

Creating MS/MS User Libraries in NIST26

Video/Handout

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Creating User Libraries in NIST26

Using the built-in Library Editor for LC-MS/MS

 Tips for easier entry of parameters and sample history

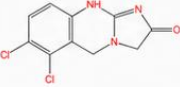
 mzinterpretation.com

Time (min)

NIST MS Search 4.0 (Any mode) - [Librarian]

File Search View Tools Options Window Help

#	Src	MW	Formula	Name
1	lib	255	C10H7	Anagrelide
2	A	0		Checkout_TestMc_AMSMS.1649.1...
3	A	0		+ESI Production (rt 5.426-5.521 min...



Mass spectrum plot showing relative intensity vs m/z. Key peaks labeled: 55.0288, 61.9789, 75.0226, 89.0382, 100.0178, 106.961, 124.0444, 137.0023, 151.0180, 164.0007, 173.9682, 200.9790, 212.9613.

(hr_msms_nist#2) Anagrelide [M+H-CO+2]+ HCD 90% P=230

Name: Anagrelide
Precursor type: [M+H-CO+2]+
Instrument type: HCD
Collision energy: NCE=90% 41eV
Precursor m/z: 230.0861
Formula: C10H7Cl2N3O
MW: 255 Exact Mass: 254.996617 CAS#: 68475-42-3 NIST#: 419612 ID#: 385401 DB: hr_msms_nist#2
Comment: NIST Mass Spectrometry Data Center
Related CAS#: 58579514
Ion mode: P
Instrument: Thermo Finnigan Elite Orbitrap
Ionization: ESIu
Collision gas: N2
Sample inlet: direct flow injection
Spectrum type: MS2
Notes: m/z(formol)(L in water/acetone/nitrile/formic acid (50/50/0.1); Spec+Consensus Nreps=24/24 Mz_diff=2.2ppm Mz_exact_mono=228.00888 Via_ID=44680 Data_source=Cla_r_uw:Elite_2623_isotopesr=2021 FFW_P2_isotopes
InChIKey: QTRXOFAQVRKIND-UHFFFAOYSA-N Non-steroid
12 m/z Values and Intensities:

m/z	Rel. Int.	Ref. m/z	Ref. Rel. Int.	Ref. Date
55.0276	1.9	12/24	7.1	24/24
61.9789	2.0	24/24	1.4	16/24
75.0226	3.3	24/24	57.2	24/24
89.0382	7.1	24/24	18.1	24/24
90.0315	1.4	16/24	13.1	16/24
100.0178	57.2	24/24	100.0	24/24
106.961	17.1	16/24	93.3	24/24
124.0444	17.1	16/24	93.3	24/24
137.0023	17.1	16/24	93.3	24/24
151.0180	18.1	24/24	18.1	24/24
164.0007	13.1	16/24	13.1	16/24
173.9682	100.0	24/24	100.0	24/24
200.9790	93.3	24/24	93.3	24/24

Names Structures Lib Search Other Search Names Compare Librarian Chromatogram

Accurate Identification | LC-MS/MS Confidence | High Quality Libraries | Organized & Reusable

See **Full Course** on NIST26 with new **Integrated** Deconvolution/Library Searching for **LC-MS/MS**!

[link to course](#)

Value of User LC-MS/MS Libraries in NIST26

- Usually smaller than public or commercial libraries
- Often contain **unique, highly relevant compounds** not found elsewhere
- May include:
 - Proprietary products
 - Metabolites
 - Intermediates
 - Impurities and degradation products
 - Biomarkers
- Can provide **important identifications** in:
 - Industry
 - R&D
 - Pharmaceutical and medical labs
 - Environmental and other specialized fields

Library size is not the same as library value — a small, highly relevant user library may contain exactly the compounds that matter most to that laboratory.

Steps for Library Entry

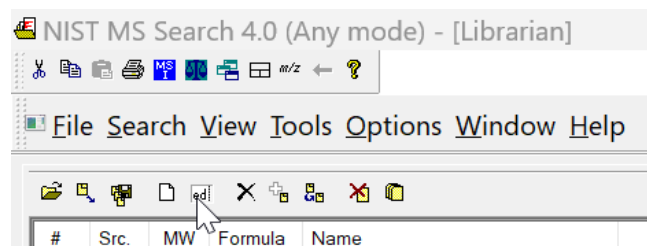
- Spectra from Vendor Software “Send to Lib Search” approach using proper Spectrum Import Options
- Or, Spectra as from Chromatogram Tab sent to Lib Search Tab in NIST26
- Structures from Drawing Program via windows clipboard
- Standard parameters in notepad document
- Sample history in notepad
- Can use tags if desirable

