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Using MZmine

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BBRB 711

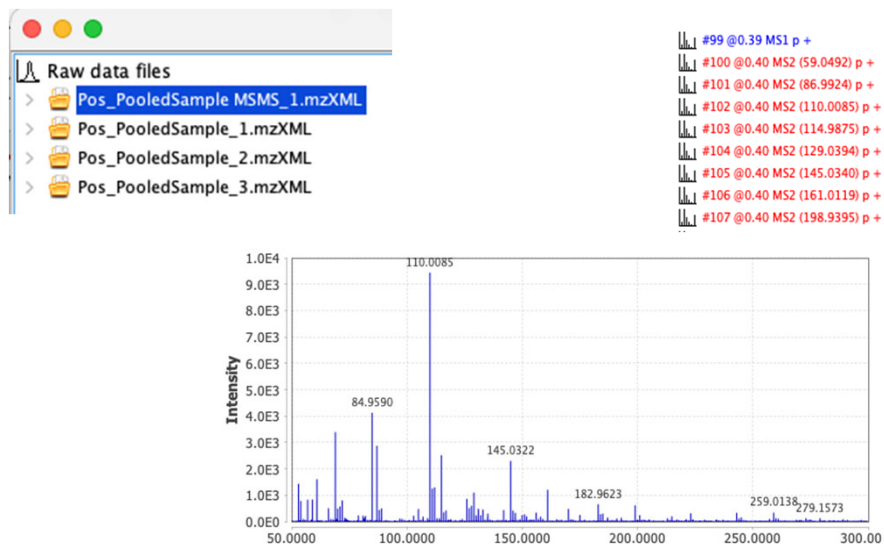
1

Loading MZmine

- **After downloading and opening Mzmine version 2.53 from GitHub, try to launch it.**
 - Mac Users will encounter security problems
 - Overcoming them takes time – you have to approve each module in the security pane – you can only do one at time, so it takes multiple launches of the software
- **Now load the three pooled samples and the pooled MSMS sample**
 - Do not alter the title of the uploaded files
- **Highlight the four files, and sort them**

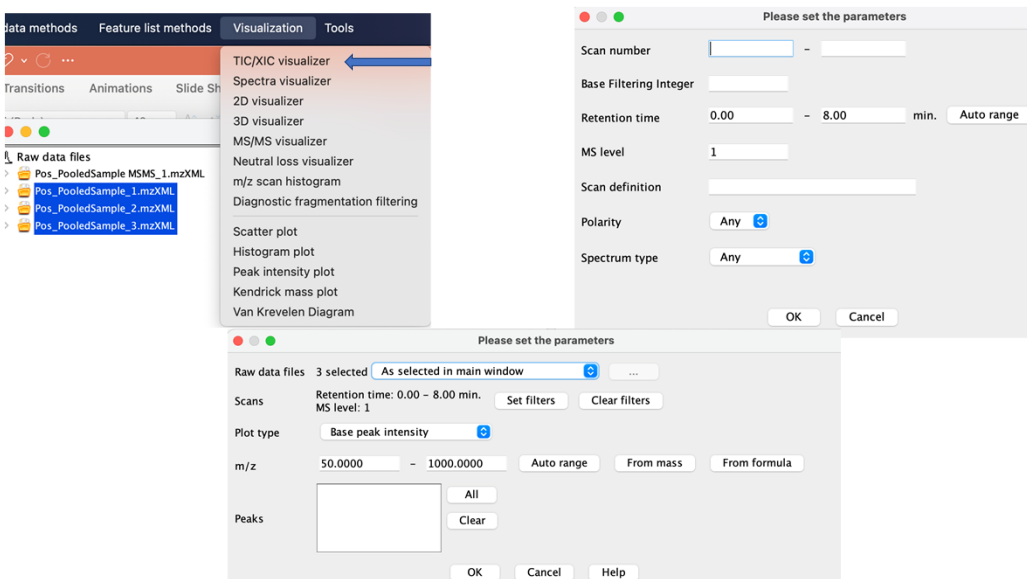
2

What is in each file?



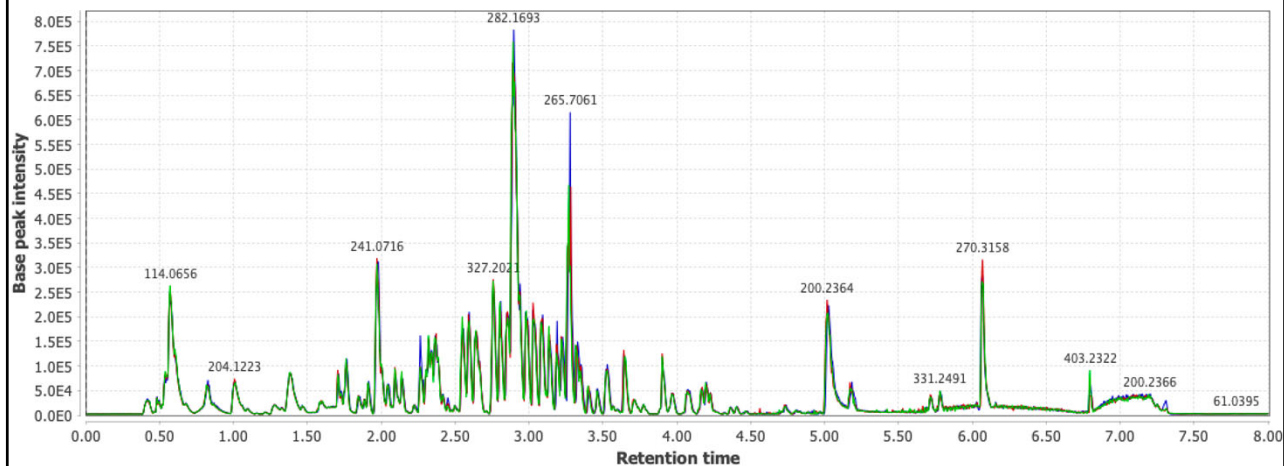
3

Under "Visualization", load pooled samples



4

Total ion current of the three pooled samples



5

Looking at the data with 2D-plots

The screenshot shows a software interface with a menu for visualization options and a dialog box for setting parameters for a 2D plot.

