LC/MS Unknown Identifications Using MSMS Libraries
Part IV: Importing MSMS Spectra

12/27/20

James Little
tvasailor@gmail.com
https://littlemsandsailing.wordpress.com/
Kingsport, TN

- *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,[3] Accurate Mass, Derivatization,[4,5] MS library management, SciFinder[6], Chemspider[6], 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

>50 Mass Specs Networked
Worldwide

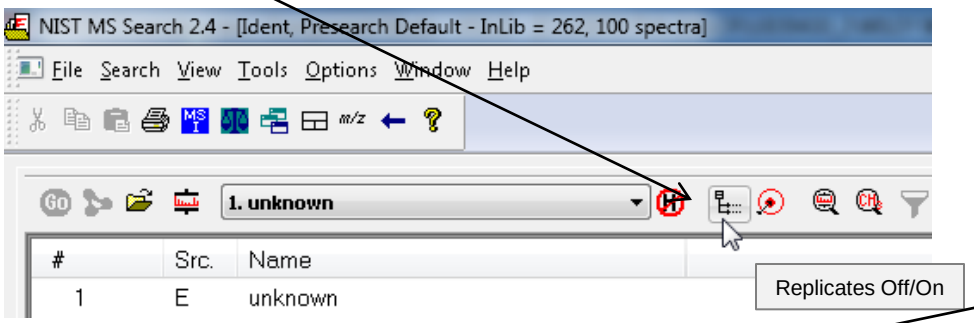
* https://en.wikipedia.org/wiki/Eastman_Chemical_Company

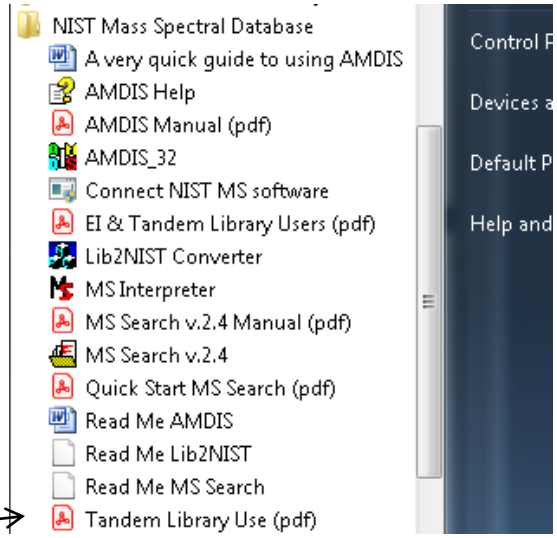
Table of Contents

Topic **Slide #**

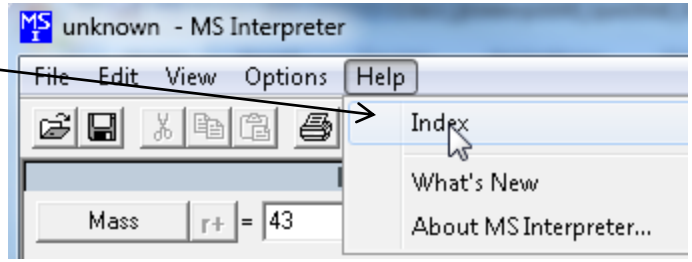
Help Files for NIST Search

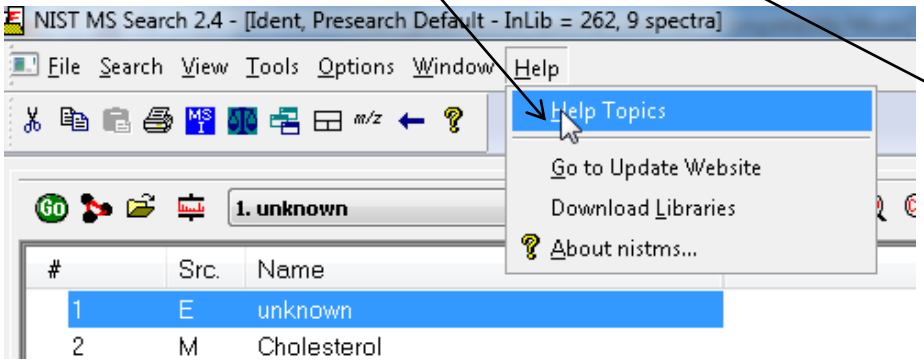
-"Hover" over Program Icon with mouse and function description displayed

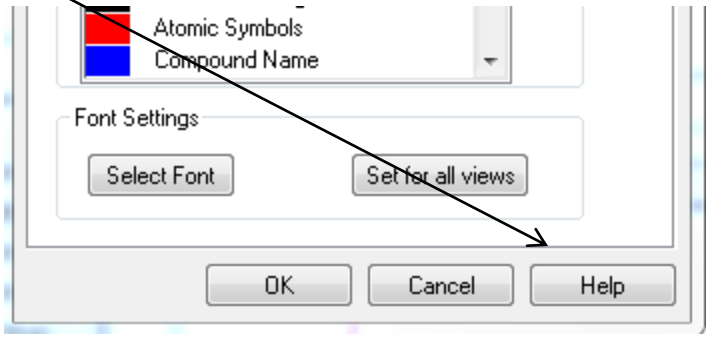




-***Detailed*** documentation for NIST Search[8]
-MS Interpreter included in NIST manual[8]
-Windows Program Group
-NIST Tandem Search ***Quick Start Guide***[9]
-"In program" assistance for both programs
-***Help button*** at corner of all windows



