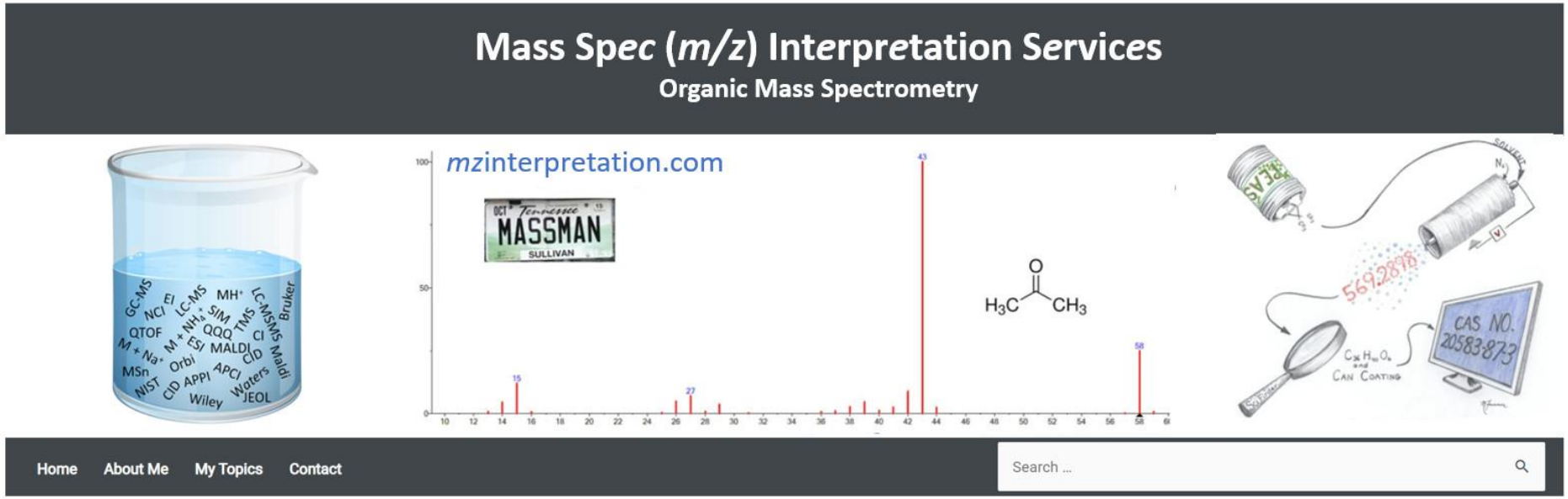
Processing MS/MS Data in NIST26 Chromatogram Window
Video/Handout

James Little
Mass Spec Interpretation Services
April 24, 2026
mzinterpretation.com

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



Important Skills

- Assume familiar with configuring and navigating Chromatogram NIST26
- ***If not***, review following video and associated handout

Configuring and Navigating Chromatogram Window NIST26

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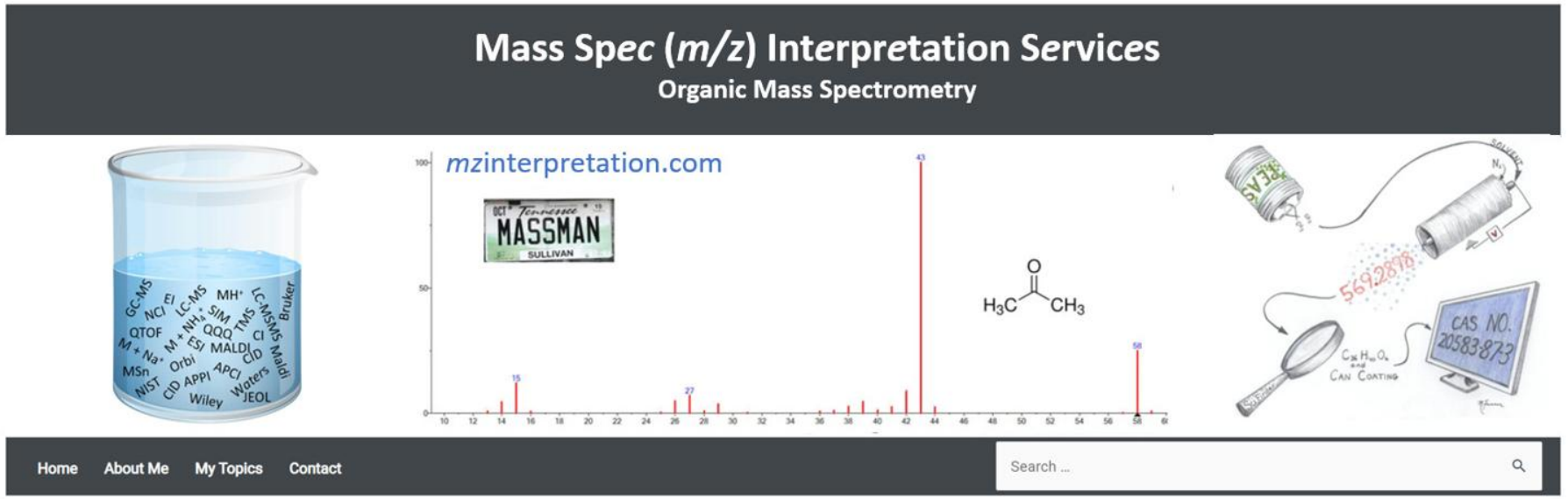
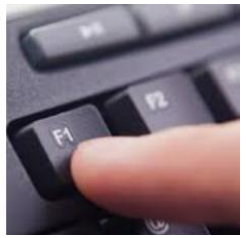


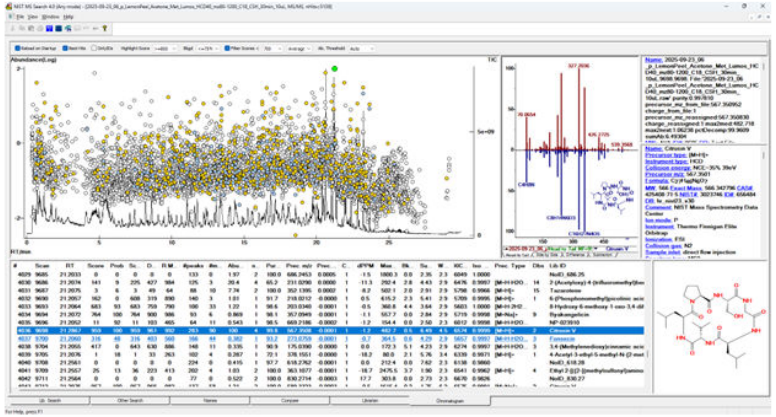
Lots of *Useful Help* in Chromatogram Window

F1 while in Chromatogram Window



The NISTMS MS/MS Chromatogram Window

A new, versatile Chromatogram Window is provided as part of the NISTMS Search software (Version 4.0) provided with the 2026 version of the NIST Mass Spectral Libr

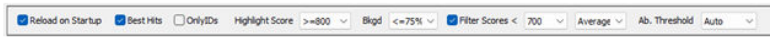


For a LC-MS/MS (dynamic data acquisition, DDA) chromatogram, this window enables searching of all MS2 spectra against the NIST library using the latest NIST mspeps XICanalyzer. The chromatograph and hit list are linked so that the same spectra are shown in both windows. Following an analysis, the display shows results of analysis and li the NISTMS program though options available from the right mouse button (RMB). This includes the *XICAnalyzer* Program which shows the underlying MS1 data, multiple library. It is also possible to save spectra in a user library through the *Librarian* tab. NISTMS provides spectral filtering functions to discard low quality, generally unidentifiat allows multiple zooming levels, with the hitlist showing results only for spectra appearing in the display.

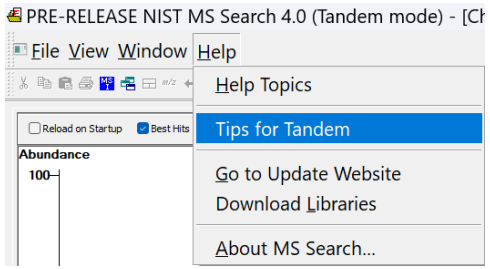
The standard input format is *mzML*. This format may be directly output by instrument company software or translated by the *MSConvert* program from Proteowizard. All file data was not centroided. The ability to directly read certain proprietary formats may also be available from instrument makers.