Table of User LC-MS/MS Tags in NIST26

Syn.tag	MSP file tag	MS Search display
\$:00	Spectrum_type	Spectrum type
\$:01	Compound_type	Compound type
\$:02	Ion_name	Ion name
\$:03	Precursor_type	Precursor type
\$:04	PrecursorMZ	Precursor m/z
\$:05	Collision_energy	Collision energy
\$:06	Instrument_type	Instrument type
\$:07	Instrument	Instrument
\$:08	Special_fragmentation	Special fragmentation
\$:09	Sample_inlet	Sample inlet
\$:10	Ionization	Ionization
\$:11	Ion_mode	Ion mode
\$:12	Collision_gas	Collision gas
\$:13	Pressure	Pressure
\$:14	Mass_range	Mass range
\$:15	Maximum_intensity	Maximum intensity
\$:16	Cone_voltage	Cone voltage
\$:17	AUX	AUX
\$:18	Link	Link <= never displayed
\$:19	Ion_Formula	Ion Formula
\$:20	Ion_MW	Ion MW
\$:21	Charge	Charge
\$:22	Salt	Salt
\$:23	Known_impurity	Known impurity
\$:24	Related_CAS#	Related CAS#
\$:25	Salt/mix_CAS#	Salt/Mix CAS#
\$:26	Peptide_sequence	Peptide sequence
\$:27	Peptide_mods	Peptide mods

Inserting Values of Tags with Library Entry Manually

- “Edit Spectrum” in “Librarian” Tab
- Select “Additional Info”
- Enter in information one at a time



Spectrum Information

Name:

Formula: From structure

Mol. Weight: CAS Number:

Library: Spec. List

ID Number: 1

RI: Edit RI

Other Names (Synonyms):

Additional Info

Comments



Spectrum Info

accurate m/z

Q-TOF MS

ESI

Spectrum type

Instrument type

Ionization

Compound type

Ion name

Precursor type

Precursor m/z

Collision energy

Instrument

Special fragmentation

Sample inlet

Ion mode

Collision gas

Pressure

Mass range

Maximum intensity

In-source voltage

Notes

Ion Formula

Ion MW

Charge

Salt

Known impurity

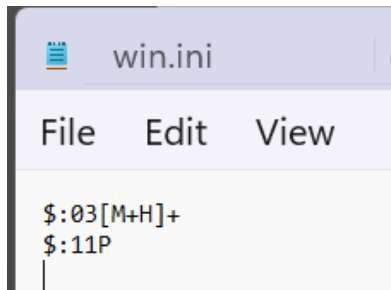
Related CAS#

OK

Cancel

Inserting Values of Tags with Library Entry as a Group

- Can paste them into the synonym field as a group, then “Add to List” to insert them into table
- Enter ones NOT imported from Vendor Software
- For mine from Agilent MassHunter, see to the right
- If you add ones already there will get duplicate
- Can keep a list of different ones in Notepad, then copy and paste, see typical example list to right
- Example in video, only needed ion adduct and polarity, all rest supplied by MassHunter export to NIST



\$:03[M+H]+
\$:11P

Name: +ESI Product Ion (rt: 5.461 min) Frag=150.0V CID@26.3 (372.1577[z=1] -> **) ForTox_TestMix_AMSMS.d
Instrument type: Q-TOF MS
Collision energy: 26.33 V
Precursor m/z: 372.1577
MW: N/A ID#: 29 DB: Text File
Spectrum type: ms2
Ionization: ESI
406 m/z Values and Intensities:

40.9318	0.15	46.9737	0.20	51.0196	0.57	54.0349	0.43	56.0530	0.40
66.0374	0.41	70.0666	1.44	77.0395	0.57	79.0341	0.66	79.1200	0.16

\$:07Thermo Exploris 120
 \$:0515,30,45,60,75
 \$:03[M-H]-
 \$:11N

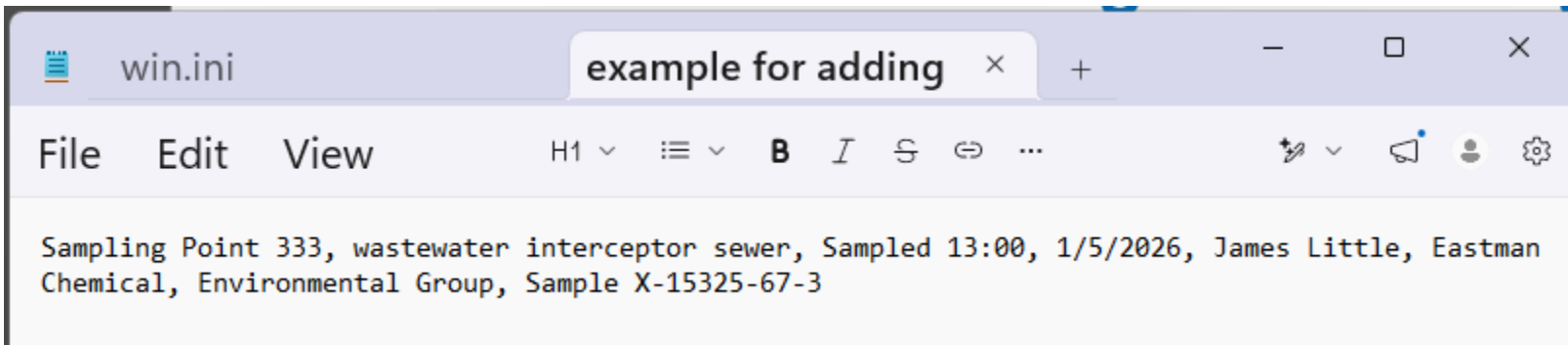
\$:07Thermo Exploris 120
 \$:0515,30,45,60,75
 \$:03[M+H]+
 \$:11P

