

Wiley Spectral Webinar

Part I: Spectral Searches with NIST MS Search

12/27/20

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Kingsport, TN

- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
- *Now Consultant, MS Interpretation Services*
- *Specialties¹ EI GC-MS, LC-MS/MS, Chemical Ionization,⁷ Accurate Mass, Derivatization,^{8,9} MS library management, SciFinder,¹⁰ Chemspider,¹⁰ Surfactant ID,¹¹ NMR, GC-IR, organic synthesis, matrix ionization effects,^{2,1} etc.*



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

* https://en.wikipedia.org/wiki/Eastman_Chemical_Company



>50 Mass Specs Networked
Worldwide

Wiley Webinar Series on Effective Use of Mass Spectral Libraries

- Part I: Spectral Searches² with NIST MS Search
- Part II: Structure Searches² with NIST MS Search and Using MS Interpreter^{2,13-15}
- Part III: AMDIS^{3,4,12} (NIST) for Processing EI Mass Spectral Data Files
- Part IV: Advanced NIST Hybrid Search^{16-19,22} of EI and MS/MS Spectra
- Part V: Creating and Sharing⁵ User EI and MS/MS Libraries

Note:²⁰ Handouts for *All Sessions Now Online!*
Google Search “little mass spec and sailing”

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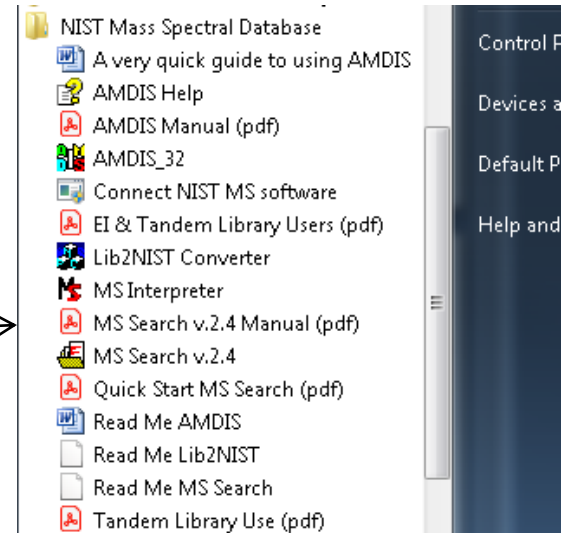
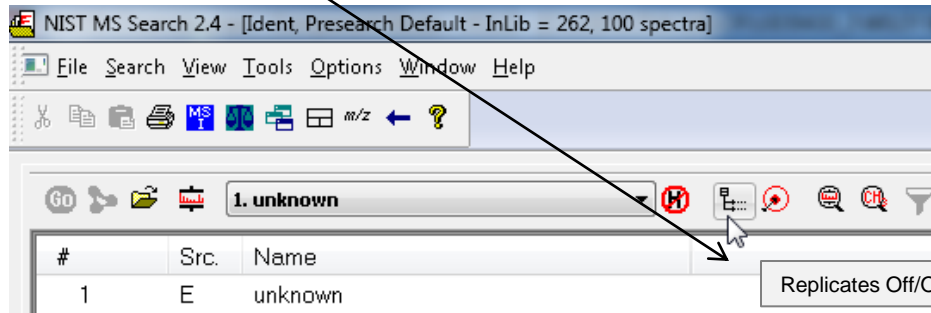
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NIST Mass Spectrometry Software and Wiley Libraries

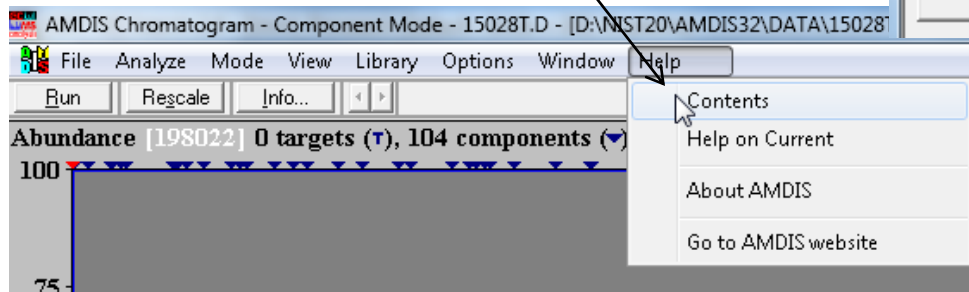
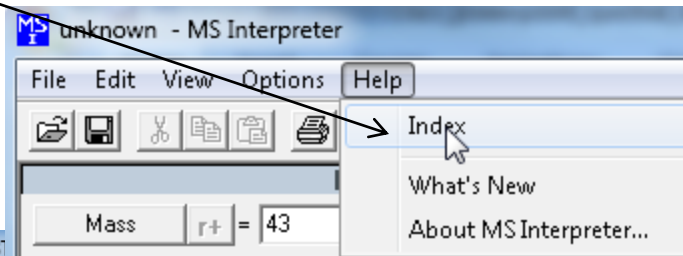
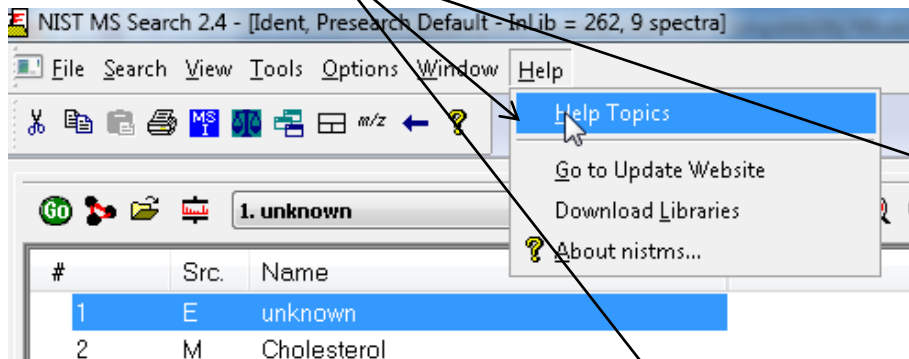
- Free software suite for processing mass spectral data
- Supplied with the purchased commercial libraries or by instrument manufacturers
- Utilized with Wiley Libraries for qualitative mass spectral analyses
- Both GC-EI and LC-MS unknown identification
- Import data^{2,6} from variety of instrument manufacturers
- Searches by spectrum, structure, name, CAS No., peaks, MW, MF, etc.
- **AMDIS**^{3,4,12} available for processing mass spectral files (*vendor and netCDF formats*)
- MS Interpreter^{2,13-15} for correlating molecular substructures to ions
- Structure Export and Import using vendor Drawing Packages²

Help Files for NIST Search

-"Hover" over Program Icon with mouse and function description displayed



- Detailed documentation for NIST Search² and AMDIS^{3,4,12}
- MS Interpreter included in NIST manual² and in posters¹³⁻¹⁵
- Windows Program Group
- "In program" assistance for all three programs



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!
- 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
- LMB** and **zoom** mass spectral windows, **RMB** then **LMB** to **zoom out**

Tip 3: **LMB** and **drag** to rearrange order of column headers

#	Lib.	Name	▼ Match	Prob. (%)	RI	R.Match	Syn	DBs
1	R	Undecane	955	44.8	1100	955	4	8
2	M	Undecane	945	44.8	1100	945	4	8
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

- LMB** on column of interest
- Can sort in lower value first or higher
- Will show use in mixtures in example later in presentation

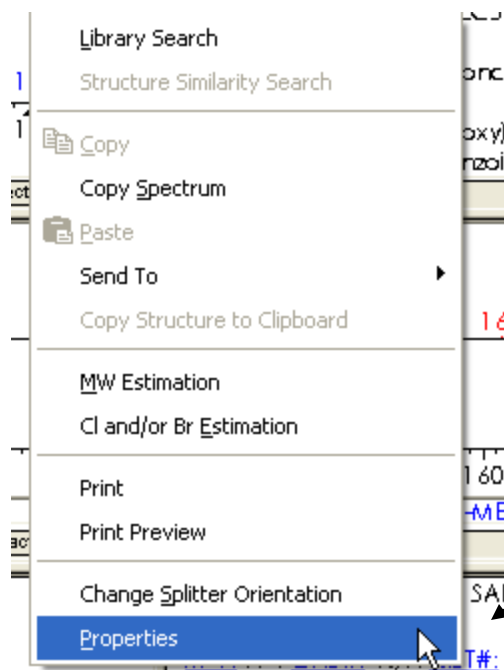
Tip 1: When reviewing search results, use up and down arrows on keyboard to quickly step through results!



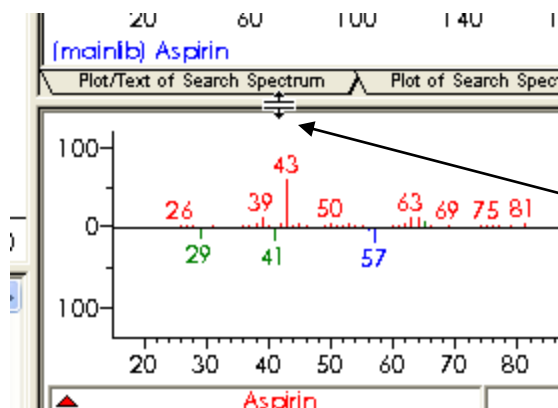
Tip 2: When viewing structures in MS Interpreter, use left and right arrows on keyboard to quickly review results!



Customizing the Windows



-**RMB** within any window of interest and select properties to change window information and/or layout



-place cursor over any bar between (**top or side**) windows and then **LMB** and drag to change the size of a window or make window so small it essentially disappears.

Customizing the Windows (continued)

-**RMB** in display windows then **LMB** to “Change Splitter Orientation”

Tip: All mass spectra in windows can be “**Boxed**” to **expand** by **LMB** and dragging to expand/enlarge; **restore** to original by **RMB** in spectrum region and selecting “**Zoom Out**”

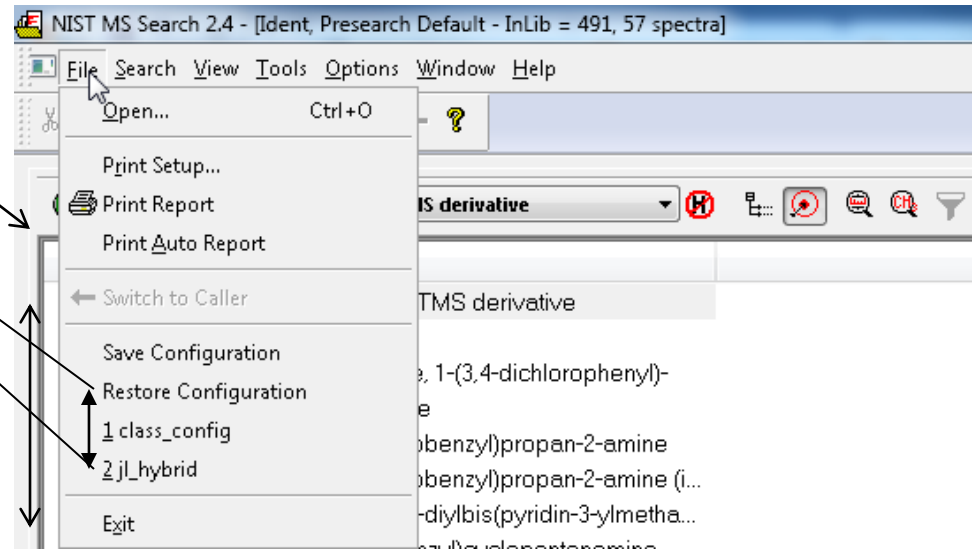
Name: example to be used to demonstrate MS interpreter
MW: N/A **ID#:** 20 **DB:** class_examples_final_2
Compound ID: 0
Compound Hash:
Comment: example to be used to link structure with spectrum, then add to list, and check fragmentation and isotope ratios with MS interpreter; also basic demonstration of adding 02-04.eva]]
InChIKey: DCQTUUYIQBVMU-UHFFFAQYSA-N **Non-stereo**
Status: Accept | 2019-03-27-21:29:47 | James Little | ok
10 largest peaks:
201 999 | 203 954 | 94 340 | 173 283 | 175 279 | 258 198 | 260 192 | 218 155 | 220 149 | 273 111 |
Synonyms:
no synonyms.
Inventory number: 30537
RI: 1698.4
RT min: 9.406
Date: 2019-02-02 (15h 29m 40s)
Sample introduction method: GC
Inlet type: Front SS Inlet He
Split ratio: 20 : 1
Inlet temp: 250C
Program type: Ramp
Start temp: 50C
Initial hold: 1 min
Heat rate: 15C/min
End temp: 300C
Final hold: 7.333 min
Column Brand name: Agilent CP6939
Active phase: VF-5ms

