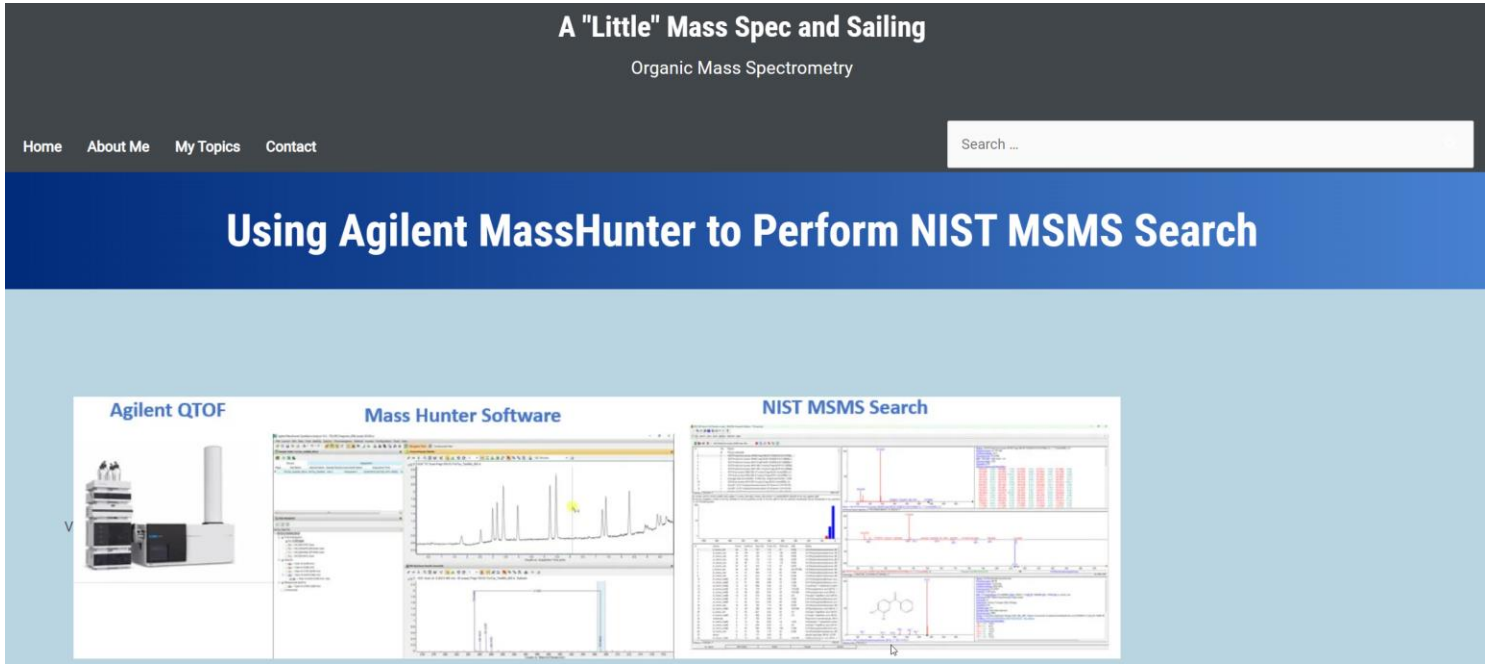


James Little
Mass Spec Interpretation Services
Sept. 9, 2024

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

Overview of Process

- Just an overview of process
- For Details, see *complete* training at websites below

MassHunter YouTube Training Videos

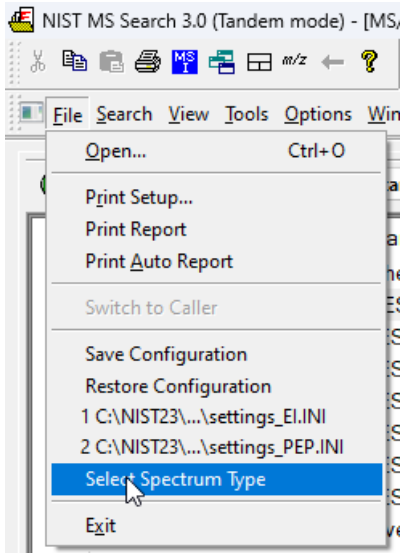
MassHunter Qualitative Analysis Training Video EP01-Basic Navigation
MassHunter Qualitative Analysis Training Video EP02-Extracting Ion Chromatograms
MassHunter Qualitative Analysis Training Video EP03-Other Miscellaneous Tips