\$:07Thermo Exploris 120
 \$:0515,30,45,60,75
 \$:03[M+NH4]+
 \$:11P

\$:07Thermo Exploris 120
 \$:0515,30,45,60,75
 \$:03[M+Na]+
 \$:11P

Inserting Comments

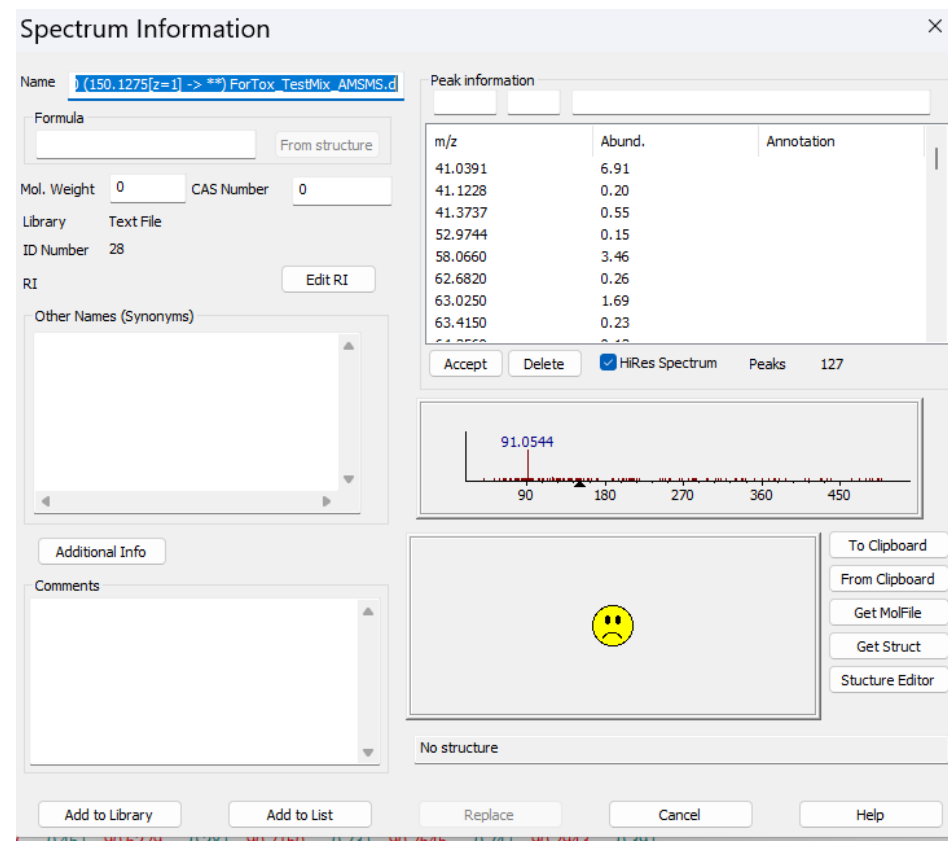
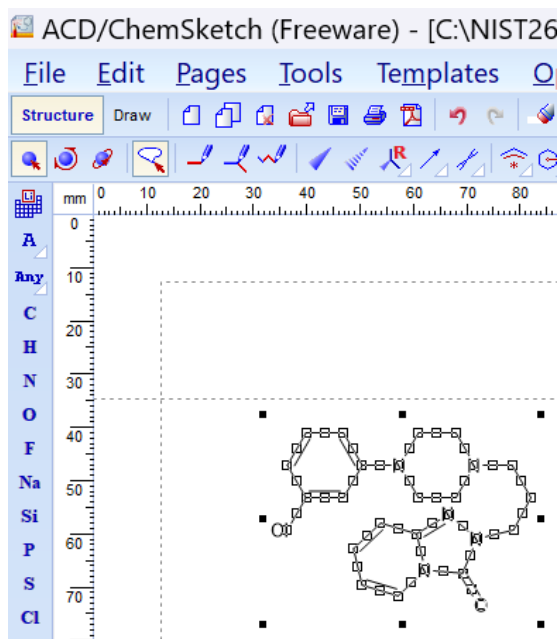
- When doing groups of samples, easier to paste in information for multiple spectra entry
- Create the comment in Notepad
- Copy and paste to Comment Field