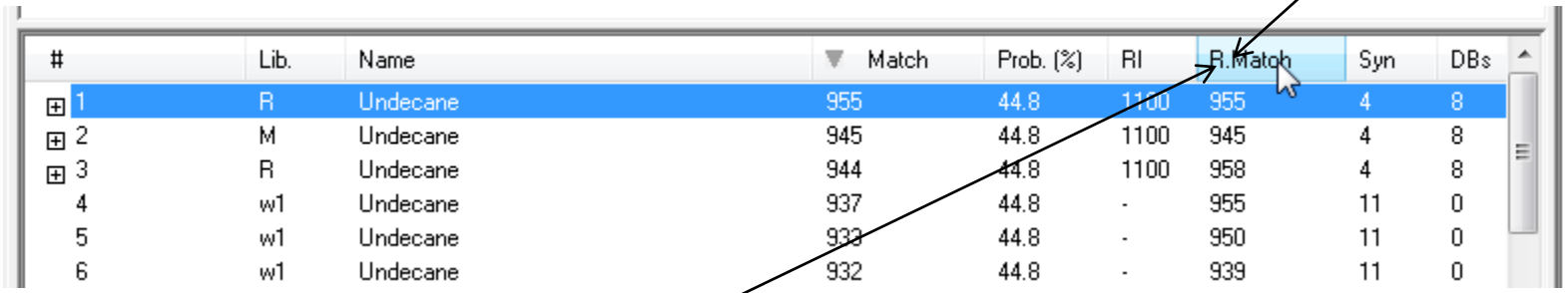




NIST Software in General is "Windows Compliant"

-***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	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

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!

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

Abbreviations and Keyboard Sequences

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

Part IV: Importing MSMS Spectra

Methods for Importing Spectra *Directly* in MSP Format to NIST Search

- The *easiest approach* is to export the spectrum to the NIST search *directly* in MSP format
- ***Simplified***[10] and ***Detailed***[11] instructions are supplied by NIST
- Approach also includes icon to return to the exporting program, Switch to Caller"

"Switch to Caller"

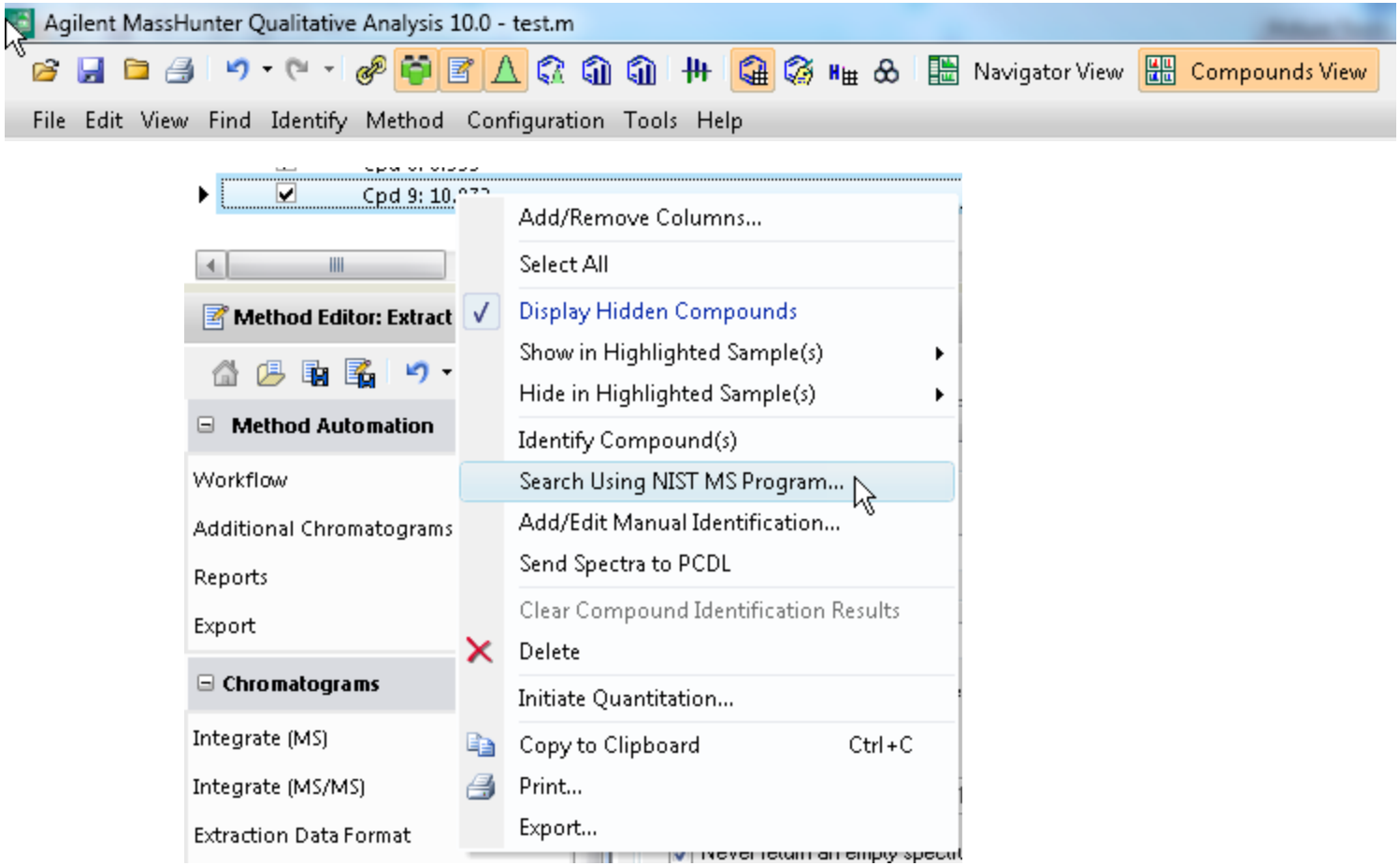
Mass Spec Manufacturers Use of NIST MS Search Software and/or NIST MSMS Libraries

