

LC/MS Unknown Identifications Using MSMS Libraries

Part VI: MS Interpreter Correlation of Substructure to MSMS Ions

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- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
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- *Specialties¹ EI GC-MS, LC-MS/MS, Chemical Ionization,³ Accurate Mass, Derivatization,^{4,5} MS library management, SciFinder⁶, Chempider⁶, Surfactant ID,⁷ NMR, GC-IR, organic synthesis, matrix ionization effects,⁸ 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

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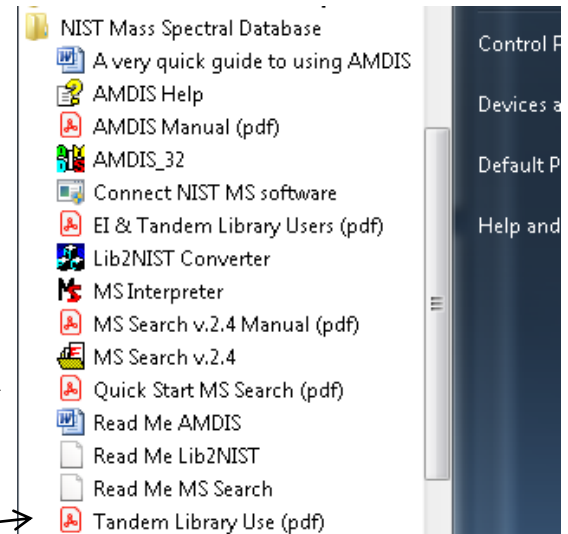
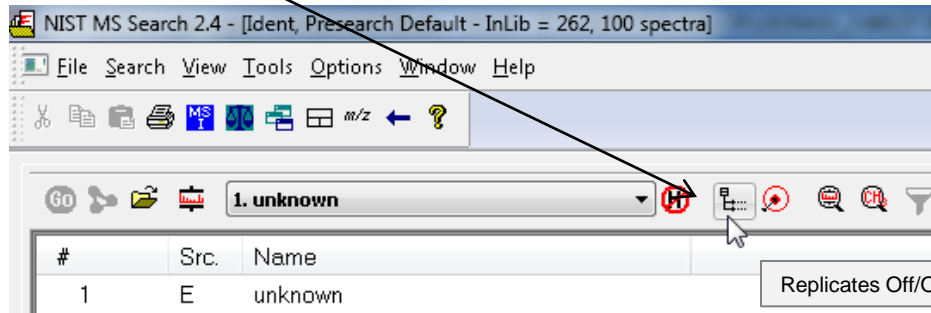
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LC/MS Unknown Identifications Using MSMS Libraries

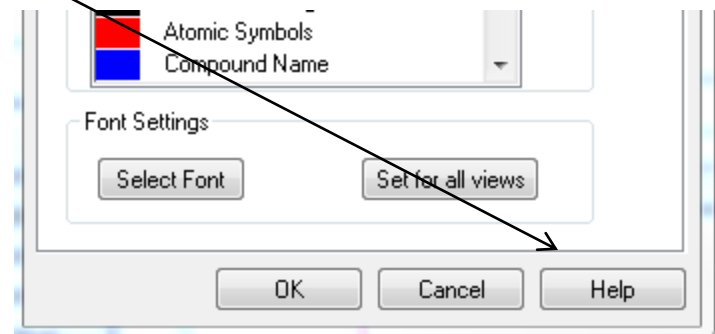
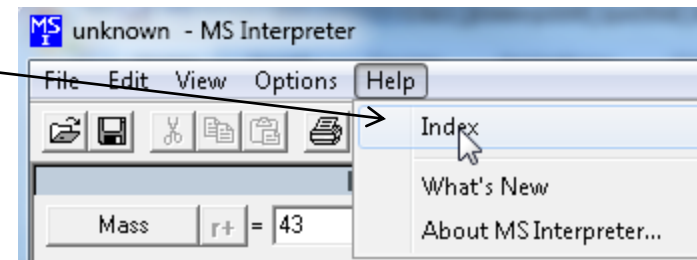
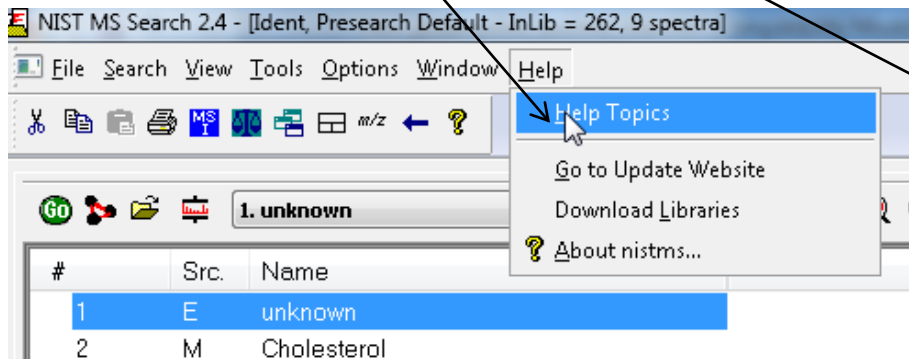
- Part I: Overview of Software and User Customized Configurations
- Part II: NIST MSMS Search Software and Libraries
- Part III: More Detailed Discussion of MSMS Hybrid Search
- Part IV: Importing MSMS Spectra
- Part V: NIST Structure Searches
- Part VI: MS Interpreter Correlation of Substructure to MSMS Ions
- Part VII: Using and Creating Other MSMS Libraries
- Part VIII: Identification of Unknowns with "Spectraless" Libraries

Help Files for NIST Search

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



- **Detailed** documentation for NIST Search⁸
- MS Interpreter included in NIST manual⁸
- Windows Program Group
- NIST Tandem Search **Quick Start Guide**⁹
- "In program" assistance for both programs
- **Help button** at corner of all windows



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

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

#	Lib.	Name	▼ Match	Prob. (%)	RI	B. 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

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!