Sampling Point 333, wastewater interceptor sewer, Sampled 13:00, 1/5/2026, James Little, Eastman Chemical, Environmental Group, Sample X-15325-67-3

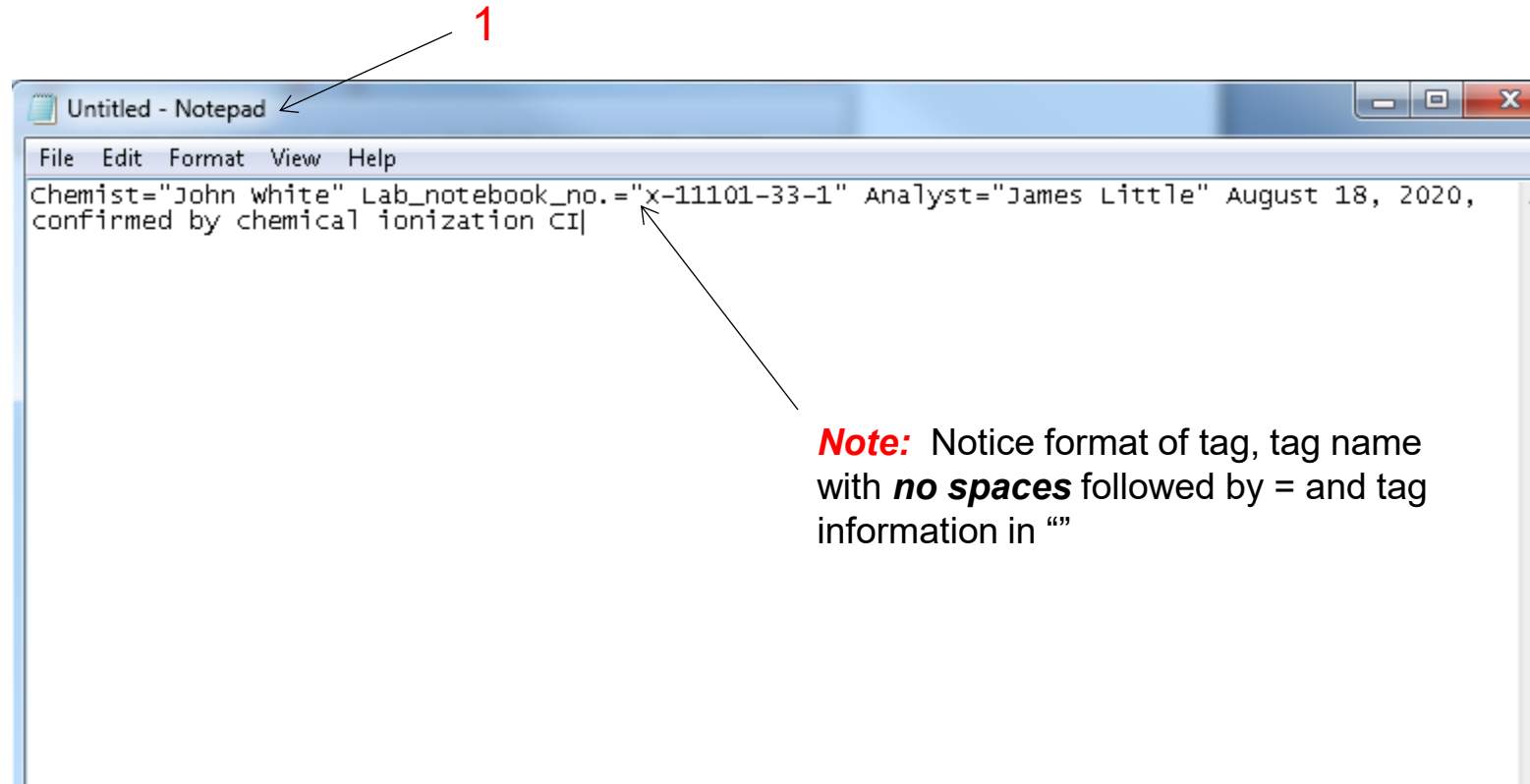
Drawing Program

- Draw from scratch in drawing program
- If possible, find one similar in NIST Search program and right click on entry and “Send to Default Structure Editor”
- Can edit to make necessary changes
- After completed, circle structure of interest in drawing program
- Copy into Windows clipboard
- Return to “Edit Spectrum” in Librarian Window
- Select “From Clipboard”
- Calculate MW and MF using “From Clipboard” button



