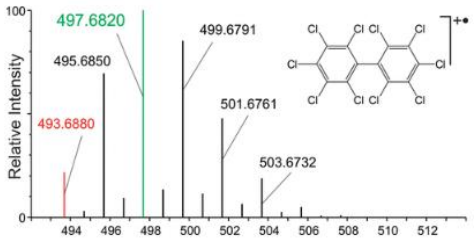
Usually follows such a decreased pattern with a Max int for MIM and lowest mz for a small molecules

Exceptions:

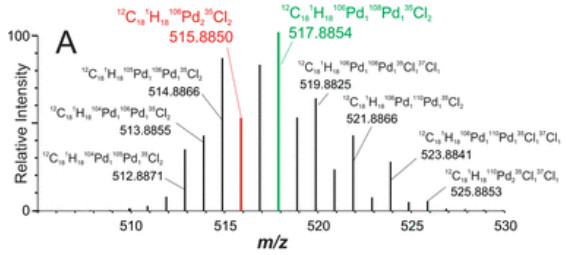
Large Peptide/Protein



Halogenated molecules



Metals

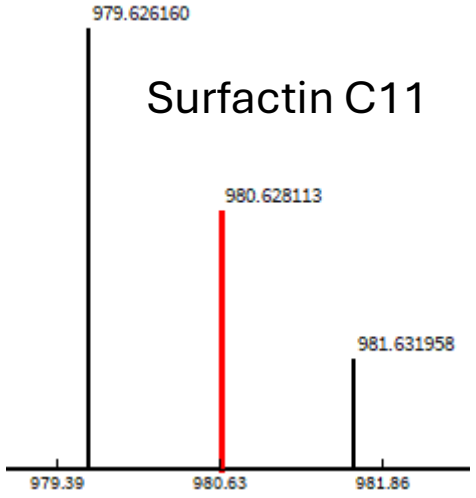


Basic Spectra Interpretation

Monoisotopic Mass

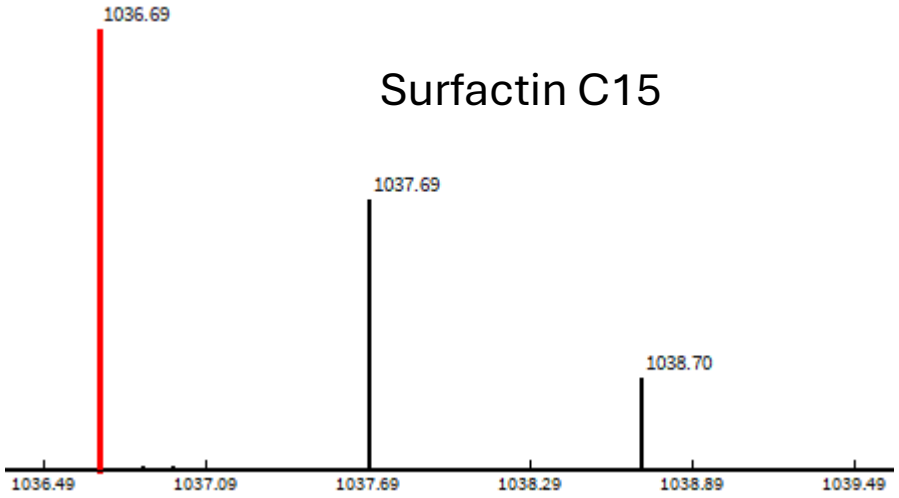
- Search by Monoisotopic Mass (with a specific adduct) by default $[M+H]^+$ or $[M-H]^-$ and Z = ±1
- Compare with Theoretical Pattern (specific adduct, Z)
- Somehow prove structure by Isotopic Pattern
- The most intense in MS1 spectra if clean, or not if fragments, other peaks co-eluted
- The lowest mass in isotopic pattern usually
- The highest m/z value in MS1 (excluding isotopic pattern) if clean peak w/o adducts

