Wiley Spectral Webinar
Part II: Structure Searches with NIST MS Search and Using MS Interprete

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- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
- *Now Consultant, MS Interpretation Services*
- *Specialties[1] 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,[21] etc.*

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

>50 Mass Specs Networked
Worldwide

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

Wiley Webinar Series on Effective Use of Mass Spectral Libraries

Part I: Spectral Searches[2] with NIST MS Search
Part II: Structure Searches[2] 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 Sharing[5] User EI and MS/MS Libraries

Note:[20] Handouts for ***All*** Sessions ***Now Online***!
Google Search "little mass spec and sailing"

Table of Contents

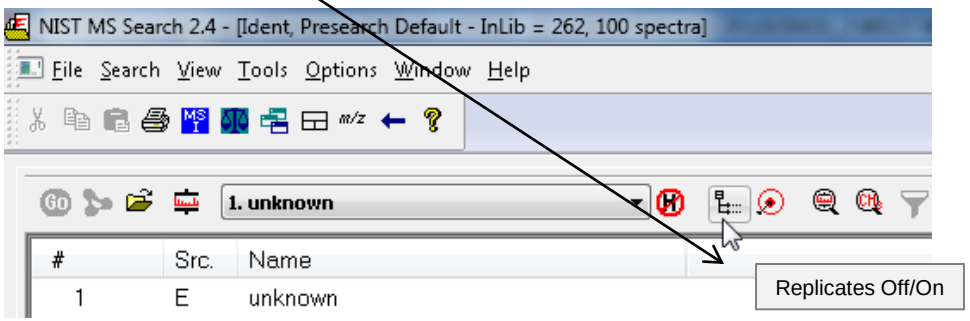
Part II: Structure Searches with NIST MS Search[2] and Using MS Interpreter[2,13-15]

Webinar Topics:

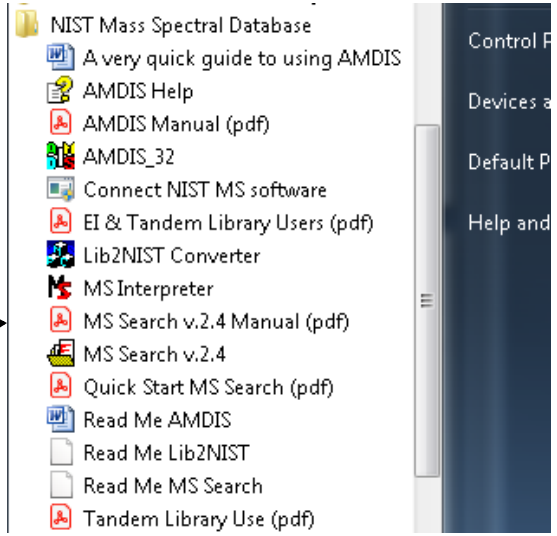
-Setting up ***Library Search Options*** for Structure Searches
-Importing structures from vendor programs[2]
-Searching for ***similar*** structures using ***Structure Search***
-Searching for ***exact*** structure using ***Library Search*** with InChIKey
-Using “Compare” window to view series of spectra
-MS Interpreter for correlating substructure and isotope ratios to ions in spectrum
-Finding model compounds
-Accurate mass spectra in MS Interpreter

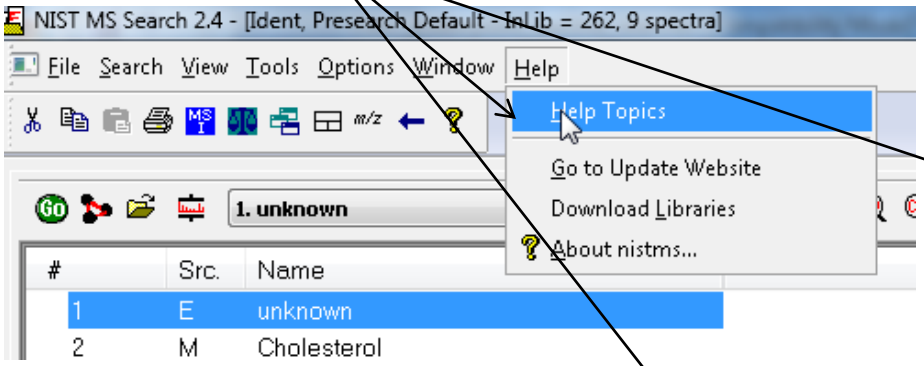
Help Files for NIST Search

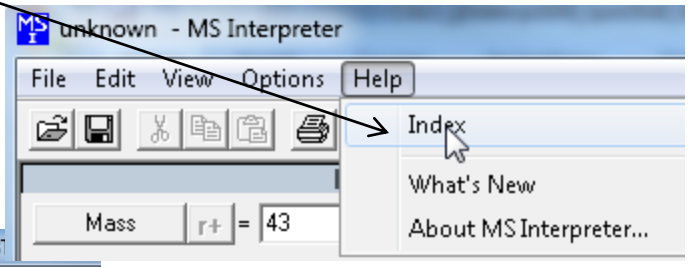
-"Hover" over Program Icon with mouse and function description displayed

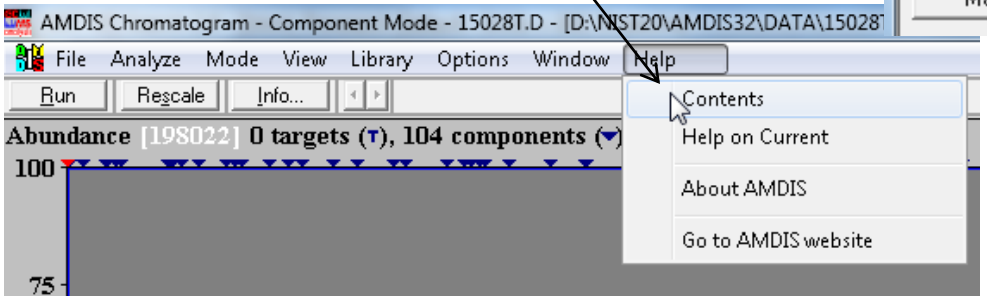


-Detailed documentation for NIST Search[2] and AMDIS[3,4,12]
-MS Interpreter included in NIST manual[2] and in posters[13-15]
-Windows Program Group
-"In program" assistance for all three programs





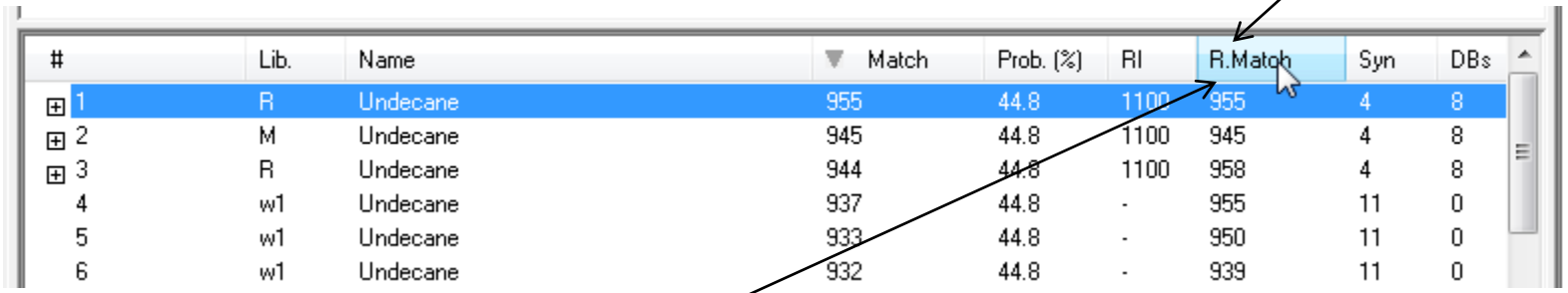




NIST Software in General is "Windows Compliant"

-***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

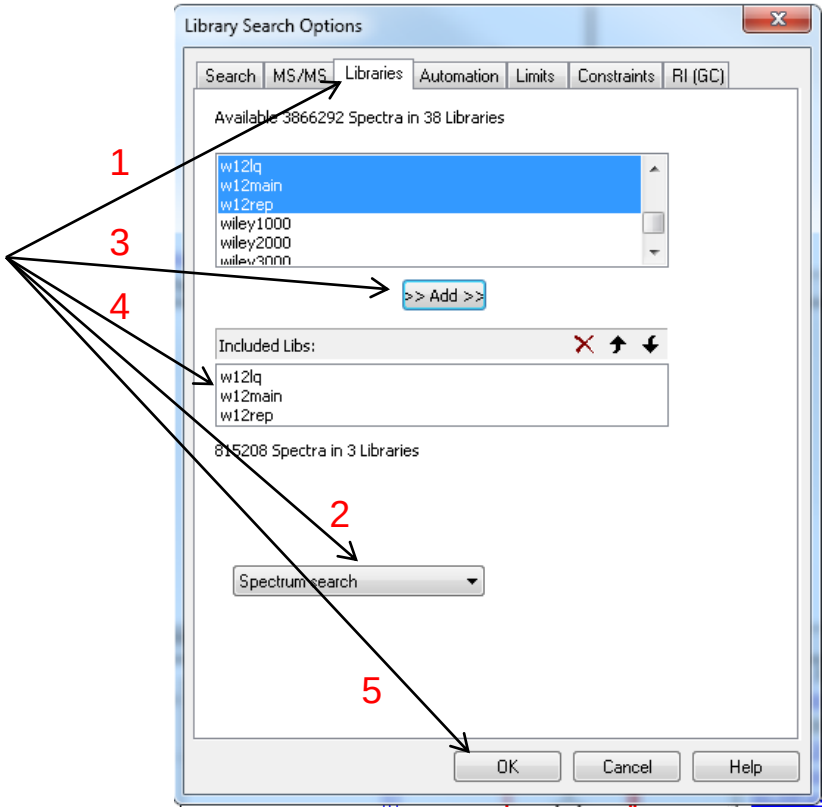
User Libraries: *e.g.* Eastman, >50K entries, automatically updated *nightly*[5]

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

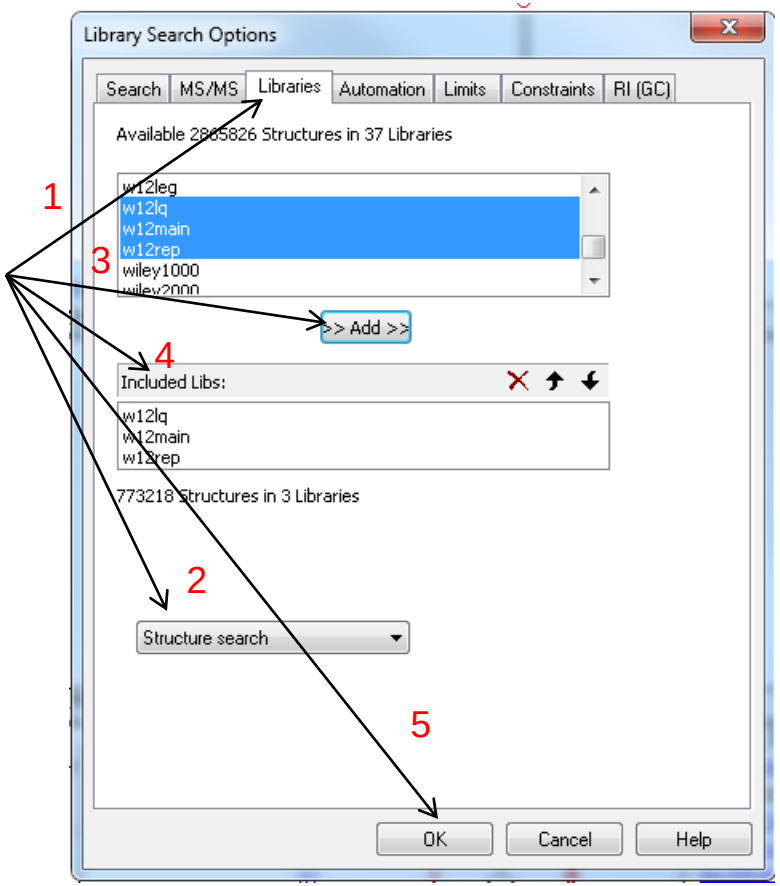
Critical Settings: Select the Libraries Used for *Exact Structure* Searches

- ***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



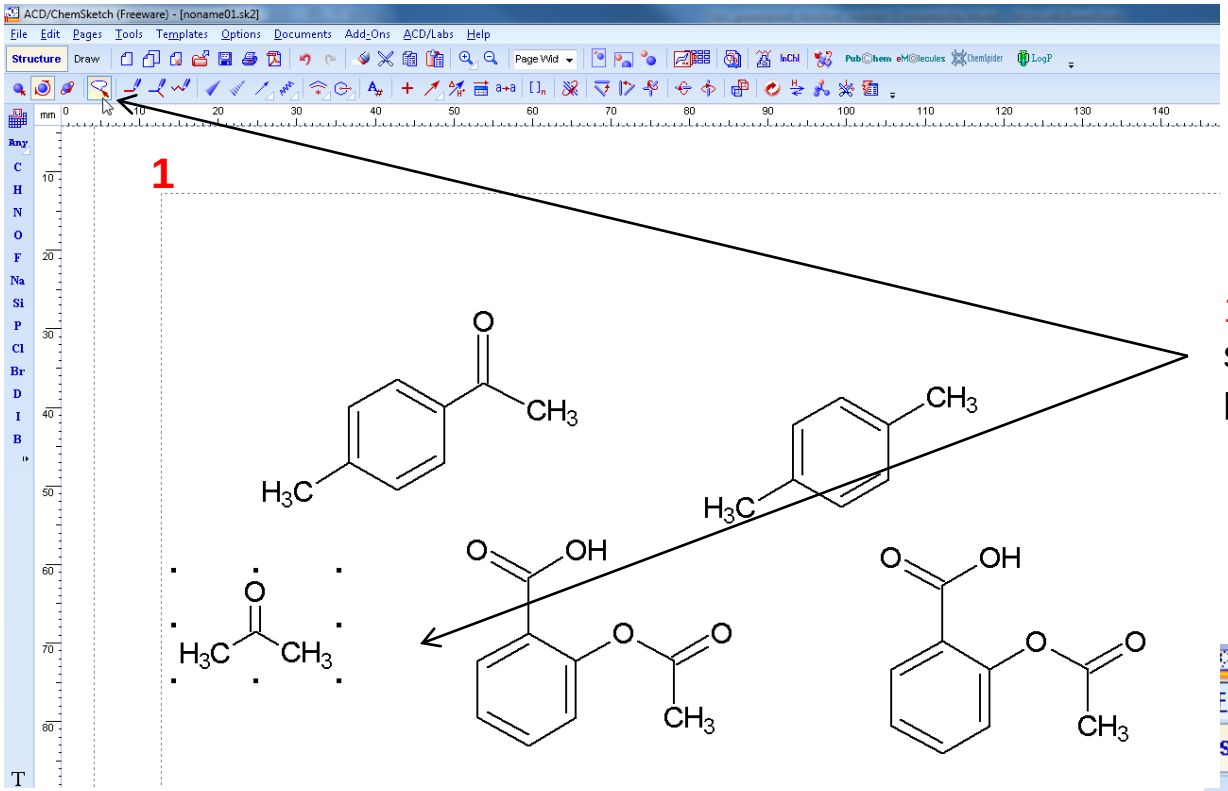
Critical Settings: Select the Libraries Used for *Similar Structure* Searches

- ***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



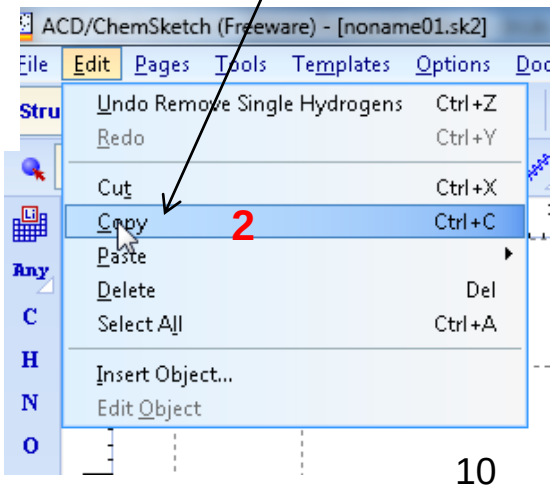
Structure Capabilities Within NIST Search Program