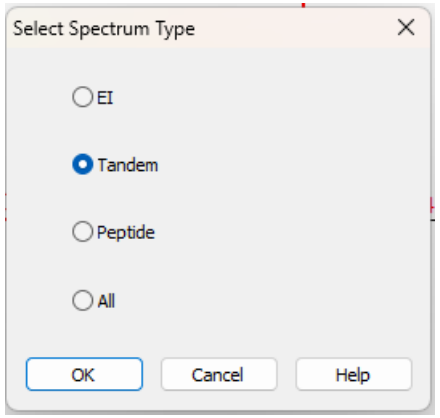
NIST MSMS Full Course

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

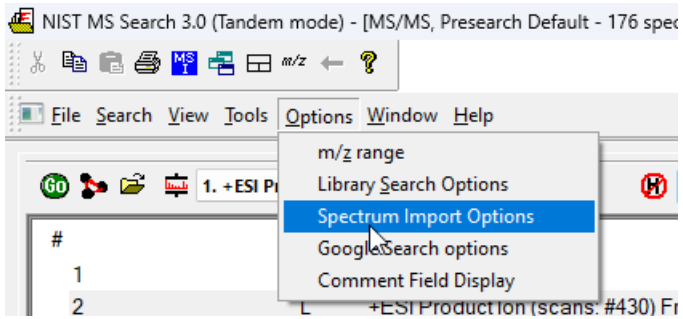
Some Typical User Options and Setting for NIST MSMS 2023 Search Version 3