☒ Misidentification

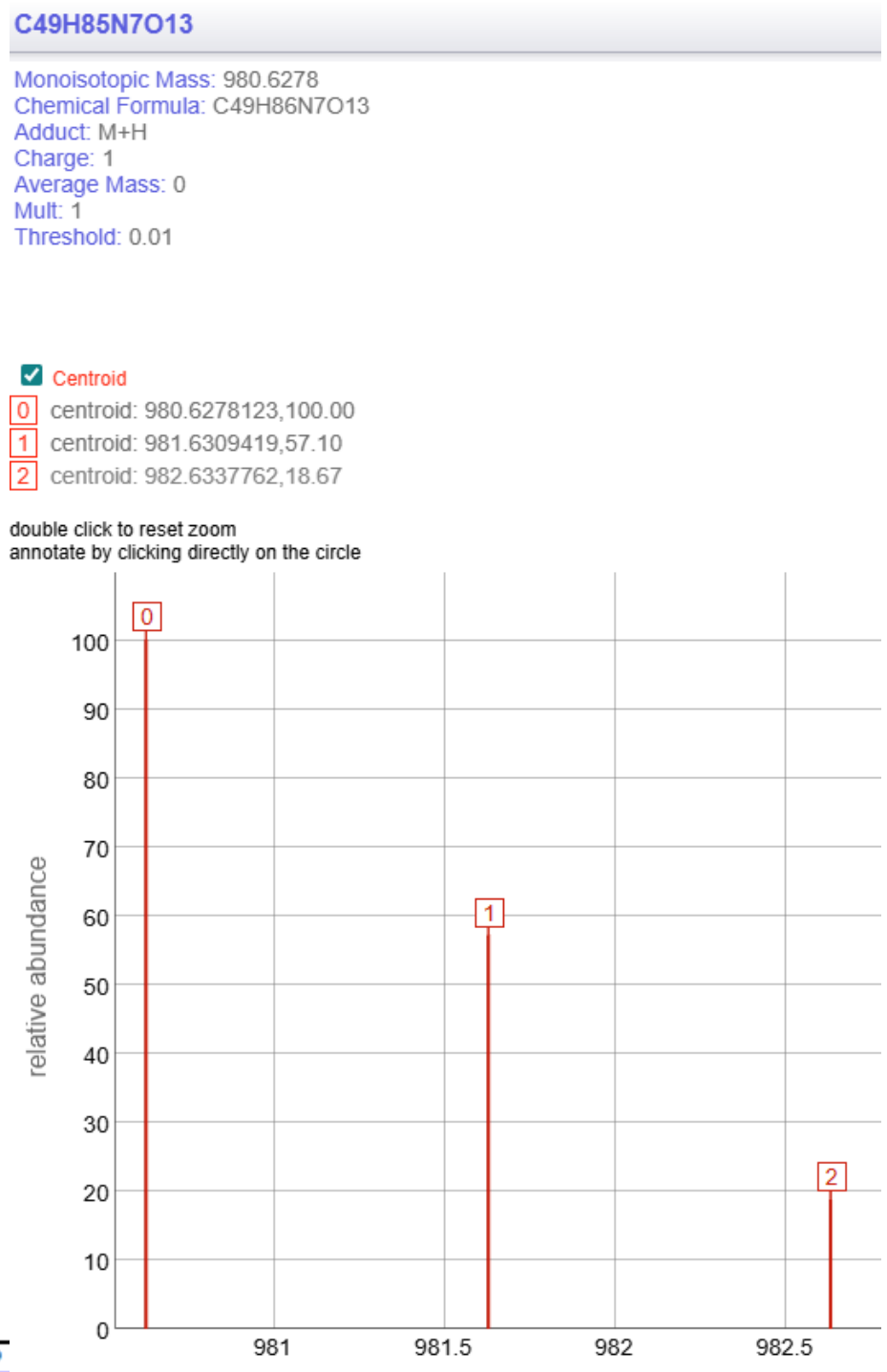


Surfactin C11

☑ Identification



Surfactin C15



Basic Spectra Interpretation

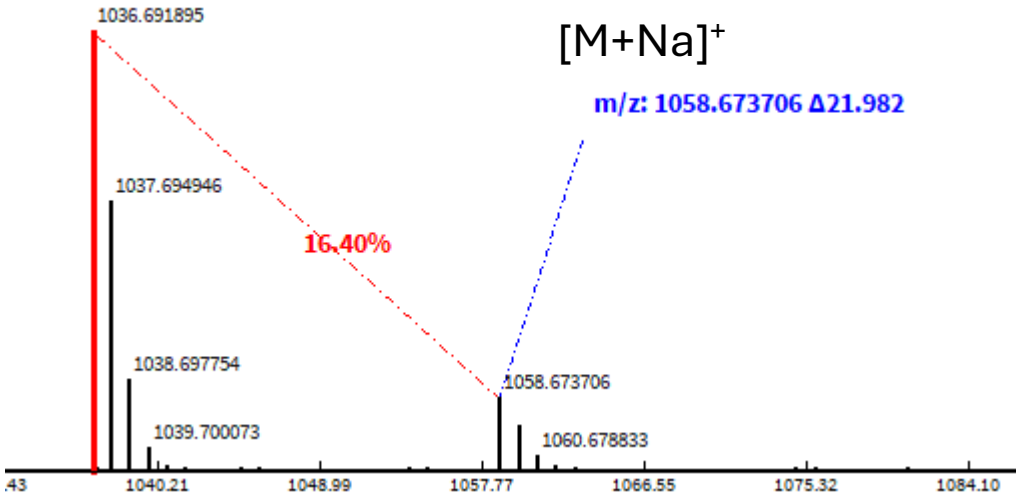
Adducts

