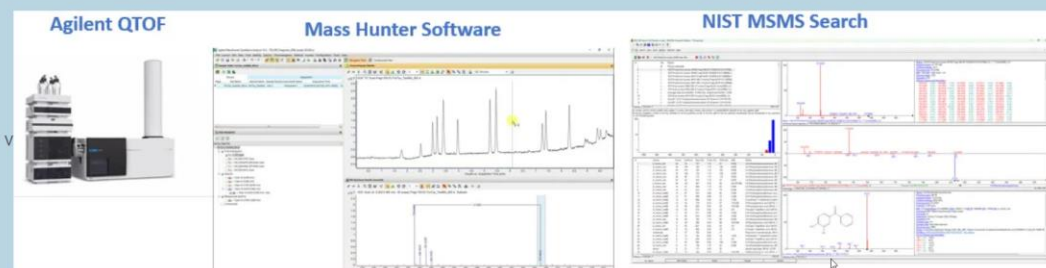


## Using Agilent MassHunter to Perform NIST MSMS Search



**James Little**  
**Mass Spec Interpretation Services**  
**Sept. 14, 2024**

<https://littlemsandsailing.com/2024/10/using-agilent-masshunter-to-perform-nist-msms-search/>  
<https://littlemsandsailing.com/>

## Overview of Process

- This is **only** an overview of the process
- For Details, see complete training at websites below

### MassHunter YouTube Training Videos

[MassHunter Qualitative Analysis Training Video EP01-Basic Navigation](#)

[MassHunter Qualitative Analysis Training Video EP02-Extracting Ion Chromatograms](#)

[MassHunter Qualitative Analysis Training Video EP03-Other Miscellaneous Tips](#)

