#### LCMS Unknown Identifications Using MSMS Libraries Part II: NIST Search Software and Libraries

Updated 12/27/20

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- ■Retired\* Research Fellow, Eastman Chem. Co.
- ■42 years experience unknown identification
- Now Consultant, MS Interpretation Services

Specialties<sup>1</sup> EI GC-MS, LC-MS/MS, Chemical Ionization,<sup>3</sup> Accurate Mass, Derivatization,<sup>4,5</sup> MS library management, SciFinder<sup>7</sup>, Chemspider<sup>7</sup>, Surfactant ID,<sup>8</sup> NMR, GC-IR, organic synthesis, matrix ionization effects,<sup>6</sup> etc.



Eastman Chemical Company, Main Site, Kingsport, TN 50 Manufacturing Sites Worldwide, ~14,500 Employees

\*<u>https://en.wikipedia.org/wiki/Eastman\_Chemical\_Company</u>



>50 Mass Specs Networked <u>Worldwide</u>

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#### **LCMS Unknown Identifications Using MSMS Libraries**

- Part I: Overview of Software and User Customized Configurations
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- Part VII: Using and Creating Other MSMS Libraries
- Part VIII: Identification of Unknowns with "Spectraless" Libraries



## **NIST Software in General is "Windows Compliant"**

-left click (LMB) to select an item, double LMB on that item to perform operation

-*right click (RMB)* in area or item to see operations that can be performed or to change properties of window

-LMB on first item and last item to select group while holding shift key

-LMB to select/deselect individual items while holding Ctrl button

-use up and down arrows on keyboard to step between entries

-some NIST windows such as librarian have no delete button to delete ions, *must* use delete key on keyboard!

Tip 3: LMB and drag to

headers

rearrange order of column

-control a (select all), control x (delete selected), control c (copy); control v (paste)

-control k copies entries into windows in tab-separated text format, e.g., paste into Excel

-F1 MS Search help

-F9 send spectrum to MS Interpreter

									_
#	Lib.	Name	Matc	h Prob. (%)	RI	B Matoh	Syn	DBs	
<b>⊕</b> 1	R	Undecane	955	44.8	1190	955 😽	4	8	
<b>⊞</b> 2	М	Undecane	945	44.8	1100	945	4	8	-
<b>⊞</b> 3	R	Undecane	944	44.8	1100	958	4	8	=
4	w1	Undecane	937	44.8	-	955	11	0	
5	w1	Undecane	933	44.8	-	950	11	0	
6	w1	Undecane	932	44.8		939	11	0	
L <b>MB</b> on c Can sort i	olumn of n lower v	interest alue first or higher	<i>Tip 1:</i> When revier results, use up an on keyboard to que through results!	ewing search d down arrows ickly step	<b>Tij</b> in l rig qu	D 2: When MS Interpre ht arrows o ickly review	viewing s eter, use n keyboa r results!	structur left and ard to	es

# *Note:* Very Useful information in NIST Tandem Quick Start Guide<sup>28</sup> and NIST Users' Manual<sup>2</sup>

#### Users' guide<sup>28</sup> includes:

- >Search settings for normal applications
- Result scoring explanations
- ➢Filtering information
- Suggested hit list columns to display
- >Introductory paragraph below, etc.

# Information for NIST 20 Tandem Library Users

This document describes special features of the NIST Mass Spectral Search Program v.2.4 (NISTMS.exe) of interest to the Tandem Library users. General software features are presented in separate documentation.

## **NIST Mass Spectrometry Software and MSMS (Tandem) Libraries**

•Free software program for searching and processing MSMS (tandem) libraries

- Aggressive NIST in-house program for obtaining new spectra of purchased compounds<sup>11,13</sup>
- Supplied with purchased NIST MSMS libraries
- Developed by NIST for curating and searching MSMS libraries
- Import data from variety of instrument manufacturers
- Searches by spectrum, structure, name, CAS No., peaks, MW, MF, etc.
- •MS Interpreter<sup>10,17-19</sup> for correlating molecular substructures to fragment ions
- Structure export and import using vendor drawing packages
- Searches other libraries including user, Wiley, MoNA, etc.