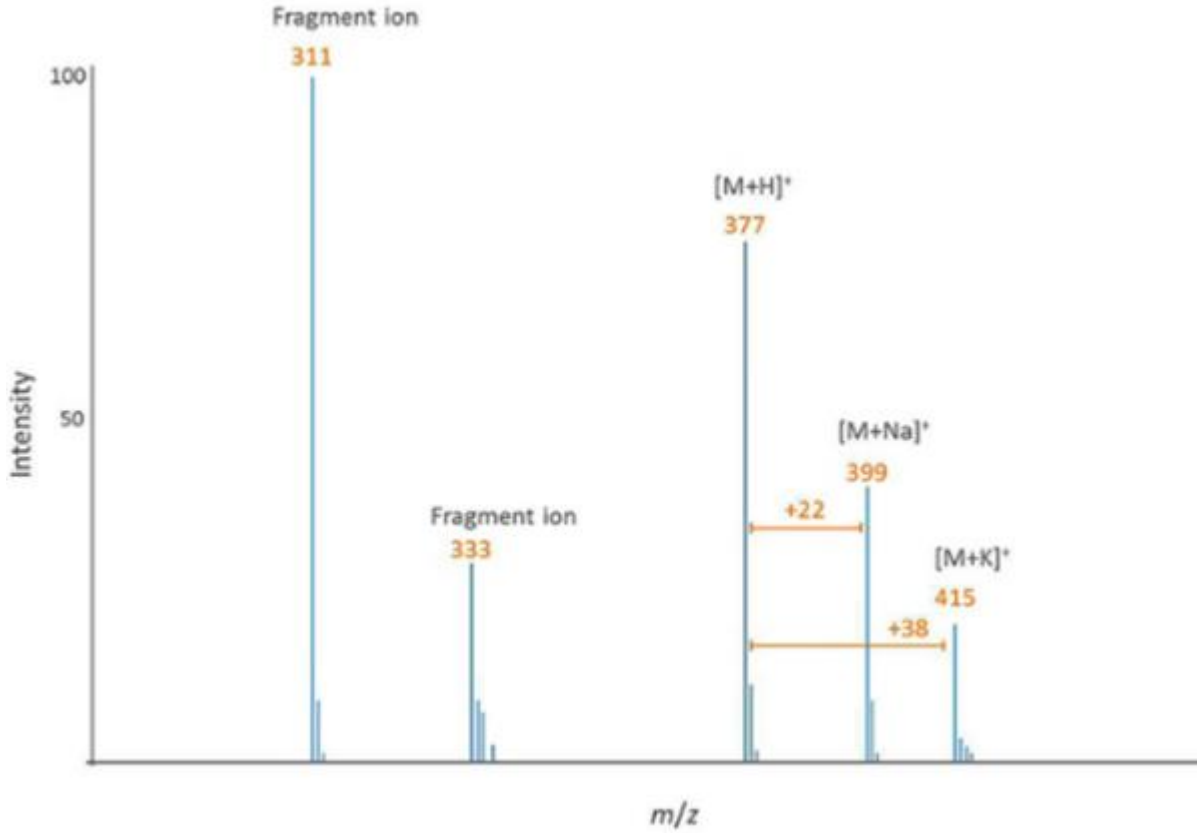
- Usually $[M+H]^+$ or $[M-H]^-$
- Adducts with Na/K and double chared commonly occurred for peptides
- With NH_3, Organic Acids depending on Mobile Phase