The *File* menu allows file analysis, re-opening or deletion of previous results using *Open MS/MS Input File*, *Reopen MS/MS Results* or *Delete MS/MS Results*, respectively hybrid search in addition to the standard search. The background selection conducts processing until complete, when user is prompted to view or save results for later examin

The control bar is below the menu. It sets filters and display features:



Chromatogram Window



Tandem Chromatogram Tips

How to Display Hits, Analyze Data, Export Data:

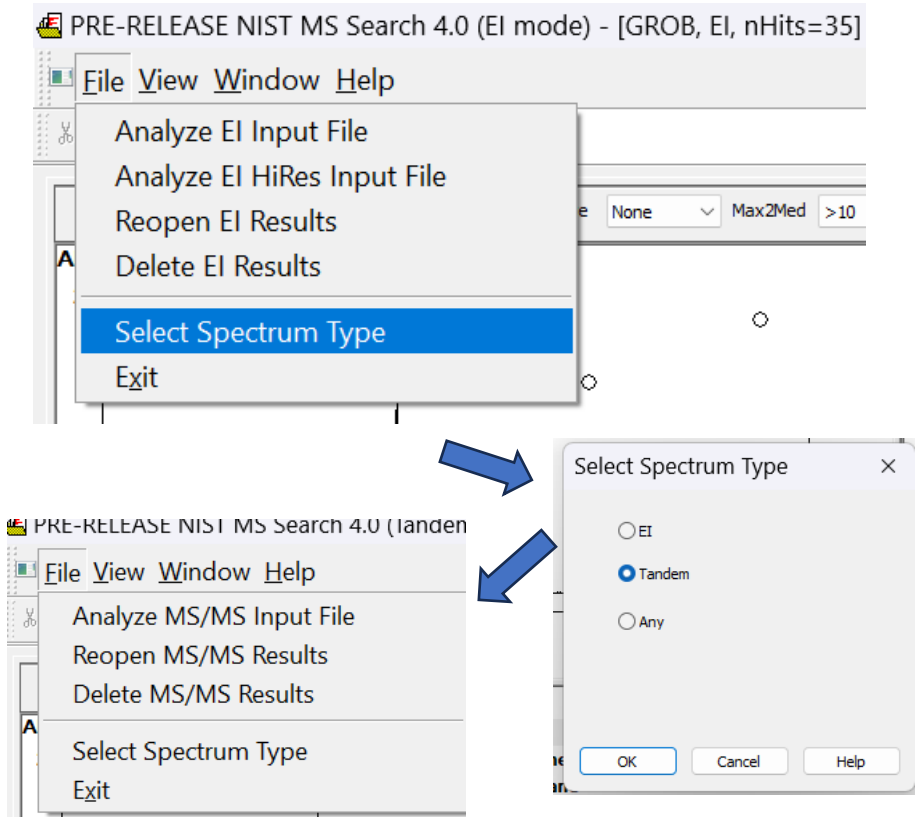
Display Hits

1) Show only IDs above a selected score
   a) On bar above chromatogram, turn *Filter Scores* button on
   b) Enter minimum score threshold into text box after *Filter Scores*
   c) Change selection in combo box to *Fixed*
   d) Only IDs with score above entered value will appear in chromatogram and hit list
   e) May set at 900 for a clear view of the TIC
2) Examine in-source ions to look for precursor ion
   a) Ensure *Best Hits* is selected
   b) Draw rectangle around all IDs (circles) in a narrow retention range
   c) Use zoom (draw rectangle) and unzoom (RMB) isolate set of in-source IDs
   d) Only IDs (circles) shown will appear in the hit list
   e) Sort by abundance (*Abund.Rel.*) to peruse IDs in order of abundance, or
   f) Sort by precursor mass to peruse from highest to lowest precursor m/z
3) Examine all spectra from a single XIC (single precursor m/z of a chromatograph peak)
   a) Turn *Best Hits* off
   b) Select a hit list item or circle in chromatogram
   c) Sort *XIC_num.* column (requires *Advanced Results* to be selected in *Properties* dialog box)
   d) Select (highlight) all XIC_num. values that match the highlighted value
   e) The number of MS2 spectra for an XIC is shown in column *nSpec*
   f) Press the right mouse button (*RMB*) in the hit list and select *Show Selected*
   g) All MS2 spectra for a single XIC will appear in the chromatogram
   h) Sort by *Abund.Rel* or *Prec. m/z* to peruse
   i) For detailed analysis of MS1 data, select *Send To* by *RMB*, then *XIC Analyzer*
      - this may also be selected when *Best Hits* is ON.
4) Examine all identifications of a compound (or precursor mass)

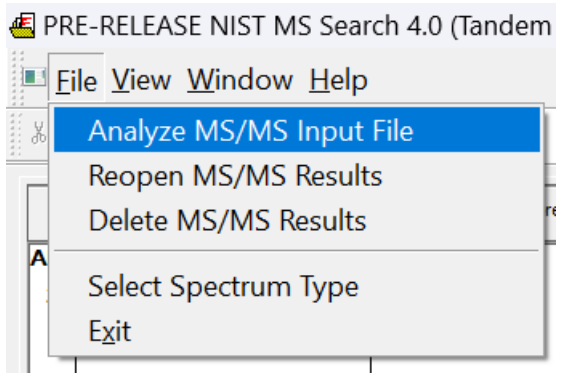
Set Processing for Tandem Mode

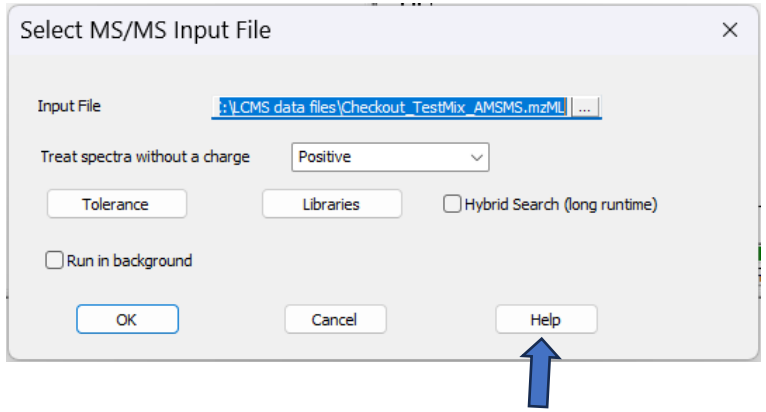
- Useful to select mode if both EI and Tandem NIST software installed
- Simplifies menu navigation

File Menu in Chromatogram window

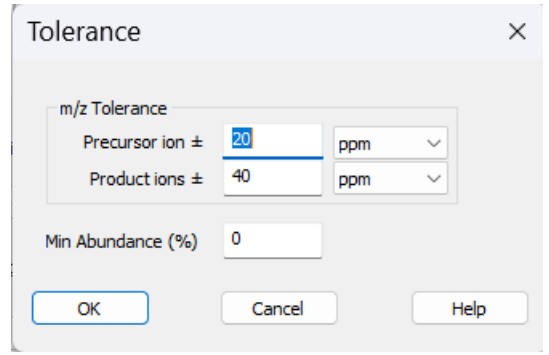


Selecting Files and Processing Parameters, ***Only mzML Files Processed***, Use ProteoWizard/msconvert for file conversions



