

LC/MS Unknown Identifications Using MSMS Libraries

Part IV: Importing MSMS Spectra

12/27/20

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- *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,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



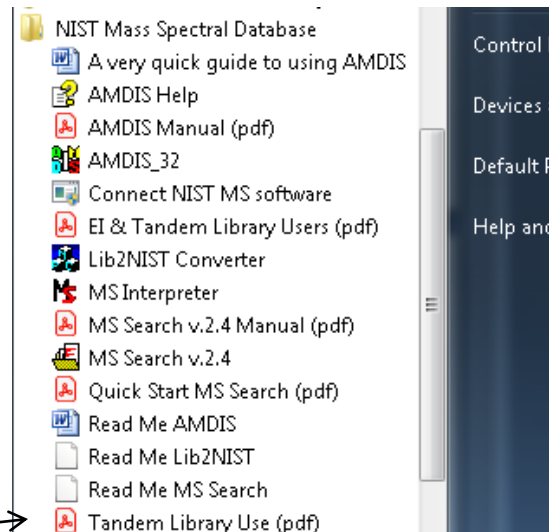
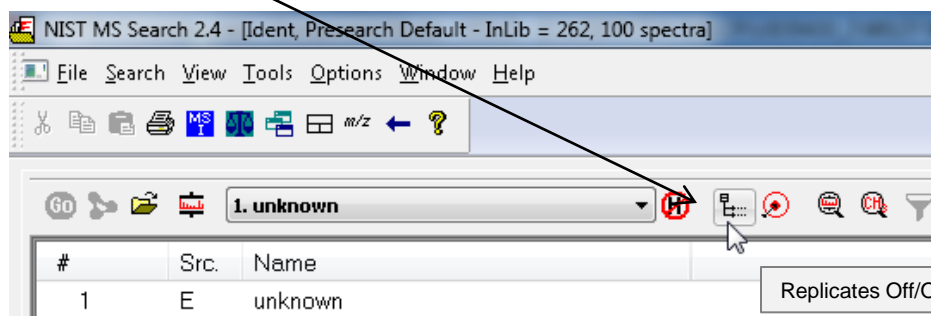
>50 Mass Specs Networked
Worldwide

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Help Files for NIST Search

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



- **Detailed** documentation for NIST Search⁸

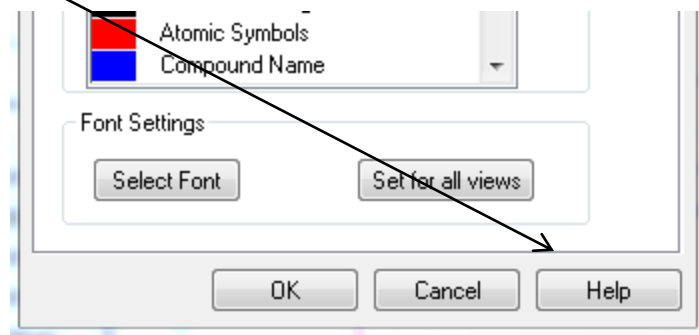
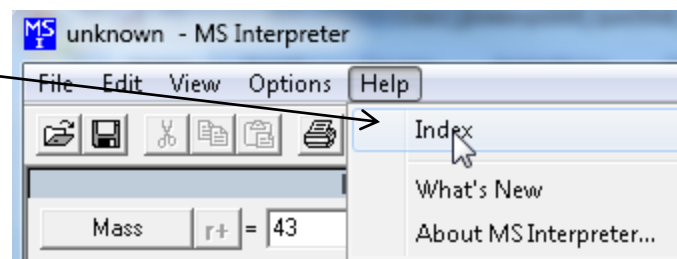
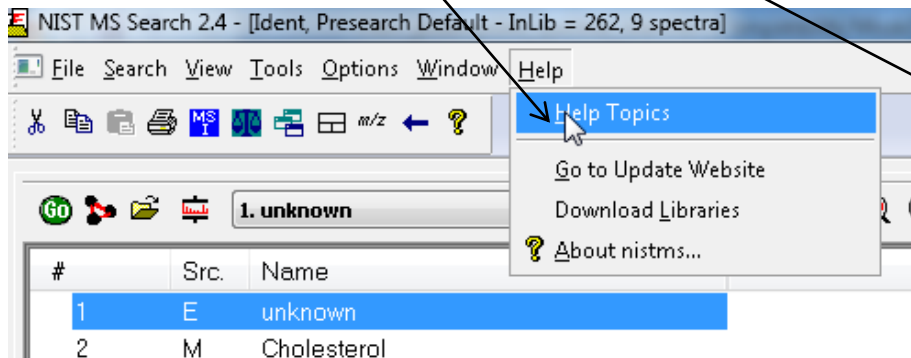
- MS Interpreter included in NIST manual⁸

