

Searching Low-Resolution Spectra and Low-Resolution Libraries in NIST26

Video and Associated Handout

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Mass Spec Interpretation Services

8/14/2026



NATIONAL INSTITUTE OF
STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

ADVANCED TECHNIQUES
NIST26 Advanced Workflows

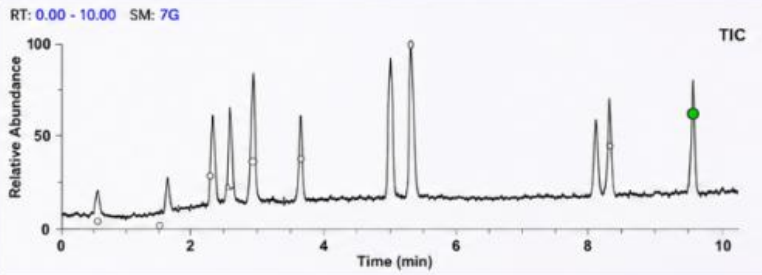
NIST26 Accurate Mass LC-MS/MS

Advanced Unknown Identification

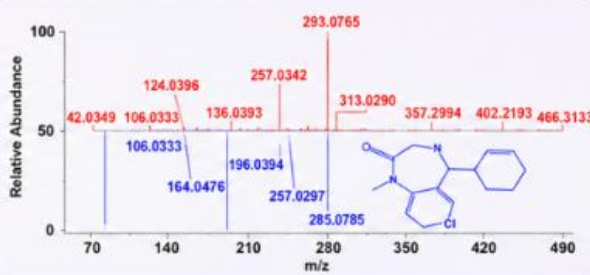
Advanced strategies for identifying challenging unknowns using tandem mass spectral libraries.

Featuring 3.2 MILLION MS/MS spectra, multiple library searching, hybrid search, structure search, MS Interpreter and the new NIST26 workflow.

RT: 0.00 - 10.00 SM: 7G

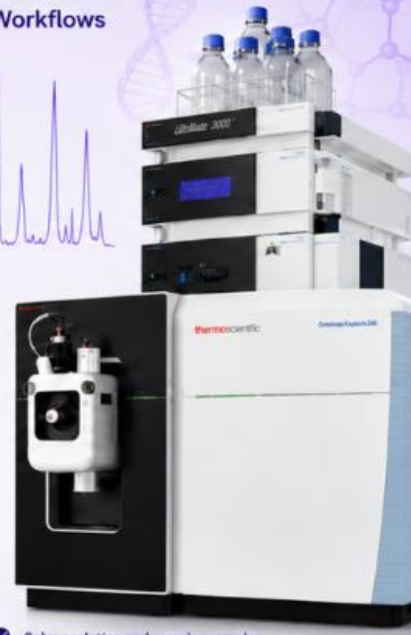


TIC



Relative Abundance

m/z





Hybrid Search
Find structurally related compounds

Structure Search
Molecular formula & substructure searching

MS Interpreter
Relate fragment ions to chemical structure

Multiple Libraries
Search NIST, MoNA, SpectraST & more

Unknown Identification
Integrate all evidence for confident ID



HYBRID SEARCH
Find structurally related compounds with confidence



STRUCTURE SEARCH
Molecular formula and substructure searching



MS INTERPRETER
Relate fragment ions to chemical structures



CONFIDENT IDENTIFICATION
Integrate accurate mass, isotope patterns, MS/MS libraries, and structural evidence

Integrating accurate mass, isotope patterns, tandem mass spectral libraries, hybrid searching, and structural interpretation for advanced unknown identifications.

MS/MS Low Resolution with MS/MS (Tandem) Libraries

Introduction:

- NIST said that the effectiveness of Low-resolution identity searches quite good if the component is in the library
- ***A fact not advertised by manufacturers of both low-resolution and high-resolution instruments!***
- *In my limited studies to date, I agree with that statement.*
- Thus, utilize with low-resolution single quads with in-source CID and triple quads with a collision cell
- Both low-resolution and high-resolution libraries utilized
- This makes many of the techniques applicable to a much wider range of instruments than accurate-mass LC-MS/MS alone!

