

Using Free Public Libraries in NIST26 Chromatogram and Lib Search

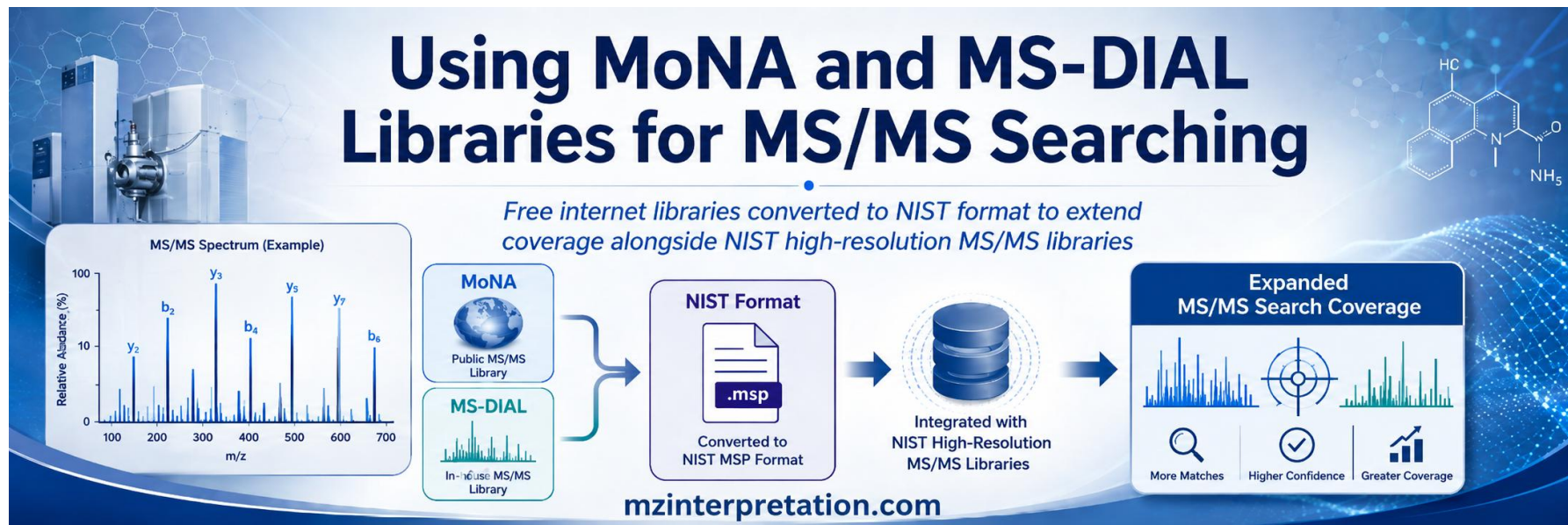
Video/Handout

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Mass Spec Interpretation Services

