

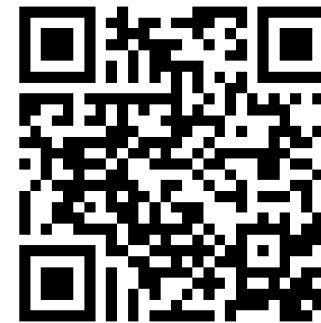
LC-MS-Cheet-Sheet



[https://github.com/plyush1993/
LC-MS-Cheet-Sheet/tree/main](https://github.com/plyush1993/LC-MS-Cheet-Sheet/tree/main)

MSConvert

Convert raw LC-MS to mzXML, mzML



MSConvertGUI (64-bit)

☒ List of Files ☐ File of file names

File:

Output Directory:

Options

Output format: Extension:

Binary encoding precision: ☒ 64-bit ☐ 32-bit

Write index: ☒ Use zlib compression: ☒

TPP compatibility: ☒ Package in gzip: ☐

Use numpress linear compression: ☐

Use numpress short logged float compression: ☐

Use numpress positive integer compression: ☐

Combine ion mobility scans: ☐

SIM as spectra: ☐ SRM as spectra: ☐

Filters

MS levels: - Charge states: -

Scan number: - Number of data points: -

Scan time (seconds): - Collision energy: - ...

Scan event: - Activation type:

Scan polarity: Analyzer type:

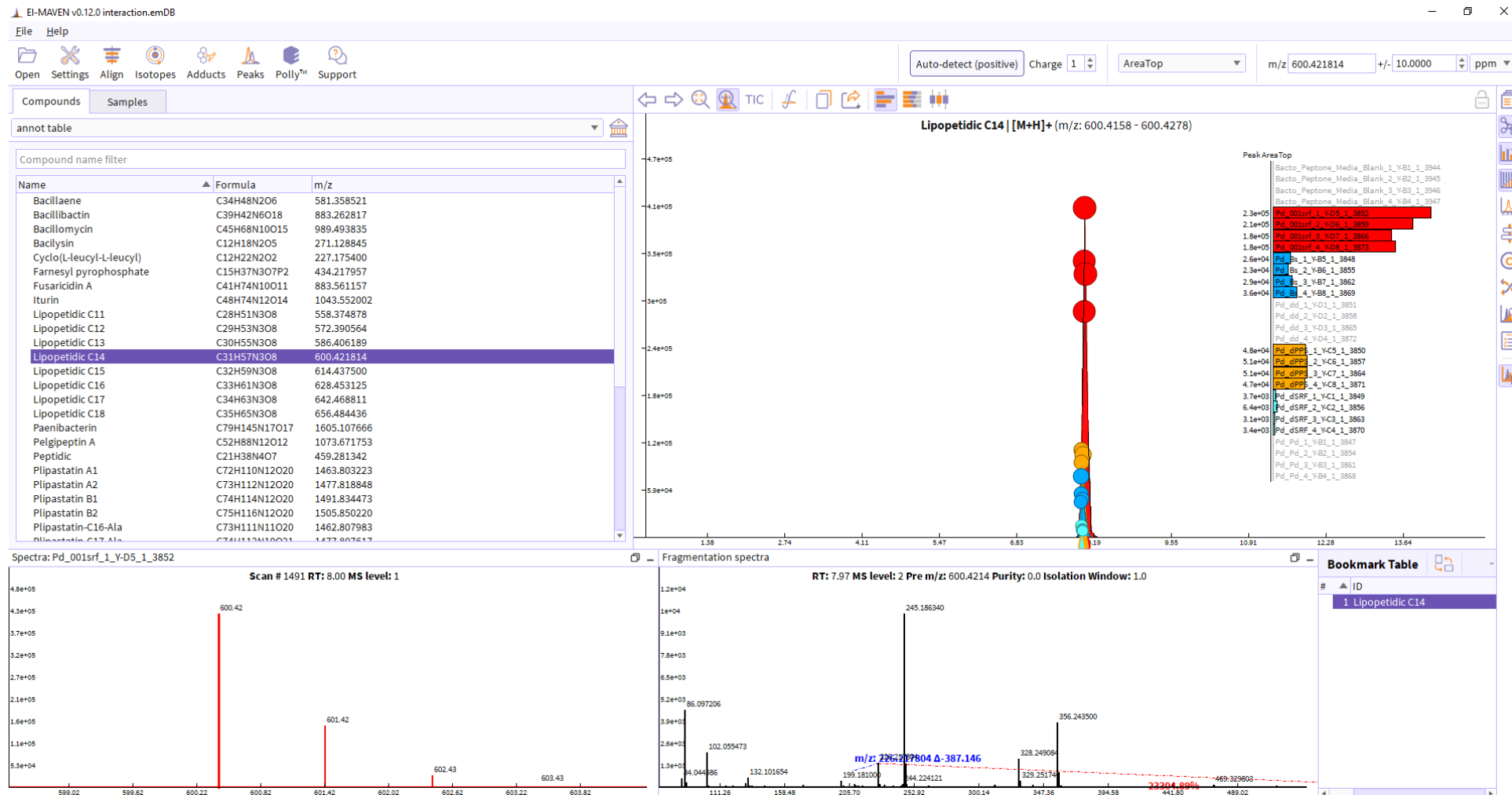
Filter	Parameters
peakPicking	vendor msLevel=1-2
msLevel	1-2

Presets:

Files to convert in parallel:

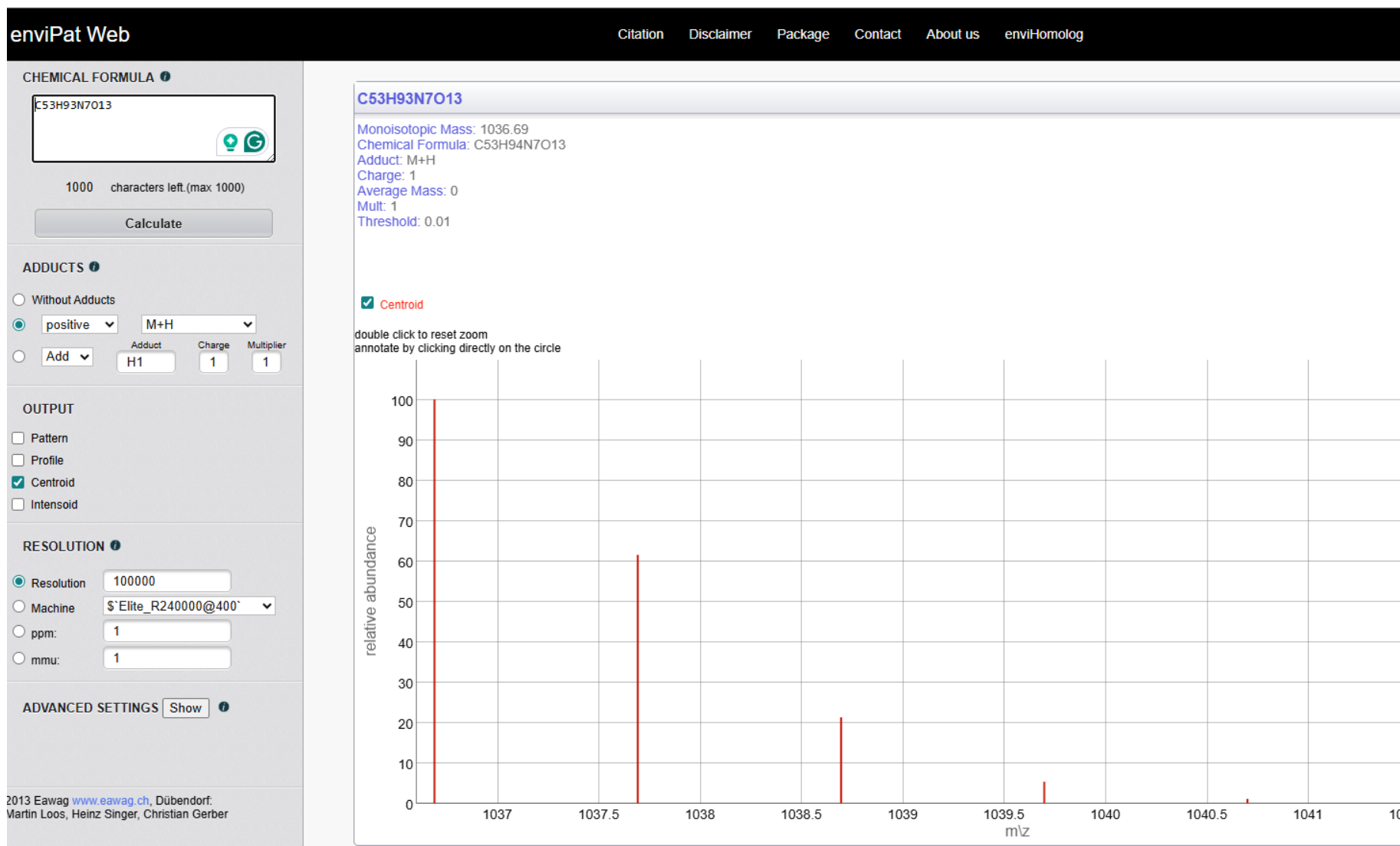
El-Maven

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



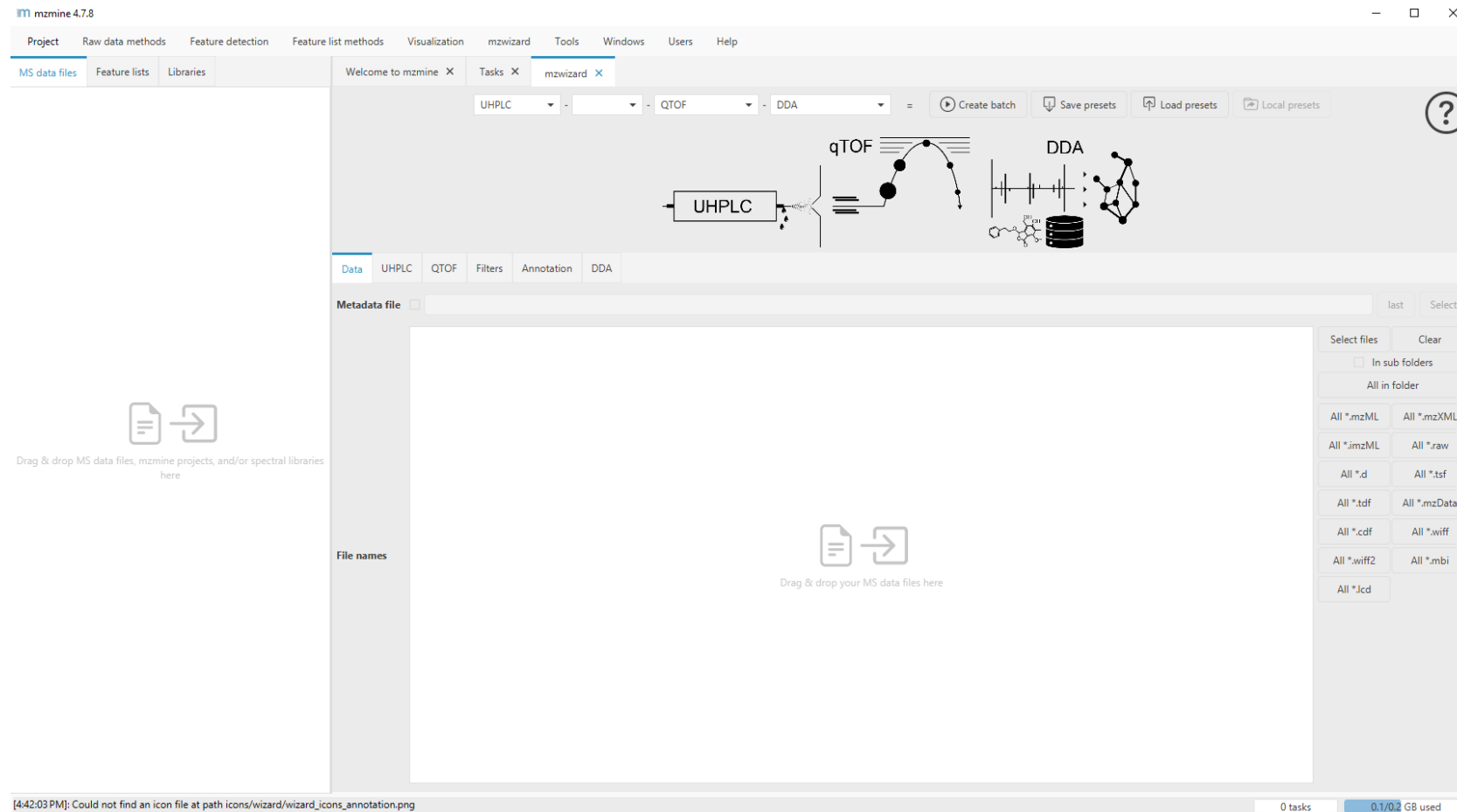
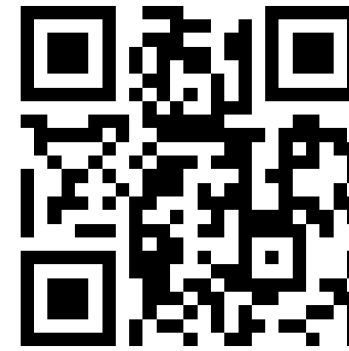
EnviPat

MS1 Isotopic Pattern Generating



MzMine

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



SIRIUS

Annotation / Identification (by mgf from mzmine)



Compute

Preset: **Default** Save Save as Remove Export Import

Advanced

Global Configuration

Instrument: **Q-TOF**

MS2 mass accuracy (ppm): **10**

Possible Adducts

- ☒ [M + H]⁺
- ☒ [M + Na]⁺
- ☒ [M + K]⁺
- ☐ [M]⁺
- ☐ [M + H₃N + H]⁺

all none

Search DBs

- ☐ test
- ☐ PubChem
- ☒ Biocyc
- ☒ Blood Exposome
- ☒ ChEBI

all none bio

Spectral Library Search

Spectral Matching

Identity Search

Precursor Deviation (ppm): **20**

Analogue Search

Perform Analogue Search: ☒

SIRIUS - Molecular Formula Identification

SIRIUS

Strategy

De novo + bottom up (recommended)

Perform de novo below m/z: **400**

Element Filter

Apply 'allowed elements' filter to: **De novo**

Allowed elements: **H,C,N,O,P** ... Re-detect

Predict properties: CSI:FingerID - Fingerprint Prediction & CANOPUS - Compound Class Prediction