#### Limitations of MSMS Searches for Unknown Identification:

•Overall process of LCMS more difficult than EI GC-MS due to large number of experimental variables of former method

- MSMS spectra are much less reproducible than EI spectra
- MSMS spectra very instrument dependent
- •NIST MSMS search software<sup>24,25</sup> not as straightforward as EI

Recording many different spectra for each species at a variety of collision energies greatly improves the usefulness of tandem searches

•High resolution LC-MS-MS with isotopic fidelity, mass accuracy, and accessibility very beneficial

### **Three Basic Types of NIST MSMS (Tandem) Searches**

 "EI Simple Similarity," no weighting of m/z intensities, *direct peak matching approach Use* for finding similar compounds;

*however,*<sup>28</sup> can use "Identity In-source HiRes," particularly good for accurate mass data, does not exclude low-resolution spectra in results

- 2) "MS/MS Identity," search of fragment ions with specified precursor ion *Use* for finding exact compound or isomer
- 3) "MS/MS Hybrid," *novel search method* <sup>20-27</sup> *Use* for identifying unknown components
- > Finds compounds that differ by a single or multiple chemical moieties
- > Key parameter is "Delta-Mass" which is mass difference between query and library components
- Significantly *extends* the scope of a library for identifications
- Combines "direct peak matching" score with the logical equivalent of "neutral loss matching" score to yield a "hybrid" score
- Precursor ion *must* be specified
- More discussion included in *Part III* of series

# **Overall Description for Searching Libraries and Displaying Results**



## NIST MSMS (Tandem) Libraries 2020 Release<sup>9-13</sup>

hr\_msms\_nist: 1,026,712 high resolution accurate mass of small molecules
lr\_msms\_nist: 215,649 low resolution spectra of small molecules
apci\_msms\_nist: 3,555 high resolution APCI spectra of extractables and leachables

# **31K** Compounds, **2X** More than 2017 186K Precursor Ions - 1.3M Spectra

Fragmentation Methods
27,840 HRAM (High Res Accurate Mass) Compounds
29,890 QTOF, HCD, IT-HRAM, QqQ Compounds

29,444 Ion Trap Compounds (Low Res., up to MS<sup>4</sup>) 246 APCI HRAM 'Extractables and Leachables' **Precursor Ion Types** 

26,575 Protonated

- 12,589 Deprotonated
- 10,032 Water/Ammonia Loss
- 24,167 Other In-Source Generated



**NOTE:** Above numbers do not include **biopep\_msms\_nist**<sup>14-16</sup> *specialty library* of >4.3 million spectra of 1.26 millions species which utilizes special search program

#### Minimizing Number of Results After Search Performed Process is Reversible without Repeating Search



#### For Example: Two Types of lons Filtered with MS/MS Hit List Filter Option

Name: Metosulam 176.9921 100-Precursor type: [M+H+2i]+ Instrument type: HCD Collision energy: NCE=50% 42eV Precursor m/z: 420.011 Formula: C14H13Cl2N5O4S MW: 417 Exact Mass: 417.00653 CAS#: 139528-85-1 NIST#: 1584905 ID#: 349076 DB: hi Other DBs: None Comment: NIST Mass Spectrometry Data Center Notes: Consensus spectrum; Nreps=10/10; Mz\_diff=-2.6ppm; Mz\_exact\_mono=418.013 Ion mode: P Instrument: Thermo Finnigan Elite Orbitrap 50-Ionization: ESI Collision gas: N2 Sample inlet: direct flow injection Spectrum type: MS2 InChIKey: VGHPMIFEKOFHHQ-UHFFFAOYSA-N Non-stereo 55 m/z Values and Intensities: 82.0399 1.20 7/10 83.0239 1.10 6/10 94.0034 1.20 7/10 96.0555 228.0312 283.1067 356.0496 10/10 96.0555 6.49 200 80 120 160 240 280 320 360 400 104.0494 1.40 9/10 (hr\_msms\_nist) Metosulam [M+H+2i]+ HCD 50% P=420 < □ 111 Plot/Text of Hit / Plot of Hit /