Overview:

- The MoNA MSMS and MS-DIAL library entries primarily accurate mass. However, some low-resolution spectra mixed with the accurate mass entries (high resolution)
- MoNA probably contains on the order of 25,000–30,000 experimental low-resolution LC-MS/MS spectra, and the MS-DIAL public libraries probably contain roughly 20,000–25,000. Very difficult to get an accurate estimate
- Many earlier user libraries in NIST format low-resolution with no precursor
- Some older commercial MS/MS libraries in low-resolution mode
- New NIST user libraries created in either accurate mass mode or low-resolution mode
- Former searchable with MS/MS searches, latter with only EI type

Restatement of Usefulness of Low-Resolution Spectra and Libraries

NIST26 MS/MS Libraries Are Valuable Even Without Accurate-Mass Instrumentation

An important point that may be overlooked: the **NIST26 MS/MS libraries can be very useful even with low-resolution LC-MS instrumentation.**

This includes:

- **In-source CID spectra from single-quadrupole LC-MS instruments**
- **Product-ion spectra from triple-quadrupole collision-cell instruments**

You do **not** need an accurate-mass instrument to benefit from a large reference MS/MS library.

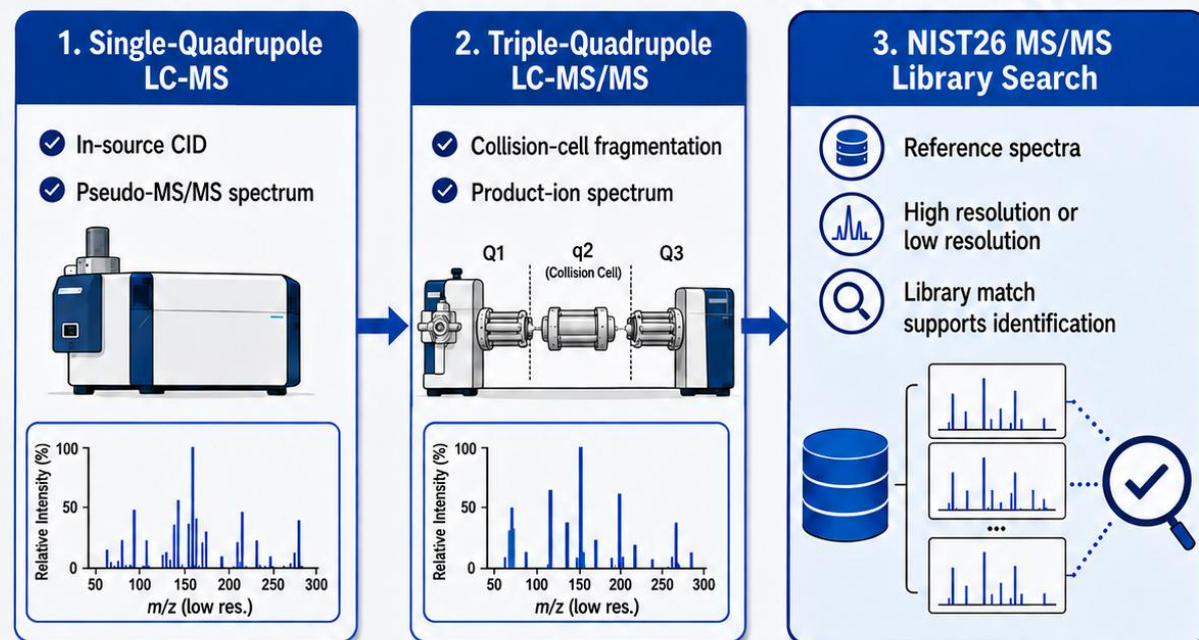
If an unknown produces a fragmentation spectrum that is represented in the reference library, it can be identified whether the reference spectrum was acquired at **high resolution or low resolution with NIST search software.**

Accurate mass certainly provides additional selectivity and confidence, but the **fragmentation pattern itself can carry substantial identification information.**

This makes the NIST26 MS/MS library useful to a much broader range of laboratories than just those operating QTOF or Orbitrap instruments.

NIST26 MS/MS Libraries Are Valuable Even Without Accurate-Mass Instrumentation

Useful for low resolution LC-MS data from multiple instrument types



You do not need accurate mass if the unknown spectrum is found in the reference MS/MS library.



