NIST Standard Reference Database 1A

NIST Mass Spectral Search Program (Version 4.0)
Mass Spectral Libraries (NIST/EPA/NIH EI & NIST Tandem 2026)
For Use with Microsoft® Windows
User's Guide

This provides instructions for the use of NIST/EPA/NIH and Tandem Libraries, which are provided under separate Licenses, with MS Search v.4.0. Some sections are for individual Libraries and others are for use of both Libraries.

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Distribution of the NIST\EPA\NIH EI Library includes the current versions of MS Search, MS Interpreter, and Lib2NIST and the NIST Automated Mass spectral Identification System (AMDIS) program. Distribution of the NIST Tandem Library includes the current versions of MS Search, MS Interpreter, and Lib2NIST.

Use of any NIST Mass Spectral Library is limited to a single computer accessible by no more than a single user at any one time. There is no restriction as to multiple copies in the same or multiple formats on the same single-user computer or to backup copies which are for management purposes and not for use on any other computer. In the event of an upgrade from a previous version of any Mass Spectral Library, that previous version of that Library may not be installed on another computer and used in the same time period as the upgrade. The previous version may continue to be used on the same single-user computer on which the upgrade is installed. Both the EI and Tandem Library can be used on the same computer with a single version of MS Search. A separate License is required for both to be used on separate computers beginning with NIST 26.

Certain trade names and company products are mentioned in the text to specify adequately the computer products and equipment needed to use this software. In no case does such identification imply endorsement by the National Institute of Standards and Technology of these computer products and equipment, nor does it imply that the products are necessarily the best available for the purpose.

A License to either of the NIST Libraries is only available through Authorized NIST Distributors. A current list of these Distributors can be found at: *https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:distributors*.

Users of any previous version of a NIST Library can purchase an upgrade to that Library license through one of these Distributors. Each Distributor sets their own price.

Contents

New Features in NIST 26 Libraries & the MS Search Program

NIST 26 Libraries

The 2026 version of the **NIST /EPA/NIH Electron Ionization (EI) Library** includes two main mass spectral libraries, along with a database of GC methods and retention indices. In addition, the **Tandem Library** contains three separate libraries.

Contents of the NIST 26 Mass Spectral Libraries*

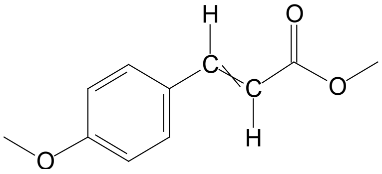
Library Name	Library Description	Library Contents
mainlib	Main EI MS Library	382,180 compounds, 10.1% increase
replib	Replicate spectra Library	49,095 Alternate spectra of compounds in mainlib, 9% increase
nist_ri	Retention Index/GC Method Library	527K values for 216K compounds (188,198 in mainlib)
hr_nist_msms	Accurate-mass Tandem Library of small molecules	2,639,049 Accurate-mass Tandem spectra for 66,782 compounds
lr_nist_msms	Low-resolution Tandem Library of small molecules	573,673 Low-resolution Tandem spectra for 66,566 compounds
apci_msms_nist	Library for ‘Extractables and Leachables’	6,403 Accurate-mass Tandem spectra for 623 compounds

* The spectra from the former biopep_msms_nist library (available in previous releases) were reorganized into the hr_nist_msms and lr_nist_msms libraries with the NIST 23 release and continue to be organized this way.

Overview:

- The first two libraries (mainlib and replib) include EI spectra with corresponding chemical structures and metadata.
- The nist_ri database provides GC methods and gas-phase retention indices.
- The remaining three libraries contain Tandem (MS/MS) spectra with molecular structures and acquisition details.

Since the 2020 release, all libraries have expanded at a rate comparable to the growth observed in NIST 23. The NIST/EPA/NIH EI Library has increased by **35,079** Compounds, with a proportional increase in the **GC Method/Retention Index Database**. The Tandem Library has had a significant increase in coverage, now including **68,635** compounds, including **3.2 million** spectra (low- and high-resolution) derived from **567K** precursor ions in both negative and positive modes.



As was the case with the previous last three releases, most new spectra were measured at NIST based on their analytical importance and then evaluated by at least two evaluators with the aid of newly developed software.

There is an increased display of chirality in the structures. Over the last several releases of the NIST Libraries, more structures have been provided with chirality. Increased chirality also brought about the introduction of the cross-double bond used for the representation of unspecific *E/Z* configurations (see figure at left).

What's New in MS Search Program v.4.0

The following features are added to the current version 4.0 (2026), distributed with NIST 26.

- Chromatogram tab view has been added allowing for the processing of GC/MS, or LC-MS/MS [DDA (Dynamic Data Acquisition, aka Data Dependent Analysis)] data files including automatic deconvolution and identification, based on comparison of components with the NIST Libraries using AMDIS for GC/MS and the NIST Mspepsearch for LC-MS/MS. Multiple controls are available to filter identifications to focus on the most relevant results. Each hit is identified in the chromatographic display and provided with a range of relevant data.

- Chromatogram Analysis values include Purity, multiple score types, signal to noise, relative abundance, and agreement with isotope abundances (Tandem only).

- In addition to the devolution of LC-MS data, the chromatogram tab view can perform deconvolution, conventional identification, and the hybrid search, simultaneously.
- The Hybrid Search has been updated to report the most chemical difference between the searched spectrum and each library compound (dForm) along with relative likelihood of each dForm.
- For the Hybrid Search (EI Only), the Retention Index (semi-standard non-polar columns) differences between the searched spectrum and library compound are estimated (dRI) as well as the differences in observed and estimated retention index values (ddRI).
- Retention Index (EI only) differences and their resulting score corrections are computed for each library Hit, using AIRI estimates for library compounds without experimental values.
- When searching an individual EI spectrum that is of a TMS derivative, the search can be constrained to reject compounds containing OH or NH with no TMS derivatization, reject all TMS derivatized compounds, or not to filter TMS derivatives.
- Addition of the *XICanalyzer* program for use with Tandem Spectrum Type in Chromatogram tab view.
- Redesigned dialog boxes making the ability to change various display aspects easier. These too are Spectrum Type Specific.
- A number of ***NEW*** column Headers have been added to the Hit List windows.
- A **Show Structure** option has been added to the **View** menu which will display the structure of the currently selected Hit in a floating window, easily turned on and off.
- Several features added to the Right Mouse Button (RMB) menu for selected spectra.
  - Switching to the matching name entry of the library identification in the Name tab of the current library
  - Exporting selected spectra to an Excel-compatible *.TSV file or to the Windows Clipboard
- Penalize rare compounds added for scoring based on a new algorithm (Compound Ubiquity with respect to non-mass spectrometry databases)
- Some **Main Menu** items have been moved. Exploring is recommended.

Additional Information on the ***NEW*** Features of MS Search v.4.0 can be found at (*https://mzinterpretation.com/*).

What was New in Version 3.0 of the MS Search Program

The following features were added to the version 3.0 of MS Search, distributed with NIST 23.

- Major Spectrum Type choice (EI, Tandem, Peptide, All) helps to suppress display of search parameters most probably not used.
- Name search has been improved by increasing search field from 16 to 249 characters; the list of names has been split into two windows: compound list in the upper window and spectra list in the lower window.
- The list of **Other Databases** (non-mass spectra) was significantly extended.
- ***NEW***. A Partial Spectrum Search method was added.
- AI estimated RI are displayed in EI Hit List and is used for EI search with RI **Match Factor** correction instead of missing semi-standard non-polar RI in a Hit.
- Hovering a mouse pointer around a peak in a spectrum plot displays peak annotation.

Changes

- Term “In-source/EI with accurate ion *m/z*” replaced with “HiRes No Precursor”.
- “Penalize rare compounds” EI library search option has been retired.
- Clicking the InChIKey in the Text Information window takes you to an Internet Google Search rather than to PubChem.

New Features MS Search Program v.2.4

Since the release of Version 2.3 with NIST 17, no substantial changes were made to the fundamental Search Routines. There have been several display changes which allow for better control of the display of **Hit Lists** and the handling of the many Tandem spectra that can be associated with a single compound.

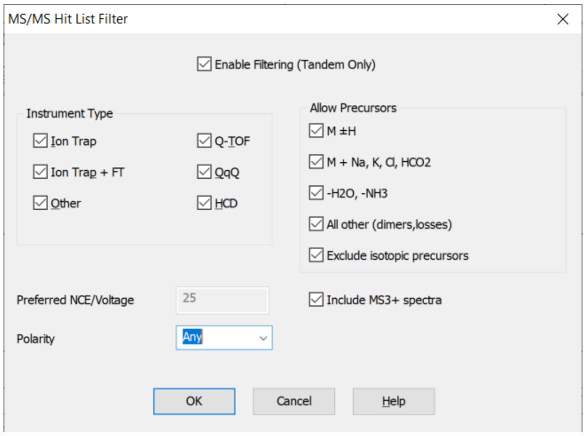
- *Names and orders of some library searches have been changed*

Identity Search		**Similarity Search**	
NIST 20	*NIST 17*	*NIST 20*	*NIST 17*
EI Normal*	**Quick***	EI Hybrid	Hybrid
EI Quick	Normal	EI Simple	Simple
MS/MS	MS/MS	EI Neutral Loss	Neutral Loss
In-Source Hi Res	In-source Hi-Res	MS/MS in EI	MS/MS in EI
		MS/MS Hybrid	MS/MS Hybrid

* It is important to note that the **Normal** search, NOT the **Quick** search, is the default **EI Identity Search**. This may cause a difference in Hit List between NIST 20 and older versions for the same search.

EI searches are strictly unit mass resolution and MS/MS (Tandem) searches allow the specification of high mass accuracy peaks.

- *Tandem Mass Spectra Filtering for all **Hit Lists** (Spectrum and Other Searches)*
  The NIST Tandem Library contains spectra over a range of energies, precursor types, and fragmentation methods. This means that a search of the Tandem Libraries can produce many Hits generated from a single compound, which can be tedious to sort through. To facilitate this task, a filtering process is available that can easily control the types of spectra shown in a **Hit List**. This is invoked by pressing the filter (▽) button in the **Button Bar** above the **Hit List** showing results. This is available for both the **Lib. Search** and **Other Search** tab views. The filtering process may be turned on and off with the control at the top of this dialog box (below). When the filter is enabled, the ▽ button will appear to be pressed. Upon pressing OK, the **Hit List** will reflect the selections in this dialog box. These controls match those in the Constraints section; except they do not permanently remove hits – all original hits can be seen by turning off the 'Enable Filtering' checkbox. This filter has no effect on EI searches. (*Figure 1*)
- Corresponding changes are made in **Constraints** used when searching Tandem Libraries.



MS/MS Hit List Filter

☑ Enable Filtering (Tandem Only)

Instrument Type
☑ Ion Trap
☑ Ion Trap + FT
☑ Other
☑ Q-TOF
☑ QqQ
☑ HCD

Allow Precursors
☑ M ±H
☑ M + Na, K, Cl, HCO2
☑ -H2O, -NH3
☑ All other (dimers,losses)
☑ Exclude isotopic precursors

Preferred NCE/Voltage 25
☑ Include MS3+ spectra
Polarity Any

Figure 1. Tandem Hit List Filter Used to Limit Hits Displayed from Library and Other Searches Only Applicable to the Tandem Libraries.

Enhancements to Utility Programs Since NIST 11

Mass Spectral Interpreter has significantly evolved over the past three years. There was a major release in April of 2020 that included a considerable number of enhancements from earlier versions. The major areas involve mechanisms and their relative rates for fragmentation of all types of ions (EI and Tandem MS, both positive and negative; integer and accurate mass) as well as various interface enhancements. This program connects mass spectral peaks to their probable chemical structure origin and can be quickly invoked from the NIST MS Search Program with the F9 key.

New Features in MS Search v.2.3 Released with NIST 17

Following Version 2.2, distributed with NIST 14, several important features were added to the NIST Mass Spectral Search Program. These are summarized below.