- Some information difficult for me to confirm
- I have only used Thermo, Agilent, and Waters software
- ***Please*** let me know if incorrect!
- References (Internet links to documents) included at ***end*** of this handout

Company	NIST MSMS Libraries Internally	Export NIST Search Automatically MSP Format	Precursor m/z tag imported	Comments
Thermo Fisher Scientific	Yes	Yes	Yes	NIST tandem library included with all instrument ,purchases; Reference[13,14,16]
Agilent	No	Yes	Yes	Reference[12]
Waters Corporation	No	Yes	No	Reference[15]
Sciex	Yes	No	?	create merged spectrum of energies; exports to MGF format
OpenChrom	?	Yes	?	
Shimadzu	?	No	?	mzXML export
Bruker	Yes?	?	?	?
Perkin Elmer	No	No	No	

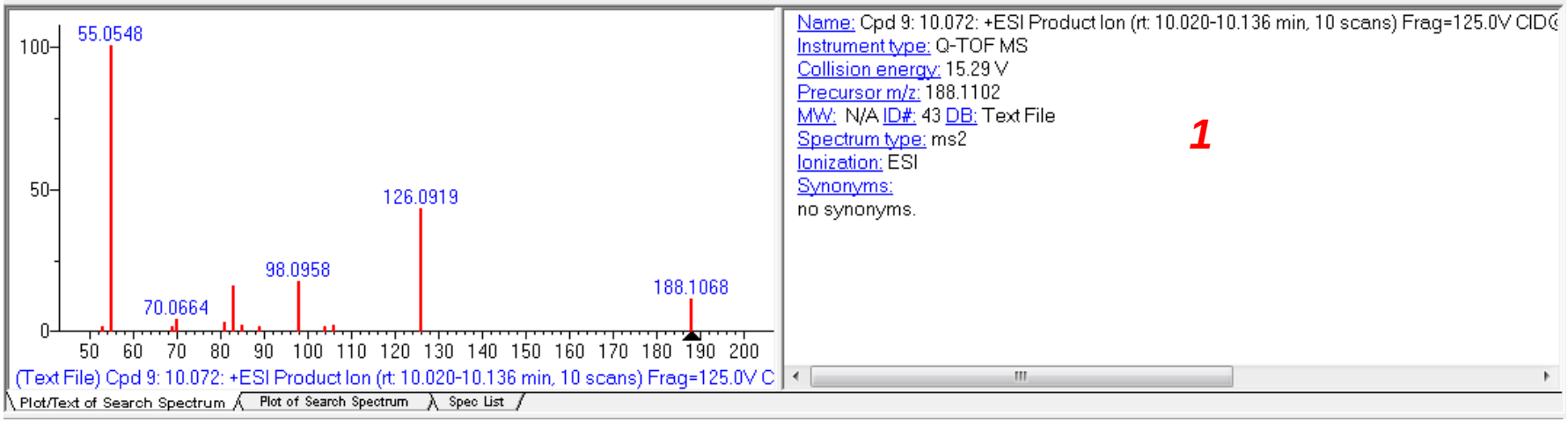
Example of Capability to Export MS/MS Spectra to NIST Search Program Agilent MassHunter

- Several companies do a good job of exporting files in MSP format to the NIST Search
- I currently only have access to Agilent MassHunter
- The program does a good job of exporting spectra in NIST MSP format to the NIST Search
- To perform, ***RMB*** to obtain pull down menu and select “Search Using NIST MS Program”