Estimated LC-MS installed base:

Single-quad + triple-quad systems
≈ 2–3× HRMS LC-MS instruments

Approximate market-based estimate

Low-resolution quadrupole systems
(Single-quad + Triple-quad)



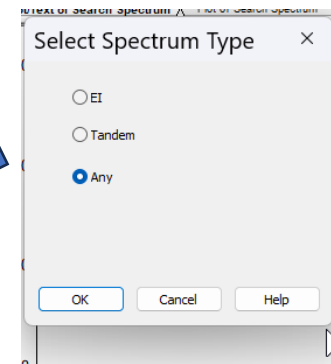
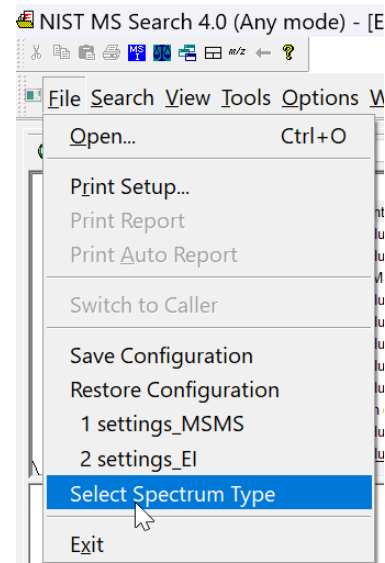
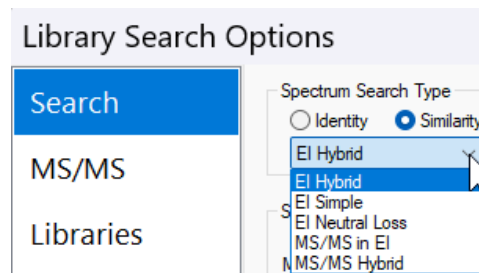
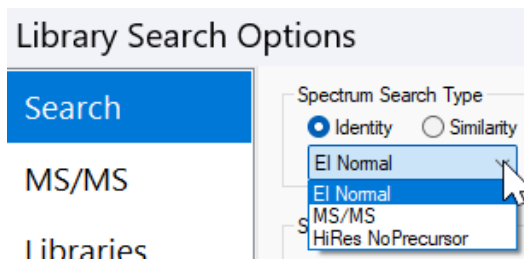
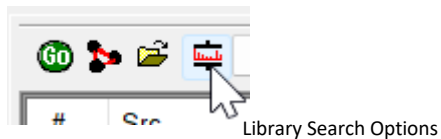
HRMS LC-MS instruments



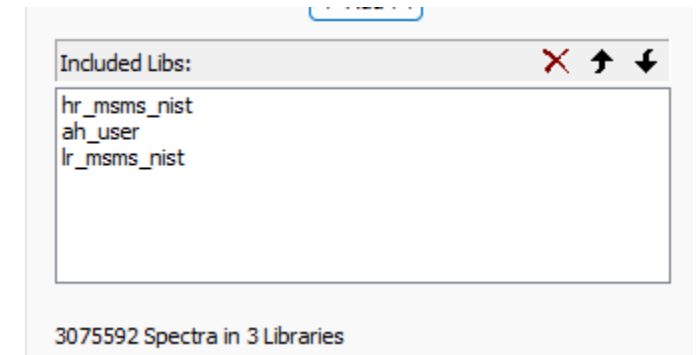
Fragmentation patterns can provide strong identification value even on low resolution instruments.

General Information on High-Resolution (Accurate Mass) and Low-Resolution Searches

- The lower resolution spectra included in the search results when employing EI type searches such as Identity Normal and Similarity EI Simple
- Lower resolution results with above searches included when either high resolution (accurate mass) or low-resolution searched
- Only accurate mass spectra included in search results when using Identity MS/MS and HiRes NoPrecursor
- When lower resolution searched with above search, no low-resolution spectra included in the results
- Only exception, lr_msms, the NIST low-resolution library included with high-resolution search results
- This NIST library includes special indices not accessible by either other commercial or user low-resolution libraries
- Library Processing easier if Spectrum Type selected Any