- 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	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

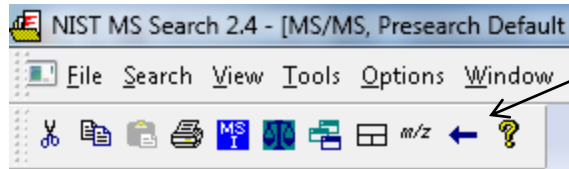
Abbreviations and Keyboard Sequences

- 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
- **Simplified¹⁰** and **Detailed¹¹** instructions are supplied by NIST
- Approach also includes icon to return to the exporting program, Switch to Caller”



“Switch to Caller”

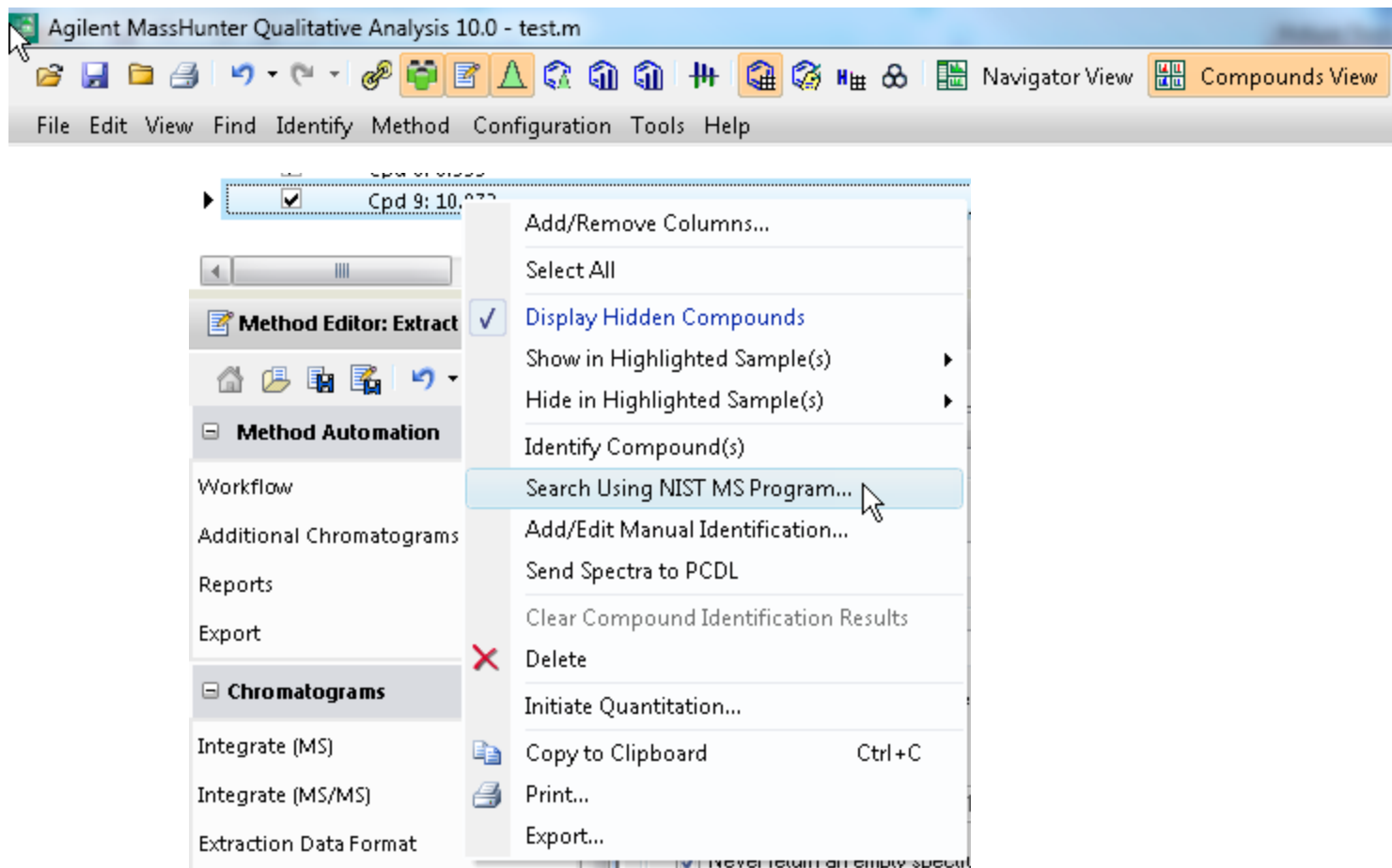
Mass Spec Manufacturers Use of NIST MS Search Software and/or NIST MSMS Libraries

