

Wiley Spectral Webinar

Part II: Structure Searches with NIST MS Search and Using MS Interpret

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”

Table of Contents

Topic	Slide #
▪ Webinar topics	4
▪ Help Files for NIST Search	5
▪ General Windows commands/functions	6
▪ Primary Libraries of EI Spectra and Associated Structures	7
▪ Select the libraries used for Exact Structure searches	9
▪ Obtaining structures from chemical drawing program	10
▪ Search for similar structures after inserting clipboard structure ..	11
▪ Sending Structure from MS Search to drawing program	12
▪ Tips for displaying structure search results	13
▪ Tips for displaying related structures in structure searches	14
▪ Search for exact structures	15
▪ Using compare window to display multiple spectra	16
▪ View of hit list structure search options utilized	20
▪ MS interpreter for correlating structure to spectrum	21
▪ Do the 3 isomers of methyl hydroxybenzoate fragment differently? ...	34
▪ Any structure and associated spectrum sent to MS Interpreter	38
▪ Accurate mass spectrum correlated to structure in MS Interpreter ...	39
▪ Webinar references	40
▪ Acknowledgements	41

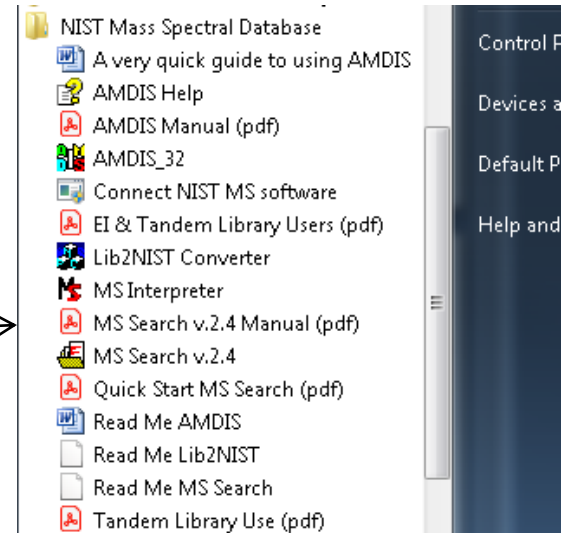
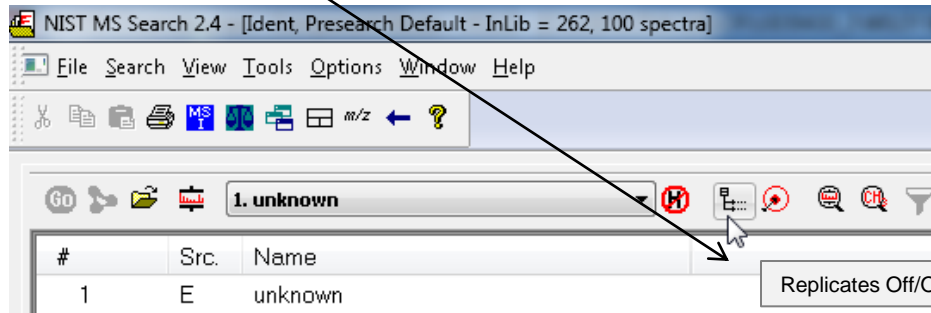
Part II: Structure Searches with NIST MS Search² and Using MS Interpreter^{2,13-15}

Webinar Topics:

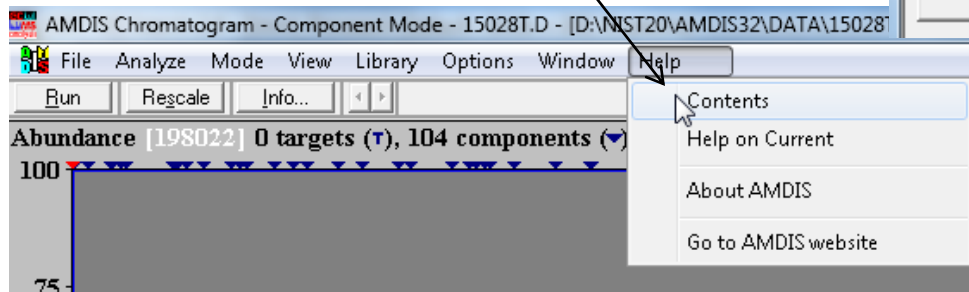
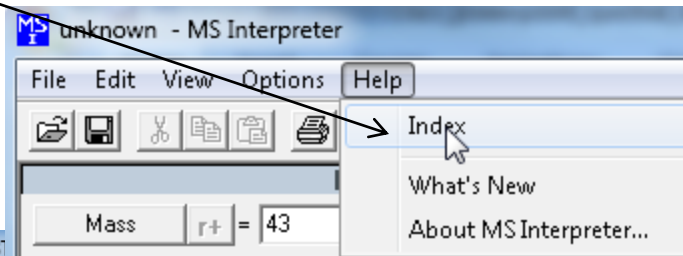
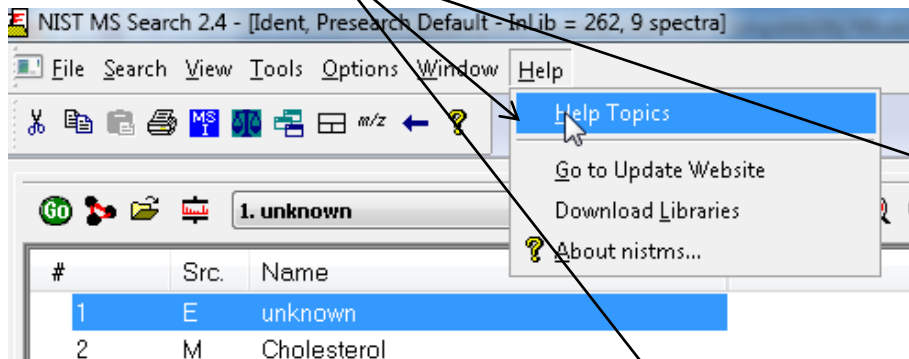
- Setting up **Library Search Options** for Structure Searches
- Importing structures from vendor programs²
- Searching for **similar** structures using **Structure Search**
- Searching for **exact** structure using **Library Search** with InChIKey
- Using “Compare” window to view series of spectra
- MS Interpreter for correlating substructure and isotope ratios to ions in spectrum
- Finding model compounds
- Accurate mass spectra in MS Interpreter

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!



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

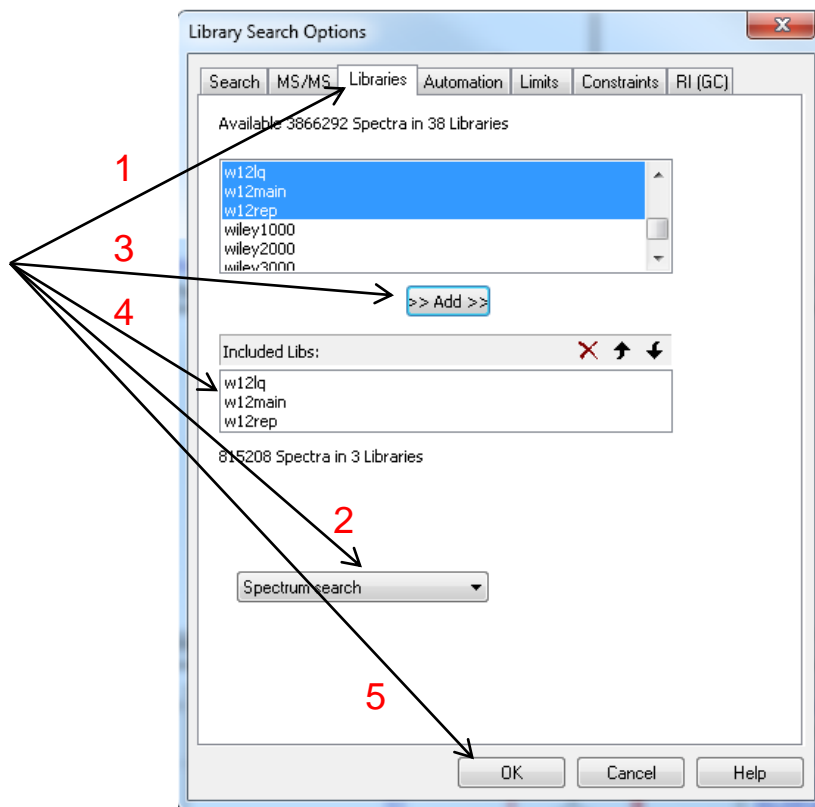
-**main (NIST):** one best entry selected (subjective) for component

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

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

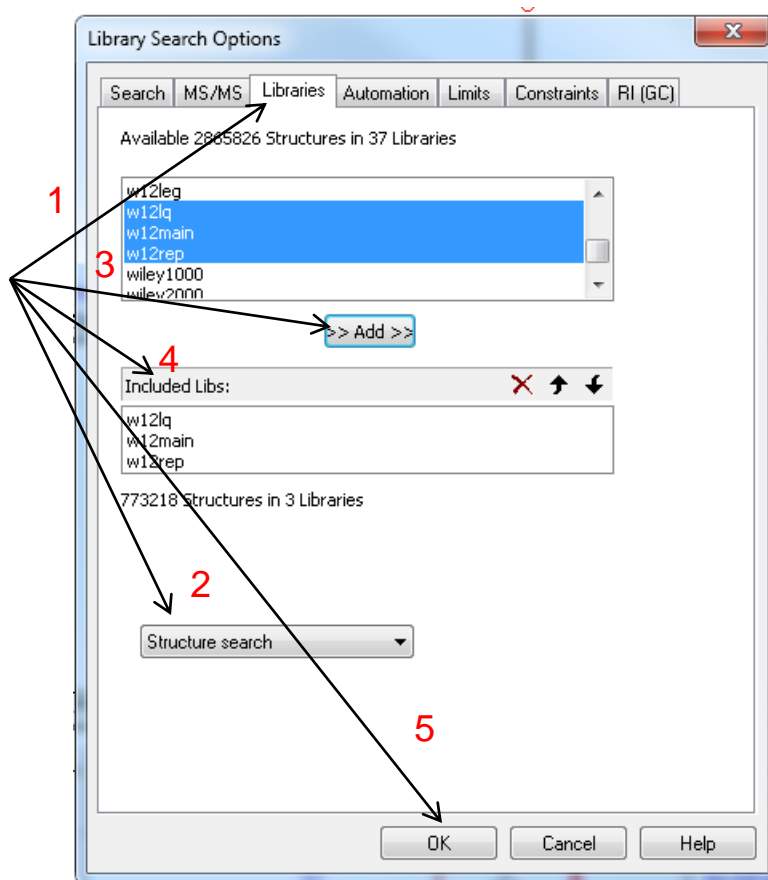
Critical Settings: Select the Libraries Used for *Exact Structure* Searches

- **Exact structure searches** are driven by libraries in **Spectrum Search**
- Somewhat surprising, but because InChIKey not actual structure used
- **LMB** on the **Libraries** tab and make sure **Spectrum Search** is selected
- Select the group of libraries to be searched by Exact Structure
- **>>Add>>** all libraries to be searched
- **Order** of libraries normally **only important** in Other Search Tab which return **maximum no.** of hits



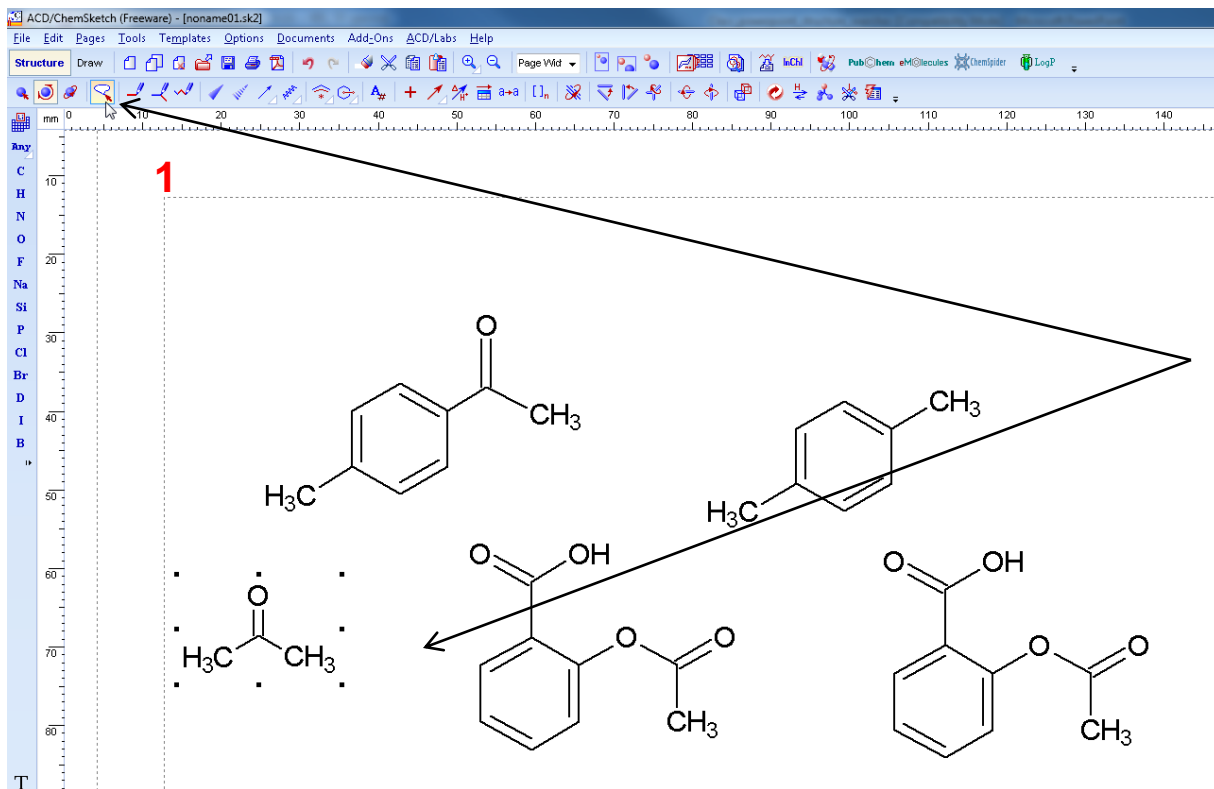
Critical Settings: Select the Libraries Used for Similar Structure Searches