Agilent MassHunter Export of Spectrum to NIST Search

- Spectrum is ***automatically transferred*** and ***searched***
- Example of spectrum imported is shown (***1***)
- ***Very important*** for the "MSMS Hybrid" and "MSMS Identity" that the Precursor m/z value is included
- If not, the precursor m/z value would require ***manual insertion*** for each search
- Many values ***are included*** with the NIST library entries (***2***) in "MS/MS Hit List Filter Options" (***3)*** which are ***not found*** in spectra imported by mass spec companies



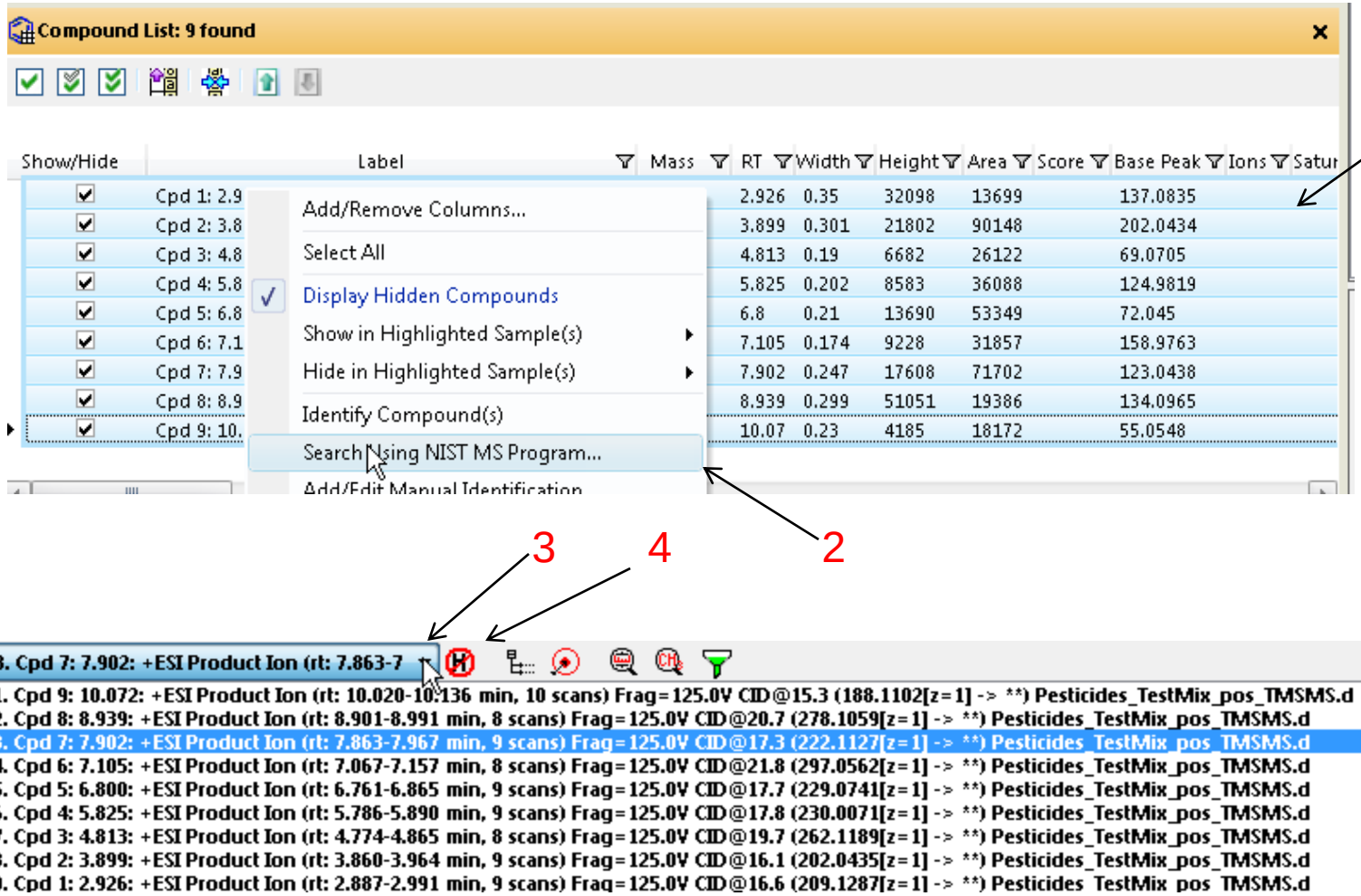
3

2

Name: 5-(3,4-Dichlorobenzyl)-1,3-thiazol-2-amine
Precursor type: [M+H]+
Instrument type: HCD
Collision energy: NCE=80% 41eV
Precursor m/z: 258.9858
Formula: $C_{10}H_8Cl_2N_2S$
MW: 258 Exact Mass: 257.978525 CAS#: 420102-86-9 NIST#: 1847875 ID#: 1 DB: hybrid_demo_3_unknowns
Other DBs: None
Comment: NIST Mass Spectrometry Data Center
Ion mode: P
Instrument: Thermo Finnigan Elite Orbitrap
Ionization: ESI
Collision gas: N2
Sample inlet: direct flow injection
Spectrum type: MS2