Features Relevant to Electron Ionization Mass Spectra (Low Resolution)

- A new **EI Hybrid Search** (**Similarity**/**EI Hybrid**).
- Presearch for EI Similarity/Neutral Loss search

Features Relevant to All Mass Spectra

- InChIKey presearch for all Library spectrum searches (finds all spectra, which have the same first InChIKey segment as the search spectrum, and compares them to the search spectrum)
- When a spectrum is associated with a structure in the **Spectrum Information** dialog box of the **Librarian** tab view, the InChIKey is automatically associated with the spectrum.
- Columns in any **Hit List** can be positioned as desired.
- Update list of libraries may be performed without exiting MS Search
- Indexing old libraries for EI Hybrid, EI Loss, HiRes MS/MS Hybrid, HiRes Any-peaks Peak and Loss searches, and InChI presearch. This includes third-party libraries like the Wiley Registry.

Features Relevant to Tandem and High-Resolution Mass Spectra

- **HiRes MS/MS Hybrid Search**
- Types of **Any Peak Search**: HiRes peak and HiRes loss
- Display additional MS/MS information in **Hit Lists**
- Edit accurate peak *m/z*, intensities, and peak annotations in the built-in mass spectrum editor
- MS/MS Precursor *m/z* search
- Precursor type constraint
- Ion polarity filter

New Features in MS Search v.2.2

The following features were added to version 2.2, distributed with NIST 14 as compared to ver. 2.0g, distributed with NIST 11 or with NIST 12 Tandem Library.

Features Relevant to Electron Ionization Mass Spectra (Low Resolution)

- Use of Retention Index in library searching: Deviation of experimental RI from the library value optionally used to penalize the **Match Factor**.
- Older user libraries with RI values can be indexed to allow the RI values to be used in library searching
- Display RI values in a **Hit List** column
- Import, export, and edit compound RI value(s)
- New definitions of derivatives are used for finding replicate spectra
- Optionally display derivative precursor in the Lib. Search **Hit List**

Features Relevant to All Mass Spectra

- All small molecule mass spectra in NIST libraries have **InChIKey**(s) linked to PubChem (NIH). These **InChIKey**(s) may also be used for searching compound information on the Web or for exact structure searches within NIST libraries.
- Set Prepend/Overwrite/Ask automatic mass spectrum import option.
- Import and display Glycan structures in KEGG Chemical Function (KCF) format and add them to user library mass spectra.

Features Relevant to Tandem and High-Resolution Mass Spectra

- Display additional MS/MS information in **Hit Lists**
- Edit accurate peak *m/z*, intensities, and peak annotations in the built-in mass spectrum editor
- In-source tandem spectra or EI with accurate *m/z* spectra - High resolution mass spectra
  - Import of ‘in-source/EI with accurate *m/z*’ spectra, which does not include a defined *m/z* of the precursor ion. To import these spectra, select "In-source /EI (accurate *m/z*)" in Spectrum Import Options.
  - In-source/EI with accurate *m/z* spectra have accurate ion peak *m/z* and intensities. The accuracy is set in Spectrum Import Options.
  - In-source/EI with accurate *m/z* spectra may be searched in In-source/EI with accurate *m/z* and/or MS/MS library with the new In-source HiRes search (ion peak *m/z* tolerance down to 0.015 ppm or $6x10^{-5}$ *m/z* units), Similarity Simple, Identity Normal or MS/MS Presearch OFF search options. For this purpose, adding Reverse Search option may be useful.
  - In-source/EI with accurate *m/z* spectra may be added to a user library. Currently, in-source/EI with accurate *m/z* spectra added with NIST MS Search to a user library may be searched with In-source HiRes search only with Presearch OFF option. Rebuild the library with Lib2NIST to get in-source HiRes search benefits[1].
- A new MS/MS search for small molecules
  - Improved ability to identify compounds with a few dominant peaks – a common situation for Tandem MS.
  - More robust scoring for spectra acquired on imperfectly tuned instruments
- New Mass Spectrum Import Options
  - Control the number of decimal places in the input *m/z*
  - Set peak intensity threshold
  - Import in-source or EI spectrum with accurate ion peak *m/z* and intensities.
  - Import MS/MS and in-source/EI with accurate *m/z* spectra from JCAMP files (October 2012, nistms32.dll version 2.1.5.3 or later)

GC Methods / Retention Indices Database in NIST/EPA/NIH EI Libraries

The NIST GC Methods/Retention Indices database introduced in NIST 05 continues to be expanded with new entries for existing spectra as well as entries for newly added spectra. When spectra are measured by NIST, they are only added if RI data is available as part of the measurement.

In NIST 17, the GC Methods/Retention Indices library was extended by including summary statistical analysis of all data in the record. This information will not be displayed in the spectrum **Text Information** window if the **GC Retention Indices** box on the **Hit List Info** page of the **Library Search Properties’** dialog box is not selected. The summary data are given in terms of the type of column (standard non-polar (DB-1), semi-standard non-polar (DB-5), or polar (carbowax)), the **median** value of the retention data for that column type, the deviation, and the number of experimental determinations. Note the deviation is calculated as the median absolute deviation. In the example below (for 6,6-Dimethyl-2-methylene, bicyclo[3.1.1]heptan-3-ol or pinocarveol), there are 13 values for the DB5 series of data, 9 for the DB 1 and 12 for the polar column with median absolute deviations of 4,7 and 8, respectively.

Experimental RI median±deviation (#data)	
Semi-standard non-polar:	1138±4 (13)
Standard non-polar:	1135±7 (9)
Polar:	1654±8 (12)

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[1] Library searchable with In-source HiRes search has files peak_em0.inu and peak_em0.dbu

The averaged data will also optionally be shown in the **Hit List** of the **Lib. Search** tab view based on the column type selection on the RI(GC) page of the Library Search Properties dialog box. This selection also determines what is optionally displayed in the **Hit Lists** of the **Other Search** tab view when the **Spectrum Type** is **EI**. The column position for the RI values is user selectable.

NEW to the Hit List in v.4.0 of MS Search (NIST 26) of the **Lib. Search** tab view is dRI. This is the difference between the average of the database values and that associated with the searched spectrum.

The Retention Indices checkbox controls the display of detailed experimental data. The number of GC Methods/Retention Indices displayed is controlled by selecting **All** or **First** and entering the number of values to be displayed in the properties dialog box. The default is to display the first 2 values. Displaying all values may cause the display to be slow if there are a very large number of method values.

In addition, for most compounds, values are shown using two theoretical methods:

1) **Estimated retention index** is displayed based on the structure associated with the spectrum. This is based on a chemical group additive approach. These values are reported in linear alkane retention index units **(iu)** with a confidence interval, e.g., “Confidence interval (Ketones): 57(50%) 246(95%) iu” reported for acetone. This means that there is a 50% probability that the true value falls within 57 iu of the estimated value and 95% of the time the true value falls within 246 iu of the estimated value. The analysis is based on error measurements of all the data for the class of the compound given, in this example, ketones. These estimates are only shown if **Retention Indices** are checked in the properties box. The estimates are not displayed in the **Hit Lists**. If spectra in a user-library have associated structures, this Estimated RI value will also be listed for these spectra if the calculation is possible.
2) **AI predicted semi-standard non-polar retention index** values from a newly developed approach by NIST scientist Lewis Geer are also shown. These predicted non-polar linear alkane retention index indices [1] are more accurate than the Estimated non-polar values provided for NIST/EPA/NIH EI Library spectra in this and previous releases as well as user-library spectra with associated structures. AI estimated values are NOT available for user-libraries with structures. The AI values were calculated for semi-standard non-polar (DB-5) columns and are expressed in index units (iu). These indices are generated by feeding the chemical structure of compounds into a path-augmented graph transformer network [2]. This network was trained on experimental retention indices from the NIST EI Library, minus a test and validation set. The test set was used to estimate 50% and 90% confidence intervals for all compounds that have predictions. AI Retention Index values have been estimated for all the compounds with EI spectra in NIST 23.

If the semi-standard non-polar column is selected on the **RI(GC)** page of the **Library Search Options** dialog box and there are no experimental values for the compound, the AI predicted semi-standard non-polar retention index value will be reported. This is indicated with an (*) following the value. No values for other column types result in no value reported.

References:

[1] Kováts, E.: Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone. Helvetica Chimica Acta 41(7), 1915{1932 (1958). doi:10.1002/hlca.19580410703

[2] Chen, B., Barzilay, R., Jaakkola, T.: Path-Augmented Graph Transformer Network (2019). https://arxiv.org/abs/1905.12712

The NIST Tandem Library

Tandem spectra, along with an increased number of fields relevant to data obtained using MS/MS, are provided in three separate libraries (high res [hr_nist_msms], low res [lr_nist_msms], and APCI [apci_msms_nist]). Most of the spectra in these libraries have been measured at NIST, though some have been acquired from other sources. The preparation of the Tandem Library revealed that at sufficiently high signal-to-noise measurement conditions, modern instruments can provide very reproducible “library-searchable” spectra. Although collision energy is an important variable in collision cell instruments, spectra vary in an understandable way depending on the compound and instrument type as well as collision-energy conditions. Energy-dependent variation is accounted for by including spectra, for most compounds, over a wide range of collision energies, ranging from slight to complete dissociation of the precursor ion. The **Score** reported for the best matching spectrum is used for identification (use the **Best matching only** button to show only closest matching spectra).

The **MSMS** tab view, included in previous releases, has been discontinued. The tandem mass spectra are treated like spectra found in any NIST MS Search Program compatible Library. Simply select the NIST library or libraries you want to search for CAS#, Formula, molecular weight range, etc. All that is necessary is to include the appropriate library or libraries in the **Include Libs** section of the **Search** dialog box for the **Other Search** tab view or as the single library to be searched in the **Incremental Name Search**. The **Library Spectrum Search** and **Structure Similarity Search** are also applicable. One of the challenges when looking at the tandem ion mass spectra for a given compound is that there can be several different precursor ions for that compound and spectra for several different collision energies. Some filtering can be done with the Tandem Filter feature that has been added. This filter is not available when searching by name.

It is important that the user understand that tandem spectra in general are different from EI spectra as well as the fact that structures in the MS/MS Library refer to the form of the compound before the precursor ions are formed. It should also be noted that the integers in the MW field of the Text Information window for the spectrum are the nominal (integral) mass of the neutral molecule. The Exact Mass field shows the monoisotopic mass.

For beam-type instruments (e.g., triple quadrupoles, HCD, and Q-tof instruments), the **MS2** designation in the **Spectrum Type** field refers to spectra generated by dissociation in a collision cell of a selected precursor ion. For ion trap instruments, the **MS2** designation refers to dissociation in the trap after following the isolation of the precursor ion.

In the case of data acquired using an ion trap mass spectrometer, there may also be data designated as **MS3**, and **MS4** spectra, which were obtained by the isolation of a precursor-ion for a subsequent iteration of MS/MS.

As can be seen, there is a great deal more information provided in a text format for the spectra in the tandem libraries. The **Library Spectrum Search** and **Structure Similarity Search** are also applicable. Note that in all cases, the structure shown is that of the compound prior to ionization.

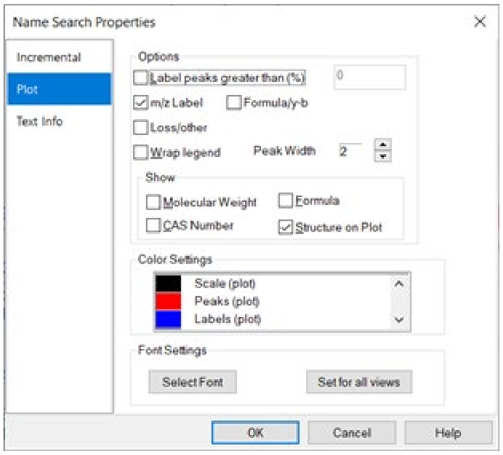
More Information from the NIST Data

A ***NEW*** feature has been added to MS Search v.3.0 by which hovering the Mouse pointer near a peak in a mass spectrum display will show the items selected in the **Options** section of the Plot tab **Properties** dialog box for display, e.g., ***m/z* Label**, **Formula/y-b**, and/or **Loss/other** in a box floating on the spectrum.