Visualization Menu Options:

- TIC/XIC visualizer
- Spectra visualizer
- 2D visualizer (highlighted with a blue arrow)
- 3D visualizer
- MS/MS visualizer
- Neutral loss visualizer
- m/z scan histogram
- Diagnostic fragmentation filtering
- Scatter plot
- Histogram plot
- Peak intensity plot
- Kendrick mass plot
- Van Krevelen Diagram

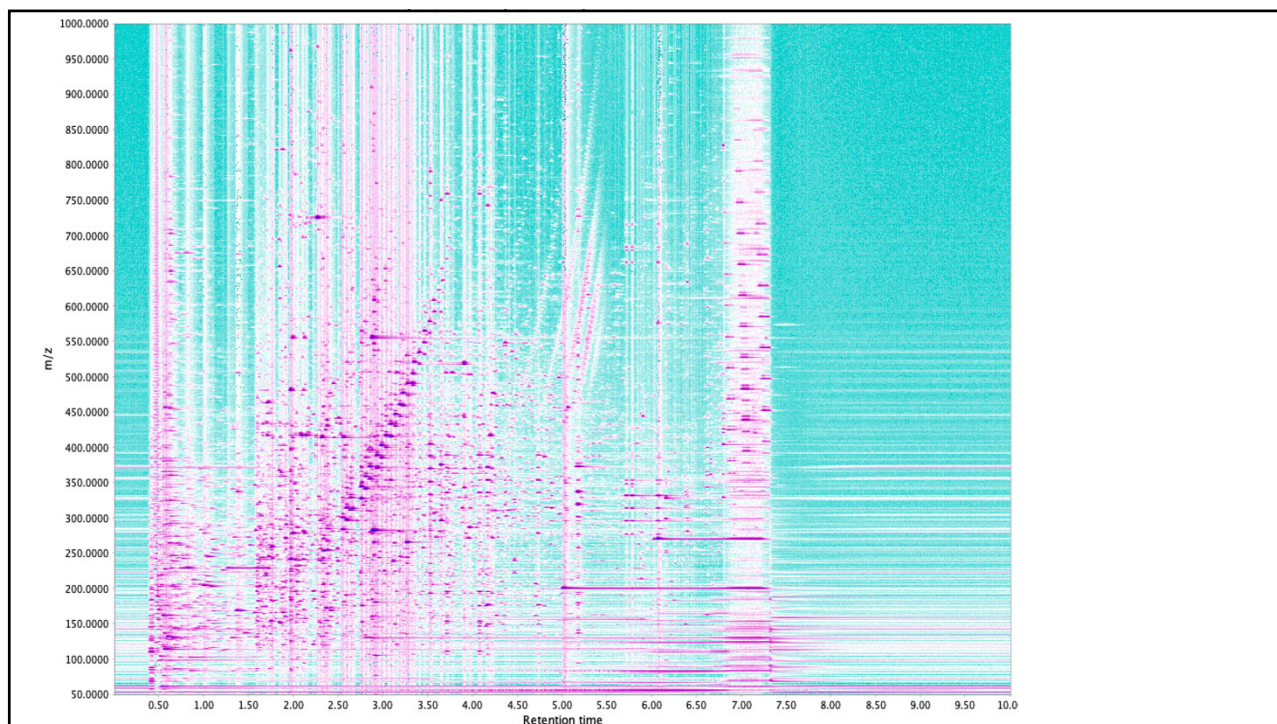
Raw data files list:

- Raw data files
 - Pos_PooledSample MSMS_1.mzXML
 - Pos_PooledSample_1.mzXML
 - Pos_PooledSample_2.mzXML (highlighted)
 - Pos_PooledSample_3.mzXML

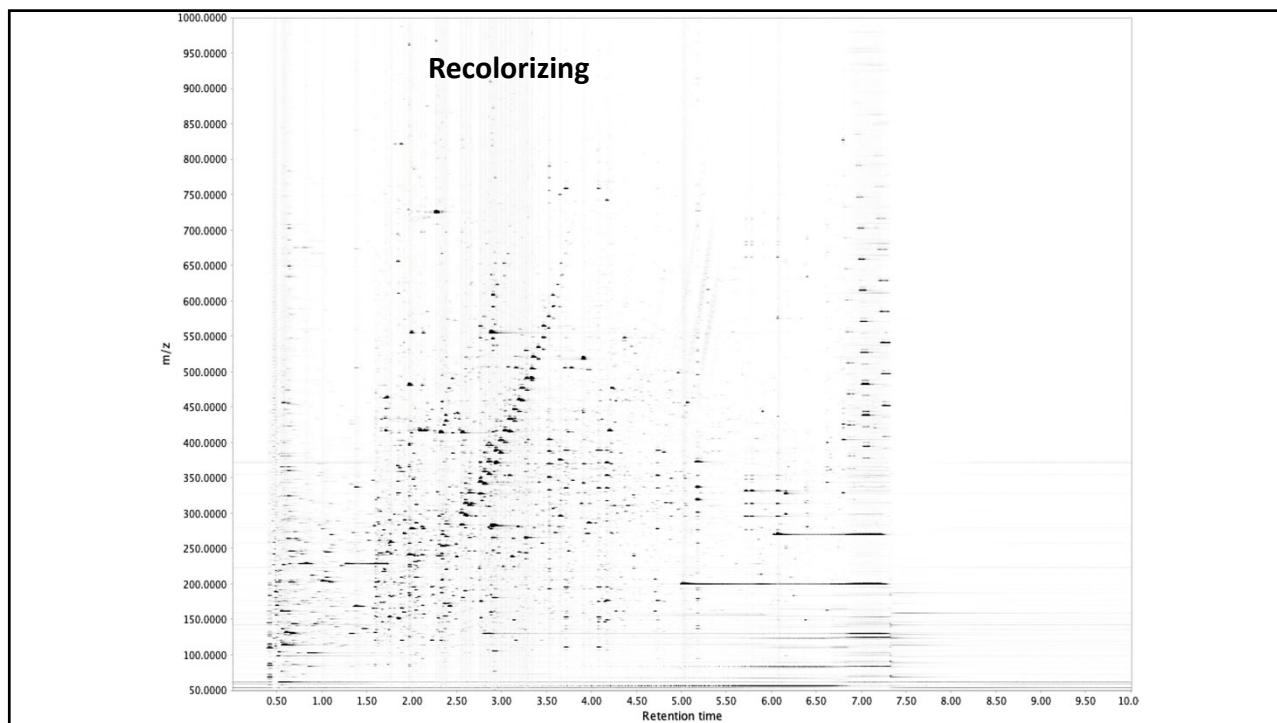
Please set the parameters dialog box:

- Type of plot: Resampled data
- Raw data files: Pos_PooledSample_2.m... As selected in main window
- Scans: MS level: 1 Set filters Clear filters
- m/z: 50.0000 - 1000.0000 Auto range From mass From formula
- Buttons: OK Cancel Help

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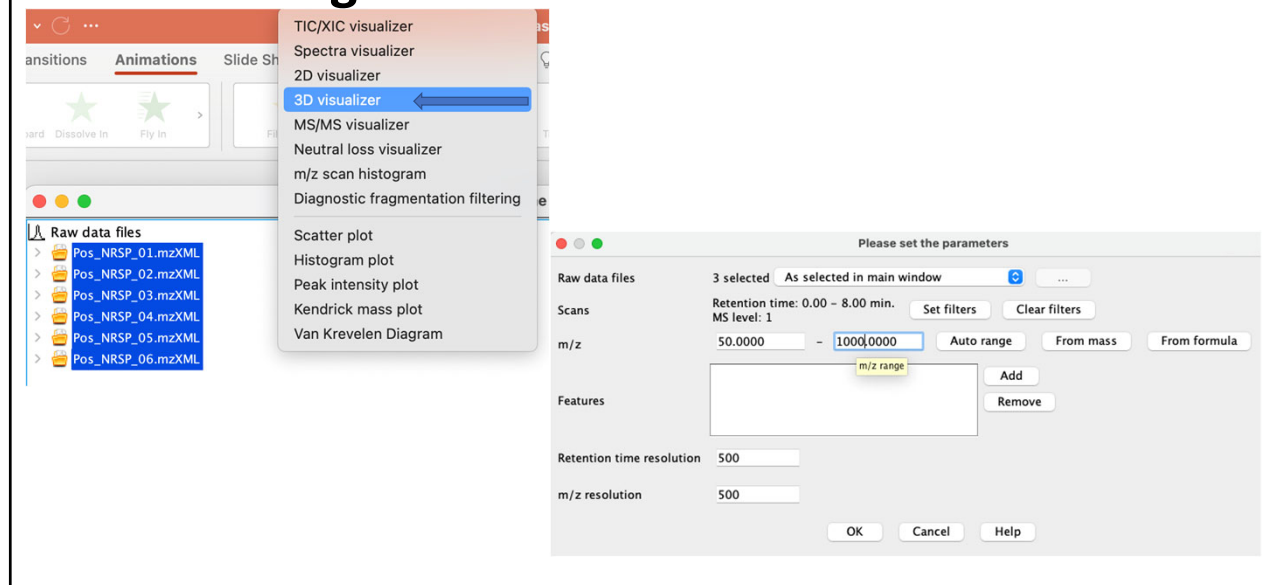


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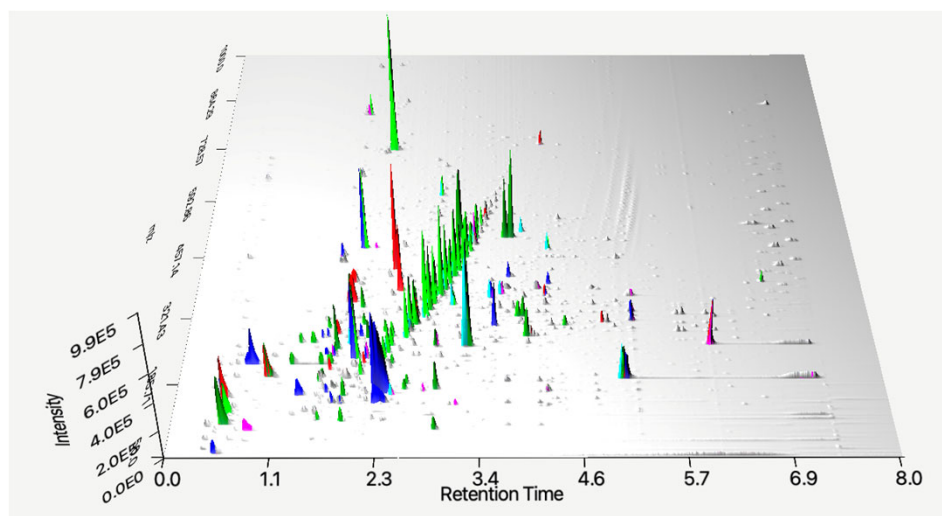
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Examining the data in 3D



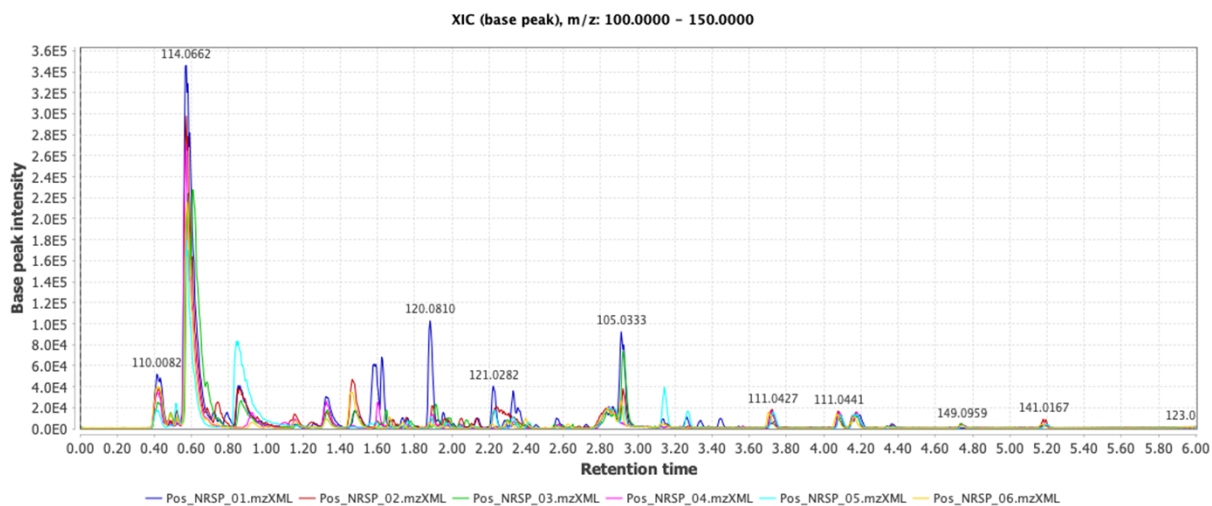
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3D-plot of samples 1-6