- **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



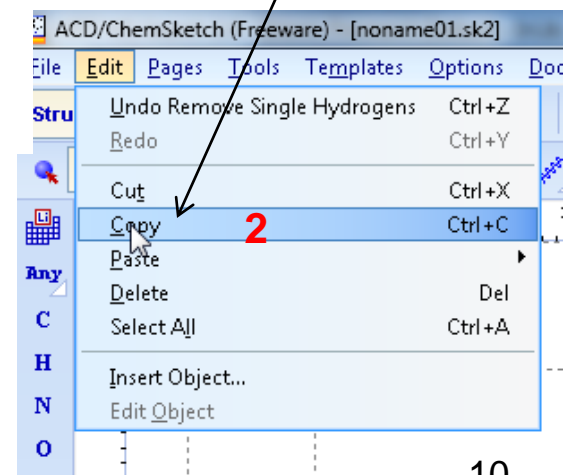
Structure Capabilities Within NIST Search Program

Obtaining Structure from ChemSketch



1) Use “lasso” tool to select structure to import into NIST program

2) Then copy into clipboard



Search for Similar Structures after “Insert Clipboard Structure”

- 1) **RMB** and **Insert Clipboard Structure not Paste**
- 2) **LMB** on **structure search icon** to search after highlighting entry
- 3) Results reported in lower left window **sorted** by similarity (1000 good fit)

NIST MS Search 2.4 - [Structure Similarity Search - 400 spe...]

File Search View Tools Options Window Help

Go 1. Structure: Clipboard #1

Src. Name

Library Search
Structure Similarity Search

Cut
Copy
Paste
Select All

Send To
Import
Export Selected
Insert Clipboard Structure
Copy Structure to Clipboard
Insert Clipboard Spectra
Copy Selected to Clipboard

Print
Print Preview
Properties

mainlib: 30
100
10
1
1000

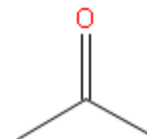
NIST MS Search 2.4 - [Structure Similarity S...]

File Search View Tools Options Win...

Go 1. Structure: Clipboard

Src. Name

1 E Clipboard #2



Name: Clipboard #2

Formula: C₃H₆O

MW: 58 Exact Mass: 58.0418649 ID#: 3 DB: Spec. Edit

InChIKey: CSCPPACGZOO CGX-UHFFFAOYSA-N [Nc](#)

Synonyms:

no synonyms.

Estimated non-polar retention index (n-alkane scale):

Value: 455 iu

Confidence interval (Ketones): 57(50%) 246(95%) iu

#	Lib.	DotProd	Distance	RI	Name
1	M	1000	1000	267	Formaldehyde
2	M	1000	1000	-	Carbon monoxide
3	M	1000	1000	401	Acetaldehyde
4	M	1000	1000	487	Acetone
5	M	1000	1000	-	Acetone-D6

Reverse Process: Sending Structure from MS Search to Drawing Program

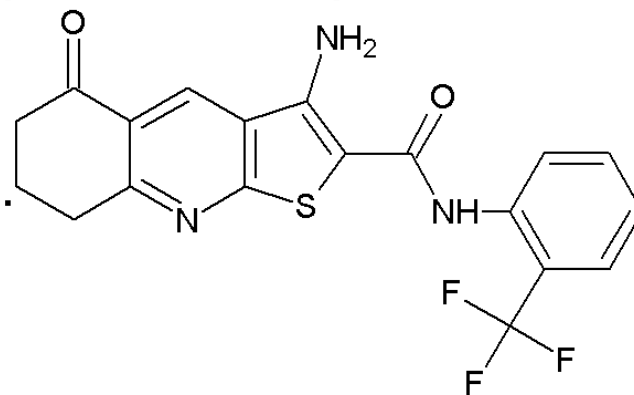
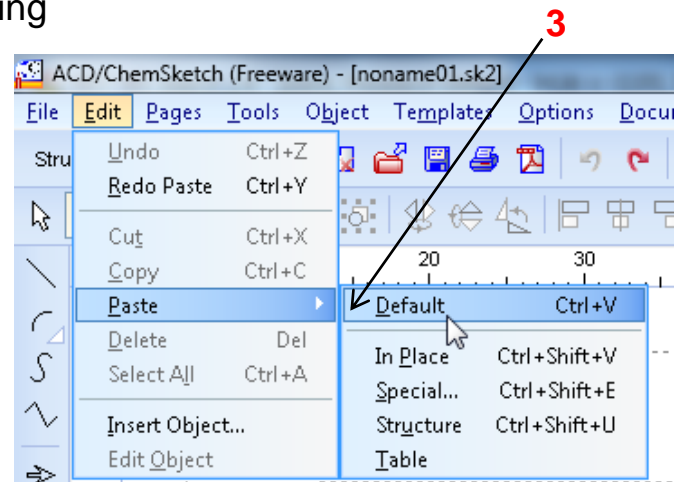
1. Any structure in MS Search Program can be sent to drawing program
2. **LMB** on entry, then **RMB** to "**Copy Structure to Clipboard**"
3. Then paste from Windows Clipboard into Drawing Program
4. Useful for modifying an existing structure for searching

#	Lib.	Match	Prob. (%)	Name
1	w1	593	53.1	1-iodo-4-isocyanatobenzene
2	M	568	16.2	3-Hydroxy-4-iodobenzonitrile
3	w1	530	3.88	3-Amino-5-keto-NH2-(trifluoromethyl)phe...
4	M	522		Library Search pyrimidine
5	w1	521		Structure Similarity Search ylthio)-4-(4-hydro...
6	M	512		benzoate
7	w1	512		ro-5-methyl-N[3...
8	M	511		idole
9	M	511		ro-5-methyl-N[3...
1..	M	511		nitrile
1..	M	508		c)pyridine
1..	M	504		benzoate
1..	M	497		
1..	R	490		
1..	M	484		-dicyanophenyl)-
1..	w1	484		-1-yl)phthalonitrile
1	M	480		l)-1,3-benzoxaz...

Names Structures InLib = -1101, Hit

Lib. Search Other Search Name

2



Tips for Displaying Structure Search Results

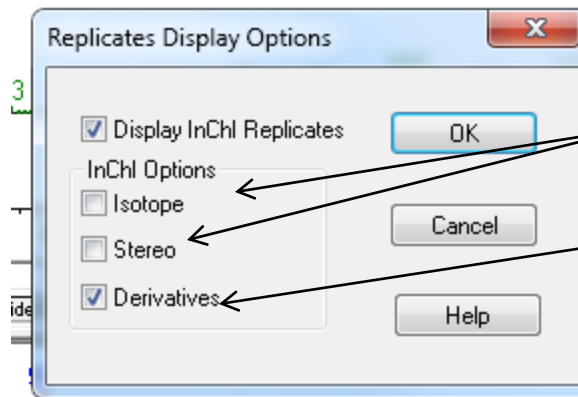
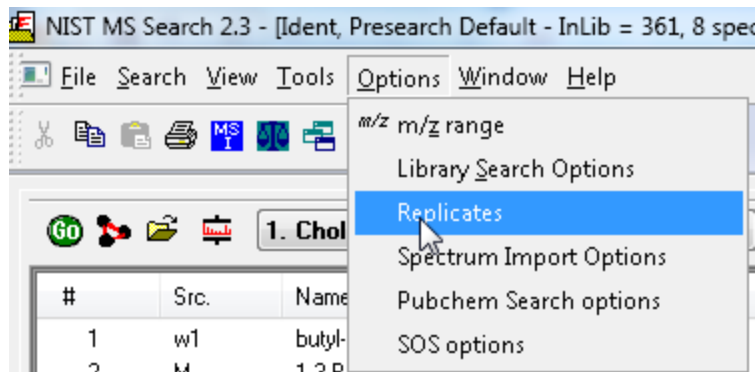
- 1) **RMB** on display box and select properties
- 2) add **delta mass** to table
- 3) **run** similarity search again and then **sort** by clicking on DeltaMass header
- 4) **Scroll down** until group with DeltaMass=0 found

The image illustrates the process of displaying structure search results. It shows a 'Hit List' table with columns for Distance, RI, and Name. A context menu is open over the 'Acetone' entry, with 'Properties' selected. This leads to the 'Library Search Properties' dialog, where 'DeltaMass' is checked under 'Items to Display'. The final screenshot shows the results table sorted by DeltaMass, with the 'DeltaMass' column header highlighted.

Distance	RI	Name
1000	267	Formaldehyde
1000	-	Carbon monoxide
1000	401	Acetaldehyde
1000	487	Acetone
1000	-	Acetone
1000	598	2-Butanone
1000	688	3-Pentanone
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	408	Acetaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	-	Formaldehyde
1000	408	Acetaldehyde
1000	408	Acetaldehyde
1000	408	Acetaldehyde
1000	408	Acetaldehyde

#	Lib.	DotProd	Distance	RI	DeltaMass	Name
25	w1	1000	1000	408	14	Acetaldehyde
26	w1	1000	1000	408	14	Acetaldehyde
27	M	1000	1000	487	0	Acetone
28	w1	1000	1000	455	0	Acetone
29	w1	1000	1000	455	0	2-Propanone
30	w1	1000	1000	455	0	2-Propanone
31	w1	1000	1000	455	0	2-Propanone
32	w1	1000	1000	455	0	2-Propanone
33	w1	1000	1000	455	0	2-Propanone
34	w1	1000	1000	455	0	2-Propanone

Tips for Displaying Related Structures in Structure Searches



Inconsistent!

“Do see” **unchecked**

“Do see” **checked**

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

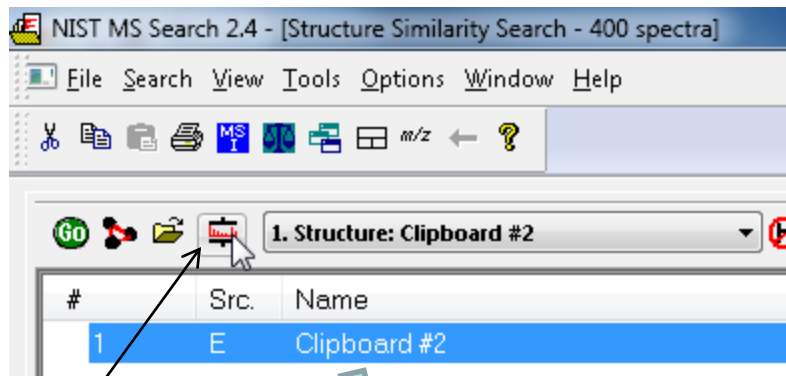
-Can toggle by “left-clicking” on + or – box

-**Also** affects Names Search Display

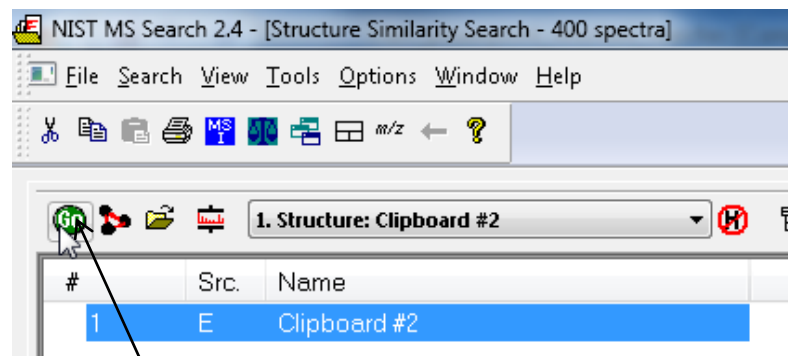
#	Lib.	Name	Match	R.Match
1	M	Cholesterol	999	999
...	R	Cholesterol		
...	R	Cholesterol		
*	m	3a-Cholesterol acetate		
*	m	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	m	Cholesterol, TMS derivative		
*	r	Cholesterol, TMS derivative		
*	r	Cholesterol, 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		