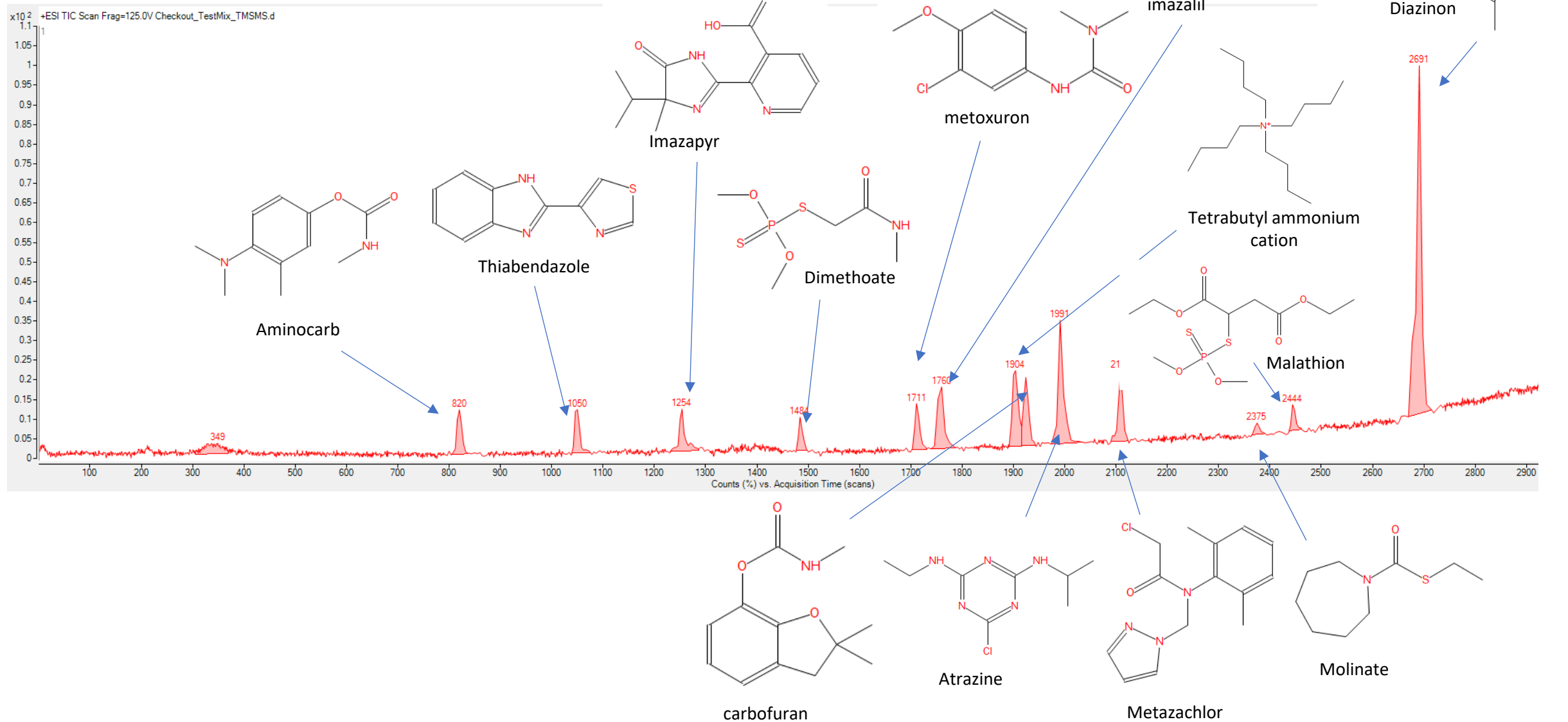
### NIST MSMS Full Course

<https://littlesandsailing.com/2020/12/lcms-unknown-identification-with-nist-search-using-msms-libraries/>

# Results for Pesticide Checkout Test Mix Tandem MSMS File

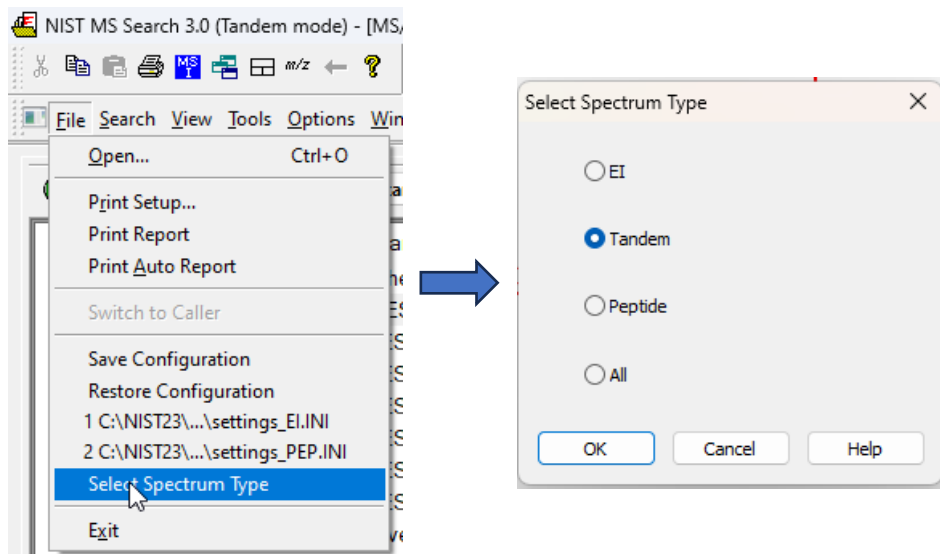
## Targeted Positive Ion TOF MS

Extracted Spectra Averaged (10, 20, 40 V)