This is especially advantageous for viewing spectra from one of the Tandem Libraries. Only the data for the selected items will be displayed.

In an accurate *m/z* value Tandem mass spectrum, selecting only the ***m/z* Label** checkbox will cause the peaks to be labeled only with the accurate *m/z* value of the ion. Selecting **Formula/y-b** checkbox only causes the peaks to be labeled with the elemental composition of the ion. Selecting only the **Loss/other** checkbox results in a label that indicates the elemental composition of the neutral loss from the precursor ion, e.g., **p-C15H28**, where **p** represents the precursor ion.

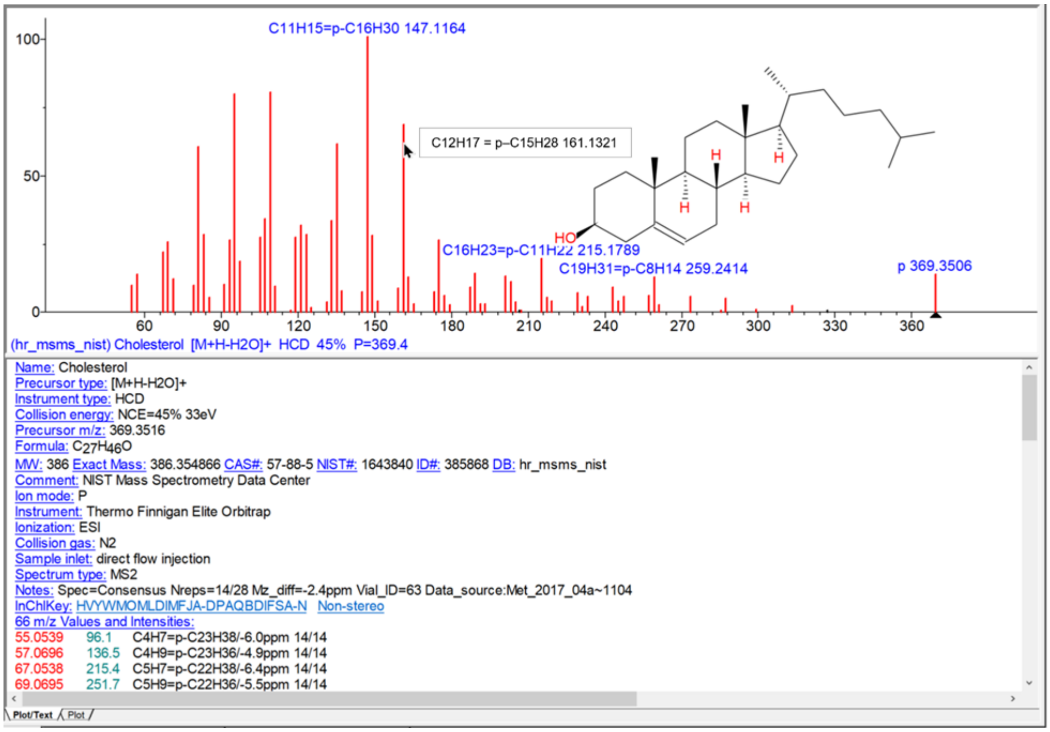
Figure 3 is an illustration of the selection of all three checkboxes. In order to have this information displayed, it had to be entered at the time the mass spectral record was created.



☐ Label peaks greater than (%) 0
☑ m/z Label ☐ Formula/y-b
☐ Loss/other
☐ Wrap legend Peak Width 2
☐ Molecular Weight ☐ Formula
☐ CAS Number ☑ Structure on Plot

■ Scale (plot)
🟥 Peaks (plot)
🟦 Labels (plot)

Figure 2. Plot tab view of the Name Search Properties Dialog Box as is in Version 4.0.



```
(hr_msms_nist) Cholesterol  [M+H-H2O]+  HCD  45%  P=369.4
Name: Cholesterol
Precursor type: [M+H-H2O]+
Instrument type: HCD
Collision energy: NCE=45% 33eV
Precursor m/z: 369.3516
Formula: C27H46O
MW: 386 Exact Mass: 386.354866 CAS#: 57-88-5 NIST#: 1643840 ID#: 385868 DB: hr_msms_nist
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
Notes: Spec=Consensus Nreps=14/28 Mz_diff=-2.4ppm Vial_ID=63 Data_source:Met_2017_04a~1104
InChIKey: HVYWMOMLDIMFJA-DPAQBDIFSA-N   Non-stereo
66 m/z Values and Intensities:
55.0539     96.1    C4H7=p-C23H38/-6.0ppm 14/14
57.0696     136.5   C4H9=p-C23H36/-4.9ppm 14/14
67.0538     215.4   C5H7=p-C22H38/-6.4ppm 14/14
69.0695     251.7   C5H9=p-C22H36/-5.5ppm 14/14
```

Figure 3. Mass Spectrum from the NIST 26 Tandem Library Showing Hover Display for Unlabeled Peak.

Software Changes from earlier versions of the MS Search Program

Version 2.0g of the MS Search Program (NIST 11)

Below is a list of features new to the previous version, 2.0g, which was distributed with NIST 11 and the NIST 12 Tandem Library.

- A new **Exact Mass Search** allows searching for exact mass of a precursor (exact molecular weight) using its mass or exact *m/z* of a product ion. It considers the presence or absence of an electron, adducts or losses; accepts uncertainty in ppm or millimass units; and allows searching for isotopic or monoisotopic peaks.
- A new **Exact Mass** Constraint similar to **Exact Mass Search**.
- Ability to use accurate mass to search exact fragment ion mass values in NIST libraries using the **Any Peaks Search**.
- Display of the exact mass with any spectrum that has an associated chemical formula. This even works for low resolution EI peaks – these are processes using the separate MS Interpreter formula to assign chemical formula to most peaks.
- Manage and search as many as 127 separate MS libraries instead of 16, which was the limit in previous versions.
- Optional display of columns in **Hit Lists** containing the number of synonyms and the number of **Other Databases** in which the Hit appears. The **Hit List** can be sorted according to the values in these columns.
- Full compatibility with the most recent NIST Peptide MS/MS Libraries.
- Copy selected lines from **Hit List** or **Spec List** into Windows Clipboard as tab-delimited Unicode text; this may be conveniently pasted into Excel.
- Spectra in the MSP text format can be imported from the Windows Clipboard into the **Spec List** via a Right Mouse button (**RMB**) menu option.
- Searching for words in mass spectrum **Text Information** Window.
- Optional exclusion of Homologues from the results of **Structure Similarity Search**.
- Import spectra from mzXML and mzData files.
- Multiple CAS Registry numbers for a spectrum. A small fraction of spectra in NIST 14 has more than one CASrn. Each CASrn may be used in a **CASrn Search**.

- The MS Interpreter program now enables high mass accuracy for both molecular ions and product ions.
- An alternative peak matching method using ‘time warping’ was implemented to better match peaks in noisy MS/MS spectra.
- Several convenience features.[2]

Version 2.0f of the MS Search Program (NIST 08)

Below is a list of features new to the previous version, 2.0f, which was distributed with NIST 08.

- A new spectrum search for MS/MS spectra in MS/MS libraries, including the NIST MS/MS libraries.
- Spectra of stereo isomers, tautomers, and derivatives are displayed as replicate spectra.
- Tags (which can function as Field Names) can be entered into the “Comments” field of a user library along with the Field contents.
- **Hit List** can be sorted alphabetically.
- Five new Constraints have been added: **Tags in Comment**, **Peptide Sequences**, **Peptide Mobile Protons**, **Peptide Charge**, and **Peptide Number of Residues**. **Tags in Comment** Constraint, for example, allows searching for the name of the contributor in the EI Library.
- A search for information in the “Contributor/Comment” field, which may be particularly useful for certain types of information relevant to MS/MS.
- Flexibility has been added to searches using a “Name Fragment in Constraints”. It is now possible to specify the presence or absence of any arbitrary part or parts of a name.
- The **Program** does not use or require the installation of special NIST fonts.

---

[2] New convenience features added to version 2.0g including:

- Default view of **Constraints** and **Other Searches** dialog boxes changed to provide more convenience to the users.
- Ability to Sort the **Spec List** alphabetically, by number of **Synonyms**, by number of **Other Databases**.
- Toggle between Tab views (**Lib. Search**, **Other Search**, **Name**, **Compare**, **Librarian**, and **MSMS** Windows) using Ctrl-Tab or Ctrl-Shift-Tab.
- When a Tag from the “Comment” field is displayed separately, its display in the “Comment” field is suppressed.

OS Compatibilities

- **The NIST Mass Spectral Search Program, Version 4.0 is ONLY COMPATIBLE** with the 64-bit versions.
- **MS Search v.4.0** is a 64-bit Unicode application that requires Windows Vista, or Windows 7, 8, 8.1, 10, 11.
- **Adobe PDF** reader is required to access the **NIST 26** Manual.
- **MS Search v.4.0** has only been tested with Windows 10 and Windows 11.
- It is best to turn off the custom scaling level feature of Windows 10 and 11 as issues may be observed.

Notes On Installation

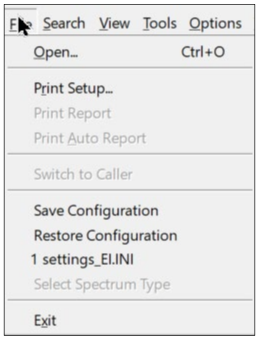
The installation program for the Tandem and EI versions of NIST 26 has different appearances.

The Installation Program does not create any **Desktop** or **Taskbar** icons. To run MS Search the first time, select the **Windows** icon on the far left of the **Button Bar** right after running the installation. The display will have an icon for MS Search at top left. Select this icon to run MS Search for the first time. This will place an icon for MS Search in the **Taskbar**. This will cause the icon for MS Search v.4.0 to be displayed. Put the Mouse pointer on the icon; click the Right Mouse button once. This will display an RMB menu. Select **Pin**. The icon is now pined to the Taskbar.

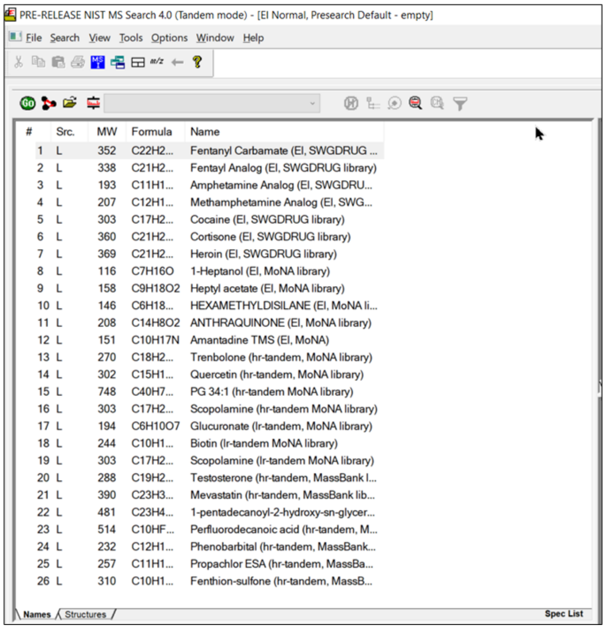
To create a Desktop icon, select the windows icon on the lower left of the display put the Mouse pointer on the NIST icon at the top of the display and drag it to the Desktop.

Nota Bene:

Each individual tab view has a Main Menu. Each Main Menu has a **File** selection. Each **File** menu has a **Select Spectrum Type** option. If only one of the two available library types (EI or Tandem) is installed, the Select Spectrum Type option will remain perpetually grayed (unavailable), as shown in the figure on the right. Once the other library (EI or Tandem) is installed, this option becomes available.