Library Search
Structure Similarity Search
Zoom Out
Neutral Loss Display
Set Anchors
Copy Plot
Copy
Paste
Send To
Copy Structure to Clipboard
MW Estimation
Cl and/or Br Estimation
Print
Print Preview
Change Splitter Orientation
Properties

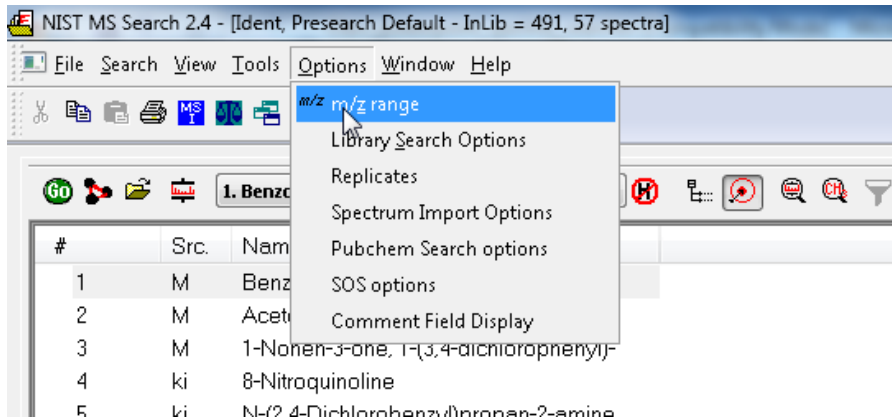
Tour of NIST Search Program (Top Links)

-Restore and Create
user Configurations

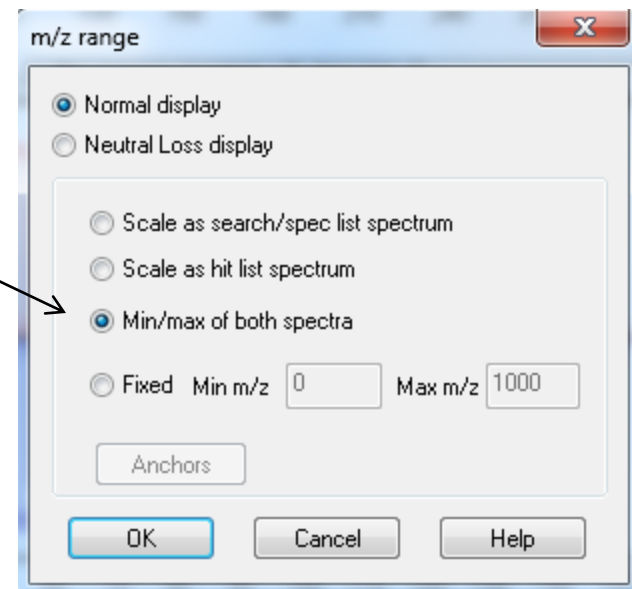
- Most used listed



Tour of NIST Search Program (continued) (Top Links)



-I prefer min/max of both spectra



Tour of NIST Search Program (continued) (Top Links)

NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 534, 31 spectra]

File Search View Tools Options Window Help

Go 1. Acetone

#	Src.	Name
1	M	Benzoic Acid, TMS derivative
2	M	Acetone

Tip: When reviewing results, use up and down arrows on keyboard to quickly review result



Only shows best hit with same CAS number

Turns on or off replicates in search results shown below

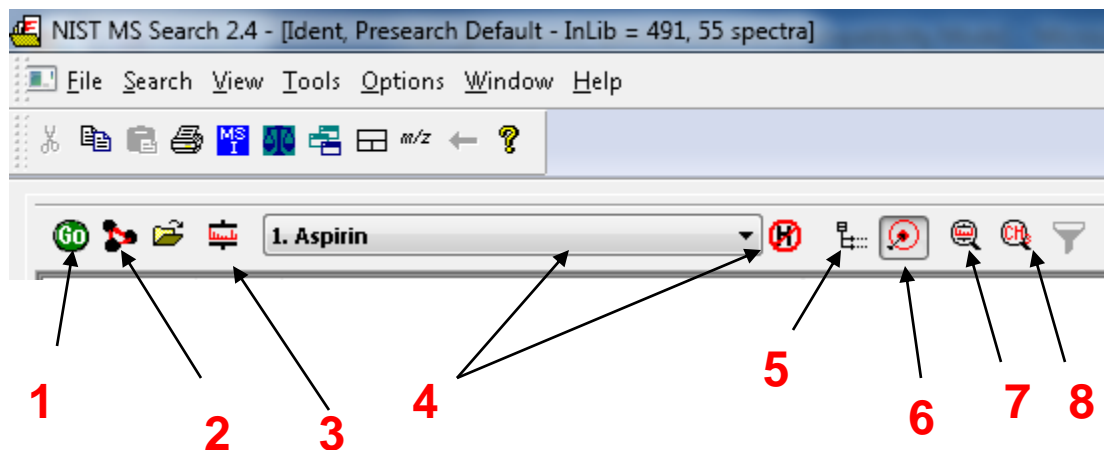
Demonstrated by Search Results for Acetone:

-Can toggle viewing replicates by "left-clicking" on + or - box here **or** icon on toolbar shown above

#	Lib.	Match	R.Match	Syn	DBs	Name
1	M	999	999	19	9	Acetone
...	R					Acetone
...	R					Acetone
...	R					Acetone
...	R					Acetone
...	R					Acetone
...	R					Acetone
...	R					Acetone

Tour of NIST Search Program (continued)

Icons in Lib Search Window



1. **LMB** to start search or **double LMB** on entry in spec list window
2. **LMB** to do structure search
3. **Critical** search criteria for structure and spectra search
4. Search results stored and ability to clear list
5. **“Show/Don’t Show”** replicate entries in NIST library
6. Only show best hit of entry with same CAS number, minimizes looking at redundant entries in search window
7. View Hit List Search Options (*see details next slide*)
8. **After** search, shows sub-structural information based on search results

Tour of NIST Search Program

View of Hit List Normal Search Options *Utilized*

1. **LMB** the “View Hit List Search Options”
2. See details of the last search performed
3. Also, an abbreviated description of the last search noted at **top** of NIST search window
4. Furthermore, at the **bottom** of the screen, *respectively* displayed, are the types of searches that will be performed and the type of results currently displayed

The image displays two screenshots of the NIST MS Search 2.4 software interface. The top screenshot shows the main application window with the search bar containing the text "1. unknown MS interpreter no structure". A red arrow labeled "1" points to the search bar. A red arrow labeled "2" points to the "Hit List Search Options" dialog box, which is open and shows the search type as "Identity : Normal". A red arrow labeled "3" points to the search bar in the main window. A red arrow labeled "4" points to the status bar at the bottom of the main window, which displays "Type of Search: Ident" and "Displayed: Ident".

1. unknown MS interpreter no structure

Hit List Search Options

Search Type

Identity : Normal

Search Options

Presearch default
Limits : Minimum m/z equals to 10. Minimum abundance 1.
No constraints

Library Involved

mainlib
replib
w12lq
w12main
w12rep

NIST MS Search 2.4 - [Ident, Presearch Default - InLib = -1101, 100 spectra]

File Search View Tools Options Window Help

1. unknown MS interpreter no structure

Type of Search: Ident

Displayed: Ident

2:52 PM
8/30/2020

Tour of NIST Search Program (continued)

(General Window in Lib Search View)

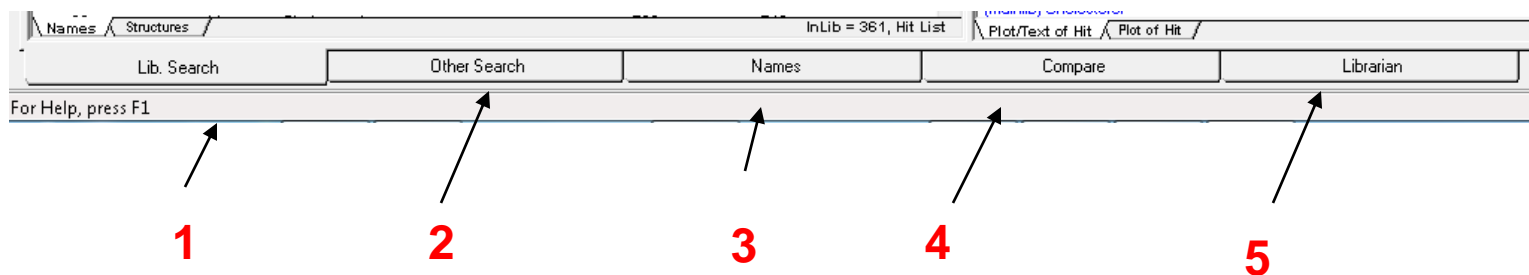
The screenshot displays the NIST MS Search 2.4 interface. The main window is titled "I. Acetone". On the left, there is a "Spec List" window (1) showing a list of search results. Below it is a histogram (2) showing the distribution of search results. In the center, there is a "Hits List" window (3) showing a table of search results. On the right, there are two comparison windows (4, 5, 6) showing mass spectra and associated information for Acetone. Red arrows point to these features, and a red number 7 is placed near the bottom navigation buttons.

#	Lib	Match	R.Match	Syn	DBs	Name
1	M	999	999	19	9	Acetone
2	M	846	869	1	0	Diisobutyl cellosolve
3	M	938	941	23	0	2-Propenal, 3-(2-furanyl)-
4	wl	984	985	0	0	Acetone-oxime
5	wl	819	875	39	6	4-methylpentane-2-one
6	wl	799	799	20	0	1-Propen-2-yl acetate
7	wl	791	946	2	0	2-Mentanone, 5-methyl-
8	wl	791	794	4	0	1-Propen-2-yl

1. Spec list window for import of spectra and structures from other programs
2. Histogram, Statistics on search
3. Hits list, step through by **LMB** then **up** and **down arrows on keyboard**
4. Unknown spectra and info
5. Comparison of unknown to selected hit result, **many different display options** with tabs at bottom left of window
6. Spectrum of hit and other associated information
7. Accessing other windows and associated functions

Tour of NIST Search Program (continued)

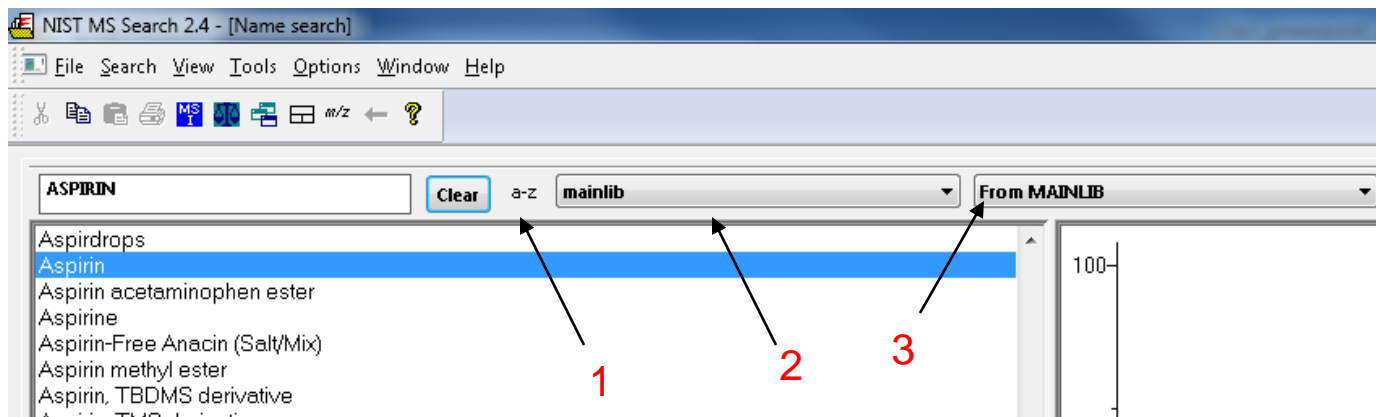
Tabs for Other Functions Accessed at **Bottom** of Main Library Page (Many Detailed Discussions in **Future Webinars**)



1. Lib Search-main window for searching spectra and structures
2. Other Search-search by CAS, MW, ID no., partial name, MF, etc.
3. Names-search by name, often 2-100 names for same compounds, e.g., check out *aspirin on next slide*
4. Compare-Window used to compare spectra, also can display best hits from search
5. Librarian-window used to edit spectra, correlate spectra with structure, create user libraries, type in spectra manually, etc.