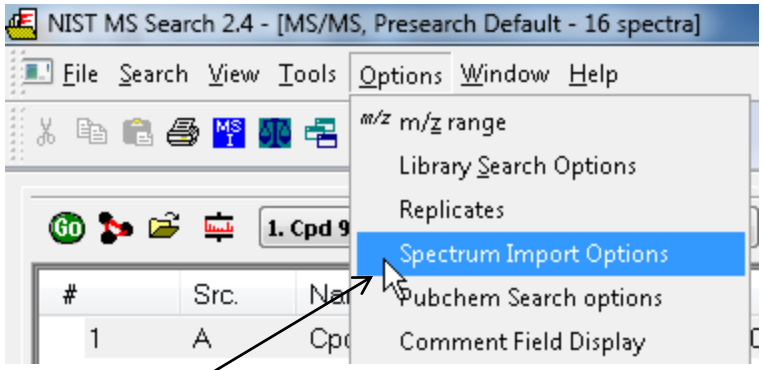
Agilent MassHunter "Multi-Export "of Spectra to NIST Search

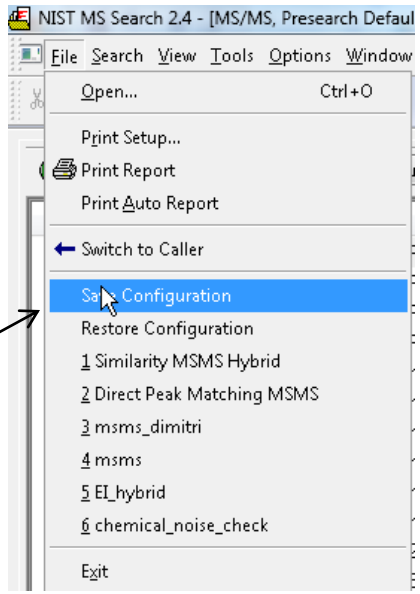
- More than one spectrum can be exported at a time
- Use the "**shift key LMB**" to select a group of spectra (1)
- Then ***RMB*** to obtain pull down menu and select "Search Using NIST MS Program" (2)
- The spectra are sent and the searches completed and stored in ***History Function*** of NIST for viewing (3)
- One can ***clear*** the history if necessary (4)
- One can ***AutoReport***, but I have not explored that capability, for assistance contact David Sparkman

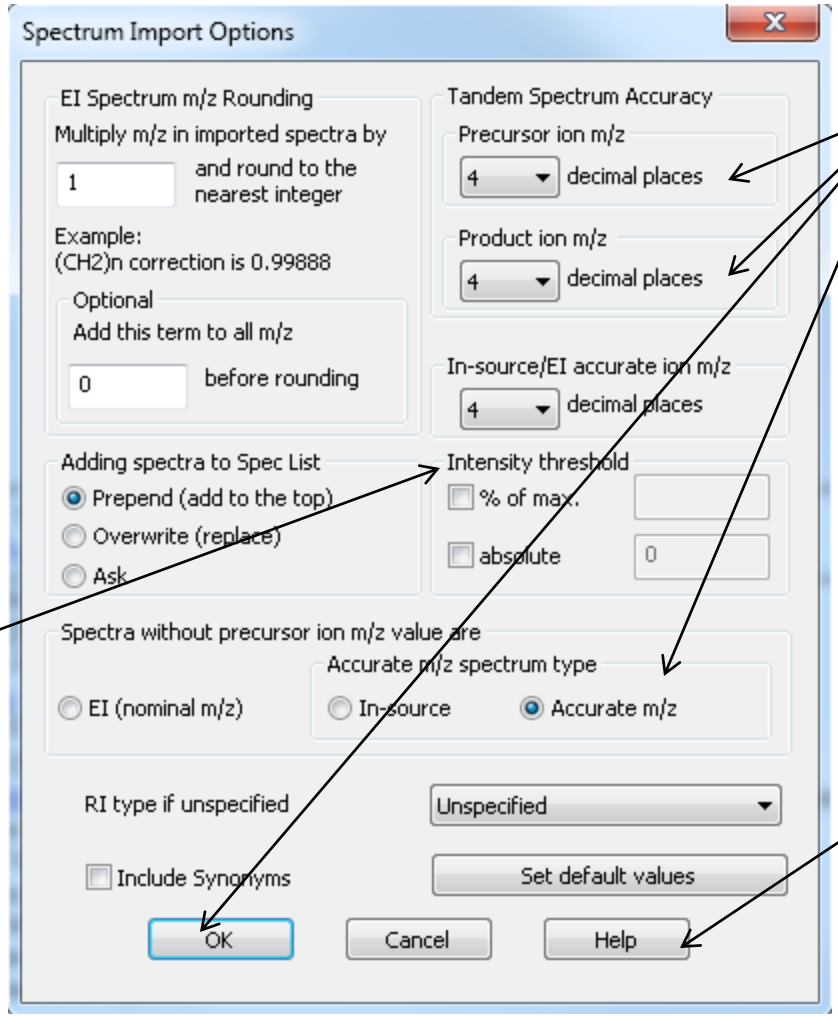


Importance of Proper Settings in NIST Search "Spectrum Import Options"