Part VI: MS Interpreter⁹⁻¹² Correlation of Substructure to MSMS Ions

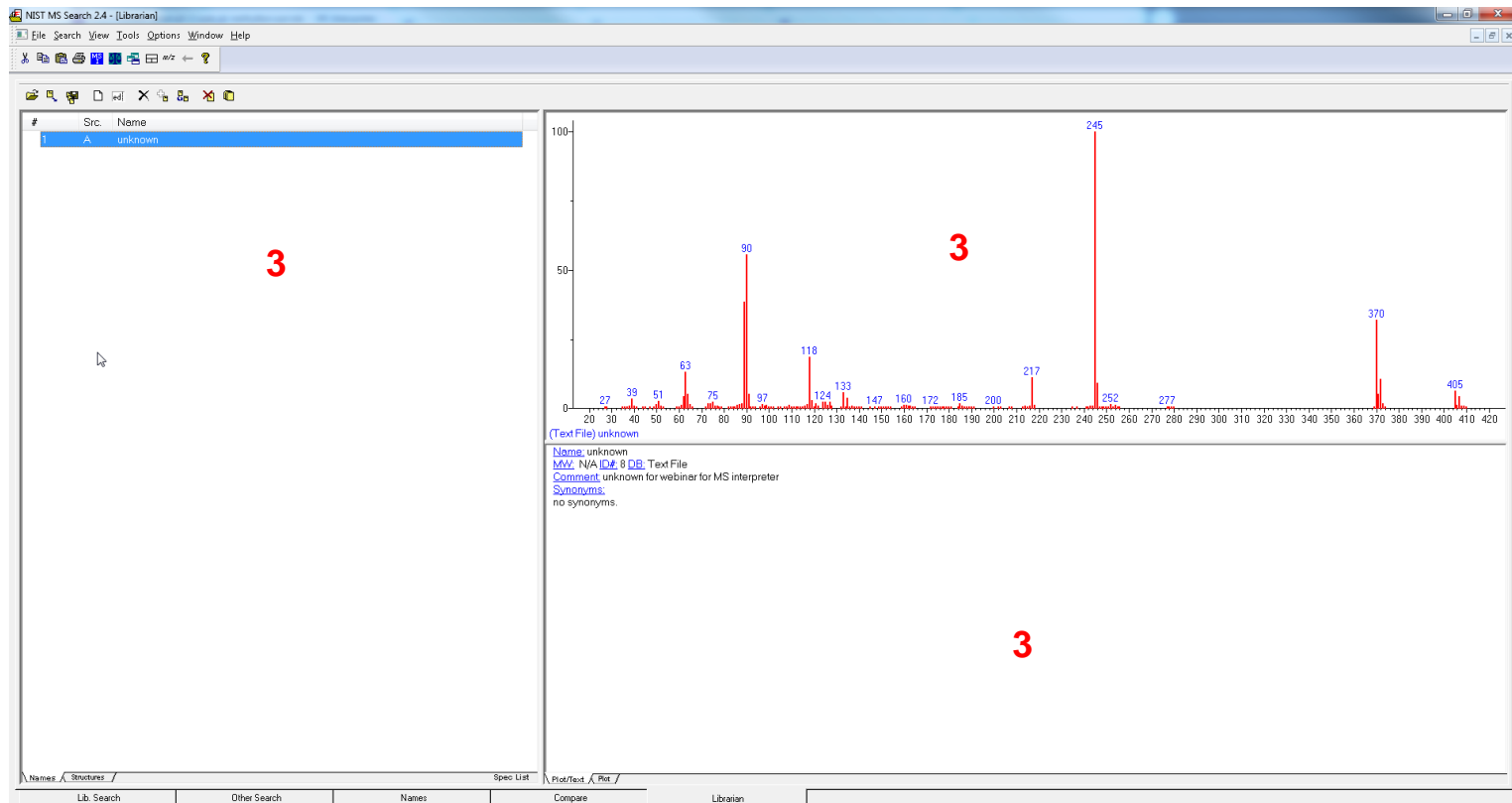
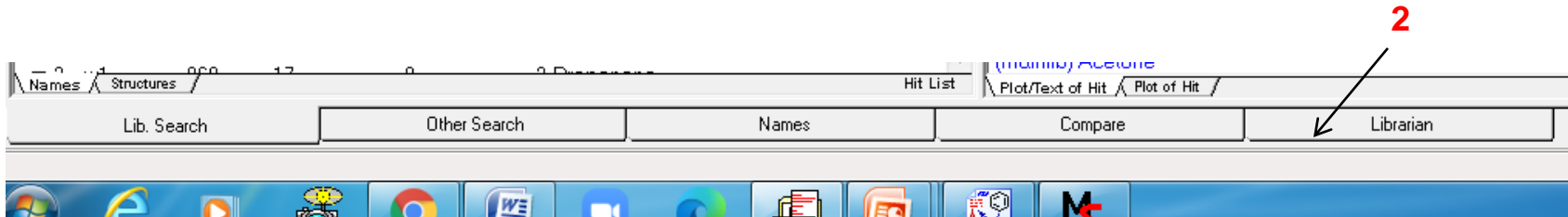
Session Topics:

- Setting up ***Library Search Options*** MS Interpreter
- Associating structure with spectrum
- Processing data

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 Presentation VII 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.