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mzinterpretation.com



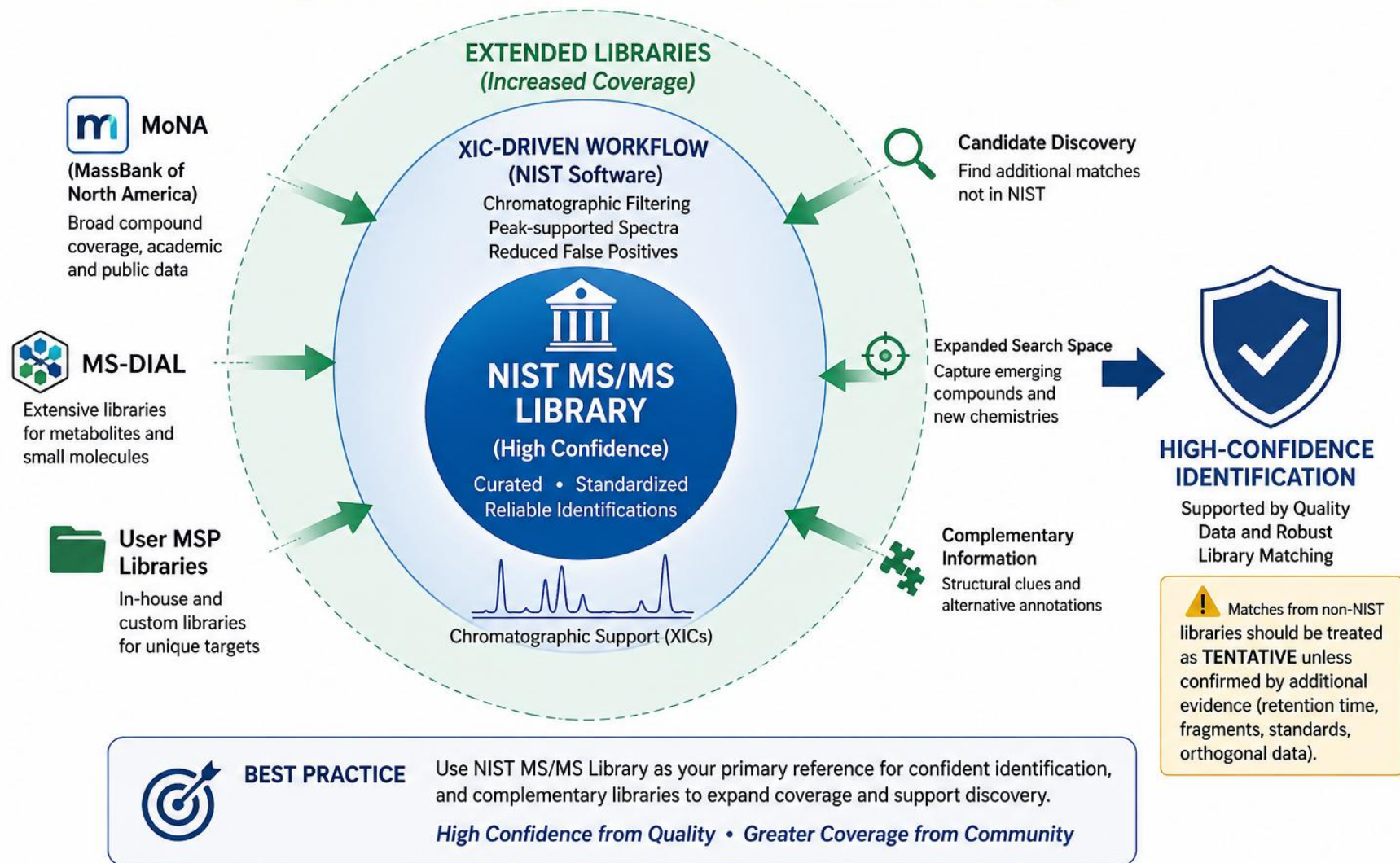
See **Full Course** on NIST26 with new **Integrated** Deconvolution/Library Searching for **LC-MS/MS**!

[link to course](#)