Predict

General

Score threshold: ☒

CSI:FingerID - Structure Database Search

Search DBs

Search strategy

PubChem as fallback: ☒

Confidence mode: **APPROXIMATE**

MSNovelist - De Novo Structure Generation

MSNovelist

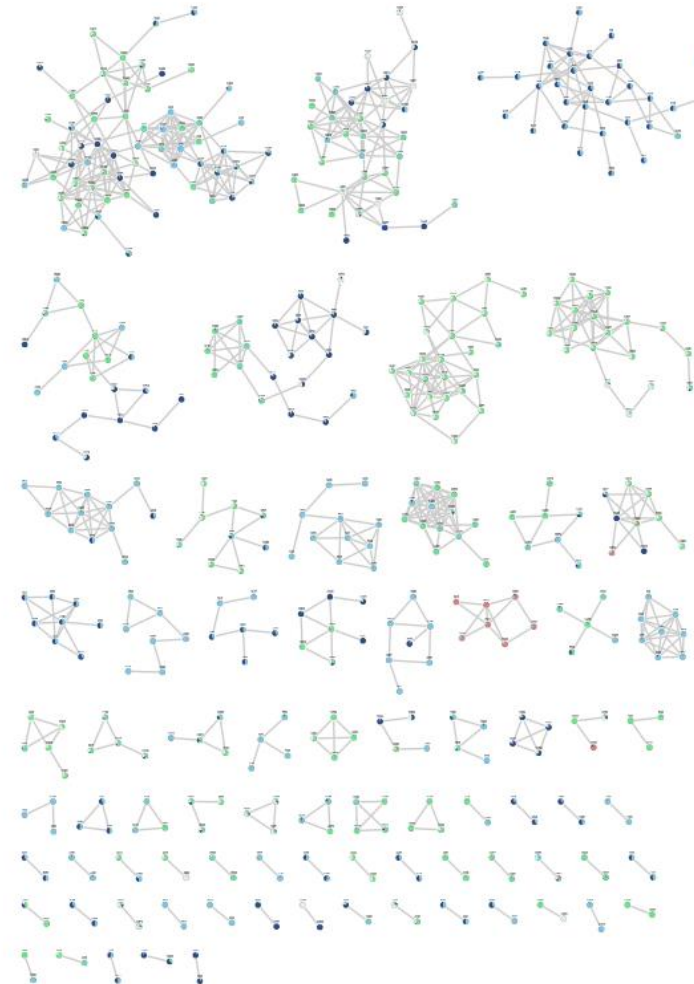
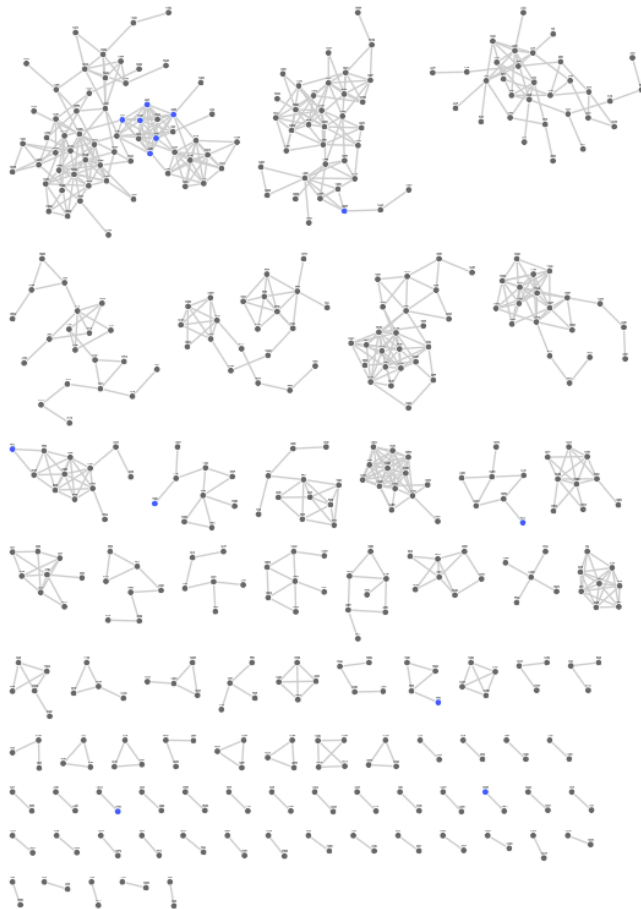
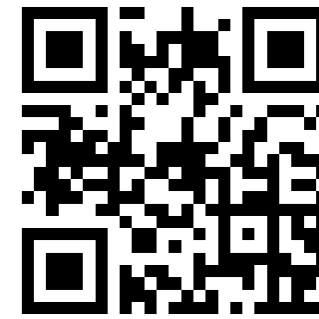
☒ Recompute already computed tasks?

Show Command Show JSON

Compute Cancel

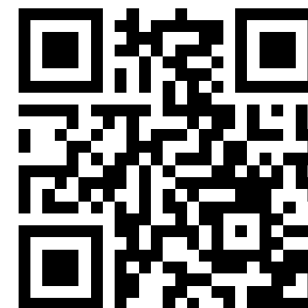
GNPS










Molecular Networking
(by mgf, peak table from mzmine)

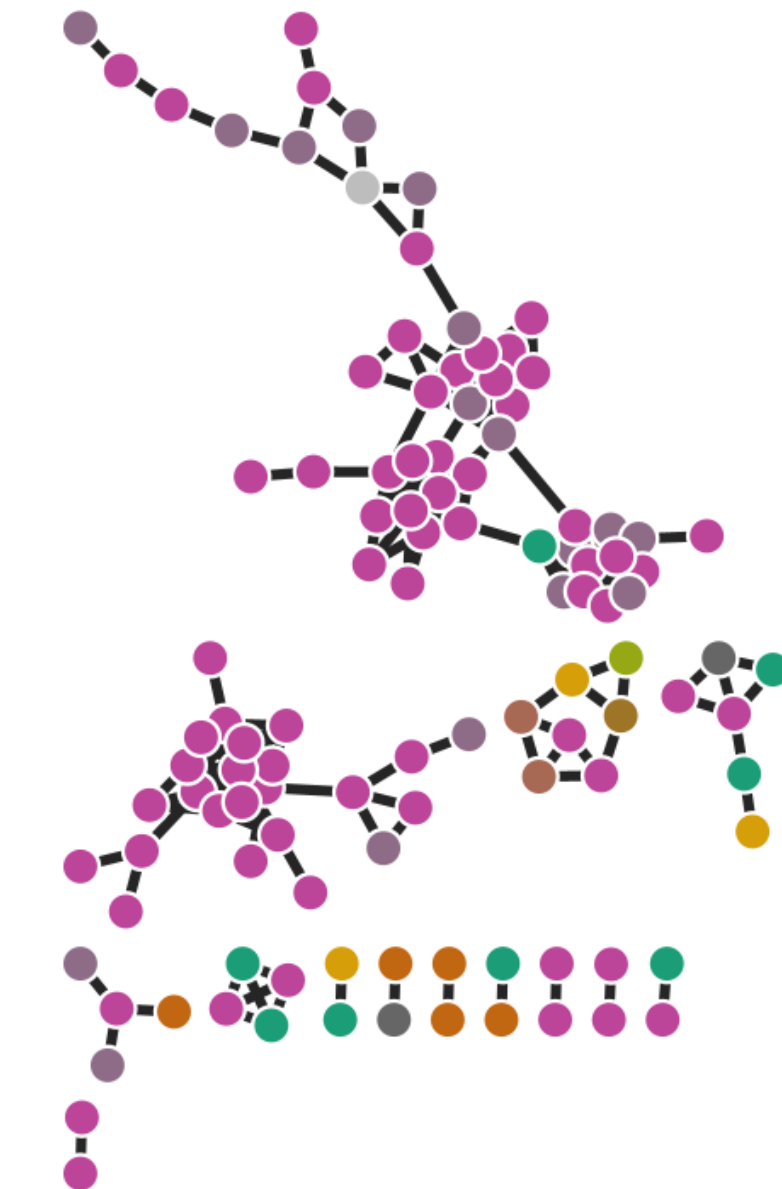


Cytoscape

Combine GNPS + Sirius



Benzenoids		f
Lipids and lipid-like molecules		f
Organic Polymers		f
Organic acids and derivatives		f
Organic oxygen compounds		f
Organohalogen compounds		f
Organoheterocyclic compounds		f
Organometallic compounds		f
Phenylpropanoids and polyketides		f