Multistage "MS4" Spectrum below, select "Include MS3+ Spectra"



MS/MS of M+2 for <sup>37</sup>Cl isotope, select "Exclude Isotopic Precursors"

#### Setting Up Default Parameters for Similarity Search (Step 1) <u>Critical Step</u>

-EI Simple Similarity Search, "direct peak matching"

-Basic search for *similar* spectra, no weighting of intensities

-Select the following settings

Two ways to access:	Library Search Options
Two ways to access:	Library Search Options         Search MS/MS Libraries Automation Limits Constraints RI (GC)         Spectrum Search Type         Identity         Identity         Similarity         Precursor MW         El Simple         Image: Spectrum Search Options         Reverse Search         Penalize rare compounds         Match Ion Mode (T andem)         Presearch         Default         Fast         Off         MW         436         InChIKey         blank = match search spectrum InChIKey
#     Src.     Name     Pubchem Search options       1     L     Clipbo     SOS options       2     L     Clipboard #1       3     M     Acetone	Other Options Auto Report Apply Limits Use Constraints Structure Similarity Search Options Match Number of Rings Show Homologues

**NOTE:** If you want to use "Identity In-Source HiRes" instead of "Similarity EI simple," **must** set-up values in MS/MS Tab,<sup>28</sup> use same value for Product ions +/- of 0.01 *m/z* found in slide 22, takes advantage of hi resolution accurate mass information

Help

OK.

Cancel

#### Setting Up Default Parameters for Similarity Search (Step 2) Select the Libraries Used for Spectral Searches in Library Search Options

•LMB on the Libraries tab and make sure Spectrum Search is selected

Select the group of libraries to be searched

>>Add>> all libraries to be searched

• Order of libraries normally only important in Other Search Tab which return maximum no. of hits in Sequential Search Function

Add *same* libraries for *structure* search, discussed in detail in *Part V* of series

![](_page_14_Figure_6.jpeg)

# Setting Up Default Parameters for Similarity Search (Step 3) Selecting Up Limits and Automation Settings in Library Search Options

Library Search Options	Library Search Options
Library Search Options  Search MS/MS Libraries Automation Limits Constraints RI (GC)  Number of Hits to Print  Include Spectrum Plot in Report  Draw Structure in Plots  Apply Maximum Spectrum Length 2000  Return Focus to Caller upon Completion  Automatic Search On	Library Search Options         Search MS/MS Libraries Automation Limits Constraints RI (GC)         Apply Limits         Minimum Abundance Off         Minimum m/z         never greater than         50         Maximum m/z         Off         Set Default
OK Cancel Help	OK Cancel Help

#### Setting Up Default Parameters for Similarity Search (Step 4) Constraints Applied to Hit List *After* the List Is Determined

- 1. I very seldom use these to refine the search results
- 2. However, *very useful* when finding spectra in the libraries using *Sequential Method* under *Other Search* Tab

2

1
Library Search Options
Search         MS/MS         Libraries         Automation         Limits         Crostraints         RI (GC)           Use Constraints         Clear All         Selected:0         Selected:0         Selected:0
MW(Nominal Mass) Exact Mass Name Fragment Elements Value Elements Present Peaks Other Databases
Molecular Weight 1 2000

УM	693	0.01	Butanoic ad	aa, z-etnyi-, i	,2,3-K
1 M	685	0.01	Hexanoic a	cid, 2,2-dime	ethyl-
بەر، ،		0.01		and the second	
Names A 🌣	structures /				
	Lib. Search		Other Se	arch	
lelp, press	F1				
		Y			
	NIST MS Sea	rch 2.4 - [ID:	s range 1-100000, 9		
	📃 <u>F</u> ile <u>S</u> earch	n <u>V</u> iew <u>T</u> o	ols <u>O</u> ptions <u>W</u> ind	¢	
	X 🖻 🖬 🖨	i 🕂 🚰	暑 🖂 m/z 🔶 🢡		
	**		······		
	(P) Sequent	ial Method		1	
		7	Y		
Se	equential Search	1			x
	Options Constru	aints			
ľ	V Use Constra	iints	Clear All	Selected:0	
	MW(Non	ninal Mass)			<b>^</b>
	Exact Ma	ass			
	📃 Name Fr	agment			=
	Elements	: Value			
	Elements	: Present			
	📄 Peaks				
	🔲 Other Da	itabases			*
	Molecular V	Weight	1	2000	