NIST MS Search 2.4 - [Librarian]

File Search View Tools Options Window Help

Src. Name

#	Src.	Name
1	A	unknown

Spectrum Information

Name: unknown

Formula: [] From structure

Mol. Weight: 0 CAS Number: 0

Library: Text File

ID Number: 8 Inventory#: []

RI: [] Edit RI

Other Names (Synonyms): []

Additional Info Experimental Data

Comments: unknown for webinar for MS interpreter

Peak information

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

Accept HiRes Spectrum Peaks: 164

100 0 50 100 150 200 250 300 350 400

90 245 370

To Clipboard From Clipboard Get MolFile Get Struct Structure Editor From Inventory

Add to Library Add to List Replace Cancel Help

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

#	Src.	Name
1	E	unknown

#	Src.	Name
1	E	unknown

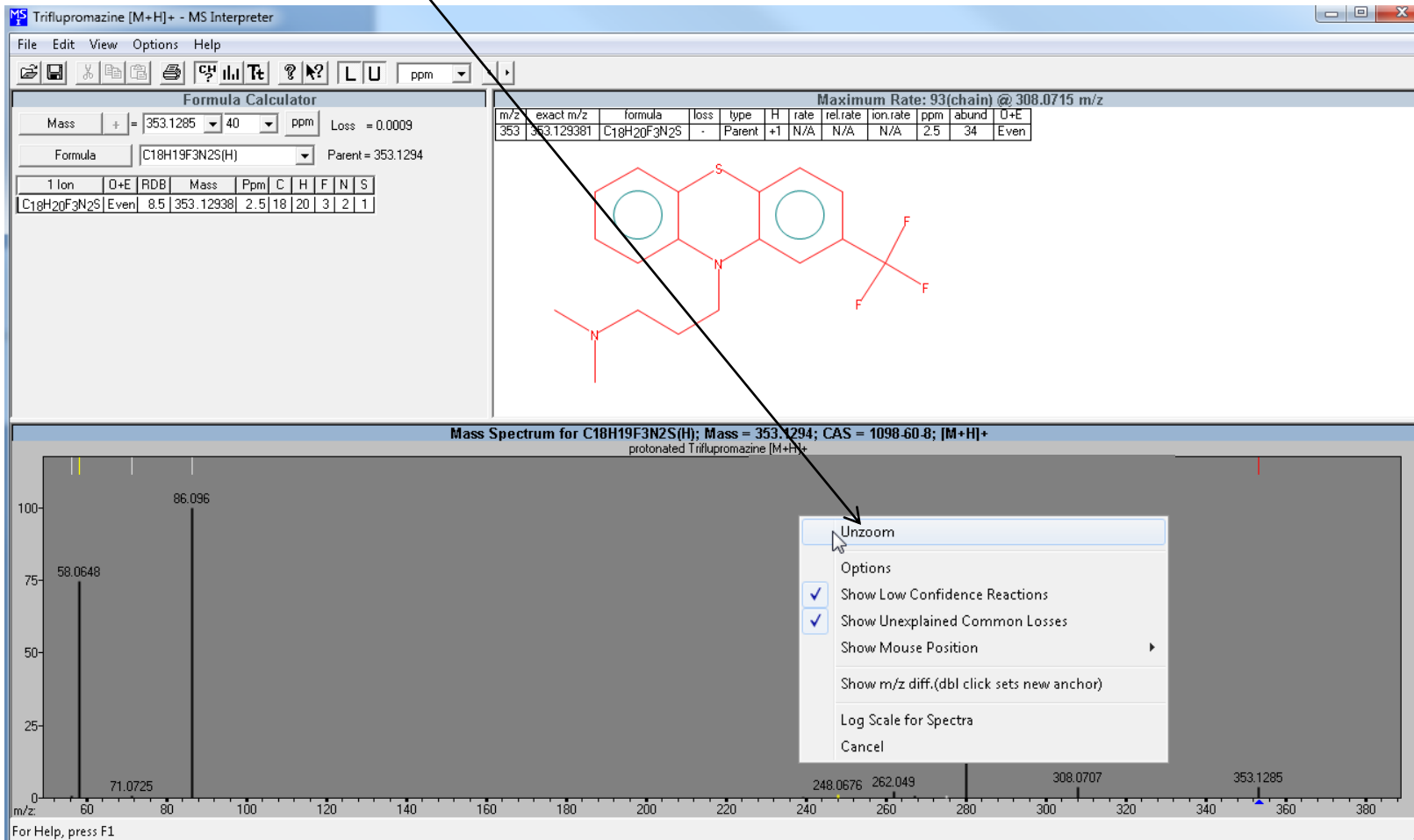
Mass Spectrum Data:

m/z	Relative Intensity
277	Low
370	High
405	Low

MS Interpreter for Correlating Structure to Spectrum:

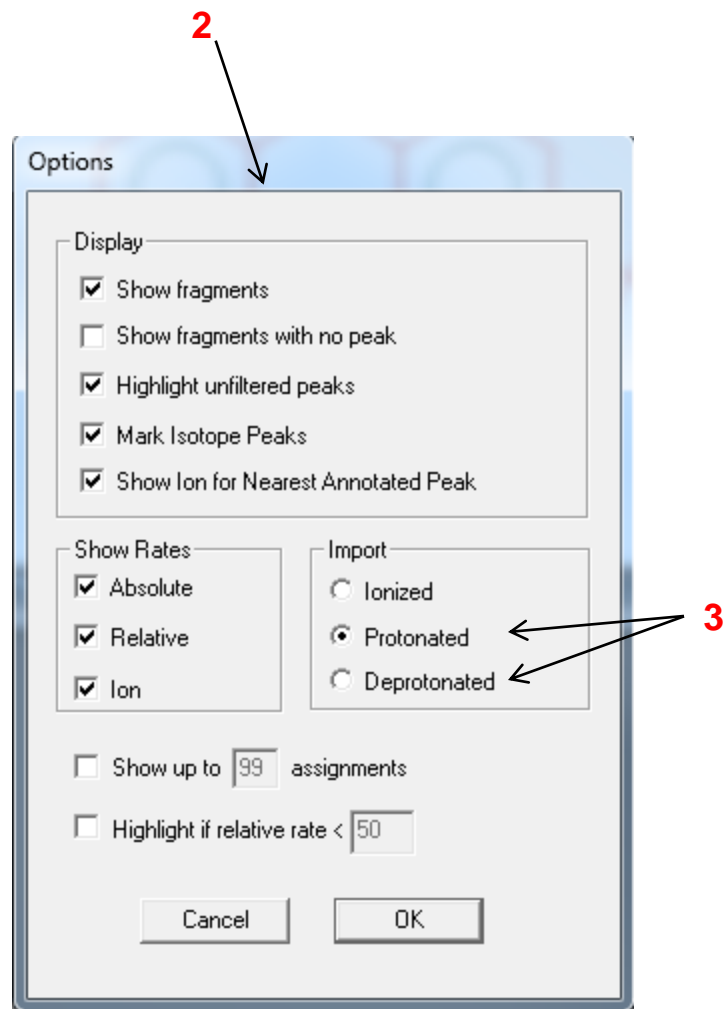
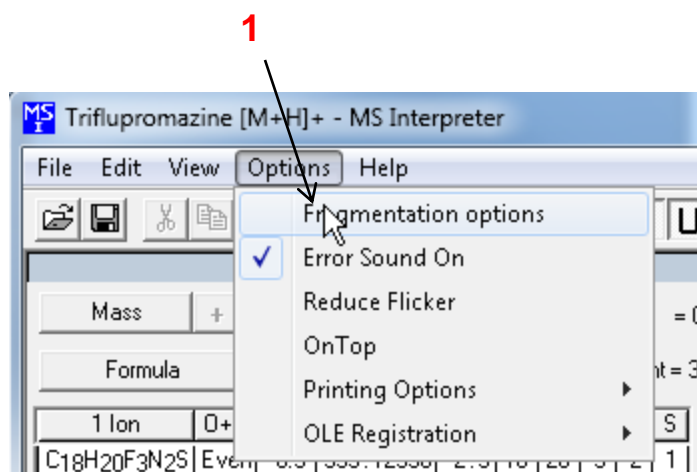
Processing Data in MS Interpreter

- All ions in black, yellow, and red correlated to structure 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: *Suggested Default Settings for MS/MS (Tandem)*

- 1) Select **Options** with **LMB** click then **Fragmentation options**
- 2) Setup options as noted in window
- 3) *In this case*, “Protonated”, if M-H, select “Deprotonated”



MS Interpreter for Correlating Structure to Spectrum: *Suggested Default Settings for MS/MS (Tandem)*

- 1) Select **Isotopes Calc** icon *if your* spectrum has isotopes
- 2) Select the Defaults as appropriate
- 3) This spectrum has **no** isotopes, just precursor

1

Trifluorpromazine [M+H]⁺ - MS Interpreter

File Edit View Options Help

Formula Calculator

Mass + = 353.1295 40 ppm Loss = 0.0009

Formula C₁₈H₁₉F₃N₂S(H) Parent = 353.1294

1 Ion	O+E	RDB	Mass	Ppm	C	H	F	N	S
C ₁₈ H ₂₀ F ₃ N ₂ S	Even	8.5	353.12938	2.5	18	20	3	2	1

Maximum Rate: 93(chain) @ 308.0715 m/z

m/z	exact m/z	formula	loss	type	H	rate	rel.rate	ion.rate	ppm	abund	O+E
353	353.129381	C ₁₈ H ₂₀ F ₃ N ₂ S	-	Parent	+1	N/A	N/A	N/A	2.5	34	Even

Mass Spectrum for C₁₈H₁₉F₃N₂S(H); Mass = 353.1293 protonated Trifluorpromazine [M+H]⁺

58.0648 86.096

Isotope Calculator

Max 100 C₁₈H₂₀F₃N₂S Recalculate

Mass	Abundance	*Max
353.1299	100.00000	100.00
354.1330	21.21855	21.22
355.1290	6.61554	6.62
356.1302	1.05074	1.05
357.1319	0.10531	0.11
358.1335	0.00778	0.01
359.1351	0.00045	0.00