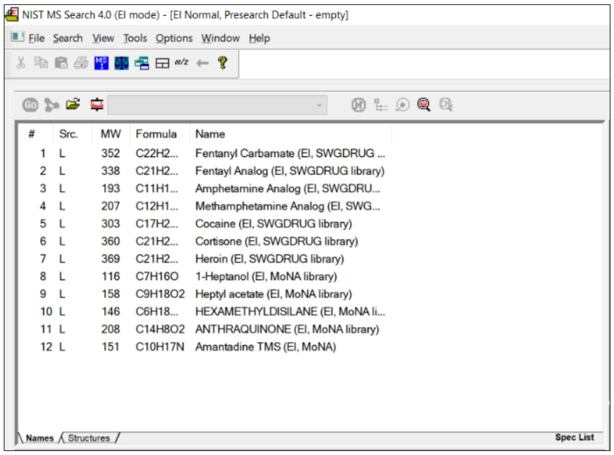


When MS Search is first run, the default display is the **Lib Search** tab view. The Spec List window (upper-left) will be populated with individual spectra that can be searched. There are 12 in the EI installation and 26 in the tandem installation; the first 12 of which are also included the EI installation.



#	Src.	MW	Formula	Name
1	L	352	C22H2...	Fentanyl Carbamate (EI, SWGDRUG ...
2	L	338	C21H2...	Fentayl Analog (EI, SWGDRUG library)
3	L	193	C11H1...	Amphetamine Analog (EI, SWGDRU...
4	L	207	C12H1...	Methamphetamine Analog (EI, SWG...
5	L	303	C17H2...	Cocaine (EI, SWGDRUG library)
6	L	360	C21H2...	Cortisone (EI, SWGDRUG library)
7	L	369	C21H2...	Heroin (EI, SWGDRUG library)
8	L	116	C7H16O	1-Heptanol (EI, MoNA library)
9	L	158	C9H18O2	Heptyl acetate (EI, MoNA library)
10	L	146	C6H18...	HEXAMETHYLDISILANE (EI, MoNA li...
11	L	208	C14H8O2	ANTHRAQUINONE (EI, MoNA library)
12	L	151	C10H17N	Amantadine TMS (EI, MoNA)
13	L	270	C18H2...	Trenbolone (hr-tandem, MoNA library)
14	L	302	C15H1...	Quercetin (hr-tandem, MoNA library)
15	L	748	C40H7...	PG 34:1 (hr-tandem MoNA library)
16	L	303	C17H2...	Scopolamine (hr-tandem MoNA library)
17	L	194	C6H10O7	Glucuronate (lr-tandem, MoNA library)
18	L	244	C10H1...	Biotin (lr-tandem MoNA library)
19	L	303	C17H2...	Scopolamine (lr-tandem MoNA library)
20	L	288	C19H2...	Testosterone (hr-tandem, MassBank l...
21	L	390	C23H3...	Mevastatin (hr-tandem, MassBank lib...
22	L	481	C23H4...	1-pentadecanoyl-2-hydroxy-sn-glycer...
23	L	514	C10HF...	Perfluorodecanoic acid (hr-tandem, M...
24	L	232	C12H1...	Phenobarbital (hr-tandem, MassBank...
25	L	257	C11H1...	Propachlor ESA (hr-tandem, MassBa...
26	L	310	C10H1...	Fenthion-sulfone (hr-tandem, MassB...

Spec List window for Tandem Installation



#	Src.	MW	Formula	Name
1	L	352	C22H2...	Fentanyl Carbamate (EI, SWGDRUG ...
2	L	338	C21H2...	Fentayl Analog (EI, SWGDRUG library)
3	L	193	C11H1...	Amphetamine Analog (EI, SWGDRU...
4	L	207	C12H1...	Methamphetamine Analog (EI, SWG...
5	L	303	C17H2...	Cocaine (EI, SWGDRUG library)
6	L	360	C21H2...	Cortisone (EI, SWGDRUG library)
7	L	369	C21H2...	Heroin (EI, SWGDRUG library)
8	L	116	C7H16O	1-Heptanol (EI, MoNA library)
9	L	158	C9H18O2	Heptyl acetate (EI, MoNA library)
10	L	146	C6H18...	HEXAMETHYLDISILANE (EI, MoNA li...
11	L	208	C14H8O2	ANTHRAQUINONE (EI, MoNA library)
12	L	151	C10H17N	Amantadine TMS (EI, MoNA)

Spec List window for EI Installation

Example data files are provided for the examples included in this manual. These data files are found in the C:\NIST26\MSSearch\test-files\ folder. This folder will contain an \EI folder and a \tandem folder depending on the installation performed. The \EI folder has both a hi-res\ and a \low-res\ folder. The \low-res\ folder has two GC/MS data files. The **C7-40.D** file is used to create a calibration file. The other file (**Hemp-flower-DCM-conc.D**) is used to demonstrate the capabilities of the **Analysis EI Input File** selection of the File menu displayed by selecting **File** from the Main Menu. The \hi-res\folder contains two CDF files. One is for RI calibration and the other (SRM3268-Kudzu.cdf) is for demonstration purposes. There are also EI GC/MS Data files in the C:\NIST26\AMDIS32\ folder.

The \tandem\ folder contains the **For_ToxMix_ASMS.msML** and **NTBLS2956_AC_01_PP_1.mzML** LC-MS/MS data files for use with the **Analyze MS/MS Input File** selection of the File menu displayed by selecting **File** from the Main Menu.

Installation

Windows 10, 11

NEW INSTALLATION on a computer that does not have a previous version of NIST Mass Spectral Data or software. NIST 26 is provided with two different installations:

NIST 26 EI Installation which includes

NIST MS Search Program. (c:\nist26\mssearch)
NIST 26 NIST/EPA/NIH Electron Ionization Library (c:\nist26\mainlib and c:\nist26\replib).
Lib2NIST Converter (c:\nist26\mssearch).
MS Interpretation Program (c:\nist26\mssearch).
GC/MS Analysis Program (c:\nist26\amdis32).
GC Method\Retention Index database (c:\nist26\nist_ri).

The NIST 26 Tandem Library

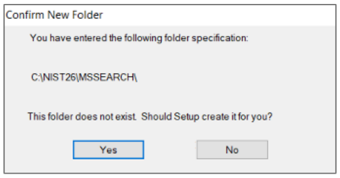
NIST MS Search Program. (c:\nist26\mssearch)
NIST 26 MS/MS HiRes Library (c:\nist26\mssearch\hr_nist_msms), Windows 10 or 11; 64 -bit Only.
NIST 26 MS/MS LowRes Library (c:\nist26\mssearch\lr_nist_msms).
NIST 26 MS/MS APCI Library (c:\nist26\mssearch\apci_msms_nist).
Lib2NIST Converter (c:\nist26\mssearch).
MS Interpretation Program (c:\nist26\mssearch).

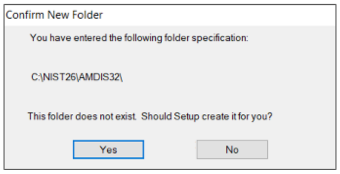
Each of the above installations is provided on its own media. The **NIST 26 EI Installation** media installs the NIST software and the EI Library on a single computer. The **NIST 26 Tandem Installation** media installs the NIST software and the Tandem Library on a single computer. These two installation programs have different appearances and perform somewhat differently.

If both the Tandem and EI Library will be used on the same computer, with a single version of MS Search, it is recommended that the **EI Installation** be done first. This will be followed by the **Tandem Installation**. Run the Tandem Installation as if it was being used as a standalone. This will overwrite some components that were installed with the EI; however, it ensures that all components of both installations are installed. If both libraries are installed on a single computer, the license prohibits installing either Library on another computer.

To install the NIST Software and the desired Library from the **NIST 26 EI** or the **NIST 26 Tandem** installation media, it is only necessary to run the **SETUP.EXE** program and accept the defaults.

- Open Windows Explorer and navigate to the location of the NIST 26 installation. This may be a folder, a CD drive, a USB drive, or in a folder on your computer, if you have done an FTP download of the installation.
- In the folder or on the device which contains the NIST 26 install software, highlight **SETUP.EXE** and double-click the left Mouse button to run the program. Depending on your computer settings, you may not see the **EXE** extension when using **Windows Explorer**. If this is the case, there are as many as three different files with the name SETUP, each having a different extension. Make sure the one with **Application** in the **Type** column is selected.
- Follow the instructions presented by the **SETUP.EXE** program.
- If installing the EI Version on a new computer, the **NIST/EPA/NIH MS Library and AMDIS Setup** dialog box will display the two dialog boxes (**Confirm New Folder**), sequentially overlaid, shown at the right, asking to create the C:\NIST\MSSEARCH\ and C:\NIST26\AMDIS32\ folders. Select the **Yes** button for both.


Confirm New Folder
You have entered the following folder specification:
C:\NIST26\MSSEARCH\
This folder does not exist. Should Setup create it for you?


Confirm New Folder
You have entered the following folder specification:
C:\NIST26\AMDIS32\
This folder does not exist. Should Setup create it for you?

- There will be a dialog box asking if you want to Copy or Link existing libraries. If this is a new installation on a new computer or an upgrade where user-libraries were backed up, there will be no existing libraries, select the **Next** button.
- If performing an EI Installation and the Agilent ChemStation Data Analysis software is on the computer, the next dialog box asks if the NIST Seach should be the default search, retain the PBM search, or do nothing. It is recommended to select the NIST Search (the default if the **Next** button is selected).
- The **Select Program Folder** dialog box is displayed next prompting to enter a Program Folder Name. The Default is NIST Mass Spctral Database. It is recommended that this name be edited to something similar to NIST 26 Mass Spectral Database or NIST 26 Mass Spectral Library.
- Allow the Setup.exe to continue, accepting the default for any further prompts. A dialog box will be displayed when the installation is complete.

The Installer may behave differently under different versions of Windows due to User Account Control security features.

Upgrading from a Previous Version of the NIST Data and Software

RECOMMENDED: If upgrading from a previous version of the NIST Library, there is no need to keep the previous version. First go to the \MSSearch\ folder of the previous version. This is usually in the c:\NISTxx\MSSearch\ folder, where xx is the version number of the previous version. Move any user-libraries (which are folders) to a secure backup location. The NIST Library folders for the previous versions (\mainlib, \replib, \ri_nist, or those listed for the Tandem Library) DO NOT need to be moved or backed up from the C:\NISTxx\MSSearch\ folder.

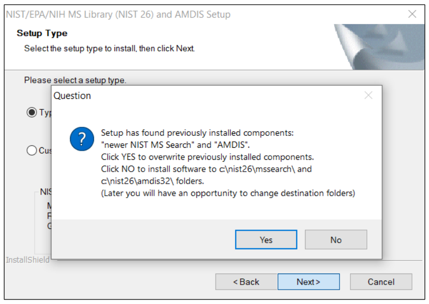
BEFORE running one of the **SETUP.EXE** programs on the installation media, the previous version of the NIST Software and Data should be uninstalled using the **Add or remove program**s feature of Windows.

If there are user-libraries (these are folders within the c:\NISTxx\MSSsrarch\ folder) to be retained, copy these to a different location. After the new version of the NIST software and Data are installed, they can be copied to the c:\NIST26\MSSearch\ folder.

To uninstall the previous version, after backing-up any user-libraries to be saved, select the magnifying glass 🔍 icon in the **Taskbar** at the bottom left of the Windows Desktop. Type "Add" in response to the **Type here to search** prompt. When **Add or remove programs** appears (upper left of displayed dialog box, select it. In the resulting Window, scroll down until **NIST xx MS EI and Tandem Library** (or something similar) comes into view. Then select the **Uninstall** button to uninstall the previous version of the NIST software and data. If installing both versions, install the EI version before the Tandem version.

After performing the uninstall, return to the c:\nistxx folder and delete this folder. You may have to delete the residual contents of the \mssearch folder and the \amdis32 folders inside the c:\nistxx folder to accomplish this total deletion. Now it is the time to install the distributed version of the NIST 26 Mass Spectra Data (EI or Tandem), MS Search, and its associated software.