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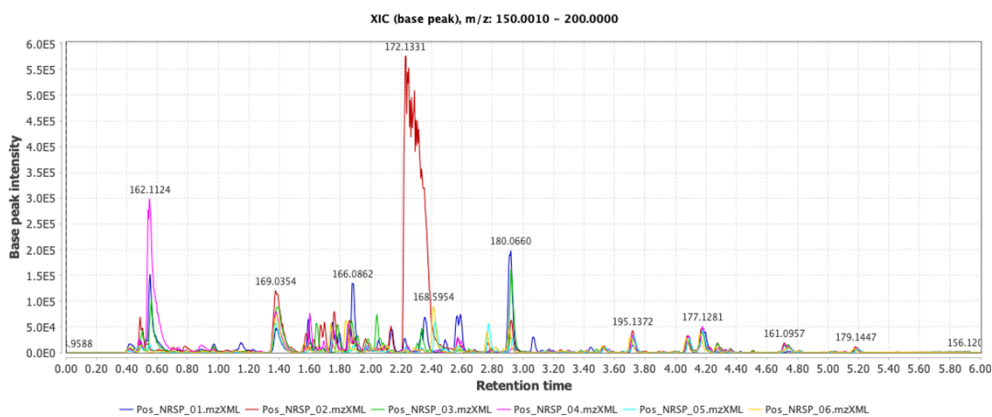
More narrow mass window for the XIC plot



Students: What is m/z 114.0662?

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Here's another limited mass window

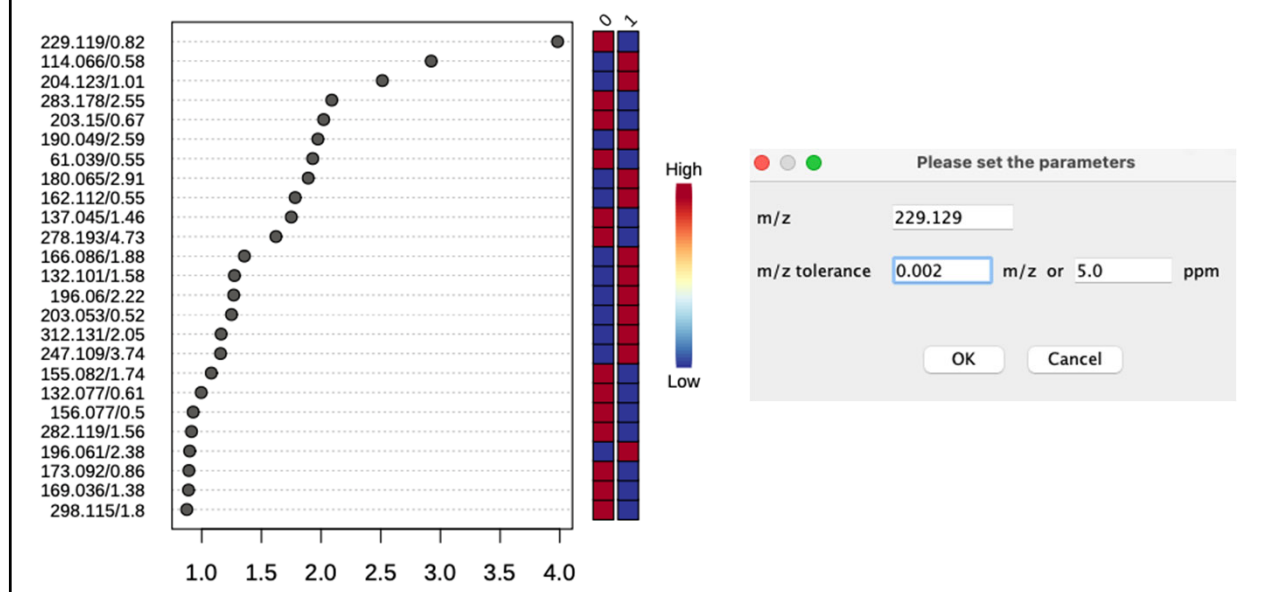


Students: What is m/z 172.133? Why does it have this shape?

Students: What is m/z 180.066?

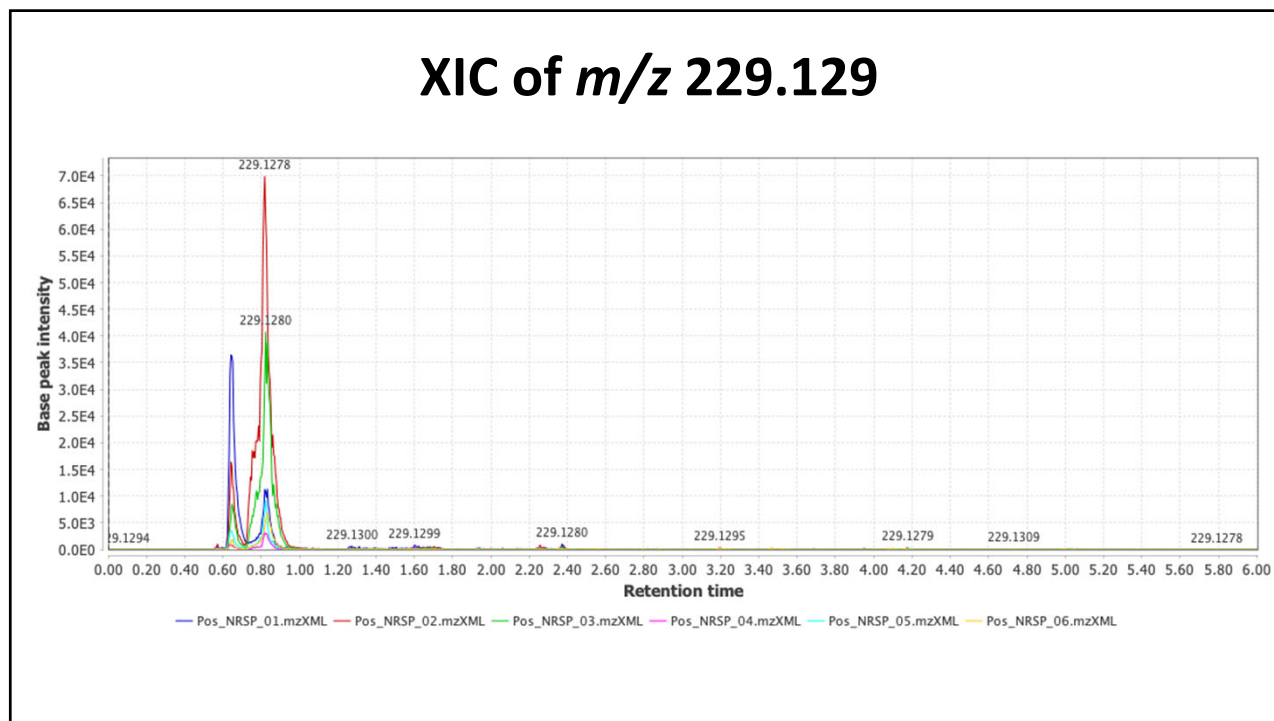
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Important ions from PLS-DA analysis