Surfactin C15

$[M+H]^+$

$[M+Na]^+$





Common Adducts:

$[M+H]^+$; $[M+Na]^+$; $[M+K]^+$; $[M+NH_4]^+$

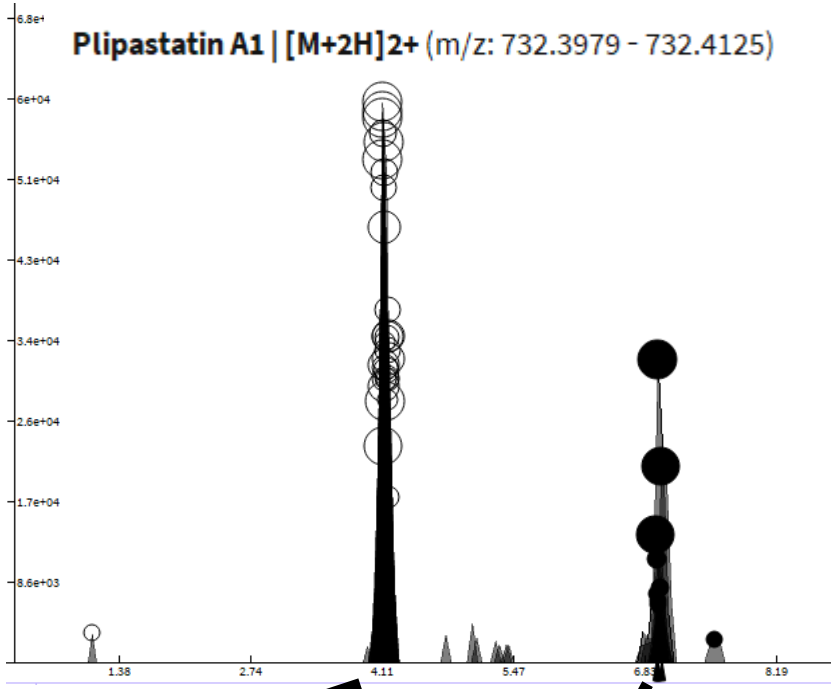
$[M+2H]^{2+}$; $[M+2Na]^{2+}$; $[M+H+Na]^{2+}$

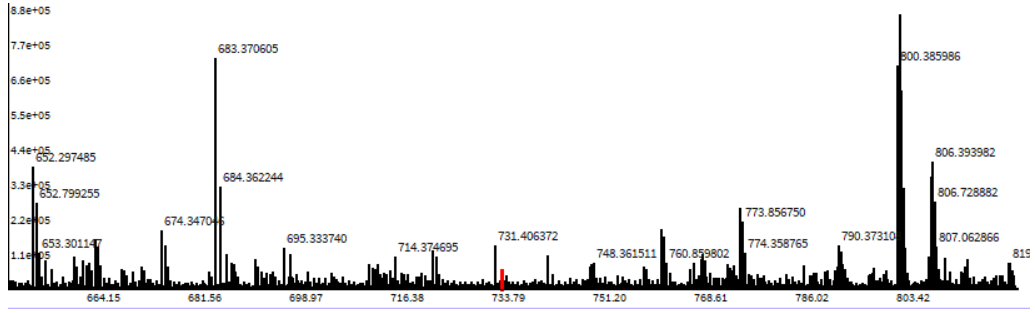
$[M-H]^-$; $[M+Cl]^-$; $[M+HCOO]^-$; $[M+CH_3COO]^-$