Obtaining Structure from ChemSketch



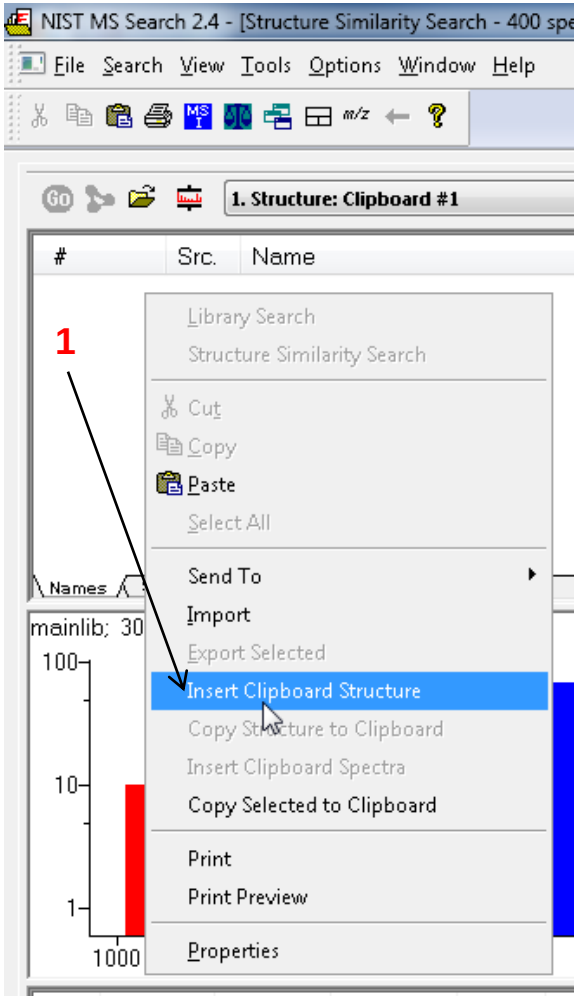
1) Use “lasso” tool to select structure to import into NIST program

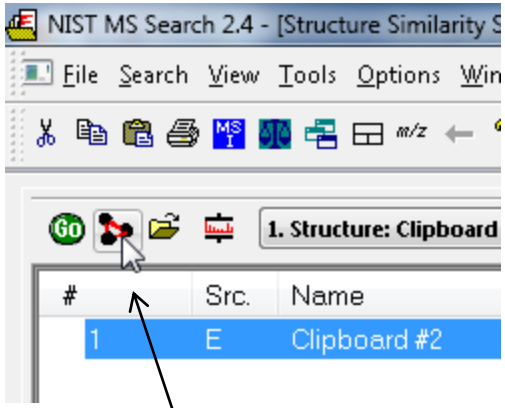
2) Then copy into clipboard

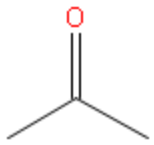


Search for Similar Structures after “Insert Clipboard Structure”

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)*







Name: Clipboard #2
Formula: C_3H_6O
MW: 58 Exact Mass: 58.0418649 ID#: 3 DB: Spec. Edit
InChIKey: CSCPPACGZOOCGX-UHFFFAOYSA-N Nc
Synonyms:
no synonyms.

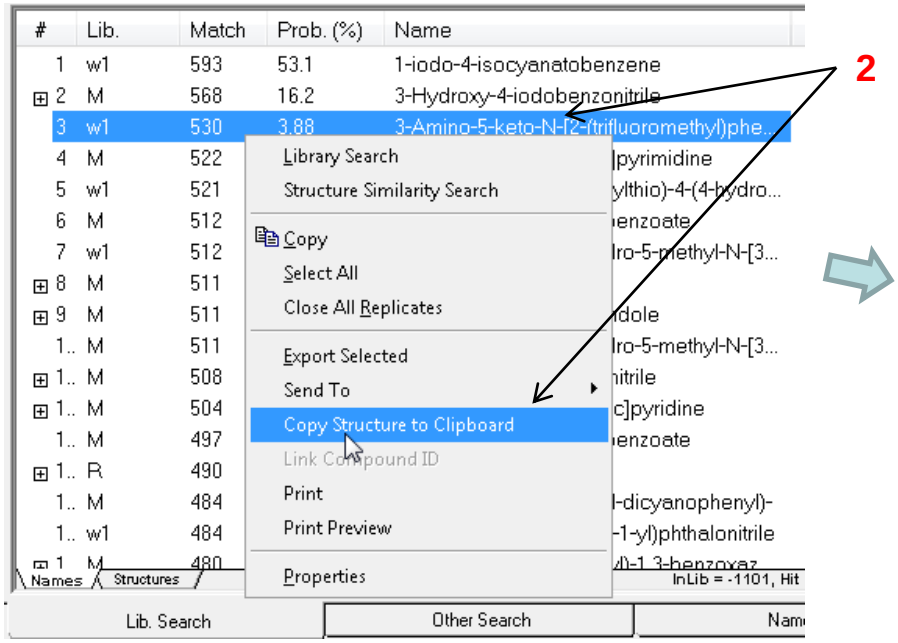
Estimated non-polar retention index (n-alkane scale):
Value: 455 iu
Confidence interval (Ketones): 57(50%) 246(95%) iu

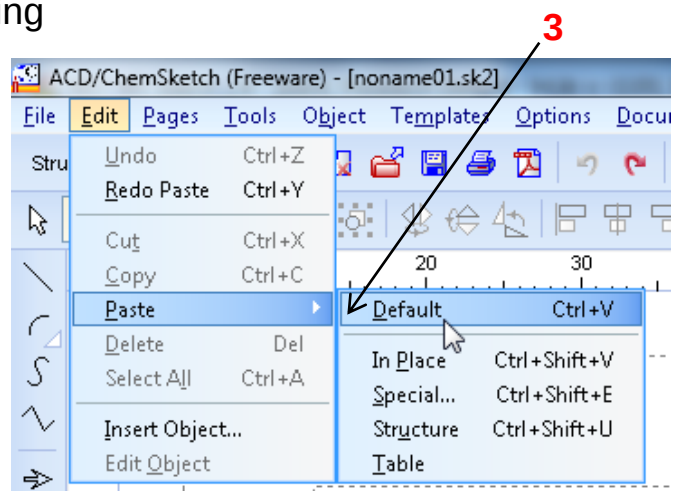
3

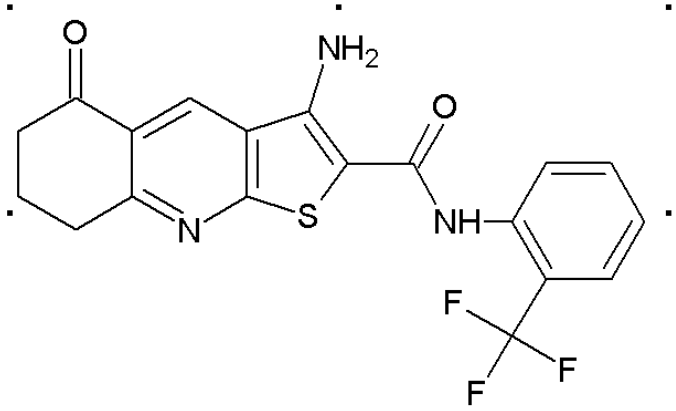
#	Lib.	DotProd	Distance	RI	Name
⊞ 1	M	1000	1000	267	Formaldehyde
⊞ 2	M	1000	1000	-	Carbon monoxide
⊞ 3	M	1000	1000	401	Acetaldehyde
⊞ 4	M	1000	1000	487	Acetone
⊞ 5	M	1000	1000	-	Acetone-D6

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

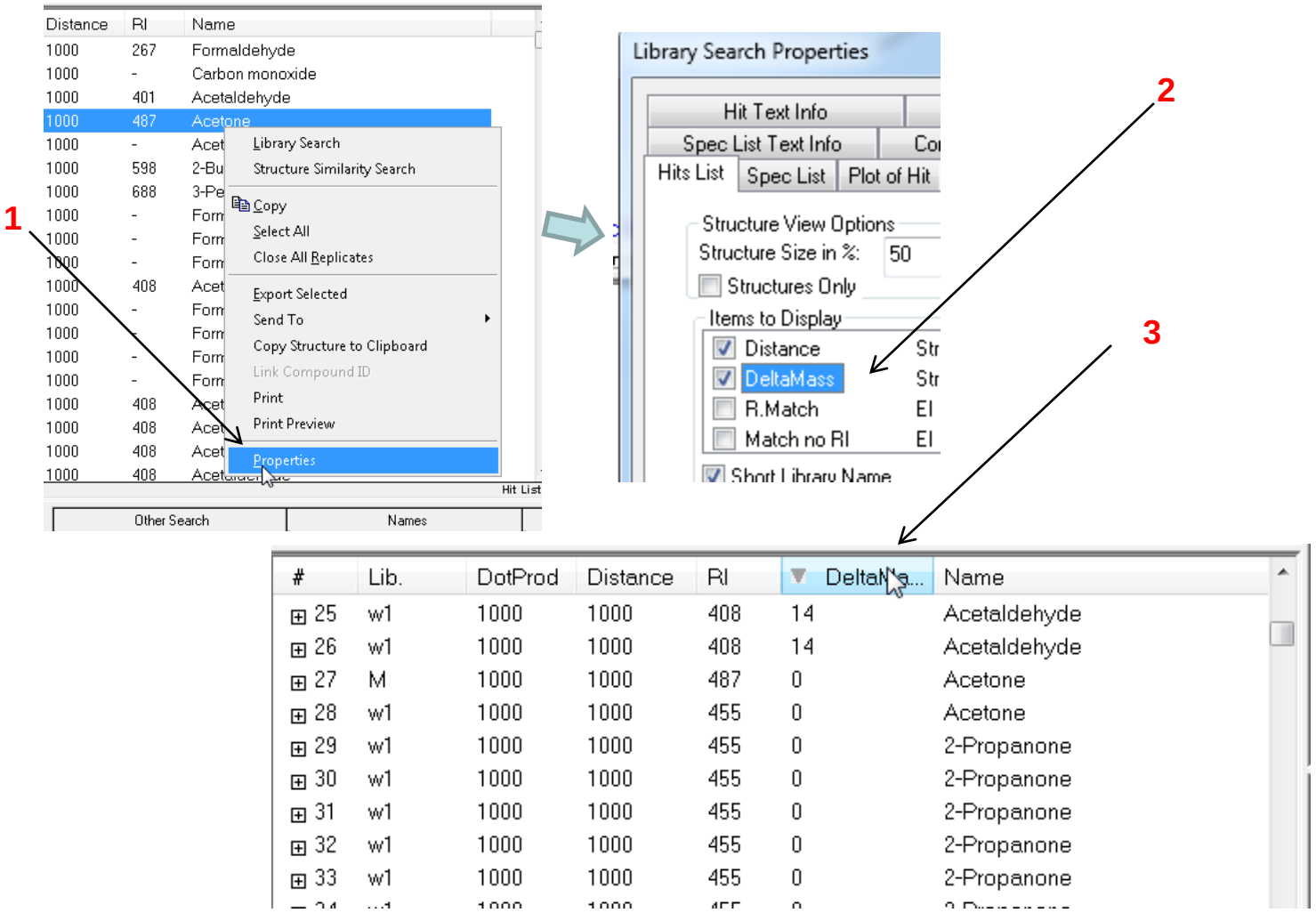




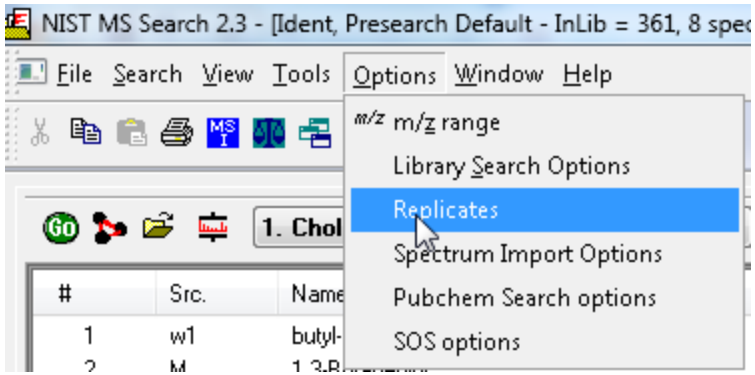


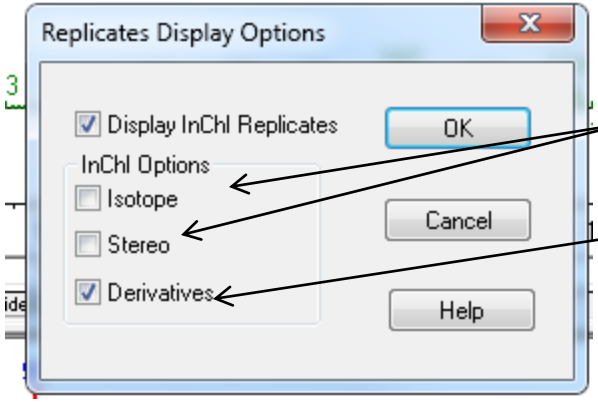
Tips for Displaying Structure Search Results

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



Tips for Displaying Related Structures in Structure Searches





Inconsistent!