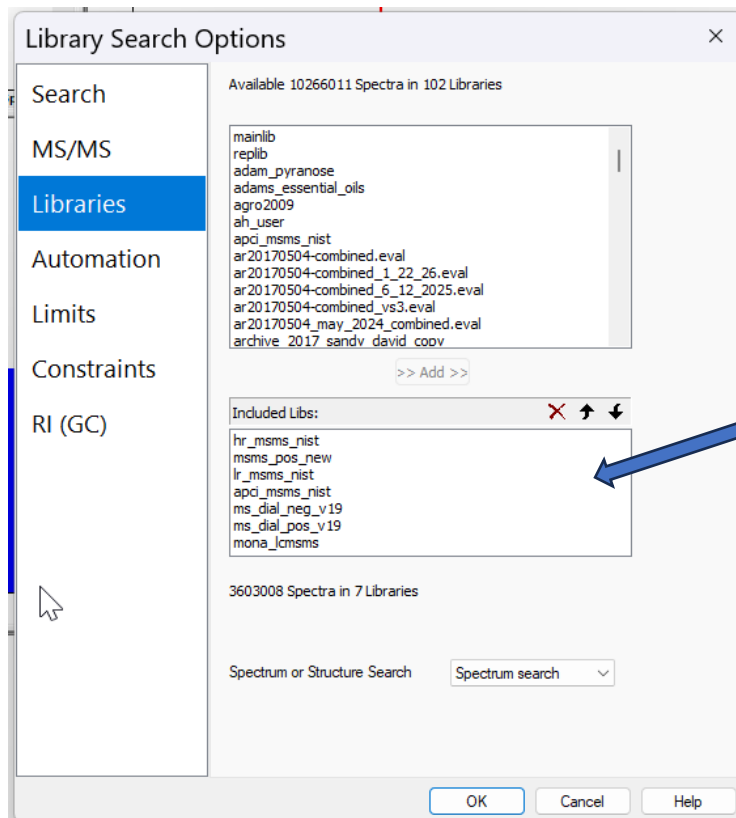


NIST MSMS Libraries



Libraries Selected

- Can actually select all the libraries when switching between Identity EI Normal and MS/MS Functions
- Usually in Any Spectrum mode so all search library functions displayed



NIST, High Resolution, Accurate Mass

- hr_msms_nist
- apci_msms

NIST, Low-Resolution

- lr_msms_nist

Public Libraries, Mainly Accurate mass, some low-resolution

- ms_dial_neg_v19
- ms_dial_pos_v19
- mona_lcmsms

Personal User Library, Low-resolution Only

- msms_pos_new

Detailed Information on EI Type Searches of Low-Resolution Libraries

<u>NIST mode</u>	<u>What happens with your low-resolution MS/MS query</u>	<u>Practical consequence</u>
Identity EI Normal	Uses the conventional EI identity-search weighting, which gives relatively greater importance to higher-m/z ions	Often useful for MS/MS because the higher-mass product ions can be structurally important and more diagnostic
Similarity → Simple EI	Uses a more straightforward/balanced comparison of the spectral peaks	Good general-purpose choice when you don't want the higher-mass emphasis of Identity
Similarity → MS/MS EI	Treats your query as an MS/MS-type spectrum but searches it against EI library spectra	Useful for asking: <i>does this MS/MS fragmentation resemble the EI fragmentation of a compound?</i> It is not simply another way of searching your MS/MS library against itself

Identity EI Normal → favors the more diagnostic higher-mass region somewhat.

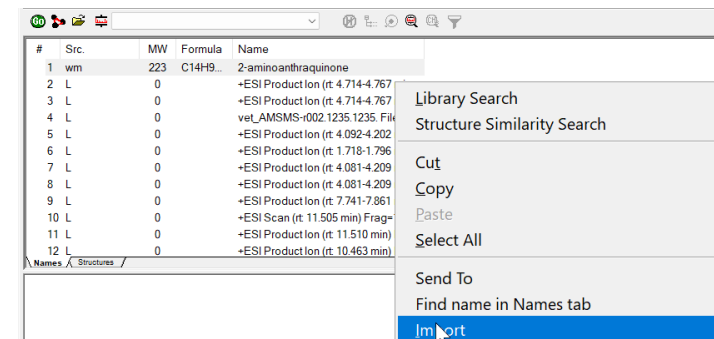
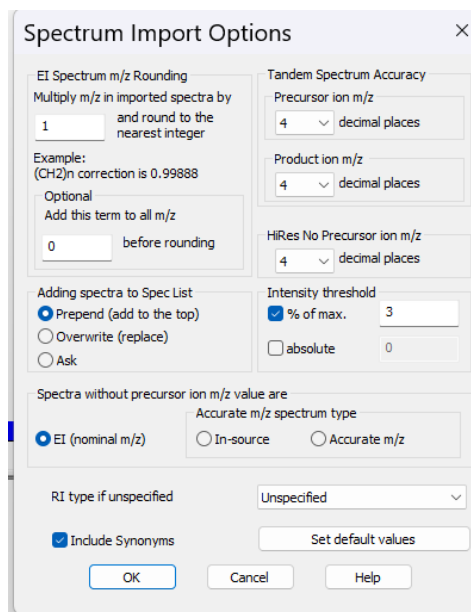
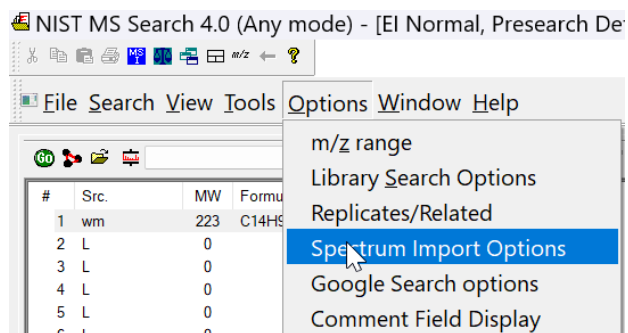
Simple EI Similarity → more balanced spectral comparison.