2023 Version NIST Search Simplifies Menus for Different Modes

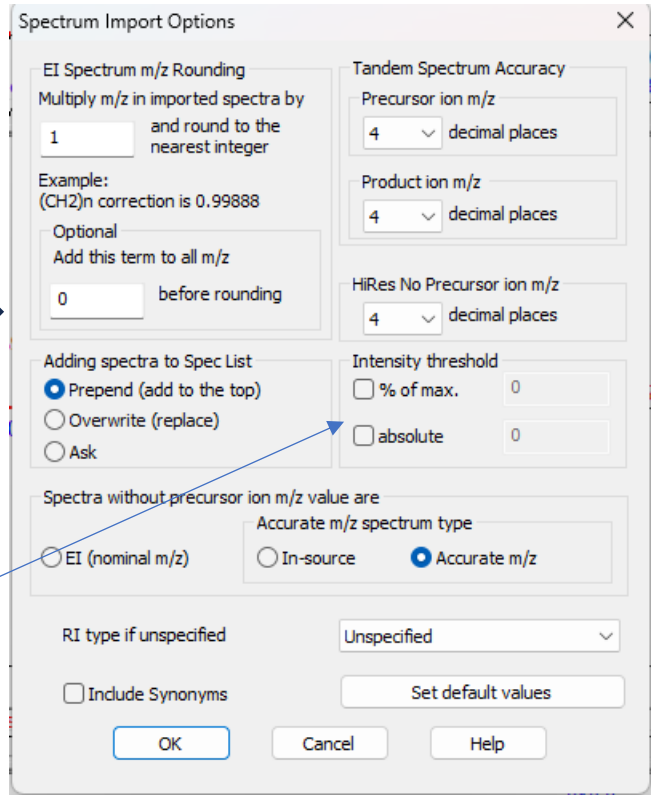




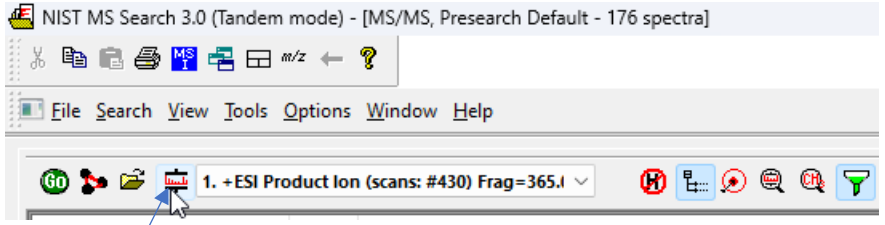
Typical Import Options Used



NOTE: Very useful for removing low intensity ions and electronic noise!



Library Search Menu in NIST MSMS Search

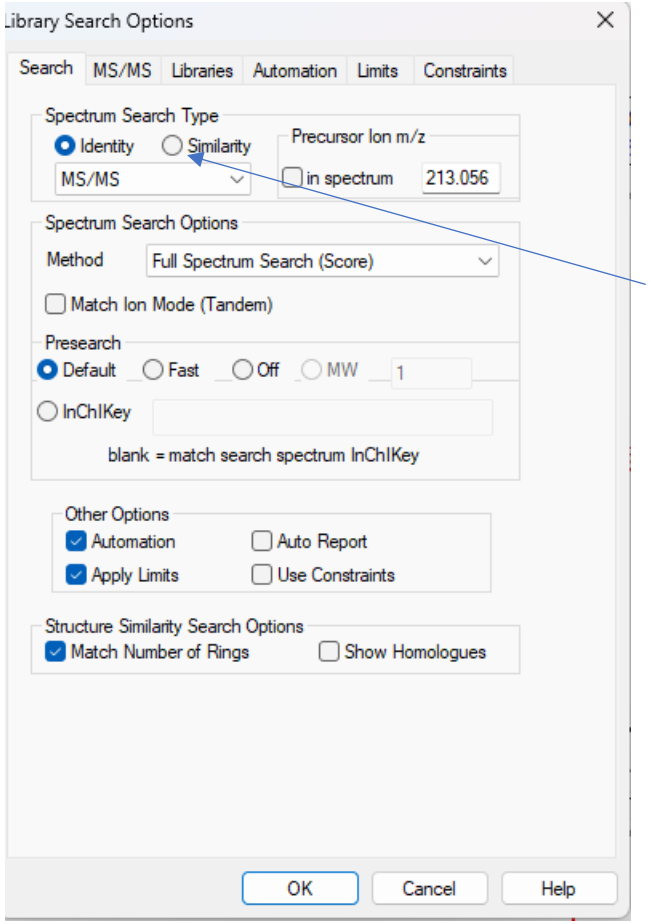


Click here

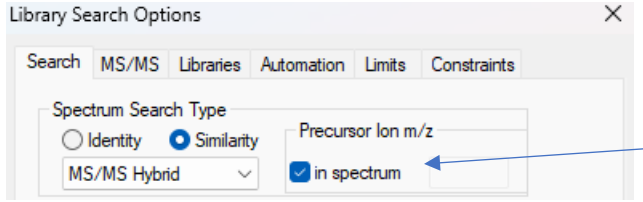
Can do Identity MS/MS which just searches spectra restricting by the precursor ion and its specified *m/z* Tolerance set in MS/MS tab (this one returns fewer but likely more useful results if compound's spectrum is present)

Or

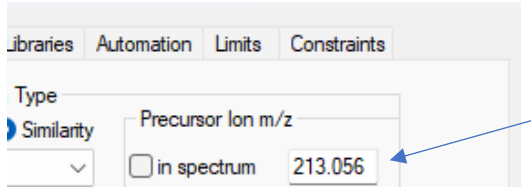
Identity/High Resolution No Precursor, just compares the overall fit of unknown spectra to reference spectra