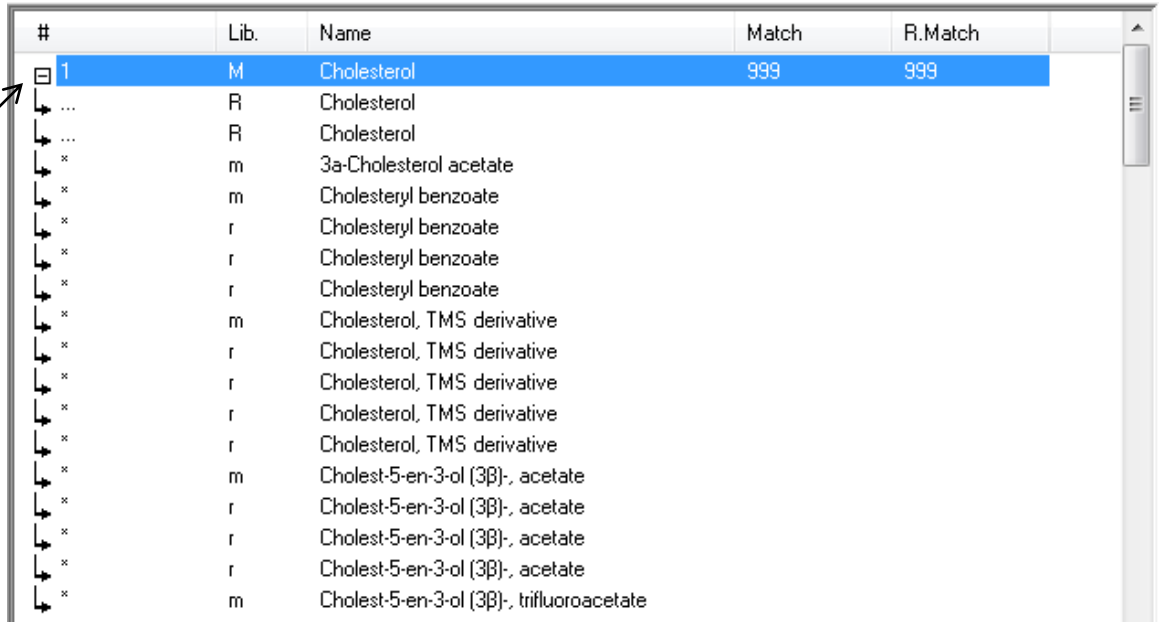
"Do see" ***unchecked***

"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

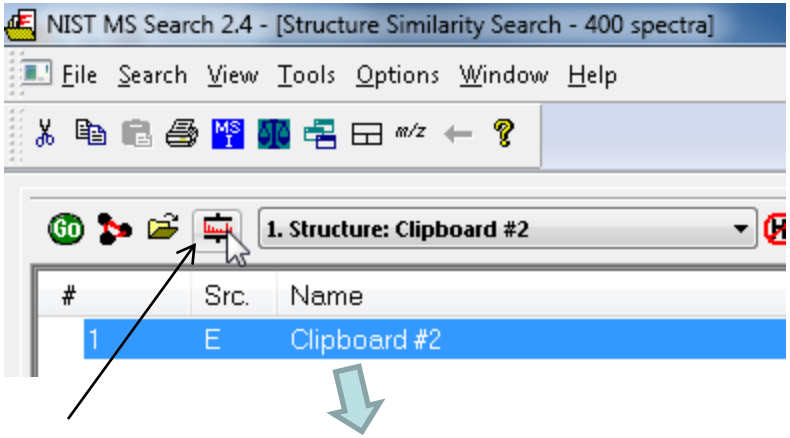
-***Also*** *affects Names Search Display*

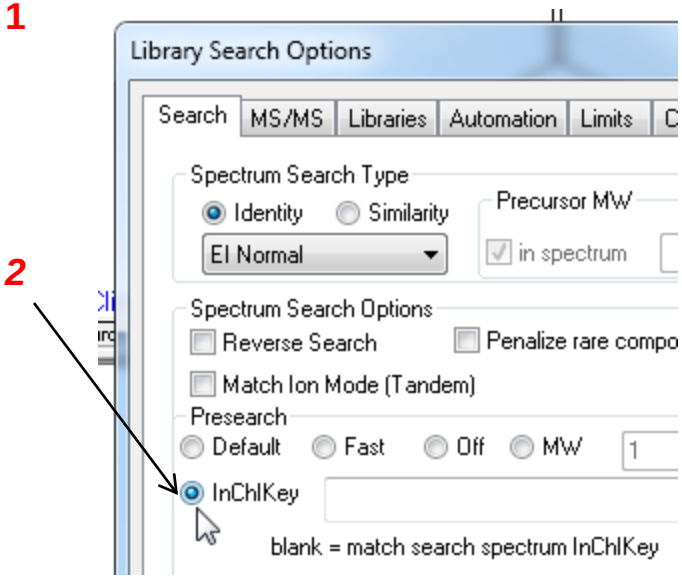


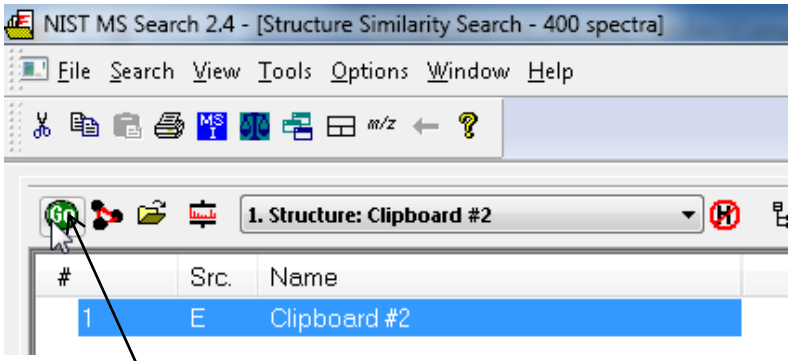
#	Lib.	Name	Match	R.Match
1	M	Cholesterol	999	999
...	R	Cholesterol		
...	R	Cholesterol		
*	m	3a-Cholesterol acetate		
*	m	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	r	Cholesteryl benzoate		
*	m	Cholesterol, TMS derivative		
*	r	Cholesterol, TMS derivative		
*	r	Cholesterol,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

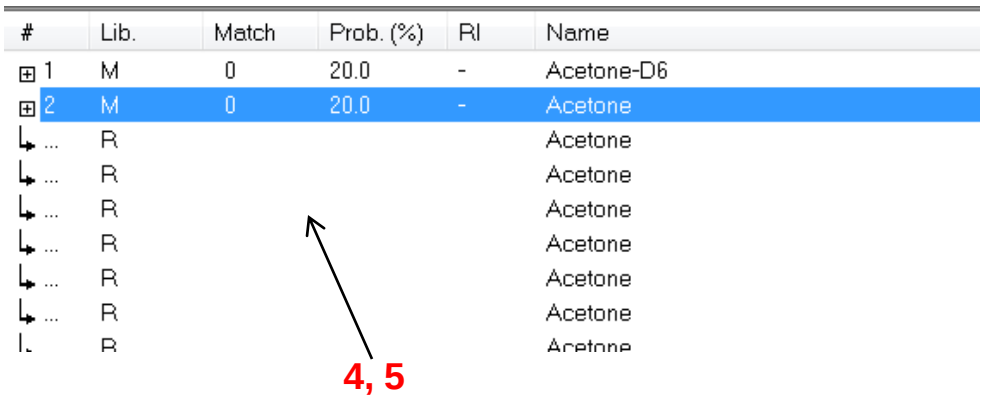
Search for Exact Structures

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







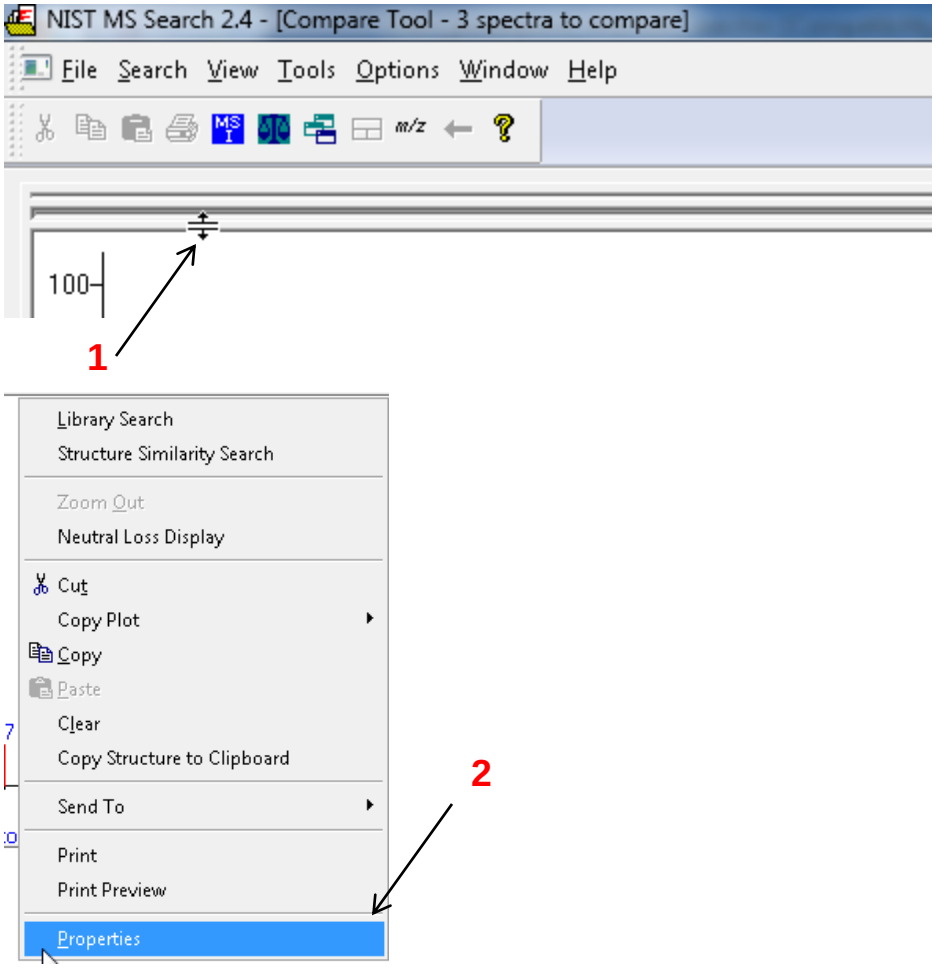


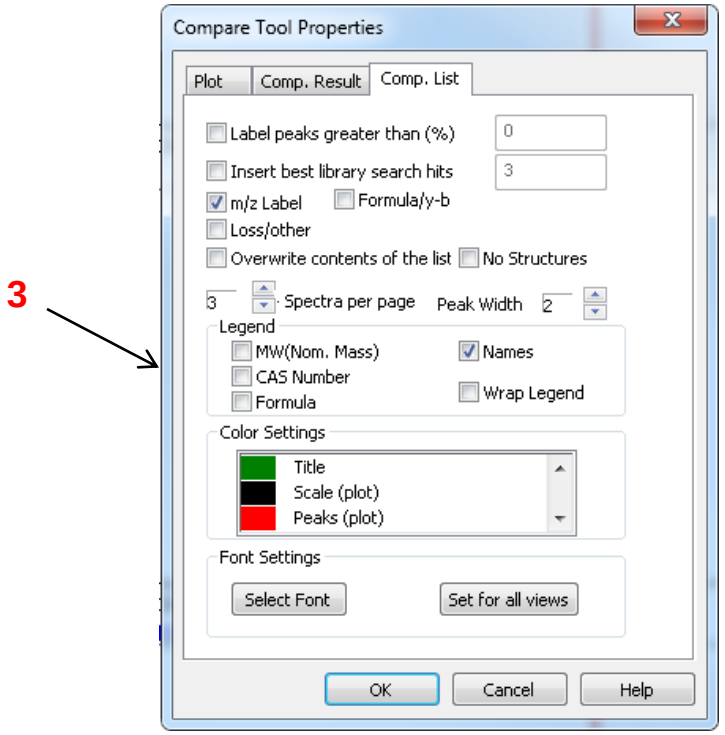
Using *"Compare"* Window to Display Multiple Spectra

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

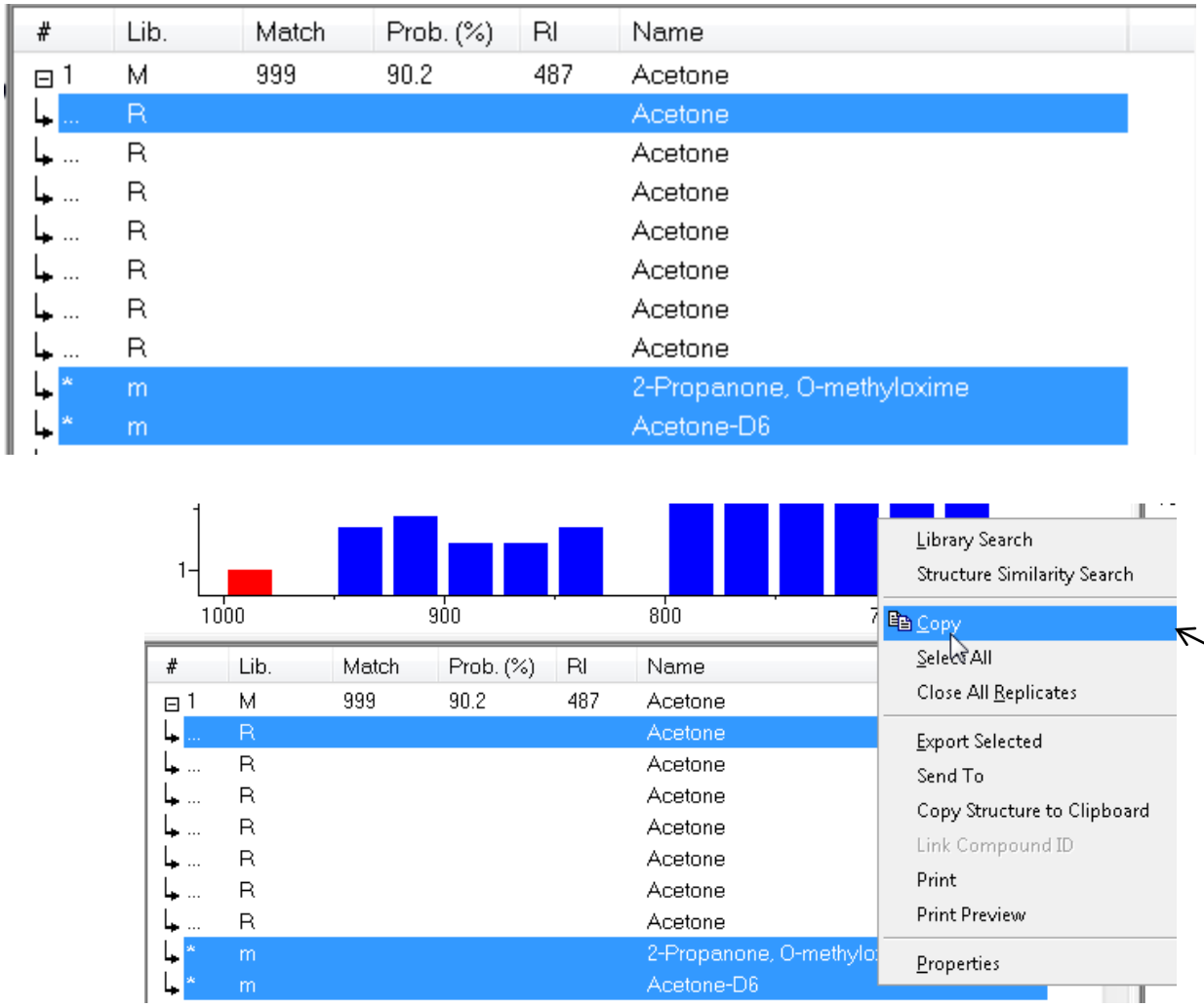
1) ***Compress top two*** windows by **selecting bar** with left mouse click and ***scrolling closed*** with mouse
2) Select prosperities window in bottom windows and then select ***Properties*** with ***LMB***
3) Setup options as shown below





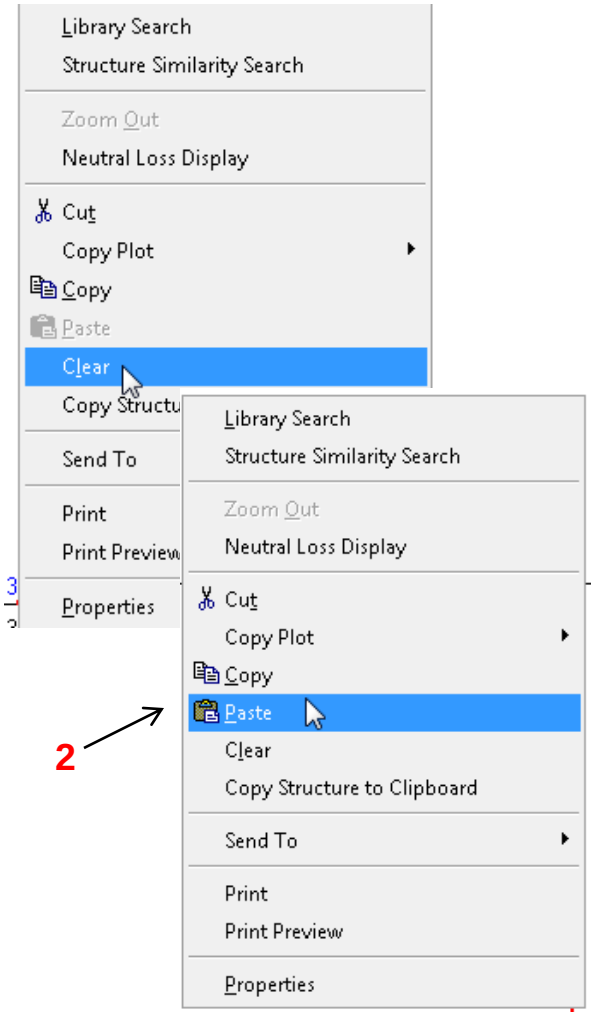
Using *"Compare"* Window to Display Multiple Spectra