Tour of NIST Search Program (continued)

Name Search Tab



1. *Toggle: letters only or letters/numbers*
2. *Search **one library** at a time*
3. *Replicates, derivatives, isotopes, stereoisomers in pull-down menu*

Name: Aspirin
Formula: C₉H₈O₄
MW: 180 **Exact Mass:** 180.042258 **CAS#:** 50-78-2 **NIST#:** 250572 **ID#:** 125480 **DB:** mainlib
Other DBs: Fine, TSCA, RTECS, USP, HODOC, NIH, EINECS
Contributor: Virginia Division of Forensic Science
InChIKey: BSYNRYMUTXBXSQ-UHFFFAOYSA-N **Non-stereo**
Related CAS#: 11126-35-5, 26914-13-6, 98201-60-6, 2349-94-2
Synonyms:
 1. Benzoic acid, 2-(acetyloxy)-
 2. Salicylic acid acetate
 3. o-Acetoxybenzoic acid
 4. o-Carboxyphenyl acetate
 ...
 190. Talacen (Salt/Mix)
 191. TheraFlu (Salt/Mix)
 192. Triaminic Sore Throat Formula (Salt/Mix)
 193. Tylenol Allergy Sinus (Salt/Mix)
 194. Tylox (Salt/Mix)
 195. Vanquish (Salt/Mix)
 196. Vicodin (Salt/Mix)
 197. Zydone (Salt/Mix)
 198. Wygesic (Salt/Mix)

198 associated names!!

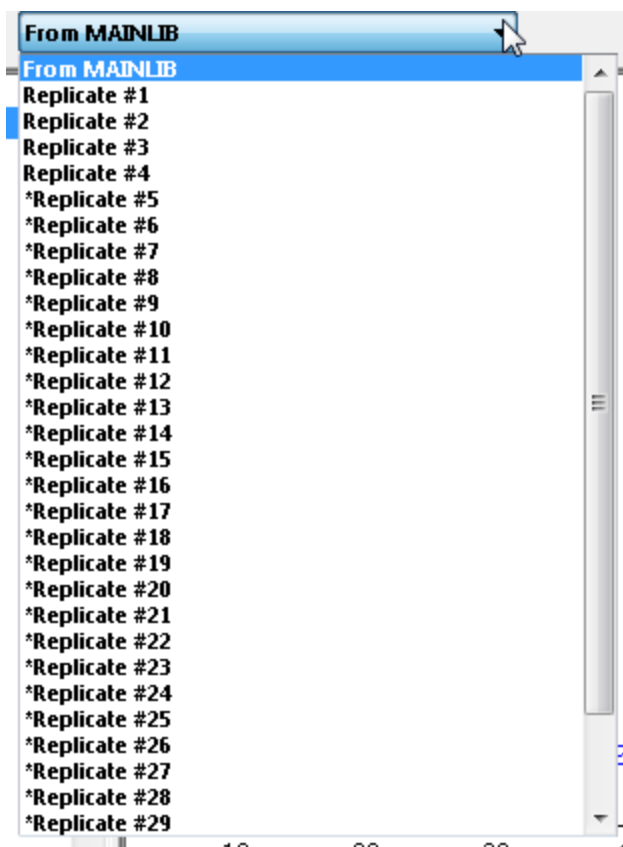
Tip: Difficult to see components in pull down menu, thus **RMB** on "Aspirin" in name list, then *either* **Send To/ Spec List** and search or **Lib Search** directly; See improved display in Library tab on next page

Tour of NIST Search Program (continued)

Improved View in Library Tab Compared to Name Tab

-**increase** from just displaying replicates in both views to **including** isotopes, stereoisomers, and derivatives as explained in **slide 32**

Limited view for aspirin in Name Tab

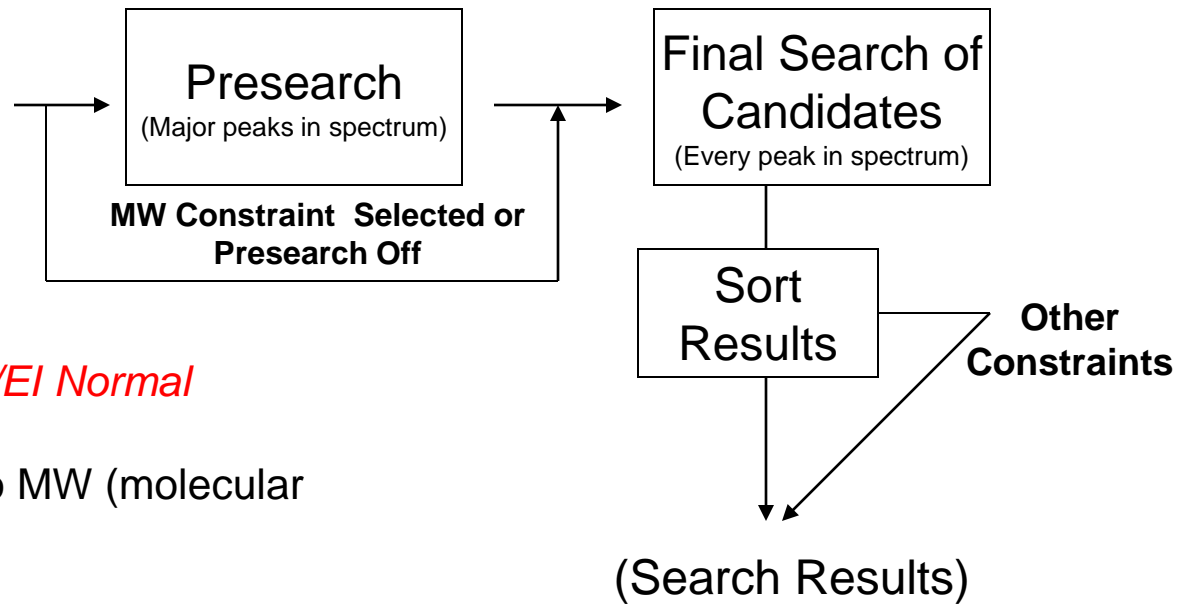
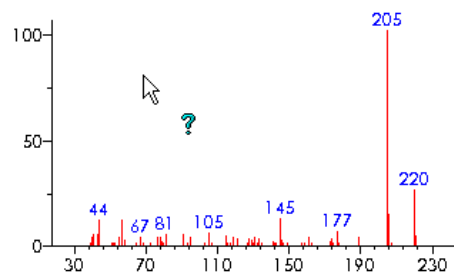


Improved view after sending to Spec List and searching in library window

A screenshot of the Library Tab in the NIST search program. The window title is "Hit List". The table shows search results for aspirin and its derivatives. The first row is highlighted in blue.

#	Lib.	Match	NumMP	DeltaMass	Name
1	M	999	49	0	Aspirin
	R				Aspirin
	R				Aspirin
	R				Aspirin
	R				Aspirin
	m				Salicylic acid, 2TMS derivative
	r				Salicylic acid, 2TMS derivative
	r				Salicylic acid, 2TMS derivative
	r				Salicylic acid, 2TMS derivative
	r				Salicylic acid, 2TMS derivative
	r				Salicylic acid, 2TMS derivative
	m				Salicylic acid, 2TBDMS derivative
	m				Salicylic acid
	r				Salicylic acid
	r				Salicylic acid
	r				Salicylic acid
	r				Salicylic acid
	r				Salicylic acid
	m				2-(1-Piperidinyloxy)phenol
	m				Salicylic acid, tert-butylidimethylsilyl ester
	m				Salicylic acid, TMS derivative
	m				Aspirin, TMS derivative
	r				Aspirin, TMS derivative
	r				Aspirin, TMS derivative
	m				Aspirin, TBDMS derivative
	r				Aspirin, TBDMS derivative
	m				2-(1-Piperidinyloxy)phenol, Ac deri...
	m				2-(1-piperidinyloxy)phenol, TMS de...
	m				Benzoic acid, 2-trifluoroacetyloxy-, trimet...
	m				Benzoic acid, 2-trifluoroacetyloxy-, tert-b...
	m				Benzoic acid, 2-pentafluoropropionyloxy...
	m				Benzoic acid, 2-heptafluorobutyryloxy-, ...
	m				Benzoic acid, 2-heptafluorobutyryloxy-, t...

Critical Parameters for Searching Libraries



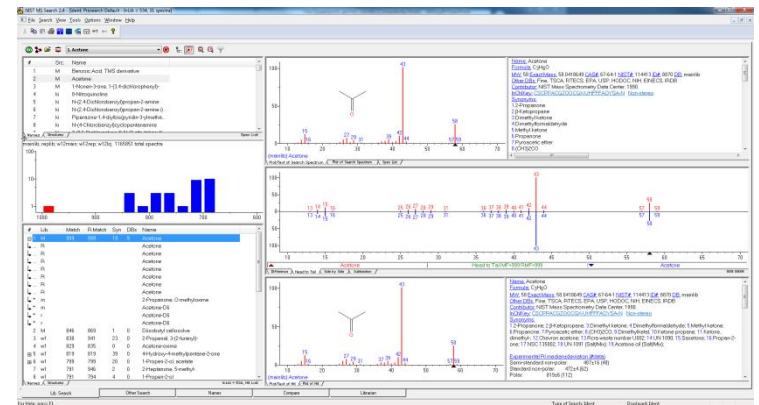
Spectrum Search Type-Identity/EI Normal

MW-skips presearch and limits to MW (molecular weight) specified

Presearch Default-*best setting for optimum results*

Presearch off-skips presearch, >1 minute per search

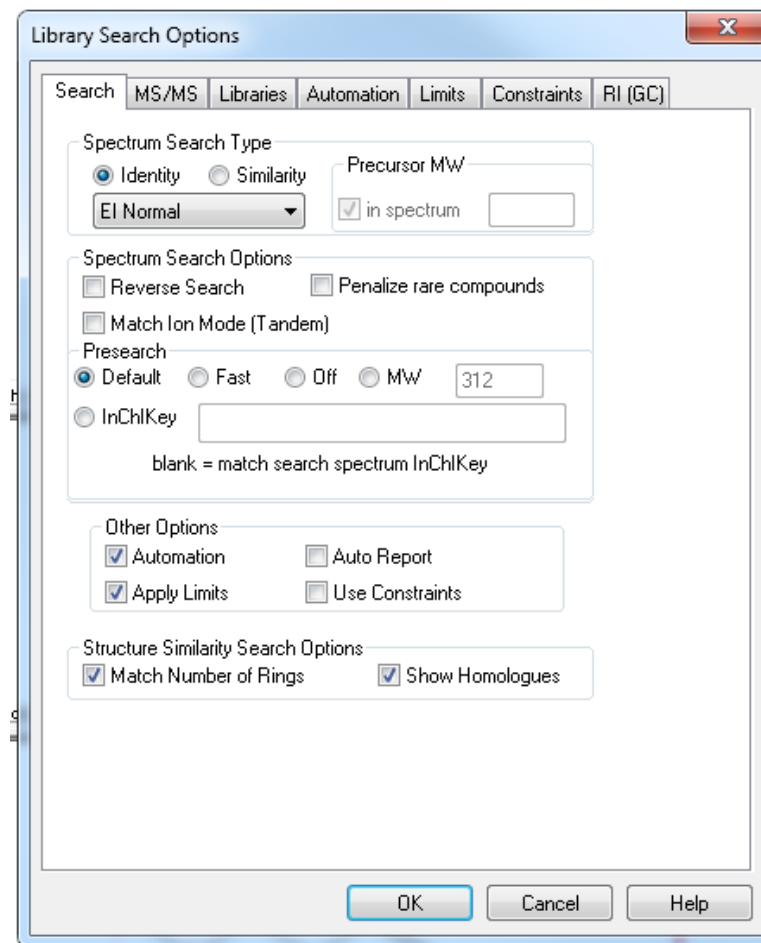
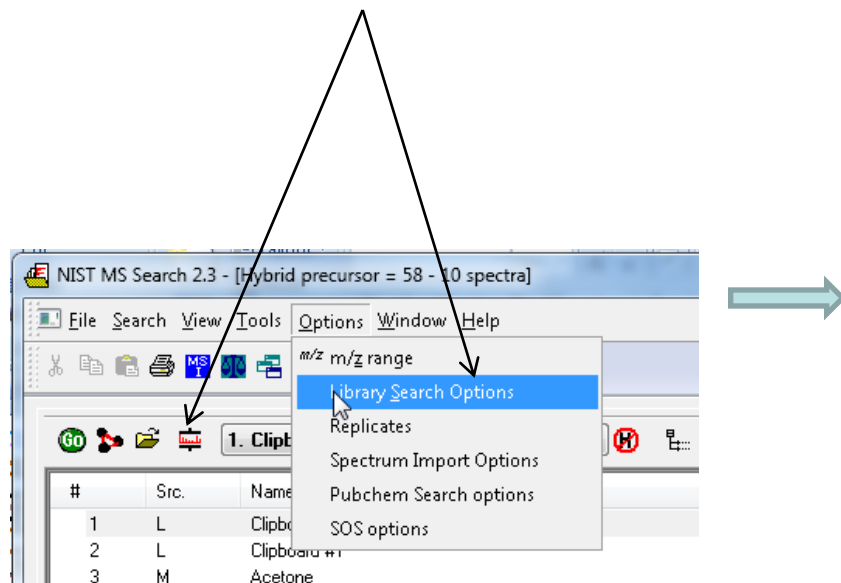
Constraints-results filtered *after* Final Search