Another type search would be Similarity/Hybrid MS/MS



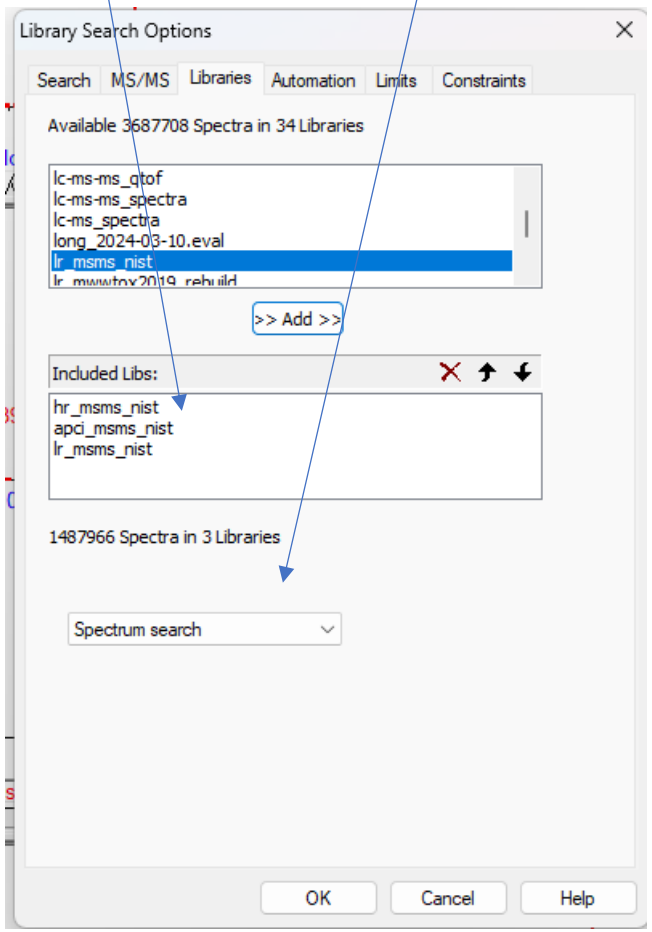
If precursor *m/z* imported correctly with spectrum, can have Precursor Ion m/z "in spectrum"



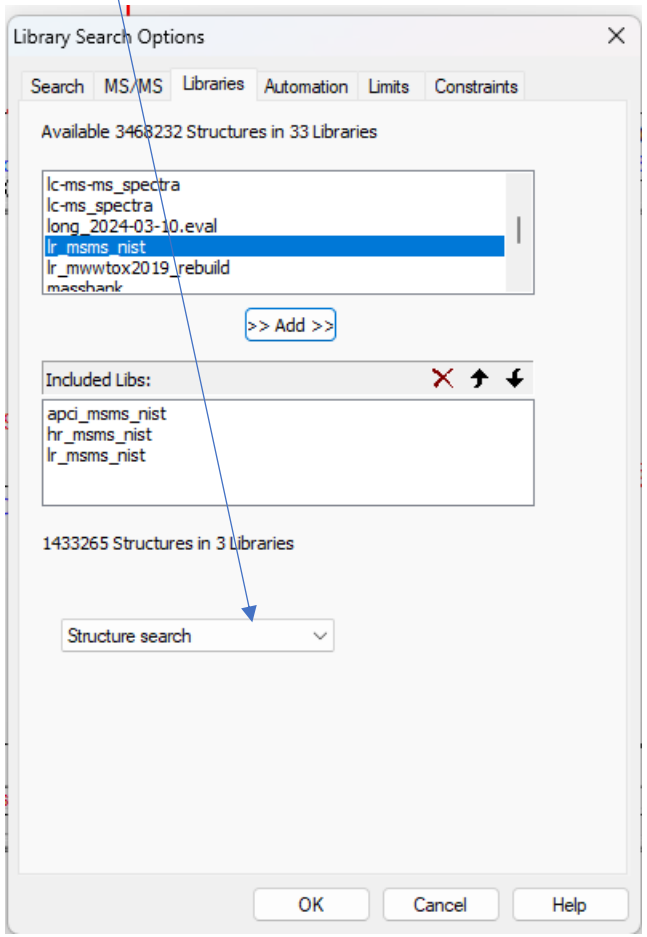
If precursor m/z not imported properly, user can unclick "in spectrum" and then enter manually