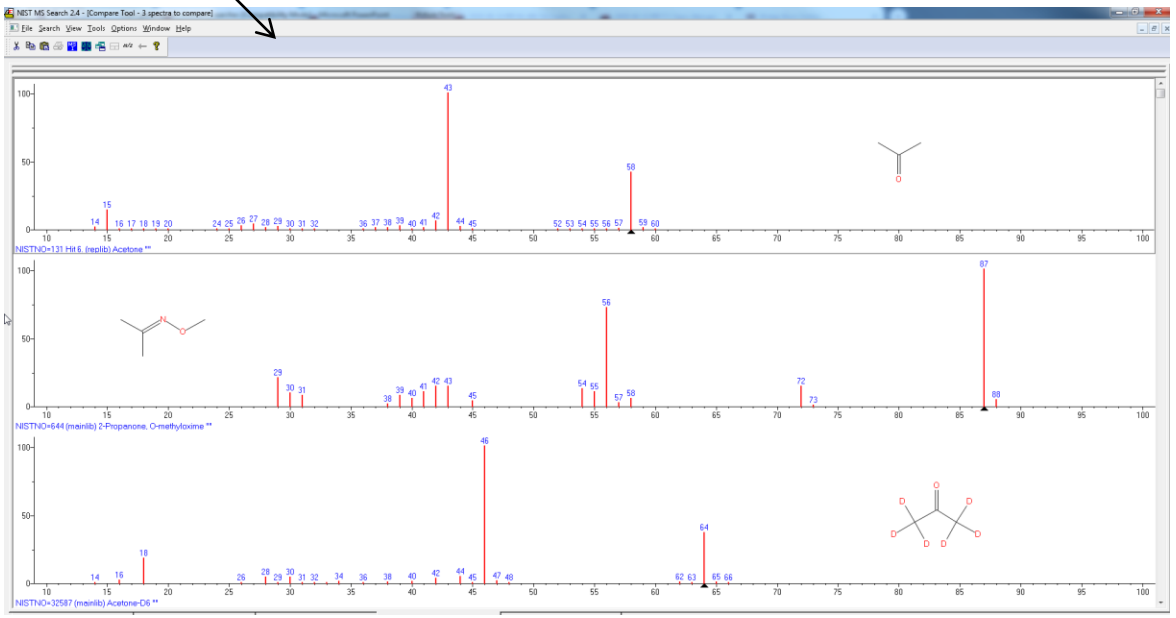
1) ***Select*** three spectra, *e.g.*, from your exact search of acetone using control ***left mouse click*** while ***holding control key*** on keyboard
2) ***RMB*** somewhere within ***blue highlighted area*** and select ***copy with left mouse*** button



Using *"Compare"* Window to Display Multiple Spectra

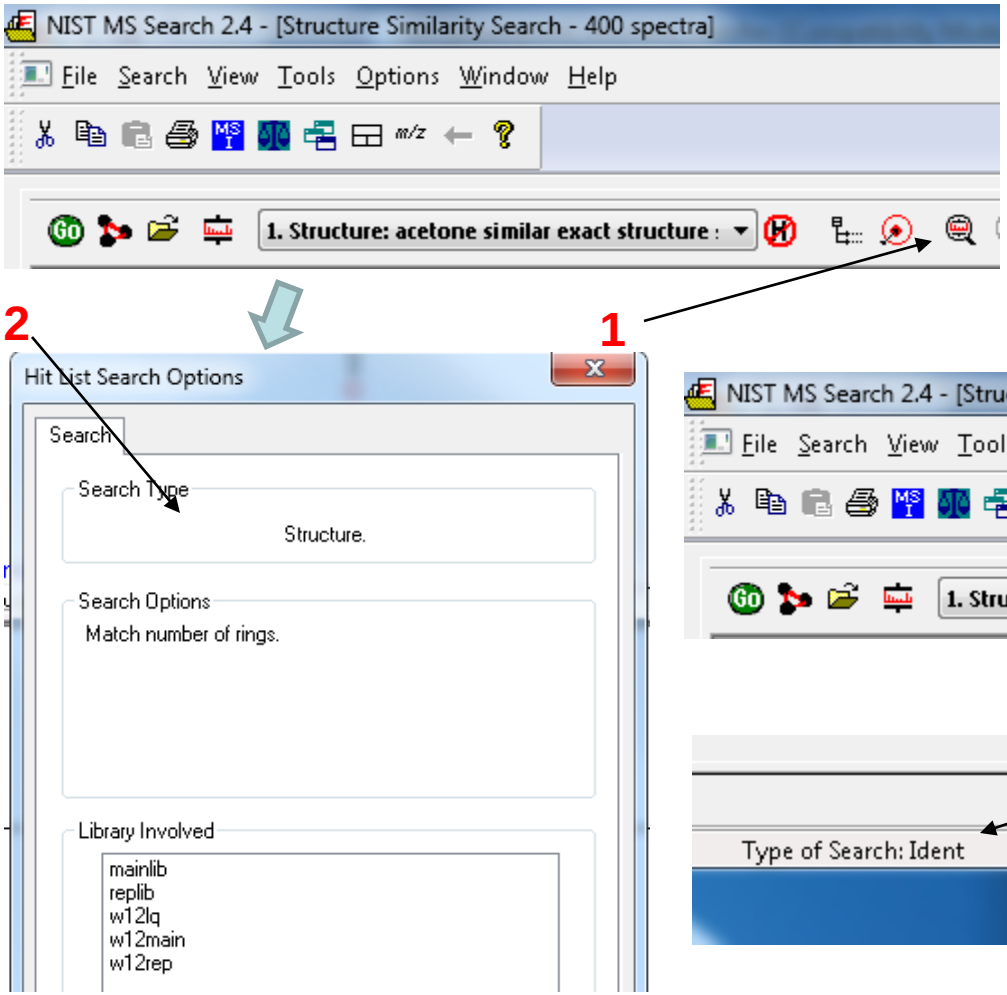
1) ***Go to Compare window*** by selecting ***tab at bottom*** of Search Display Window
2) ***RMB*** in window and then ***LMB*** on ***Clear*** to delete spectra present
3) ***RMB spectra*** of acetone related species ***should appear*** in window for comparison





View of Hit List Structure Search Options *Utilized*

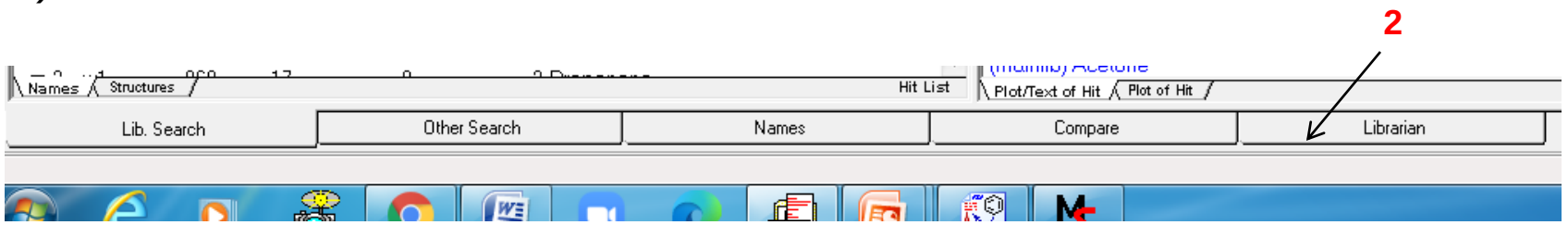
1. ***LMB*** the “View Hit List Search Options”
2. See details of the last search performed
3. Also, an abbreviated description of the last search noted at ***top*** of NIST search window
4. Furthermore, at the ***bottom*** of the screen, *respectively* displayed, are the types of searches that will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

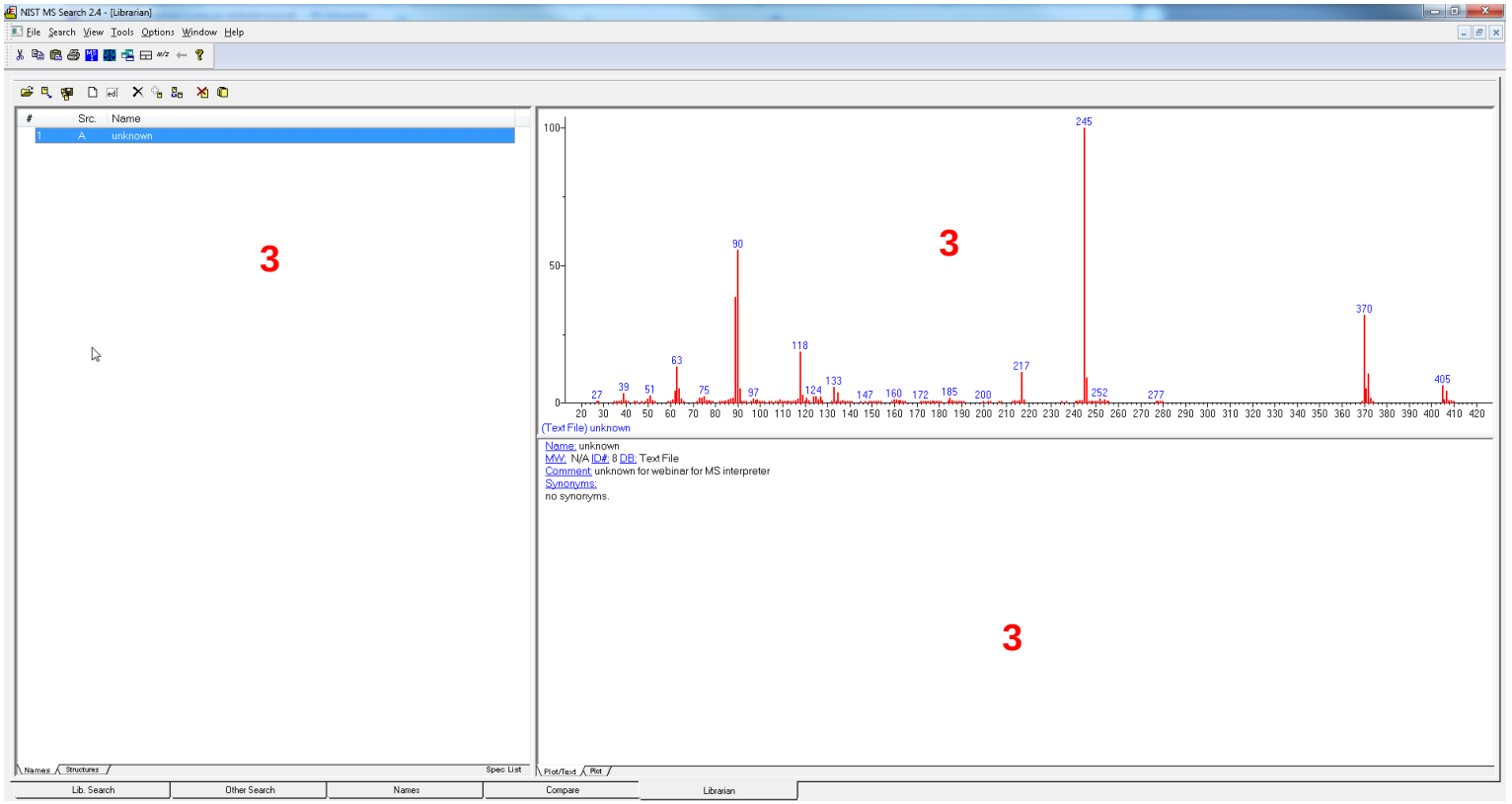


MS Interpreter for Correlating Structure to Spectrum:

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



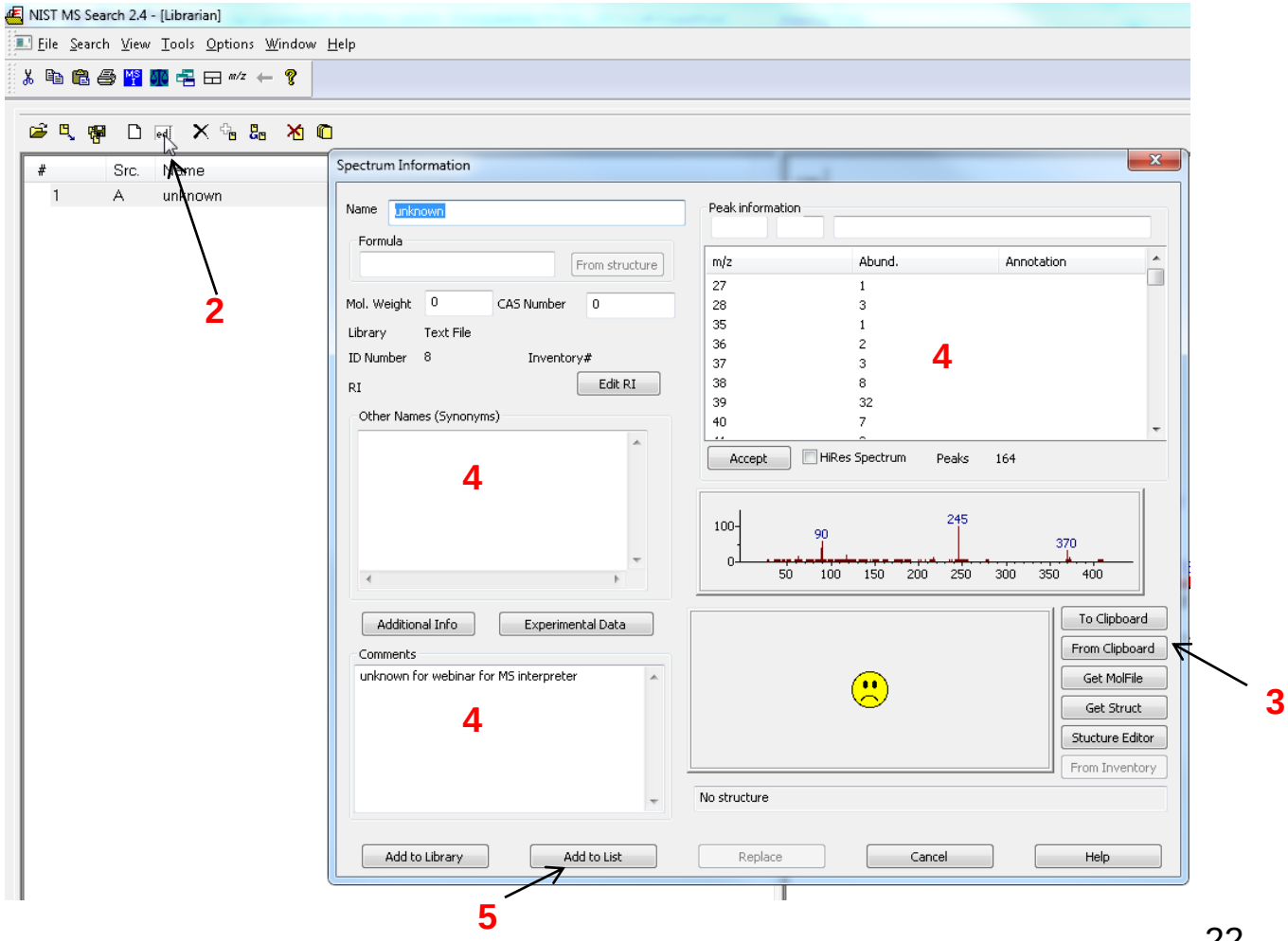


MS Interpreter for Correlating Structure to Spectrum:

Adding a Structure to a Spectrum

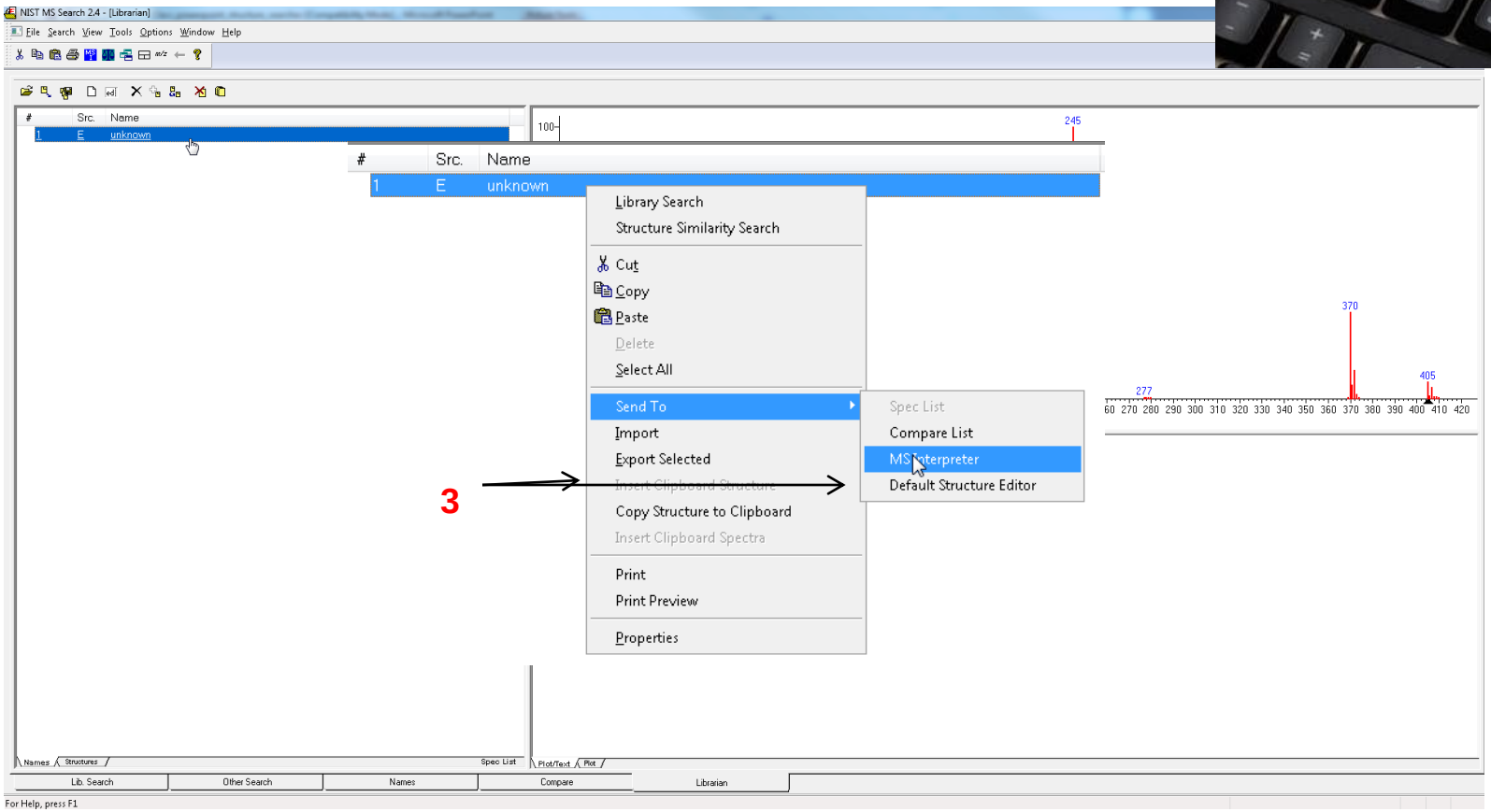
1) ***Draw*** the proposed structure with drawing program and ***copy*** into clipboard
2) ***Open Librarian tab*** and select ***"ed "*** icon with **left mouse click**
3) ***LMB*** on **From Clipboard** to associate structure with spectrum
4) ***Modify*** name fields, synonyms, comments fields, or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



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

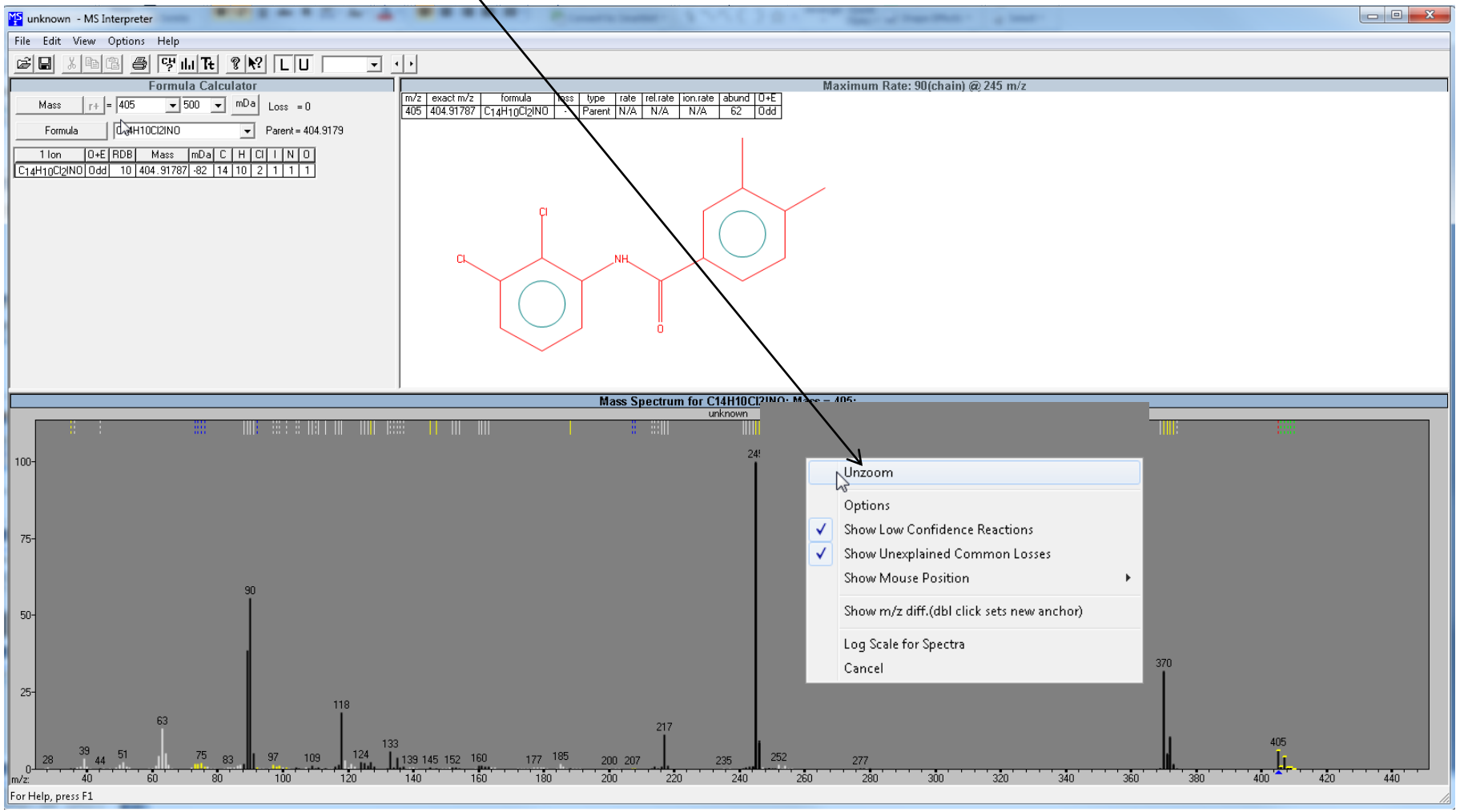
1) Entry is now ready to send to MS interpreter
2) Program has automatically calculated nominal molecular weight, exact mass, molecular formula, and InChIKey
3) ***Right mouse*** click on selected entry, then LMB on Send to/MS Interpreter
4) An ***alternative*** to step 3, is to ***send*** the entry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



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*

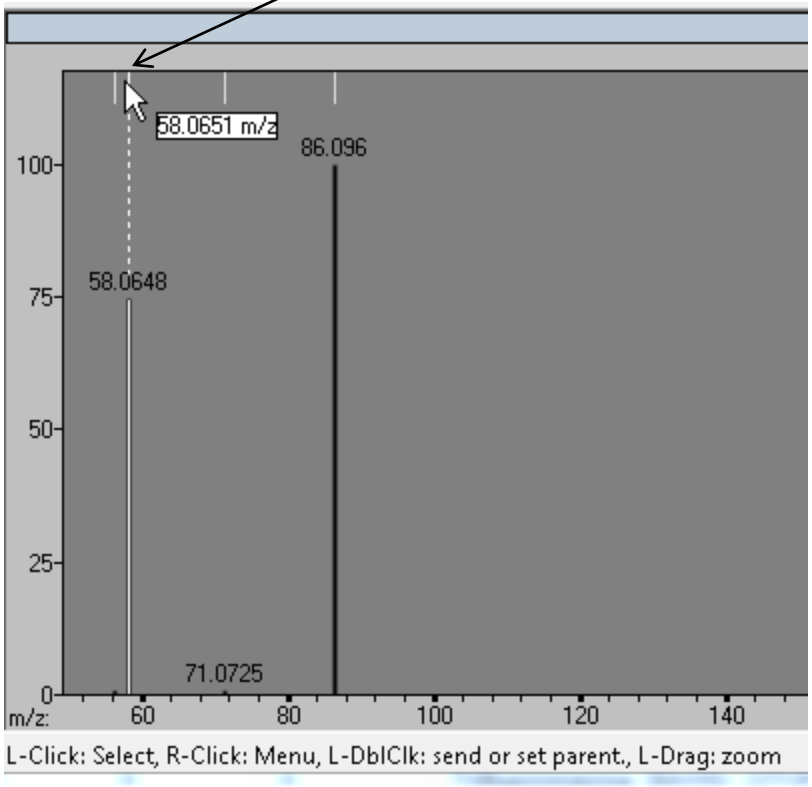
1) ***LMB*** on the ***tick mark*** for ion of interest , the display will toggle through ***all 4*** in this example

2) If ***more than one*** is present, the box will indicate the number, in this case ***¼***

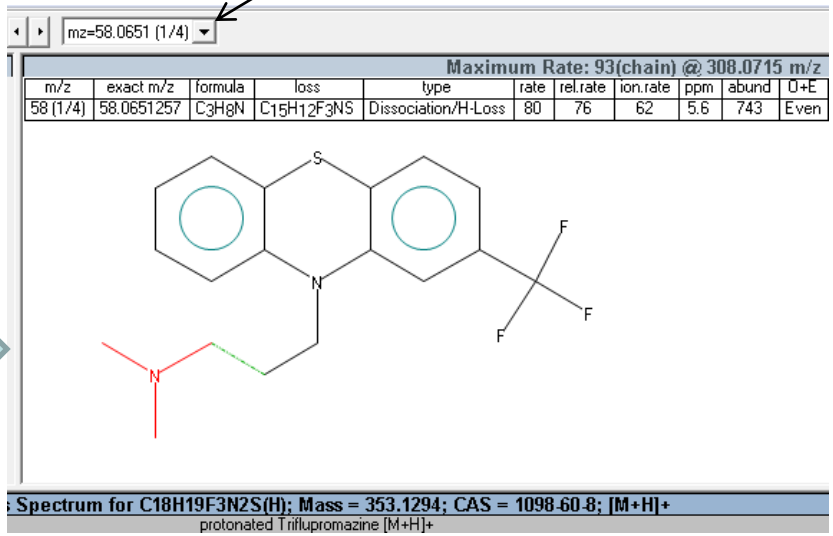
3) Also, you can select one of the tick marks with LMB then use your ***left*** and ***right*** arrows ***on keyboard*** to step through the assigned ions in the mass spectrum