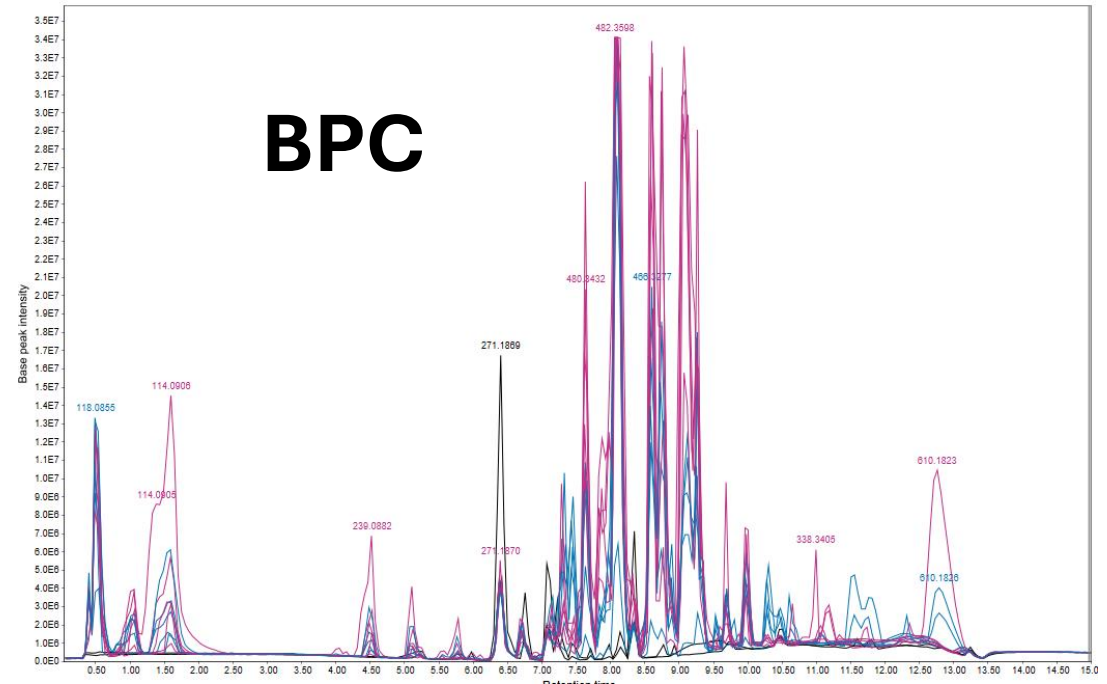
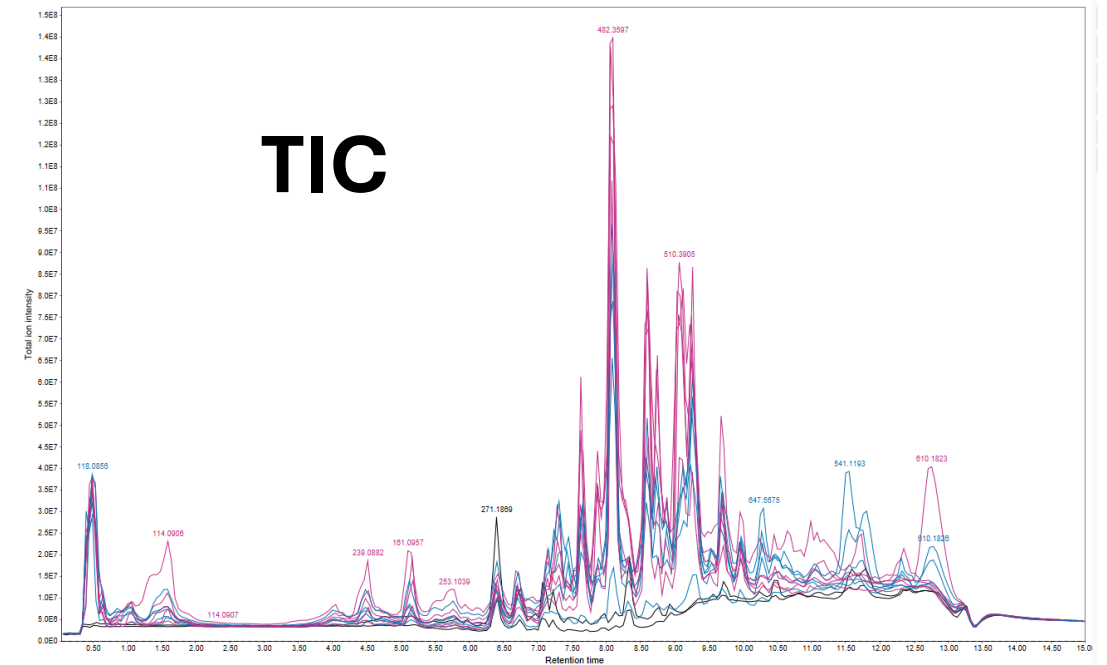
Chromatography (LC-MS)

TIC & BPC

Check:

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

Blank_2_R-C1_1_5
Blank_1_R-C1_1_5
Sample_10 sw_R-
Sample_1_R-D1_1
Sample_2_R-D2_1
Sample_3_R-D3_1
Sample_4_R-D4_1
Sample_5_R-D5_1
Sample_6 sw_R-D
Sample_7 sw_R-D
Sample_8 sw_R-D
Sample_9 sw_R-D

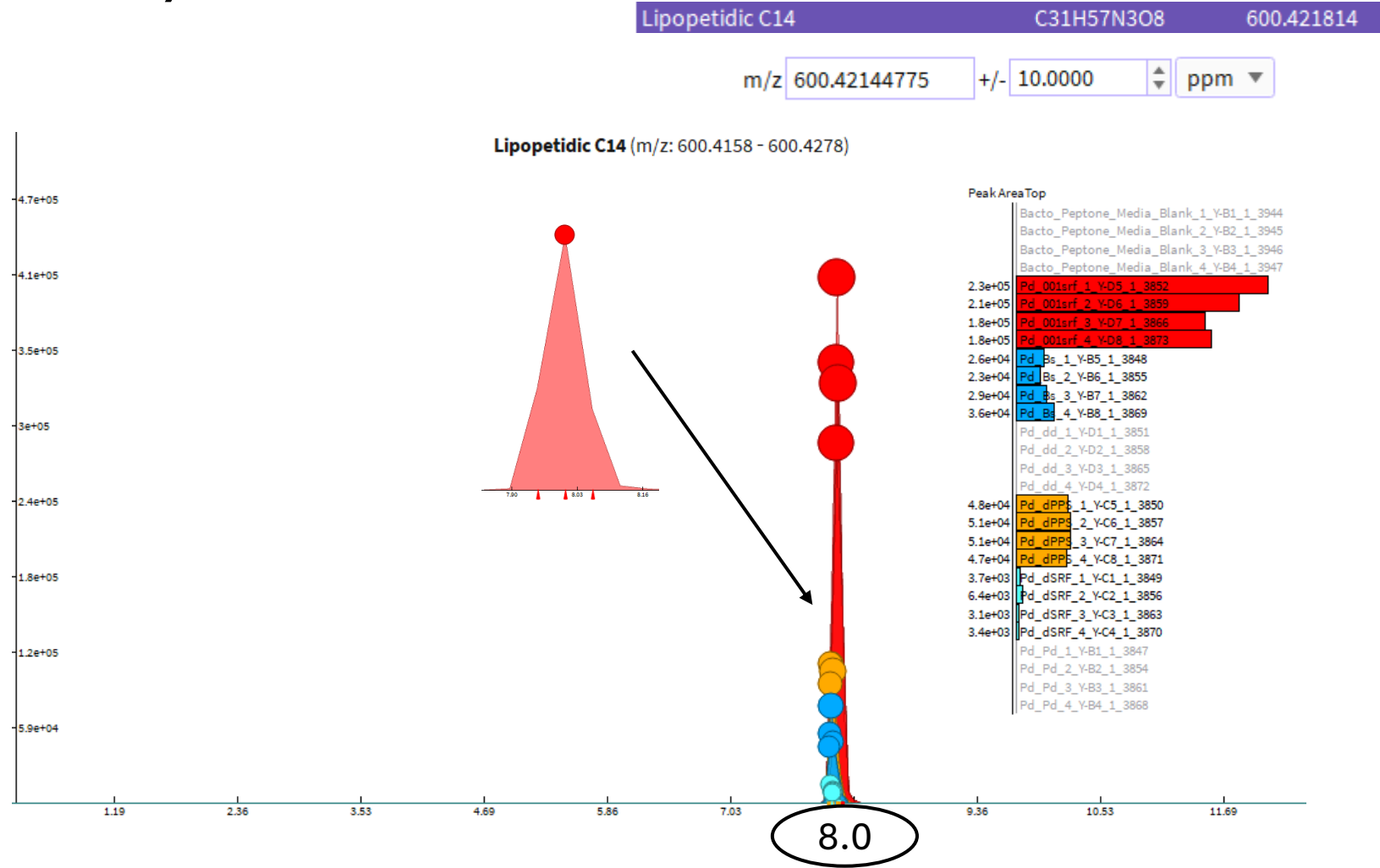


Chromatography (LC-MS)

EIC (MS1)

Check:

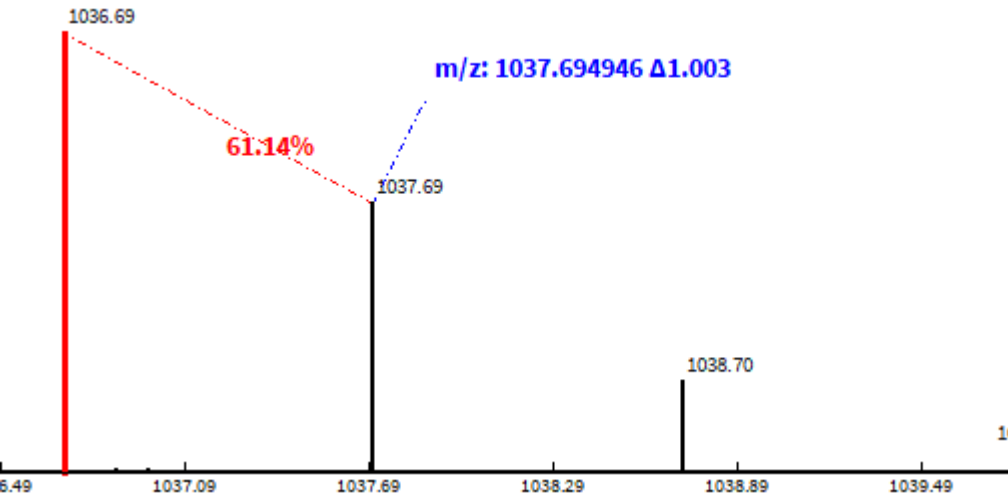
- Peak shape
- EIC in 10 ppm range
- Retention time
- Noise level
- Compare with Blanks / Replicate