NIST-CENTERED MS/MS LIBRARY STRATEGY

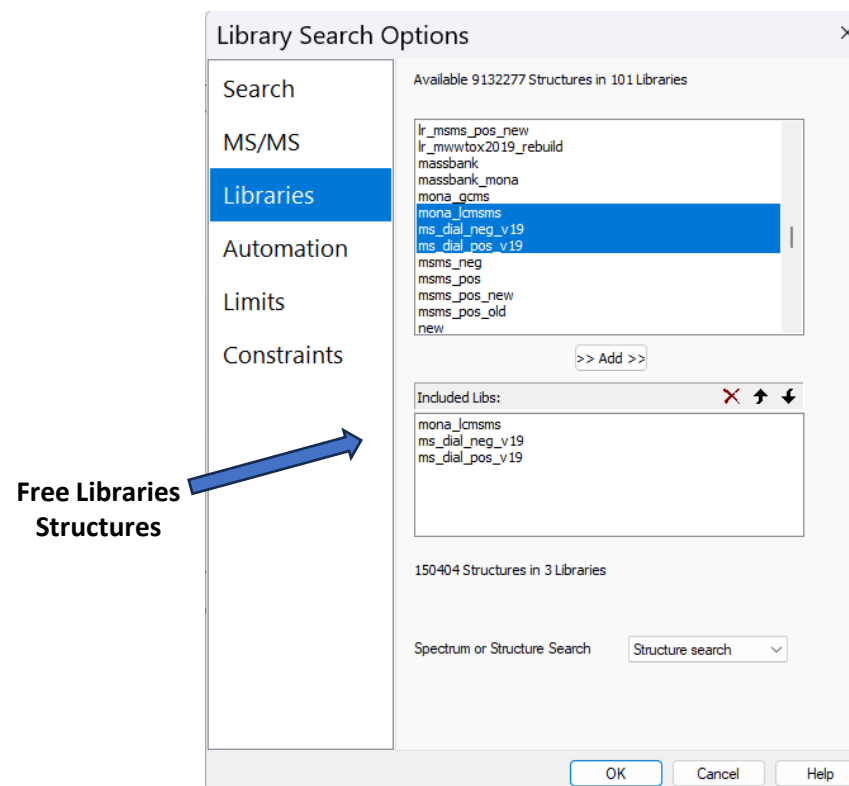
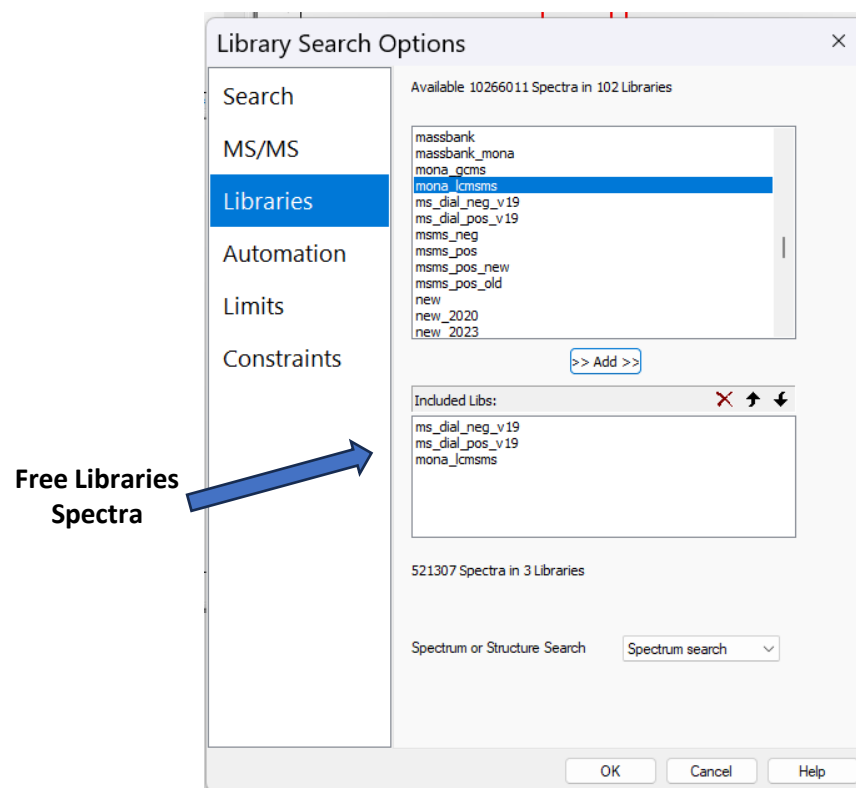
High Confidence Identifications with Maximum Coverage

- NIST Library is the gold standard for MS/MS Identifications
- Price is very reasonable, shop around! Prices vary Dramatically, [see link](#)
- However, free public libraries and user libraries are useful