1

click on tick marks



2

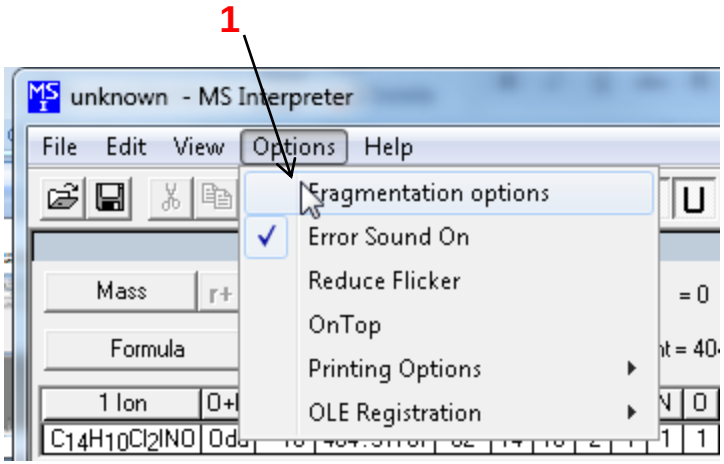


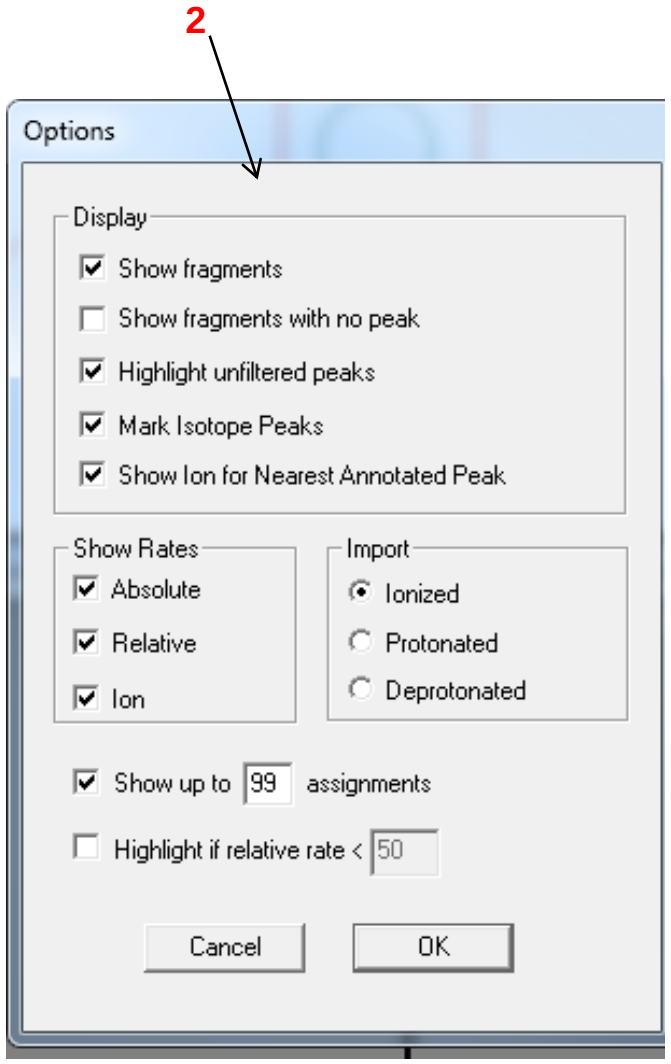
3

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

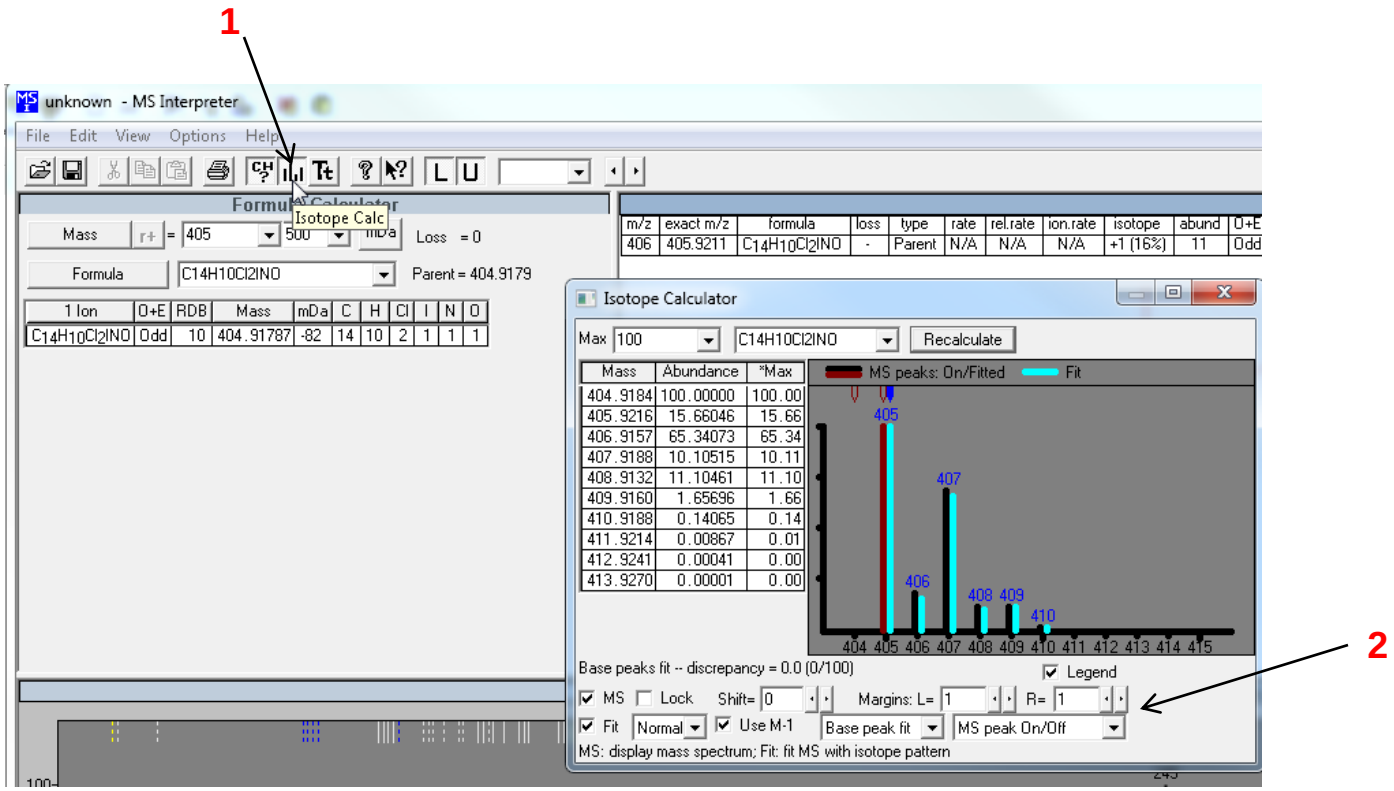




MS Interpreter for Correlating Structure to Spectrum:

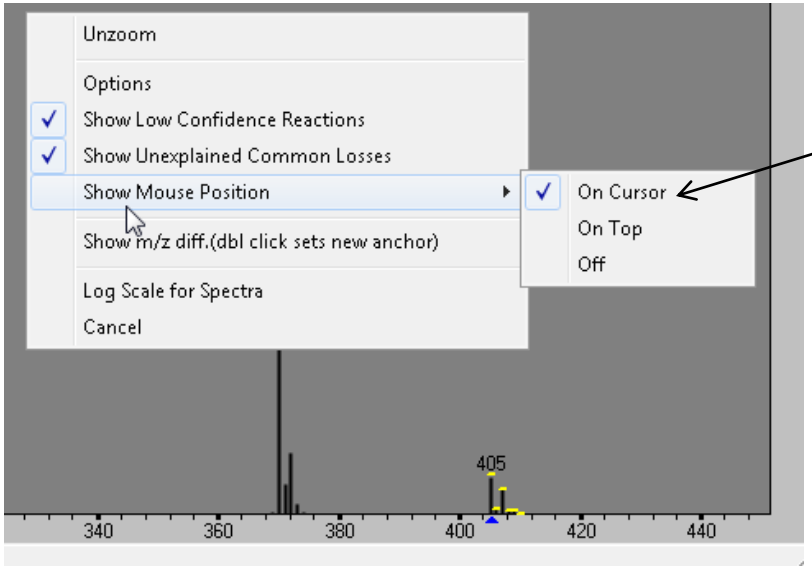
Suggested Default Settings for Electron Ionization

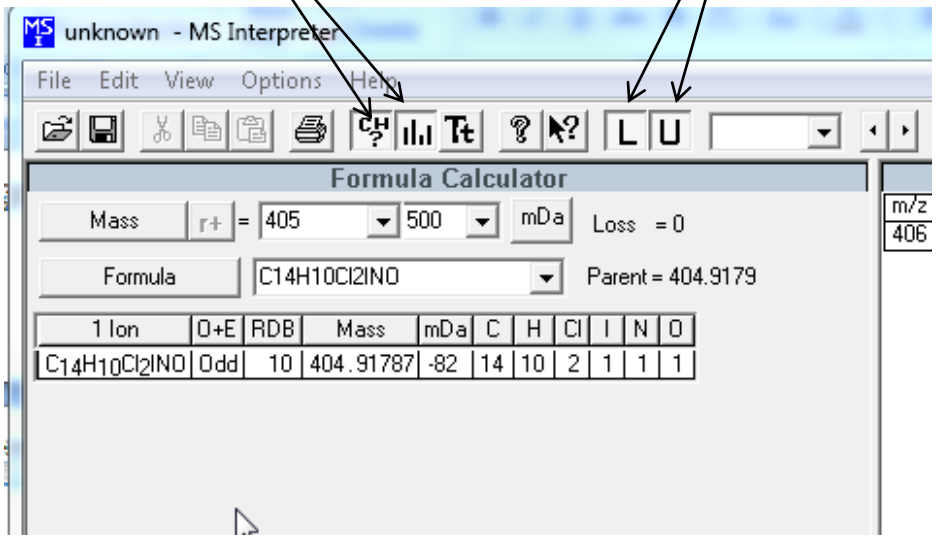
1) Select ***Isotopes Calc*** icon
2) Carefully set the menu options as noted in displayed Isotope Calculator Window below



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

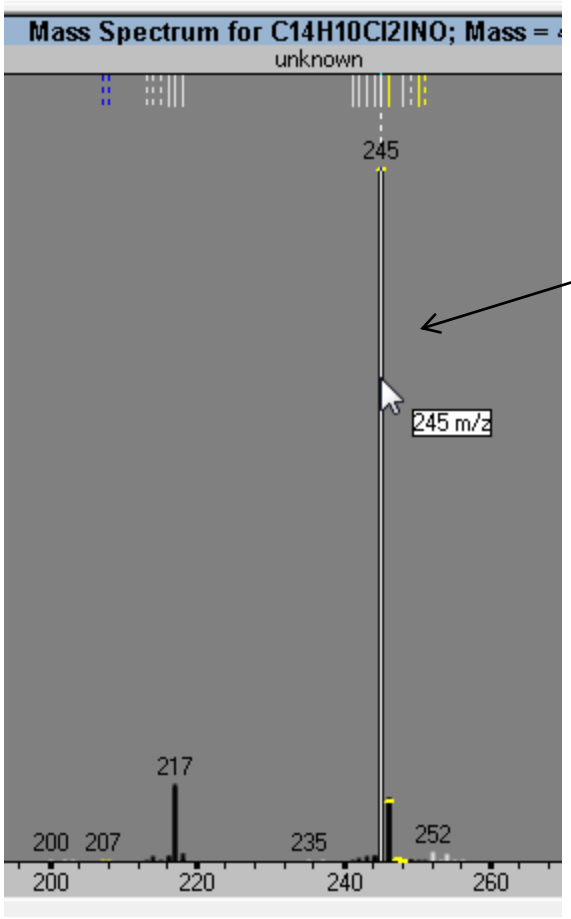




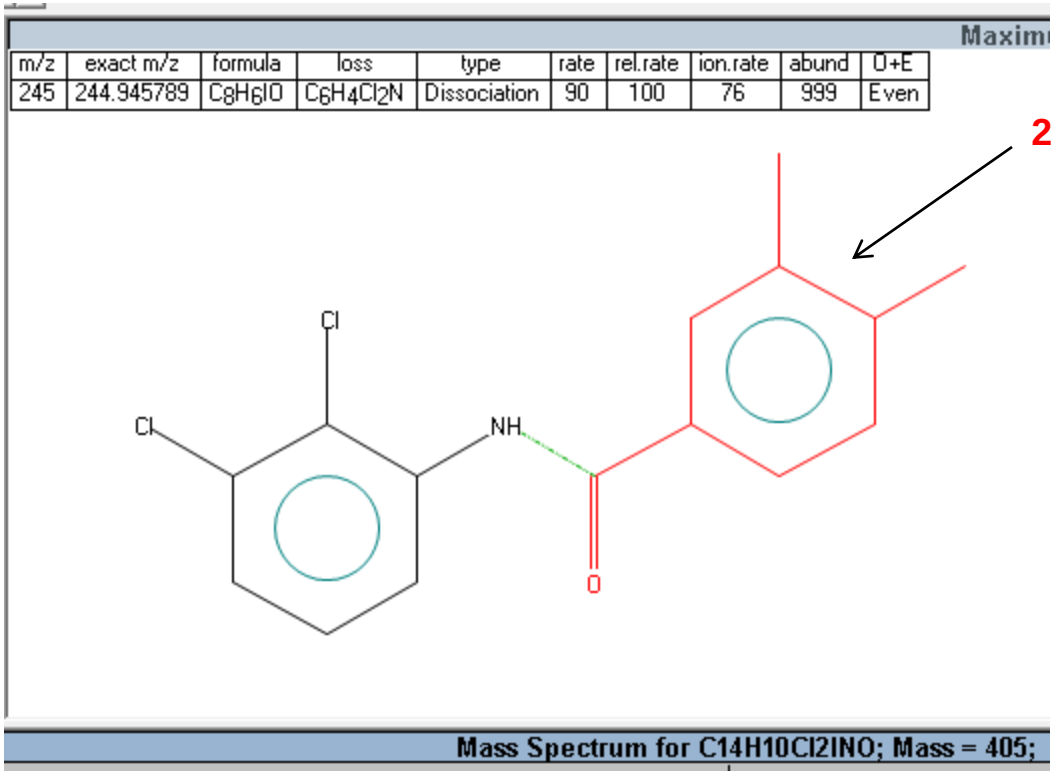
MS Interpreter for Correlating Structure to Spectrum:

Displaying the Proposed Structures

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



1

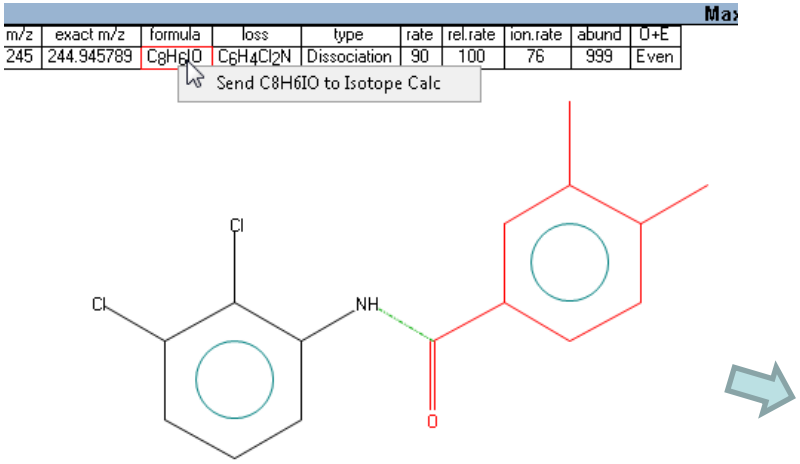


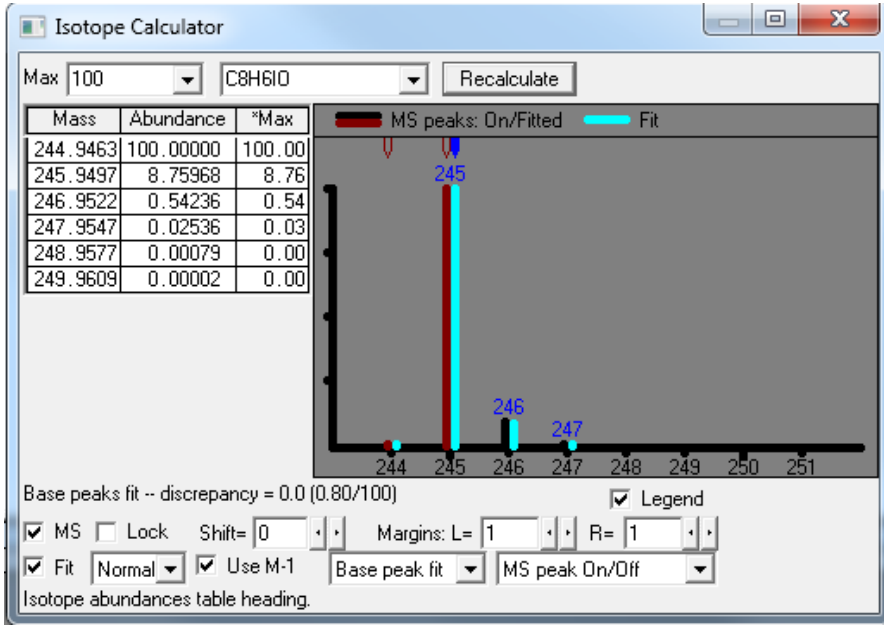
MS Interpreter for Correlating Structure to Spectrum:

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

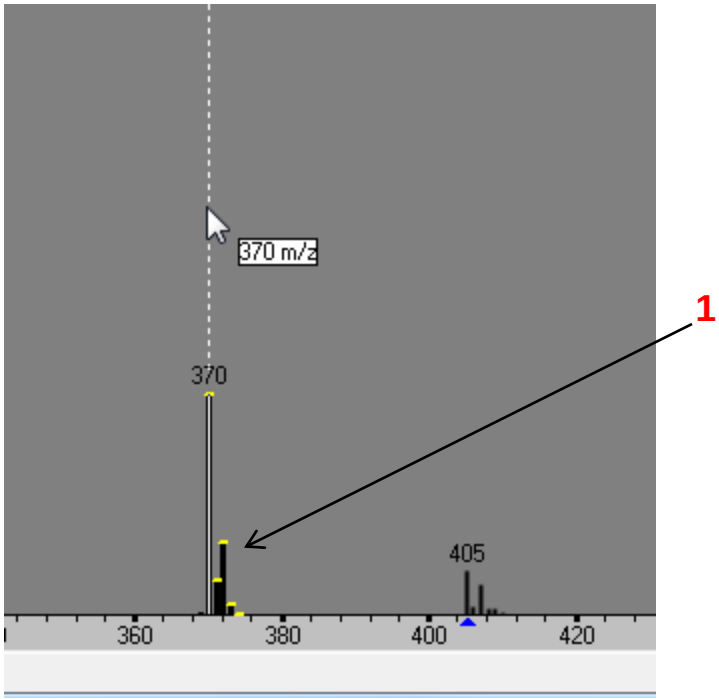


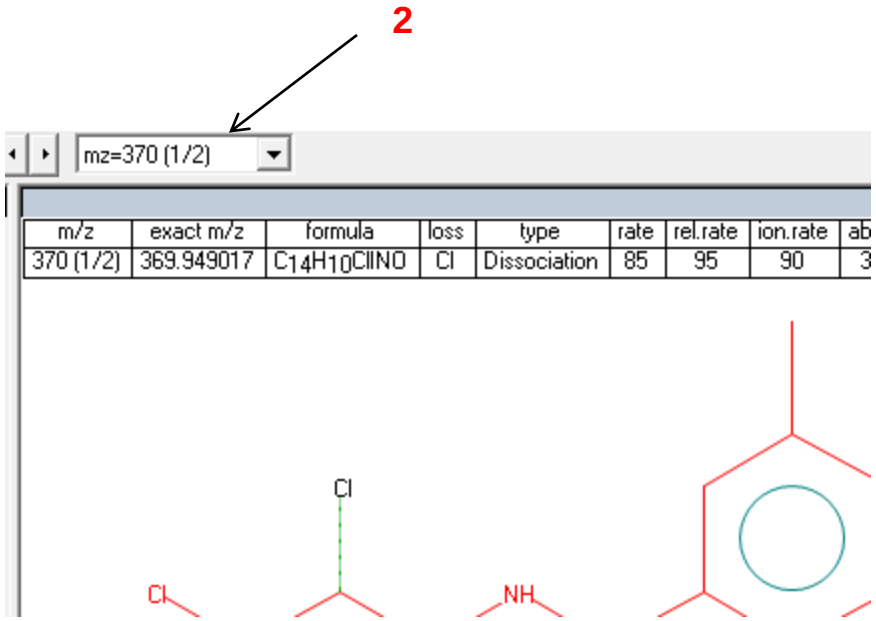


MS Interpreter for Correlating Structure to Spectrum:
Checking the Isotope Ratios for a Proposed Fragment