Setting Up Presearch Parameters

Critical Step

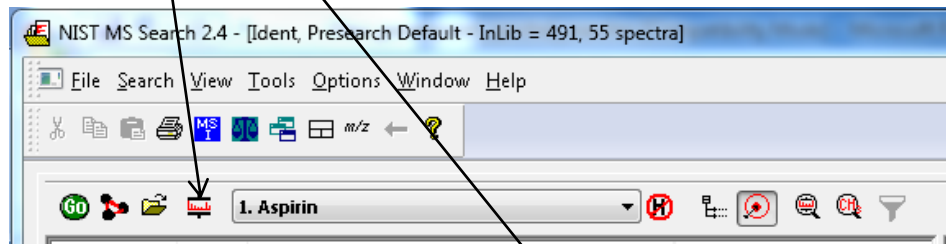
Two ways to access:



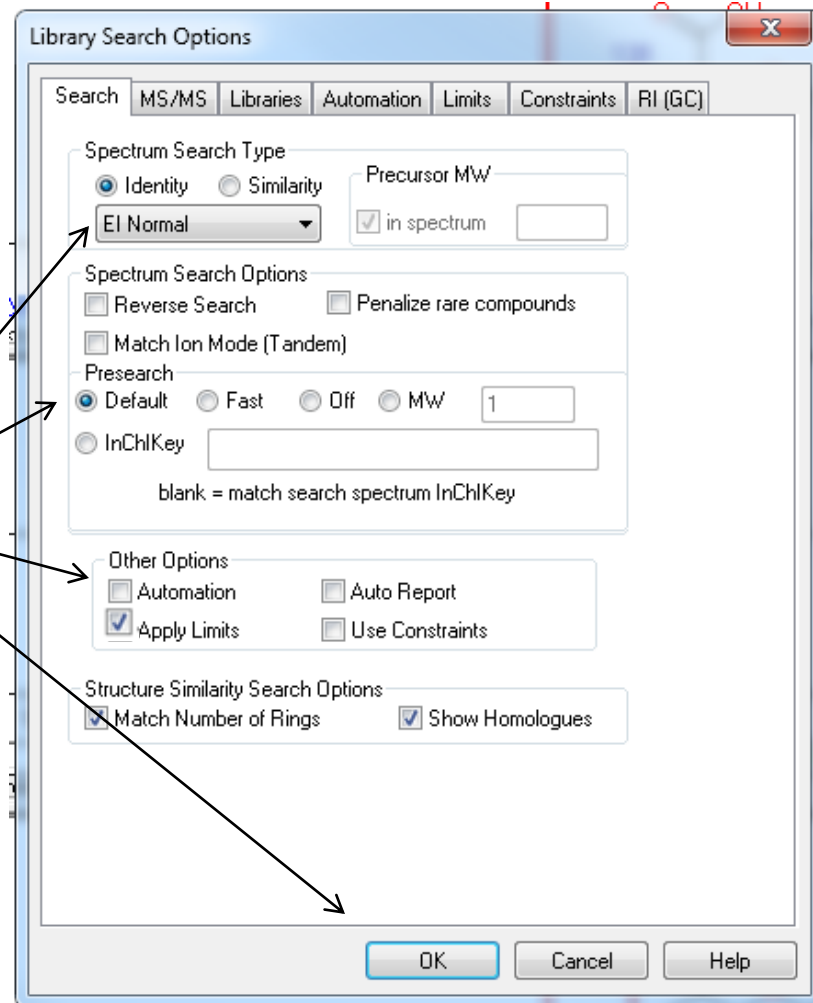
Setting Up Presearch Parameters

Critical Step

- **LMB** on icon to open **Library Search Options** window
- Then select appropriate options with **LMB**



then...



Note: Previous to Version 2.4 (2020), EI Normal was just "Normal"!

Primary Libraries of EI Spectra and Associated Structures

Wiley: >815k entries

NIST: >350K entries

User Libraries: e.g. Eastman, >50K entries, automatically updated *nightly*⁵

Which Ones to Search? (My opinion, all >1,100K **excluding** w12leg)

-**w12main:** best spectra for component

-**w12rep:** replicates (spectra can be instrument dependent)

-**w12lq:** lower quality, <4 ions per spectrum

-**w12leg:** spectra once present in main and rep, but removed for various reasons

-**main (NIST):** one best entry selected (subjective) for component

-**rep (NIST):** replicates for main spectrum

-**user libraries:** individual's or company's personal libraries

Other EI Libraries: Wiley/NIST Combined and Specialty

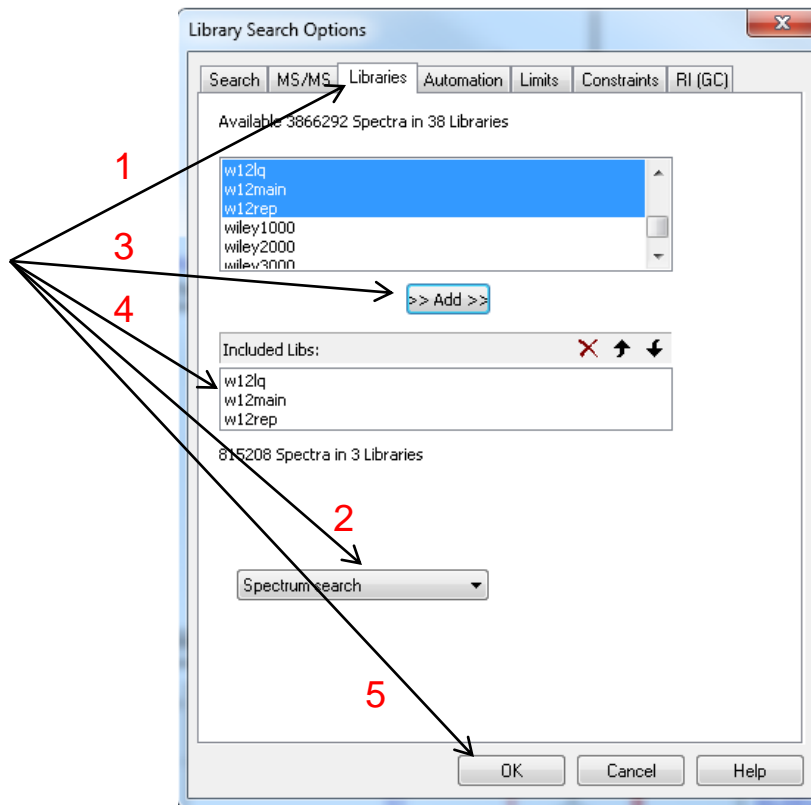
Combined Wiley/NIST (>1.1 M spectra)

Wiley Specialty (>91 K spectra):

- Lipids (430)
- Pesticides from Prof. Mondello (1,300)
- Designer Drugs (28,032)
- Fragrances (3,462)
- Drugs, Poisons, Pesticides, Pollutants, and Their Metabolites (10,430)
- FAMES: Fatty Acid Methyl Esters (240)
- Physiologically Active Substances: Drugs, Steroid Hormones, and Endocrine Disruptors (4,182)
- Pesticides from Rolf Kuhnle (1,238)
- Androgens, Estrogens, Steroids and Derivatives (3,722)
- Organic Compounds for Drug Discovery/Combinatorial Synthesis (37,055)
- Petrochemicals and Biomarkers (1,100)
- Online Search Wiley and NIST Libraries (Check for Inclusion, free)
<https://www.sisweb.com/software/ms/nistsearch.htm>

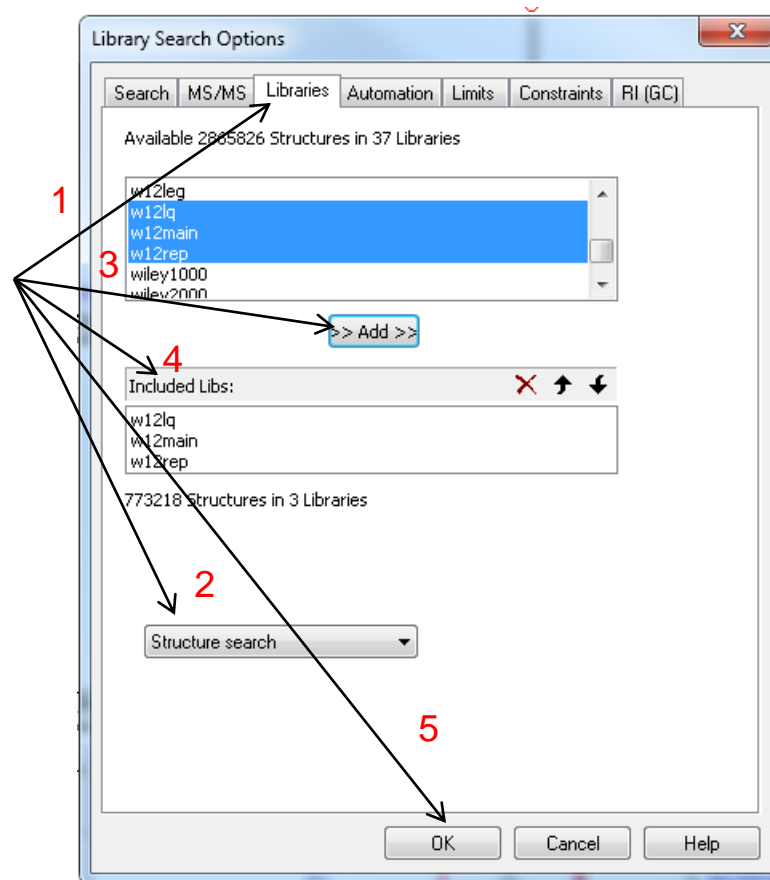
Select the Libraries Used for Spectral Searches