#### Setting Up Default Parameters for Similarity Search (Step 5) Ways of Simplifying Search Results Display

These settings used to minimize spectra shown in library search results
 *Turn* them *off initially*, but use as needed to refine the results
 They can be used to limit the results *without repeating* the search
 The 1<sup>st</sup> setting removes hits from list with same CAS number
 The 2<sup>nd</sup> group of *filter settings* uses tags in library spectra to filter results

![](_page_17_Picture_2.jpeg)

### Setting Up Default Parameters for Similarity Search (Step 6) Setting Default *m*/*z* Range Displayed in Spectra

Æ	NIST N	MS Searc	:h 2.4	- [Ident,	Presearch Default - InLib = 491, 5	7 spectra				
	🖳 <u>F</u> ile	<u>S</u> earch	<u>V</u> iew	<u>T</u> ools	<u>Options</u> <u>W</u> indow <u>H</u> elp					
	X 🗈	R &	MS	<b>STN =</b> =	‴∕z m∕z range					
			-		Library <u>S</u> earch Options					
	@ 9	🖌 🚅	line b	1 Benze	Replicates	B	P. 🕥	a	<u>m</u>	
	•••		-	I. DEHZC	Spectrum Import Options	90	#: 💌	•= <b>(</b>	~	
	#		Src.	Nam	Pubchem Search options					
	1		М	Benz	SOS options					
	2		М	Aceti	Comment Field Display					
	3		M	1-No	nen-3-one, 1-(3,4-aichiorophen	у <u>л</u> -				
	4		ki	8-Nitr	oquinoline					
	5		Ŀi	N-72 -	1-Dichlorohenzy/Noronan-2-am	ino				

	m/z range
-I prefer min/max of both spectra	<ul> <li>Normal display</li> <li>Neutral Loss display</li> </ul>
	<ul> <li>Scale as search/spec list spectrum</li> <li>Scale as hit list spectrum</li> </ul>
	<ul> <li>Min/max of both spectra</li> <li>Fixed Min m/z</li> <li>Max m/z</li> </ul>
	Anchors
	OK Cancel Help

# Setting Up Default Parameters for Similarity Search (Step 7) Saving Setup of Standard Search Parameters

After all the parameters are setup for search, save them for future use
 Example setup below was named *Direct Peak Matching MSMS* In the future, this can be "restored" by selecting it from this menu

![](_page_19_Figure_2.jpeg)

#### Setting Up Default Parameters for Identity MS/MS Search (Step 1) <u>Critical Step</u>

*Many* of settings same as in first type of search

Searches are restricted by precursor ion m/z

Check "in spectrum" if precursor field is *not listed* in spectrum header information

>User *must* then enter value in "Precursor Ion m/z" field appropriate for each spectrum searched

Library Search Options           Search         MS/MS         Libraries         Automation         Limits         Constraints         RI (GC)
Spectrum Search Type Identity Similarity Precursor Ion m/z MS/MS Spectrum Search Options Reverse Search Penalize rare compounds Match Ion Mode (T andem) Presearch Default Fast Off MW 436 InChIKey
Diank = match search spectrum InLhIKey         Other Options         Automation         Auto Report         Apply Limits         Use Constraints         Structure Similarity Search Options         Image: Match Number of Rings         Show Homologues
OK Cancel Help

### Setting Up Default Parameters for Identity MSMS Search (Step 2) MS/MS Limits Tab

#### *Excerpt* from NIST Tandem Quick Start Guide<sup>28</sup>

The precursor mass tolerance should be set to reflect the accuracy of your instrument - NIST Tandem Library spectra always have the exact mass value for the precursor ion. It is generally recommended that the product-ion tolerance be set at 0.01 *m/z* units to ensure that lower mass peaks are matched. Due to the fact that spurious peaks commonly appear near the precursor ion, a setting of 20 ppm is recommended for the **Ignoring peaks around precursor** specification. **DO NOT** select **Peptide Scoring** unless using peptide libraries.