Basic Spectra Interpretation

Charge $z = +1$

- Difference
- Ratio

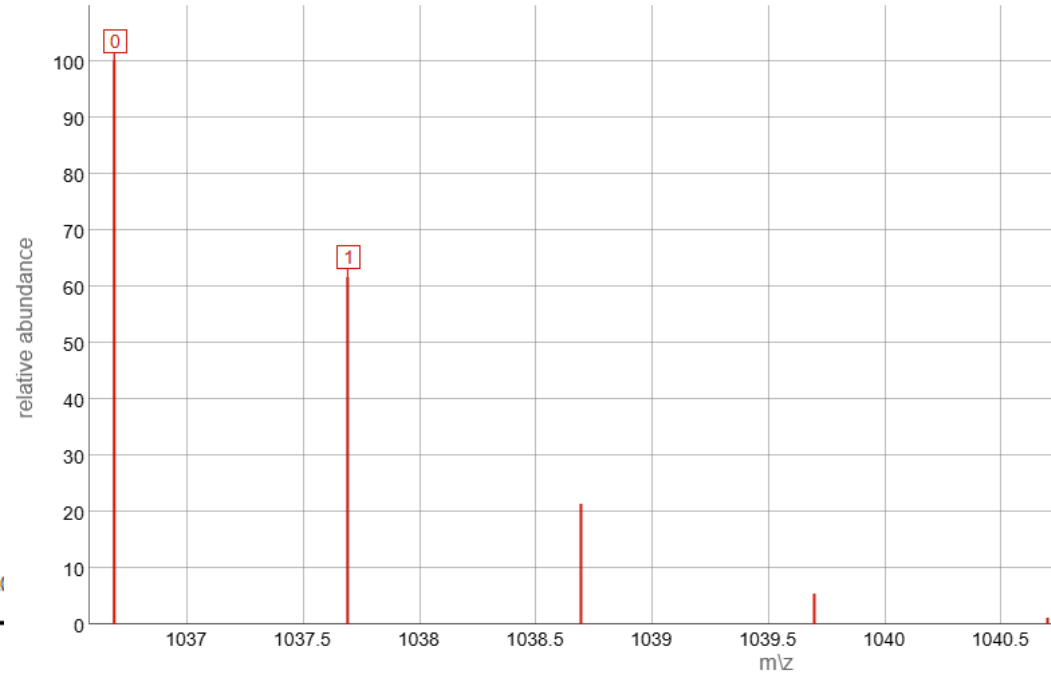


Monoisotopic Mass: 1036.69
Chemical Formula: C₅₃H₉₄N₇O₁₃
Adduct: M+H
Charge: 1
Average Mass: 0
Mult: 1
Threshold: 0.01

Surfactin C15

☒ Centroid
☐ centroid: 1036.6904126,100.00
☐ centroid: 1037.6935628,61.52

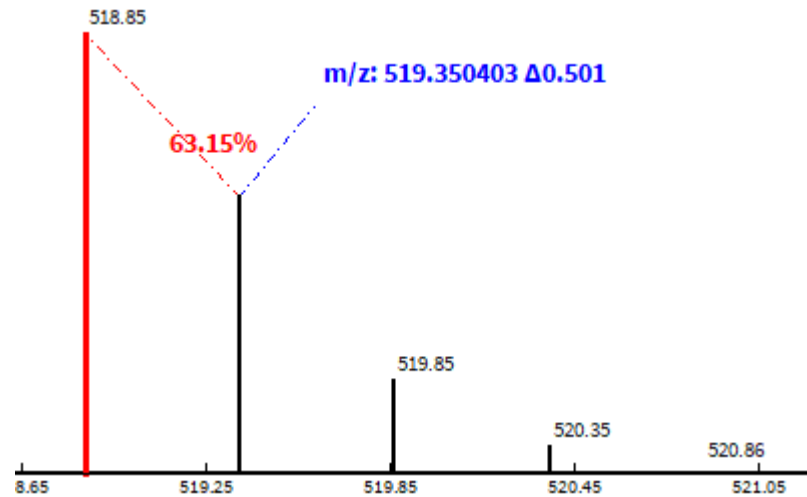
double click to reset zoom
annotate by clicking directly on the circle



Basic Spectra Interpretation

Charge $z = +2$

- Difference
- Ratio

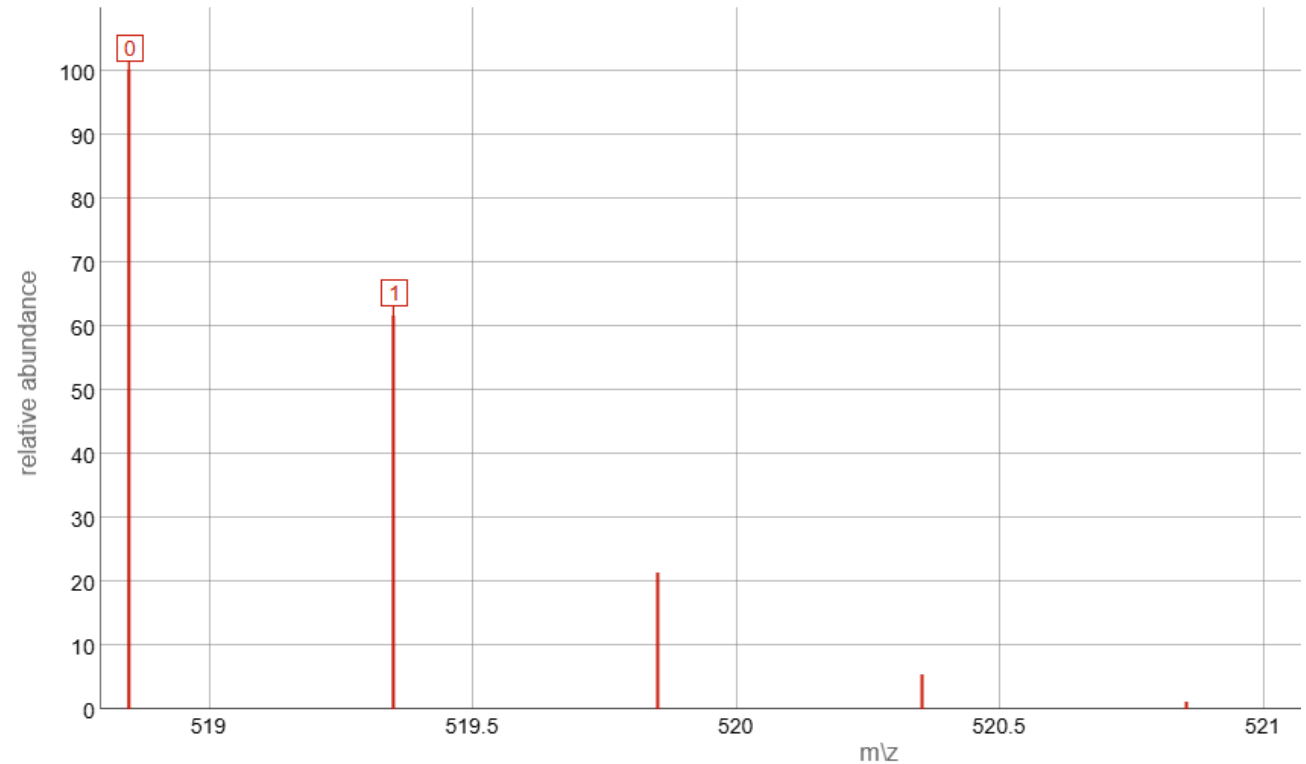


Monoisotopic Mass: 518.8488
Chemical Formula: C₅₃H₉₅N₇O₁₃
Adduct: M+2H
Charge: 2
Average Mass: 0
Mult: 1
Threshold: 0.01