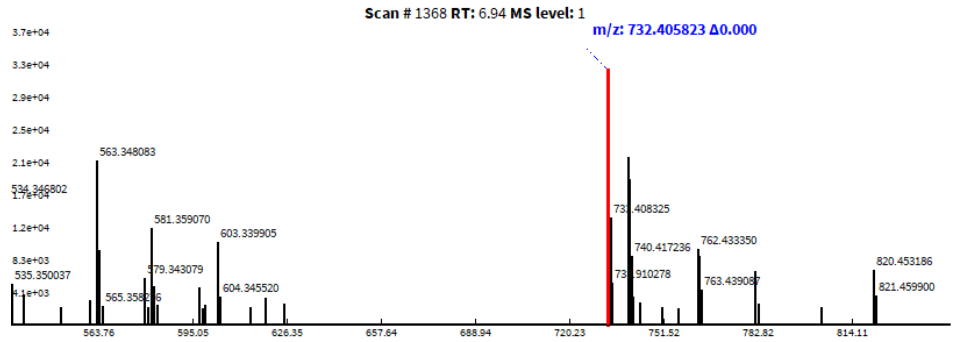
Basic Spectra Interpretation

Abundance

- Higher than noise
- Expected isotopic pattern
- The highest signal if clean peak and no intense fragments
- The highest m/z value (excluding isotopic pattern) if clean peak w/o adducts / fragments / coeluting peaks