- **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



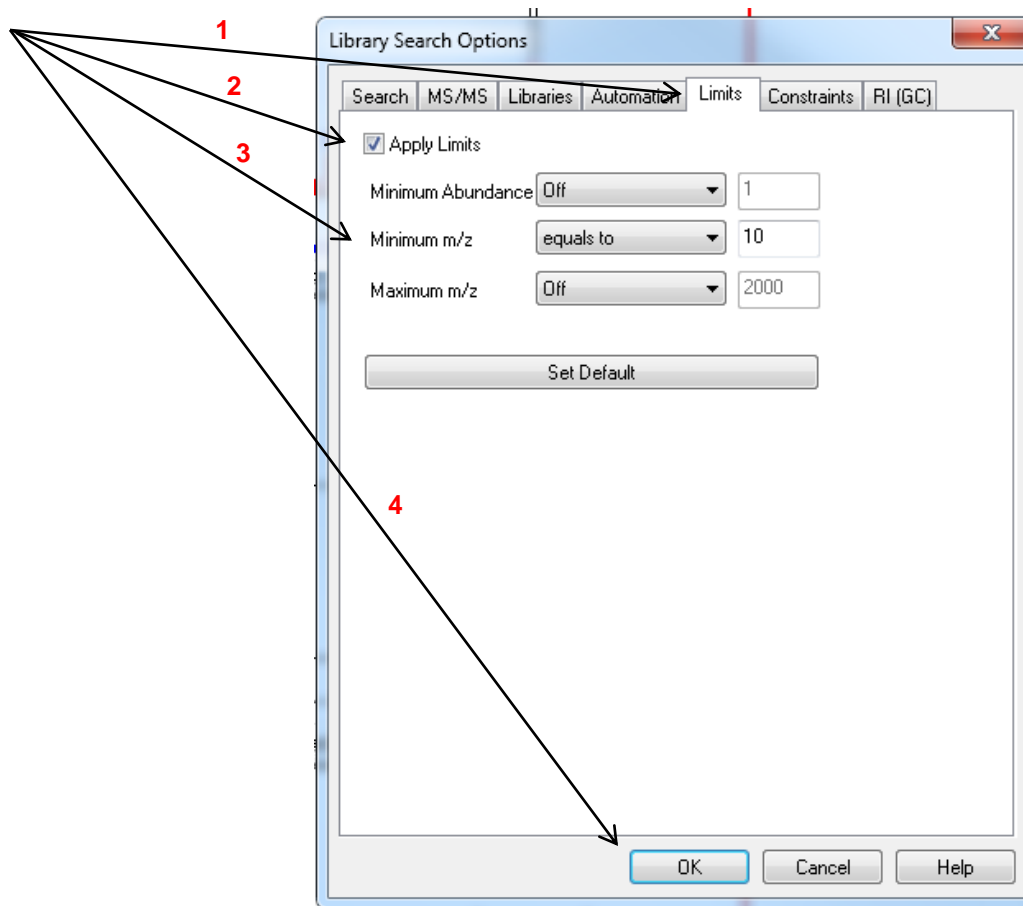
Select the Libraries Used for *Similar Structure* Searches

- **Similar structure searches** are driven by libraries in **Structure Search**
- **LMB** on the **Libraries** tab and make sure **Structure Search** is selected
- Select the group of libraries to be searched by Similar Structure
- **>>Add>>** all libraries to be searched
- **Order** of libraries normally **only important** in Other Search Tab which return **maximum no.** of hits



Selecting Limits to Avoid High Match Factors for Partial Spectra in Libraries

- **LMB** on the **Limits** tab
- Select **Apply Limits**
- Enter the value for “Minimum m/z”
- This is necessary to force the search to compare the whole spectrum when calculating a match factor
- Library spectra with only a few ions **are minimized** in the search results by using limits



Tip: The partial spectra in libraries would be hard to find. One way to accomplish is to do a normal search limiting the MW. This will skip the presearch. Then **sort** the results by clicking on Rmatch to sort by “Reverse Search.”

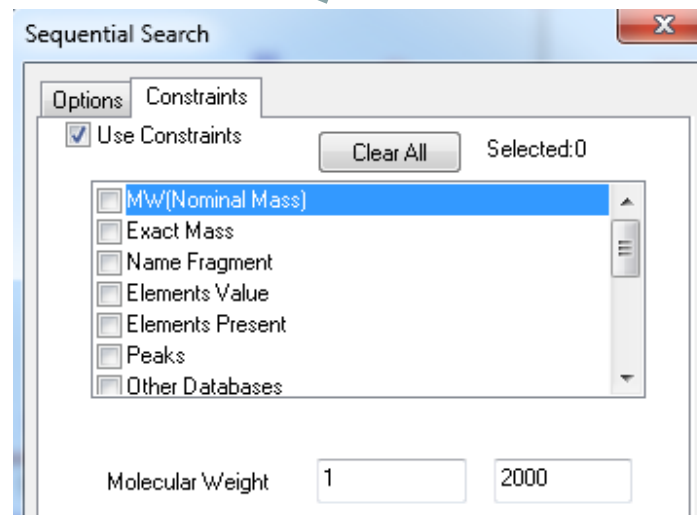
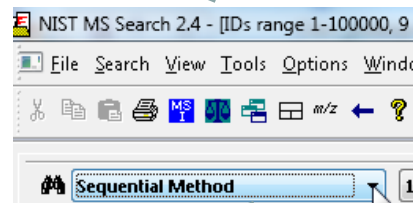
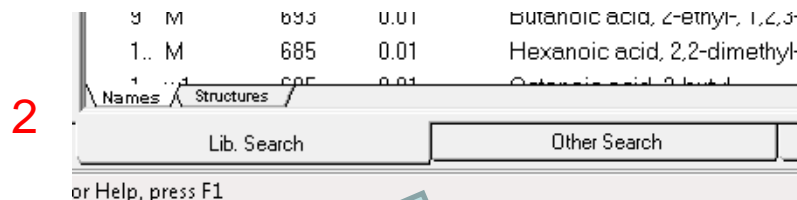
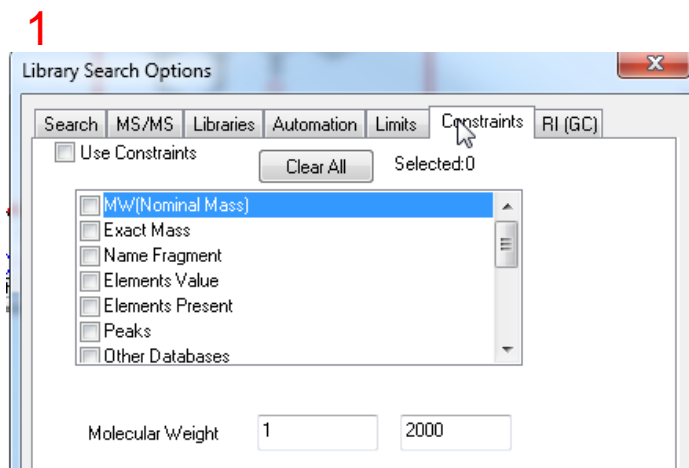
The screenshot shows the 'Library Search Options' dialog box with the 'Limits' tab selected. Red arrows labeled 1 and 2 point to the following elements:

- 1: The 'MW' radio button in the 'Presearch' section.
- 2: The 'R.Match' column header in the results table.

#	Lib.	Match	R.Match	Syn	DBs	Name
1	M	999	999	19	9	Acetone
2	M	846	869	1	0	Diisobutyl
3	w1	838	941	23	0	2-Propena
4	w1	829	835	0	0	Acetone-o-

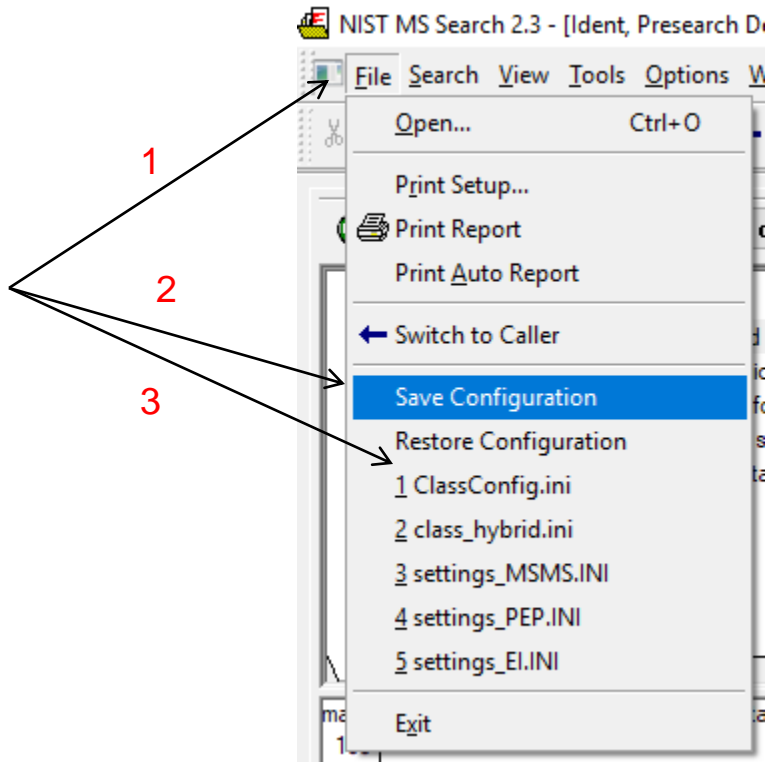
Constraints Applied to Hit List *After* the List Is Determined

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



Saving Setup of Standard Search Parameters

- After all the parameters are setup for search, save them for future use
- Example setup below was named **ClassConfig**
- In the future, this can be “re-called” by selecting it from this menu



Searching Demonstration

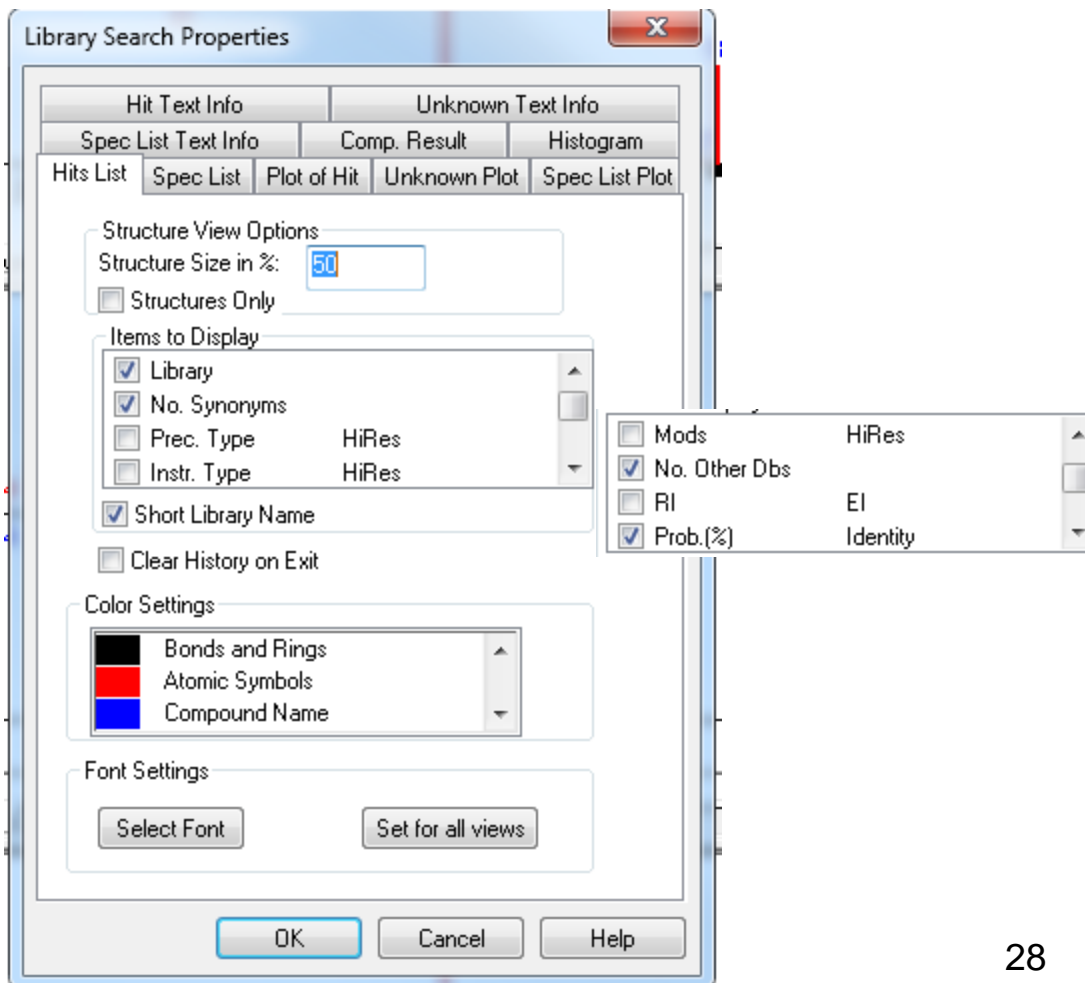
-Example searches demonstrated in live presentation

Tip1: When reviewing results, use up and down arrows on keyboard to quickly review results!



Tip2: When viewing results of search, consider adding **No. Synonyms** and **No. of Other Databases** to columns displayed.

The number of associated synonyms and databases makes a candidate more likely to be correct structure in many cases!