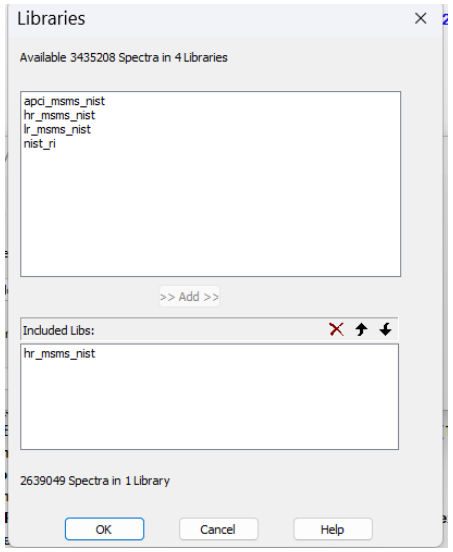


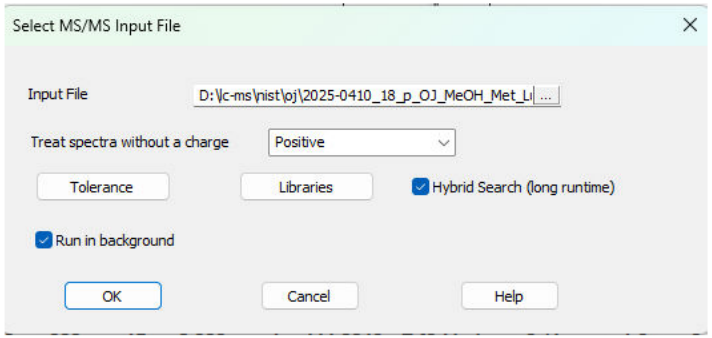
Click Help



20 and 40 ppm for Tolerance Reasonable

Best hr_msms_nist,
but can use apci and lr library or
crowd-sourced free libraries
Search Many Libraries at Once!





Input file: Select file in mzML format or other if translator software is loaded

Treat spectra without a charge: If no charge is given in data file, use this polarity.

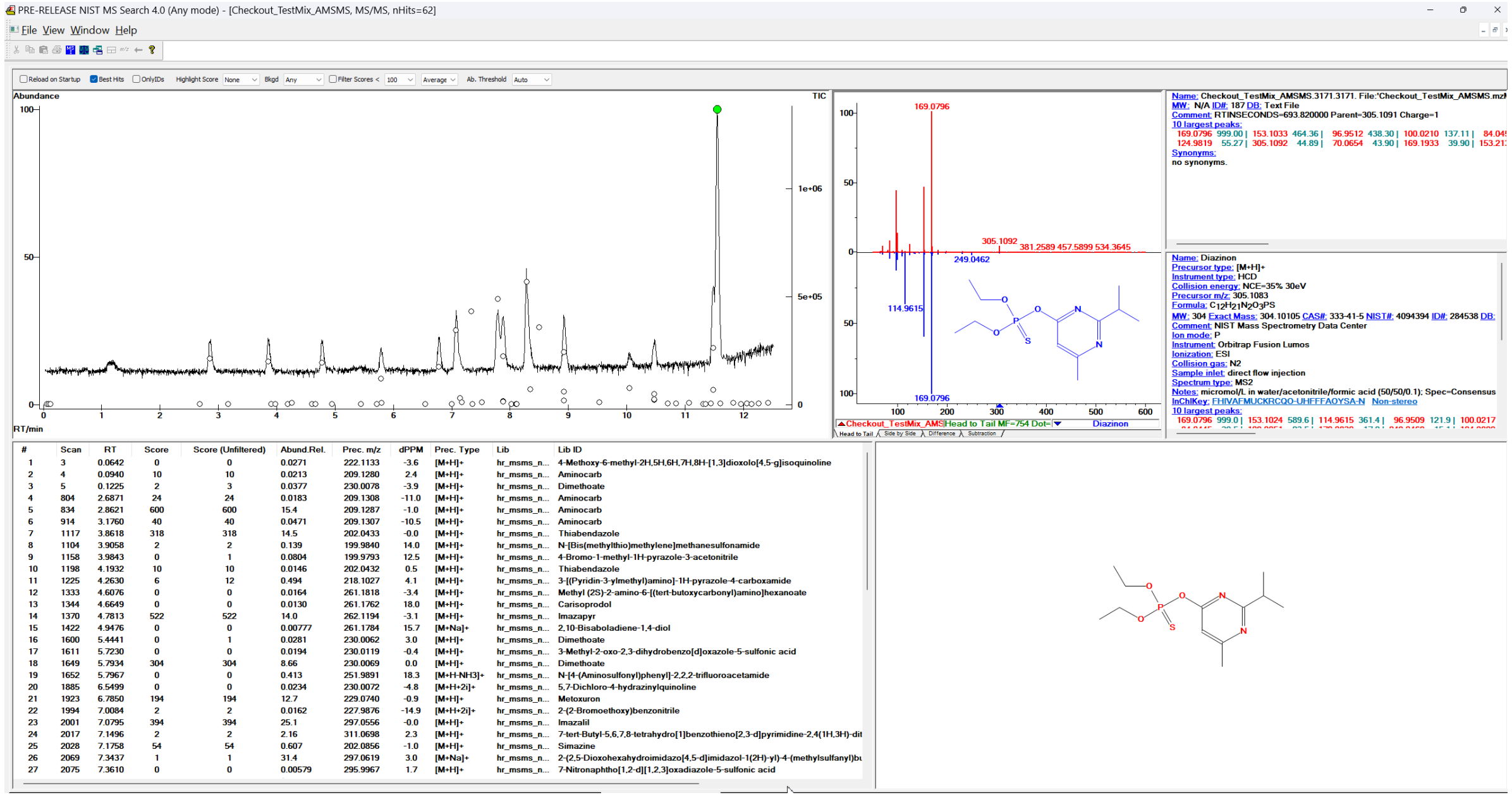
Tolerance: Shows dialog that specifies mass accuracy tolerances for precursor and product ions (ppm or mz) along with minimum abundances.

Libraries: Select NIST formatted tandem libraries

Hybrid Search: Perform a hybrid search with results added to simple Tandem search results. This can take some time, so it is often preferable to run in background (next control).

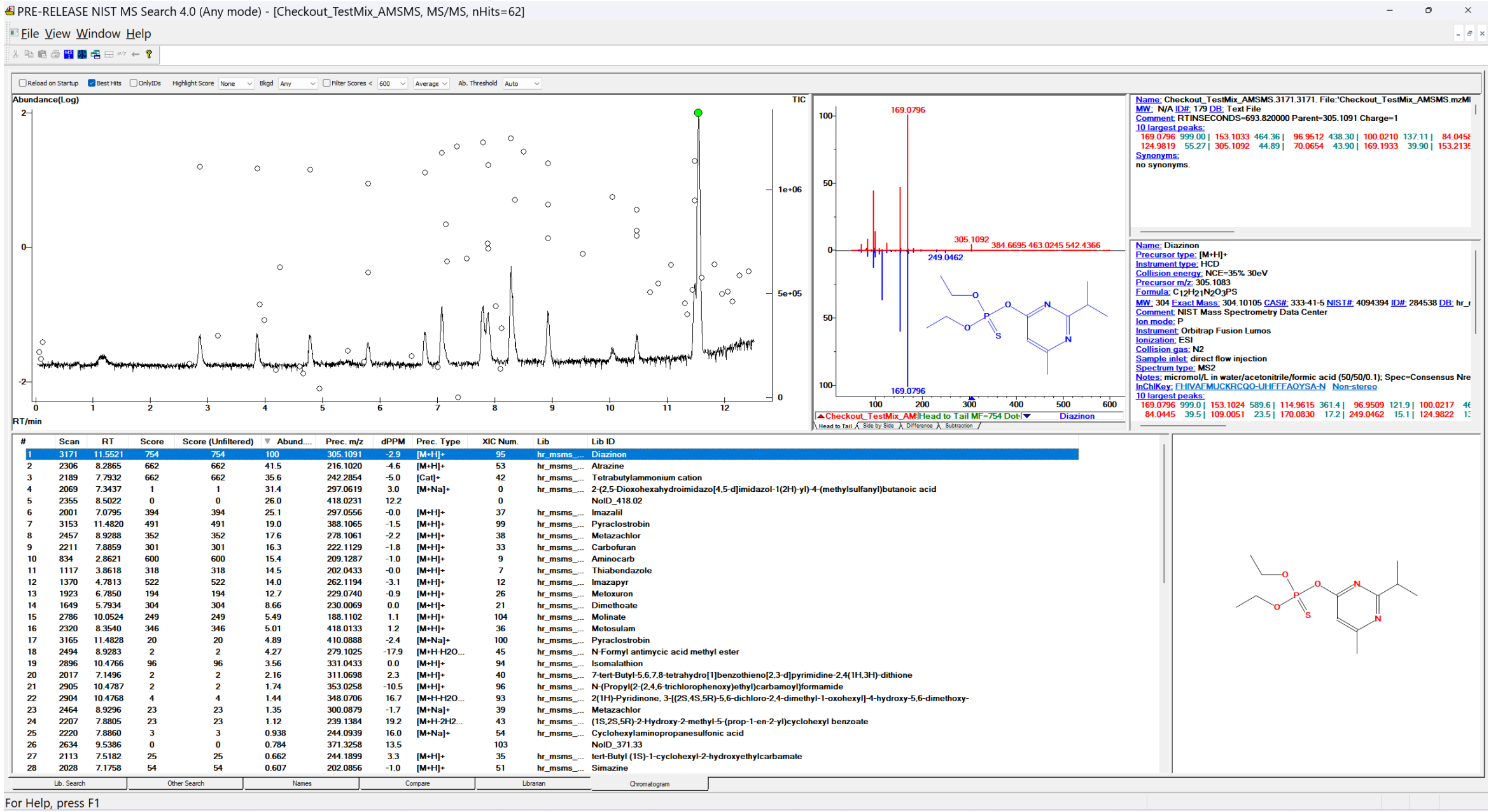
Run in Background: Analysis will proceed without affecting user interface until done, when user will be prompted to save or load.