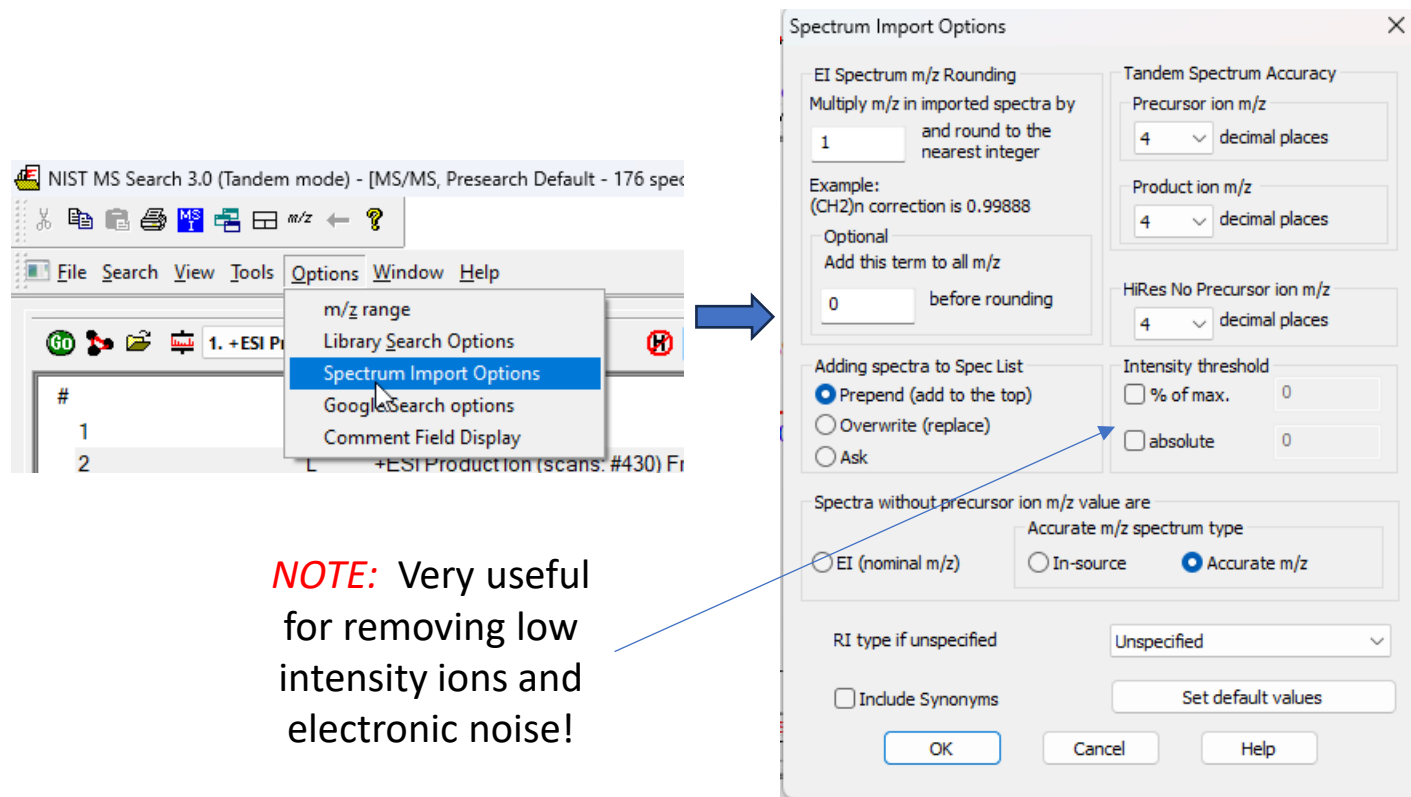


## Typical MS Search v.3.0 (2023) Settings for This Data Evaluation

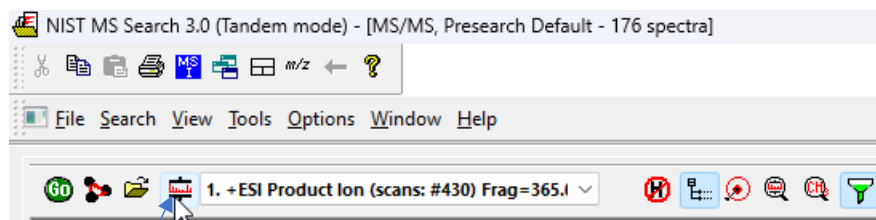
### v.3.0 NIST Search *Simplifies* Menus for Different Modes