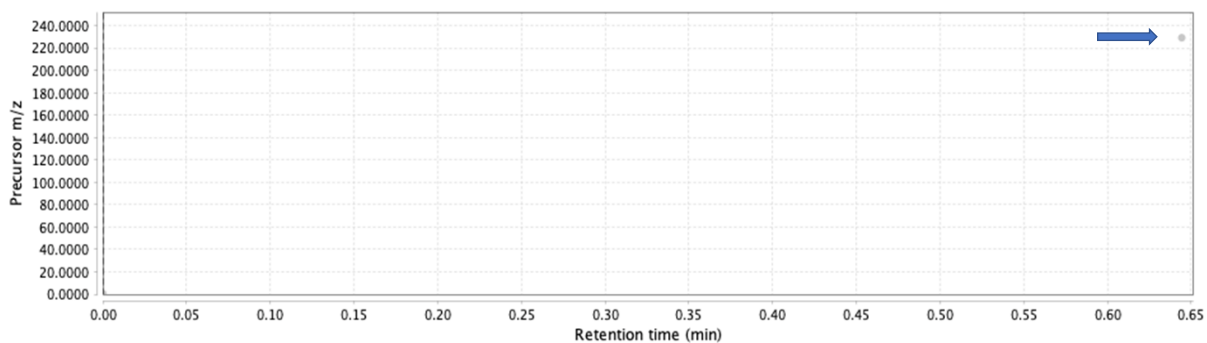
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XIC of m/z 229.129



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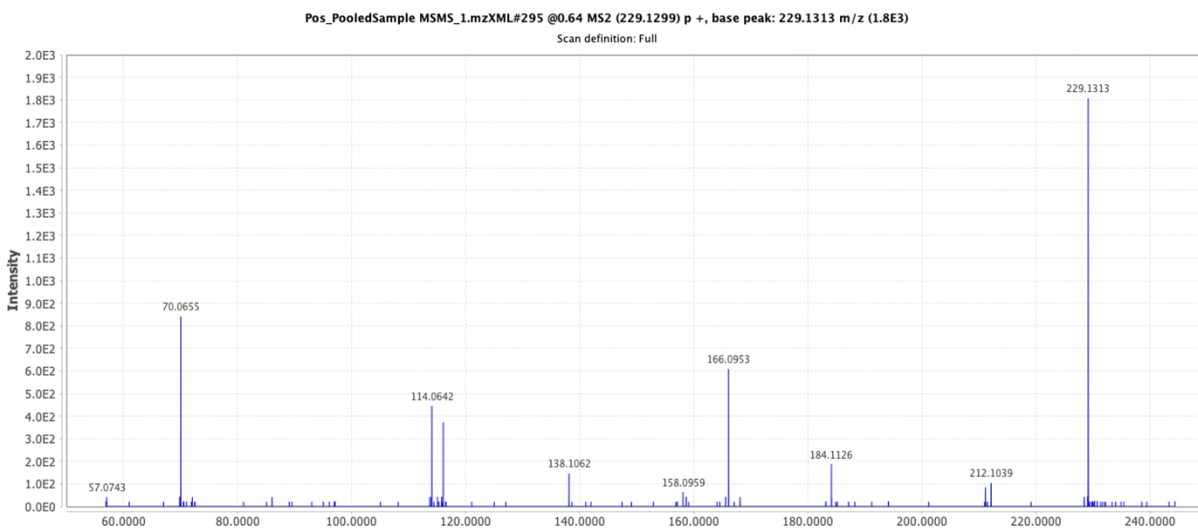
Searching for MSMS spectra for m/z 229.129



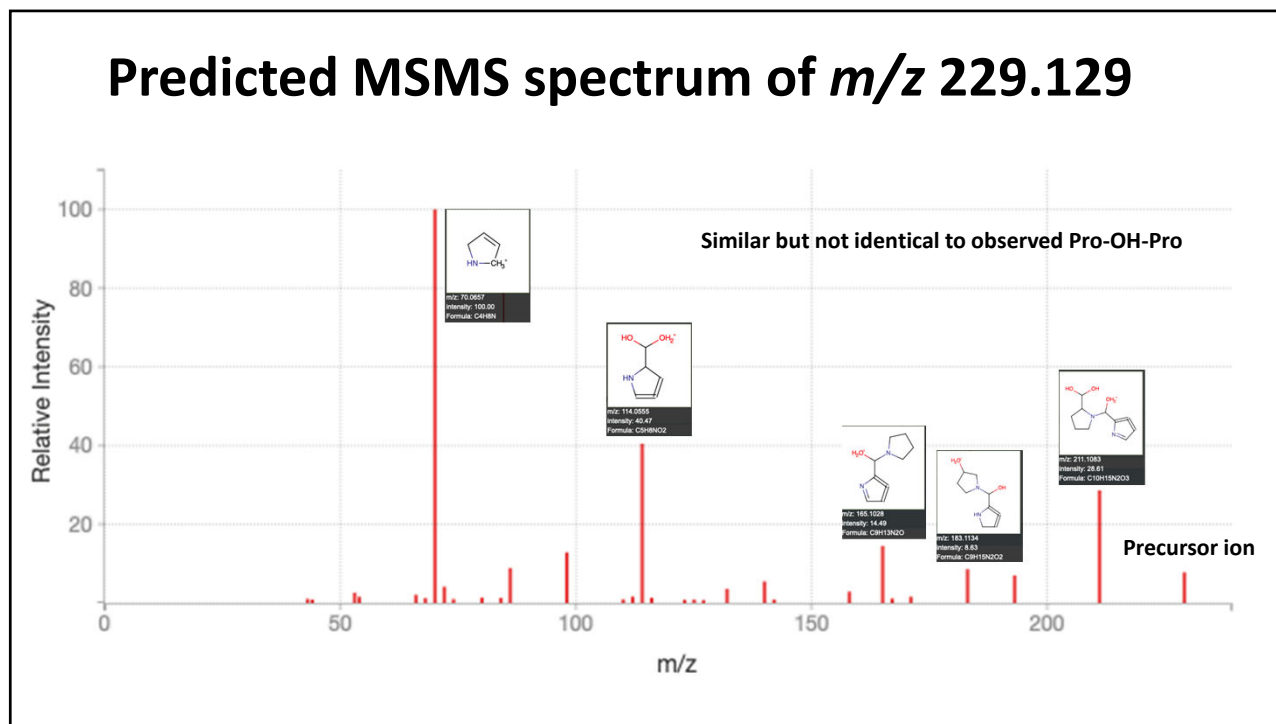
On my Mac I got a divide-by-zero error. Nonetheless, the MSMS spectrum was found.