- ***LMB*** the Options/Spectrum Import Options on the toolbar (1)
- The typical settings for "*Accurate m/z spectrum type" and "decimal places" are selected (2)*
- *This must be done **before*** spectra are imported
- Useful information found in Help menu (3)
- Save with the configuration (4) with the MSMS search methods
- Intensity Threshold "% of max" or "absolute" very useful for filtering noisy spectra! (5), normally set with no filtering, filter as needed

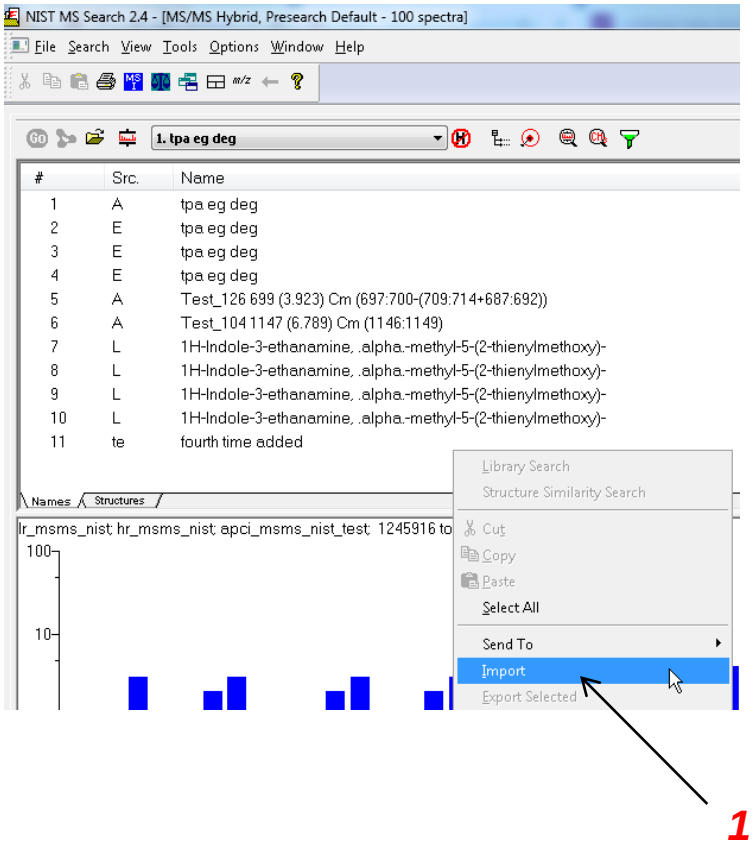


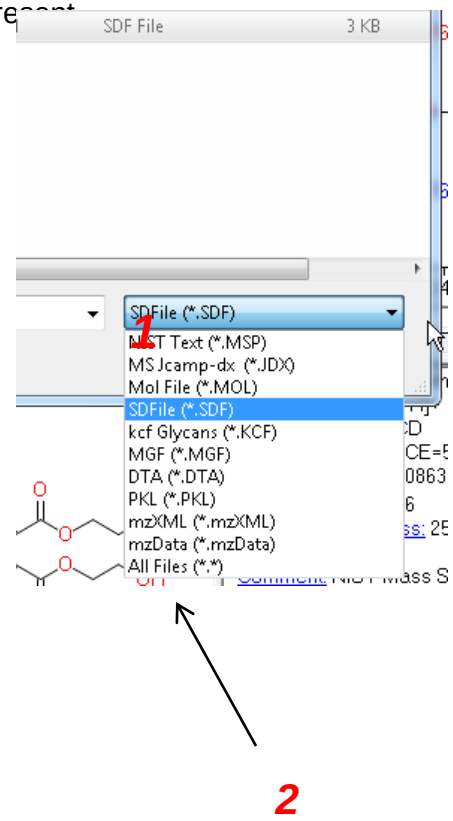


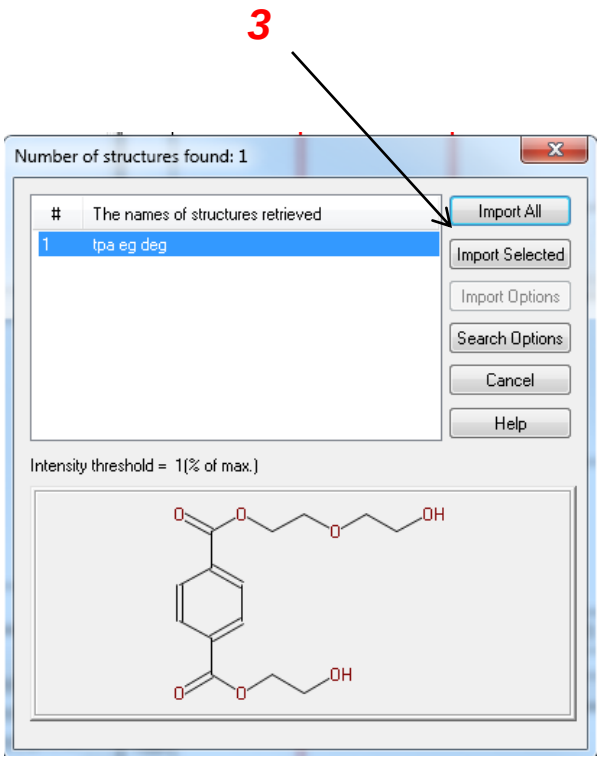


Importing Spectra Manually *Indirectly* in MSP and Other Formats

- Spectra can be imported in ***other formats*** besides MSP
- This ***indirect*** approach ***less convenient***
- Just ***RMB*** in the ***Spec List*** and ***LMB*** the ***Import*** Option (***1***)
- ***Pick*** the desired File ***format*** and the file of interest (***2***)
- ***Import Selected*** or ***Import All*** depending the file type (***3***)
- Items will be imported into Spec List window for processing
- ***In some ca***ses if not recognized format, possibly try ***All files?***
- ***SDF*** will import spectrum with associated structure if present