### Typical Spectrum Import Options



## Library Search Options for This MSMS Search v.3.0 (2023)

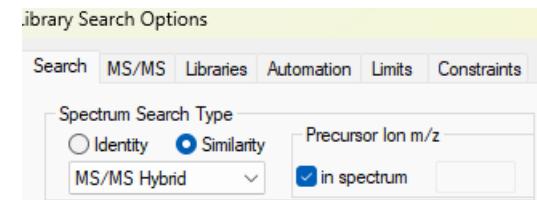
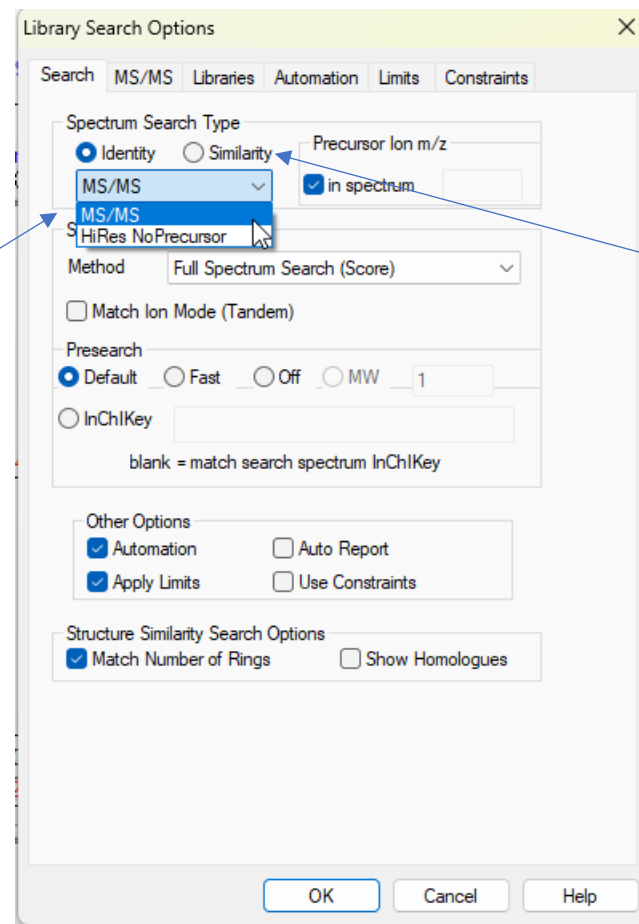


Click here

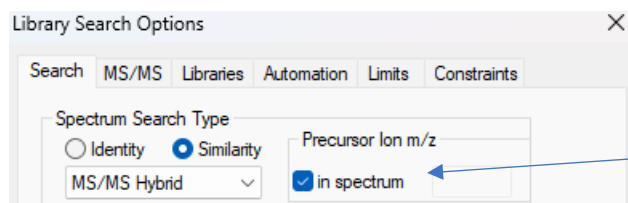
Can do Identity MS/MS which searches spectra restricting by the precursor ion and its specified  $m/z$  Tolerance set in MS/MS tab (this one returns fewer but likely more useful results if compound's spectrum is present in accurate mass format)

Or

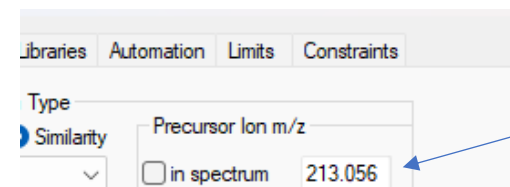
Identity/High Resolution No Precursor, just compares the overall fit of unknown spectra to reference spectra



- Another type search is Similarity/Hybrid MS/MS which can be **very** useful if a related compound is present in the reference libraries, but the actual compound is **not** present
- See NIST MSMS **Full** Course for details on Hybrid Search



If precursor  $m/z$  imported correctly with spectrum, will have Precursor Ion  $m/z$  "in spectrum" selected