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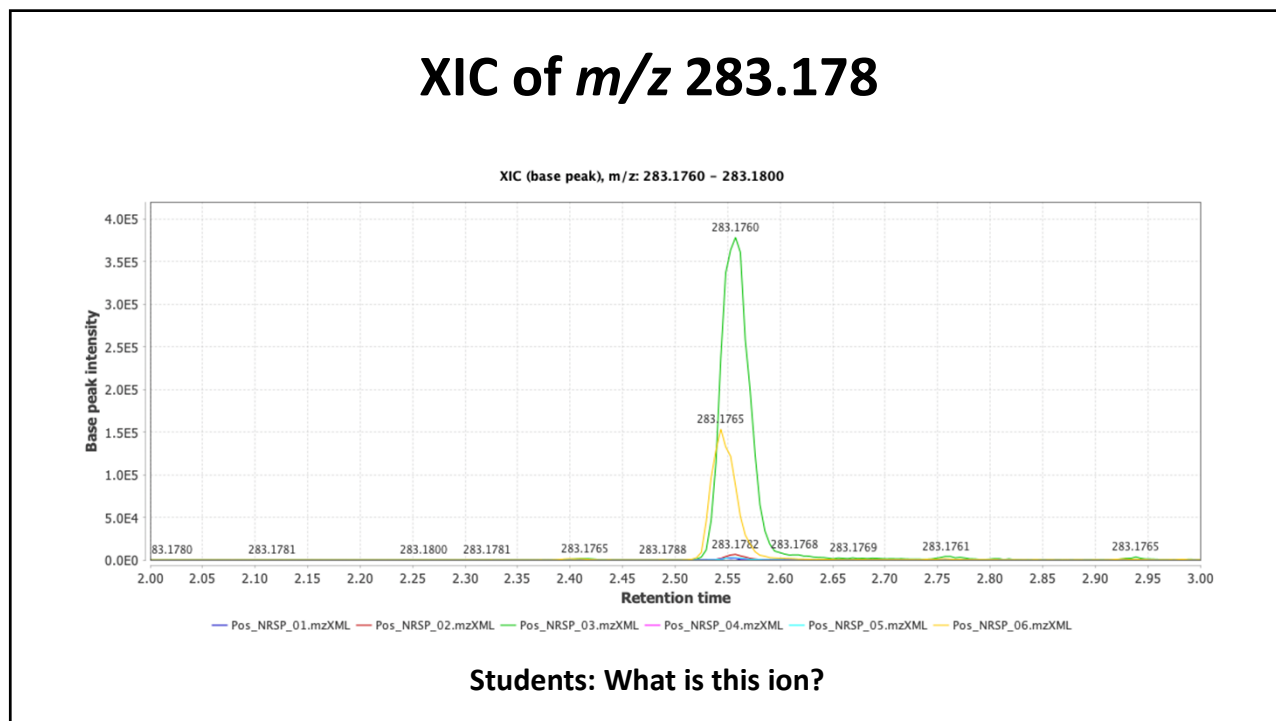
MSMS spectrum of prolylhydroxyproline, m/z 229.129



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Let's look at the MSMS spectrum of m/z 283.178

Please set the parameters

Raw data files Pos_PooledSample MSM... As selected in main window

Scans Retention time: 0.00 – 6.00 min. MS level: 2

Plot type Base peak intensity

m/z 283.1760 – 283.1800 Auto range From mass From formula

Peaks

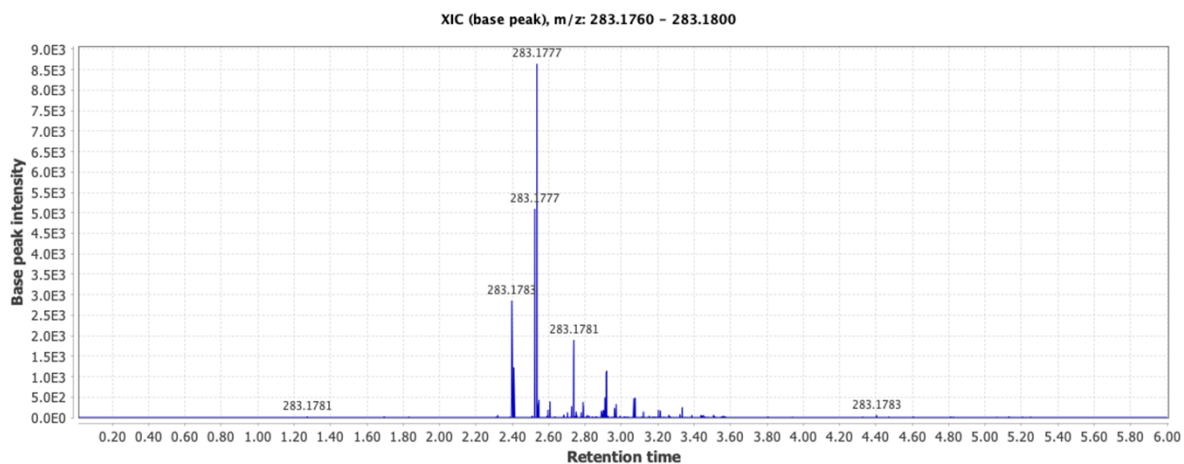
All

Clear

OK Cancel Help

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All MSMS spectra containing m/z 283.178



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Selecting MSMS spectra of m/z 283.178