Other Settings in NIST MSMS Search

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



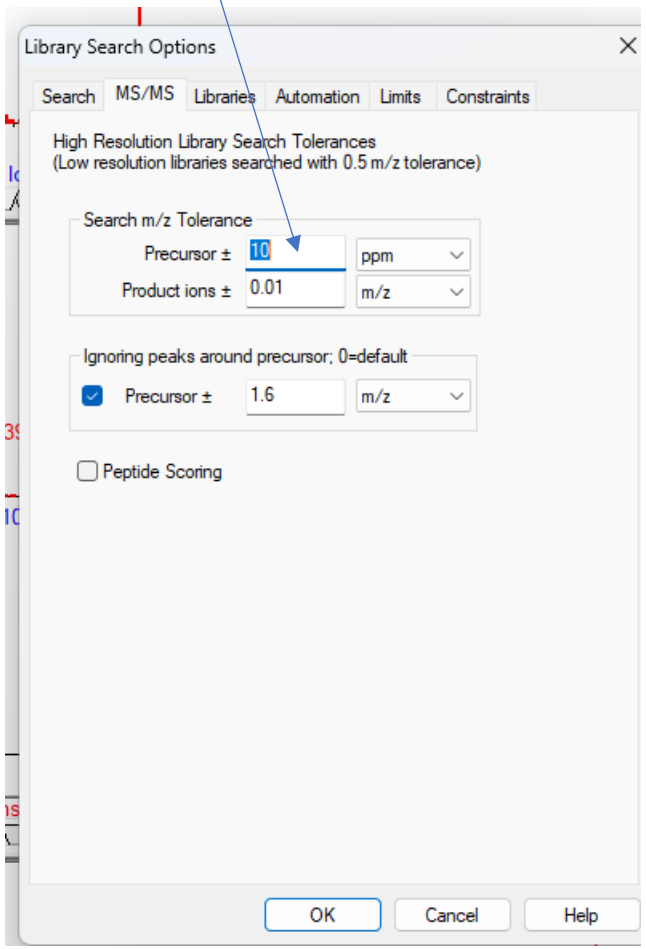
Libraries to search by Structure are also selected after toggling to ***Structure Search***