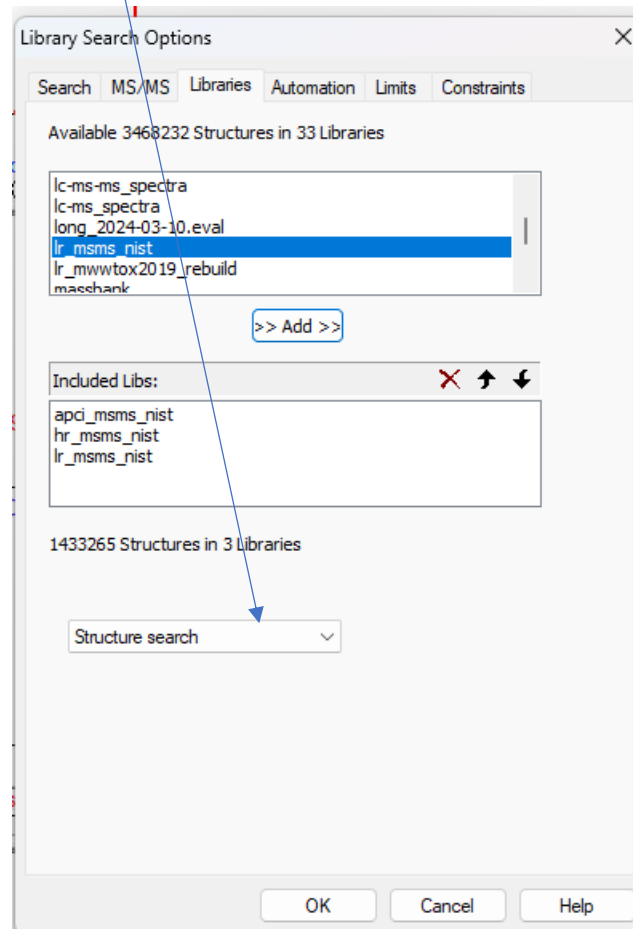
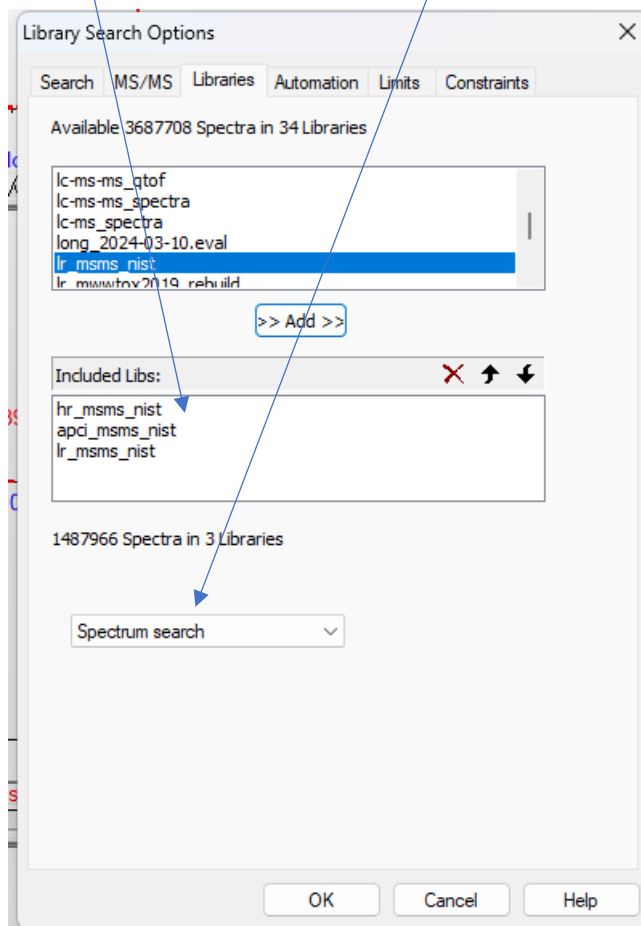


If precursor  $m/z$  **not** imported properly, user can deselect "in spectrum" and then enter value manually

## Other Settings in This NIST MSMS Search v.3.0 (2023)

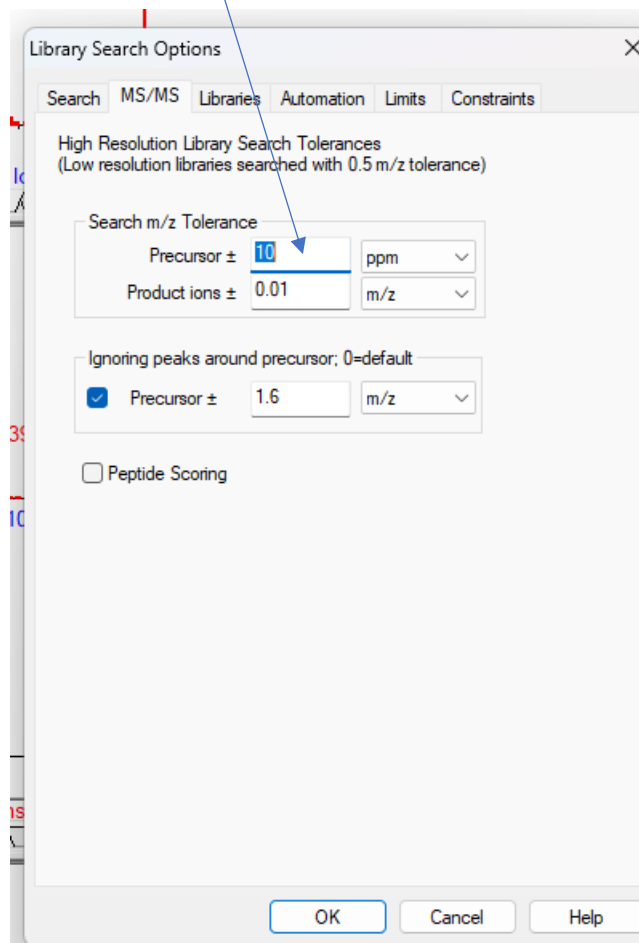
Select whichever MSMS Libraries you want to search, the ***standard ones*** with NIST 2023 Software are selected below for ***spectrum search***