DISCOURAGED: It is possible to keep both the existing versions and NIST 26. If the setup program finds a previous version of the MS Search program and the NIST Data, the **Question** dialog box on the right will overlay the **NIST Setup** dialog box. Read the contents carefully. If the **Yes** button is selected, the existing version MS Search and the NIST Data will be overwritten. The name of the previous version's folder will be retained (e.g., \NIST14) and the contents of the previous version will be overwritten. If **No** is selected, no changes to the existing folder will occur and NIST 26 will be installed in the C:\NIST26 folder. If **No** is selected, prompts will appear to allow setup to create the C:\NIST26\mssearch and C:\NIST26\AMDIS32\ folder as in the case for a clean installation. If user-libraries to be retained were moved to a folder and the plan is to move them to C:\NST26\MSSearch after the installation is complete, this dialog box saying to **Copy** or **Link** user-libraries by selecting them can be skipped by selecting the NEXT button. After the backed-up user-libraries are copied to the C:\NIST26\MSSEARCH folder and MS Search subsequently started, all will be available.



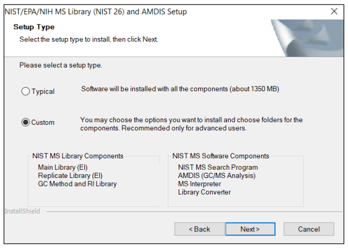
Setup has found previously installed components:
"newer NIST MS Search" and "AMDIS".
Click YES to overwrite previously installed components.
Click NO to install software to c:\nist26\mssearch\ and
c:\nist26\amdis32\ folders.
(Later you will have an opportunity to change destination folders)

A new **Program Folder** should be created (selected) for the **Start Programs** shortcuts (see New Installation, page *14*). The default name displayed when the **Program Folder** dialog box appears is **NIST Mass Spectral Database** and should be changed to something similar to **NIST 26 Mass Spectral Libraries**. If this is not done, the contents of the existing Program Folder, **NIST Mass Spectral Database**, will be overwritten, and the previous version of MS Search and its Libraries will no longer be accessible from the Start menu.

Select the defaults for the remaining dialog boxes.

INSTALLING THE TANDEM LIBRARY AFTER INSTALLATION OF EI LIBRARY

Locate and run the **SETUP.EXE** program on the Tandem distribution media. The dialog box on the top right is displayed. **Typical** is selected by default. Select **Custom**, then select the **Next** button. From the displayed dialog box (below, right) deselect all items except for those associated with the Tandem Library. After selecting the **Next** button on this second dialog box, follow the prompts as they appear on subsequent displayed dialog boxes to complete the installation of the Tandem Library.

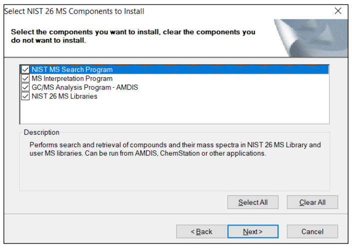


○ Typical — Software will be installed with all the components (about 1350 MB)

● Custom — You may choose the options you want to install and choose folders for the components. Recommended only for advanced users.

NIST MS Library Components
Main Library (EI)
Replicate Library (EI)
GC Method and RI Library

NIST MS Software Components
NIST MS Search Program
AMDIS (GC/MS Analysis)
MS Interpreter
Library Converter



☑ NIST MS Search Program
☑ MS Interpretation Program
☑ GC/MS Analysis Program - AMDIS
☑ NIST 26 MS Libraries

Description
Performs search and retrieval of compounds and their mass spectra in NIST 26 MS Library and user MS libraries. Can be run from AMDIS, ChemStation or other applications.

History & Evolution of NIST Mass Spectral Search Program

The NIST Mass Spectral Search Program began in the late 1980s as an IBM PC DOS application. It was developed by Steve Stein at NIST, then the National Bureau of Standards (NBS), to allow users of the EPA/NIH electron ionization (EI) mass spectral library to recall spectra without needing to measure a compound's spectrum and submit it to a search system to view its library spectrum with related data. At that time, instrument vendors' data systems required users to measure a spectrum that closely matched a specific compound in the library before being able to view the reference spectrum. Most of these systems did not display chemical structures, a limitation that set MS Search apart early on. MS Search's inclusion of structural displays and superior spectrum-matching algorithms made it more effective than many manufacturer-provided search tools.

Around 1988, the National Institute of Standards and Technology (then NBS) assumed stewardship of the EPA/NIH EI mass spectral library, which contained spectra for approximately 45,000 compounds. Soon after, NIST began a comprehensive project to evaluate the existing spectra and collect additional data from donated sources. In the early 1990s, NIST started measuring EI mass spectra and acquiring large datasets from organizations such as Chemical Concepts and the Japanese Mass Spectrometry Society. As the evaluation process advanced, features were added to the MS Search Program to support NIST's internal data reviewers—features that also became popular with end-users.

By 1994, NIST adopted a policy of including replicate EI spectra for frequently encountered compounds, a practice that continues today.

In 1998, NIST released the first version of the NIST/EPA/NIH Mass Spectral EI Library (NIST 98) in which every spectrum had been evaluated by trained mass spectrometrists. This release also introduced AMDIS (Automated Mass Spectral Deconvolution and Identification System), a tool used to extract pure mass spectra from complex GC-MS data where compounds often coelute or are present at trace levels.

The next major release, NIST 02, was initially accompanied by an updated DOS-based search program. Shortly afterward, a Windows version replaced the DOS interface. The data had expanded to 147,200 compounds, with 27,500 additional replicate spectra.

With the release NIST 05, a new era began. It included a library of product-ion (MS/MS) spectra derived from electrospray ionization (the NIST Tandem Library), along with a database of GC methods and retention index (RI) values from scientific literature. Many of the compounds in the RI database were represented in the EI mass spectral library. The MS Interpreter program was also introduced in this release, allowing users to correlate structural fragments with spectral peaks—an invaluable tool for both evaluators and end-users.

Subsequent releases (NIST 08, 11, and 14) saw continued growth in both EI and tandem spectral data, as well as expansion of the GC Method/RI database.

NIST 17 marked another milestone. It included a substantial growth in the number of spectra in both Libraries, a significantly updated version of MS Interpreter, now applicable to EI, product-ion, and accurate *m/z* spectra, and a Hybrid Search algorithm for both EI and MS/MS spectra, which modifies reference spectra to resemble query spectra when exact matches are unavailable. The algorithm identifies mass differences to help infer structural modifications. Statistical RI information, including average values, standard deviations, and citation counts for each column type were also introduced.

NIST 20 brought further expansion of the EI and Tandem datasets and a new version of MS Interpreter, enhancing its utility for Hybrid Search users.

By 2023, the MS Search Program had become increasingly complex due to diverging requirements for EI and Tandem data. MS Search v3.0 introduced a data-type selection system—EI, Tandem, or Peptide—allowing users to filter features based on the data they're working with, reducing confusion, and streamlining the interface.

Most search types had supported the display of multiple related spectra for a compound (e.g., from the EI replicate library, stereoisomers, or isotope variants); however, this was not the case for the Incremental Name Search. A significant improvement in NIST 23 was the improvement to the Incremental Name Search. When using NIST-provided libraries, all associated spectra are now displayed in a dedicated window below the Names List. This makes it much easier to view related compound spectra. NIST 23 also added a special names library for both low- and high-resolution tandem spectra, allowing name searches across all Tandem data regardless of resolution.

With the EI library growing more than tenfold since NIST assumed stewardship, finding new compounds to measure became challenging. Starting with NIST 14, a new acquisition strategy was implemented using the InChIKey (International Chemical Identifier Key). By comparing chemicals of interest across non-spectral databases and available commercial inventories, NIST identifies compounds to acquire and measure using MS/MS or EI techniques. Resulting spectra are added to the appropriate libraries, with associated GC methods and RI values for EI spectra. This approach contributed to an increase of over 40,000 spectra in the EI Library between NIST 20 and NIST 23, with substantial growth in the Tandem Library.

Another key development in NIST 23 was the expansion of non-mass spectral database coverage. The number of searchable external databases grew from 9 (a number unchanged for over 30 years) to seven classes comprising 59 generic titles.

NIST 23 not only delivered dramatic growth in both EI and MS/MS data coverage but also introduced significant software improvements.

NIST 26 represents the next major leap forward. It is now divided into two distinct products. One contains the NIST\EPA\NIH EI Mass Spectra Library and the other contains the Tandem Library. The Spectrum Type (EI, Tandem, Peptide, or All) is defaulted to EI or Tandem depending on the installation. Both the EI and Tandem Libraries are provided with software (MS Search and MS Interpreter).

Much of the investigative use of NIST's Data results from chromatography; either gas or liquid. A new feature in the form of a Chromatography tab has been added to the MS Search Desktop. This incorporates the features of AMDIS and the mzML file which contains LC-MS/MS data. The process performs an AMDIS deconvolution on EI GC-MS and a similar process on LC-MS/MS data. The processed files are saved and can easily be recalled. Changes have been made to the appearance of various dialog boxes that enhance their usability.

Nominal Mass for Libraries

In the early 1940s, before the introduction of the first commercial mass spectrometers, individuals and organizations began collecting reference mass spectra. These spectra displayed (first tabularly and later graphicly) the *m/z* values of ions as integers, whole numbers. This was partly because of the limited accuracy of the instruments used to measure the mass of organic compounds at that time. Even though the mass spectrometer measures the actual *m/z* value of ions, the upper limit was such that cumulative effects on the mass of an ion were inconsequential.

Since 1961, the masses of all elements have been measured relative to the most abundant isotope of carbon (^{12}C), which has been assigned an exact value of 12 Da[3]. Importantly, mass spectrometers do not measure ion mass directly; instead, they measure the mass-to-charge ratio (*m/z*)—the ion's mass divided by its charge. In electron ionization (EI), nearly all ions carry a single positive charge. Therefore, the *m/z* value of the **molecular ion** (**$M^{+\bullet}$**), formed by the loss of a single electron, is effectively the same as the molecular mass on an integer scale, because the electron's mass (~1/2000 of a proton or neutron) is negligible.

EI mass spectra of organic compounds consist of a series of peaks, often starting with a cluster of peaks representing the $M^{+\bullet}$ and proceeding to lower *m/z* values toward the left, the lowest of which is usually a function of the *m/z* acquisition range. These peaks represent ions formed by the fragmentation of the $M^{+\bullet}$. The various ions are usually represented by a cluster rather than a single peak. In most cases, the lowest *m/z* peak in the cluster represents an ion where all atoms of each element in the molecular formula are the same isotope (the **monoisotopic peak**). Each of the peaks at higher *m/z* values represent ions where one or more of the atoms of an element is an isotope with a higher mass. These are called **isotope peaks** (ions of the same elemental composition but with different isotopic makeups).

EI mass spectrometry of organic compounds typically involves 11 elements: C, H, N, O, Si, S, P, and the halogens. Some of these elements have multiple stable isotopes (atoms of the same element differing in the number of neutrons[4]), while others—such as F, P, and I—are mononuclidic, meaning they possess only one stable nuclide.

The exact mass of an element's most abundant nuclide is the element's **monoisotopic mass**. The integer value for the most abundant nuclide is its **nominal mass**. The **nominal mass** of an ion is the sum of the **nominal masses** of each of its elements' times the number of atoms of that element.

The difference between the integer mass of a nuclide and its exact mass is called the **mass defect**. The **mass defect** of ^{1}H, ^{2}H, ^{13}C, and the two isotopes of nitrogen is a positive number; meaning that the exact mass is a little greater than the integer mass. The mass defect of the nuclides of all other nuclides listed above is negative; the exact mass is a little less than the integer mass. The **mass defect** of a nuclide is always less than 100 milliDa regardless of it being positive or negative. The two elements with the greatest **mass defect** relative to their exact masses are hydrogen (7.825 milliDa relative to an exact mass of 1.007825, ~0.8%) and bromine (21.7 milliDa relative to an exact mass of 78.9183, ~0.3%).