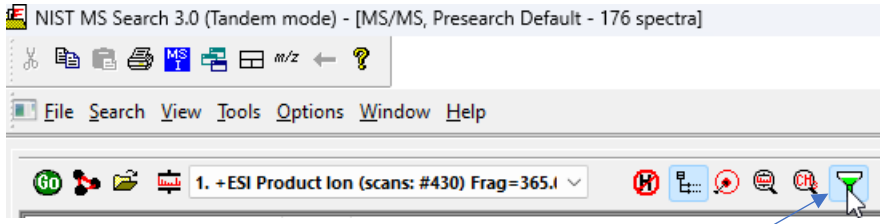


Other Settings in NIST MSMS Search

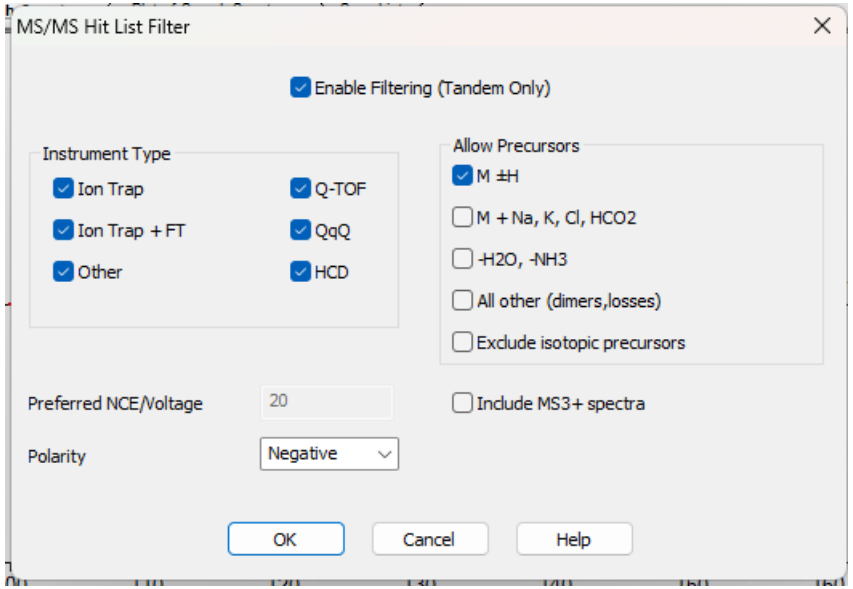
Check the settings in Library Search Options/MS/MS Tab

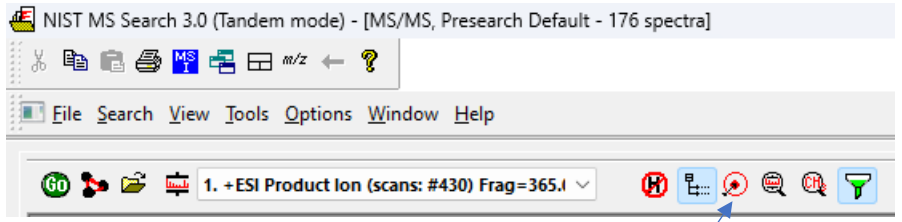
Set the tolerance for precursor m/z which is appropriate for your instrument





The “funnel” settings affect the results displayed from MSMS search such as polarity of MSMS spectrum and many others if “*Enable Filtering (Tandem Only)* is selected



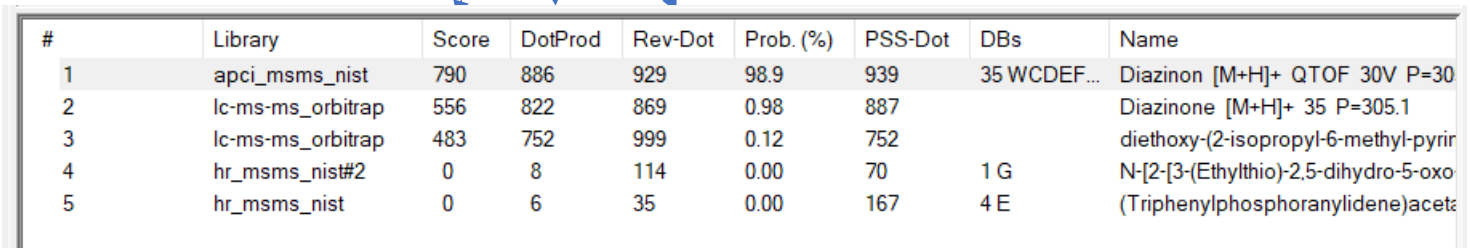


The “Best Matching” only can simplify the results, removing all but the best matching spectrum using the CAS Number as a filter