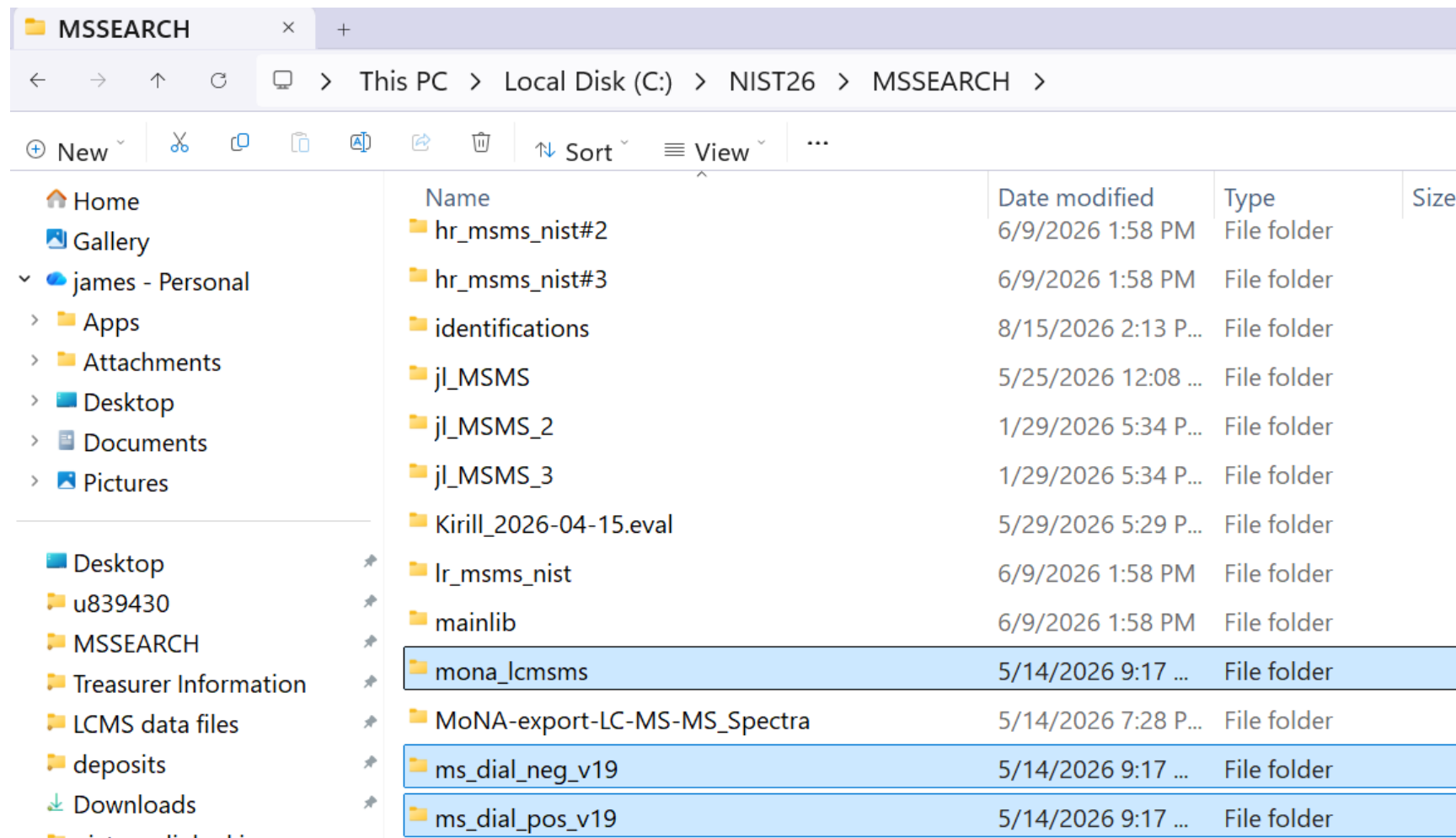
Free Public Libraries in NIST Format

- NIST commercial MS/MS (Tandem) Libraries are the “gold-standard”
- Majority of the most useful and diverse components in my identification evaluations
- However, there are useful MS/MS spectra in free public libraries on the internet
- Initially, use free copies of Free Public Libraries in NIST format found on my website
- If useful, learn to create your own copies from more current versions
- Definitely create your own unique user library
- >500,000 free public MS/MS spectra downloaded at the [following link](#)



Directory for Storing Free Public Libraries in NIST Search

- >500,000 free public MS/MS spectra downloaded at the [following link](#)
- After downloading zip file, unzip them to MSSEARCH folder with other NIST libraries
- Note in screen capture below, I have many other libraries that appear as folders