Search for Exact Structures

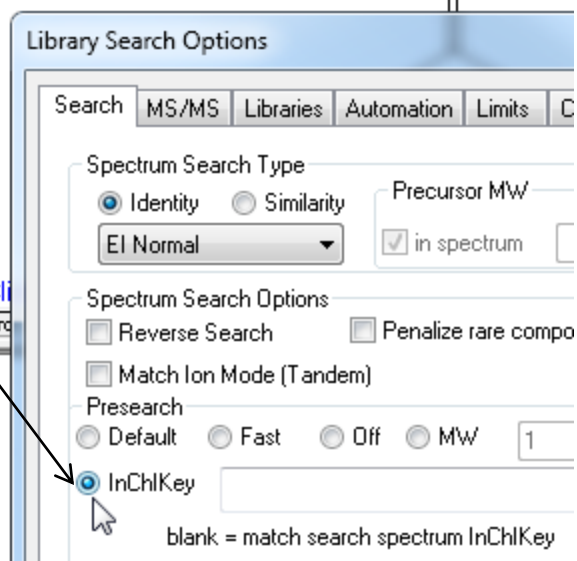
- 1) **LMB** on **Library Search Options**
- 2) select **Search tab** and select **InChIKey**
- 3) **Click** on **Library Search** **not Structure Search** icon
- 4) Process results using tips used for similarity search
- 5) **Much simpler** results when **NOT** looking for similar structures



1



3



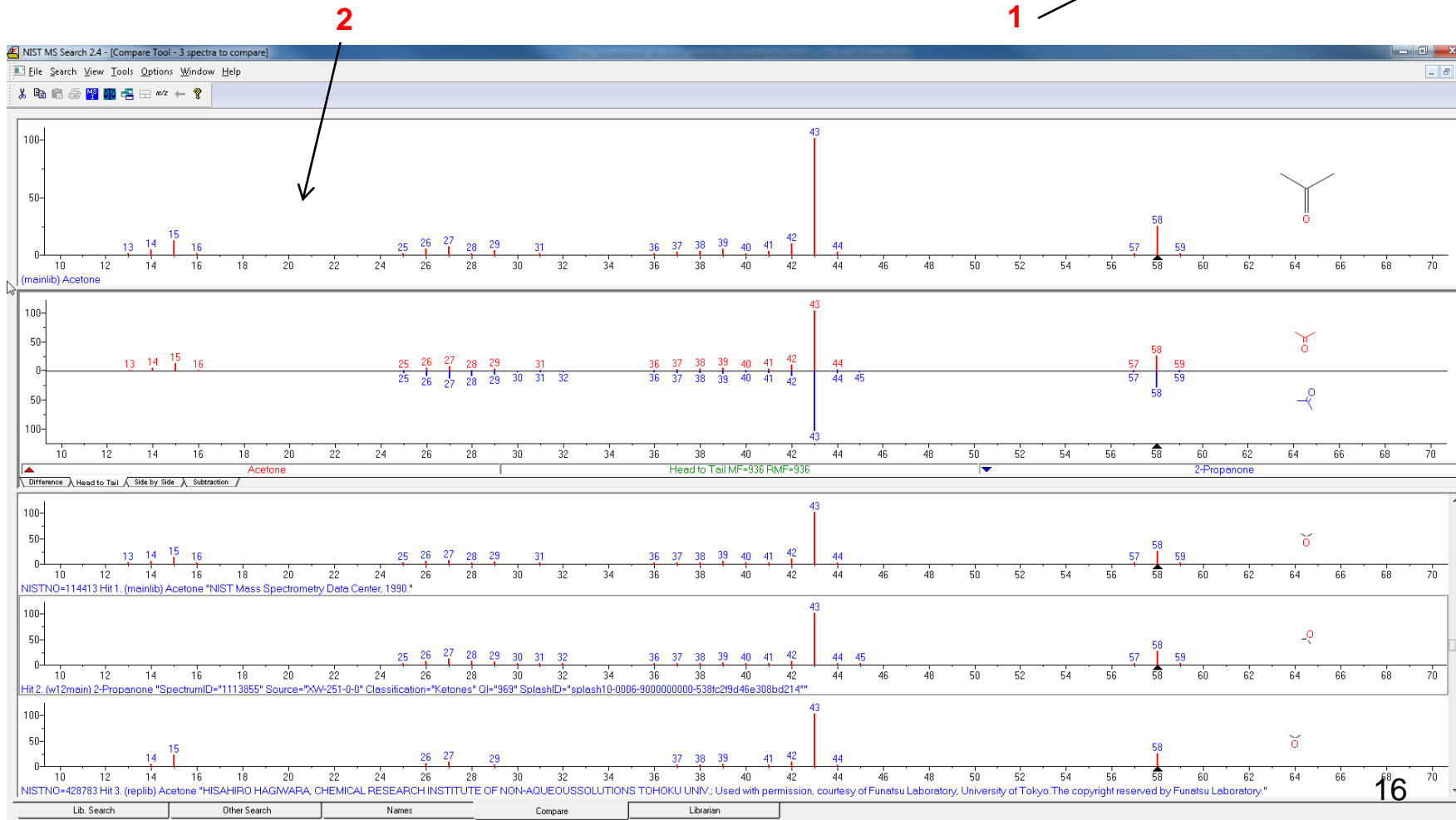
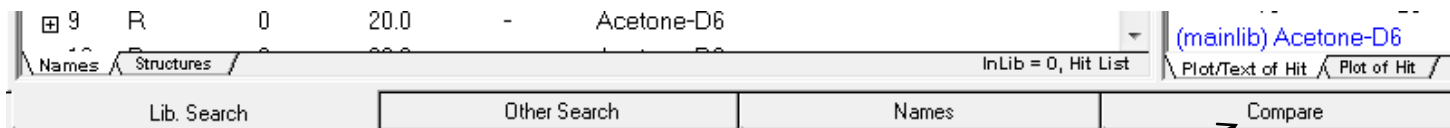
2

#	Lib.	Match	Prob. (%)	RI	Name
1	M	0	20.0	-	Acetone-D6
2	M	0	20.0	-	Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone

4, 5

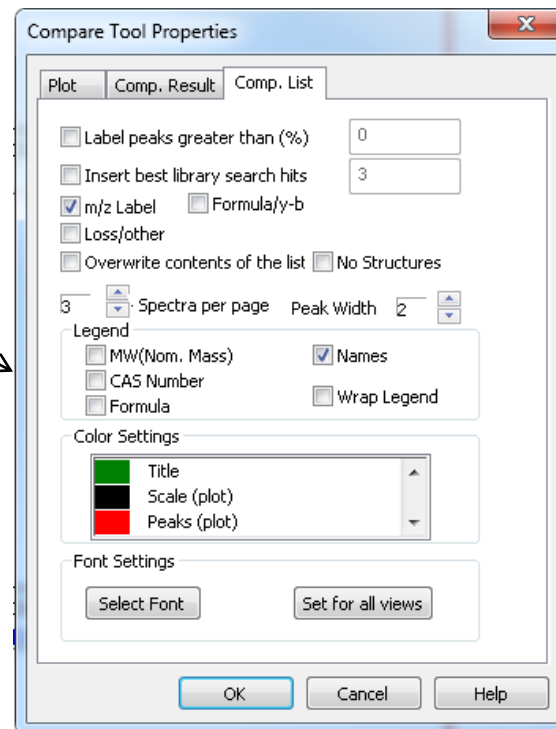
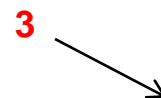
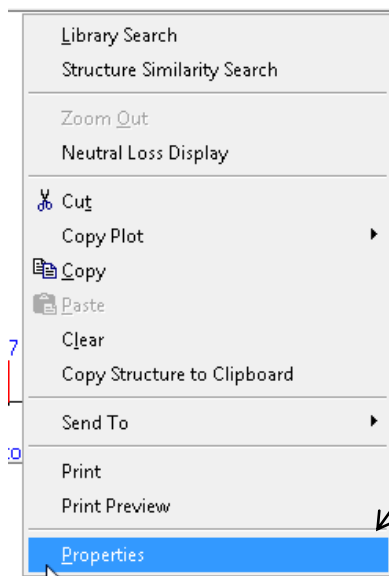
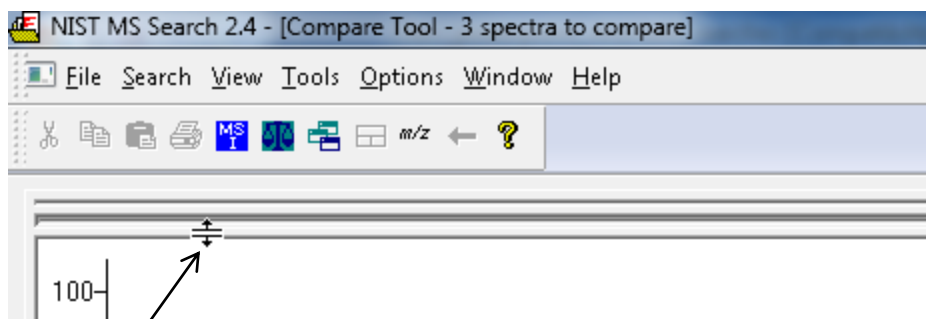
Using “Compare” Window to Display Multiple Spectra

- 1) Select **Compare** tab at bottom of program
- 2) Normally setup to transfer the 3 best library hits for comparison as shown below



Using “Compare” Window to Display Multiple Spectra

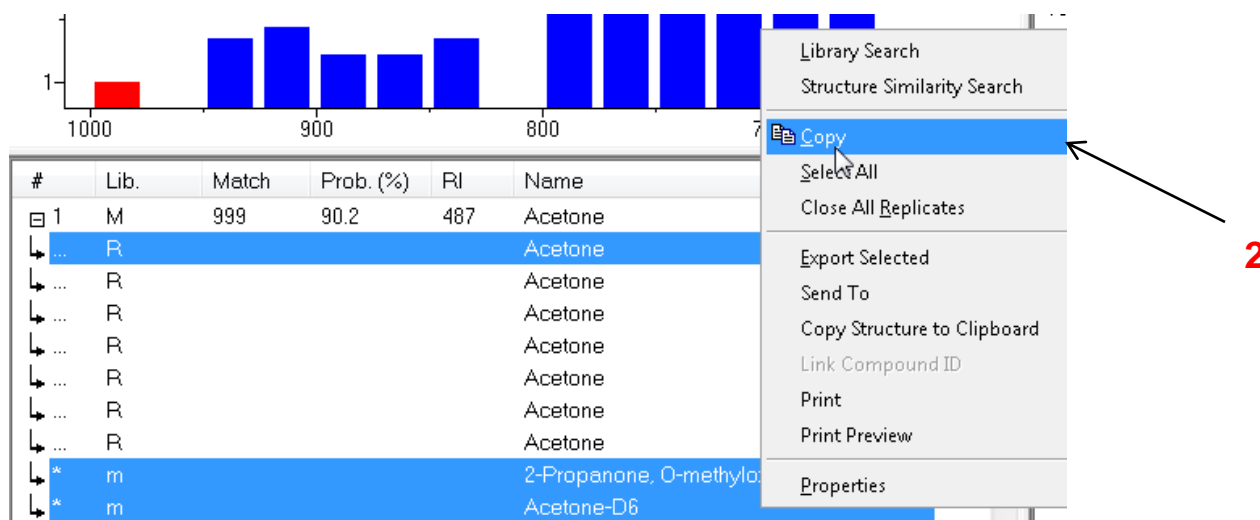
- 1) **Compress top two** windows by **selecting bar** with left mouse click and **scrolling closed** with mouse
- 2) Select prosperities window in bottom windows and then select **Properties** with **LMB**
- 3) Setup options as shown below