Reverse Match for Mixtures Versus Standard Match

- Also “Head to Head” display of unknown versus best hit shows many additional ions in unknown
- can **LMB** on R. Match (Reverse Header) to resort results
- Reverse match factor is calculated **disregarding** any peaks in the unknown that **not** in the library spectrum
- Make sure “Best Matching Only” is **not selected** on toolbar

The screenshot displays the NIST MS Search 2.3 interface. At the top, the search results table shows three entries:

#	Src.	Name
1	A	Manual Component in C:\NIST17\AMDIS32\DATA\DATA_FILES\WG1.D\DATA.MS
2	A	Manual Component (7.733 min) in C:\NIST17\AMDIS32\DATA\DATA_FILES\WG1.D\DATA.MS
3	E	t-Butyl 3-Bromo-4-fluorobenzamide

Below the table is a bar chart showing the distribution of spectra. The x-axis represents m/z from 1000 to 500, and the y-axis represents the number of spectra on a log scale from 1 to 100. A red bar is visible at approximately m/z 600.

The main search results table is as follows:

#	Lib	Name	Match	Prob. (%)	RI	R.Match	Syn	DBs
1	R	Undecanoic acid, methyl ester	688	33.8	1409	853	5	6
2	w1	Undecanoic acid, methyl ester	686	33.8	-	836	7	0
3	w1	Undecanoic acid, methyl ester	671	33.8	-	801	7	0
4	R	Undecanoic acid, methyl ester	667	33.8	1409	902	5	6
5	R	Suberic acid monomethyl ester	665	12.3	-	694	1	1
6	w1	8-keto-8-methoxy-caprylic acid	665	12.3	-	694	3	0
7	M	Undecanoic acid, methyl ester	660	33.8	1409	791	5	6
8	M	Suberic acid monomethyl ester	658	12.3	-	695	1	1
9	w1	8-keto-8-methoxy-caprylic acid	658	12.3	-	695	3	0
10	w1	Undecanoic acid, methyl ester	652	33.8	-	838	7	0
11	M	Nonanoic acid, 9-hydroxy-, methyl ester	652	7.96	-	692	1	0
12	w1	Nonanoic acid, 9-hydroxy-, methyl ester	652	7.96	-	692	6	0
13	w1	Undecanoic acid, methyl ester	649	33.8	-	776	7	0
14	R	Undecanoic acid, methyl ester	649	33.8	1409	774	5	6
15	w1	Methyl 11-(2,3-dideuteriocyclohexan-1-yl)und...	648	6.72	-	692	0	0
16	w1	Cyclohexanamine, N-cyclohexyl-	633	4.07	-	877	21	0
17	M	Undecanoic acid, 2-methyl-	633	4.07	-	824	1	3
18	w1	Decanoic acid, methyl ester	626	3.12	-	776	16	0
19	w1	Cyclopropanononanoic acid, methyl ester	626	3.12	-	645	3	0
20	R	Decanoic acid, 2-methyl-	625	3.00	-	758	1	4
21	w1	Decanoic acid, 2-methyl-	625	3.00	-	758	3	0
22	R	Cyclohexanamine, N-cyclohexyl-	623	4.07	1404	892	16	6
23	w1	Undecanoic acid, methyl ester	623	33.8	-	871	7	0

The interface also shows two mass spectra plots. The top plot is the search spectrum, and the bottom plot is the library hit spectrum for Cyclohexanamine, N-cyclohexyl-. The library hit spectrum includes the following data:

- Name: Cyclohexanamine, N-cyclohexyl-
- Formula: C₁₂H₂₃N
- MW: 181 Exact Mass: 181.16305 CAS#: 101-83-7 ID#: 13649 DB: w11rep
- Other DBs: None
- Compound ID: 0
- Comment: SpectrumID=1177677 Source="JZ:1992:1567-0" QI=417
- InChIKey: Z8FCUJwBYRCDP-UHFFFAOYSA-N Non-stereo
- 10 largest peaks: 138 999 | 56 401 | 41 2251 | 55 1381 | 181 1281 | 57 124 | 43 1221 | 139 1081 | 82 731 | 98 611
- Synonyms: (none listed)

Reverse Match for Mixtures Versus Standard Match (continued)

- The top Reverse match is 873 for the dicyclohexylamine
- The next highest is 849 for methyl undecanoate
- These are the correct identities for the components in the mixture

NIST MS Search 2.4 - [Ident, Presearch Default - InLib = -1149, 33 spectra]

File Search View Tools Options Window Help

1. mixture of species

#	Src.	Name
1	E	mixture of species
3	M	Benzoic Acid, TMS derivative
4	M	Acetone
5	M	1-Nonen-3-one, 1-(3,4-dichlorophenyl)-
6	ki	8-Nitroquinoline
7	ki	N-(2,4-Dichlorobenzyl)propan-2-amine
8	ki	N-(2,4-Dichlorobenzyl)propan-2-amine (l...
9	ki	Piperazine-1,4-diybis(pyridin-3-yl)metha...

mainlib: replib; w12main; w12rep; w12lq: 1165951 total spectra

#	Lib.	Match	R....	Syn	DBs	Name
1	w1	635	873	21	0	Cyclohexanamine, N-cyclohexyl-
2	R	680	849	5	6	Undecanoic acid, methyl ester
3	w1	640	841	1	0	n-undecanoic acid methyl ester
4	w1	579	834	6	0	Pentadecanoic acid, methyl ester
5	M	626	819	1	3	Undecanoic acid, 2-methyl-
6	M	612	814	1	1	(Bicyclohexyl)-2-amine
7	w1	599	793	13	0	Hexanoic acid methyl ester
8	R	586	778	8	8	Octanoic acid, methyl ester
9	M	595	774	2	0	Cyclopentaneundecanoic acid, methyl e...
1.	w1	622	773	16	0	Decanoic acid, methyl ester
1.	R	585	769	9	6	Hexadecanoic acid, methyl ester
1.	w1	584	766	2	0	Methyl Palmitate
1.	w1	582	762	11	0	Eicosanoic acid, methyl ester
1.	R	618	753	1	4	Decanoic acid, 2-methyl-
1.	w1	580	740	8	0	Tridecanoic acid, methyl ester
1.	R	663	692	1	1	Suberic acid monomethyl ester
1.	M	649	688	1	0	Nonanoic acid, 9-hydroxy-, methyl ester
1.	M	589	686	1	0	Butanoic acid, 4-dicyclohexylamino-4-ox...
1.	w1	641	685	0	0	Methyl 11-(2,3-dideuterocyclopentan-1-y...

Plot of Search Spectrum

Name: mixture of species
MW: N/A ID#: 3 DB: Spec. Edit
Synonyms:
no synonyms.

Plot of Search Spectrum

Name: Cyclohexanamine, N-cyclohexyl-
Formula: C₁₂H₂₃N
MW: 181 Exact Mass: 181.183049 CAS#: 101-83-7 ID#: 96341 DB: w12main
Other DBs: None
Comment: SpectrumID="1177675" Source="AD-0-3311-0" Classification="*Cyclohexylamines* QI="900"
SplashID="splash10-052r-980000000-ad7da2bab861db07e15d"
InChIKey: XZPCUCUWBYBCDF-UHFFFAOYSA-N Non-stereo
Synonyms:
1 Dicyclohexylamine; 2 Aminodicyclohexane; 3 Bis(cyclohexyl)amine; 4 Cyclohexylcyclohexanamine;
5 DCH; 6 DCHA; 7 DCHA; 8 Dicyclohexyl-amine; 9 Dicyclohexylamin; 10 Dodecahydrodiphenylamine;
11 N,N-Dicyclohexylamine; 12 N,N-dicyclohexyl-amine; 13 N-Cyclohexyl-cyclohexylamine; 14 N-
Cyclohexylcyclohexanamine; 15 A13-15334; 16 BRN 0605923; 17 CCRIS 6228; 18 EINECS 202-980-7;
19 HSDB 4018; 20 NSC 3399; 21 UN2565;
Experimental RI median±deviation (#data)
Unspecified: 1540±N/A (1)

Chemical structure: C1CCC(CC1)N(C2CCCCC2)C3CCCCC3

Plot of Hit

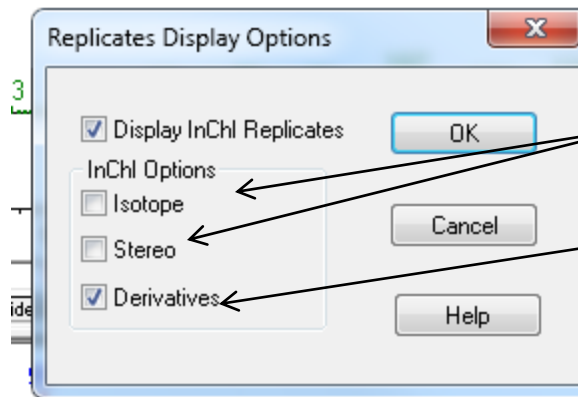
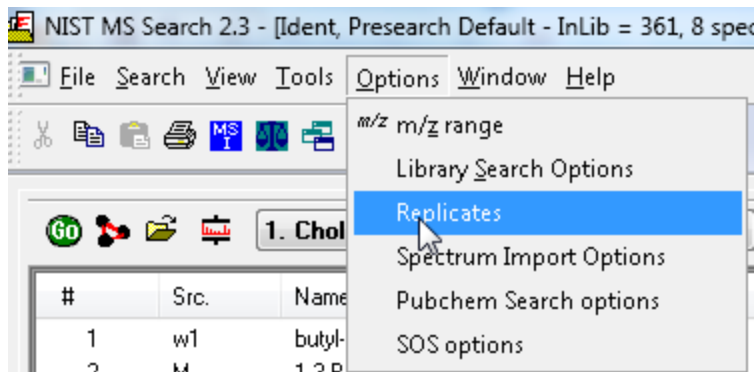
Reverse Match for Mixtures Versus Standard Match (continued)

- One can switch the display of unknown versus the selected hit to the “subtraction” mode (1)
- Thus the dicyclohexylamine result spectrum is *subtracted* from the unknown spectrum
- The difference or subtracted spectrum, using a “right-click,” can then be “Send to” “Spec List” window *or* Library Search (2)
- This subtracted spectrum can then be searched again (3) to yield a good “Match” for methyl undecanoate from SpecList

The screenshot displays the NIST MS Search 2.4 interface with several key components:

- Top Panel:** Shows the search parameters and a list of results. A red arrow labeled '3' points to the 'mixture of species' entry in the list.
- Left Panel:** A bar chart showing the distribution of spectra across different mass ranges (1000 to 500).
- Bottom-Left Panel:** A table of search results with columns for #, Lib, Match, R..., Syn, DBs, and Name. The top entry is Cyclohexanamine, N-cyclohexyl-.
- Top-Right Panel:** A mass spectrum plot of the 'mixture of species' with major peaks at m/z 74 and 138.
- Middle-Right Panel:** A mass spectrum plot of the 'Subtraction' mode, showing the difference between the mixture and the selected hit. A red arrow labeled '1' points to the peak at m/z 74.
- Bottom-Right Panel:** A mass spectrum plot of the 'Cyclohexanamine, N-cyclohexyl-' hit, with a chemical structure shown. A red arrow labeled '2 (two choices)' points to the 'Send To' menu option in the context menu.
- Context Menu:** A 'Library Search' menu is open, showing options like 'Send To', 'Compare List', and 'MS Interpreter'. The 'Send To' option is highlighted, and a sub-menu is visible with 'Spec List' selected.

Display of Related Species



Inconsistent!