Name	Date modified	Type	Size
hr_msms_nist#2	6/9/2026 1:58 PM	File folder	
hr_msms_nist#3	6/9/2026 1:58 PM	File folder	
identifications	8/15/2026 2:13 P...	File folder	
jl_MSMS	5/25/2026 12:08 ...	File folder	
jl_MSMS_2	1/29/2026 5:34 P...	File folder	
jl_MSMS_3	1/29/2026 5:34 P...	File folder	
Kirill_2026-04-15.eval	5/29/2026 5:29 P...	File folder	
lr_msms_nist	6/9/2026 1:58 PM	File folder	
mainlib	6/9/2026 1:58 PM	File folder	
mona_lcmsms	5/14/2026 9:17 ...	File folder	
MoNA-export-LC-MS-MS_Spectra	5/14/2026 7:28 P...	File folder	
ms_dial_neg_v19	5/14/2026 9:17 ...	File folder	
ms_dial_pos_v19	5/14/2026 9:17 ...	File folder	

Free Public Libraries

Source	File Name	MS/MS Spectra	Structures
MoNA (May 2025)	mona_lcmsms	~150K (~70% pos, ~30 neg)	150K
MS-DIAL (2025-2026)	ms_dial_pos_v19	~300K (~88% pos)	0
MS-DIAL (2025-026)	ms_dial_neg_v19	~40K (~12% neg)	0

Approximately 30K unique compounds

Total ~500K

Total ~150K

NIST 2026 MS/MS Libraries

Source	File Name	MS/MS Spectra	Structures
NIST Hi-Resolution	hr_msms	~2,600K (~80%, ~20 neg)	~2,60K
NIST Low-Resolution	lr_msms	~400K (~75% pos, ~25% neg)	~400K
NIST APCI	apci_msms_nist	6K (~75% pos, ~25% neg)	~6,000

Approximately 70K unique compounds

Total ~3,000K

Total ~3,000K

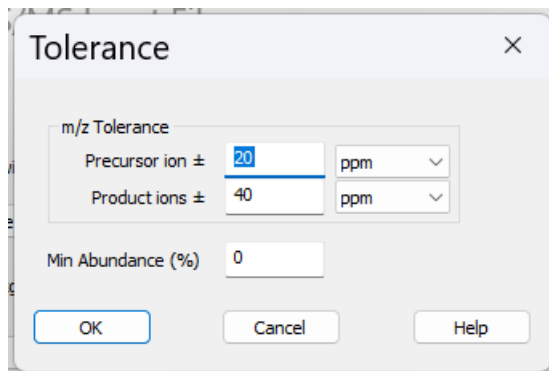
- NIST 2026 tandem library ***substantially*** larger and more much ***higher quality***
- Use NIST26 as primary MS/MS library, relatively inexpensive
- Using NIST and Free Public libraries together with integrated processing unlocks the full potential!

Suggested Tolerances for Free Public MS/MS Library Searches

Identity MS/MS and Similarity MS/MS Hybrid

- **See** the high-resolution tolerances suggested below by NIST for their libraries
- The NIST `lr_msms_nist` (low-resolution) library corrected automatically even with high-resolution tolerances
- Both public libraries supplied on my website contain some low-resolution spectra
- See separate video/handout finding low-resolution spectra present in high-resolution libraries
- Precursor ion can be added manually if necessary

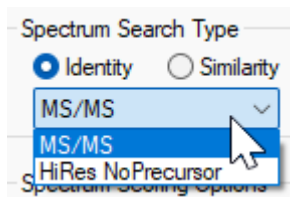
NIST Suggested High Resolution Settings



The 'Tolerance' dialog box shows the following settings:

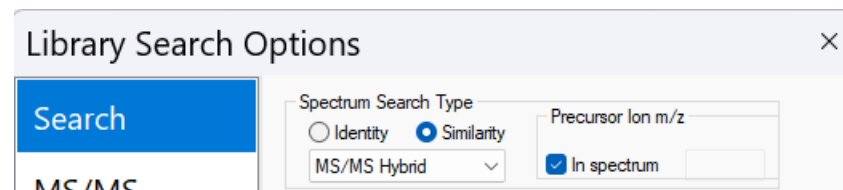
- m/z Tolerance: 20 ppm
- Precursor ion ±: 40 ppm
- Product ions ±: 40 ppm
- Min Abundance (%): 0

Buttons: OK, Cancel, Help



The 'Spectrum Search Type' dropdown menu is open, showing the following options:

- Identity (selected)
- Similarity
- MS/MS
- MS/MS
- HiRes NoPrecursor

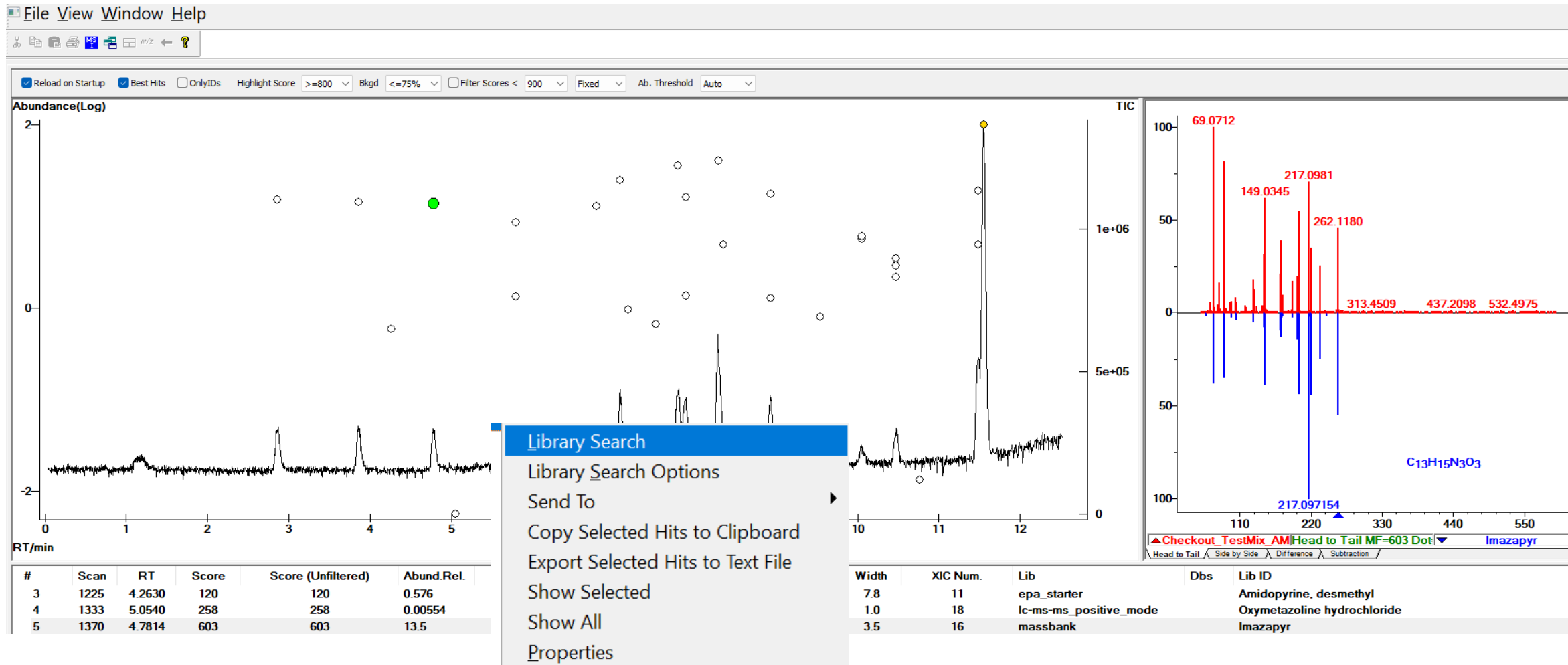


The 'Library Search Options' dialog box shows the following settings:

- Search: MS/MS
- Spectrum Search Type: Identity (selected), Similarity
- MS/MS Hybrid
- Precursor Ion m/z: In spectrum (checked)