MS/MS EI Similarity → specifically useful for comparing an **MS/MS spectrum to EI spectra**.

That makes **Identity EI Normal and Similarity Simple EI** particularly interesting for what you're doing with your **low-resolution MS/MS personal library**, whereas **MS/MS EI** has a somewhat different purpose.

Importing Low-Resolution Spectra into Spec List in Librarian Tab for Processing

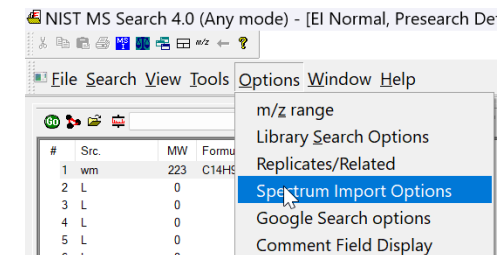
- Low-resolution spectra primarily sent from Vendors program using “Send NIST Lib option”
- Process for adding this function to vendor software, [click link](#)
- When importing low-resolution spectra, use radio button for “EI (normal m/z)” selected
- If you select “In-source” or “Accurate m/z” radio buttons, it will cause ions in Chromatogram window to be nominal mass set no matter what the Tandem Spectrum Accuracy boxes show
- Can set Intensity Threshold to minimize noise imported, BUT BUG will cause all imported spectra from Vendors program to be nominal mass no matter what Tandm Spectrum Accuracy values employed!
- Waters, Agilent ([MassHunter](#) and [Open Lab](#)), and [Thermo](#) (names to left linked to my site info) incorporate NIST “Send NIST Lib option”
- Don’t think Sciex or Bruker employ, might have to export from them as txt or msp ASCII format and import into Spec List in Lib Search



Importing High-Resolution (Accurate Mass) Spectra into Spec List in Librarian Tab for Processing

- High-resolution sent several ways
- Sent from Chromatogram Tab in NIST 26 with right click select Library Search
- From vendors software using “Send NIST Lib option” like Low-resolution spectra on previous slide
- Process for adding this function to vendor software, [click link](#)
- When importing high-resolution spectra, set decimal places as shown below with “Accurate m/z” radio button selected
- Some instruments include “grassy” random noise levels deleteriously affecting search results, however, when trying to set a value there, BUG in software took spectra to nominal mass when imported!
- Waters, Agilent ([MassHunter](#) and [Open Lab](#)), and [Thermo](#) (names to left linked to my site info) incorporate NIST “Send NIST Lib option”
- Don’t think Sciex or Bruker employ, might have to export from them as txt or msp ASCII format and import into Spec List in Lib Search

17	2306	8.2865	662	68	662	812	40.9	216.1020		Atrazine
18	2320	8.3540	346	99	346	685	4.93	418.0133		Metosulam
19	2457	8.9288	352	98	352	499	17.3	278.1061		Metazachlor
20	2464	8.9296	23	7	23	100	1.33	300.0879		Metazachlor
21	2786	10.0524	249	66	249	464	5.40	188.1102		Molinate
22	2896	10.4767	96	31	96	503	3.51	331.0433		Isomalathion
23	2904	10.4769	4	8	4	63	1.40	348.0706		2(1H)-Pyridinone, 3-[(2S,4S,5R)-5,6
24	2905	10.4787	2	15	2	33	1.67	353.0258		N-(Propyl(2-(2,4,6-trichlorophenoxy
25	3153	11.4820	491	99	491	726	18.8	388.1065		Pyraclostrobin
26	3165	11.4829	20	30	20	162	4.80	410.0888		Pyraclostrobin
27	3171	11.5522	754	100	754	838	100	305.1091		Diazinon
28	3197	11.7519	4	9	4	75	0.0132	388.1100		Pyraclostrobin