As the *m/z* range of instruments increased and the accuracy of measuring the *m/*z values improved a decision had to be made as to what would be the standard for ions in reference libraries. Would it be based on **nominal mass** or the mass measured by the mass spectrometer. Consider the mass spectrum of Hexacontane ($C_{60}H_{122}$). The measured integer *m/z* value for the $M^{+\bullet}$ would be 843 (842.995 Da); whereas the **nominal mass** of the $M^{+\bullet}$ is 842 Da. It was decided that all reference spectra would have peaks which corresponded to the ion **nominal mass**. Some instrument companies' data systems will adjust the EI mass spectrum to accommodate the mass defect. If compensation is not done by the acquiring instrument, then when the spectrum is imported into the NIST MS Search Program, MS Search can be set to make the correction. All spectra in the NIST/EPA/NIH EI Reference Library are based on **nominal mass**. There are spectra in the NIST Tandem Library that are based on measured mass, and these are what are called accurate *m/z* value spectra, sometimes called high-resolution spectra.

Mass defect only becomes an issue when the mass of the analyte is >500 Da. Care must be taken when analyzing such a compound that the appropriate correction be applied for the mass defect. The value for the hydrogen mass defect correction entered in the numeric enter box at the top left of the **Spectrum Import Options** dialog box is 0.99888. The bromine mass defect value is 1.00087.

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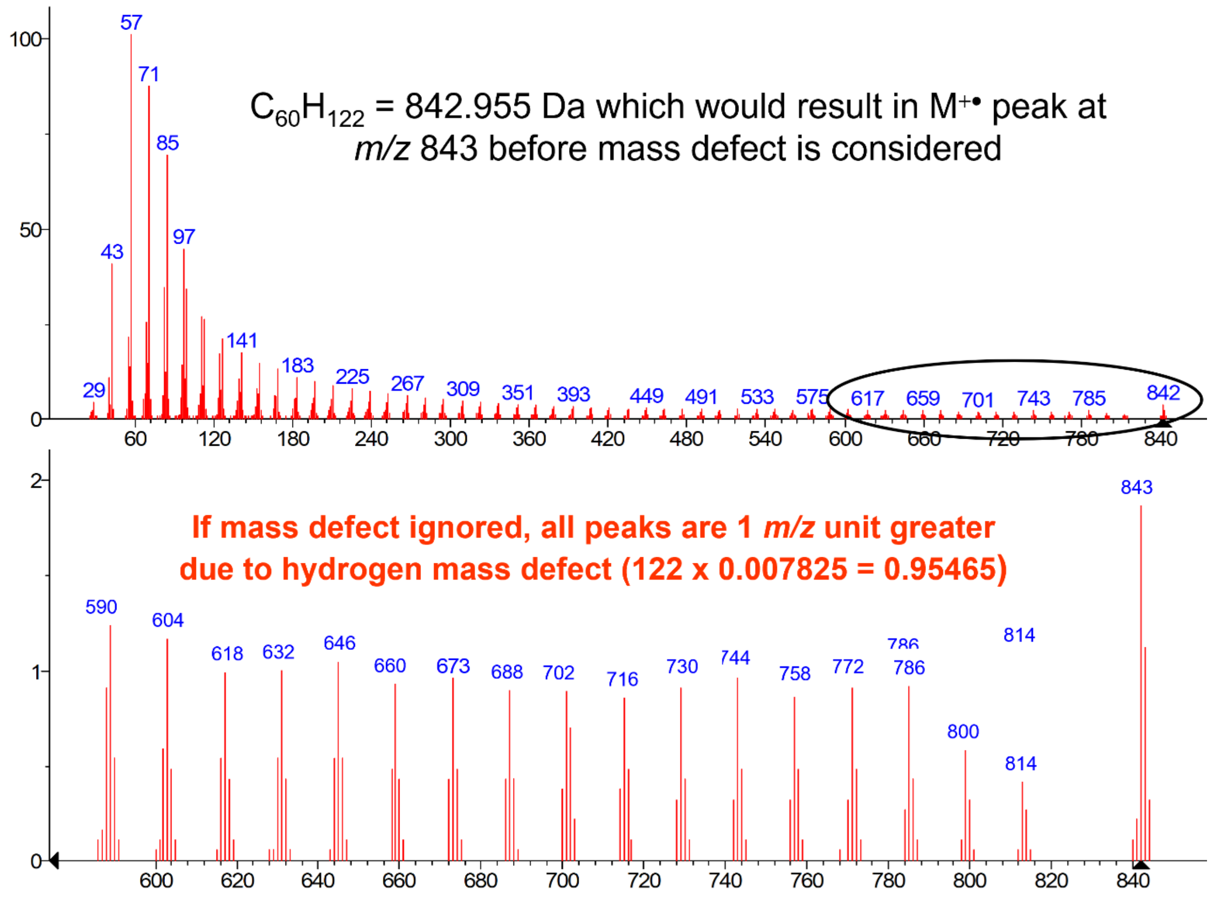
[3] At the time the +C standard the symbol for atomic mass was changed from amu (atomic mass unit) to u (unified atomic mass unit).Many people had difficulty with this translon and in the 2005, the official unit became the Dalton (Da).

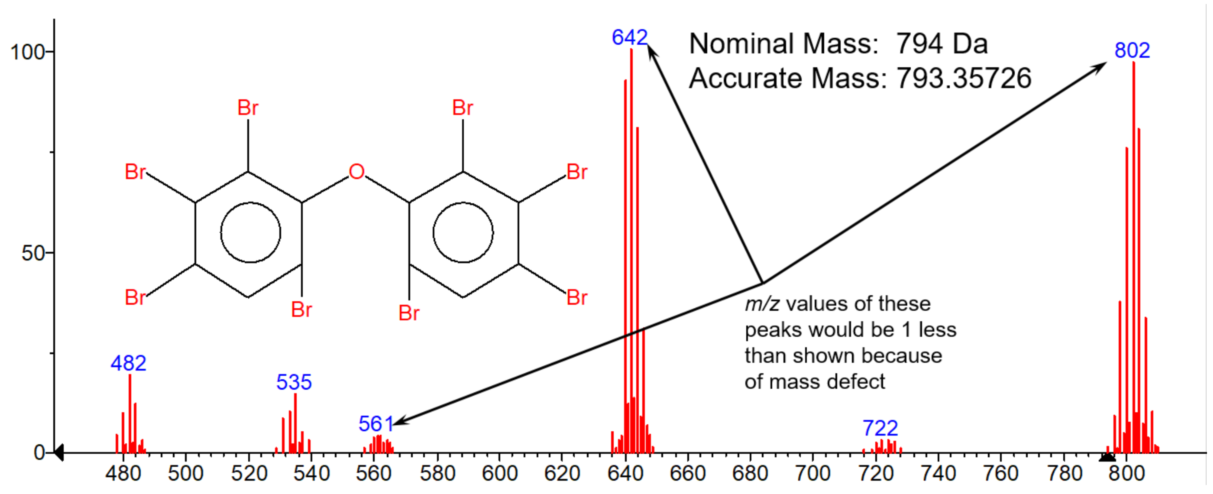
[4] In order for an element to have isotopes it must have two or more species with the same number of electrons and neutrons, but different numbers of neutrons. Each of these is also referred to as a nuclide of the element. Elements with only a single nuclide are NOT referred to as monoisotopic, but rather mononuclidic.



$C_{60}H_{122}$ = 842.955 Da which would result in $M^{+\bullet}$ peak at *m/z* 843 before mass defect is considered

If mass defect ignored, all peaks are 1 *m/z* unit greater due to hydrogen mass defect (122 x 0.007825 = 0.95465)

Figure 4. NIST/EPA/NIH NIST 26 Spectrum of Hexacontane (C60H122).



Nominal Mass: 794 Da
Accurate Mass: 793.35726

m/z values of these peaks would be 1 less than shown because of mass defect

Figure 5. NIST/EPA/NIH NIST 26 Spectrum of 2,2',3,3',4,4',6,6'-Octabromodiphenyl Ether.

As stated above, in EI most ions have a single charge; however, there can be peaks representing ions that are double charged. These are typically observed with compounds that are highly aromatic. Linear quadrupole mass spectrometers measure ions every 0.05 *m/z* units. This means that the observed *m/z* value of such ions with the mass of 205 Da will be 102.50. If the instrument being used is capable of reporting to the nearest 0.05 *m/z* units, have it do so. This way, you will be able to spot double charge ions. Otherwise, errors may occur because the integer *m/z* value of the ion in the spectrum can be 102 or 103.

FIRST QUESTION: How To Get Spectra into MS Search

The first question always asked after installing the **NIST Mass Spectra Search Program** is, “How do spectra get into the Program?”

Prior to NIST 26 and MS Search v.4.0, it was necessary to import spectra from a file containing individual spectra (MSP/MSPEC) or have spectra exported from manufacturers’ data analysis programs while that program called MS Search. It has always been possible to send EI GC/MS spectra from AMDIS, which could open netCDF and some manufacturers’ proprietary data file types. With the addition of the **Chromatogram** tab view to the MS Search Desktop, it is now possible to open chromatographic data files in MS Search (GC/MS low resolving-power files as *netCDF*, or the *mzML* format and several instrument company proprietary formats, and high resolving-power (accurate *m/z* values) GC/MS files in the Thermo Fisher© RAW*, mzML, netCDF, and mzData* formats. LC-MS/MS data files as Thermo Fisher© *RAW*, Bruker TIMS and TOF *tdf*, and *mzML* can also be opened and processed.[*] When a chromatographic file is opened in the **Chromatogram** tab view, the reconstructed total ion current (RTIC) chromatogram is displayed in the **Chromatogram** window and individual spectra, purged of mass spectral peaks not related to the component, are added to a **Hit List** window. These deconvoluted spectra are displayed in a **Sample/Lib Match Spectrum** window in a comparison with a library search result. The deconvoluted spectra can be searched using conventional spectrum comparison library searches of **Lib. Search** tab view or transferred to other parts of MS Search or MS Interpreter along with the structure from the library search match.

Spectra in the **Hit List** window of the **Chromatogram** tab view can be added to the **Spec List** window of **MS Search** (**Lib. Search** or **Librarian** tab views) or added to the **Compare** tab view (see cautionary statement in the **Compare** tab view) by use of a **Right Mouse Button** (**RMB**) menu in the **Hit List** window. By default, when these spectra are sent to the other tab views, the views do NOT automatically come into focus. A regular library search of the spectrum in the **Hit List** window of the **Chromatogram** tab view can be initiated by selecting **Library Search** from this **RMB** menu. This action will bring the **Lib. Search** tab view into focus as is the case when **Library Search** is selected from a **RMB** menu in tab view.

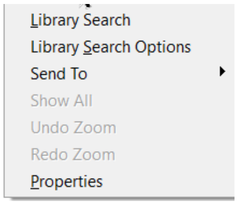
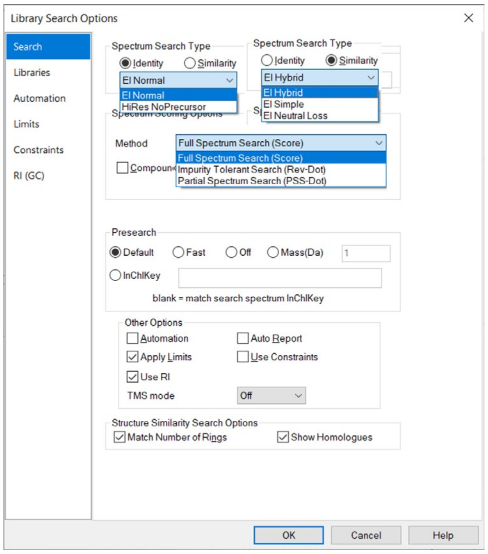


Figure 6. RMB menu displayed in Chromatogram window of Chromatogram tab view.



Spectrum Search Type: ● Identity ○ Similarity — EI Normal
Spectrum Search Type: ○ Identity ● Similarity — EI Hybrid
Method: Full Spectrum Search (Score)
☐ Compound
Presearch: ● Default ○ Fast ○ Off ○ Mass(Da) 1
○ InChIKey
blank = match search spectrum InChIKey
Other Options:
☐ Automation ☐ Auto Report
☑ Apply Limits ☐ Use Constraints
☑ Use RI
TMS mode: Off
Structure Similarity Search Options:
☑ Match Number of Rings ☑ Show Homologues

Figure 7. Displayed Library Search Options dialog box from RMB menu in Chromatogram or Hit List window.