(		
t	Search MS/MS     Libraries     Automation     Limits     Constraints     RI (GC)       MS/MS     and In-source HiRes     search options       Search     Search     Toler area	<i>Additional</i> information available within program "Help Topics" on MSMS parameters
	Brecilisor + 20	
	Product ions ± 0.01	Help Topics NIST MS Search 2.4 Help
1	Ignoring peaks around precursor; 0=default	Go to Undate Website 🛛 🖓 🖓 🖓
	Precursor ± 20 ppm •	Download Libraries
	Peptide Scoring	Y About nistms     Type in the keyword to find:
		msms
		For example:
4 ) -		<u>Note.</u> For Low Resolution library (LR_*) product ions tolerance is fixed at unit mass resolution.
1	OK Cancel Help	22

#### Setting Up Default Parameters for Identity MSMS Search (Step 3) Select the Libraries Used for Spectral Searches in Library Search Options

•LMB on the Libraries tab and make sure Spectrum Search is selected

Select the group of libraries to be searched

>>Add>> all libraries to be searched

 Order of libraries normally only important in Other Search Tab which return maximum no. of hits in Sequential Search Function

•Add *same* libraries for *structure* search, discussed in detail in *Part V* of series

![](_page_22_Figure_6.jpeg)

# Setting Up Default Parameters for Identity MSMS Search (Step 4) Selecting Up Limits and Automation Settings in Library Search Options

Library Search Options	Library Search Options
Search MS/MS Libraries       Automation       Limits       Constraints       RI (GC)         Number of Hits to Print       Include Spectrum Plot in Report       Include Spectrum Plots       Apply Maximum Spectrum Length       2000         Return Focus to Caller upon Completion       Automatic Search On       Automatic Search On	Search MS/MS Libraries Automation Limits Constraints RI (GC) Apply Limits Minimum Abundance Off I Minimum m/z Never greater than 50 Maximum m/z Off Set Default
OK Cancel Help	OK Cancel Help

### Setting Up Default Parameters for Identity MSMS Search (Step 5) Constraints Applied to Hit List *After* the List Is Determined

- 1. I very seldom use these to refine the search results
- 2. However, *very useful* when finding spectra in the libraries using *Sequential Method* under *Other Search* Tab

2

1
Library Search Options
Search         MS/MS         Libraries         Automation         Limits         Crostraints         RI (GC)           Use Constraints         Clear All         Selected:0         Selected:0         Selected:0
MW(Nominal Mass) Exact Mass Rame Fragment Elements Value Elements Present Peaks Other Databases
Molecular Weight 1 2000

эм	ხყე	0.01	Butanoic ac	:ia, 2-etnyi-, i	,2,3-K
1 M	685	0.01	Hexanoic a	cid, 2,2-dime	thyl-
1 <del>1</del>	cor	0.01	O-+i	المعديدا فالمت	
ames A oud	ictures /				
Li	ib. Search		Other Sea	arch	
elp, press F1					
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	🗐 NIST MS Se	arch 2.4 -	[IDs range 1-100000. 9		
	🛄 File Sear	:h View	Tools Options Wind	(	
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		37 🔢 🛚			
	AA Secure	tial Math			
	ara <u>seque</u> i	ILIAI IYICLIN			
			Y		
Seq	uential Searc	h			x
	ptions Const	raints			
	V Use Constr	aints			
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	MW(No	minal Ma	ss)		*
	📄 Exact M	lass			=
	Name F	ragment			_
	Elemen	ts Value			
	Elemen	is Presen	it i		
	Other D	atabases	1		-
					_
	Molecular	Weight	1	2000	