In Video, Showing Initial Results and Demonstrating Some Window Changes with *Abundance Linear*



#	Scan	RT	Score	Score (Unfiltered)	Abund.Rel.	Prec. m/z	dPPM	Prec. Type	Lib	Lib ID
1	3	0.0642	0	0	0.0271	222.1133	-3.6	[M+H]+	hr_msms_n...	4-Methoxy-6-methyl-2H,5H,6H,7H,8H-[1,3]dioxolo[4,5-g]isoquinoline
2	4	0.0940	10	10	0.0213	209.1280	2.4	[M+H]+	hr_msms_n...	Aminocarb
3	5	0.1225	2	3	0.0377	230.0078	-3.9	[M+H]+	hr_msms_n...	Dimethoate
4	804	2.6871	24	24	0.0183	209.1308	-11.0	[M+H]+	hr_msms_n...	Aminocarb
5	834	2.8621	600	600	15.4	209.1287	-1.0	[M+H]+	hr_msms_n...	Aminocarb
6	914	3.1760	40	40	0.0471	209.1307	-10.5	[M+H]+	hr_msms_n...	Aminocarb
7	1117	3.8618	318	318	14.5	202.0433	-0.0	[M+H]+	hr_msms_n...	Thiabendazole
8	1104	3.9058	2	2	0.139	199.9840	14.0	[M+H]+	hr_msms_n...	N-[Bis(methylthio)methylene]methanesulfonamide
9	1158	3.9843	0	1	0.0804	199.9793	12.5	[M+H]+	hr_msms_n...	4-Bromo-1-methyl-1H-pyrazole-3-acetonitrile
10	1198	4.1932	10	10	0.0146	202.0432	0.5	[M+H]+	hr_msms_n...	Thiabendazole
11	1225	4.2630	6	12	0.494	218.1027	4.1	[M+H]+	hr_msms_n...	3-[(Pyridin-3-ylmethyl)amino]-1H-pyrazole-4-carboxamide
12	1333	4.6076	0	0	0.0164	261.1818	-3.4	[M+H]+	hr_msms_n...	Methyl (2S)-2-amino-6-[(tert-butoxycarbonyl)amino]hexanoate
13	1344	4.6649	0	0	0.0130	261.1762	18.0	[M+H]+	hr_msms_n...	Carisoprodol
14	1370	4.7813	522	522	14.0	262.1194	-3.1	[M+H]+	hr_msms_n...	Imazapyr
15	1422	4.9476	0	0	0.00777	261.1784	15.7	[M+Na]+	hr_msms_n...	2,10-Bisaboladiene-1,4-diol
16	1600	5.4441	0	1	0.0281	230.0062	3.0	[M+H]+	hr_msms_n...	Dimethoate
17	1611	5.7230	0	0	0.0194	230.0119	-0.4	[M+H]+	hr_msms_n...	3-Methyl-2-oxo-2,3-dihydrobenzo[d]oxazole-5-sulfonic acid
18	1649	5.7934	304	304	8.66	230.0069	0.0	[M+H]+	hr_msms_n...	Dimethoate
19	1652	5.7967	0	0	0.413	251.9891	18.3	[M+H-NH3]+	hr_msms_n...	N-[4-(Aminosulfonyl)phenyl]-2,2,2-trifluoroacetamide
20	1885	6.5499	0	0	0.0234	230.0072	-4.8	[M+H+2i]+	hr_msms_n...	5,7-Dichloro-4-hydrazinylquinoline
21	1923	6.7850	194	194	12.7	229.0740	-0.9	[M+H]+	hr_msms_n...	Metoxuron
22	1994	7.0084	2	2	0.0162	227.9876	-14.9	[M+H+2i]+	hr_msms_n...	2-(2-Bromoethoxy)benzonitrile
23	2001	7.0795	394	394	25.1	297.0556	-0.0	[M+H]+	hr_msms_n...	Imazalil
24	2017	7.1496	2	2	2.16	311.0698	2.3	[M+H]+	hr_msms_n...	7-tert-Butyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-2,4(1H,3H)-dit
25	2028	7.1758	54	54	0.607	202.0856	-1.0	[M+H]+	hr_msms_n...	Simazine
26	2069	7.3437	1	1	31.4	297.0619	3.0	[M+Na]+	hr_msms_n...	2-(2,5-Dioxohexahydroimidazo[4,5-d]imidazol-1(2H)-yl)-4-(methylsulfanyl)bu
27	2075	7.3610	0	0	0.00579	295.9967	1.7	[M+H]+	hr_msms_n...	7-Nitronaphtho[1,2-d][1,2,3]oxadiazole-5-sulfonic acid