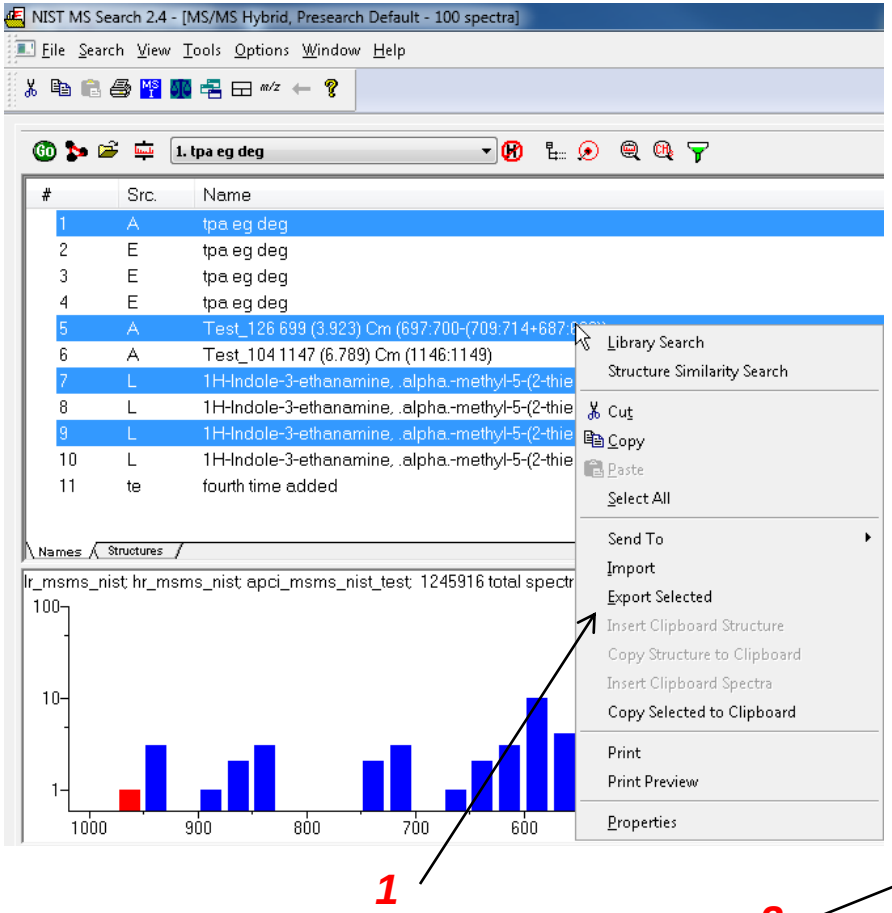


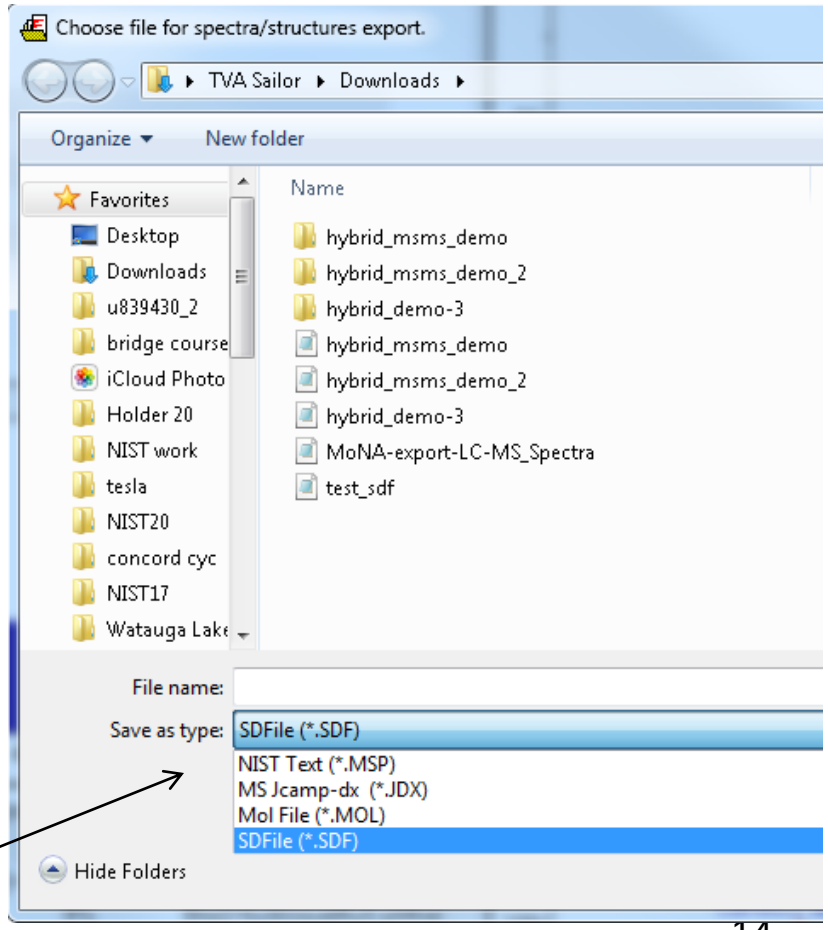




Exporting Spectra Manually in *MSP* and Other *Formats*

- Spectra can be exported in other formats besides MSP
- For example, a group of spectra can be exported in SDF as shown below
- Select a group or even one to be exported using ***Ctrl-LMB,*** then RMB on one of the spectra highlighted in blue
- ***LMB*** on ***Export Selected (1)***
- ***LMB*** to pick ***Save as type: (2)***
- ***SDF*** is a useful format because the associated ***structure is saved in same file as spectrum***





1

2

Example of Typical *.MSP of NIST Entry with Fields That Can be *Filtered After* Searching

```
Name: Bis(2-hydroxyethyl) phthalate
Ion_mode: P
Instrument: Orbitrap Fusion Lumos
Instrument_type: HCD
Ionization: ESI
Collision_energy: NCE=5%
Collision_gas: N2
Sample_inlet: direct flow injection
Spectrum_type: MS2
Precursor_type: [M+H]+
PrecursorMZ: 255.0863
Notes: micromol/L in water/acetonitrile/formic acid (50/50/0.1);
Vial_ID=31887 Spec=Consensus Nreps=31/31 Mz_diff=2.4ppm
Data_source:Met_Lumos_2019_11b_FTMSn~1129
InChIKey: CAKVXHUYTFYBPK-UHFFFAOYSA-N
Synon: Benzene-1,2-dicarboxylic acid bis(2-hydroxyethyl) ester
Synon: 1,2-Benzenedicarboxylic acid, 1,2-bis(2-hydroxyethyl)
ester
Formula: C12H14O6
MW: 254
ExactMass: 254.079039
CAS#: 84-73-1;  NIST#: 3268373