Using “Compare” Window to Display Multiple Spectra

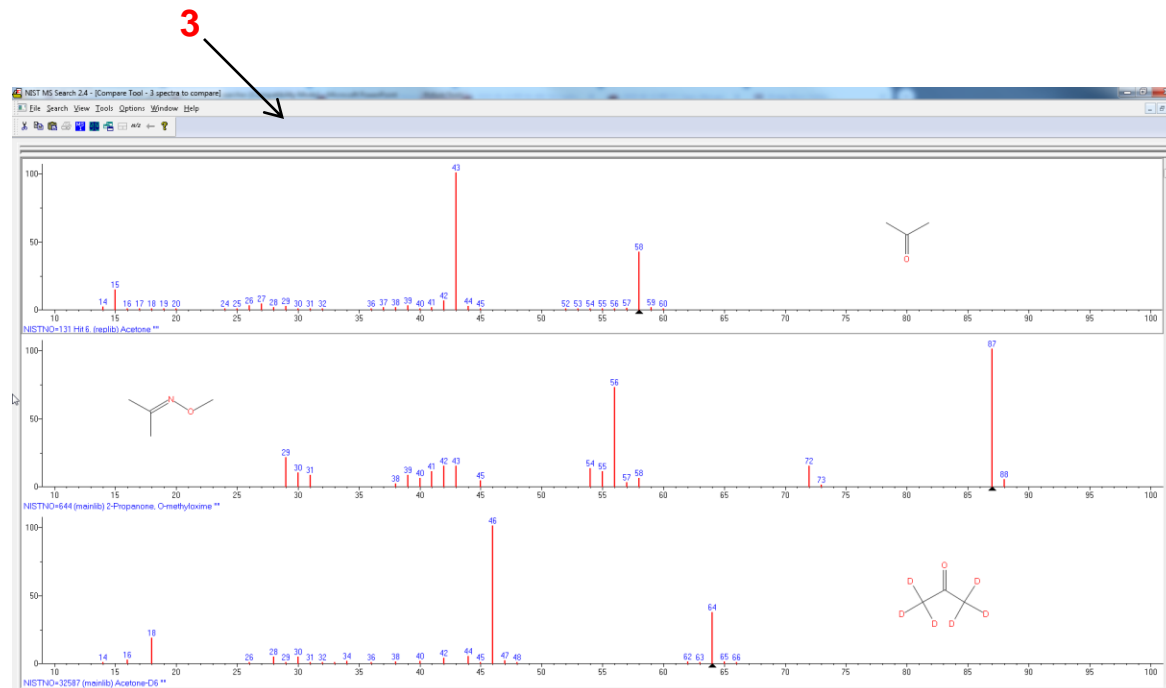
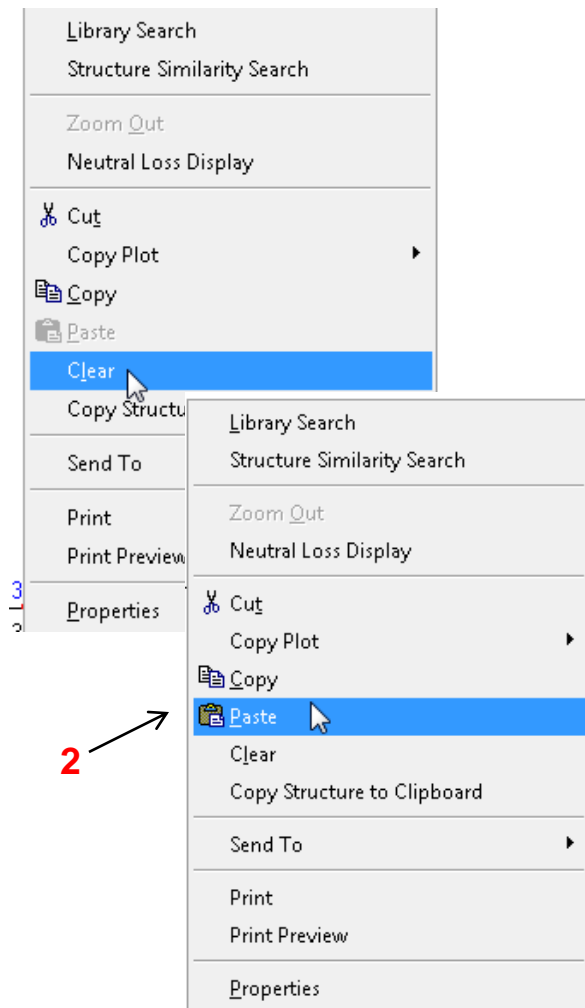
- 1) **Select** three spectra, e.g., from your exact search of acetone using control **left mouse click** while **holding control key** on keyboard
- 2) **RMB** somewhere within **blue highlighted area** and select **copy with left mouse** button

#	Lib.	Match	Prob. (%)	RI	Name
1	M	999	90.2	487	Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
...	R				Acetone
*	m				2-Propanone, O-methyloxime
*	m				Acetone-D6



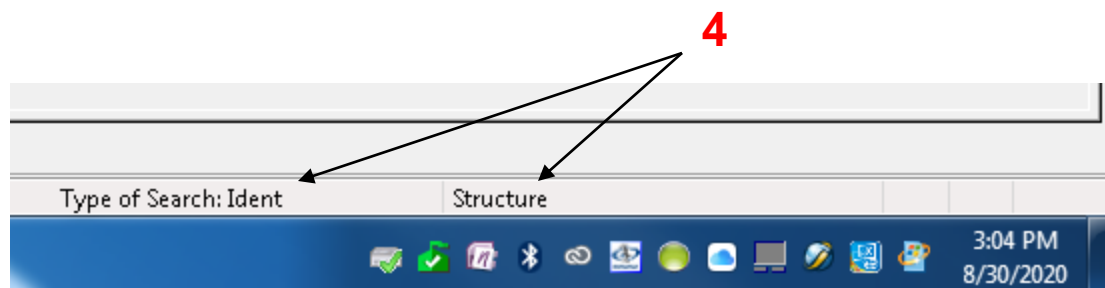
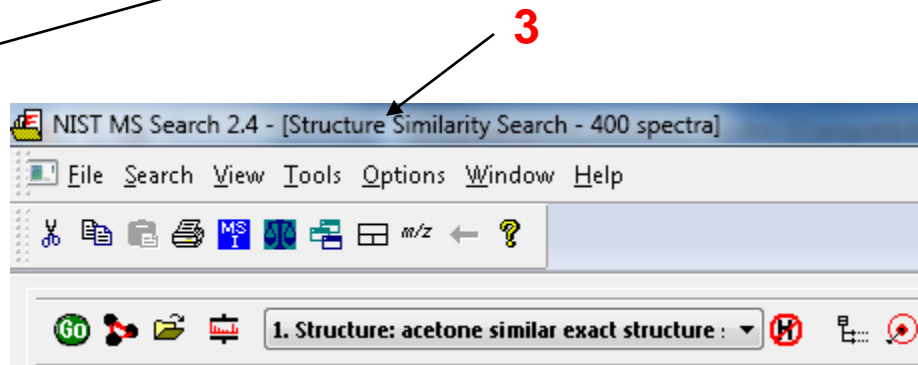
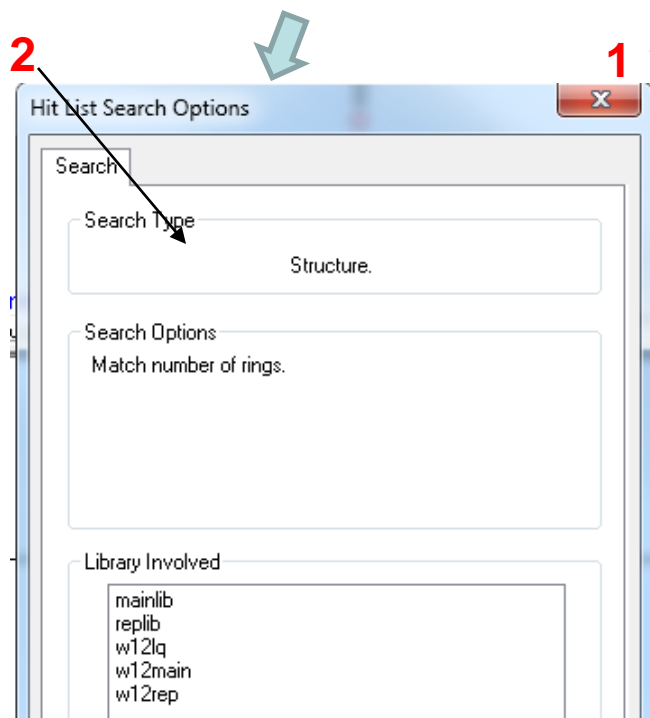
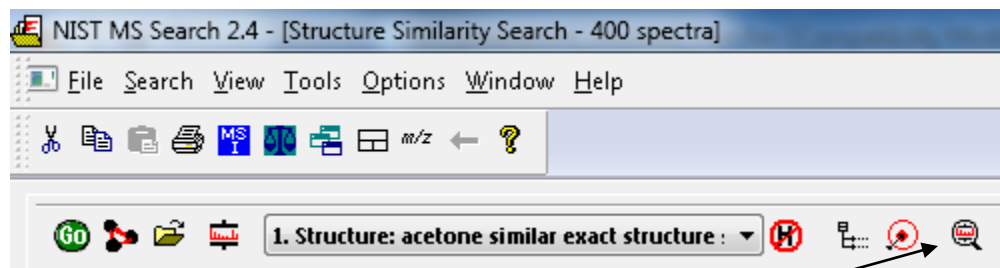
Using “Compare” Window to Display Multiple Spectra

- 1) **Go to Compare window** by selecting **tab at bottom** of Search Display Window
- 2) **RMB** in window and then **LMB** on **Clear** to delete spectra present
- 3) **RMB spectra** of acetone related species **should appear** in window for comparison



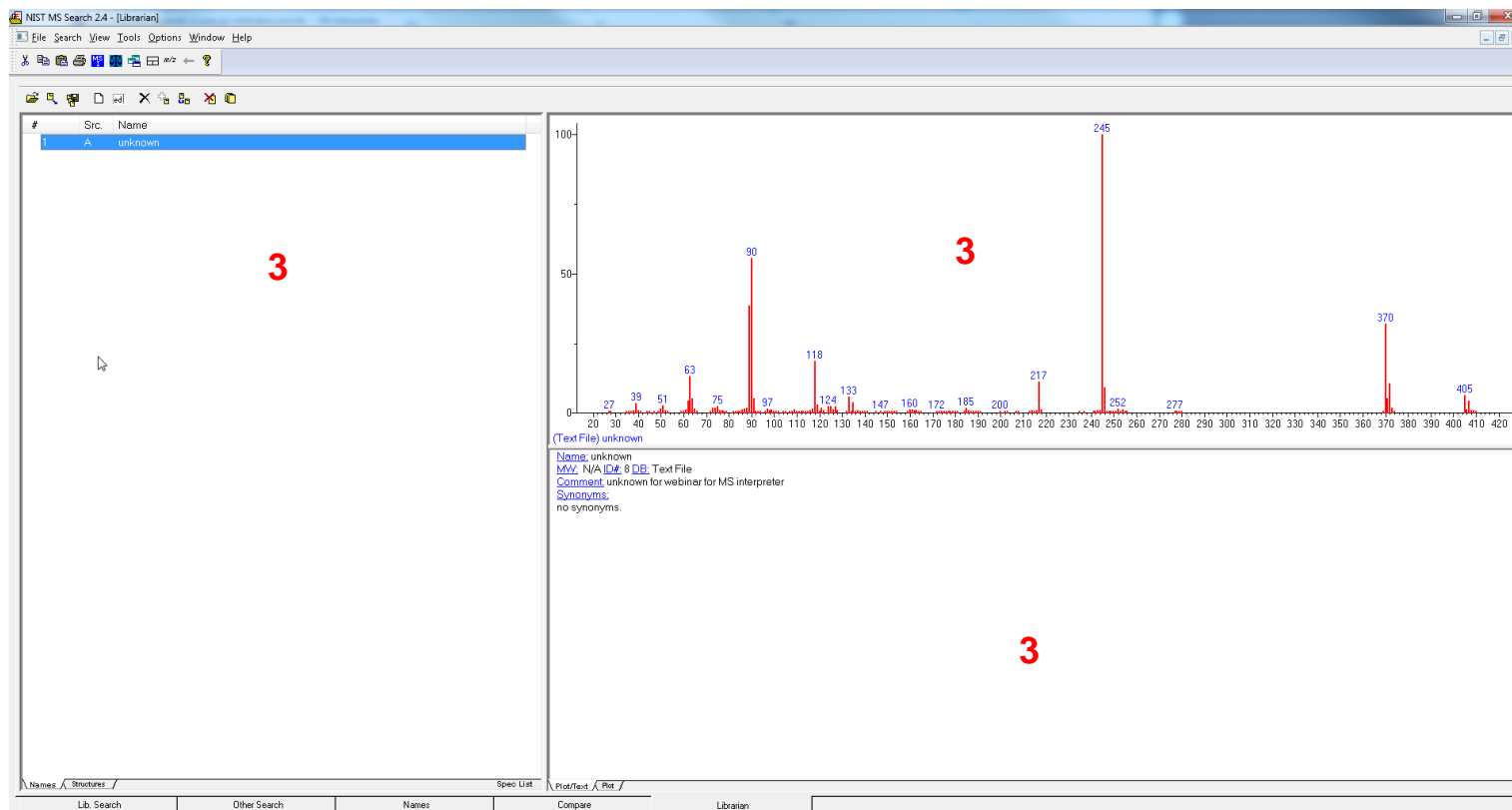
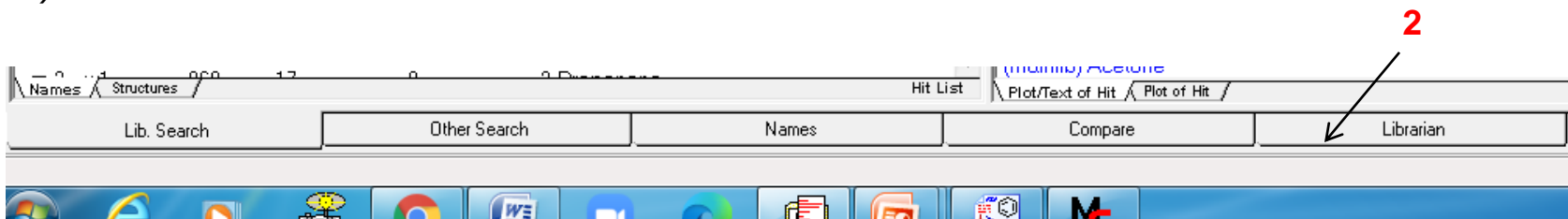
View of Hit List Structure Search Options *Utilized*

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



MS Interpreter for Correlating Structure to Spectrum: *Adding a Structure to a Spectrum*

- 1) **Import** the mass spectrum from instrument data system
- 2) **Open Librarian tab** at bottom of MS Search Window
- 3) **View** in **Librarian** tab



MS Interpreter for Correlating Structure to Spectrum:

Adding a Structure to a Spectrum

- 1) **Draw** the proposed structure with drawing program and **copy** into clipboard
- 2) **Open Librarian tab** and select “**ed**” icon with **left mouse click**
- 3) **LMB** on **From Clipboard** to associate structure with spectrum
- 4) **Modify** name fields, synonyms, comments fields, or even **edit** spectrum
- 5) **Add to List**

Tip: This is basically the approach used to **create a user library** and/or user library **entry**. This will be discussed in detail in Webinar 5 in this series. **Instead** of “Add to List”, the “Add to library” button will be selected. Also, one can “Replace” a current spectrum in user library after correcting it.