“Do see” ***unchecked***

“Do see” ***checked***

-Determines if replicates, isotopically labeled species, stereoisomers, and/or derivatives displayed with search results

-Can toggle by “left-clicking” on + or – box

-***Also*** affects Names Search Display

#	Lib.	Name	Match	R.Match
1	M	Cholesterol	999	999
...	R	Cholesterol		
...	R	Cholesterol		
*	m	3a-Cholesterol acetate		
*	m	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	m	Cholesterol, TMS derivative		
*	r	Cholesterol, TMS derivative		
*	r	Cholesterol, TMS derivative		
*	r	Cholesterol, TMS derivative		
*	r	Cholesterol, TMS derivative		
*	m	Cholest-5-en-3-ol (3β)-, acetate		
*	r	Cholest-5-en-3-ol (3β)-, acetate		
*	r	Cholest-5-en-3-ol (3β)-, acetate		
*	r	Cholest-5-en-3-ol (3β)-, acetate		
*	m	Cholest-5-en-3-ol (3β)-, trifluoroacetate		

InChIKey Field Link to PubChem on Web

Name: Pentabromophenyl ether
Formula: C₁₂Br₁₀O
MW: 950 Exact Mass: 949.17829 CAS#: 1163-19-5 NIST#: 376366 ID#: 267338 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, NIH, EINECS
Compound ID: 0
Compound Hash:
Contributor: R.A. Hites, Indiana Univ., Bloomington, IN
Related CAS#: 145538-74-5; 109945-70-2
InChIKey: [WHHGLZMJPXIBIX-UHFFFAOYSA-N](#) Non-stereo
10 largest peaks:


-InChIKey is ASCII “hashed” representation of structure* and is searchable

-Double **LMB** on **any** InChIKey in NIST software and you will be taken to PubChem Web Page on internet

NCBI Resources How To

PubChem Compound PubChem Compound "USIUUVZYUHIAEV-UHFFFAOYSA-N"[InChIKey] Create alert Limits Advanced

Summary

 [DIPHENYL ETHER; Diphenyl oxide; 101-84-8 ...](#)
MW: 170.210 g/mol MF: C₁₂H₁₀O
IUPAC name: phenoxybenzene
Create Date: 2005-03-26
CID: 7583
[Summary](#) [Similar Compounds](#) [Same Parent, Connectivity](#) [Mixture/Component Compounds](#)

Selecting PubChem Options:

NIST MS Search 2.4 - [Ident, Presearch Default - InLib = 617, 51 spectra]

File Search View Tools Options Window Help

m/z m/z range
Library Search Options
Replicates
Spectrum Import Options
Pubchem Search options
SOS options

#	Src.	Name
1	M	Diph

* https://en.wikipedia.org/wiki/International_Chemical_Identifier

Sending data to PubChem

The structural data may be sent to pubchem (<https://pubchem.ncbi.nlm.nih.gov>) to find matching structures.

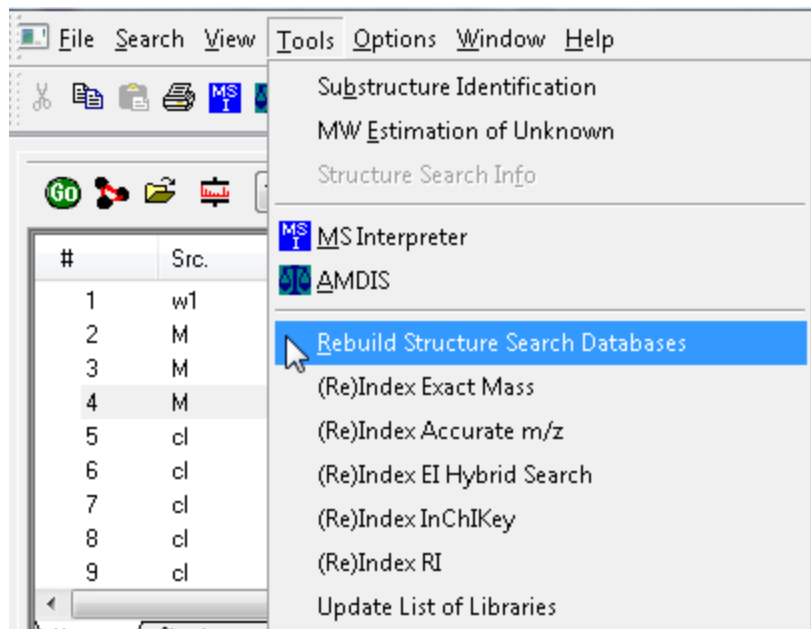
How should the program proceed?

Ask each time
 Always allow data to be sent
 Never send data

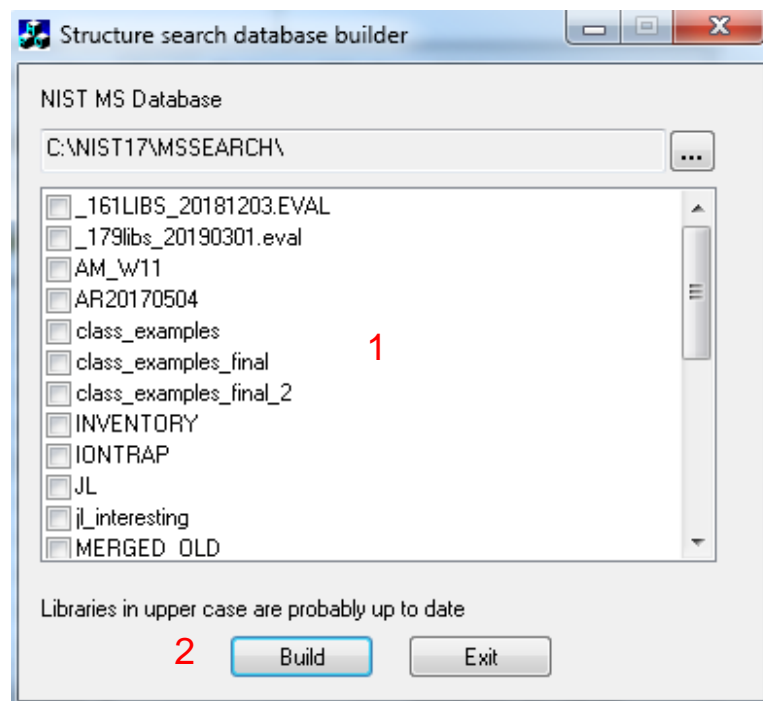
OK Cancel

Updating Indices in Older Libraries

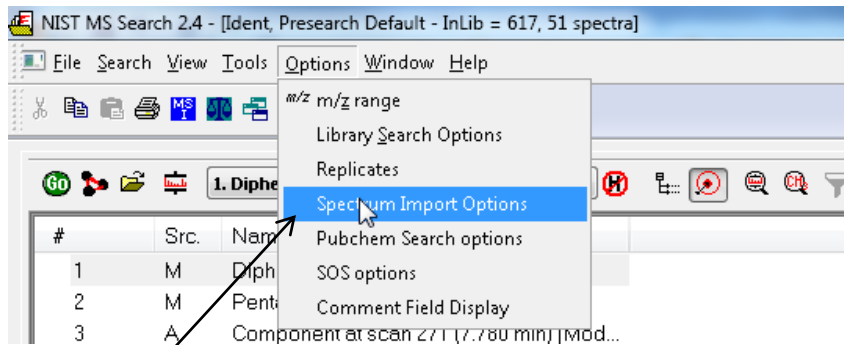
-Upgrades older versions of libraries to newer functions such as hybrid search, structures InChIKey, etc.



e.g. Updating indexes for a library
Ones in lower case probably *not* up to date, 1)
select, 2) then Build

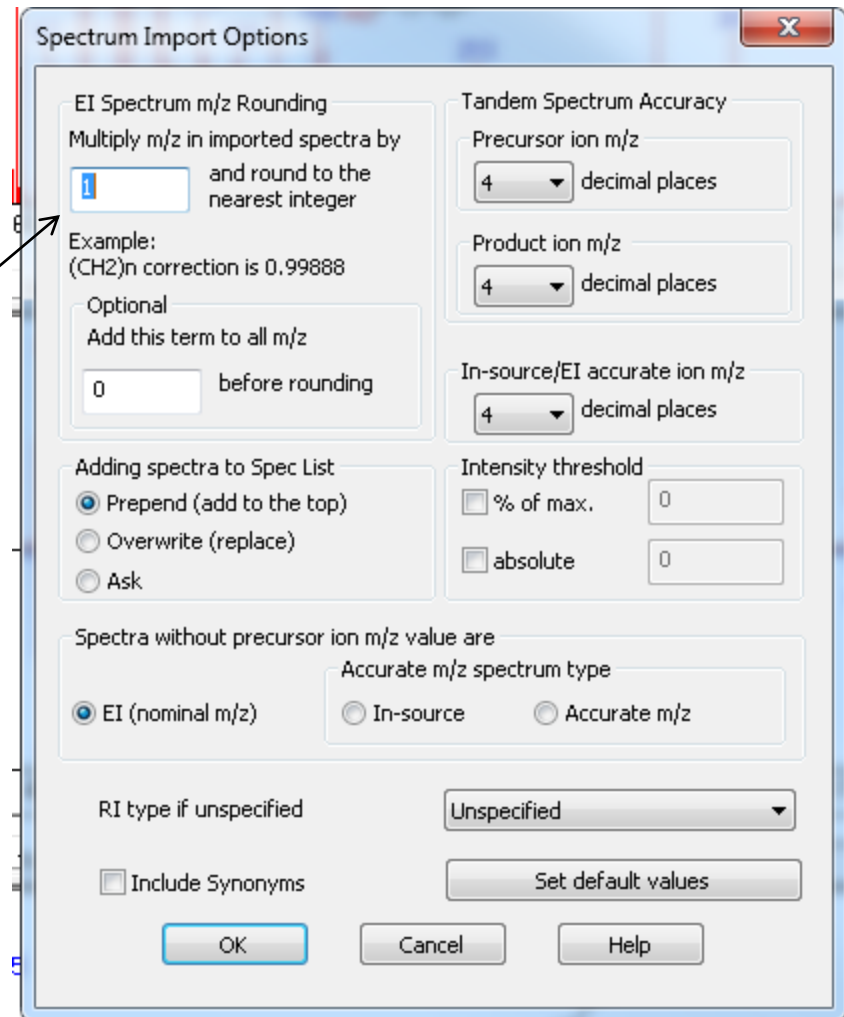


Library Spectra m/z Values by Nominal *not* Exact Mass



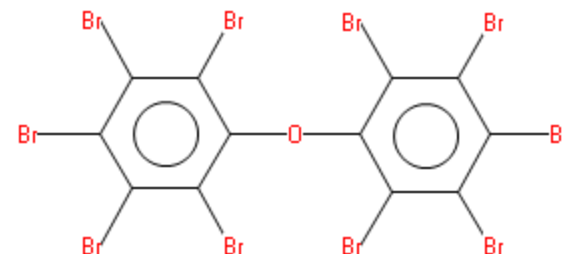
- Select "Spectrum Import Options"
- Spectra in NIST library are corrected to nominal mass*
- Use **this option** to "Multiply m/z in imported spectra by.."
- More problematic for compounds $MW > 500$, see examples **next** slide
- Negative mass defect a problem for multi brominated and chlorinated, species
- Positive mass defect a problem for compounds with large number of hydrogens

* [https://en.wikipedia.org/wiki/Mass_\(mass_spectrometry\)](https://en.wikipedia.org/wiki/Mass_(mass_spectrometry))



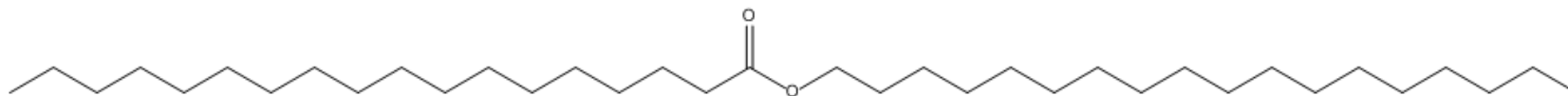
Nominal Molecular Weight Correction Examples

-NIST rounds up above 0.5, e.g. 536.5 converted to 537
-Some data systems round *before* exporting to NIST!