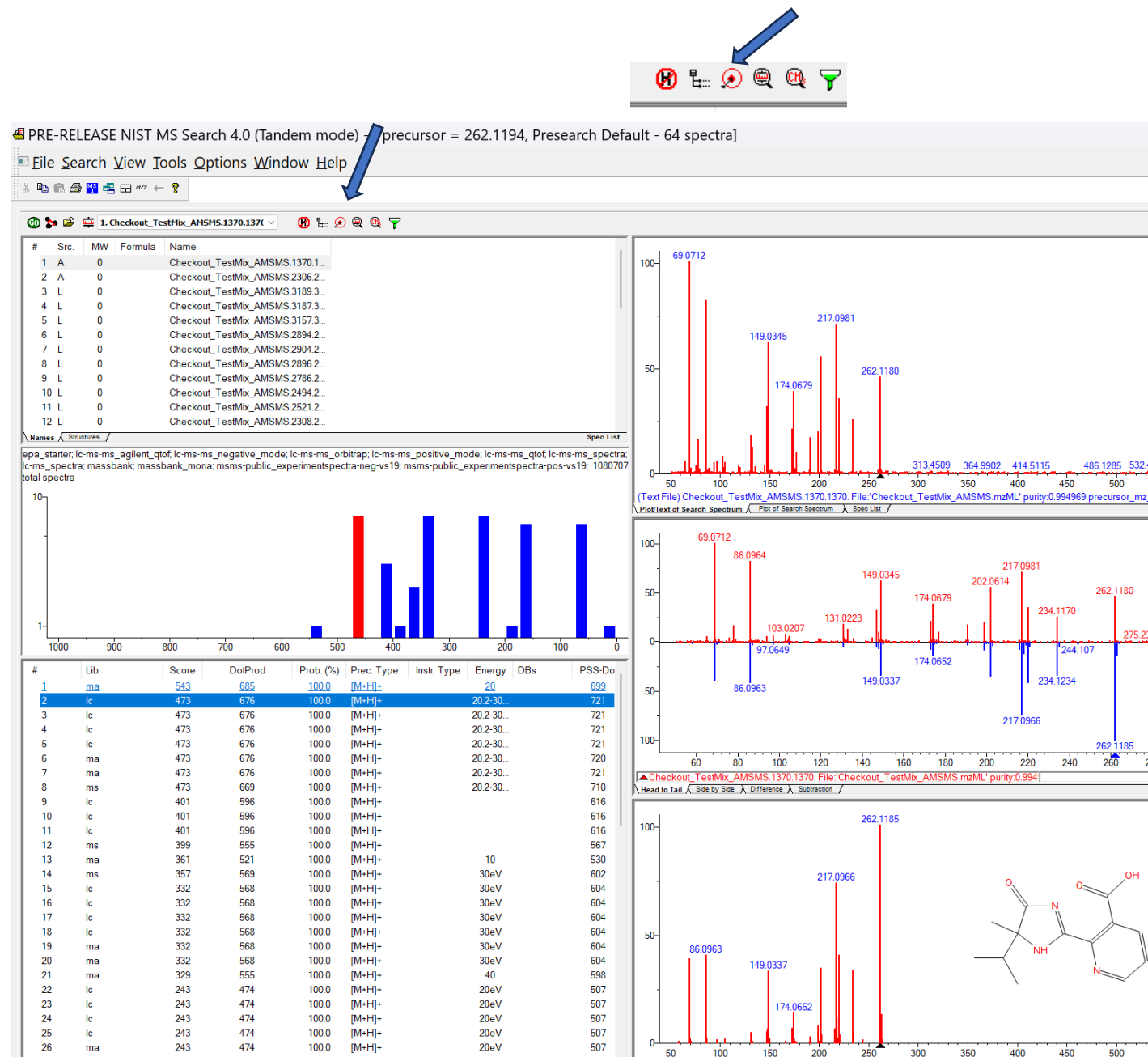
Compounds with No Structures for Many Free Public MS/MS Entries

- Many of the compounds that will be found in Chromatogram Searches using the Free Public libraries might not have structures
- e.g. Imazapyr
- Imazapyr entry below, sent to Library Search with right click



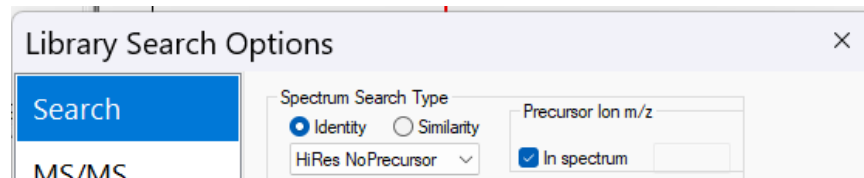
Lib Search of Imazapyr showing structures