The screenshot shows the NIST MS Search 2.4 - [Librarian] interface. The 'Spectrum Information' dialog box is open, displaying the following fields and buttons:

- Name: unknown
- Formula: [Empty] From structure
- Mol. Weight: 0 CAS Number: 0
- Library: Text File
- ID Number: 8 Inventory#: [Empty]
- RI: [Empty] Edit RI
- Other Names (Synonyms): [Empty]
- Additional Info: [Empty]
- Experimental Data: [Empty]
- Comments: unknown for webinar for MS interpreter
- Buttons: Add to Library, Add to List, Replace, Cancel, Help

The Peak information table is as follows:

m/z	Abund.	Annotation
27	1	
28	3	
35	1	
36	2	
37	3	
38	8	
39	32	
40	7	

The mass spectrum plot shows peaks at m/z 90, 245, and 370. The 'From Clipboard' button is highlighted with a red arrow and number 3. The 'Add to List' button is highlighted with a red arrow and number 5. The 'ed' icon in the toolbar is highlighted with a red arrow and number 2. The 'From structure' button and the 'Other Names (Synonyms)' field are highlighted with a red arrow and number 4. The 'To Clipboard' button is highlighted with a red arrow and number 3.

MS Interpreter for Correlating Structure to Spectrum: *Sending to MS Interpreter*

- 1) Entry is now ready to send to MS interpreter
- 2) Program has automatically calculated nominal molecular weight, exact mass, molecular formula, and InChIKey
- 3) **Right mouse** click on selected entry, then LMB on Send to/MS Interpreter
- 4) An **alternative** to step 3, is to **send** the entry to MS interpreter **using F9** key

The screenshot displays the NIST MS Search 2.4 interface. A search result for 'unknown' is selected in the main table. A context menu is open over this entry, with the 'Send To' option expanded to show 'MS Interpreter' as the selected choice. A red arrow labeled '3' points to the 'MS Interpreter' option. Another red arrow labeled '4' points to the F9 key on a keyboard inset in the top right corner. The bottom of the window shows a spectrum plot with peaks at m/z 277, 370, and 405.

#	Src.	Name
1	E	unknown

#	Src.	Name
1	E	unknown

245

277 370 405

60 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420

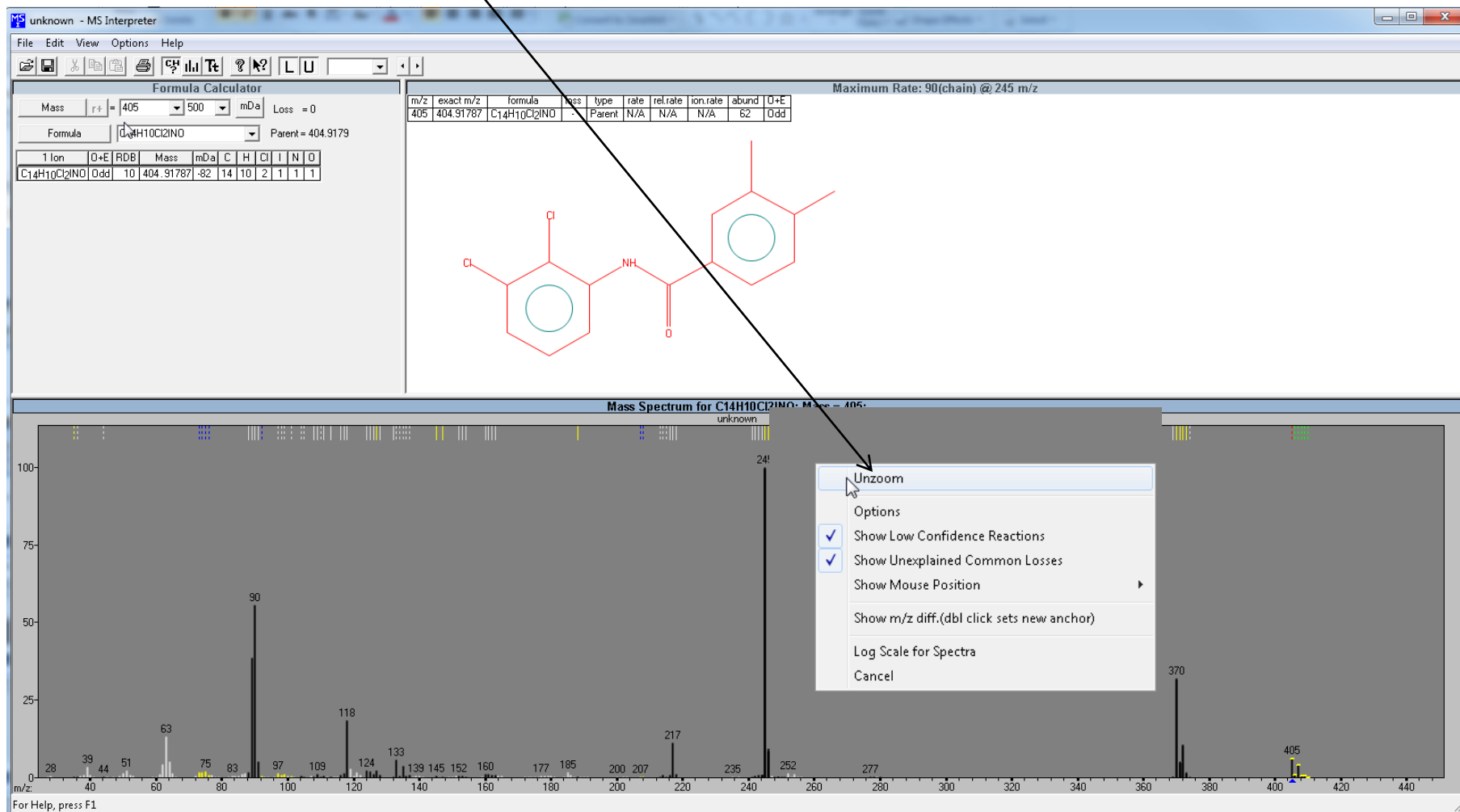
Names Structures / Spec List Plot/Text Plot / Lib. Search Other Search Names Compare Librarian

For Help, press F1

MS Interpreter for Correlating Structure to Spectrum:

Spectrum and Structure in MS Interpreter

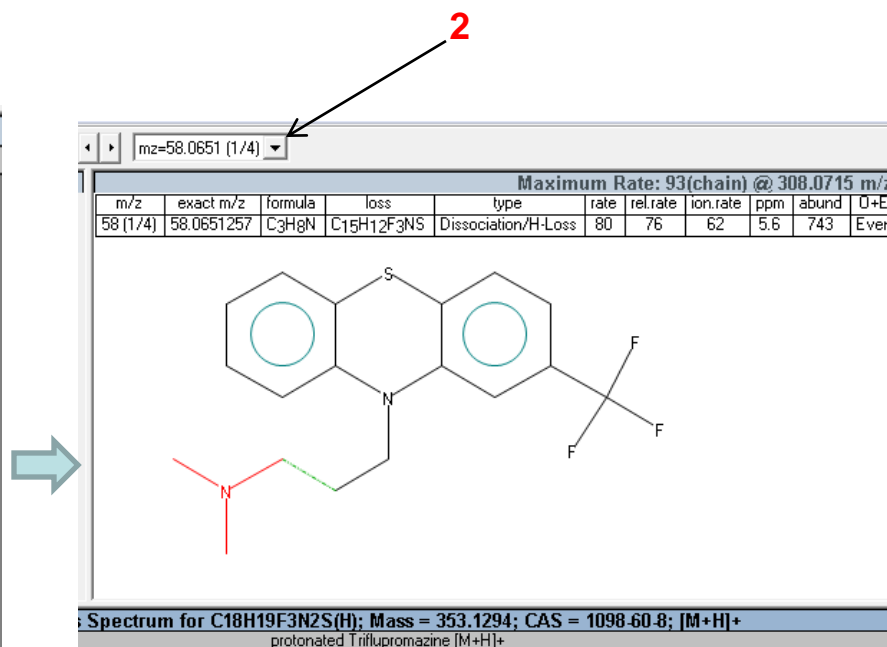
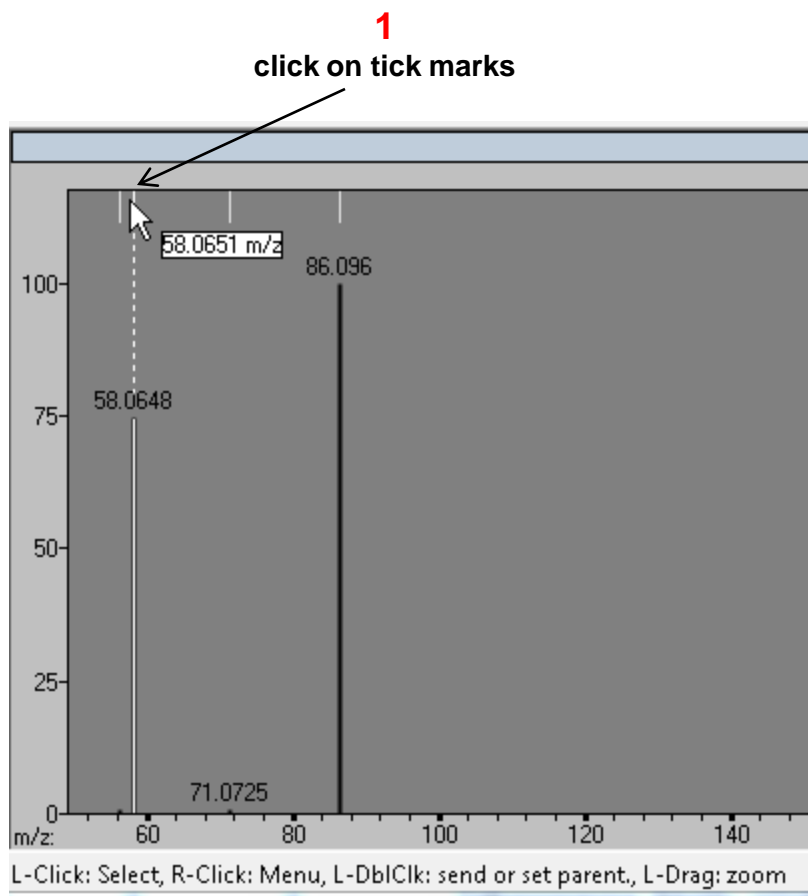
- All ions in black, yellow, and red correlated to structure including isotopes automatically
- Ions in white are not assigned
- Spectrum can be expanded like any spectrum in NIST programs by **LMB** and **dragging** to form box
- RMB** and then **LMB Unzoom** to return to original size



MS Interpreter for Correlating Structure to Spectrum:

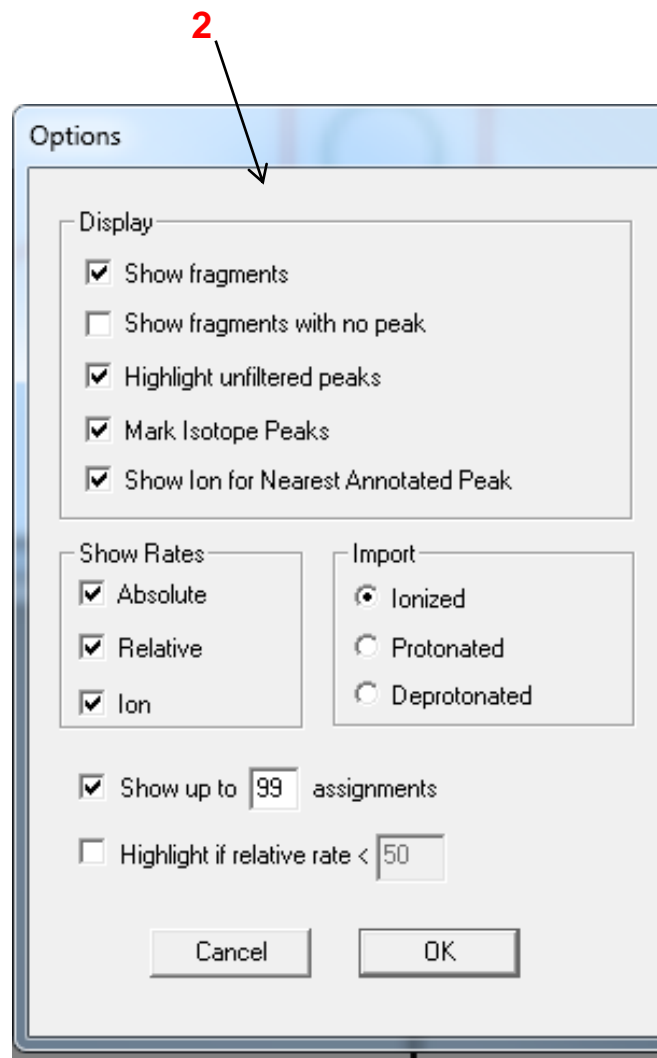
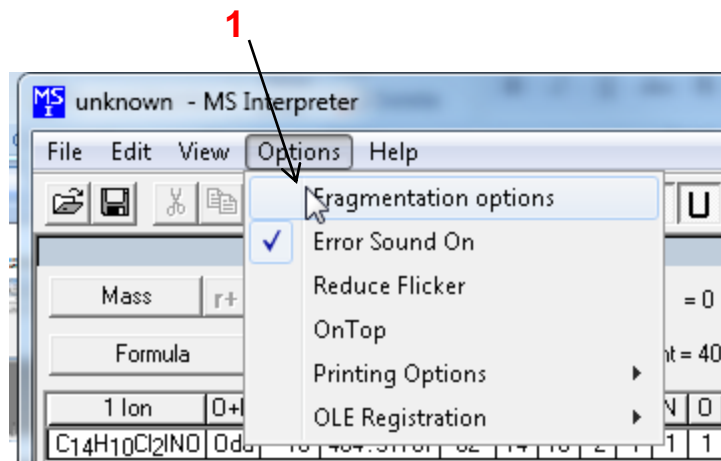
Stepping Through **More Than One Proposed Structure**