#### Setting Up Default Parameters for Identity MSMS Search (Step 6) Ways of *Simplifying* Search Results Display

These settings used to minimize spectra shown in library search results
 *Turn* them *off initially*, but use as needed to refine the results
 They can be used to limit the results *without repeating* the search
 The 1<sup>st</sup> setting removes hits from list with same CAS number
 The 2<sup>nd</sup> group of *filter settings* uses tags in library spectra to filter results

![](_page_25_Picture_2.jpeg)

## Setting Up Default Parameters for Identity MSMS Search (Step 7) Setting Default *m*/*z* Range Displayed in Spectra

NIST N	VIS Searc	:h 2.4	- [Ident,	Presearch Default - InLib = 491, 57 sp	ectra]				
🖳 <u>F</u> ile	<u>S</u> earch	<u>V</u> iew	<u>T</u> ools	Options Window Help					
X 🗈	R #	MS	STS 🚘	<sup>///</sup> z m/z range					
		_		Library Search Options					
@ 1	<b>⊾</b> @2	line la	1 Benze	Replicates	ിത	P. 🕥	a	CB.	0
<u> </u>	_	-	I. DEHZC	Spectrum Import Options	w	±			
#		Src.	Nam	Pubchem Search options					
1		М	Benz	SOS options					
2		M	Aceti	Comment Field Display					
3 M 1-No		1-No	nen-3-one, 1-(3,4-aichiorophenyi)-						
4		ki	8-Nitr	roquinoline					
5		ki 🛛	N-72 -	4-Dichlorohanzy/Ibronan-2-amina					
	NIST   Eile & Eile & Ei	NIST MS Search File Search Real Contents File Search Real Contents File Search File Search	NIST MS Search 2.4 File Search View Compared Search View Compared Search Compared Searc	NIST MS Search 2.4 - [Ident,         File       Search       View       Tools         Image: Search       View       Tools       Image: Search       View       Tools         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       Image: Search         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       Image: Search         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       Image: Search         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       Image: Search         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       Image: Search         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       Image: Search         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       Image: Search         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       Image: Search         Image: Search       Image: Search       Image: Search       Image: Search       Image: Search       I	NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 491, 57 sp         File Search View Tools       Options Window Help         Image: I	NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 491, 57 spectra]         File Search View Tools       Options Window Help         Image: Im	NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 491, 57 spectra]         File Search View Tools       Options Window Help         Image: Im	NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 491, 57 spectra]         File Search View Tools       Options Window Help         Image: Im	NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 491, 57 spectra]         File Search View Tools       Options Window Help         Image: Im

	m/z range
-I prefer min/max of both spectra	<ul> <li>Normal display</li> <li>Neutral Loss display</li> </ul>
	<ul> <li>Scale as search/spec list spectrum</li> <li>Scale as hit list spectrum</li> </ul>
	Min/max of both spectra
	○ Fixed Min m/z 0 Max m/z 1000
	Anchors
	OK Cancel Help

## Setting Up Default Parameters for Identity MSMS Search (Step 8) Saving Setup of Standard Search Parameters

After all the parameters are setup for search, save them for future use
 Example setup below was named *Identity MSMS*

>In the future, this can be "restored" by selecting it from this menu

![](_page_27_Figure_3.jpeg)

#### Setting Up Default Parameters for Similarity MS/MS Hybrid Search (Step 1) <u>Critical Step</u>

*Many* of settings same as in other types of MS/MS searches

Searches are restricted by precursor ion m/z

> **Un-**Check "in spectrum" if Precursor\_m/z field is *not listed* in spectrum header information of unknown

> Some manufacturers import Precursor\_m/z with their spectrum, others do not

>If not, user *must* then enter value in "Precursor Ion m/z" field

Library Search Options
 Search MS/MS Libraries Automation Limits Constraints RI (GC)
Spectrum Search Type Tdentity Similarity MS/MS Hybrid Frecursor Ion m/z MS/MS Hybrid Frecursor Ion m/z Ms/MS Hybrid Frecursor Ion m/z Ms/MS Hybrid Frecursor Ion m/z Precursor Ion m/z Frecursor Ion Mode Frecursor Ion
Other Options         Automation         Auto Report         Apply Limits         Use Constraints         Structure Similarity Search Options         Natch Number of Rings
OK Cancel Help