Libraries to search by Structure are also selected after toggling to ***Structure Search***

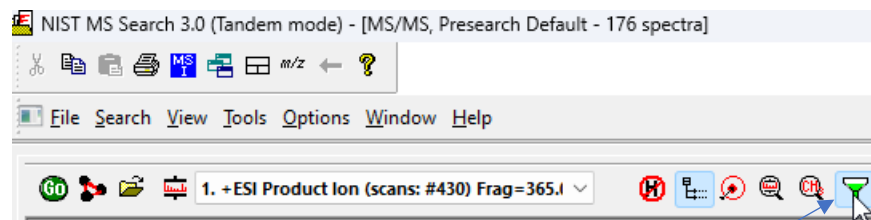


Check the settings in Library Search Options/MS/MS Tab

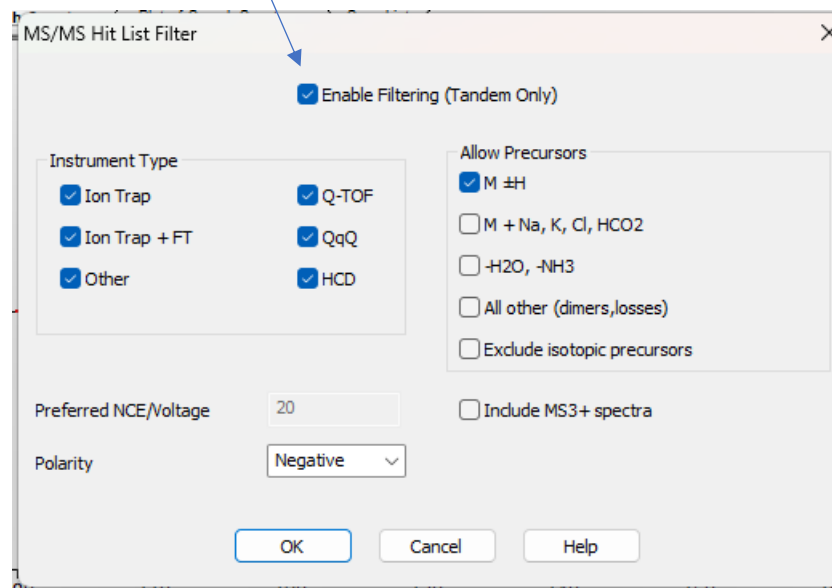
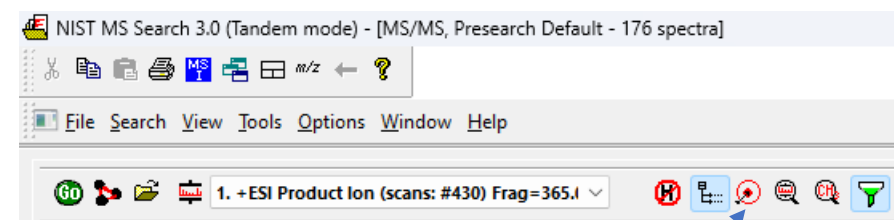
Set the tolerance for precursor  $m/z$  which is appropriate for your instrument



## Other Settings for NIST23 MS Search 3.0 (Tandem Mode)



The “filter” settings affect the results displayed from MSMS search such as polarity of MSMS spectrum and many others if “*Enable Filtering (Tandem Only)*” is selected, this filtering occurs **after** the search is completed!

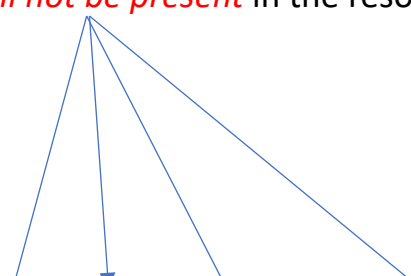


The “Best Matching” only can greatly simplify the results, removing all but the best matching spectrum using the CAS Number as a filter, this filtering occurs **after** the search is completed!