Abundance Set for Log Scale

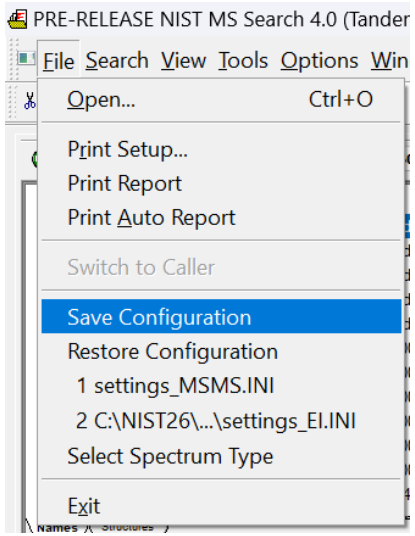


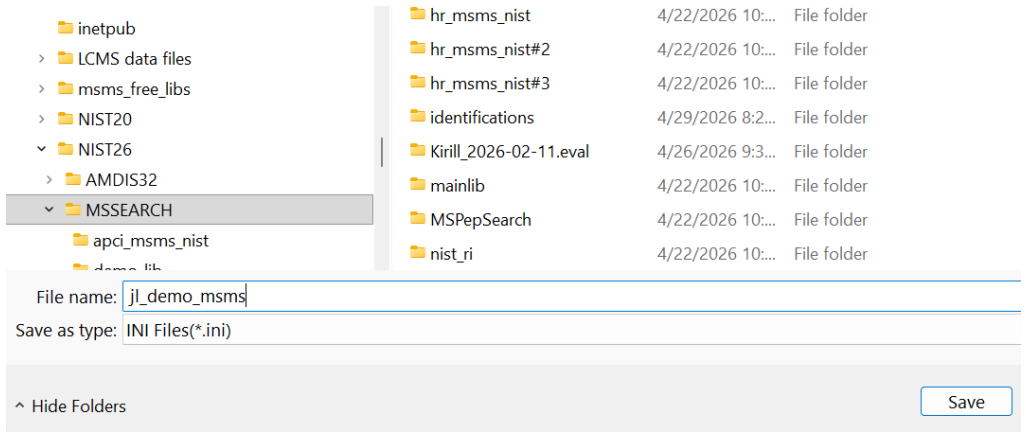
#	Scan	RT	Score	Score (Unfiltered)	Abund....	Prec. m/z	dPPM	Prec. Type	XIC Num.	Lib	Lib ID
1	3171	11.5521	754	754	100	305.1091	-2.9	[M+H]+	95	hr_msms_...	Diazinon
2	2306	8.2865	662	662	41.5	216.1020	-4.6	[M+H]+	53	hr_msms_...	Atrazine
3	2189	7.7932	662	662	35.6	242.2854	-5.0	[Cat]+	42	hr_msms_...	Tetrabutylammonium cation
4	2069	7.3437	1	1	31.4	297.0619	3.0	[M+Na]+	0	hr_msms_...	2-(2,5-Dioxohexahydroimidazo[4,5-d]imidazol-1(2H)-yl)-4-(methylsulfanyl)butanoic acid
5	2355	8.5022	0	0	26.0	418.0231	12.2		0		NoID_418.02
6	2001	7.0795	394	394	25.1	297.0556	-0.0	[M+H]+	37	hr_msms_...	Imazalil
7	3153	11.4820	491	491	19.0	388.1065	-1.5	[M+H]+	99	hr_msms_...	Pyraclostrobin
8	2457	8.9288	352	352	17.6	278.1061	-2.2	[M+H]+	38	hr_msms_...	Metazachlor
9	2211	7.8859	301	301	16.3	222.1129	-1.8	[M+H]+	33	hr_msms_...	Carbofuran
10	834	2.8621	600	600	15.4	209.1287	-1.0	[M+H]+	9	hr_msms_...	Aminocarb
11	1117	3.8618	318	318	14.5	202.0433	-0.0	[M+H]+	7	hr_msms_...	Thiabendazole
12	1370	4.7813	522	522	14.0	262.1194	-3.1	[M+H]+	12	hr_msms_...	Imazapyr
13	1923	6.7850	194	194	12.7	229.0740	-0.9	[M+H]+	26	hr_msms_...	Metoxuron
14	1649	5.7934	304	304	8.66	230.0069	0.0	[M+H]+	21	hr_msms_...	Dimethoate
15	2786	10.0524	249	249	5.49	188.1102	1.1	[M+H]+	104	hr_msms_...	Molinate
16	2320	8.3540	346	346	5.01	418.0133	1.2	[M+H]+	36	hr_msms_...	Metosulam
17	3165	11.4828	20	20	4.89	410.0888	-2.4	[M+Na]+	100	hr_msms_...	Pyraclostrobin
18	2494	8.9283	2	2	4.27	279.1025	-17.9	[M+H-H2O...	45	hr_msms_...	N-Formyl antimycic acid methyl ester
19	2896	10.4766	96	96	3.56	331.0433	0.0	[M+H]+	94	hr_msms_...	Isomalathion
20	2017	7.1496	2	2	2.16	311.0698	2.3	[M+H]+	40	hr_msms_...	7-tert-Butyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine-2,4(1H,3H)-dithione
21	2905	10.4787	2	2	1.74	353.0258	-10.5	[M+H]+	96	hr_msms_...	N-(Propyl(2-(2,4,6-trichlorophenoxy)ethyl)carbamoyl)formamide
22	2904	10.4768	4	4	1.44	348.0706	16.7	[M+H-H2O...	93	hr_msms_...	2(1H)-Pyridinone, 3-[(2S,4S,5R)-5,6-dichloro-2,4-dimethyl-1-oxohexyl]-4-hydroxy-5,6-dimethoxy-
23	2464	8.9296	23	23	1.35	300.0879	-1.7	[M+Na]+	39	hr_msms_...	Metazachlor
24	2207	7.8805	23	23	1.12	239.1384	19.2	[M+H-2H2...	43	hr_msms_...	(1S,2S,5R)-2-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexyl benzoate
25	2220	7.8860	3	3	0.938	244.0939	16.0	[M+Na]+	54	hr_msms_...	Cyclohexylaminopropanesulfonic acid
26	2634	9.5386	0	0	0.784	371.3258	13.5		103		NoID_371.33
27	2113	7.5182	25	25	0.662	244.1899	3.3	[M+H]+	35	hr_msms_...	tert-Butyl (1S)-1-cyclohexyl-2-hydroxyethylcarbamate
28	2028	7.1758	54	54	0.607	202.0856	-1.0	[M+H]+	51	hr_msms_...	Simazine

Saving Configurations

- ***After*** all ***program parameters*** set in ***all windows*** including Chromatogram, ***save the configuration***
- Makes easy to restore a configuration between different data processing requirements

Saving/Restoring Configurations (*.ini)
Note **This Menu in Lib Search Tab**



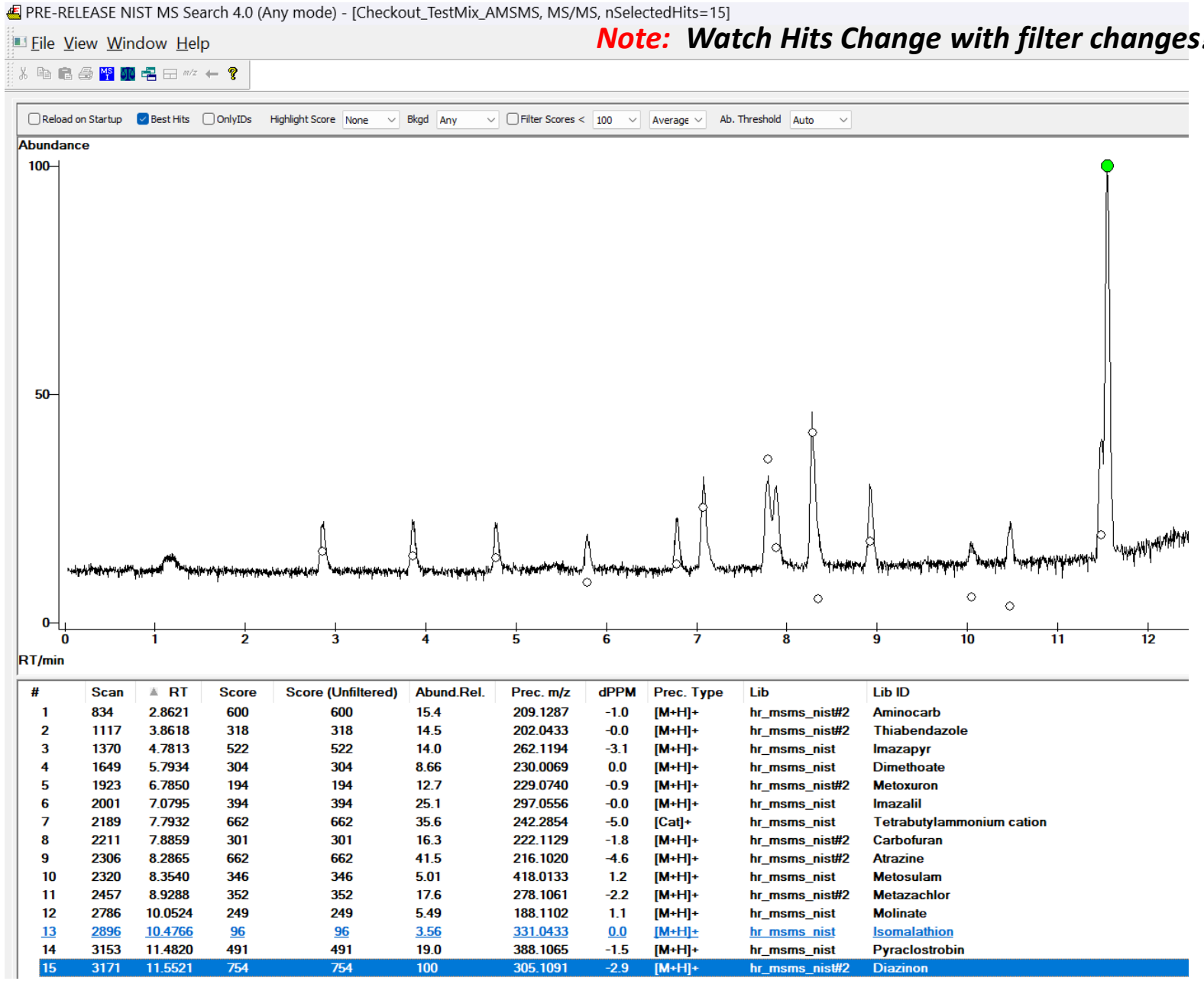


In Video, Demonstrate Effects of Various Changes in Filters on Top Menu

Video Demonstrates all such as Best Hits, Only ID's, Bkgd, expanding TIC with mouse, etc.