- 1) **LMB** on the **tick mark** for ion of interest, the display will toggle through **all 4** in this example
- 2) If **more than one** is present, the box will indicate the number, in this case $\frac{1}{4}$
- 3) Also, you can select one of the tick marks with LMB then use your **left** and **right** arrows **on keyboard** to step through the assigned ions in the mass spectrum



MS Interpreter for Correlating Structure to Spectrum: *Suggested Default Settings for Electron Ionization*

- 1) Select **Options** with **left mouse** click then **Fragment options**
- 2) Setup options as noted in window



MS Interpreter for Correlating Structure to Spectrum: *Suggested Default Settings for Electron Ionization*

- 1) Select **Isotopes Calc** icon
- 2) Carefully set the menu options as noted in displayed Isotope Calculator Window below

The screenshot displays the MS Interpreter software interface. The main window shows the formula C14H10Cl2INO and its parent ion mass of 404.9179. The Isotope Calculator window is open, showing a table of isotopes and a mass spectrum plot. The plot displays MS peaks (red) and fits (cyan) for the parent ion and its isotopes. The table below is a reproduction of the data shown in the Isotope Calculator window.

Mass	Abundance	*Max
404.9184	100.00000	100.00
405.9216	15.66046	15.66
406.9157	65.34073	65.34
407.9188	10.10515	10.11
408.9132	11.10461	11.10
409.9160	1.65696	1.66
410.9188	0.14065	0.14
411.9214	0.00867	0.01
412.9241	0.00041	0.00
413.9270	0.00001	0.00

Base peaks fit -- discrepancy = 0.0 (0/100)

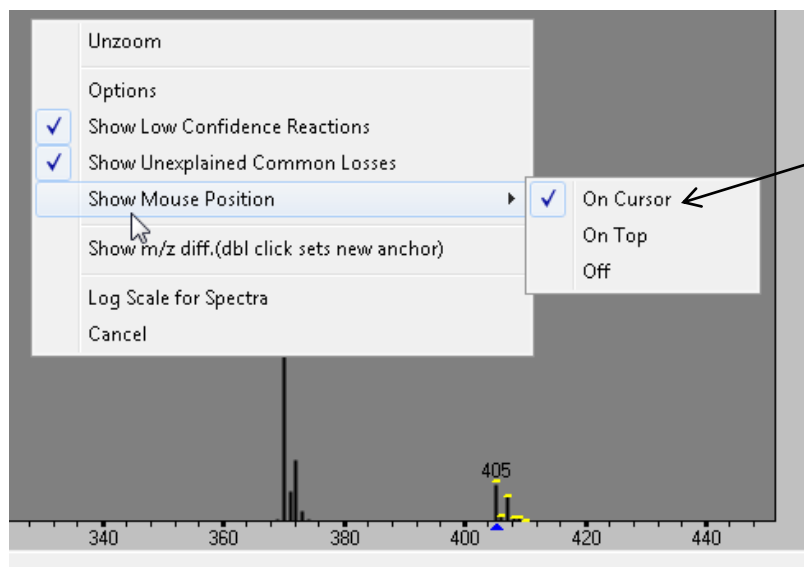
MS Lock Shift= 0 Margins: L= 1 R= 1

Fit Normal Use M-1 Base peak fit MS peak On/Off

MS: display mass spectrum; Fit: fit MS with isotope pattern

MS Interpreter for Correlating Structure to Spectrum: *Suggested Default Settings for Electron Ionization*

- 1) **RMB** in spectrum window, then **LMB** to select **Show Mouse Position/On Cursor**
- 2) Usually have the **Formula Calc** and **Isotope Calc** buttons **depressed** to display windows
- 3) **Low confidence mechanism** and **unknown mechanism** buttons **depressed** to show additional fragmentation



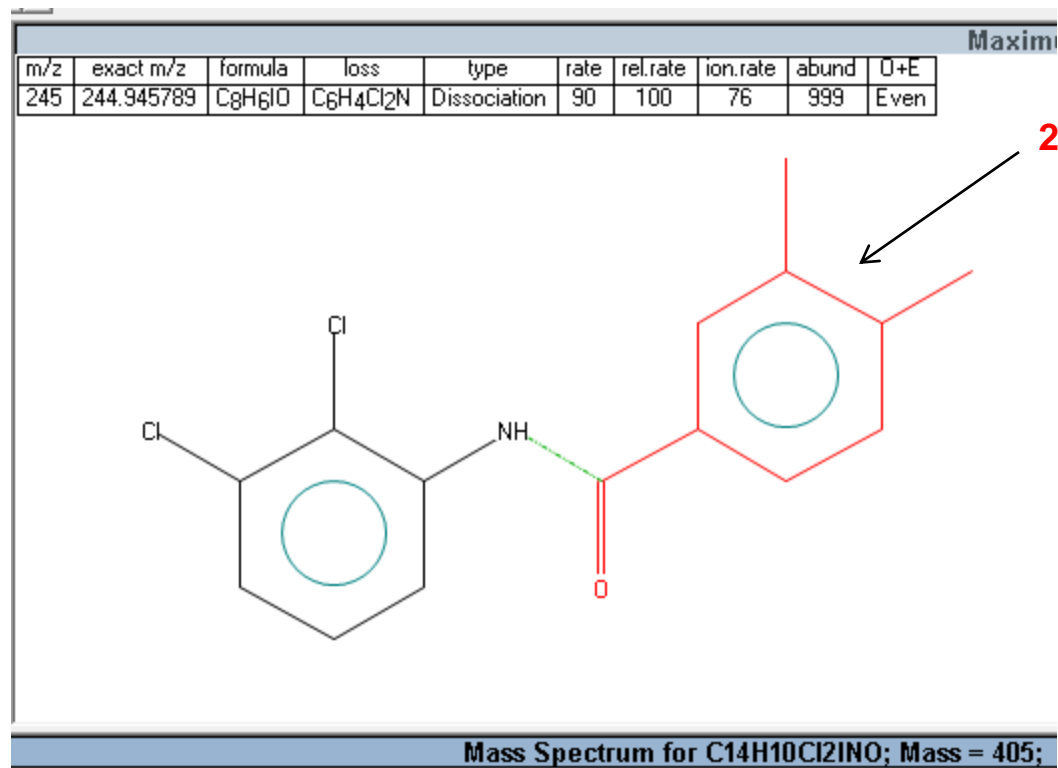
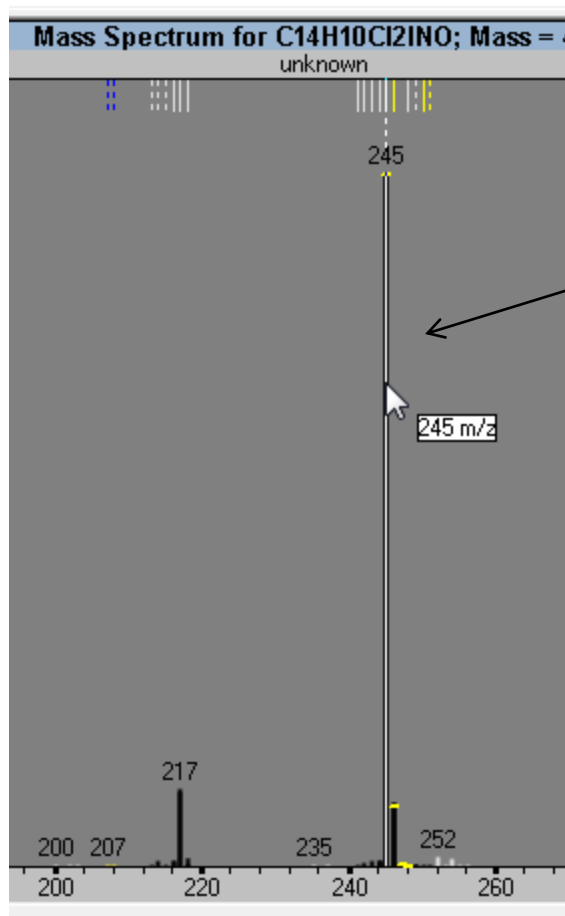
A screenshot of the MS Interpreter software interface. The main window title is 'MS unknown - MS Interpreter'. The menu bar includes File, Edit, View, Options, and Help. The toolbar contains icons for File, Edit, View, Options, Help, and several calculation buttons. A red arrow labeled '2' points to the 'Formula Calc' button (represented by a 'CH' icon) and the 'Isotope Calc' button (represented by a 'Tt' icon). A red arrow labeled '3' points to the 'Low Confidence Mechanism' button (represented by a question mark icon) and the 'Unknown Mechanism' button (represented by a question mark icon with a mouse cursor). Below the toolbar, the 'Formula Calculator' window is open, showing the following data:

1 Ion	O+E	RDB	Mass	mDa	C	H	Cl	I	N	O
C ₁₄ H ₁₀ Cl ₂ INO	Odd	10	404.91787	-82	14	10	2	1	1	1

The 'Parent' mass is listed as 404.9179. The 'Loss' is 0. The 'm/z' of the ion is 406.

MS Interpreter for Correlating Structure to Spectrum: *Displaying the Proposed Structures*

- 1) **LMB** on the ion of interest and the
- 2) Structure in **highlighted in red** in top window



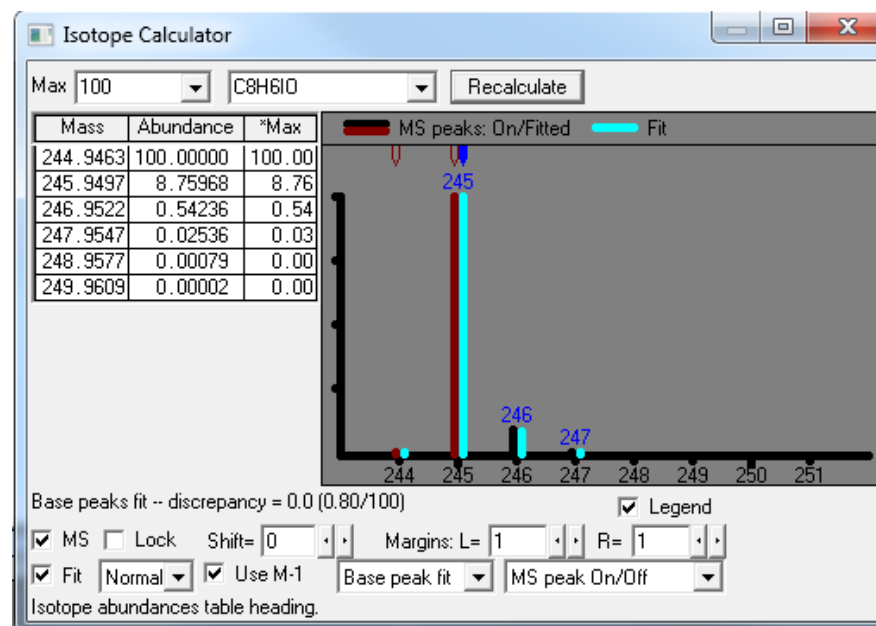
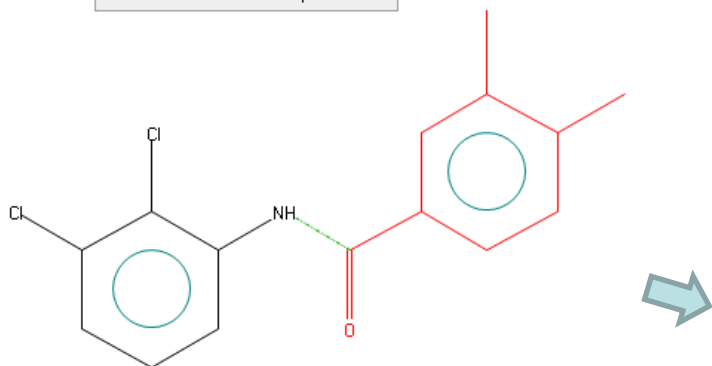
MS Interpreter for Correlating Structure to Spectrum:

Checking the Isotope Ratios for a Proposed Fragment

- 1) **RMB** on the formula for the ion, then **LMB** on **Send "x" to Isotope Calc**
- 2) Theoretical isotope ratio **compared** to observed

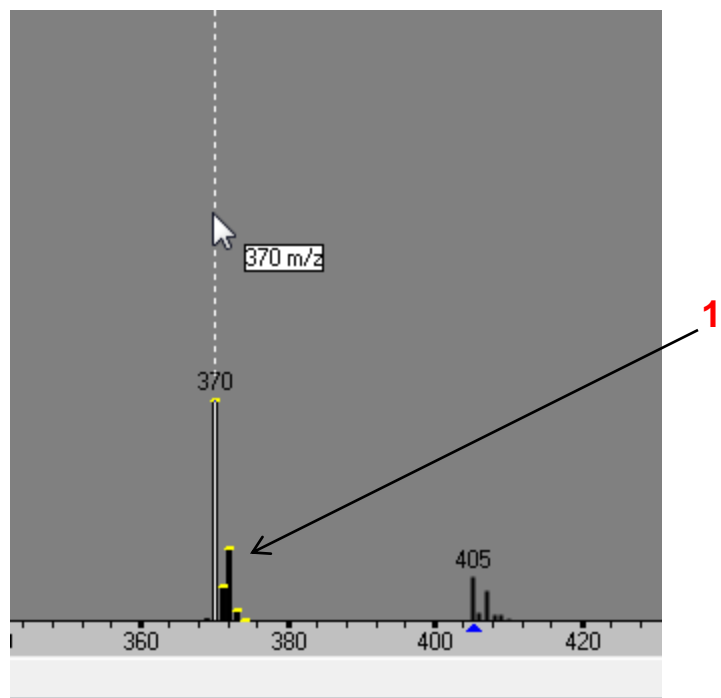
m/z	exact m/z	formula	loss	type	rate	rel.rate	ion.rate	abund	O+E
245	244.945789	C8H6O	C6H4Cl2N	Dissociation	90	100	76	999	Even

Send C8H6O to Isotope Calc



MS Interpreter for Correlating Structure to Spectrum: *Checking the Isotope Ratios for a Proposed Fragment*

- 1) **Theoretical** isotope ratio for selected also shown in spectrum with **horizontal yellow lines**
- 2) Many ions can have **multiple structures** or **mechanisms**, just **continue LMB** on the ions to **cycle display**



The software interface shows a dropdown menu with 'mz=370 (1/2)'. Below it is a table with the following data:

m/z	exact m/z	formula	loss	type	rate	rel.rate	ion.rate	ab
370 (1/2)	369.949017	C ₁₄ H ₁₀ ClNO	Cl	Dissociation	85	95	90	3

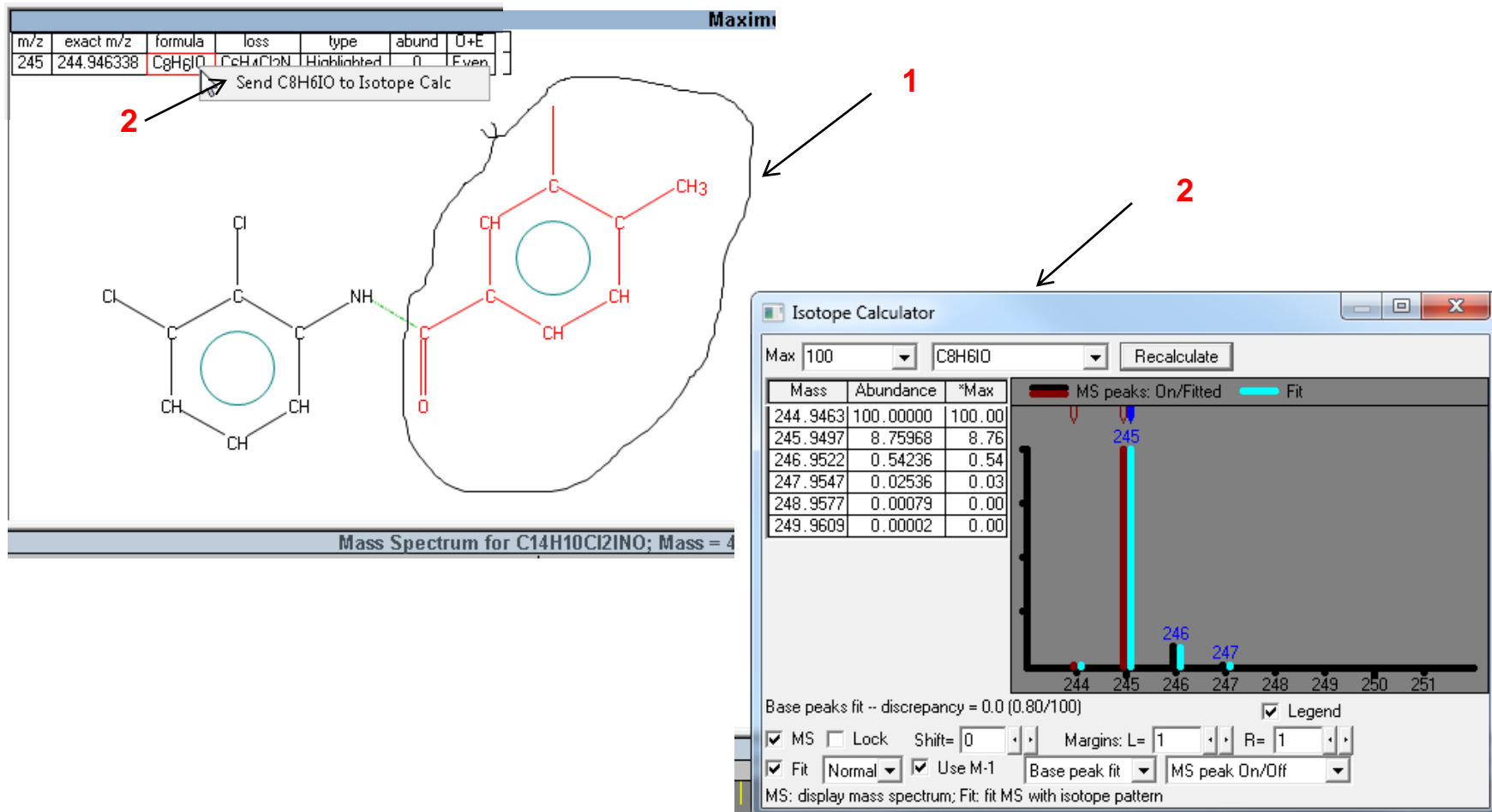
Below the table is a 'cycle display' showing four chemical structures in red: a chlorine atom (Cl), a carbon atom bonded to a chlorine atom (C-Cl), an amino group (NH), and a benzene ring with a chlorine atom attached to one of the carbons.

A red '2' with an arrow points to the dropdown menu.

MS Interpreter for Correlating Structure to Spectrum:

Other Miscellaneous Tips: Lassoing Ions

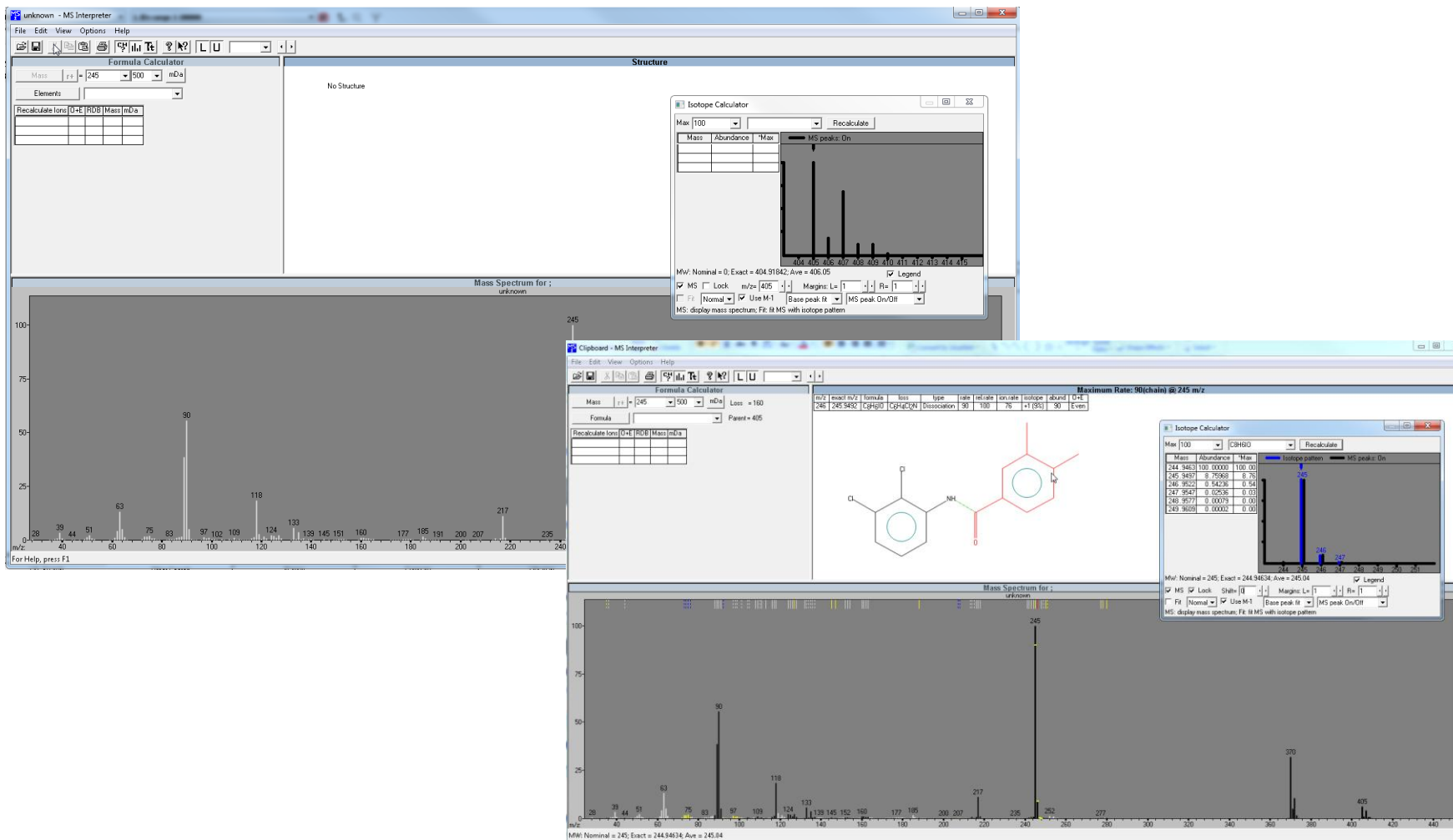
- 1) **Lasso** your own substructure by **LMB** and **circling**, shows type as Highlighted and calculates **m/z** and **formula**
- 2) **RMB** on formula, e.g. C₈H₅IO, and **LMB** and **Send "x" to Isotope Calc window**



MS Interpreter for Correlating Structure to Spectrum:

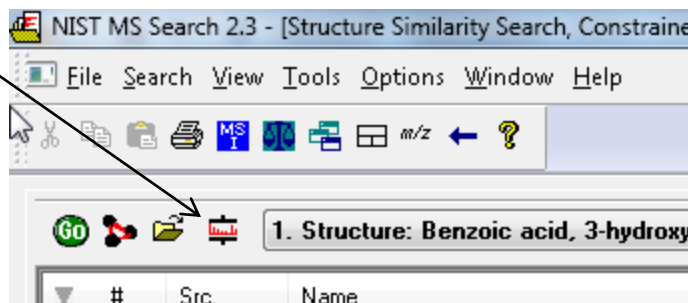
Other Miscellaneous Tips: Direct Structure Paste

- 1) **Copy structure** into clipboard **from drawing program**, then **directly paste** into MS interpreter with control V on keyboard or **pull-down menu/paste**

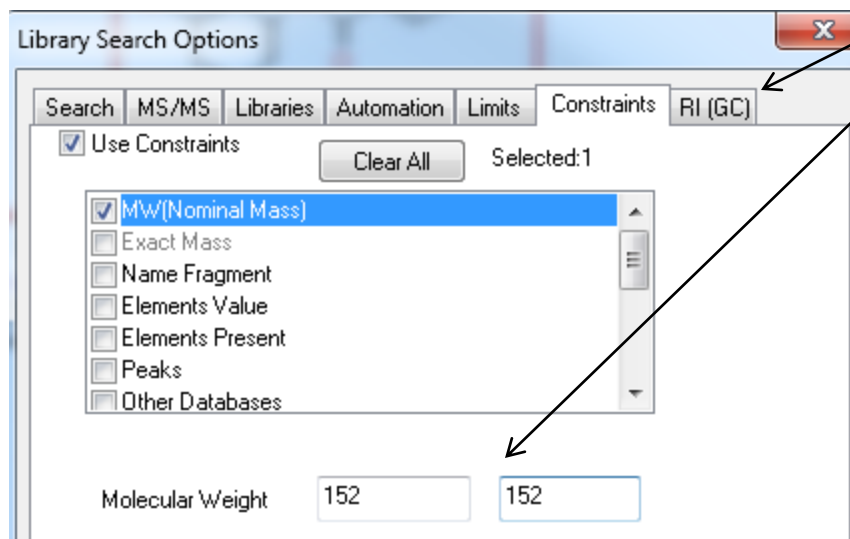


Do the 3 Isomers of Methyl Hydroxybenzoate Fragment Differently? Let's Compare Them!