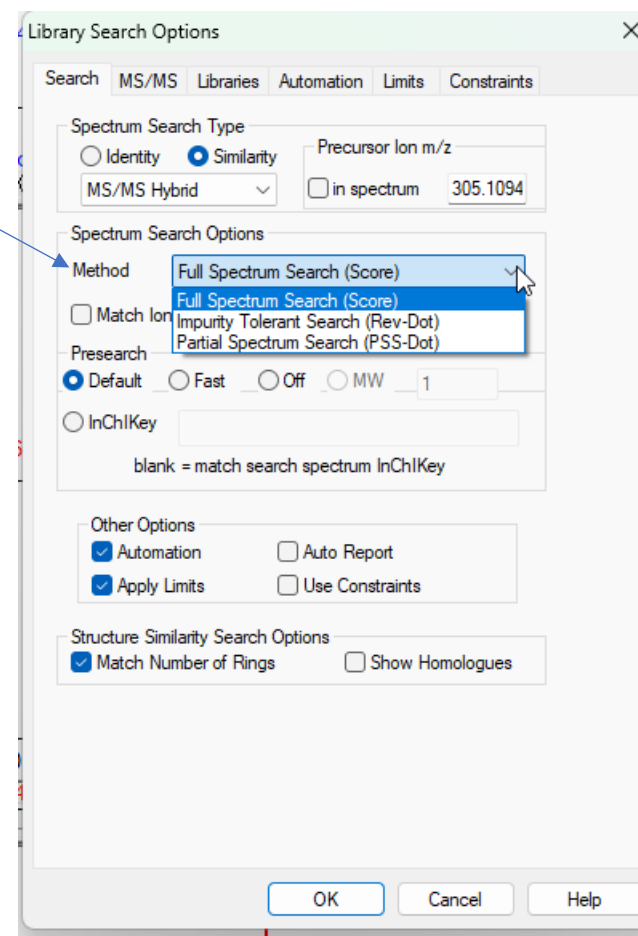
## Sorting of the MSMS Search Results v.3.0 (2023)

I usually sort by Full Spectrum (Score), but can specify Impurity Tolerant (Rev-Dot) which is tolerant if ions found in unknown spectrum have ions that are *not found* in the reference spectrum. The Partial Spectrum Search (PSS-DOT) *does not* penalize the search score if ions are absent in the unknown which are present in the reference spectrum.

One can just click on the column in the results, and they will be resorted giving preference to the other type search methods. *However*, if the spectrum of interest was eliminated in the original search because more than the specified no. of hits was exceeded, it *will not be present* in the resorted results.



| # | Library           | Score | DotProd | Rev-Dot | Prob. (%) | PSS-Dot | DBs         | Name                                      |
|---|-------------------|-------|---------|---------|-----------|---------|-------------|-------------------------------------------|
| 1 | apci_msms_nist    | 790   | 886     | 929     | 98.9      | 939     | 35 WCDEF... | Diazinon [M+H] <sup>+</sup> QTOF 30V P=30 |
| 2 | lc-ms-ms_orbitrap | 556   | 822     | 869     | 0.98      | 887     |             | Diazinone [M+H] <sup>+</sup> 35 P=305.1   |
| 3 | lc-ms-ms_orbitrap | 483   | 752     | 999     | 0.12      | 752     |             | diethoxy-(2-isopropyl-6-methyl-pyri       |
| 4 | hr_msms_nist#2    | 0     | 8       | 114     | 0.00      | 70      | 1 G         | N-[2-[3-(Ethylthio)-2,5-dihydro-5-oxo     |
| 5 | hr_msms_nist      | 0     | 6       | 35      | 0.00      | 167     | 4 E         | (Triphenylphosphoranylidene)aceta         |



Library Search Options

Search MS/MS Libraries Automation Limits Constraints

Spectrum Search Type

☐ Identity ☒ Similarity

MS/MS Hybrid

Precursor Ion m/z

☐ in spectrum 305.1094

Spectrum Search Options

Method

Full Spectrum Search (Score)

Full Spectrum Search (Score)

Impurity Tolerant Search (Rev-Dot)

Partial Spectrum Search (PSS-Dot)