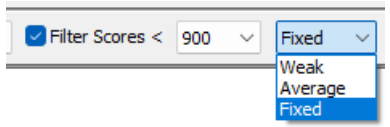
NOTE:

- Normally a background setting is *very useful; NIST suggest 75%*
- However, in a few instances was background noted to remove a few major peaks with good search scores
- These were peaks with an XIC value of 0
- See ***end*** of my video/handout on "Understanding and Using XICs,"
- Those peaks ***Bkgd = Any*** needed



#	Scan	RT	Score	Score (Unfiltered)	Abund.Rel.	Prec. m/z	dPPM	Prec. Type	Lib	Lib ID
1	834	2.8621	600	600	15.4	209.1287	-1.0	[M+H]+	hr_msms_nist#2	Aminocarb
2	1117	3.8618	318	318	14.5	202.0433	-0.0	[M+H]+	hr_msms_nist#2	Thiabendazole
3	1370	4.7813	522	522	14.0	262.1194	-3.1	[M+H]+	hr_msms_nist	Imazapyr
4	1649	5.7934	304	304	8.66	230.0069	0.0	[M+H]+	hr_msms_nist	Dimethoate
5	1923	6.7850	194	194	12.7	229.0740	-0.9	[M+H]+	hr_msms_nist#2	Metoxuron
6	2001	7.0795	394	394	25.1	297.0556	-0.0	[M+H]+	hr_msms_nist	Imazalil
7	2189	7.7932	662	662	35.6	242.2854	-5.0	[Cat]+	hr_msms_nist	Tetrabutylammonium cation
8	2211	7.8859	301	301	16.3	222.1129	-1.8	[M+H]+	hr_msms_nist#2	Carbofuran
9	2306	8.2865	662	662	41.5	216.1020	-4.6	[M+H]+	hr_msms_nist#2	Atrazine
10	2320	8.3540	346	346	5.01	418.0133	1.2	[M+H]+	hr_msms_nist	Metosulam
11	2457	8.9288	352	352	17.6	278.1061	-2.2	[M+H]+	hr_msms_nist#2	Metazachlor
12	2786	10.0524	249	249	5.49	188.1102	1.1	[M+H]+	hr_msms_nist	Molinate
13	2896	10.4766	96	96	3.56	331.0433	0.0	[M+H]+	hr_msms_nist	Isomalathion
14	3153	11.4820	491	491	19.0	388.1065	-1.5	[M+H]+	hr_msms_nist	Pyraclostrobin
15	3171	11.5521	754	754	100	305.1091	-2.9	[M+H]+	hr_msms_nist#2	Diazinon

?Filter Scores in Chromatogram Menu Bar?



- I don't really understand how to use this effectively
- Based on 5 different parameters
- Try it and see if useful in your work

The NISTMS MS/MS Chromatogram Window

[Found with F1 Key while in Chromatogram Window]

f) *Filter Scores* enables the filtering of lower quality spectra at three levels – weak, average and fixed, the latter show only IDs above the specified score. This is based on five different quality factors: *bkgd, purity, max2min, signal-to-noise* and *isotope analysis*, all of which may be optionally viewed in the hit list.

Tandem Chromatogram Tips

[Found under Help on Chromatogram pages, tips for tandem]

1) Show only IDs above a selected score

a) On bar above chromatogram, turn *Filter Scores* button on

b) Enter minimum score threshold into text box after *Filter Scores*

c) Change selection in combo box to *Fixed*

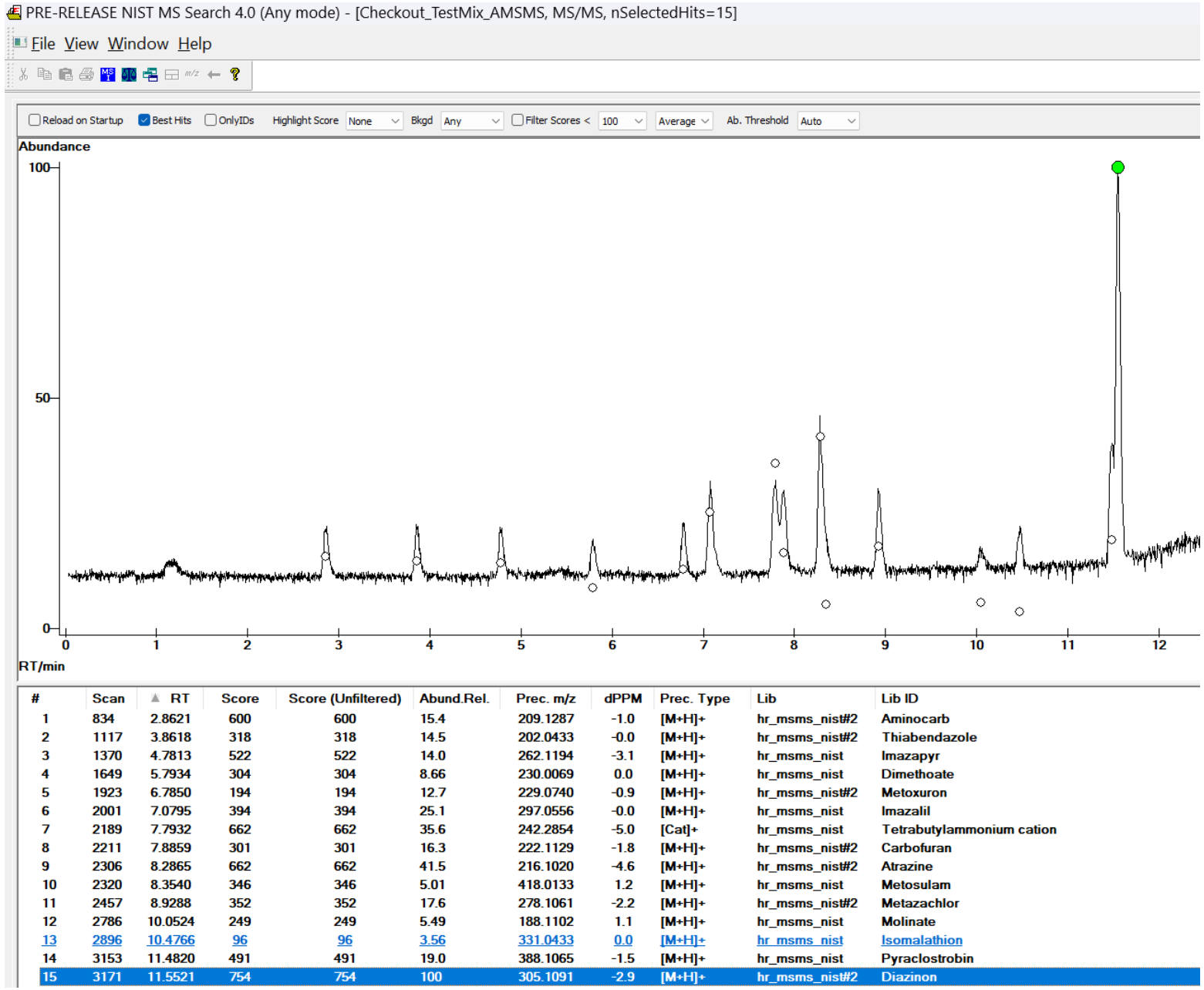
d) Only IDs with score above entered value will appear in chromatogram and hit list

e) May set at 900 for a clear view of the TIC

In Video, Demonstrate Sorting/Selecting Components of Interest from Results List

Operations Demonstrated in Video such as:

- Clicking to sort a column
- Selecting entries using keyboard Shift and Ctrl with left mouse click
- Sending selected to smaller list
- Almost endless numbers of approaches!
- Very flexible workflow to meet diverse user needs

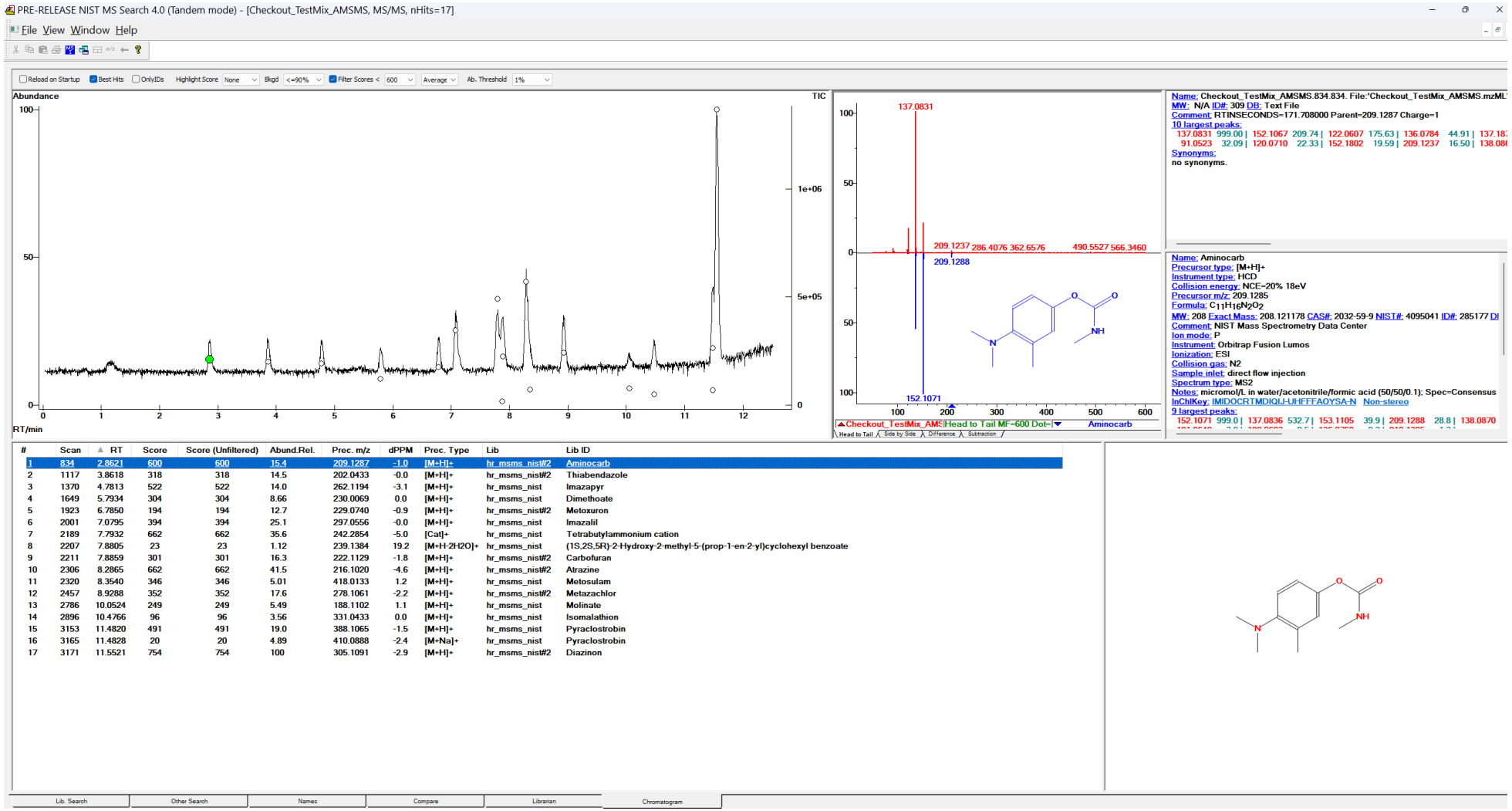


#	Scan	RT	Score	Score (Unfiltered)	Abund.Rel.	Prec. m/z	dPPM	Prec. Type	Lib	Lib ID
1	834	2.8621	600	600	15.4	209.1287	-1.0	[M+H]+	hr_msms_nist#2	Aminocarb
2	1117	3.8618	318	318	14.5	202.0433	-0.0	[M+H]+	hr_msms_nist#2	Thiabendazole
3	1370	4.7813	522	522	14.0	262.1194	-3.1	[M+H]+	hr_msms_nist	Imazapyr
4	1649	5.7934	304	304	8.66	230.0069	0.0	[M+H]+	hr_msms_nist	Dimethoate
5	1923	6.7850	194	194	12.7	229.0740	-0.9	[M+H]+	hr_msms_nist#2	Metoxuron
6	2001	7.0795	394	394	25.1	297.0556	-0.0	[M+H]+	hr_msms_nist	Imazalil
7	2189	7.7932	662	662	35.6	242.2854	-5.0	[Cat]+	hr_msms_nist	Tetrabutylammonium cation
8	2211	7.8859	301	301	16.3	222.1129	-1.8	[M+H]+	hr_msms_nist#2	Carbofuran
9	2306	8.2865	662	662	41.5	216.1020	-4.6	[M+H]+	hr_msms_nist#2	Atrazine
10	2320	8.3540	346	346	5.01	418.0133	1.2	[M+H]+	hr_msms_nist	Metosulam
11	2457	8.9288	352	352	17.6	278.1061	-2.2	[M+H]+	hr_msms_nist#2	Metazachlor
12	2786	10.0524	249	249	5.49	188.1102	1.1	[M+H]+	hr_msms_nist	Molinate
13	2896	10.4766	96	96	3.56	331.0433	0.0	[M+H]+	hr_msms_nist	Isomalathion
14	3153	11.4820	491	491	19.0	388.1065	-1.5	[M+H]+	hr_msms_nist	Pyraclostrobin
15	3171	11.5521	754	754	100	305.1091	-2.9	[M+H]+	hr_msms_nist#2	Diazinon

After User List of Interest Defined, Review

Video Demonstration of Reviewing Selected List:

- Usually sort by RT, retention time
- Left click on first hit in list to highlight
- Use up and down arrows on keyboard to step through list
- Carefully examine the butterfly display in top right box to decide if to include in final report
- Demonstrate ctrl and shift keys to modify components to report
- *etc.*