- 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	

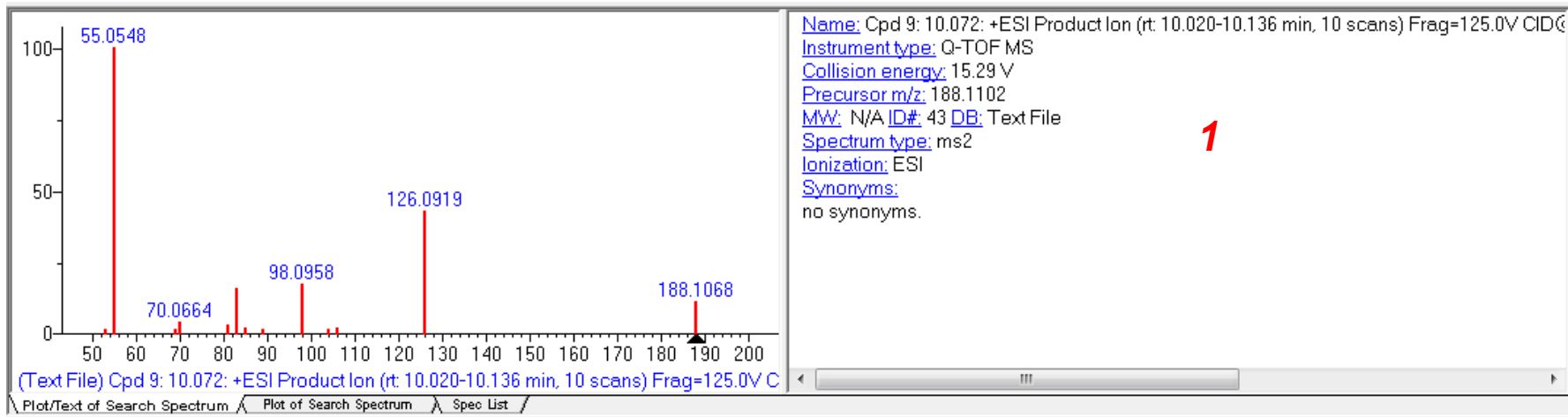
Example of Capability to Export MS/MS Spectra to NIST Search Program Agilent MassHunter

- 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: C₁₀H₈Cl₂N₂S
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

Compound List: 9 found

Show/Hide	Label	Mass	RT	Width	Height	Area	Score	Base Peak	Ions	Satur
<input checked="" type="checkbox"/>	Cpd 1: 2.9	Add/Remove Columns...	2.926	0.35	32098	13699		137.0835		
<input checked="" type="checkbox"/>	Cpd 2: 3.8	Select All	3.899	0.301	21802	90148		202.0434		
<input checked="" type="checkbox"/>	Cpd 3: 4.8	Display Hidden Compounds	4.813	0.19	6682	26122		69.0705		
<input checked="" type="checkbox"/>	Cpd 4: 5.8	Show in Highlighted Sample(s)	5.825	0.202	8583	36088		124.9819		
<input checked="" type="checkbox"/>	Cpd 5: 6.8	Hide in Highlighted Sample(s)	6.8	0.21	13690	53349		72.045		
<input checked="" type="checkbox"/>	Cpd 6: 7.1	Identify Compound(s)	7.105	0.174	9228	31857		158.9763		
<input checked="" type="checkbox"/>	Cpd 7: 7.9	Search Using NIST MS Program...	7.902	0.247	17608	71702		123.0438		
<input checked="" type="checkbox"/>	Cpd 8: 8.9	Add/Edit Manual Identification	8.939	0.299	51051	19386		134.0965		
<input checked="" type="checkbox"/>	Cpd 9: 10.0		10.07	0.23	4185	18172		55.0548		

3. Cpd 7: 7.902: +ESI Product Ion (rt: 7.863-7.967 min, 9 scans) Frag=125.0V CID@17.3 (222.1127[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

1. Cpd 9: 10.072: +ESI Product Ion (rt: 10.020-10.136 min, 10 scans) Frag=125.0V CID@15.3 (188.1102[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

2. Cpd 8: 8.939: +ESI Product Ion (rt: 8.901-8.991 min, 8 scans) Frag=125.0V CID@20.7 (278.1059[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