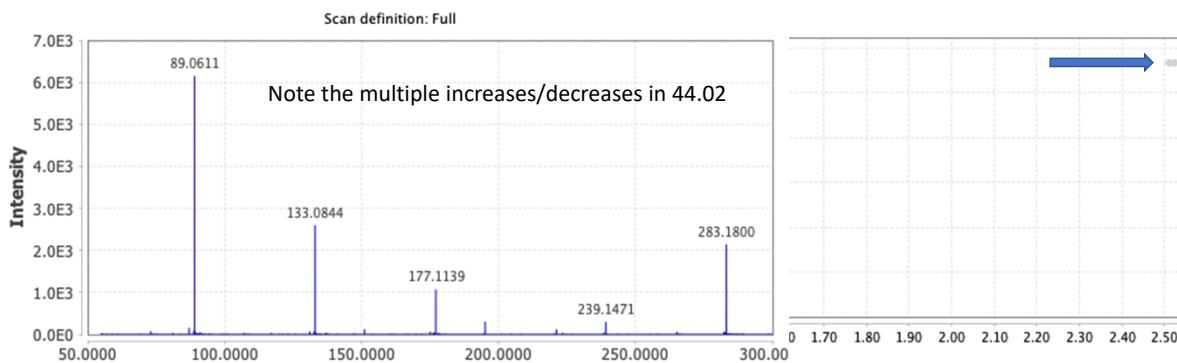
The screenshot shows a software interface with a menu on the left and a dialog box on the right. The menu includes options like 'TIC/XIC visualizer', 'Spectra visualizer', '2D visualizer', '3D visualizer', 'MS/MS visualizer' (highlighted with a blue arrow), 'Neutral loss visualizer', 'm/z scan histogram', 'Diagnostic fragmentation filtering', 'Scatter plot', 'Histogram plot', 'Peak intensity plot', 'Kendrick mass plot', and 'Van Krevelen Diagram'. The dialog box, titled 'Please set the parameters', has the following settings:

- Raw data files: Pos_PooledSample MSM... (As selected in main window)
- Retention time: 0.00 - 6.00 min. (Auto range)
- m/z: 283.1760 - 283.1800 (Auto range, From mass, From formula)
- Intensity: Total intensity in MS/MS scan
- Normalize by: All data points
- Min. MS/MS peak intensity: 10

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Recovering the MSMS spectrum of m/z 283.178

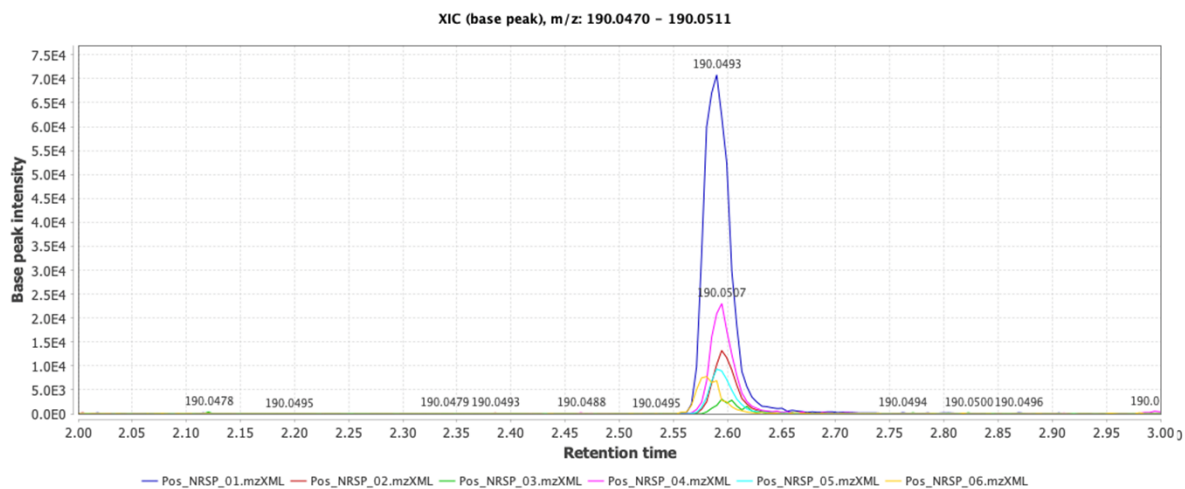
Pos_PooledSample MSMS_1.mzXML#1698 @2.52 MS2 (283.1765) p +, base peak: 89.0611 m/z (6.1 E3)



This "metabolite" is hexaethylene glycol - HO-CH2CH2-O-CH2CH2-O-CH2CH2-OH

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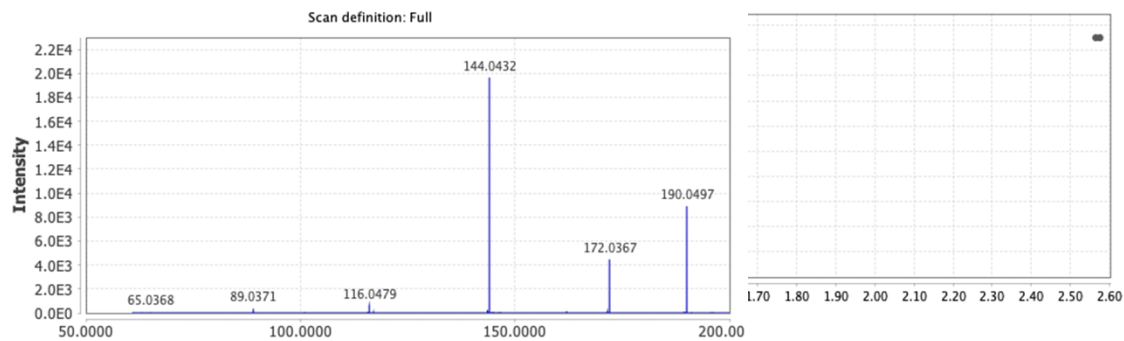
XIC of m/z 190.049