MS Search has multiple library search routines that execute different types of searches and may be performed with different constraints as well as different libraries. When the Mouse Pointer is in either the **Chromatogram** or **Hit List** window of the **Chromatogram** tab view, the **RMB** menu (*Figure 6*) has a **Library Search Options** selection that results in the display of the same **Library Search Options** dialog box that is displayed by selecting **Library Search** from the Main Menu’s **Options** menu. After making all adjustments to accommodate the desired search, select the **OK** button at the bottom of the dialog box to save all the settings. These settings will be employed when the Library Search selection is made from the **Chromatogram** or **Hit List** window’s **RMB** menu. The settings for this dialog box are described in detail beginning on *page 77*.

When spectra imported directly from manufacturers’ data analysis programs or files containing one or more spectra are searched, **Constraints** can be used which are set in the **Library Search Options** dialog box displayed by selecting the **Library Search Options** button in the **Button Bar** of the **Lib. Search** tab view or by selecting **Library Search Options** from the **Options** menu. Multiple libraries can be selected for search in this dialog box. These same **Constraints** and libraries to be searched are used when a library search is initiated with the **Library Search** selection of the **RMB** menu in the **Hit List** window of the **Chromatogram** tab view.

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[*] Proteowizard’s MS Convert (https://proteowizard.sourceforge.io/tools/msconvert.html) can be used to create a supported format from most instrument company’s proprietary format files. The *netCDF* high-resolution files produced by the JEOL AccuTOF and the Thermos Fisher© Orbitrap software will work with the **Analyze EI HiRes Input File** selection from the **File** menu.

If MS Search is installed on a computer that has Agilent ChemStation®, or MassHunter® Qual, MassHunter® Quant, or OpenLab software installed, direct links between MS Search and these Agilent programs are established. Spectra are exported from the ChemStation Data Analysis spectrum display by putting the Mouse pointer in the ChemStation spectrum window and double-clicking the Right Mouse button. One spectrum or several spectra can be sent from ChemStation Data Analysis by running one of the NIST macros called from the ChemStation **Spectrum** menu. Spectra can be sent, one at a time, from MassHunter Qual or MassHunter Quant by using the **Search using the NIST MS Program** selection on a **RMB** menu displayed with the Mouse pointer on the spectrum display. A group of spectra can be sent from MassHunter Qual by highlighting spectra shown in the **Data Navigation** window and using the same **RMB** menu command used to send individual spectra. OpenLab uses the NIST dll for its internal search and can send spectra to MS Search from an **RMB** menu.

The Thermo Fisher's FreeStyle data analysis program in Xcalibur® (or Qual Browser in older versions) can send spectra directly to MS Search with calls from menus and the use of the **RMB** menu commands. Thermo Fisher's Xcalibur data analysis software uses the NIST MS Search dll(s). A demo version of MS Search is provided with Xcalibur. This will have to be replaced when installing the current version of NIST Data (both Tandem and/or EI) and the current version of MS Search.

Accurate *m/z* data files must contain spectra with centroided mass spectra peaks.

The Perkin Elmer TurboMass® and Waters MassLynx® Data Analysis software have links that send spectra directly to the NIST MS Search Program. Consult these individual manufacturers for details. Other manufacturers may have similar capabilities.

The NIST **A**utomatic **M**ass spectral **D**econvolution and **I**dentification **S**ystem (**AMDIS**) will read the native formats of several instrument manufacturers' GC/MS data files. Spectra can easily be exported from **AMDIS** (with or without retention index values) to the MS Search Program. Manufacturers that have formats which are not readable by **AMDIS** will often have utilities to export their GC/MS data files in the netCDF format, which can be read by AMDIS.

There are several ways to export product-ion mass spectra so they can be read by MS Search. MS Search can read files containing one or more mass spectra with the following extensions: MSP, MSPEC, TXT, MOL, JDX, SDF. KCF, MGF, DTA, PKL, mzXML, and mzData. Before importing individual spectra or groups of spectra from instrument specific data or using spectra identified using the **Chromatogram** tab view of MS Search, it is a good idea to go through the **Getting Started** section of this manual.

If the mass spectrometry data analysis software in use does not have a feature allowing for the direct export of a mass spectrum to the NIST MS Search program, try using the **File**/**Open** command from the Main Menu of the data analysis program to export spectra in one of the readable NIST file types. Better yet, export the data files to a netCDF file (EI data) or an mzML file (LC-MS/MS data) and open these in the *New* **Chromatogram** tab view.

If the LC-MS or LC-MS/MS instrument does not have the ability to export the chromatography data file in an mzML format, this can be accomplished using the **msconvert** utility **ProteoWizard** downloadable from:

https://proteowizard.sourceforge.io/

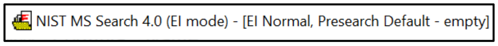
This program is reported to be able to convert all instrument manufacturers' LC-MS LC-MS/MS data files into mzML files with centroided mass spectra peaks.

The following example is for the processing of a nominal *m/z* EI GC/MS data file (**Analyze EI Input File**) in the *New* **Chromatogram** tab view. The next two sections will cover **Analyze HiRes EI Input File** processing and **Analyze MS/MS Input File**.

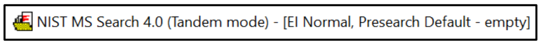
Getting Started with the *NEW* Chromatogram tab view

[*In this manual* ***Selecting*** *means to place the Mouse pointer an object and click the left Mouse button once.*]

When MS Search is opened for the first time after installation, one of two **Title Bars** will be displayed:


NIST MS Search 4.0 (EI mode) - [EI Normal, Presearch Default - empty]

or


NIST MS Search 4.0 (Tandem mode) - [EI Normal, Presearch Default - empty]

depending on whether the **NIST Tandem** or the **NIST\EPA\NIH Electron Ionization (EI) Library** was installed. The display will be that of the **Lib. Search** tab view. At the bottom of the Desktop, there are six selectable tabs.

For Help, press F1

Type of Search: MS/MS

Displayed: EI Normal

Select the tab labeled **Chromatogram** to bring the **Chromatogram** tab view into focus.

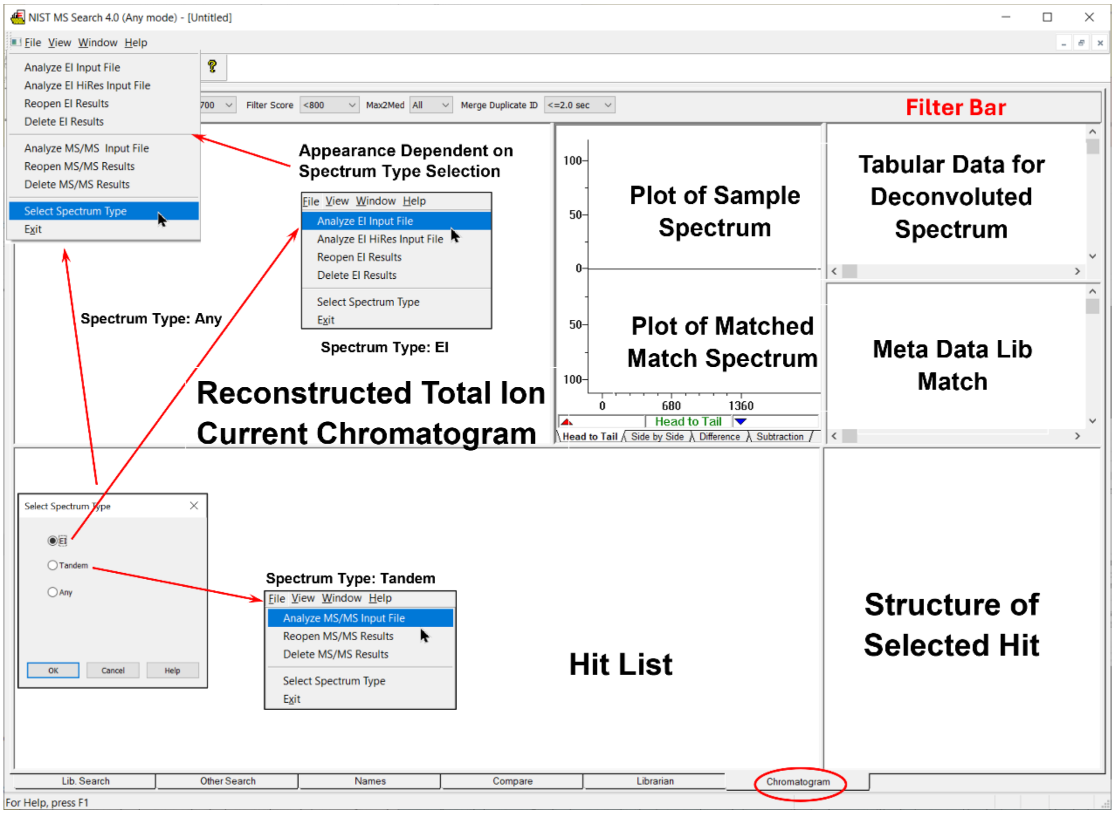
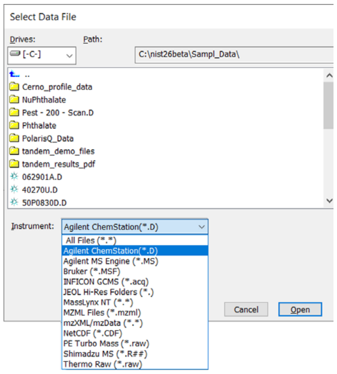

NIST MS Search 4.0 (Any mode) - [Untitled]

Figure 8. Setting up a Program for GC/MS EI from the Unpopulated Chromatogram tab view. This Changes the Appearance of the File menu as shown above.

This presentation is related to LowRes EI GC/MS. If the Tandem version of NIST 23 was installed, go to page 30.


Select Data File

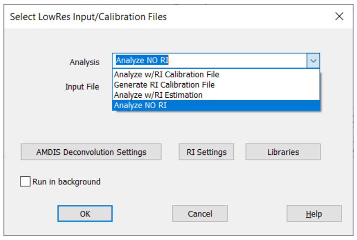
Drives: [-C-]

Path: C:\nist26beta\Sampl_Data\

Instrument: Agilent ChemStation(*.D)

Figure 9. Select Data File dialog box.

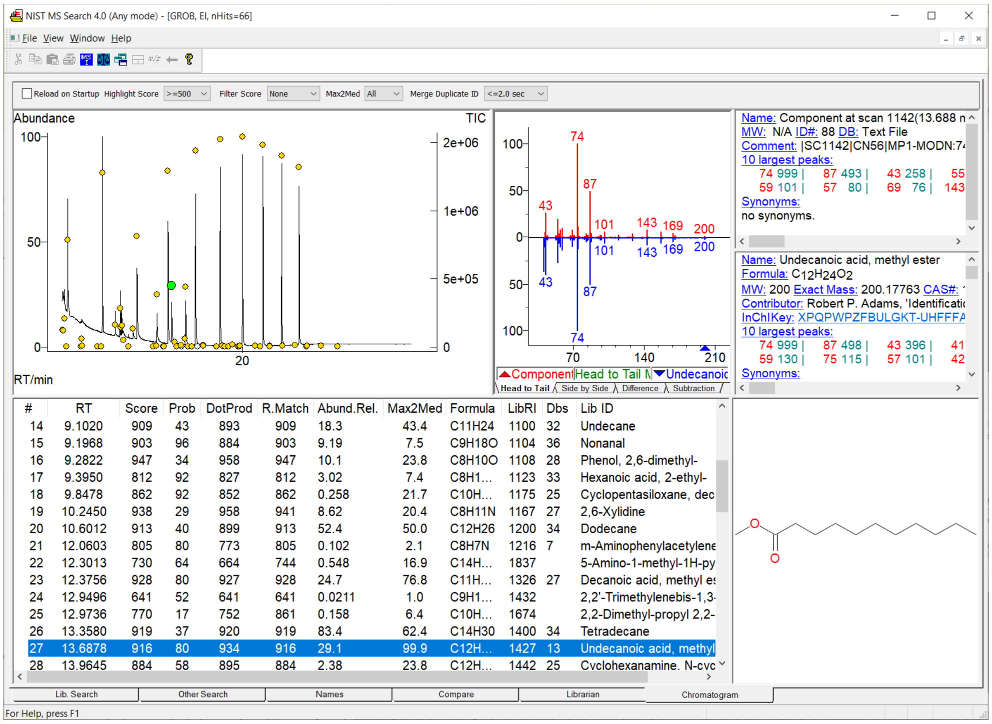
The **Spectrum Type** should be **EI** or **Any**. If it is **Tandem**, change it appropriately by selecting **File** on the Main Menu, then select **Select Spectrum Type** from the **File** menu. Selecting an item is done by placing the Mouse pointer on the item and clicking the left Mouse button. This will sometimes result in the display of a dialog box. The spectrum type is chosen by selecting the radio button next to the desired type in the **Select Spectrum Type** dialog box. Once the **Spectrum Type** has been selected, select **File** on the Main Menu, and select **Analyze EI Input File** from the **File** menu. This causes the **Select LowRes/Calibration Files** dialog box to be displayed with three list boxes (*Figure 10*). The top box specifies the type of **Analysis**.