The 'Spectrum Import Options' dialog box is shown. It contains settings for 'EI Spectrum m/z Rounding', 'Tandem Spectrum Accuracy', 'Adding spectra to Spec List', and 'Spectra without precursor ion m/z value are'. The 'Accurate m/z' radio button is selected under 'Spectra without precursor ion m/z value are'. The 'Include Synonyms' checkbox is unchecked. The 'Set default values' button is visible at the bottom right.

Types of Searches and Resulting Rankings

<u>NIST value</u>	<u>What it measures</u>	<u>What gets penalized?</u>	<u>Particularly useful when...</u>	<u>Practical interpretation</u>
Score	NIST's overall MS/MS match score . It builds on spectral similarity but incorporates additional weighting/scaling appropriate for tandem spectra.	Differences between the query and library spectrum, with additional adjustment compared with a simple dot product.	Routine MS/MS identification when you want the best overall ranking.	Usually, the best column to sort first. Then inspect the other scores and spectra.
Dot / Dot Product	Direct similarity of the two complete spectra , based mainly on matching fragment peaks and their relative intensities.	Both directions: extra peaks in the query and missing library peaks can lower it.	Query and library spectra are both reasonably complete and clean.	High Dot means the overall fragmentation patterns look very similar.
Rev-Dot	A reverse dot product . It asks primarily: <i>Are the important library peaks present in my unknown?</i>	Does NOT penalize extra peaks in the query spectrum that are absent from the library spectrum.	Query spectrum contains noise, co-fragmentation, contaminants, background ions, or mixed MS/MS spectra .	If Rev-Dot is much higher than Dot, the library pattern may be present but your experimental spectrum probably contains additional ions.
PSS-Dot	Partial Spectrum Search dot product . Essentially the opposite direction from Rev-Dot: it asks how well the observed portion of the query agrees with the library spectrum.	Does NOT penalize library peaks that are missing from the query spectrum.	Incomplete MS/MS spectra , low-energy spectra, low-abundance spectra, limited mass range, or spectra where only some expected fragments were acquired.	If PSS-Dot is much higher than Dot, the fragments you <i>did</i> observe match well, but your query spectrum is missing some library fragments.

Library Search Options

Search

MS/MS

Libraries

Automation

Limits

Constraints

Spectrum Search Type

☒ Identity ☐ Similarity

EI Normal

Precursor MW

☒ In spectrum

Spectrum Scoring Options

Method

☐ Compound

Full Spectrum Search (Score)

Full Spectrum Search (Score)

Impurity Tolerant Search (Rev-Dot)

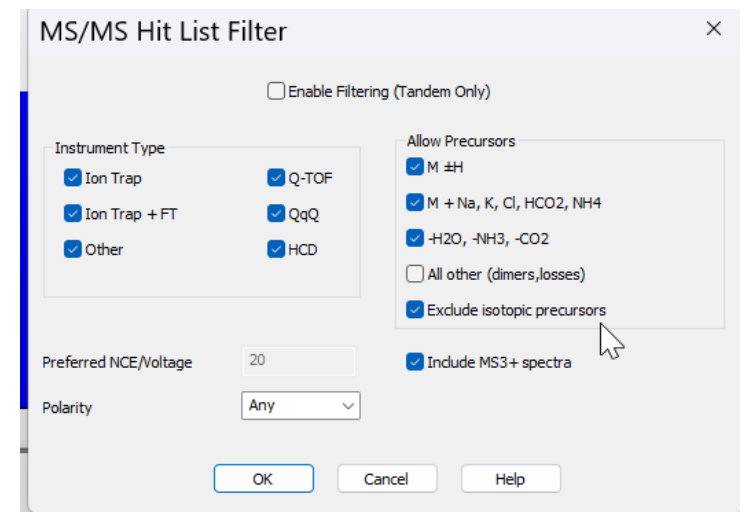
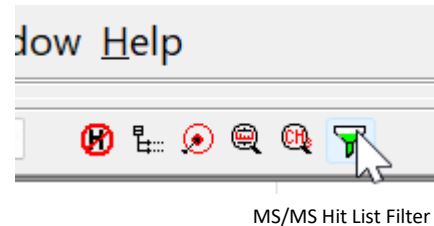
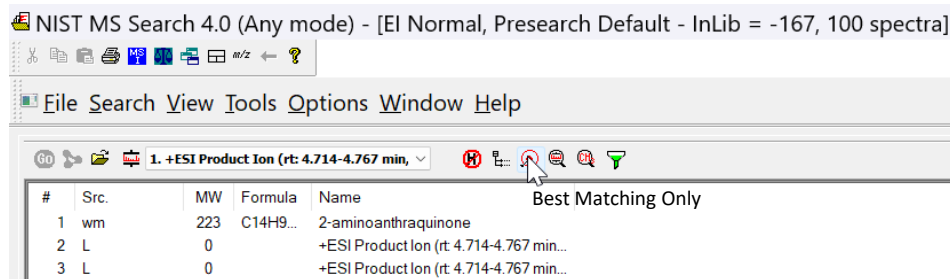
Partial Spectrum Search (PSS-Dot)