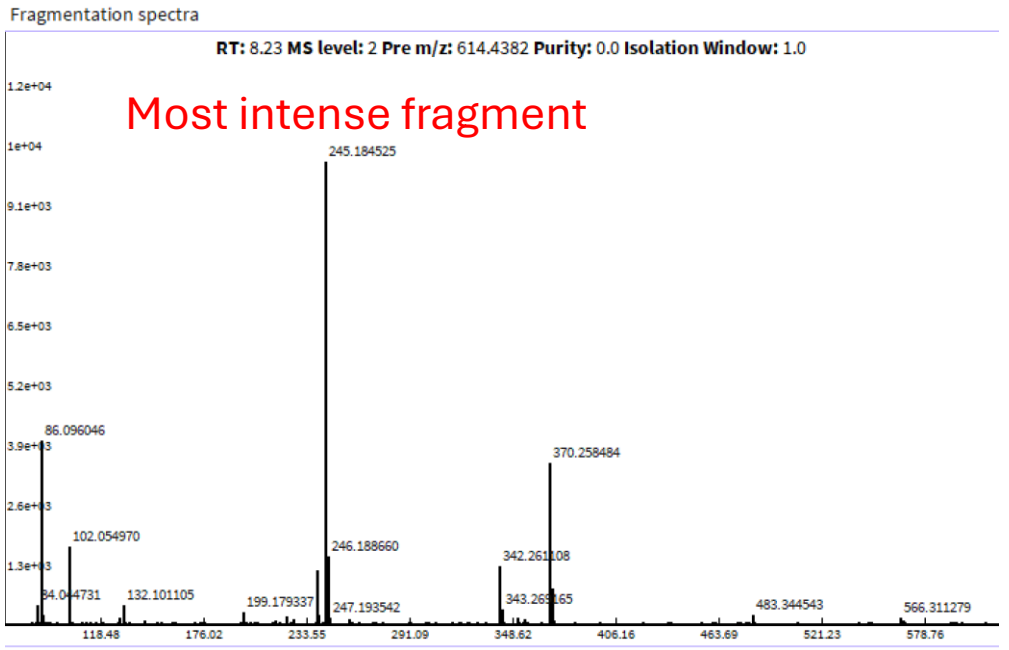


Basic Spectra Interpretation

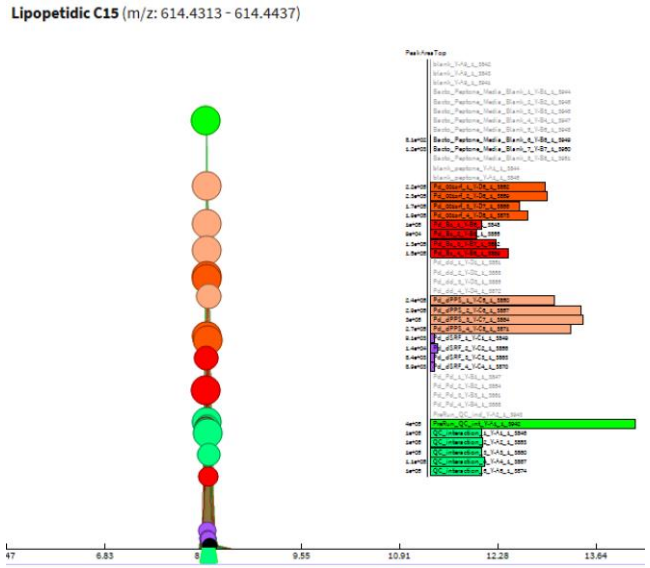
In-Source Fragments

- Perfect correlation by rt, intensity
- Present in MS2

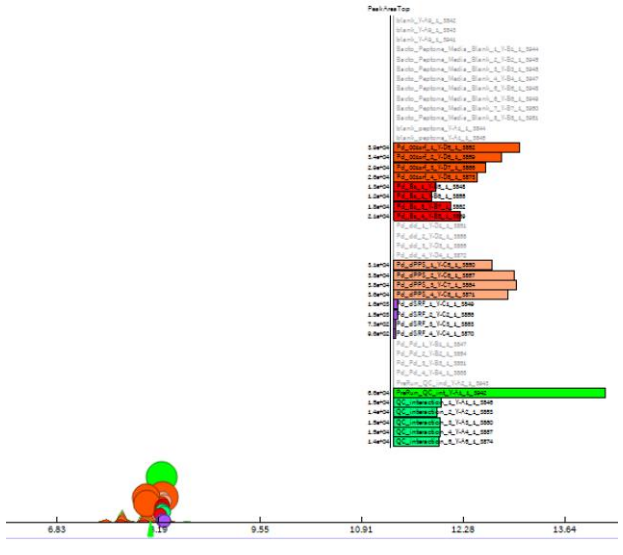
MS2 Fragmentation Spectra



Lipopeptidic C15 product
EIC 614.4832±10ppm



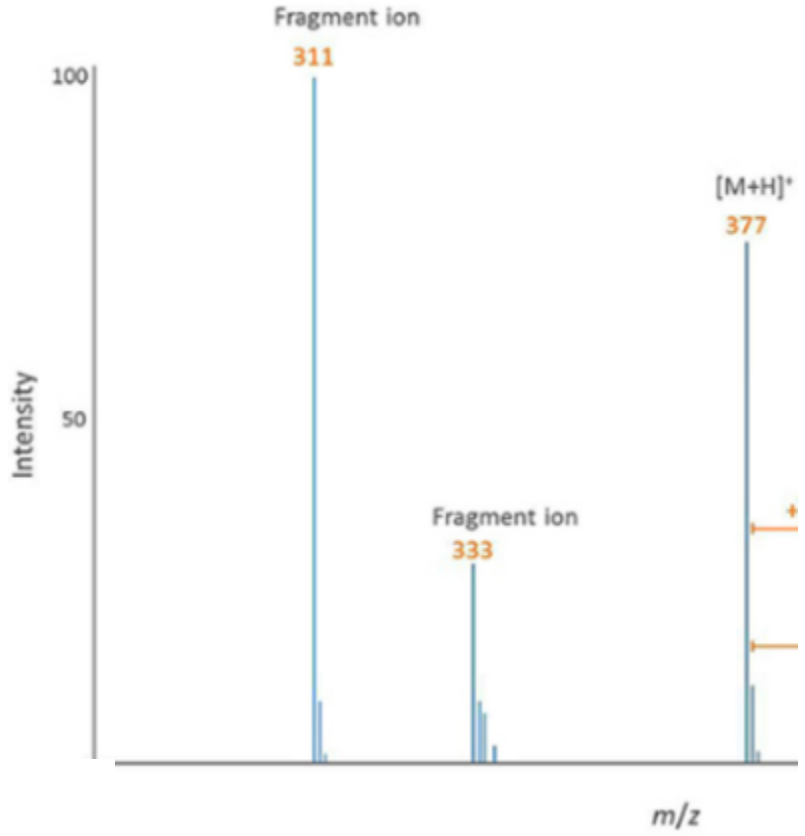
Most intense fragment
EIC 245.1825±10ppm



Basic Spectra Interpretation

MS2 (Fragmentation)

- Presence/Absence of Precursor Ion
- Spectra composition: abundance over the noise, isotopic pattern for fragments
- Used in SIRIUS / GNPS



Surfactin C15