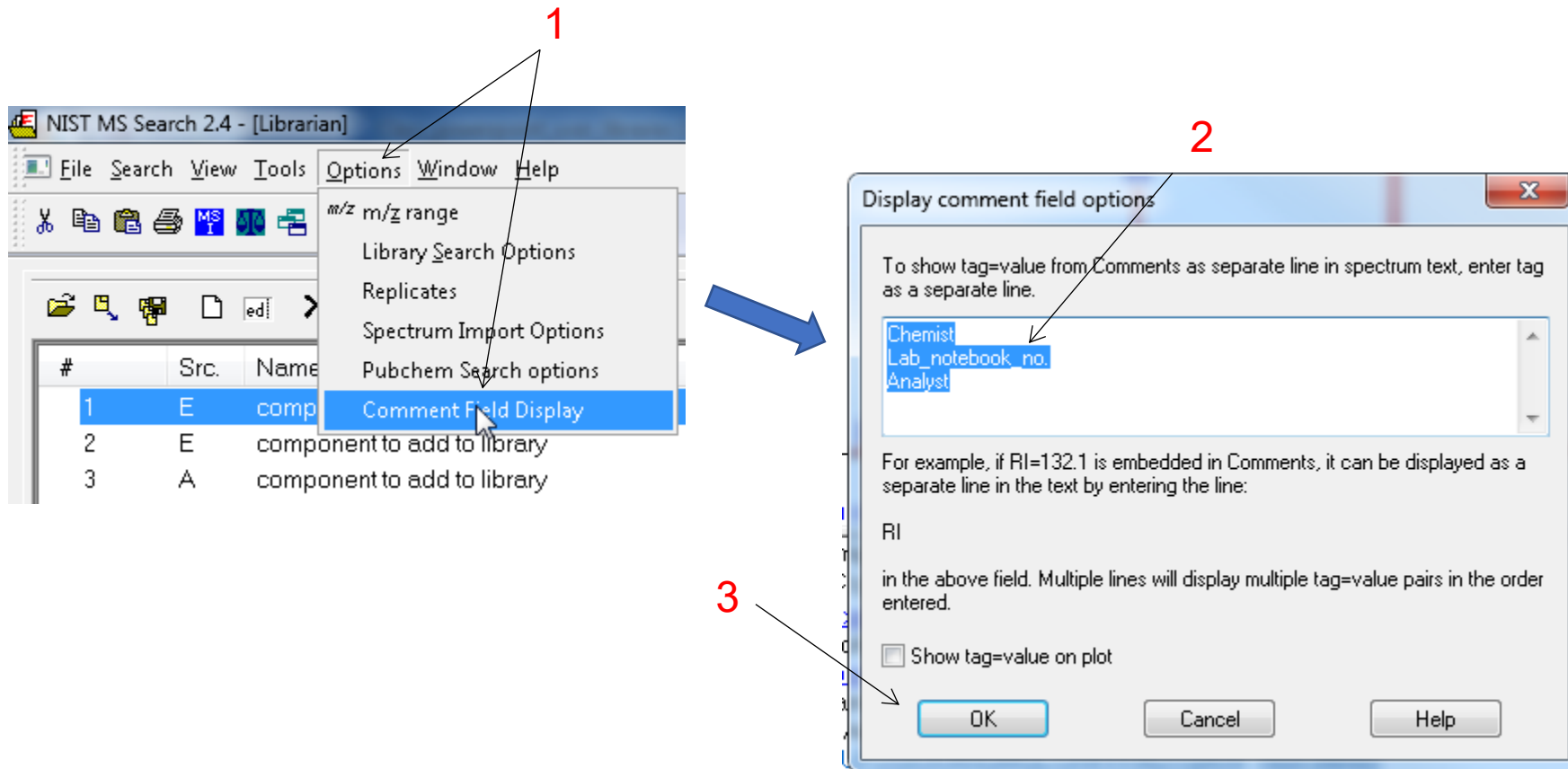
Creating a User Library: Using Windows Notepad and Tags

- 1) **Tip:** If adding the same comment many times to different spectra, **create in Windows Notepad** and paste into Spectrum Information Window using **Ctrl V** on keyboard
- 2) My Tags below are chemist, lab_notebook_no., and Analyst
- 3) When ultimately displayed, they will show up as **separate line items** and are removed from the Comments display



Creating a User Library: Comment Field Display with User Tags

- 1) **LMB** on Options, Comment Field Display
- 2) Add the **user defined Tags** Chemist, Lab_notebook_no., and Analyst to **Display comment field options** window
- 3) **LMB OK** to accept values



Creating a User Library: Comment Field Display Showing User Tags

- 1) User library comments **without** Tags
- 2) User library comments **with** Tags
- 3) The InChIKey and Estimated retention index automatically added by NIST program

1

Name: component to add to library
Formula: C₁₀H₈BrF₄NO
MW: 313 Exact Mass: 312.972538 ID#: 6 DB: Spec. Edit
Comment: Chemist="John White" Lab_notebook_no.="x-11101-33-1" Analyst="James Little" August 18, 2020, confirmed by chemical ionization CI
InChIKey: IUFOQIOKANQMQL-UHFFFAOYSA-N Non-stereo
Synonyms:
1.PM 2201 derivative
2.TFA derivative of PM 2201
3.Morrison's Amine, TFA derivative