### Setting Up Default Parameters Similarity MS/MS Hybrid Search (Step 2) MS/MS Limits Tab

#### **Excerpt** from NIST Tandem Quick Start Guide<sup>28</sup>

The precursor mass tolerance should be set to reflect the accuracy of your instrument - NIST Tandem Library spectra always have the exact mass value for the precursor ion. It is generally recommended that the product-ion tolerance be set at 0.01 *m/z* units to ensure that lower mass peaks are matched. Due to the fact that spurious peaks commonly appear near the precursor ion, a setting of 20 ppm is recommended for the **Ignoring peaks around precursor** specification. **DO NOT** select **Peptide Scoring** unless using peptide libraries.

ſ	ibran Saarah Ontiona	
t	Search MS/MS Libraries Automation Limits Constraints RI (GC) MS/MS and In-source HiRes search options	<b>Additional</b> information available within program "Help Topics" on MSMS parameters
	Precursor ± 20	Halp
	Product ions ± 0.01 m/z • Ignoring peaks around precursor; 0=default Precursor ± 20 ppm •	Help Topics       Image: Search 2.4 Help         Go to Update Website       Image: Search 2.4 Help         Download Libraries       Image: Search 2.4 Help         Image: Search 2.4 Help       Image: Search 2.4 Help         Image: Search 2.4 Help <td< th=""></td<>
	Note My Thoughts: 1) Value in precursor ion window (Search <i>m/z</i> Tolerance) <i>is not</i> critical in Hybrid search 2) Often get <i>better visual</i> results with Precursor+/- <i>unchecked</i> in "Ignoring peaks around")	For example: <u>Note.</u> For Low Resolution library (LR_*) product ions tolerance is fixed at unit mass resolution.
	OK Cancel Help	30

#### Setting Up Default Parameters for Similarity MS/MS Hybrid Search (Step 3) Select the Libraries Used for Spectral Searches in Library Search Options

•LMB on the Libraries tab and make sure Spectrum Search is selected

Select the group of libraries to be searched

>>Add>> all libraries to be searched

 Order of libraries normally only important in Other Search Tab which return maximum no. of hits in Sequential Search Function

•Add *same* libraries for *structure* search, discussed in detail in *Part V* of series

![](_page_30_Figure_6.jpeg)

# Setting Up Default Parameters for Similarity MS/MS Hybrid Search (Step 4) Selecting Up Limits and Automation Settings in Library Search Options

Library Search Options X	Library Search Options
Number of Hits to Print       Include Spectrum Plot in Report         Include Spectrum Plot in Report         Draw Structure in Plots         Apply Maximum Spectrum Length	✓ Apply Limits       Minimum Abundance Off       Minimum m/z       Never greater than       Maximum m/z       Off
Return Focus to Caller upon Completion	Set Default
OK Cancel Help	OK Cancel Help

# Setting Up Default Parameters for Similarity MS/MS Hybrid Search (Step 5) Constraints Applied to Hit List *After* the List Is Determined

- 1. I very seldom use these to refine the search results
- 2. However, *very useful* when finding spectra in the libraries using *Sequential Method* under *Other Search* Tab

2

1
Library Search Options
Search         MS/MS         Libraries         Automation         Limits         Crostraints         RI (GC)           Use Constraints         Clear All         Selected:0         Selected:0         Selected:0
MW(Nominal Mass)       Exact Mass       Name Fragment       Elements Value       Elements Present       Peaks       Other Databases
Molecular Weight 1 2000

УM	ხყე	0.01	Butanoic ai	cia, 2-ethyi-, i	,2,3-K
1 M	685	0.01	Hexanoic a	cid, 2,2-dime	ethyl-
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	Uther Da	tabases			
	Molecular \	Weight	1	2000	

#### Setting Up Default Parameters for Similarity MS/MS Hybrid Search (Step 6) Ways of Simplifying Search Results Display