Name: Pentabromophenyl ether
Formula: C₁₂Br₁₀O
MW: 950 Exact Mass: 949.17829

- Nominal MW needs to be **increased** to 950 when importing for proper searching!
- Library “thinks” bromine has MW of 79, mass spec “knows” MW is 78.9!
- Correct by multiplying *all* *m/z* values by 1.00087
- Problem **only** with higher MW species



Name: Eicosanoic acid, hexadecyl ester
Formula: C₃₆H₇₂O₂
MW: 536 Exact Mass: 536.55323 CAS#:

- Nominal MW needs to be **decreased** to 536 when importing for proper searching!
- Library “thinks” hydrogen has MW of 1, mass spec “knows” MW is 1.0078!
- Correct by multiplying observed *all* *m/z* values by 0.99888
- Problem **only** with higher MW species

Retention Indices (Kovat) Determined *by Users* in AMDIS

Name: Cholesterol
Formula: C₂₇H₄₆O
MW: 386 **Exact Mass:** 386.354866 **CAS#:** 57-88-5 **NIST#:** 332884 **ID#:** 7754 **DB:** mainlib
Other DBs: Fine, TSCA, RTECS, EPA, USP, HODOC, NIH, EINECS
Compound ID: 0
Compound Hash:
Contributor: NIST Mass Spectrometry Data Center
Related CAS#: 218965-24-3; 262418-13-3; 378185-03-6; 676322-57-9; 793670-51-6; 80356-14-5; 80356-33-8; 849593-11-9; 856708-55-9
InChIKey: HYYWMDMLDIMFJA-DPAQBDIFSA-N **Non-stereo**
10 largest peaks:
43 999 | 55 886 | 57 744 | 105 686 | 386 681 |
107 661 | 95 610 | 81 582 | 91 567 | 41 559 |
Synonyms:
1.Cholest-5-en-3-ol (3β); 2.(-)-Cholesterol; 3.Cholest-5-en-3β-ol; 4.Cholesterin; 5.Cholesterol base H; 6.Cholesteryl alcohol;
7.Cordulan; 8.Dusoline; 9.Dusoran; 10.Dythol; 11.Hydrocerin; 12.Kathro; 13.Lanol; 14.Nimco cholesterol base H; 15.Nimco
cholesterol base No. 712; 16.Provitamin D; 17.Tegolan; 18.Wool alcohols B, P.; 19.3β-Hydroxycholest-5-ene; 20.5-Cholesten-
3β-ol; 21.Cholestrin; 22.Cholesterol; 23.Super hartolan; 24.5,6-Cholesten-3β-ol; 25.DELTA.5-Cholesten-3β-ol; 26.Cholesterine;
27.Dastar; 28.Fancol CH; 29.Cholest-5-en-3-ol, (3β)- #; 30.Cholest-5-en-3beta-ol; 31.Lidinite; 32.NSC 8798;
Experimental RI median±deviation (#data)
Semi-standard non-polar: 3087±12 (2)
Standard non-polar: 3052±29 (32)
Estimated non-polar retention index (n-alkane scale):
Value: 2596 iu
Confidence interval (Low reliability): 174(50%) 752(95%) iu
Retention index.
1. Value: 3098 iu
Column Type: Capillary
Column Class: Standard non-polar
Active Phase: DB-1
Column Length: 30 m
Carrier Gas: Helium
Column Diameter: 0.25 mm
Phase Thickness: 0.25 μm
Data Type: Normal alkane RI
Program Type: Ramp
Start T: 50 C
End T: 250 C
Heat Rate: 10 K/min
Source: Steiger, S.; Haberer, W.; Muller, J.K., **Social environment determines degree of chemical signalling (Supplemented materials).** *Biol. Lett.*, 7(6), 2011, 822-824.
2. Value: 3098 iu
Column Type: Capillary
Column Class: Semi-standard non-polar
Data Type: Normal alkane RI
Program Type: Ramp
Source: Steiger, S.; Peschke, K.; Francke, W.; Muller, J.K., **The smell of parents: breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides*.** *Proc. Roy. Soc. B*, 274, 2007, 2211-2220.
3. Value: 3075 iu
Column Type: Capillary

- NIST libraries have Retention/Kovat (RI) indices *
- Converts retention times into **system-independent** constants using a hydrocarbon calibration mixture
- RI's **determined in NIST AMDIS** software^{3,4,12}
- Limit search, see Library Search Options/RI(GC) tab
- Additional orthogonal information for characterizing compounds
- MS Search results list methods and conditions for determination
- Standard display is top two to avoid “slowing” the display of search results
- Can expand to see **All** for a library entry, display First 0, 1..., or uncheck box to see none

Library Search Properties

Spec List Text Info	Comp. Result	Histo
Hits List	Spec List	Plot of Hit
Unknown Plot	Spec	

Hit Text Info

Unknown Text Info

Display

- Compound Information
- m/z/Intensity List
- Ten Largest Peaks
- Synonyms
- GC Retention Indices

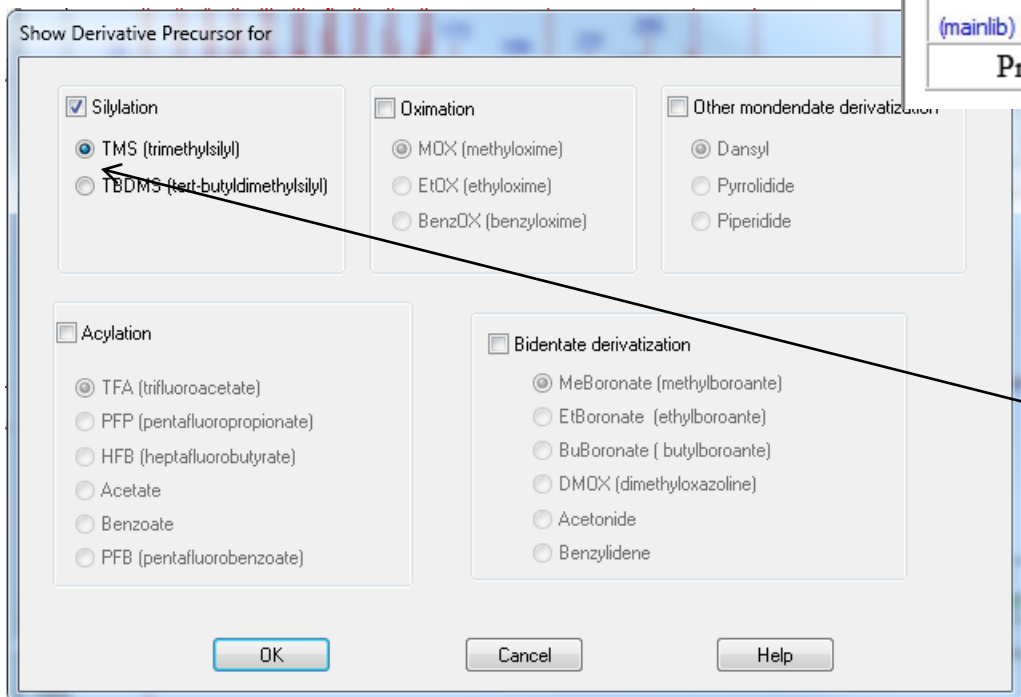
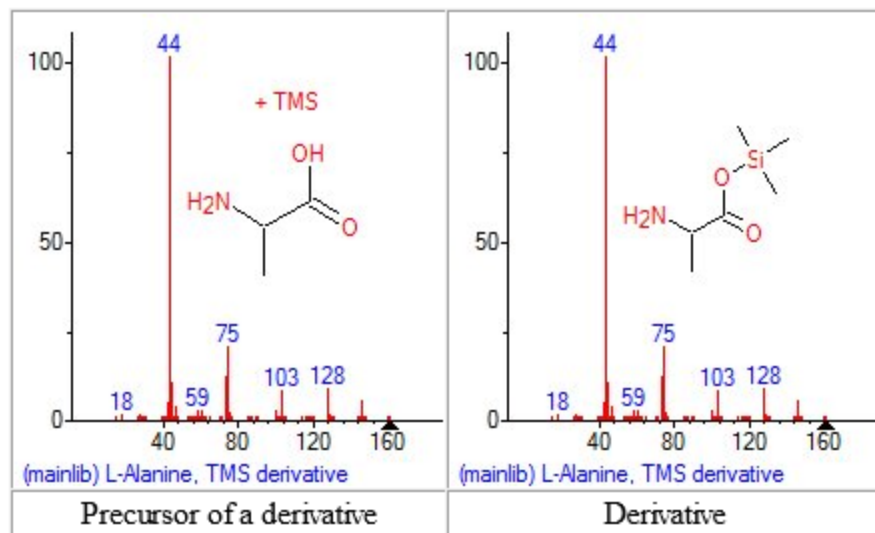
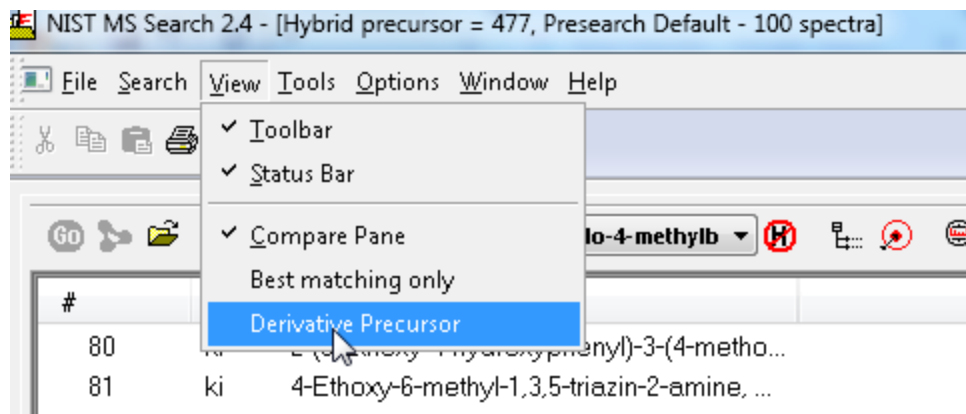
Hit Text Info: All (2) First

Arrange peaks by: Rows Columns

Wrap text Noise level % 0

* https://en.wikipedia.org/wiki/Kovats_retention_index

Different Derivative Displays (Affects Only Search Results Displays)



Original molecule or Derivative
Shown in Search Results

Limitation: Can Only select one radio button under a class

Using NIST Software to Obtain **Proposed** Nominal MW and Substructural Information

1. **First** Search spectrum in “Simple” Identity mode
2. Software uses components in hit list to determine probable nominal MW and substructural information
3. **LMB** on “View substructure Information”
4. **Scroll** through various windows in “Substructure Information” window

The image shows a screenshot of the NIST software interface. On the left, a toolbar contains several icons, with a red '3' and an arrow pointing to the 'View substructure Information' icon (a magnifying glass over a document). A light blue arrow points from this icon to the 'Substructure Information' dialog box on the right. The dialog box has a title bar 'Substructure Information' and a close button. It contains the following information:

Name of Unknown
hybrid "unknown" example

Chlorine/Bromine information
Cl=0, Br=1 Probability=87%
Probability of presence of Cl=0%, of Br=90%

MW	Prob. (%)
313	94
312	1

Substructural information

Prob. (%)	Prese	Prob. (%)	Abser
99	PhBr	99	Si
99	F	99	RDB1
99	Cl Br	99	CH-al.
99	Br	99	Ar-CO
99	halog	99	HC
99	ArBr	99	NoAr
96	AR	99	ph

Set of Substructures in use

#	Substruct
1	OH
2	CO2H
3	ArOH
4	ROH
5	SH
6	?OH
7	SiH3

Buttons: OK, Print, Help

Webinar References (*Internet Links*)

1. [James Little Mass Spectral Resource Website](#)
2. [NIST Search Software Detailed Manual](#)
3. [AMDIS Program for Data Processing Detailed Manual](#)
4. [Basic Instructions for Using AMDIS with NIST Search](#)
5. [Nightly Automatic Update of Users' Libraries](#)
6. [Using NIST Search from Instrument Manufacturers' Software](#)
7. [Chemical Ionization for MW Determination](#)
8. [Trimethylsilyl Derivatives for GC-MS](#)
9. [Methyl Ester Derivatives for GC-MS](#)
10. [SciFinder/ChemSpider and Accurate Mass LC-MS Data for Unknown ID's](#)
11. [Surfactant Identification](#)
12. [QuickGuide.rtf Supplied with AMDIS Software Installation for Retention Indices](#)
13. [New Developments in the Modeling of Ion Fragmentation by MS Interpreter Software](#)
14. [Enhancements to NIST MS Interpreter for Modeling High Mass Accuracy Tandem Mass Spectra](#)
15. [An Automated Method for Verifying Structure-Spectral Consistency Based on Ion Thermochemistry](#)
16. [Combining Fragment-Ion and Neutral-Loss Matching during Mass Spectral Library Searching: A New General Purpose Algorithm Applicable to Illicit Drug Identification](#)
17. [The Hybrid Search: A Mass Spectral Library Search Method for Discovery of Modifications in Proteomics](#)
18. [Hybrid Search: A Method for Identifying Metabolites Absent from Tandem Mass Spectrometry Libraries](#)
19. [Structure Annotation of All Mass Spectra in Untargeted Metabolomics](#)
20. [Most Current Handouts for Webinar Series, Parts I-V](#)
21. [Lipid Matrix Ionization Effects in LC-MS](#)
22. [Mass Spectral Similarity Mapping in Hybrid Searches Applied to Fentanyl Analogs](#)

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