Estimated non-polar retention index (n-alkane scale):
Value: 1422 iu
Confidence interval (Diverse functional groups): 89(50%) 382(95%) iu

2

Name: component to add to library
Formula: C₁₀H₈BrF₄NO
MW: 313 Exact Mass: 312.972538 ID#: 6 DB: Spec. Edit
Chemist: John White
Lab_notebook_no.: x-11101-33-1
Analyst: James Little
Comment: August 18, 2020, confirmed by chemical ionization CI
InChIKey: IUFOQIOKANQMQL-UHFFFAOYSA-N Non-stereo
Synonyms:
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Low Resolution Libraries

- Previous slides describe creating a high-resolution library
- Low-resolution libraries can also be very useful
- Examples would be spectra from in-source collision from a single quadrupole
- MS/MS spectra from a triple quadrupole, etc.
- Best to keep a separate user libraries of low-resolution and high-resolution spectra
- Video/Handout Available on searching low resolution libraries and low-resolution spectra
- [See link](#)
- Low-resolution spectra yield very good search results

Software Bug: Intensity Threshold Bug in Spectrum Import Options Window

- When any Intensity Threshold is used, the imported spectra from Vendor's Software are nominal
- Should be top spectrum with 4 digits to right of decimal
- Instead, nominal as shown on bottom spectrum

Spectrum Import Options

EI Spectrum m/z Rounding
Multiply m/z in imported spectra by and round to the nearest integer
Example: (CH₂)_n correction is 0.99888
Optional
Add this term to all m/z before rounding

Tandem Spectrum Accuracy
Precursor ion m/z decimal places
Product ion m/z decimal places
HiRes No Precursor ion m/z decimal places

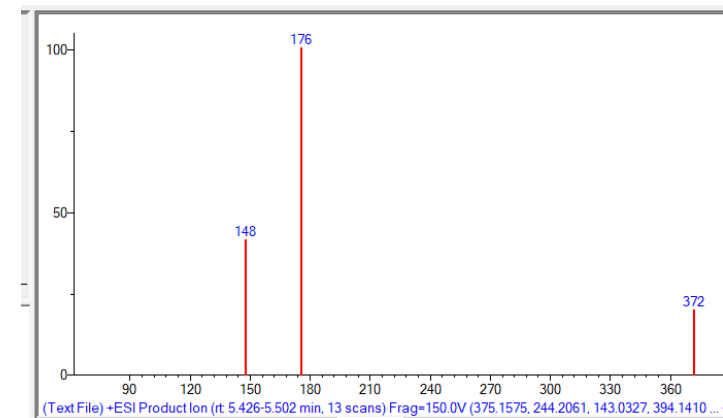
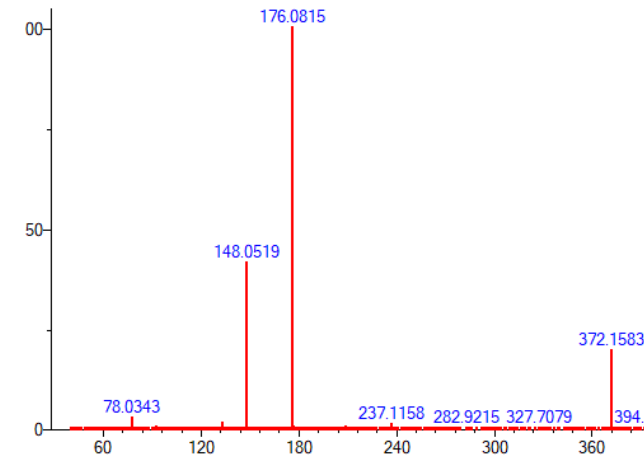
Adding spectra to Spec List
☒ Prepend (add to the top)
☐ Overwrite (replace)
☐ Ask

Intensity threshold
☒ % of max.
☐ absolute

Spectra without precursor ion m/z value are
☒ EI (nominal m/z) ☐ In-source ☐ Accurate m/z