These settings used to minimize spectra shown in library search results
 *Turn* them *off initially*, but use as needed to refine the results
 They can be used to limit the results *without repeating* the search
 The 1<sup>st</sup> setting removes hits from list with same CAS number
 The 2<sup>nd</sup> group of *filter settings* uses tags in library spectra to filter results

![](_page_33_Picture_2.jpeg)

# Setting Up Default Parameters for Similarity MS/MS Hybrid Search (Step 7) Setting Default *m*/*z* Range Displayed in Spectra

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	5		ki 🛛	N-72 -	4-Dichlo	rohenzy.	(Inronan-S	7-amino					

	m/z range
-I prefer min/max of both spectra	<ul> <li>Normal display</li> <li>Neutral Loss display</li> </ul>
	<ul> <li>Scale as search/spec list spectrum</li> <li>Scale as hit list spectrum</li> </ul>
	<ul> <li>Min/max of both spectra</li> <li>Fixed Min m/c</li> </ul>
	Anchors
	OK Cancel Help

#### Setting Up Default Parameters for Similarity MS/MS Hybrid Search (Step 8) *Adding* or *Changing* Columns in Results Display

>Useful to have "Rev-Dot" added as column in Identity MS/MS Search Results

- Can left click on column header and resort results by Rev-Dot
- >Rev-Dot score is not penalized for ions found in unknown spectrum not found in reference spectrum

![](_page_35_Figure_4.jpeg)

2) Select columns needed to reproduce display in bottom window, can sort by any column desired by clicking on heading after search is complete, the o-score is part of the hybrid score based on "direct peak matching" so similar to that obtained in first search, "El Simple Similarity"

$\rightarrow$											_
	#	Lib.	Score	DotProd	o.Score	o.DotProd	DeltaMass	Prec. Type	Energy	Name	4
	1	hr	999	999	999	999	-0.0000	[M+H]+	35%	Atrazine [M+H]+ IT-FT 35	Ξ
1	2	hr	985	997	676	974	-14.0157	[M+H]+	35%	Sebuthylazin [M+H]+ IT-F	-
1	3	hx	965	979	965	979	-0.0000	[M+H]+	35%	Atrazine [M+H]+ HCD 35	
1	4	hr A	938	982	938	982	-0.0000	[M+H]+	40%	Atrazine [M+H]+ HCD 40	
1	5	hr	936	990	3	49	-12.0267	[M+H]+	35%	Ametryne [M+H]+ HCD 3	
	6	lr	932	992	932	992	-0.0000	[M+H]+	35%	Atrazine [M+H1+ IT 35%	

# Setting Up Default Parameters for Similarity MS/MS Hybrid Search (Step 9) Saving Setup of Standard Search Parameters

After all the parameters are setup for search, save them for future use
Example setup below was named *Similarity Hybrid MSMS*In the future, this can be "restored" by selecting it from this menu

![](_page_36_Figure_2.jpeg)

# **Searching Demonstration**

-Example searches demonstrated in live presentation on YouTube video

![](_page_37_Picture_2.jpeg)

Library Search Properties			
Hit Text Info Unkno	wn Text Info		
Spec List Text Info Comp. Result	Histogram		
Hits List Spec List Plot of Hit Unknown	Plot Spec List Plot		
Structure View Options Structure Size in %: 50 Structures Only Items to Display			
Library	<u>^</u>		
Rec Tupe HiBes	Mods	HiBes	
Instr. Type Hilles	▼ No. Other Dbs		
Short Library Name		EI	
	V Prob.(%)	Identity	-
Clear History on Exit			
Color Settings			
Bonds and Rings	A		
Atomic Symbols			
Compound Name			
<ul> <li>Font Settings</li> </ul>			
Select Font Set for all v	riews		
			38
OK Cance			50

#### **Presentation References (Internet Links)**

- 1. James Little Mass Spectral Resource Website
- 2. NIST Search Software Detailed Manual
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- 4. Trimethylsilyl Derivatives for GC-MS
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- 26. Delta Mass Table Constructed from El Hybrid Searches
- 27. Handouts and Other Resources for NIST EI Search
- 28. NIST Tandem Quick Start Guide

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