1) ***Theo***retical 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***

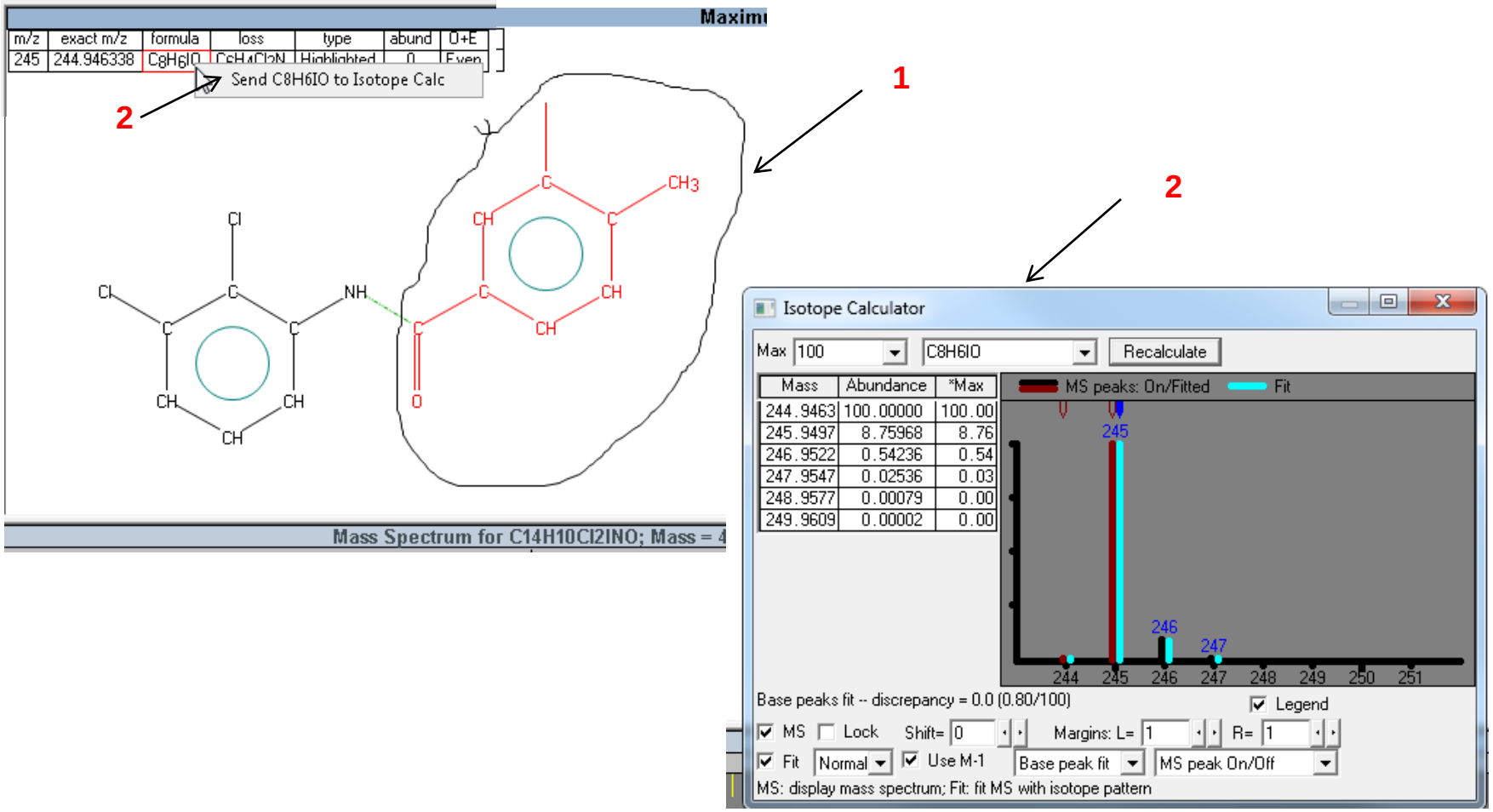




MS Interpreter for Correlating Structure to Spectrum:

Other Miscellaneous Tips: Lassoing Ions

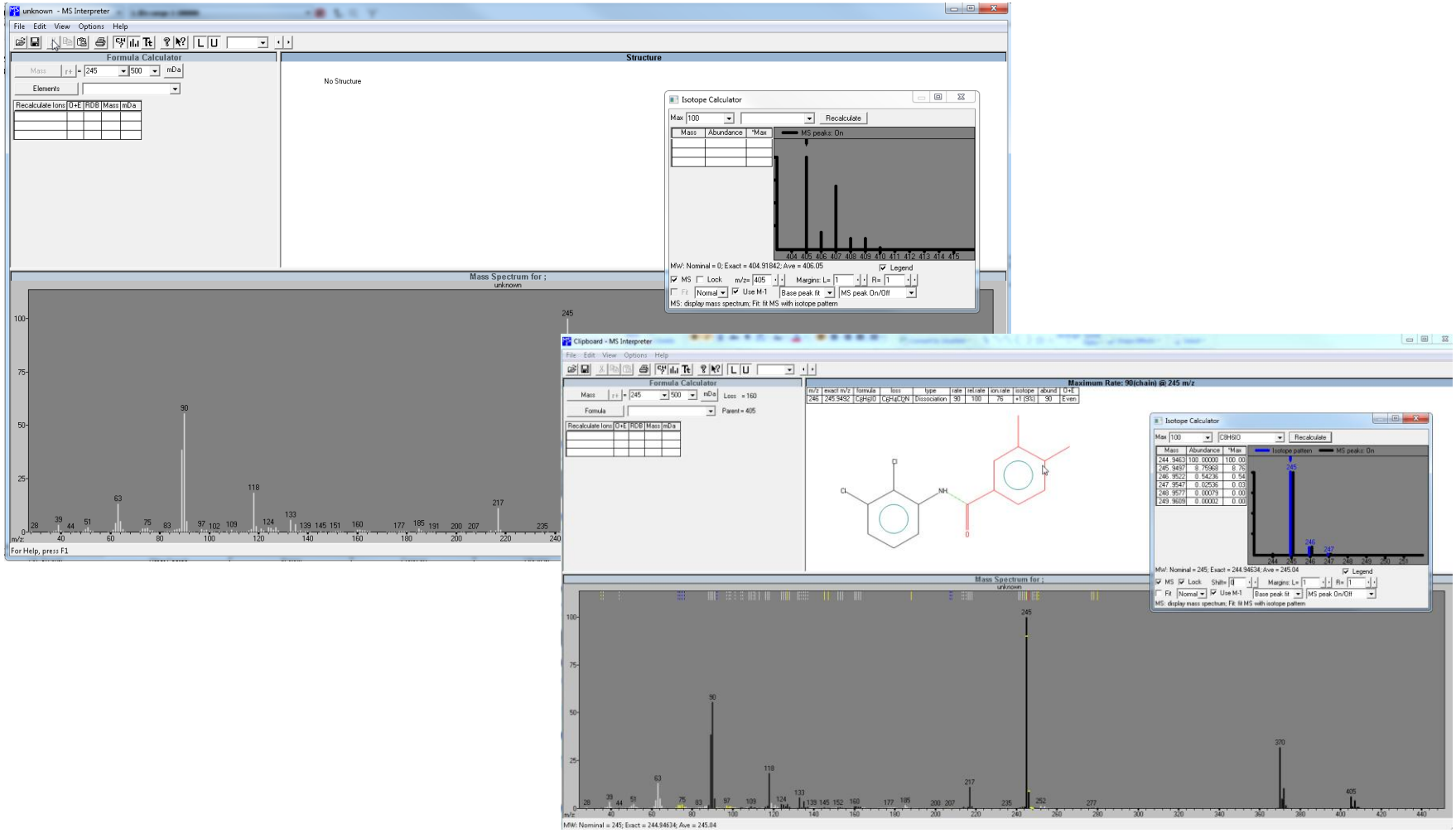
1) ***Lasso*** your own substructure by ***LMB*** and ***circling,*** shows type as Highlighted and calculates ***m/z*** and ***formula***
2) ***RMB*** on formula, e.g. C8H5IO, and ***LMB*** and ***Send "x" to Isotope Calc window***



MS Interpreter for Correlating Structure to Spectrum:

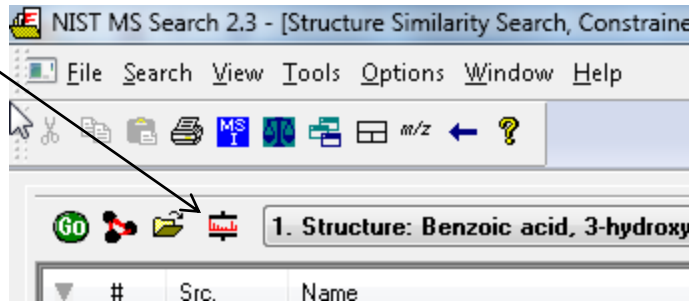
Other Miscellaneous Tips: Direct Structure Paste

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

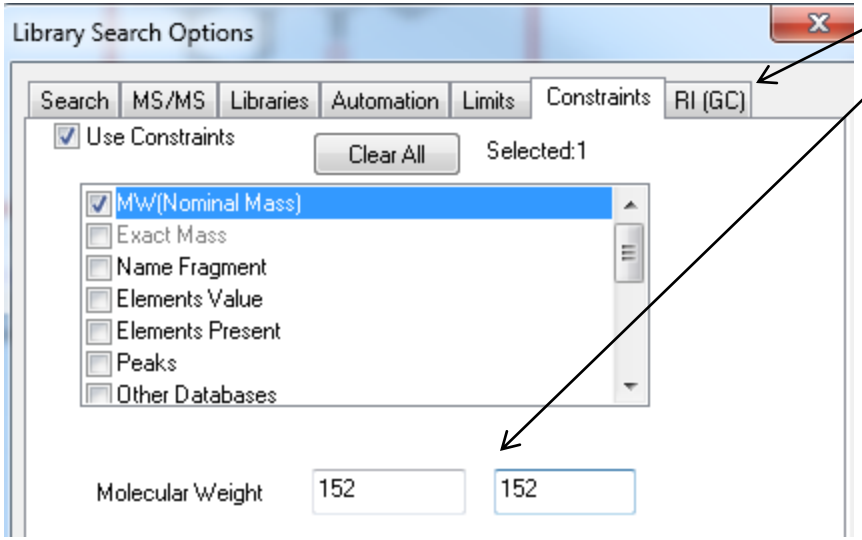


Do the 3 Isomers of Methyl Hydroxybenzoate Fragment Differently?
Let's Compare Them!

-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"

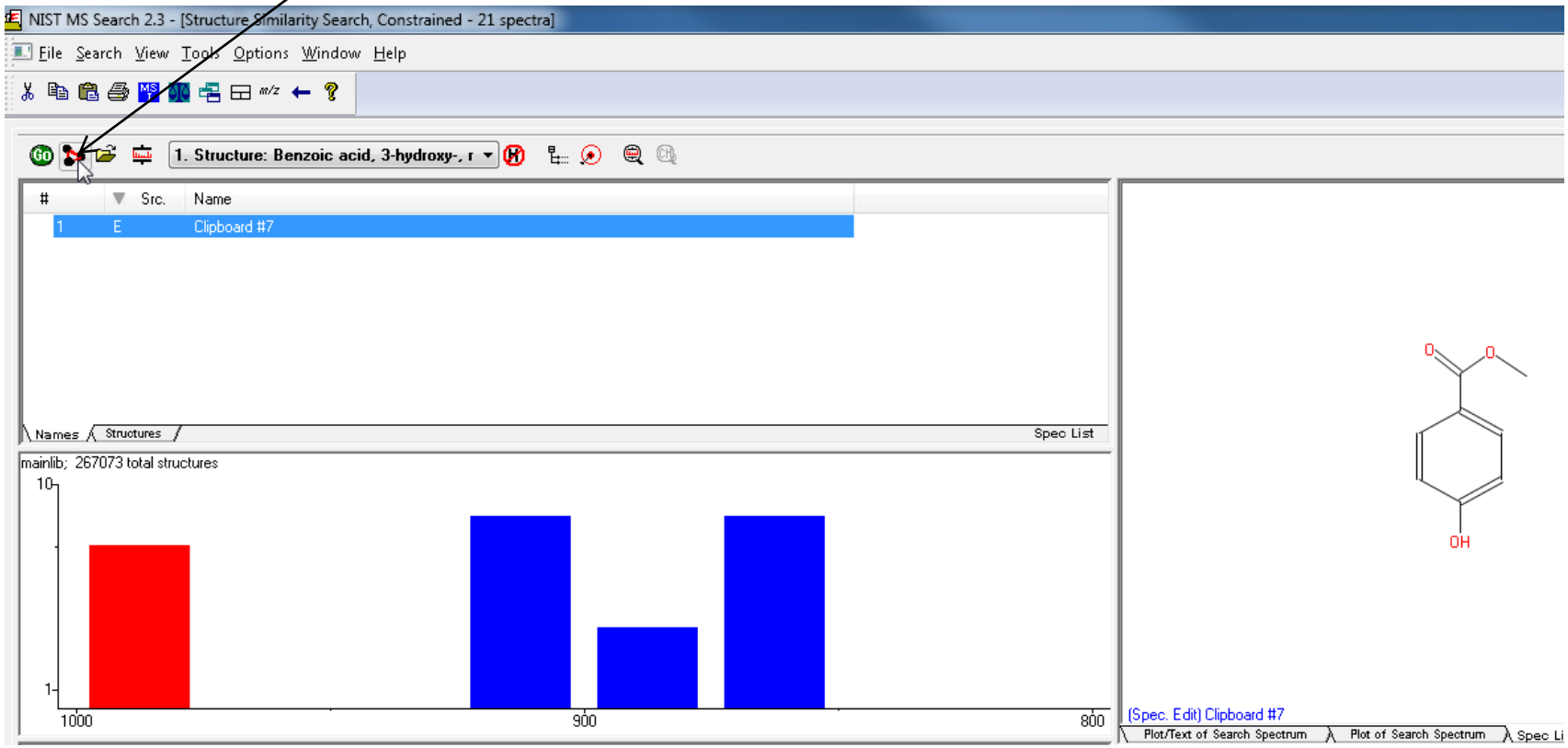


-***Constrain*** the results to be displayed after the search by ***MW(Nominal Mass)*** of 152 t0 152