DB#: 856832
Comments: NIST Mass Spectrometry Data Center
Num Peaks: 10
149.0237 999.00 "C8H5O3=p-C4H10O3/2.6ppm 31/31"
150.0271 59.54 "C8H5O3+i=p-C4H10O3+i/2.6ppm 31/31"
193.0501 664.83 "C10H9O4=p-C2H6O2/2.9ppm 31/31"
194.0534 51.35 "C10H9O4+i=p-C2H6O2+i/2.4ppm 31/31"
229.0092 14.19 "? 16/31"
237.0764 504.10 "C12H13O5=p-H2O/2.7ppm 31/31"
238.0798 48.65 "C12H13O5+i=p-H2O+i/2.7ppm 31/31"
255.0253 7.69 "? 15/31"
255.0866 2.70 "p/1.1ppm 23/31"
255.9530 23.88 "? 31/31"
```

Live Demo on YouTube
LC/MS Unknown Identifications Using MSMS Libraries
Part IV: Importing MSMS Spectra

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. *NIST Tandem Quick Start Guide*
10. *Approach for Importing MSMS Spectra to NIST Search: Simple Description*
11. *Approach for Importing MSMS Spectra to NIST Search: Detailed Description*
12. *Agilent MassHunter Importing to NIST*
13. *Thermo Fisher Scientific FreeStyle Brief Importing to NIST*
14. *Thermo Fisher Scientific FreeStyle Detailed Importing to NIST*
15. *Waters Corporation Masslynx Importing to NIST*
16. *Thermo Fisher Scientific TraceFinder Importing to NIST*

Acknowledgements

- David Sparkman (NIST consultant)
- Stephen Stein (NIST)
- Emma Rennie (Agilent)
- Andrew McEachran (Agilent)
- David Weil (Agilent)
- Aurelie Marcotte (Waters Corporation)
- Josef Ruzicka (Thermo Fisher Scientific)