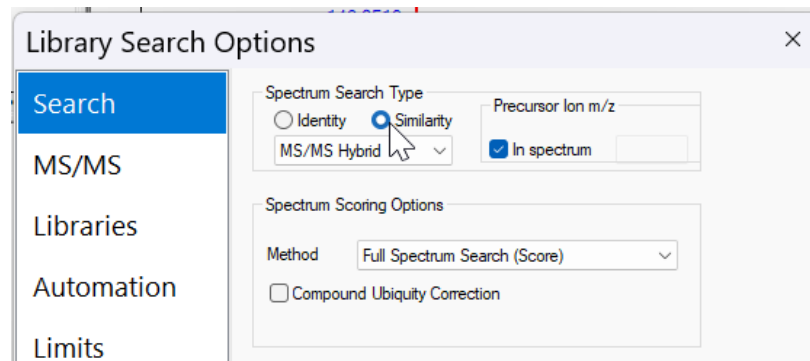
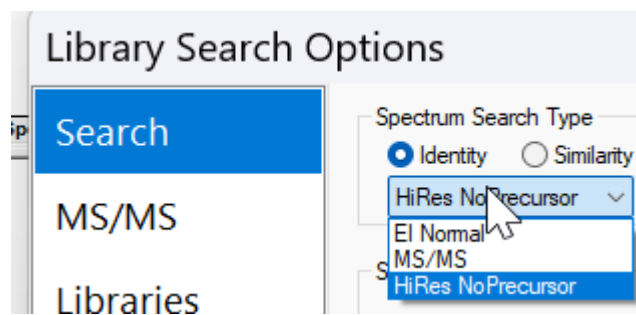
RI type if unspecified

☒ Include Synonyms



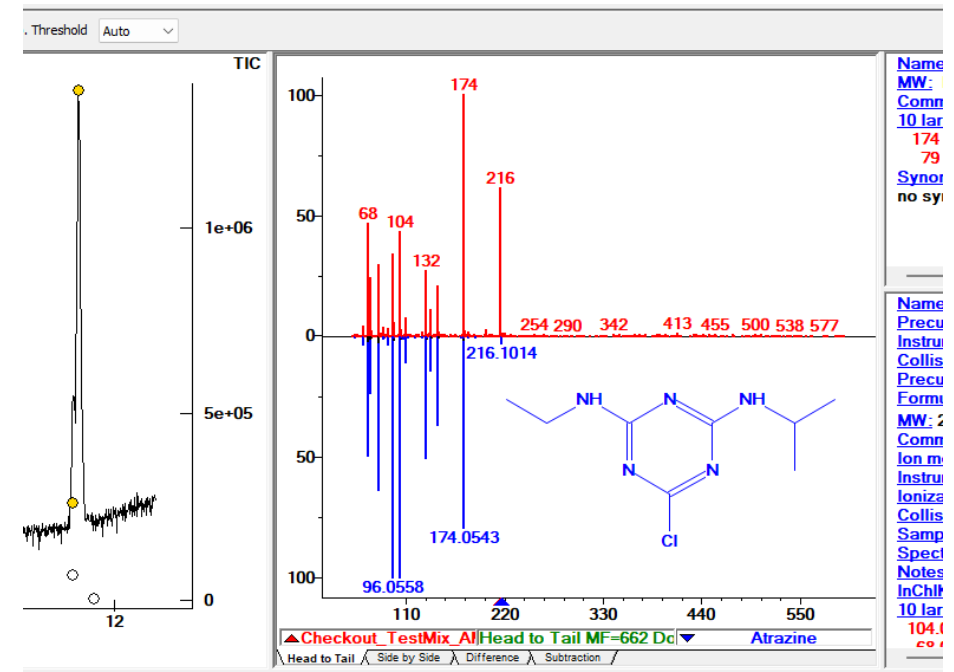
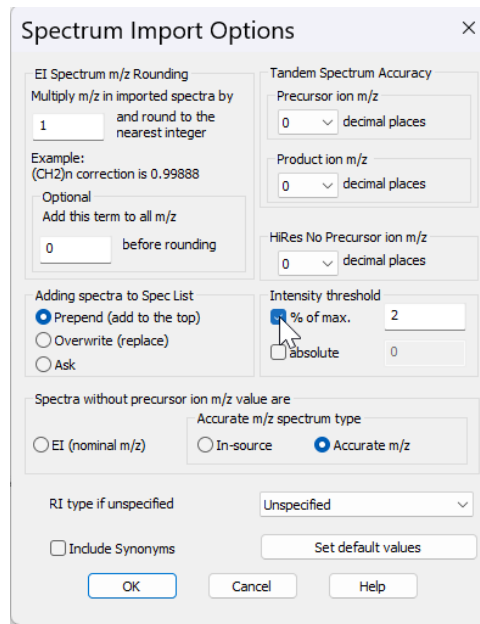
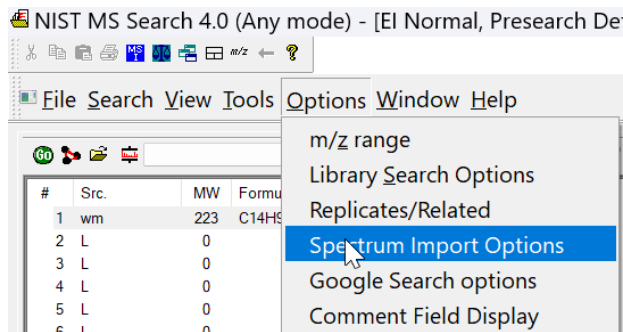
Software Bug: Identity MS/MS No Precursor and Similarity Hybrid MS/MS