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7	2189	7.7932	662	662	35.6	242.2854	-5.0	[Cat]+	hr_msms_nist	Tetrabutylammonium cation
8	2207	7.8805	23	23	1.12	239.1384	19.2	[M+H-2H2O]+	hr_msms_nist	(1S,2S,5R)-2-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexyl benzoate
9	2211	7.8859	301	301	16.3	222.1129	-1.8	[M+H]+	hr_msms_nist#2	Carbofuran
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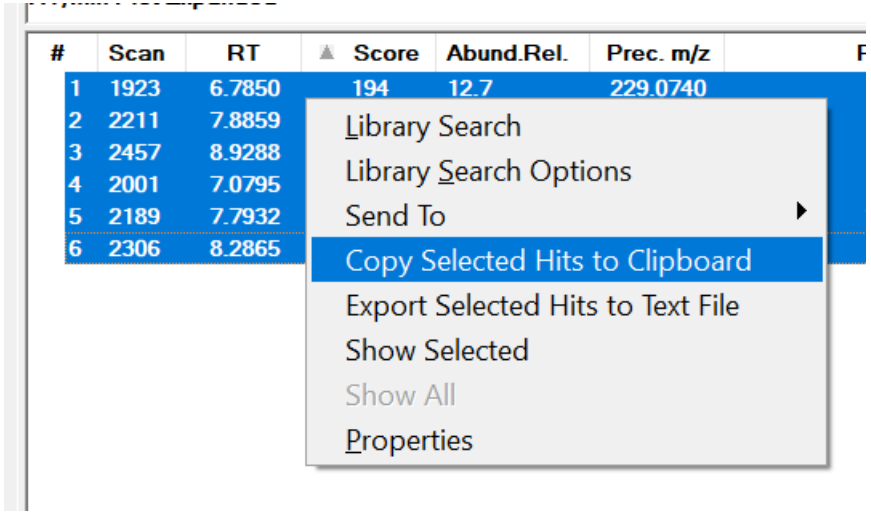
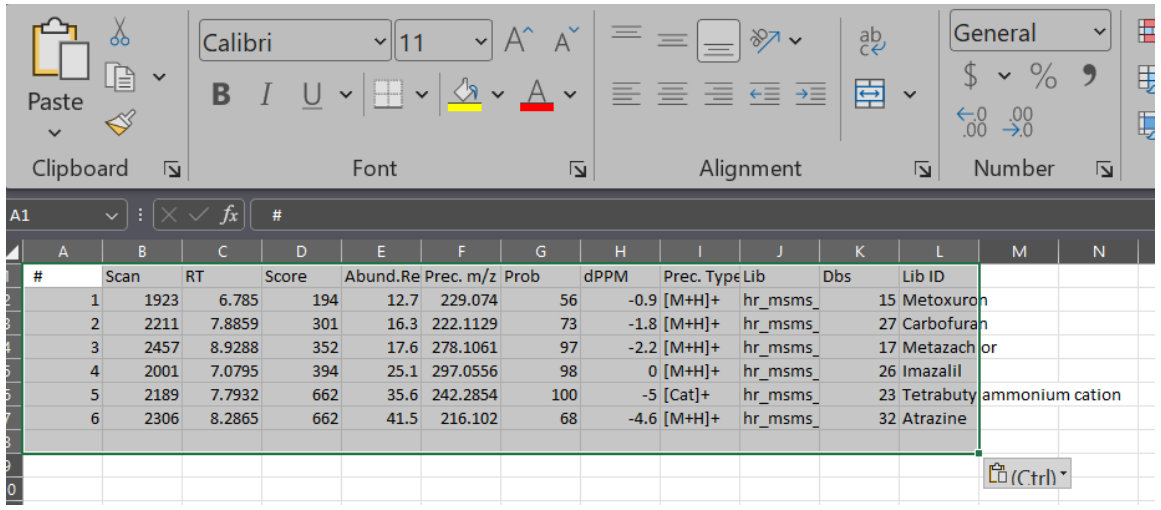
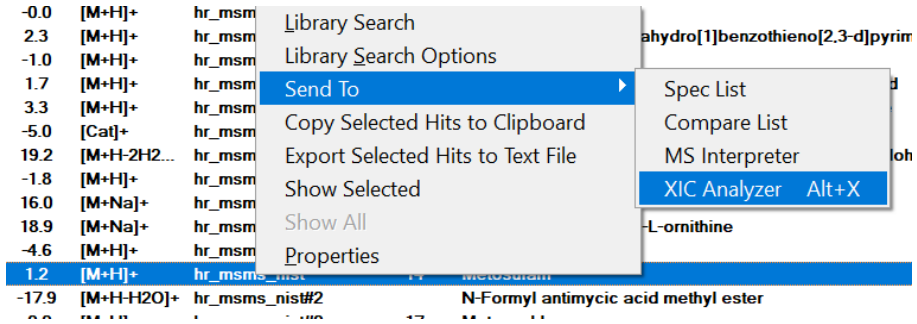
Excel Report

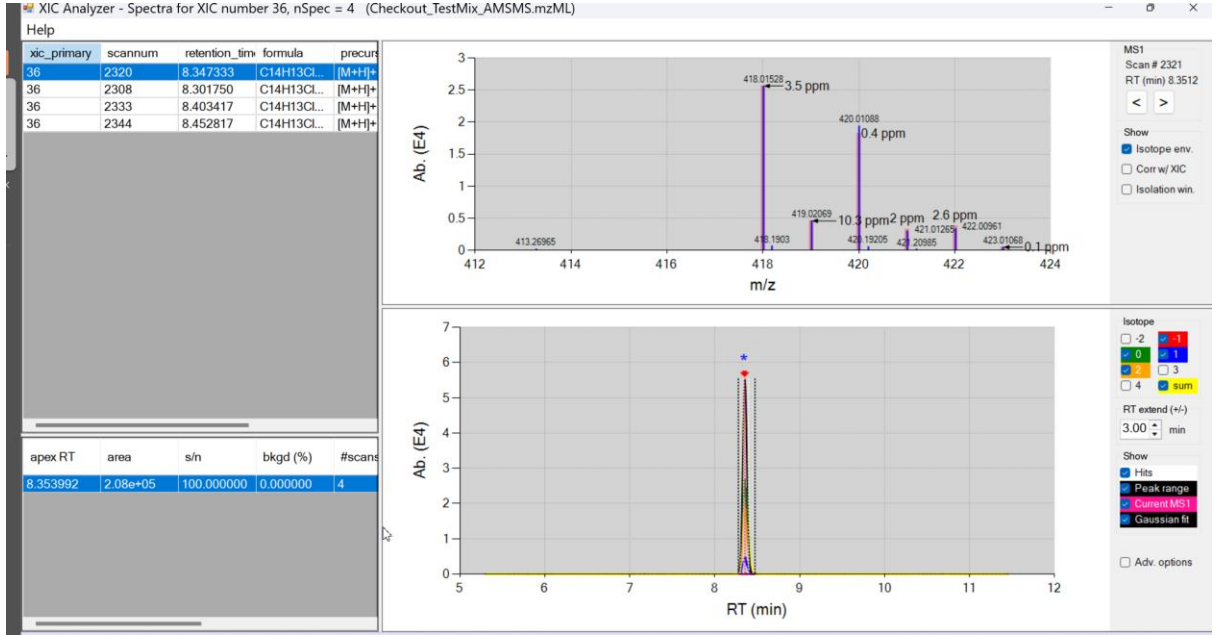
- Select Entries of Interest
- Then right click and Select “Copy Selected Hits to Clipboard”
- Paste into Excel

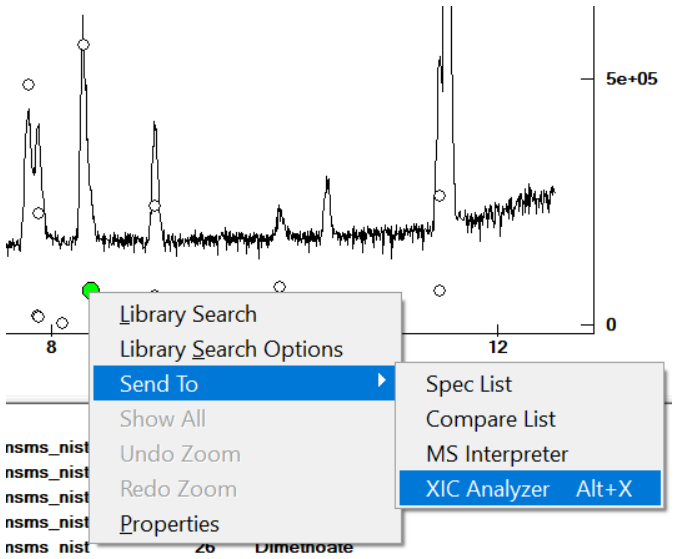


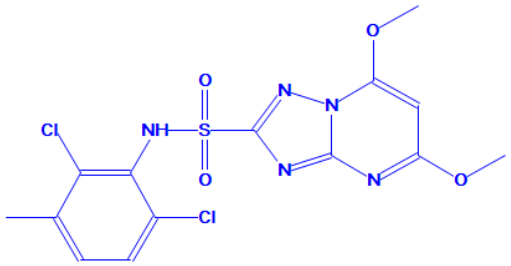


Send Spectrum to See MS2 Spectra in XIC Browser window, note isotope pattern for presence of 2 chlorines for this Identity
F1 keyboard or Help to See Details of XIC Window









Help Window
XIC Browser