4. Cpd 6: 7.105: +ESI Product Ion (rt: 7.067-7.157 min, 8 scans) Frag=125.0V CID@21.8 (297.0562[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

5. Cpd 5: 6.800: +ESI Product Ion (rt: 6.761-6.865 min, 9 scans) Frag=125.0V CID@17.7 (229.0741[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

6. Cpd 4: 5.825: +ESI Product Ion (rt: 5.786-5.890 min, 9 scans) Frag=125.0V CID@17.8 (230.0071[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

7. Cpd 3: 4.813: +ESI Product Ion (rt: 4.774-4.865 min, 8 scans) Frag=125.0V CID@19.7 (262.1189[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

8. Cpd 2: 3.899: +ESI Product Ion (rt: 3.860-3.964 min, 9 scans) Frag=125.0V CID@16.1 (202.0435[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

9. Cpd 1: 2.926: +ESI Product Ion (rt: 2.887-2.991 min, 9 scans) Frag=125.0V CID@16.6 (209.1287[z=1] -> **) Pesticides_TestMix_pos_TMSMS.d

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

The image displays the NIST MS Search 2.4 software interface. On the left, the 'Options' menu is open, with 'Spectrum Import Options' highlighted. A red arrow labeled '1' points to this menu item. Below it, the 'Save Configuration' option is highlighted, with a red arrow labeled '4' pointing to it. On the right, the 'Spectrum Import Options' dialog box is shown. Red arrows and numbers point to various settings: '2' points to the 'Tandem Spectrum Accuracy' section where 'Precursor ion m/z' and 'Product ion m/z' are both set to 4 decimal places; '3' points to the 'Help' button; '5' points to the 'Intensity threshold' section where '% of max.' is selected; and another '2' points to the 'Accurate m/z spectrum type' section where 'Accurate m/z' is selected.

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 cases** if not recognized format, possibly try **All files?**
- **SDF** will import spectrum with associated structure if present

NIST MS Search 2.4 - [MS/MS Hybrid, Presearch Default - 100 spectra]

File Search View Tools Options Window Help

Go 1. tpa eg deg

#	Src.	Name
1	A	tpa eg deg
2	E	tpa eg deg
3	E	tpa eg deg
4	E	tpa eg deg
5	A	Test_126 699 (3.923) Cm (697:700-(709:714+687:692))
6	A	Test_104 1147 (6.789) Cm (1146:1149)
7	L	1H-Indole-3-ethanamine, .alpha.-methyl-5-(2-thienylmethoxy)-
8	L	1H-Indole-3-ethanamine, .alpha.-methyl-5-(2-thienylmethoxy)-
9	L	1H-Indole-3-ethanamine, .alpha.-methyl-5-(2-thienylmethoxy)-
10	L	1H-Indole-3-ethanamine, .alpha.-methyl-5-(2-thienylmethoxy)-
11	te	fourth time added

Library Search
Structure Similarity Search

- Cut
- Copy
- Paste
- Select All
- Send To
- Import**
- Export Selected

SDF File 3 KB

- SDFile (*.SDF)
- NIST Text (*.MSP)
- MS Jcamp-dx (*.JDx)
- Mol File (*.MOL)
- SDFile (*.SDF)
- kcf Glycans (*.KCF)
- MGF (*.MGF)
- DTA (*.DTA)
- PKL (*.PKL)
- mzXML (*.mzXML)
- mzData (*.mzData)
- All Files (*.*)

Number of structures found: 1

#	The names of structures retrieved
1	tpa eg deg

Import All
Import Selected
Import Options
Search Options
Cancel
Help

Intensity threshold = 1(% of max.)

CCOC(=O)c1ccc(cc1)OCCOC

1

2

3

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**

#	Src.	Name
1	A	tpa eg deg
2	E	tpa eg deg
3	E	tpa eg deg
4	E	tpa eg deg
5	A	Test_126 699 (3.923) Cm (697.700-(709.714+687.714))
6	A	Test_104 1147 (6.789) Cm (1146:1149)
7	L	1H-Indole-3-ethanamine, .alpha.-methyl-5-(2-thienyl)-
8	L	1H-Indole-3-ethanamine, .alpha.-methyl-5-(2-thienyl)-
9	L	1H-Indole-3-ethanamine, .alpha.-methyl-5-(2-thienyl)-
10	L	1H-Indole-3-ethanamine, .alpha.-methyl-5-(2-thienyl)-
11	te	fourth time added

File name:

Save as type: SDFFile (*.SDF)

- NIST Text (*.MSP)
- MS Jcamp-dx (*.JDX)
- Mol File (*.MOL)
- SDFFile (*.SDF)

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)