- After adding high-resolution spectra, the HiRes NoPrecursor and Similarity Hybrid MS/MS Searches do not work
- This can be fixed by exporting in Lib2NIST to msp files ASCII files with associated mol structure files
- Then importing back with Lib2NIST in NIST library format
- **Do not** attempt to run the MS/MS Hybrid Search, it does not work and will crash the program giving 0005 error code
- The EI Normal *mimics the results of HiRes No Precursor* and does work after initial addition and **works very well** when both high-resolution and low-resolutions libraries selected together
- The HiRes NoPrecursor will work with presearch turned off, but the search time is **VERY** long



Software Bug: Chromatogram Window Software

- The values used in the Spectrum Import Options “echoed” in the Chromatogram Tab
- Normally these values are shown to 4 significant figures to right of decimal
- When set as zero to import low-resolution spectra, truncated to values selected
- DOES NOT affect the results in accurate mass deconvolution/library searching table!
- Just the “head-to-head” display at top of Chromatogram Tab

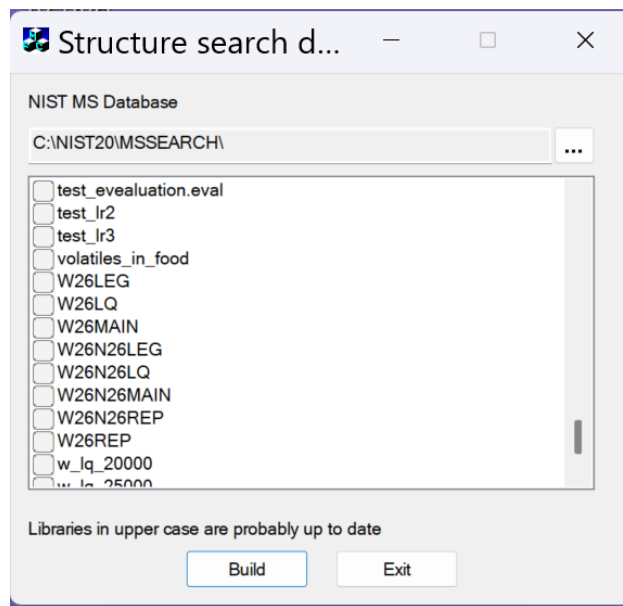
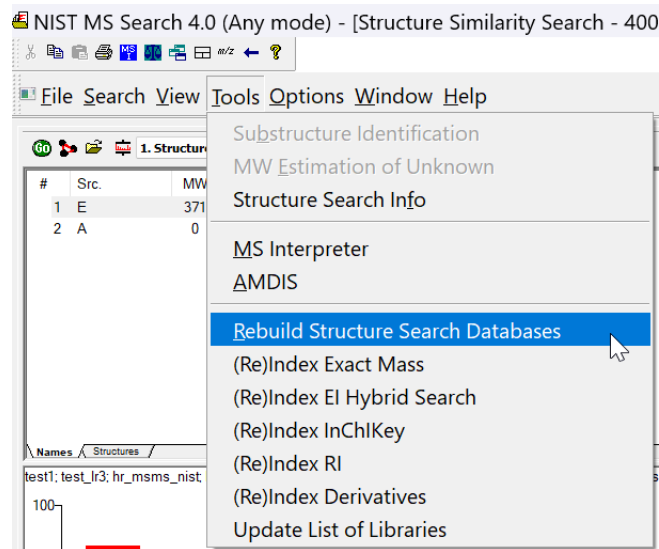


Check to See If Libraries Present and Limitations

- After creating low-resolution and high-resolution libraries, checked for proper searching
- Demonstrated in video which library searches worked
- Others that did not, needed “patching” with Lib2NIST utility
- Lib2NIST utility described in detail in video

Updates by User in Lib Search Window

- Every time a new entry is added with a structure
- Structure index must be updated to include proper search by structure of the library
- The InChIKey seems to be updated with structure
- If problems occur with duplicated entries not being removed, ReIndex the InChIKey for the library with new spectrum added
- The Update List of Libraries used when user add a library folder to c:/NIST26/MSSearch Folder while NIST program open



Lib2NIST NIST Utility

- Fixes problems with HiRes NoPrecursor and Hybrid Search in user library
- Only needed for your high-resolution libraries
- Best to close NIST Search software during these Lib2NIST conversions
- Exports library of interest with problems to e.g. hr_jl.msp with mol files in folder hr_jl.mol
- Then import back into NIST formatted library hr_jl
- Put fixed library in the C: NIST26/MSSearch folder where hr_jl is located
- *Might want to change name of original to hr_jl_old until check if the new hr_jl is working*
- If you already had NIST libraries on your system
- Software will ask you to point to them
- If so, need to know that location for specifying locations in Lib2NIST
- The location of libraries is found in nistms.INI found in C: NIST26/MSSearch folder
- It is an ASCII file and the location of the library can be changed by user