Presearch

☒ Default ☐ Fast ☐ Off ☐ Mass(Da) 201

Other Setting in Lib Search Window

- I usually keep “Best Matching Only” off initially
- Removes duplicates using the InChIKey for compound
- **InChIKey** is a fixed-length, machine-readable identifier derived from a chemical structure’s **InChI (International Chemical Identifier)**
- Hard to see if On or Off, just toggle it, should reduce the 100 spectra in most cases to smaller number
- Often helps in reviewing results
- I try to toggle back Off after using
- I usually do not “Enable” Filtering, but can toggle On or Off



Useful Columns Added

- Right Click and add MW, PSS Match, R. Match (Rev-Dot) under Properties
- Can resort results by clicking on any column header
- Usually do Full Spectrum Search (Score), see pull down menu on next page
- In this mode, R. Match (Impurity Tolerant, Rev-Dot) and PSS Spectrum Search results might not make results list *in a few cases*, best results done in some cases by selecting their particular search, but usually don't bother
- Can also left click and "drag" column header to change order of display
- See detailed definition of these searches on next page
- MW very useful if precursor ion known

#	Library	Match	PSS.Match	R.Match	MW	Prob. (%)	DBs	Name
1	hr_msms_nist#2	656	656	831	358	99.8	18	Triclabendazole
2	hr_msms_nist#2	611	611	801	358	99.8	18	Triclaben
3	hr_msms_nist#2	605	605	825	358	99.8	18	Triclaben
4	hr_msms_nist#2	538	538	714	358	99.8	18	Triclaben
5	hr_msms_nist#2	520	520	808	358	99.8	18	Triclaben
6	hr_msms_nist#2	505	505	670	358	99.8	18	Triclaben
7	hr_msms_nist#2	485	485	725	358	99.8	18	Triclaben
8	hr_msms_nist#2	482	482	811	358	99.8	18	Triclaben
9	hr_msms_nist#2	474	474	793	358	99.8	18	Triclaben
10	hr_msms_nist#2	474	474	787	358	99.8	18	Triclaben
11	hr_msms_nist#2	461	461	675	358	99.8	18	Triclaben
12	hr_msms_nist#2	457	457	750	358	99.8	18	Triclaben
13	hr_msms_nist#2	452	452	707	358	99.8	18	Triclaben
14	hr_msms_nist#2	444	444	643	358	99.8	18	Triclaben
15	hr_msms_nist#2	442	442	750	358	99.8	18	Triclaben
16	hr_msms_nist#2	441	441	702	358	99.8	18	Triclaben
17	hr_msms_nist#2	437	437	650	358	99.8	18	Triclaben
18	hr_msms_nist	432	432	506	461	0.04		N-Desme
19	hr_msms_nist#3	430	430	483	520	0.04		2-[3-(4-N
20	hr_msms_nist	426	426	503	461	0.04		N-Desme
21	hr_msms_nist#3	426	426	484	520	0.04		2-[3-(4-N
22	hr_msms_nist#2	423	423	562	358	99.8	18	Triclaben
23	hr_msms_nist#2	419	419	686	358	99.8	18	Triclaben

displays and edits the properties of the window

Library Search Options

Search

MS/MS

Libraries

Automation

Limits

Constraints

Spectrum Search Type

☒ Identity
 ☐ Similarity

El Normal

▼

Precursor MW

☒ In spectrum

Spectrum Scoring Options

Method

Full Spectrum Search (Score)