MW: Nominal = 353; Exact = 353.12993; Ave = 353.43

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

Fit Normal Use M-1 Base peak fit Mark isotope peaks

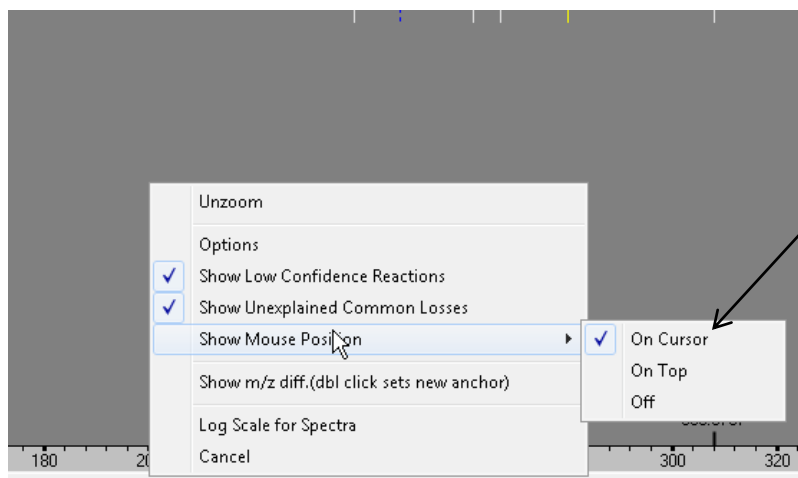
MS: display mass spectrum; Fit: fit MS with isotope pattern

2

MS Interpreter for Correlating Structure to Spectrum:

Suggested Default Settings for MS/MS (Tandem)

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



MS Triflupromazine [M+H]+ MS Interpreter

File Edit View Options Help

Mass + = 353.1285 40 ppm Loss = 0.0009

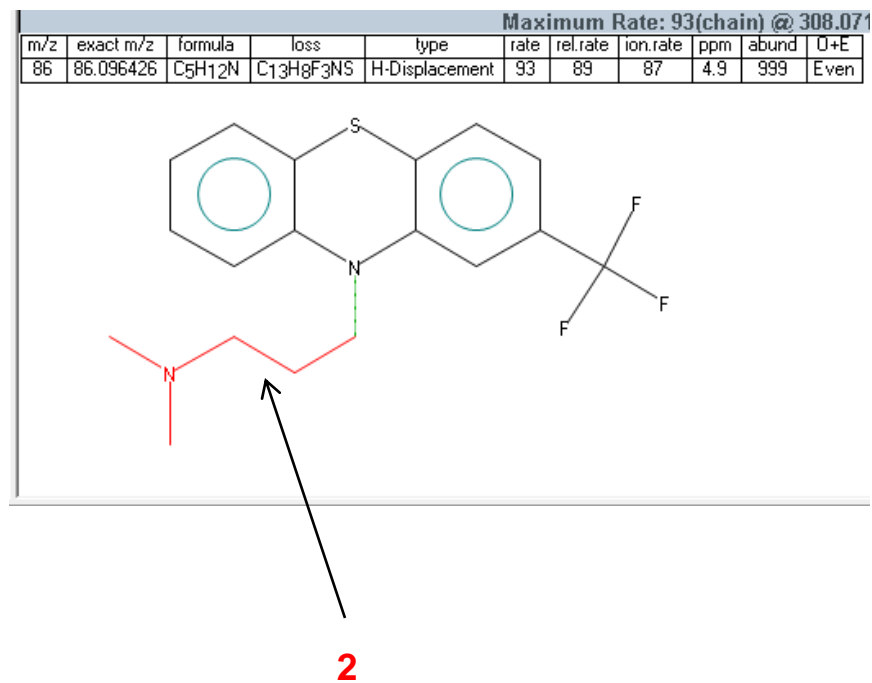
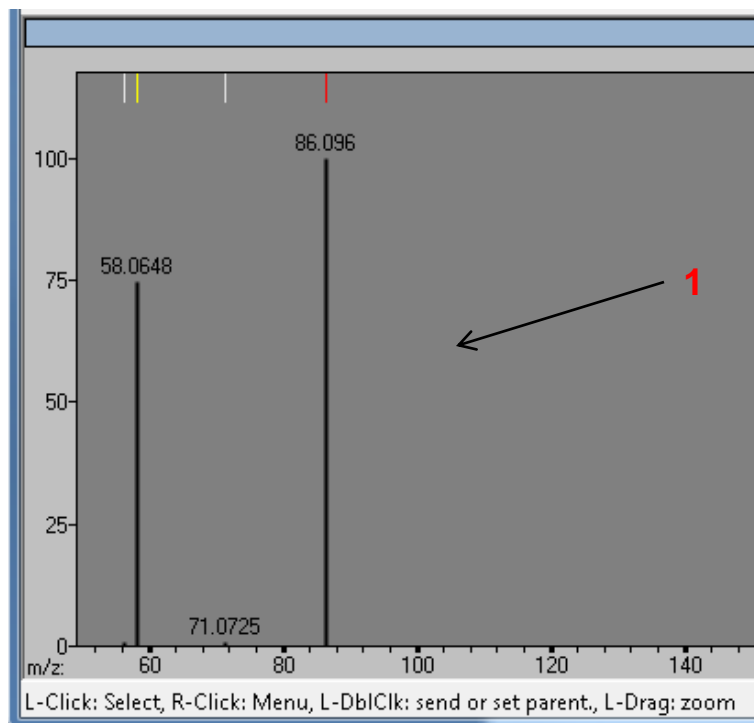
Formula C₁₈H₁₉F₃N₂S(H) Parent = 353.1294