- Draw one of the isomers, methyl 4-hydroxybenzoate in ChemDraw
- Import the structure into the NIST Spec List
- Lets **limit** the search to minimize the number to view by “constraining” the MW
- First open the “library search options menu”



-**Constrain** the results to be displayed after the search by **MW(Nominal Mass)** of 152 to 152



Do the 3 Isomers of Methyl Hydroxybenzoate Fragment Differently? Let's Compare Them! (continued)

-Select the structure search icon to perform the similar structure search constrained by MW 152

The screenshot shows the NIST MS Search 2.3 interface. The title bar reads "NIST MS Search 2.3 - [Structure Similarity Search, Constrained - 21 spectra]". The menu bar includes "File", "Search", "View", "Tools", "Options", "Window", and "Help". The toolbar contains various icons, including a structure search icon (a red circle with a white 'S') which is highlighted by an arrow from the text above. The search input field contains "1. Structure: Benzoic acid, 3-hydroxy-, r". Below the search bar is a table with columns "#", "Src.", and "Name". The first row is highlighted in blue and contains "1", "E", and "Clipboard #7". Below the table is a bar chart showing the number of structures found in the main library (267073 total structures) for different mass ranges. The x-axis is labeled "m/z" and has markers at 1000, 900, and 800. The y-axis is labeled "Number of Structures" and has markers at 1 and 10. There are four bars: a red bar at m/z 1000, and three blue bars at approximately m/z 880, 860, and 840. To the right of the bar chart is a chemical structure of methyl 3-hydroxybenzoate, which is a benzene ring with a methyl ester group (-COOCH3) at the top and a hydroxyl group (-OH) at the meta position (3-position). Below the structure is the text "(Spec. Edit) Clipboard #7". At the bottom of the interface are tabs for "Plot/Text of Search Spectrum", "Plot of Search Spectrum", and "Spec Li".

Do the 3 Isomers of Methyl Hydroxybenzoate Fragment Differently? Let's Compare Them! (continued)

-Many hits are found

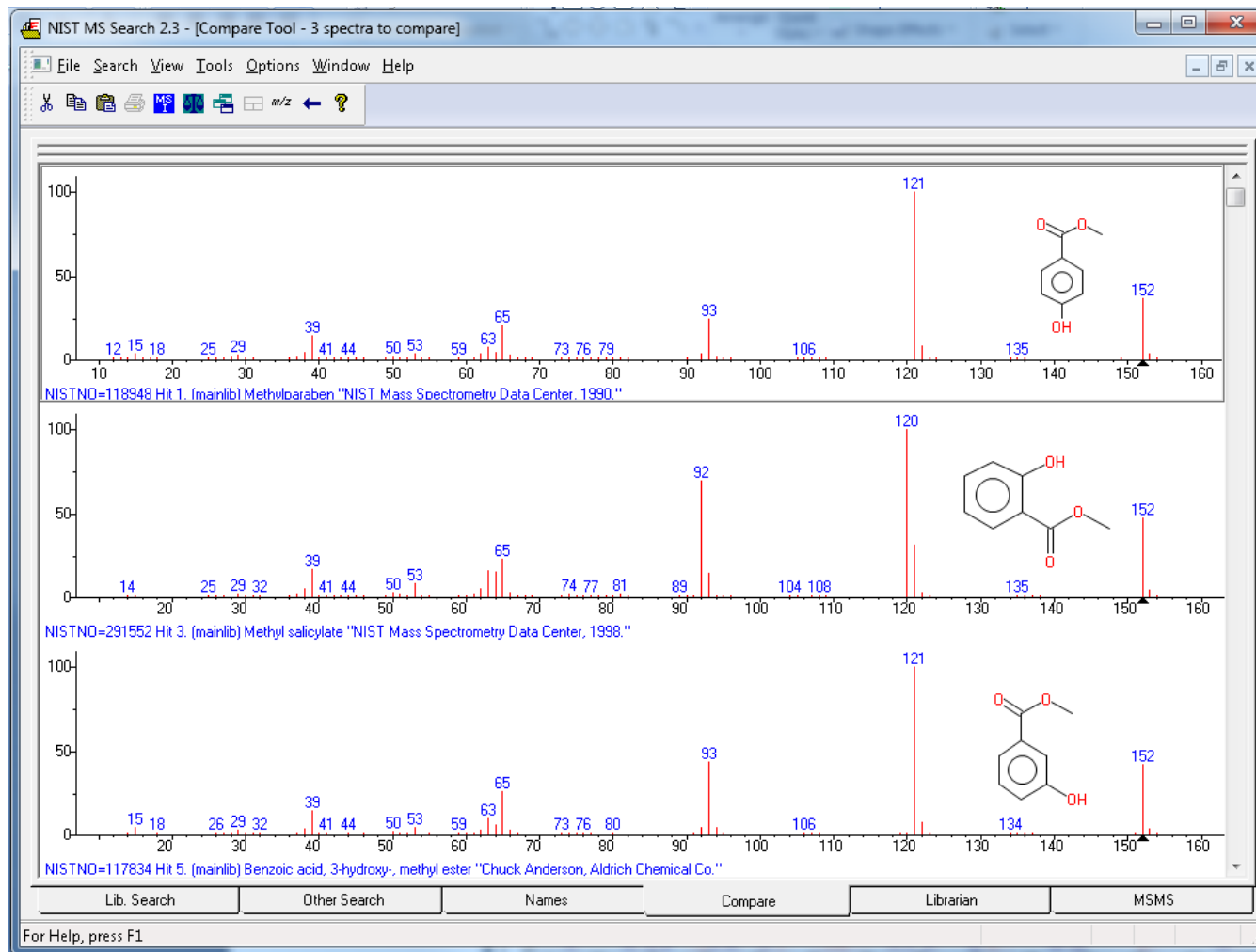
-Use **control key/LMB** to select entries 1,3, and 5 in hit list using **LMB**

#	Lib.	Name	DotProd	Distance	RI	DeltaMass	Syn	DBs
1	M	Methylparaben	1000	1000	1419	0	40	8
2	M	Resorcinol monoacetate	999	999	1383	0	19	1
3	M	Methyl salicylate	999	999	1174	0	31	1
4	M	Hydroquinone, acetate	999	999	1383	0	0	0
5	M	Benzoic acid, 3-hydroxy-, methyl ester	999	999	1389	0	11	1
6	M	1,3-Benzenediol, 4-propyl-	924	870	-	0	2	1
7	M	Phenol, 4-propoxy-	906	969	-	0	5	1
8	M	Phenol, 4-(ethoxymethyl)-	906	969	1379	0	1	1
9	M	4-(2-Methoxyethyl)phenol	906	969	-	0	4	1
10	M	Phenol, 2-propoxy-	906	969	-	0	1	1
11	M	Phenol, 4-ethyl-2-methoxy-	901	967	1252	0	1	1
12	M	2-Ethoxy-4-methylphenol	901	967	-	0	1	1
13	M	1,4-Benzenediol, 2,3,5-trimethyl-	895	830	-	0	9	1
14	M	2-Isopropoxyphenol	878	957	1164	0	3	1
15	M	1-(2,3-Dihydroxyphenyl)ethanone	866	804	-	0	1	1
16	M	3',5'-Dihydroxyacetophenone	866	804	-	0	3	1
17	M	Ethanone, 1-(2,5-dihydroxyphenyl)-	866	804	-	0	10	1
18	M	3,4-Dihydroxyacetophenone	866	804	-	0	3	1
19	M	Resorcinol, 2-acetyl-	866	804	-	0	10	1

- Library Search
- Structure Similarity Search
- Copy
- Select All
- Close All Replicates
- Export Selected
- Send To
- Copy Structure to Clipboard
- Link Compound ID
- Print
- Print Preview
- Properties

Do the 3 Isomers of Methyl Hydroxybenzoate Fragment Differently? Let's Compare Them! (continued)

- Open the "Compare" window
- Then paste the three hits in the windows clipboard into the "Compare" window
- The ortho-isomer loses 32 for methanol which is absent in the para- and meta-isomers
- Often noted in o-isomers, "ortho-effect"



Any Structure and Associated Spectrum in Program Sent to MS Interpreter

1

#	Lib	DotPr...	Distance	DeltaMass	Name
7..	w1	999	999	0	Benzoic acid, 2-hydrox
7..	w1	999	999	0	Benzoic acid, 3-hydrox
7..	w1	999	999	0	Benzoic acid, 3-hydrox
7..	w1	999	999	0	Benzoic acid, 3-hydrox
7..	w1	999	999	0	Benzoic acid, 3-hydrox
7..	w1	999	999	0	1,3-Benzenediol, monc
7..	w1	999	999	0	1,3-Benzenediol, monc
7..	w1	999	999	0	1,3-Benzenediol, monc
7..	w1	999	999	0	1,4-Benzenediol, monc
7..	w1	1000	1000	0	4-Hydroxy-benzoic aci
8..	M	1000	1000	0	Methylparaben
8..	M	999	999	0	Benzoic acid, 3-hydrox
8..	M	999	999	0	Resorcinol monoacete
8..	M	999	999	0	Methyl salicylate
8..	M	999	999	0	Hydroquinone, acetate

1. Either **LMB** select then **RMB** Send To/MS Interpreter
2. Or select **F9** on keyboard



2

Formula Calculator

Formula	Parent	Mass	mDa	C	H	O
C13H10O3	Parent	222	152.0468	13	10	3
C12H10O3		214	144.0620	12	10	3
C11H10O3		206	136.0772	11	10	3
C10H10O3		198	128.0924	10	10	3
C9H10O3		190	120.1076	9	10	3
C8H10O3		182	112.1228	8	10	3
C7H10O3		174	104.1380	7	10	3
C6H10O3		166	96.1532	6	10	3
C5H10O3		158	88.1684	5	10	3
C4H10O3		150	80.1836	4	10	3
C3H10O3		142	72.1988	3	10	3
C2H10O3		134	64.2140	2	10	3
C1H10O3		126	56.2292	1	10	3

Mass Spectrum for C13H10O3; Mass = 152; CAS = 119-36-8; Methyl salicylate

Accurate Mass Spectrum Correlated to Structure in MS Interpreter: Accurate Mass EI Mass Spectral Data from Orbitrap

Calculates molecular formula showing *either* ppm *or* mDa errors limited by MF of drawn structure

Formula Calculator

Mass r_{\pm} = 158.09949 40 ppm Loss = 87.0447

Formula C₁₁H₂₃N₀Si₁ Parent = 245.1442

3 Ions	O+E	RDB	Mass	Ppm	C	H	N	O	Si
C ₇ H ₁₆ N ₀ Si	Even	1.5	158.09957	0.5	7	16	1	1	1
C ₈ H ₁₄ O ₃	Odd	2	158.09375	-36	8	14	0	3	0
C ₁₁ H ₁₂ N	Even	6.5	158.09643	-19	11	12	1	0	0

or

Formula Calculator

Mass r_{\pm} = 158.09949 6.3 mDa Loss = 87.0447

Formula C₁₁H₂₃N₀Si₁ Parent = 245.1442

3 Ions	O+E	RDB	Mass	mDa	C	H	N	O	Si
C ₇ H ₁₆ N ₀ Si	Even	1.5	158.09957	0.1	7	16	1	1	1
C ₈ H ₁₄ O ₃	Odd	2	158.09375	-5.7	8	14	0	3	0
C ₁₁ H ₁₂ N	Even	6.5	158.09643	-3.1	11	12	1	0	0

N-Acetyl-DL-Leucine, ITMS - MS Interpreter

File Edit View Options Help

Formula Calculator

Mass r_{\pm} = 158.09949 40 ppm Loss = 87.0447

Formula C₁₁H₂₃N₀Si₁ Parent = 245.1442

3 Ions	O+E	RDB	Mass	Ppm	C	H	N	O	Si
C ₇ H ₁₆ N ₀ Si	Even	1.5	158.09957	0.5	7	16	1	1	1
C ₈ H ₁₄ O ₃	Odd	2	158.09375	-36	8	14	0	3	0
C ₁₁ H ₁₂ N	Even	6.5	158.09643	-19	11	12	1	0	0

Maximum Rate: 88(chain) @ 230.1207 m/z

CO₂

Isotope Calculator

Mass	Abundance	Max
158.1001	100.0000	100.00
159.1019	13.23653	13.24
160.0988	4.26397	4.26
161.1011	0.3140	0.32
162.1030	0.01847	0.02
163.1052	0.00081	0.00
164.1078	0.00002	0.00

Base peaks fit - discrepancy = 0.0 (0/100)

MS Lock Shift [72.00] Margins: L=1 R=1

Fit [Normal] Use M-1 Base peak fit MS peak On/Off

MS: display mass spectrum; Fit RM MS with isotope pattern

Mass Spectrum for C₁₁H₂₃N₀Si₁; Mass = 245.1442; N-Acetyl-DL-Leucine, ITMS

For Help, press F1

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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