☐ Match Ion

Presearch

☒ Default ☐ Fast ☐ Off ☐ MW 1

☐ InChIKey

blank = match search spectrum InChIKey

Other Options

☒ Automation ☐ Auto Report

☒ Apply Limits ☐ Use Constraints

Structure Similarity Search Options

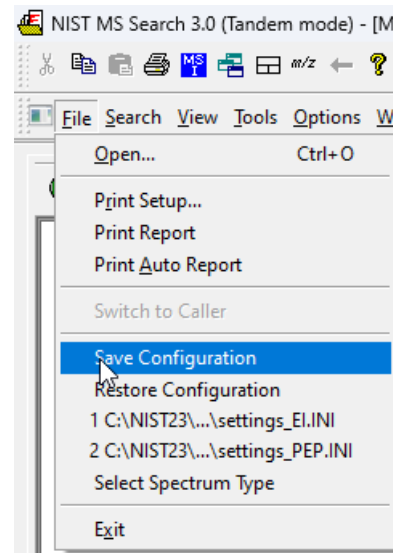
☒ Match Number of Rings ☐ Show Homologues

OK Cancel Help



## Important!! Save Your Settings for Future Sessions!!

- After initially setting up search preferences, most normally do not change significantly
- *All Settings* can be saved for future searches by **Save Configuration** with a unique name
- User can then easily **Restore Configuration**



## Libraries Typically Searched in My Work NIST v.3.0 (2023)

A variety of MSMS Libraries can be searched including NIST, Wiley, personal libraries, and MONA Libraries. The MONA libraries are *free* and I have copies in NIST format available on my website (link below). I *only* searched the NIST Purchased Libraries in my Overview Video.

| 1  | Lib No. | NIST Library           | No. of Spectra | Comment                                                                                                            |
|----|---------|------------------------|----------------|--------------------------------------------------------------------------------------------------------------------|
| 2  | 1       | hr_msms_nist           | 1,048,560      | NIST high resolution, Selecting this one, gets Lib 1 and 2, high resolution from NIST (Purchased)                  |
| 3  | 2       | hr_msms_nist#2         | 886,098        | NIST High resolution, This one used by selecting library 1, high resolution from NIST (Purchased)                  |
| 4  | 3       | epa_starter            | 4,064          | NIST, Free high resolution from NIST ( <a href="#">link</a> )                                                      |
| 5  | 4       | lr_msms_nist           | 433,692        | NIST Low Resolution (Purchased ), All libraries <i>NOT</i> beginning with LR assumed by NIST to be High Resolution |
| 6  | 5       | apci_msms_nist         | 5,714          | NIST High resolution APCI (Purchased)                                                                              |
| 7  | 6       | wmsn1                  | 12,048         | Wiley MSforID_1.5 (Purchased)                                                                                      |
| 8  | 7       | lr_mwwwtox2019_rebuild | 13,027         | Wiley Specialty rebuilt by J. Little, LR added to name for NIST Search (Purchased)                                 |
| 9  | 8       | lc-ms-ms_agilent_qtof  | 23,258         | MONA Free my website NIST format, high resolution                                                                  |
| 10 | 9       | lc-ms-ms_negative_mode | 46,459         | MONA Free mix of high and low resolution,                                                                          |
| 11 | 10      | lc-ms-ms_orbitrap      | 66,201         | MONA Free high resolution                                                                                          |
| 12 | 11      | lc-ms-ms_positive_mode | 98,154         | MONA Free mix of high and low resolution                                                                           |
| 13 | 12      | lc-ms-ms_qtof          | 47,838         | MONA Free high resolution                                                                                          |
| 14 | 13      | lc-ms-ms_spectra       | 148,509        | MONA Free, mix of high and low resolution                                                                          |
| 15 | 14      | massbank_mona          | 72,360         | MONA Free, high resolution                                                                                         |
| 16 | 15      | massbank               | 52,950         | MONA Free, no structures, high resolution                                                                          |
| 17 | 16      | lc-ms_spectra          | 153,242        | MONA Free, mix of high and low resolution                                                                          |
| 18 |         | sum                    | 3,112,174      |                                                                                                                    |

**Free MONA Libraries in NIST Format on my Website**

[713K FREE spectra and 653K Structures](#) (Be patient, will take a while to download!)