1 Ion	Q+E	RDB	Mass	Ppm	C	H	F	N	S
C ₁₈ H ₂₀ F ₃ N ₂ S	Even	8.5	353.12938	2.5	18	20	3	2	1

m/z exact
353 353.1294

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

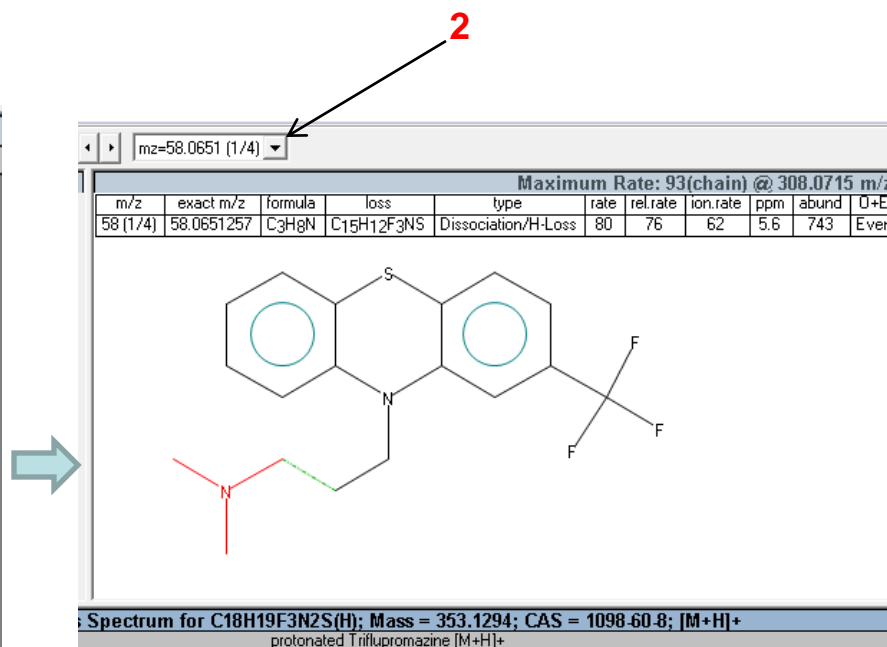
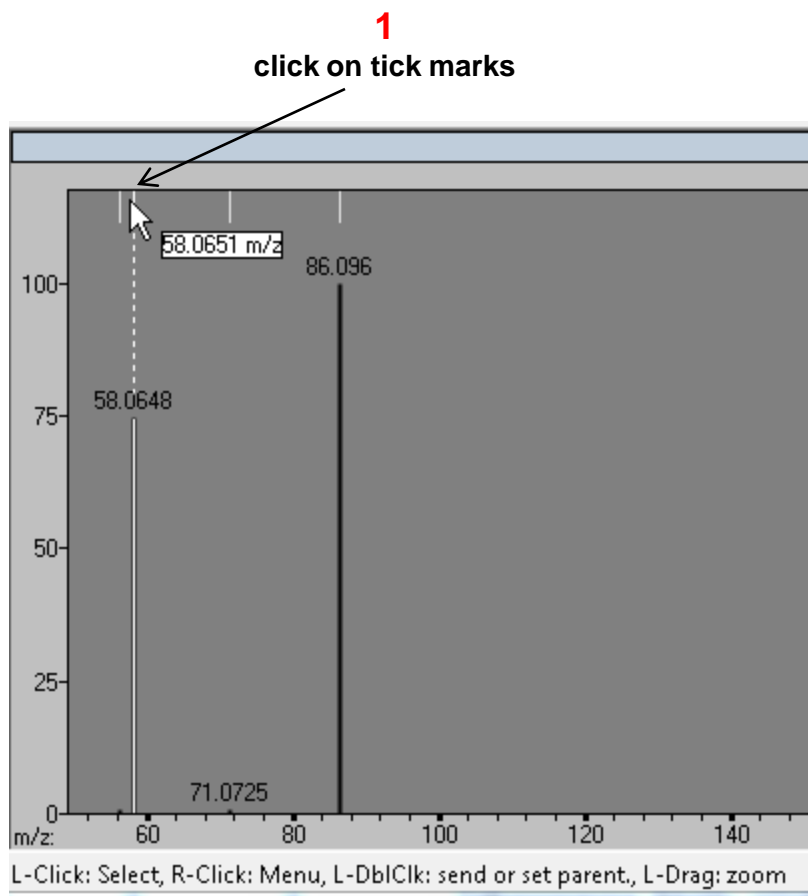
- 1) **LMB** on the ion of interest and the
- 2) Structure in **highlighted in red** in top window



MS Interpreter for Correlating Structure to Spectrum:

Stepping Through **More Than One** Proposed Structure

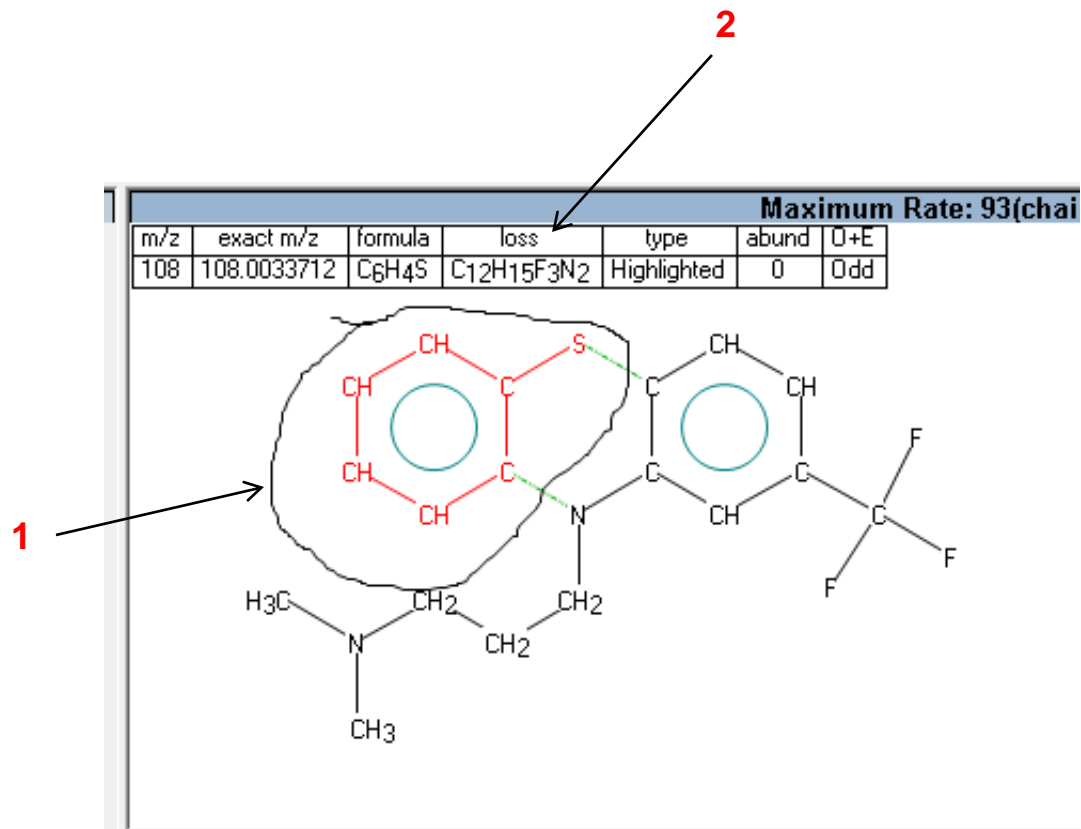
- 1) **LMB** on the **tick mark** for ion of interest, the display will toggle through **all 4** in this example
- 2) If **more than one** is present, the box will indicate the number, in this case $\frac{1}{4}$
- 3) Also, you can select one of the tick marks with LMB then use your **left** and **right**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



MS Interpreter for Correlating Structure to Spectrum:

Other Miscellaneous Tips: Lassoing Ions Manually

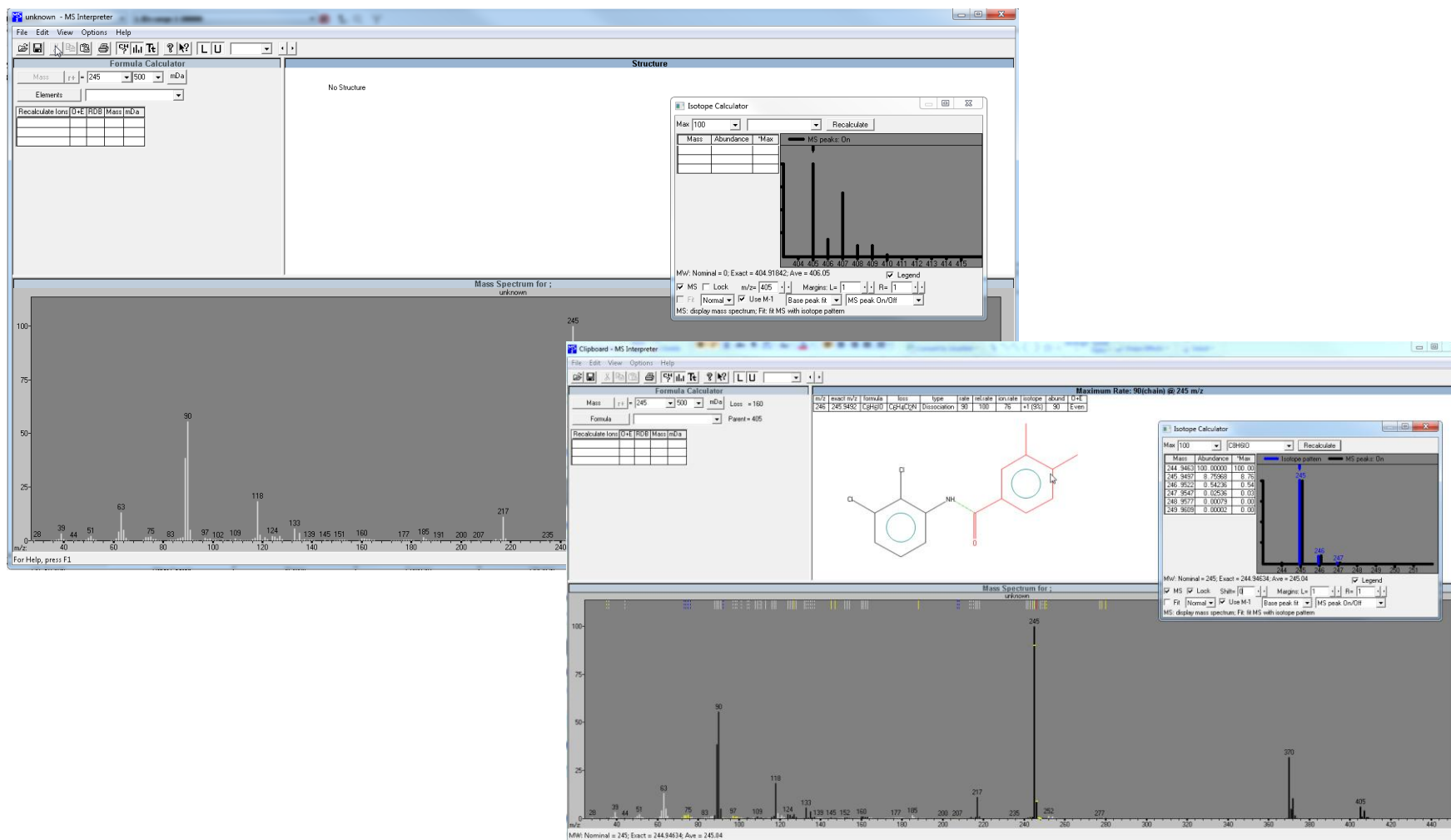
- 1) **Lasso** your own substructure by **LMB** and **circling**, shows type as **highlighted**
- 2) The formula , exact mass, and loss will be shown in the “Formula Calculator” window