Surfactin C15

☒ Centroid
0 centroid: 518.8488445,100.00
1 centroid: 519.3504199,61.53

double click to reset zoom
annotate by clicking directly on the circle

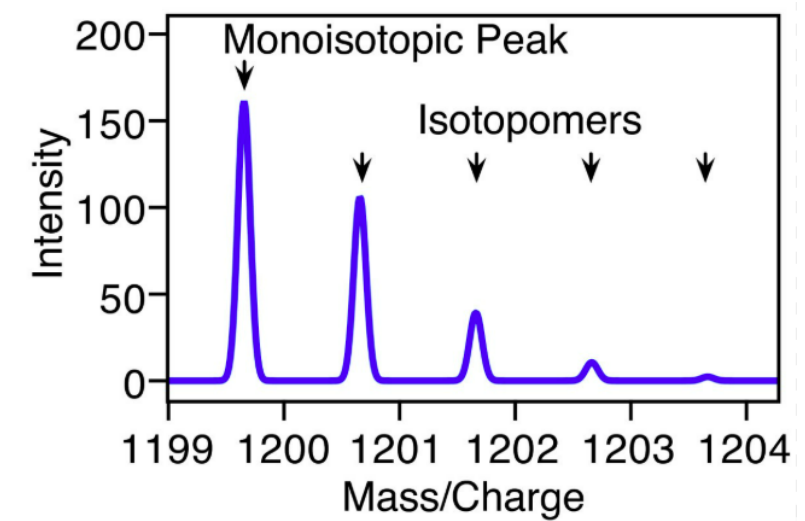
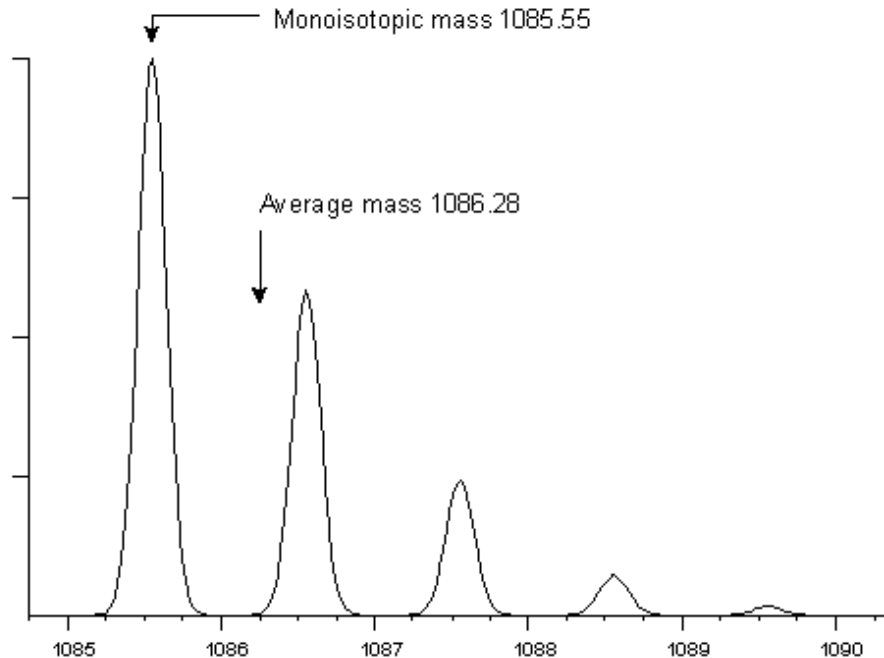


Basic Spectra Interpretation

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

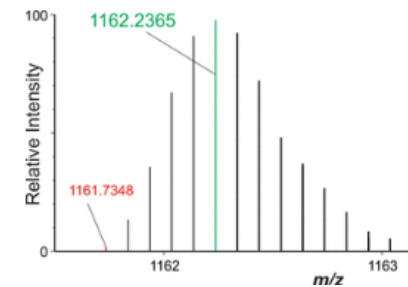
Peptide HLKTEAEMK



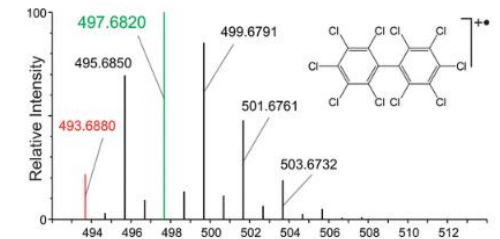
Usually follows such a decreased pattern with a Max int for **MIM** and lowest m/z for a small molecules

Exceptions:

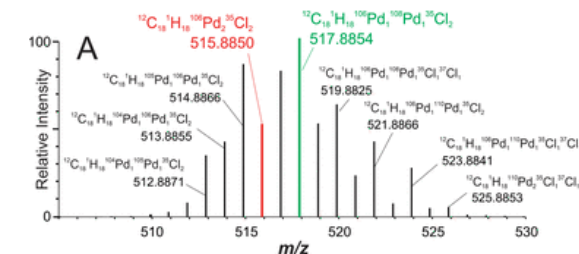
Large Peptide/Protein



Halogenated molecules



Metals

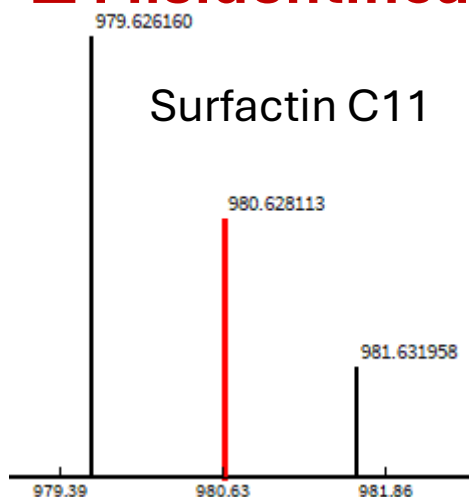


Basic Spectra Interpretation

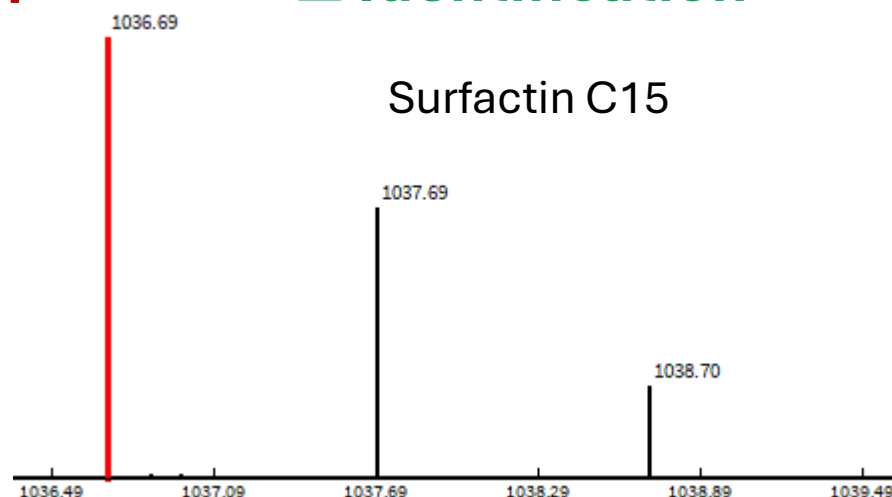
Monoisotopic Mass

- Search by Monoisotopic Mass (with a specific adduct)
by default $[M+H]^+$ or $[M-H]^-$ and $Z = \pm 1$
- 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**



✓ **Identification**



C49H85N7O13

Monoisotopic Mass: 980.6278
Chemical Formula: C49H86N7O13
Adduct: M+H
Charge: 1
Average Mass: 0
Mult: 1
Threshold: 0.01