- Many of the hits had structures
- Only the best hit found in Chromatogram Search did not
- Make sure the “Best Matching Only” *is not* selected (depressed), or the duplicates with structure *might not be* found



Suggested Tolerances for Free Public MS/MS Library Searches in Lib Search and Chromatogram Windows

- This essentially matches the observed spectrum to the library MS/MS spectrum
- Can be useful for determining the class of a compound by looking at the top 2-5 hits
- Normally, use the Product Ion +/- 40 ppm, Precursor setting not important, not utilized
- Low-Resolution spectra searched with EI type searches, see separate video/handout
- Both public libraries supplied on my website contain some low-resolution spectra
- See separate video/handout finding low-resolution spectra present in high-resolution libraries
- Precursor ion can be added manually if necessary



Library Search Options

Search

MS/MS

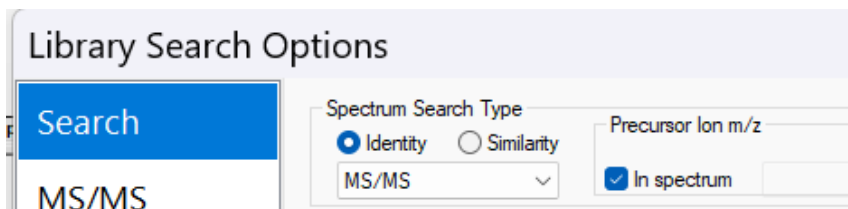
Spectrum Search Type

☒ Identity ☐ Similarity

HiRes NoPrecursor

Precursor Ion m/z

☒ In spectrum



Library Search Options

Search

MS/MS

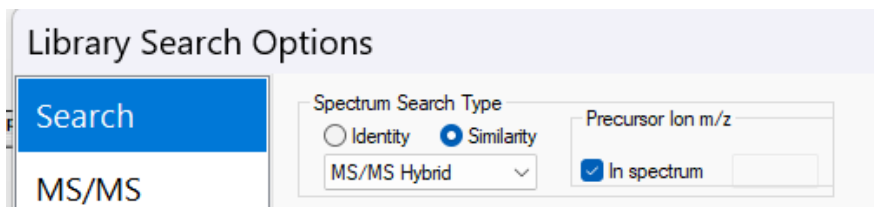
Spectrum Search Type

☒ Identity ☐ Similarity

MS/MS

Precursor Ion m/z

☒ In spectrum



Library Search Options

Search

MS/MS

Spectrum Search Type

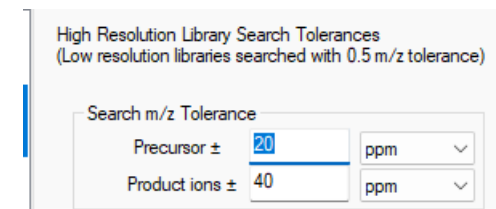
☐ Identity ☒ Similarity

MS/MS Hybrid

Precursor Ion m/z

☒ In spectrum

Standard Product ion Tolerance for High-Resolution Spectra



High Resolution Library Search Tolerances
(Low resolution libraries searched with 0.5 m/z tolerance)

Search m/z Tolerance

Precursor ± 20 ppm

Product ions ± 40 ppm

Create Your Own Updated Version of Free Public Libraries in NIST Format

- Another tutorial showing how to create your own NIST libraries from internet sources on my website
- See NIST full “List of Sources” of Free Public MS/MS Libraries in my website resources, [see link](#)

List of Sources Public Tandem Libraries/Data

NIST Compatible *msp* or *SDF* files (convertible to libraries with the *Lib2NIST* program)

MassBank-Europe - <https://github.com/MassBank/MassBank-data/releases>

MoNA - <https://massbank.us/downloads> (includes *SDF* files with chemical structures)

MS-Dial - <https://systemsomicslab.github.io/compms/msdial/main.html#MSP>

mzInterpretation.com - <https://mzinterpretation.com/2023/10/free-ei-and-msms->

Note full list, [see link](#)