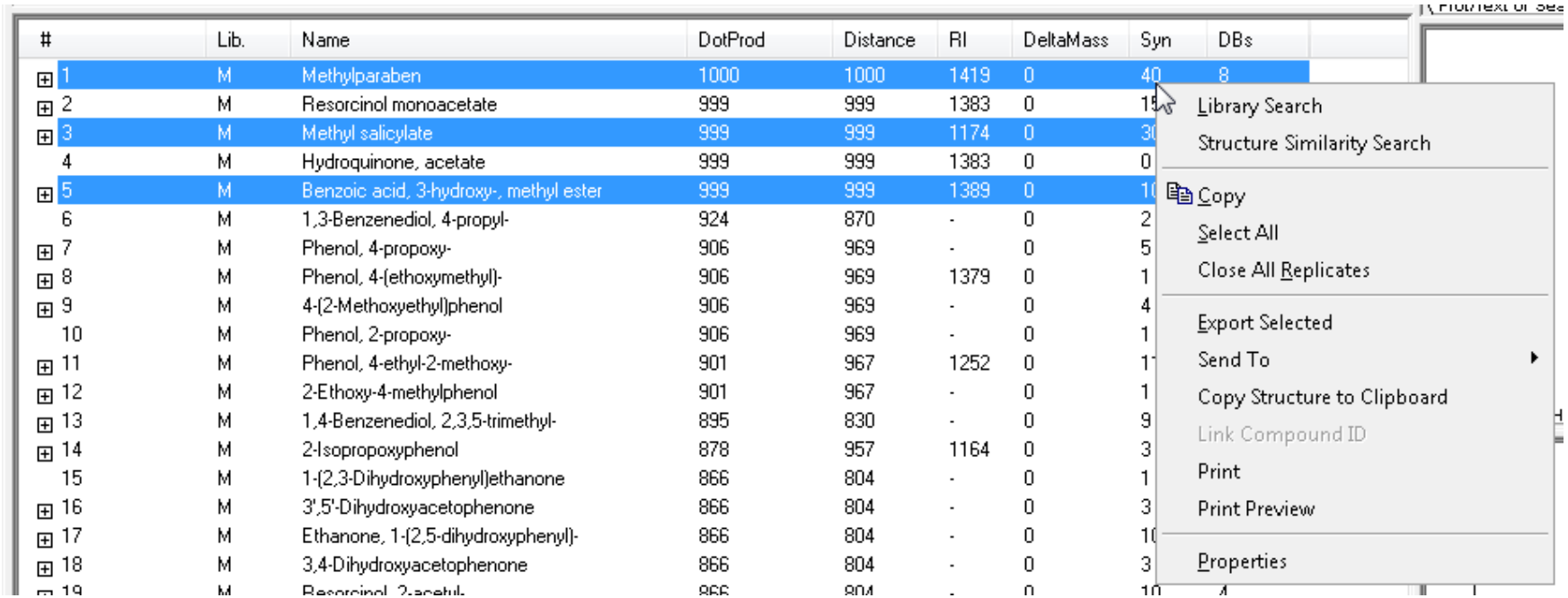
Do the 3 Isomers of Methyl Hydroxybenzoate Fragment Differently?
Let's Compare Them! (continued)

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



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

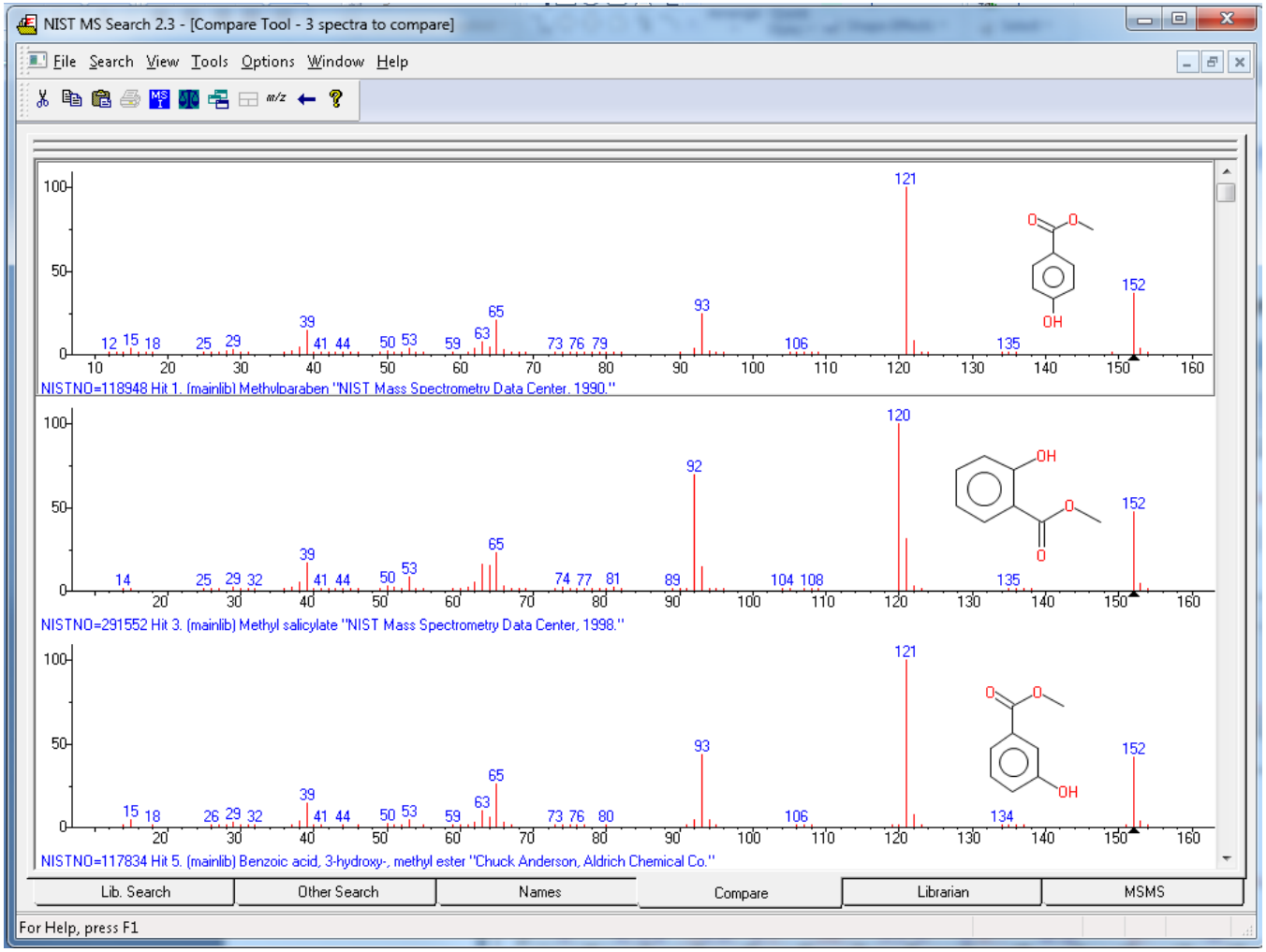
-Many hits are found
-Use ***control key/LMB*** to select entries 1,3, and 5 in hit list using ***LMB***



Do the 3 Isomers of Methyl Hydroxybenzoate Fragment Differently?

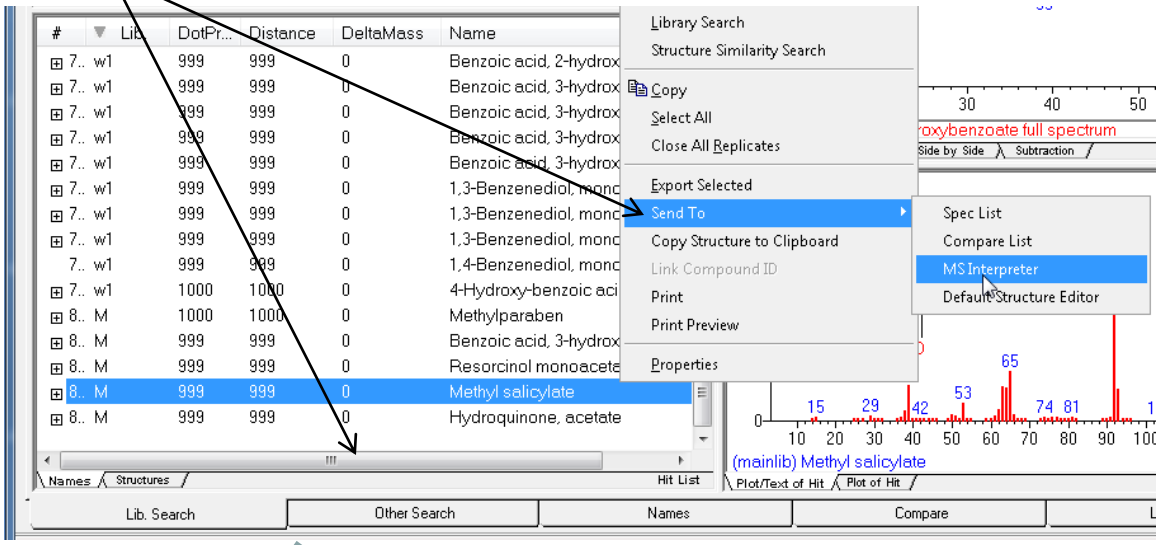
Let's Compare Them! (continued)

-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"



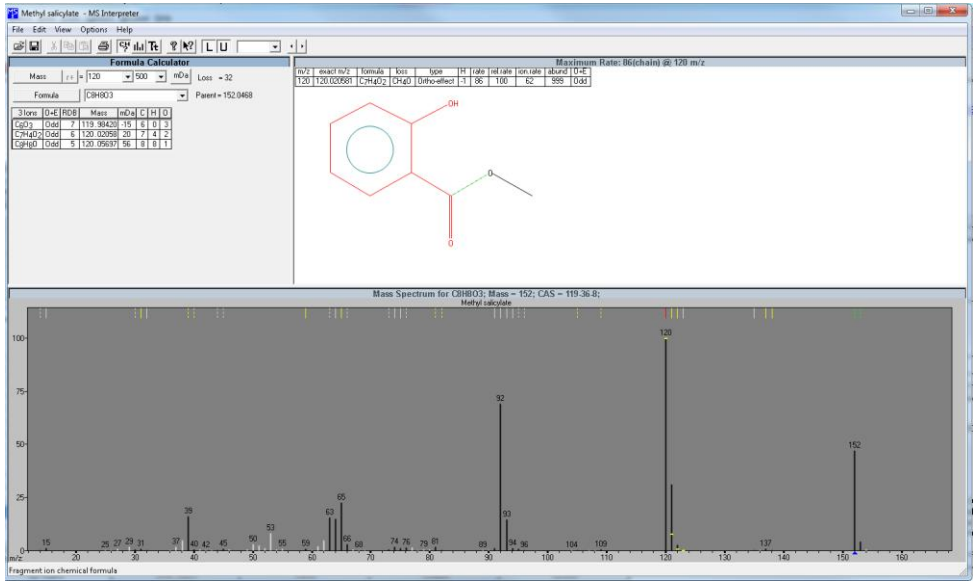
Any Structure and *Associated Spectrum* in Program *Sent* to *MS Interpreter*

1



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

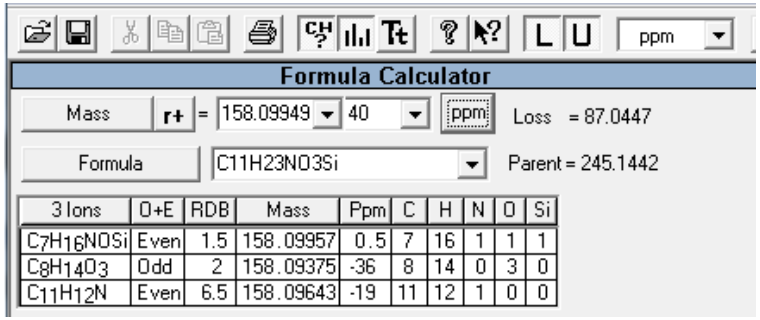
2



Accurate Mass Spectrum Correlated to Structure in MS Interpreter:

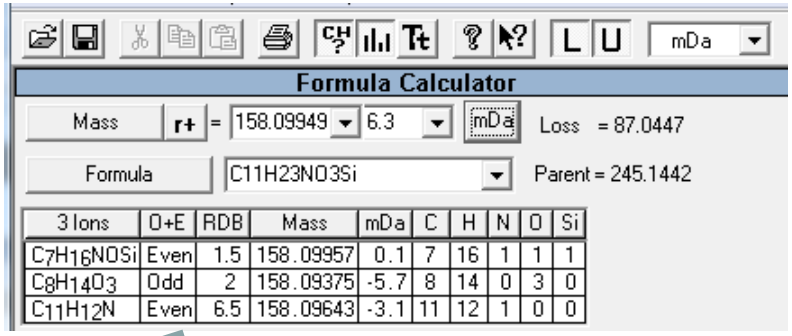
Accurate Mass EI Mass Spectral Data from Orbitrap

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

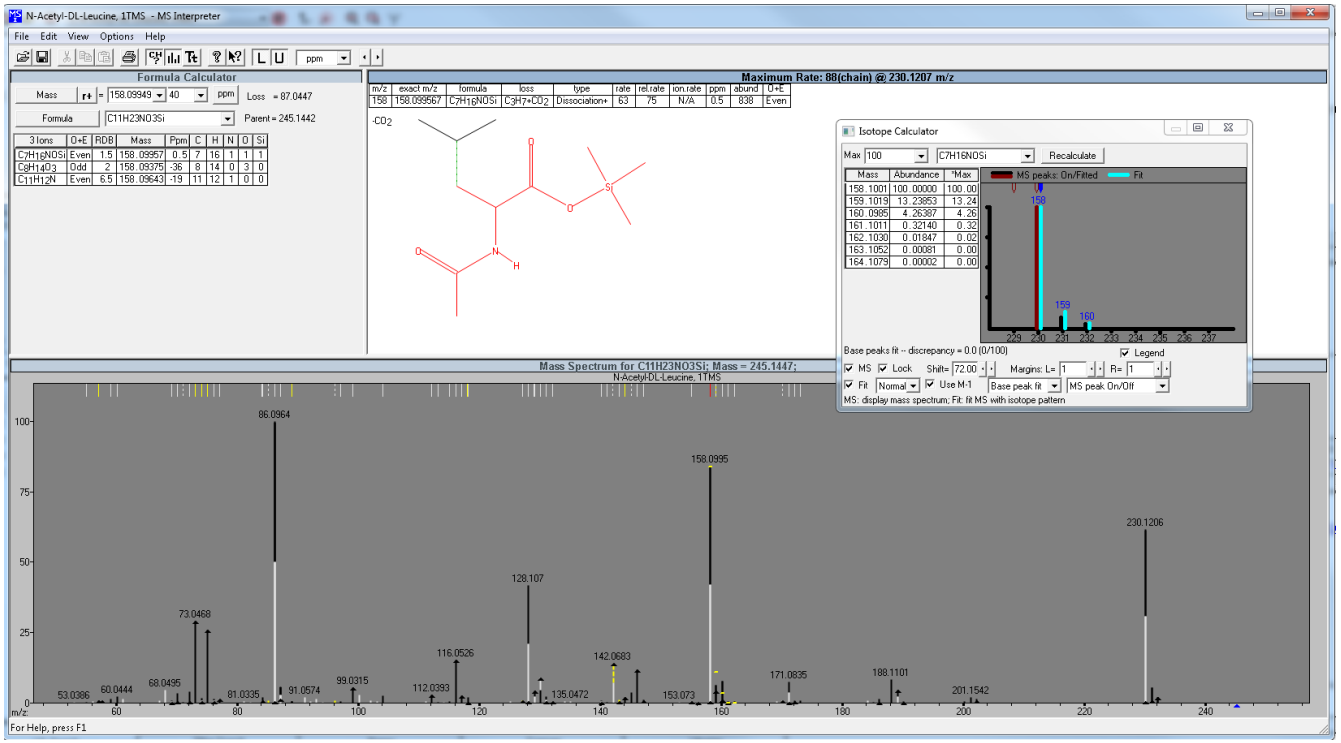


3 Ions	O+E	RDB	Mass	Ppm	C	H	N	O	Si
$C_7H_{16}NOSi$	Even	1.5	158.09957	0.5	7	16	1	1	1
$C_8H_{14}O_3$	Odd	2	158.09375	-36	8	14	0	3	0
$C_{11}H_{12}N$	Even	6.5	158.09643	-19	11	12	1	0	0

or



3 Ions	O+E	RDB	Mass	mDa	C	H	N	O	Si
$C_7H_{16}NOSi$	Even	1.5	158.09957	0.1	7	16	1	1	1
$C_8H_{14}O_3$	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



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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