Full Spectrum Search (Score)

Impurity Tolerant Search (Rev-Dot)

Partial Spectrum Search (PSS-Dot)

☐ Compound

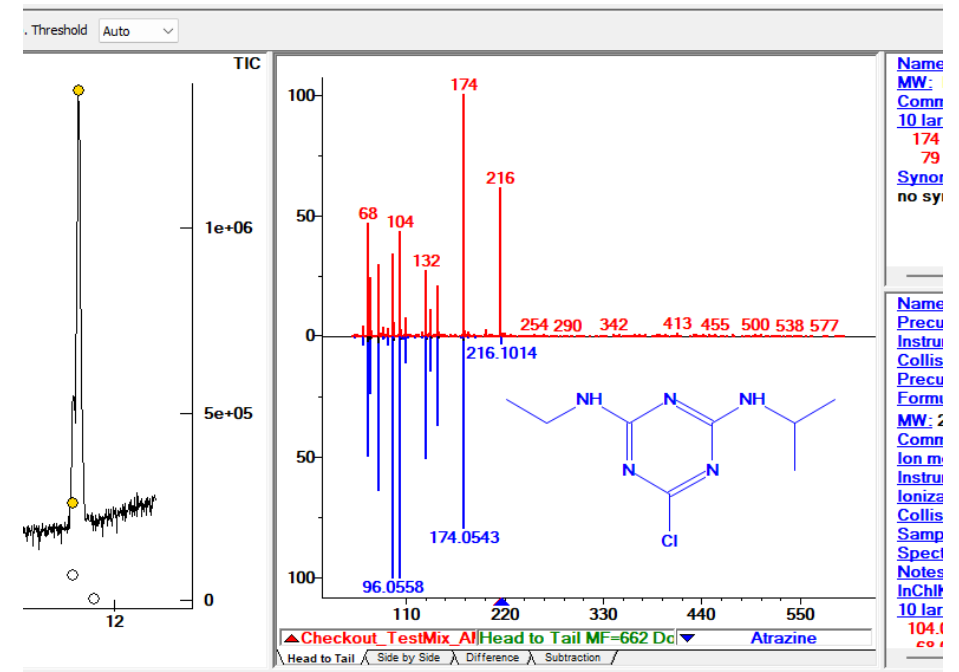
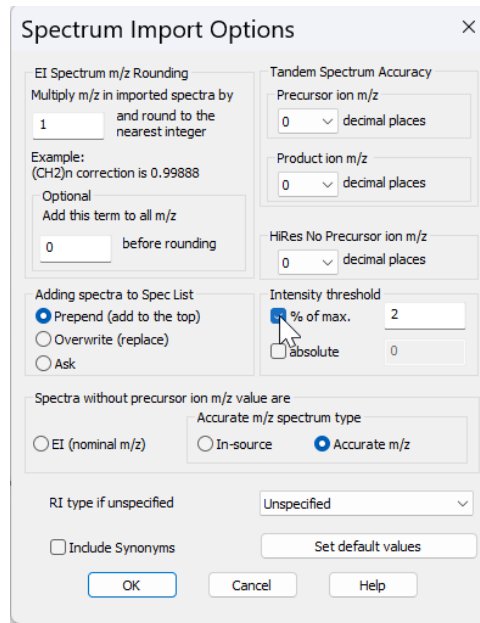
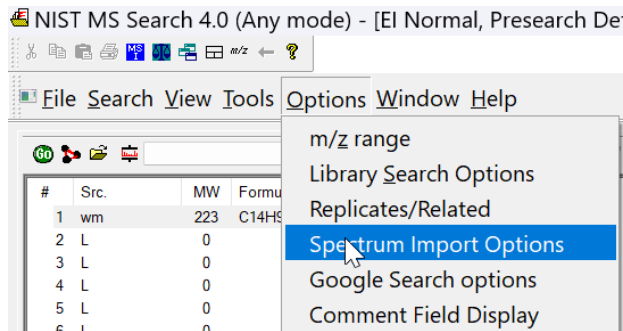
Presearch

☒ Default
 ☐ Fast
 ☐ Off
 ☐ Mass(Da)

201

Software Bug in 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



Software 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