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**
- 2) The structure **cannot be saved** with spectrum upon exiting MS Interpreter



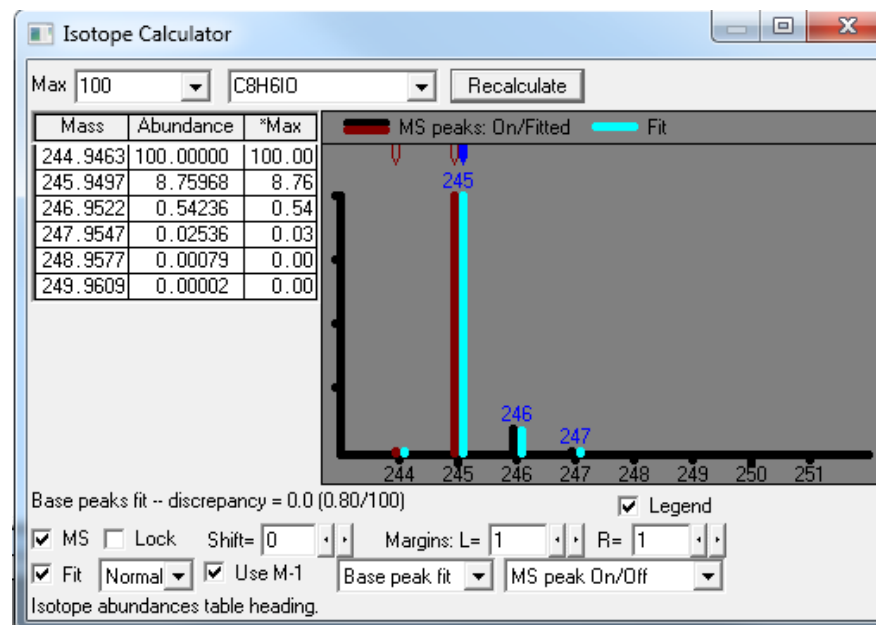
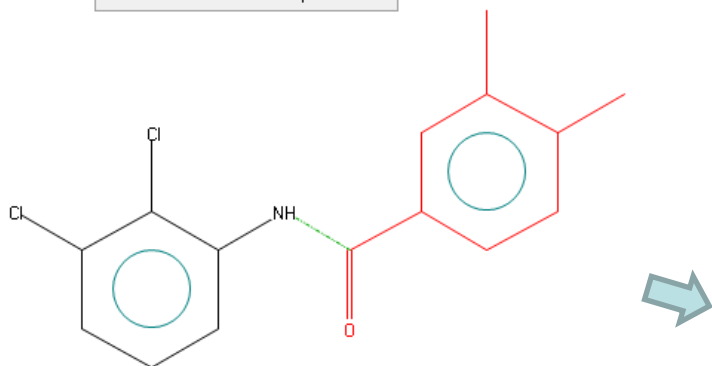
MS Interpreter for Correlating Structure to Spectrum:

If your MS/MS (Tandem) Spectrum is acquired with isotopes

- 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	C ₈ H ₆ O	C ₆ H ₄ Cl ₂ N	Dissociation	90	100	76	999	Even

Send C₈H₆O to Isotope Calc



***Live Demo* on YouTube**
LC/MS Unknown Identifications Using MSMS Libraries
NIST Structure Searches

Presentation References (*Internet Links*)

1. [James Little Mass Spectral Resource Website](#)
2. [NIST Search Software Detailed Manual](#)
3. [Chemical Ionization for MW Determination](#)
4. [Trimethylsilyl Derivatives for GC-MS](#)
5. [Methyl Ester Derivatives for GC-MS](#)
6. [SciFinder/ChemSpider and Accurate Mass LC-MS Data for Unknown ID's](#)
7. [Surfactant Identification](#)
8. [Lipid Matrix Ionization Effects in LC-MS](#)
9. [New Developments in the Modeling of Ion Fragmentation by MS Interpreter Software](#)
10. [Enhancements to NIST MS Interpreter for Modeling High Mass Accuracy Tandem Mass Spectra](#)
11. [NIST Tandem Quick Start Guide](#)
12. [NIST Flyer: New Hybrid Search and MS Interpreter for EI and Tandem Spectra](#)

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