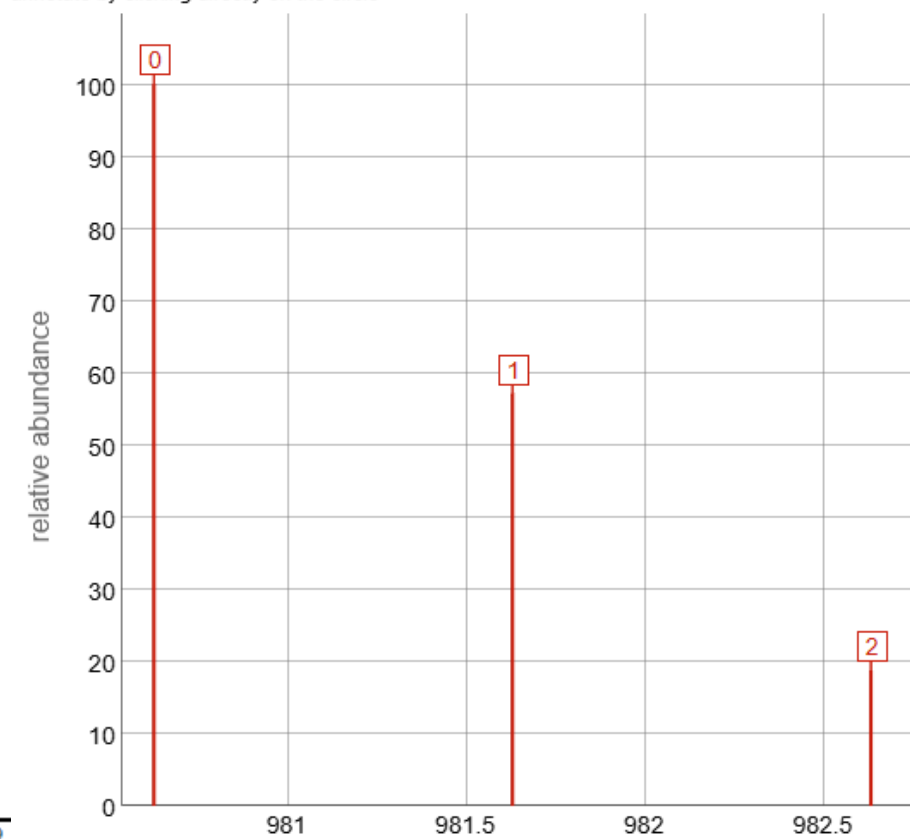
☑ Centroid

0 centroid: 980.6278123,100.00

1 centroid: 981.6309419,57.10

2 centroid: 982.6337762,18.67

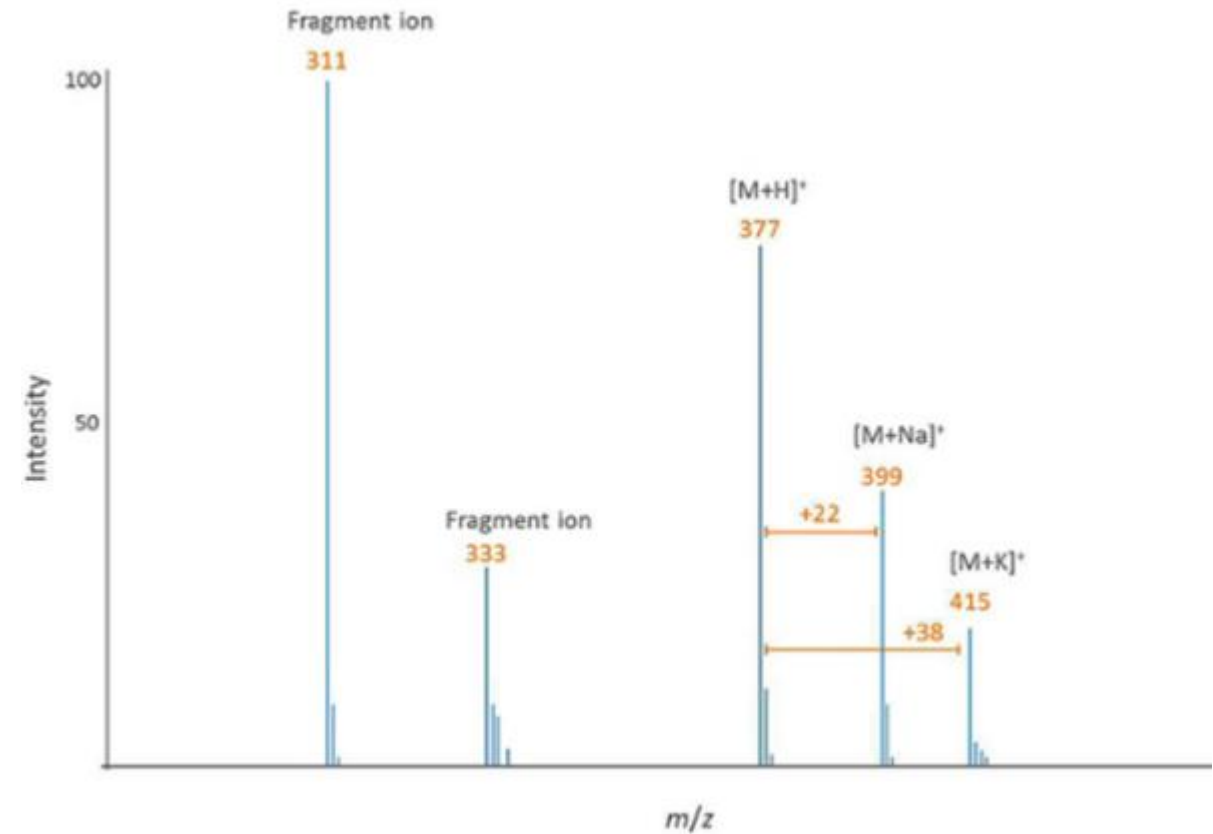
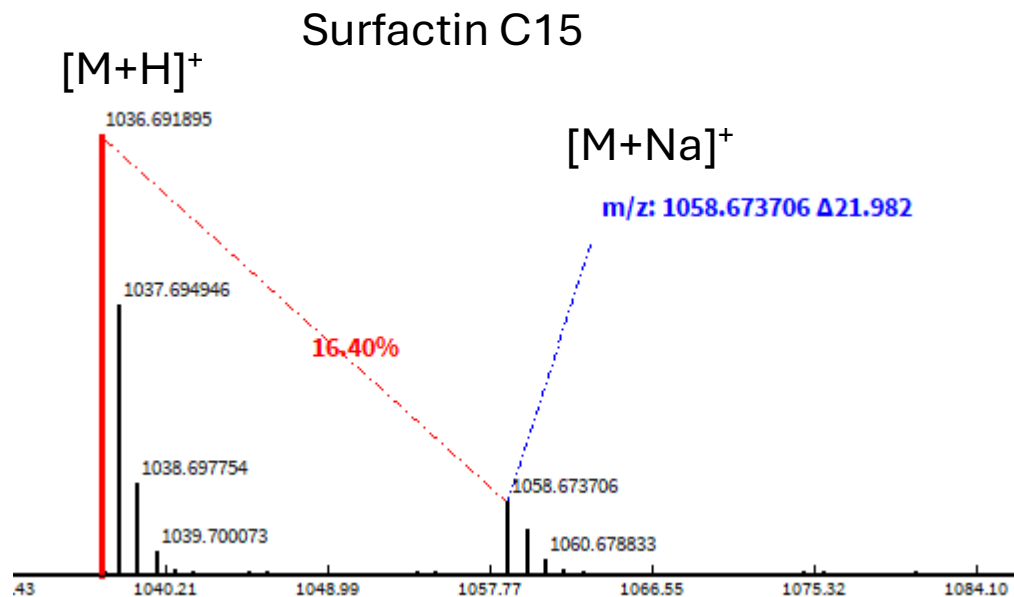
double click to reset zoom
annotate by clicking directly on the circle



Basic Spectra Interpretation

Adducts

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



Common Adducts:

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

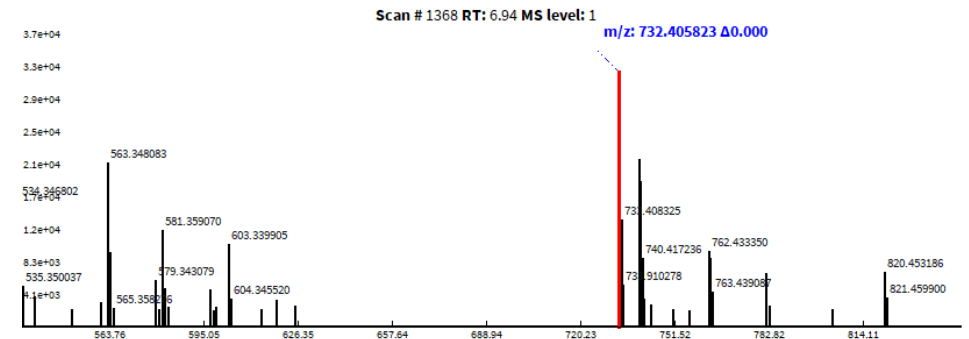
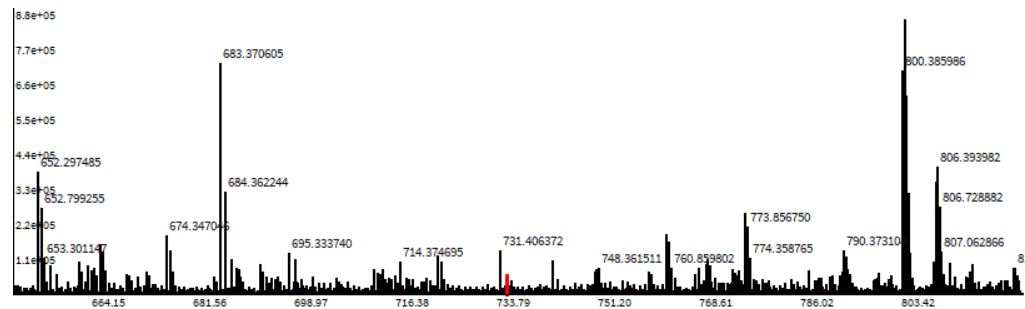
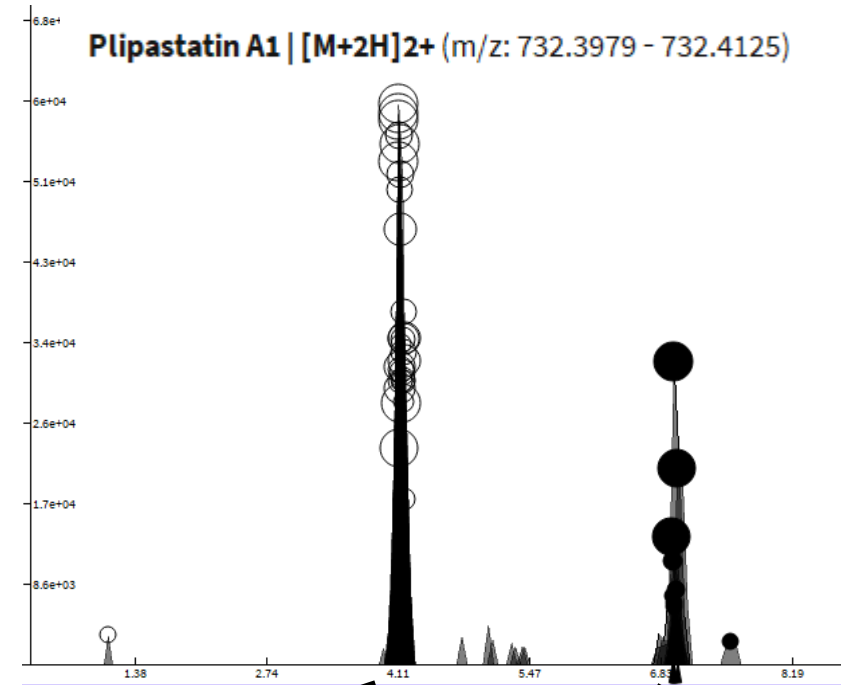
$[M+2H]^{2+}$; $[M+2Na]^{2+}$; $[M+H+Na]^{2+}$

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

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

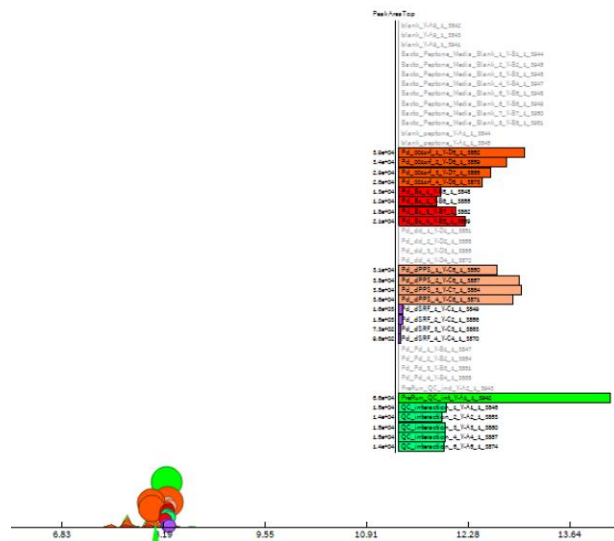
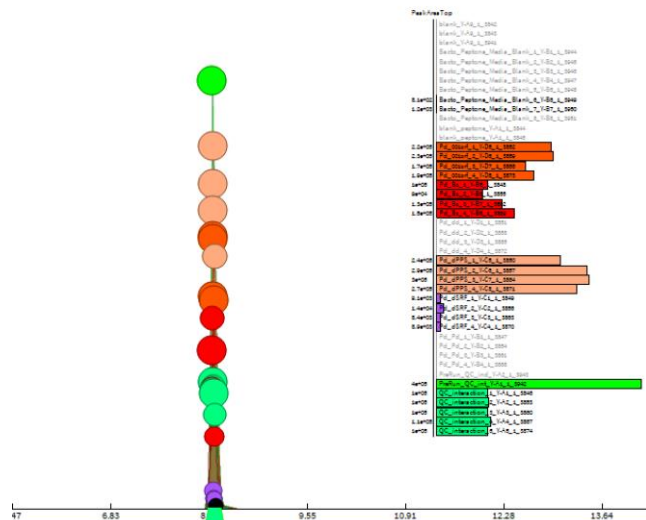
- Perfect correlation by rt, intensity
- Present in MS2

Lipopeptidic C15 product
EIC 614.4832±10ppm

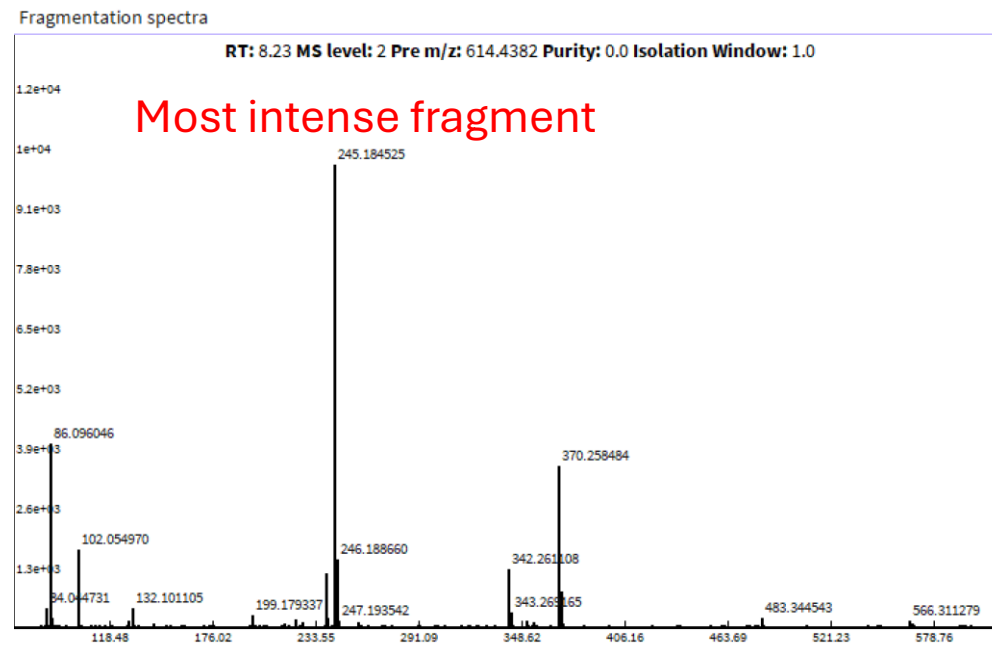
Most intense fragment
EIC 245.1825±10ppm

Lipopeptidic C15 (m/z: 614.4313 - 614.4437)

(m/z: 245.1825 - 245.1875)



MS2 Fragmentation Spectra



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