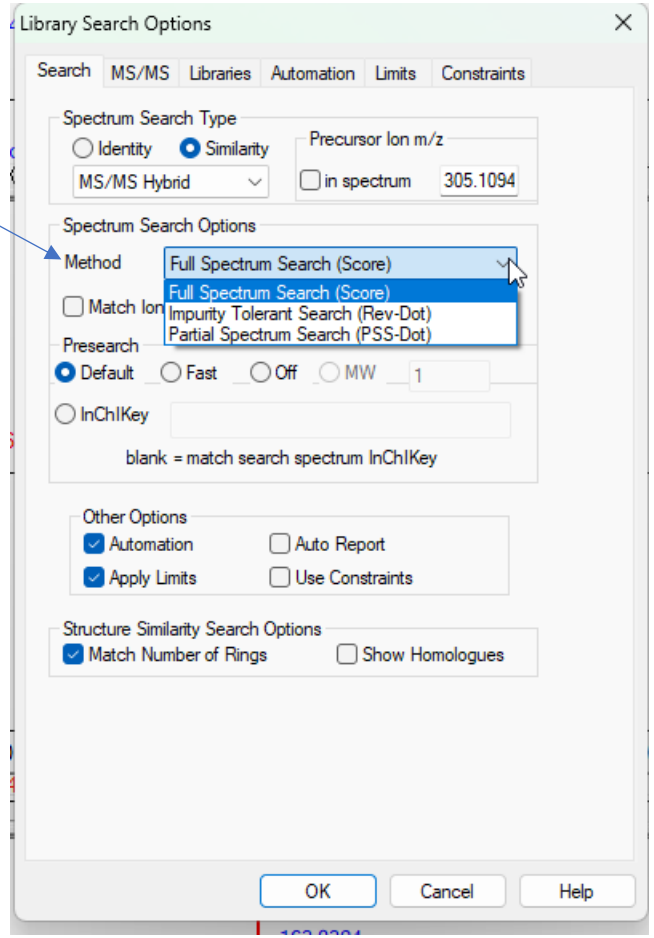
Sorting of the Search Results

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 fit 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. However, if the spectrum of interest was eliminated in the original search because more than the specified no. of hits, it will *not be present* in the resorted results.

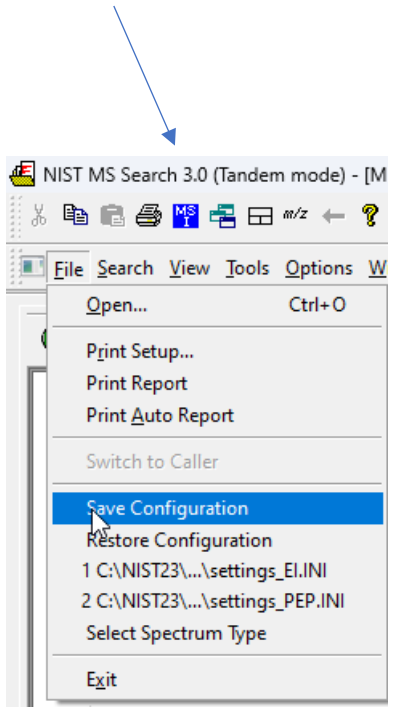


#	Library	Score	DotProd	Rev-Dot	Prob. (%)	PSS-Dot	DBs	Name
1	apci_msms_nist	790	886	929	98.9	939	35 WCDEF...	Diazinon [M+H]+ QTOF 30V P=30
2	lc-ms-ms_orbitrap	556	822	869	0.98	887		Diazinone [M+H]+ 35 P=305.1
3	lc-ms-ms_orbitrap	483	752	999	0.12	752		diethoxy-(2-isopropyl-6-methyl-pyrir
4	hr_msms_nist#2	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



Important!! Save Your Settings for Future Sessions!!

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



Libraries Searched

A variety of MSMS Libraries can be searched including Wiley licensed libraries, personal libraries, and MONA Libraries. The MONA libraries are free and I have copies in NIST format available on my website. The ones that I routinely utilize are listed below:

	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
3	2	hr_msms_nist#2	886,098	NIST High resolution, This one used by selecting library 1, high resolution from NIST
4	3	epa_starter	4,064	NIST, Free high resoluton from NIST
5	4	lr_msms_nist	433,692	NIST Low Resolution, Low ressolution from NIST, all libraries not beginnin with LR assumed by NIST to be low resolution
6	5	apci_msms_nist	5,714	NIST High resolution APCI
7	6	wmsn1	12,048	Wiley MSforID_+_1.5
8	7	lr_mwwtox2019_rebuild	13,027	Wiley Specialy rebuilt by JL, LR added to name for NIST search
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 Libraries in NIST Format on my Website

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