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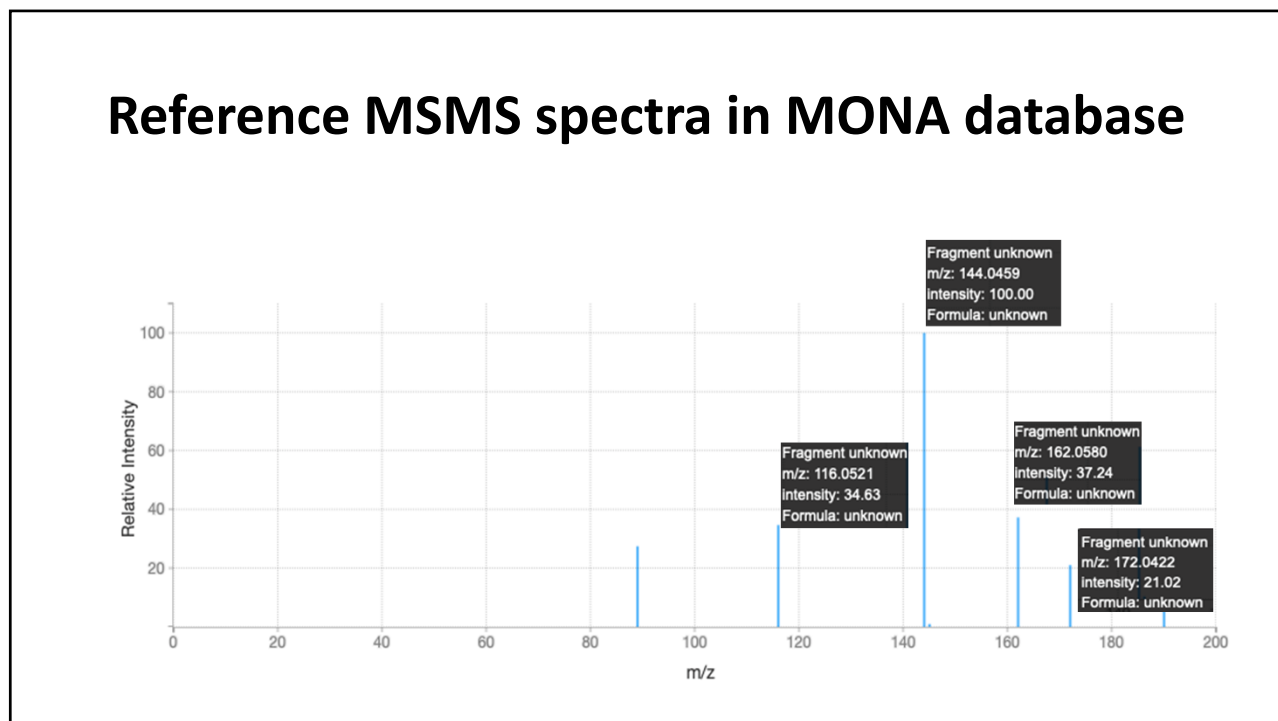
MSMS of m/z 190.049

Pos_PooledSample MSMS_1.mzXML#1741 @2.57 MS2 (190.0501) p +, base peak: 144.0432 m/z (2.0E4)



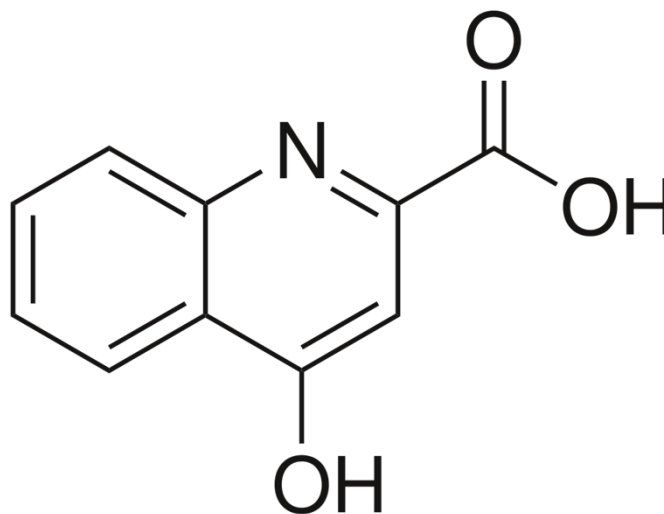
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Reference MSMS spectra in MONA database



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Kyurenic acid



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From Human Metabolome Database

- Kynurenic acid (KYNA) is a well-known **endogenous antagonist** of the glutamate ionotropic excitatory amino acid receptors N-methyl-D-aspartate (NMDA), alphaamino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate receptors and of the nicotine cholinergic subtype alpha 7 receptors.
- KYNA neuroprotective and anticonvulsive activities have been demonstrated in animal models of neurodegenerative diseases. Because of KYNA's **neuromodulatory character**, its involvement has been speculatively linked to the pathogenesis of a number of neurological conditions including those in the ageing process.
- Different patterns of abnormalities in various stages of KYNA metabolism in the CNS have been reported in **Alzheimer's disease, Parkinson's disease** and **Huntington's disease**. In HIV-1-infected patients and in patients with Lyme neuroborreliosis a marked rise of KYNA metabolism was seen. In the ageing process KYNA metabolism in the CNS of rats shows a characteristic pattern of changes throughout the life span.
- A marked increase of the KYNA content in the CNS occurs before the birth, followed by a dramatic decline on the day of birth. A low activity was seen during ontogenesis, and a slow and progressive enhancement occurs during maturation and ageing.

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More on Kynurenic acid

- This remarkable profile of KYNA metabolism alterations in the mammalian brain has been suggested to result from the development of the organisation of neuronal connections and synaptic plasticity, development of receptor recognition sites, maturation and ageing. There is significant evidence that KYNA **can improve cognition and memory**, but it has also been demonstrated that it interferes with working memory. Impairment of cognitive function in various neurodegenerative disorders is accompanied by profound reduction and/or elevation of KYNA metabolism.
- The view that enhancement of CNS KYNA levels could underlie cognitive decline is supported by the increased KYNA metabolism in **Alzheimer's disease**, by the increased KYNA metabolism in **down's syndrome** and the enhancement of KYNA function during the early stage of **Huntington's disease**.
- Kynurenic acid is the only endogenous N-methyl-D-aspartate (NMDA) receptor antagonist identified up to now, that mediates glutamatergic hypofunction. Schizophrenia is a disorder of dopaminergic neurotransmission, but modulation of the dopaminergic system by glutamatergic neurotransmission seems to play a key role.
- Despite the NMDA receptor antagonism, kynurenic acid also **blocks, in lower doses, the nicotinic acetylcholine receptor**, i.e., increased kynurenic acid levels can explain psychotic symptoms and cognitive deterioration. Kynurenic acid levels are described to be higher in the cerebrospinal fluid (CSF) and in critical central nervous system (CNS) regions of schizophrenics as compared to controls. (PMID: [17062375](#), [16088227](#)).
- KYNA has also been identified as a **uremic toxin** according to the European Uremic Toxin Working Group (PMID: [22626821](#)).

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