Select LowRes Input/Calibration Files

Analysis: Analyze NO RI

☐ Run in background

Figure 10. Dialog box displayed when Open EI Input File is selected from File



NIST MS Search 4.0 (Any mode) - [GROB, EI, nHits=66]

☐ Reload on Startup Highlight Score >=500 Filter Score None Max2Med All Merge Duplicate ID <=2.0 sec

Name: Component at scan 1142(13.688 n
MW: N/A ID#: 88 DB: Text File
Comment: |SC1142|CN56|MP1-MODN:74
10 largest peaks:
74 999 | 87 493 | 43 258 | 55
59 101 | 57 80 | 69 76 | 143
Synonyms:
no synonyms.

Name: Undecanoic acid, methyl ester
Formula: $C_{12}H_{24}O_2$
MW: 200 Exact Mass: 200.17763 CAS#:
Contributor: Robert P. Adams, 'Identificati
InChIKey: XPQPWPZFBULGKT-UHFFFA
10 largest peaks:
74 999 | 87 498 | 43 396 | 41
59 130 | 75 115 | 57 101 | 42
Synonyms:

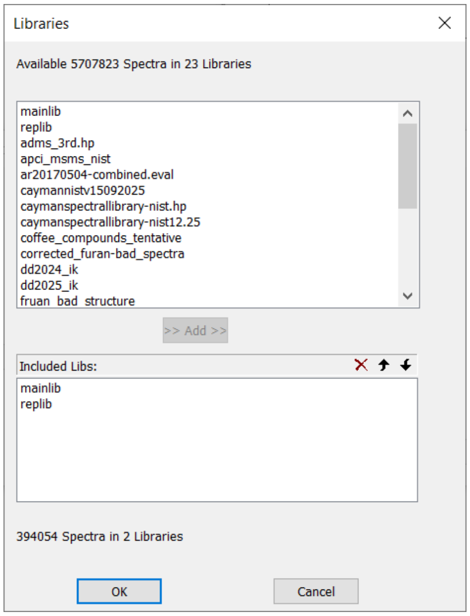
#	RT	Score	Prob	DotProd	R.Match	Abund.Rel.	Max2Med	Formula	LibRI	Dbs	Lib ID
14	9.1020	909	43	893	909	18.3	43.4	C11H24	1100	32	Undecane
15	9.1968	903	96	884	903	9.19	7.5	C9H18O	1104	36	Nonanal
16	9.2822	947	34	958	947	10.1	23.8	C8H10O	1108	28	Phenol, 2,6-dimethyl-
17	9.3950	812	92	827	812	3.02	7.4	C8H1...	1123	33	Hexanoic acid, 2-ethyl-
18	9.8478	862	92	852	862	0.258	21.7	C10H...	1175	25	Cyclopentasiloxane, dec
19	10.2450	938	29	958	941	8.62	20.4	C8H11N	1167	27	2,6-Xylidine
20	10.6012	913	40	899	913	52.4	50.0	C12H26	1200	34	Dodecane
21	12.0603	805	80	773	805	0.102	2.1	C8H7N	1216	7	m-Aminophenylacetylene
22	12.3013	730	64	664	744	0.548	16.9	C14H...	1837		5-Amino-1-methyl-1H-py
23	12.3756	928	80	927	928	24.7	76.8	C11H...	1326	27	Decanoic acid, methyl es
24	12.9496	641	52	641	641	0.0211	1.0	C9H1...	1432		2,2'-Trimethylenebis-1,3-
25	12.9736	770	17	752	861	0.158	6.4	C10H...	1674		2,2-Dimethyl-propyl 2,2-
26	13.3580	919	37	920	919	83.4	62.4	C14H30	1400	34	Tetradecane
27	13.6878	916	80	934	916	29.1	99.9	C12H...	1427	13	Undecanoic acid, methyl
28	13.9645	884	58	895	884	2.38	23.8	C12H...	1442	25	Cyclohexanamine, N-cyc

For Help, press F1

Figure 11. NIST Mass Spectral Search Program Chromatogram tab view after Processing an EI Data File.

For this example, select **Analysis NO RI**. The bottom displayed list box (labeled **Calibration File**) will disappear. This is used to select the name of the **Retention Index Calibration** file when doing analyses using RI values (explained later in this manual). Preforming analyses using Retention Index will also be described later in this manual.

The dialog box will continue to be displayed after selecting the **Analysis** type. Select the button with **···** on the right side of the middle drop-down list box. This will cause the **Select Data File** dialog box (to be displayed *Figure 9*). This dialog box has a button that will display a drop-down list of instrument manufacturers data analysis software. If not already selected, select the Agilent ChemStation (*.D). Then select the GROB.D file name (navigation to the file location in the C:\NIST26\AMDIS32\ folder is required) and select the **Open** button (lower right of the dialog box) The **Select Data File** dialog box will close leaving the **Select LowRes Input/Calibration Files** dialog box still displayed. For this example, ignore the **AMDIS Deconvolution Settings** button in the dialog box.



Available 5707823 Spectra in 23 Libraries

mainlib
replib
adms_3rd.hp
apci_msms_nist
ar20170504-combined.eval
caymannistv15092025
caymanspectrallibrary-nist.hp
caymanspectrallibrary-nist12.25
coffee_compounds_tentative
corrected_furan-bad_spectra
dd2024_ik
dd2025_ik
fruan_bad_structure

Included Libs:

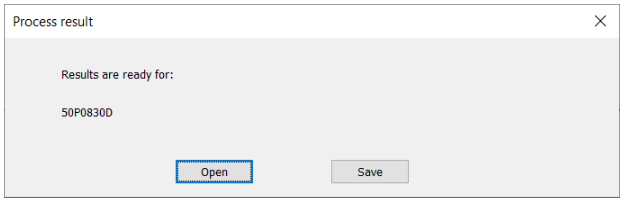
mainlib
replib

394054 Spectra in 2 Libraries

Figure 12. Libraries dialog box displayed by selecting Library on the Select LowRes Input/Calibration Files dialog box.

Any library in the NIST MS Search format and in the folder with the NIST EI mainlib and replib can be included (user-libraries or third-party libraries such as the *Wiley Registry of Mass Spectral Data*) in the search. Select the **Library** button on the **Select LowRes Input/RI Calibration Files** dialog box to display the **Libraries** dialog box (*Figure 12*) with the upper window displaying the names of available libraires and the lower window containing the names of the libraries to be included in the search. Just below this a line showing the number of spectra to be searched and the number of libraries being searched. The order of the libraries can be changed by highlighting the name and using the ↑ ↓ buttons on the right. The order of the libraries has no impact on the **Hit List** because spectra are displayed based on **Match Factor**, not search order. If the mainlib and replib are not in the lower window, select them in the upper window and then select the **Add** button. If other libraries are in the lower window (under the **Include Libs:** label), highlight and select the **Delete** button (✕).

Once the data file and libraries are selected, select the **OK** button on **Select LowRes Input/Calibration Files** dialog box. Running Task will appear in the **Filter Bar** and the hourglass icon (⌛) will display until processing is finished (this can take some time, depending on the size of the file), at which time the windows of the **Chromatogram** tab view are populated as shown in *Figure 11*, unless the **Run in background**



Process result

Results are ready for:

50P0830D

check box (☐) was selected. During this *processing* time the focus can be changed to another tab view. The focus may be changed to another tab view and other operations carried out. When the data file processing finishes, the **Process result** dialog (see next page) will be displayed, regardless of the tab view in focus. Selecting the **Open** button will cause the focus to change to the **Chromatogram** tab view and the results of the processed file will be visible. If the Save button is selected, the dialog box closes, and the focus remains with the present tab view. When the focus is changed to the **Chromatogram** tab view, the results of the processed file will be visible.

When the processing is complete the data file name will appear with the number of components identified. (**[Grob nHits=xx]**). This number is based on the settings in **Filter Bar**. If the settings are different than those shown in *Figure 11*, the **nHits=XX** will be different. The first component is automatically selected. Circles (O) on the chromatographic trace represent deconvoluted/tentatively identified components. Some of the Os are filled (colored yellow by default). The filled Os represent components that have a **Score** greater than the number in the box to the right of the **Highlight Score** label on the **Filter Bar**. Selecting one of the Os will cause it to increase in size and change color (green, by default). The entry in the **Hit List** window for that component will be highlighted and the display in the **Sample/Lib Match Spectrum** window will be the component (up) and library match (down) spectrum. The **Meta Data Lib Match** window will contain information for the library match. The meaning of the **Filter**, **Max2Med**, and **Merge Duplicate ID** are explained later in this manual or the **Help** file displayed by selecting the **Help** button in the **Hit List** page of the **Chromatogram Properties** dialog box.

> ***Nota Bene:*** The **Select LowRes Input/Calibration Files** dialog box (*Figure 9*) is displayed when **File/Analyze EI input File** is selected from the Main Menu. A checkbox next to the **Run in background** label is observed on the lower left, just above the **OK** button. Due to the time required to process some data files, this will cause the file processing in the **Chromatogram** tab view to run in background, allowing other operations to be executed. This includes moving from one tab view to another. Once the file processing is complete, a dialog box will appear offering the bringing of the processed file into focus in the **Chromatogram** tab view (Open button) or saving the results (Save button) and leaving the current tab view in focus. The checkbox remains selected until deselected.

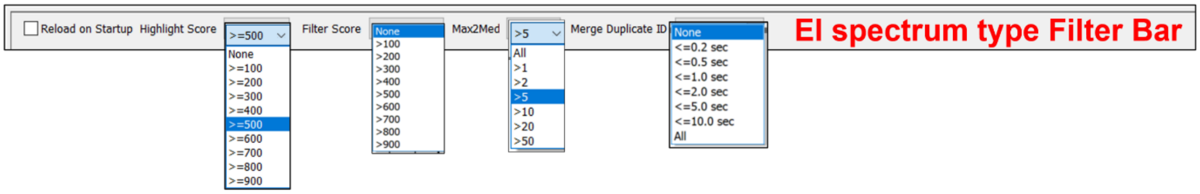


Figure 13. Hit List Controls in Bar below the Menu.

The horizontal axis of the (Reconstructed Total Ion Current) **RTIC Chromatogram** window is fixed as a linear display of Retention Time (in minutes), and the left vertical axis is, by default, a linear display of relative abundance. The right vertical axis is, by default, linear with respect to the total ion current. Displays of both the vertical axes of the RTIC **Chromatogram** window can be linear, square root or log. This setting is on the **Chromatogram View** page of **Chromatogram Properties** dialog box displayed from the **RMB** menu (Properties) with the Mouse pointer in the **Chromatogram** window. The default is linear.

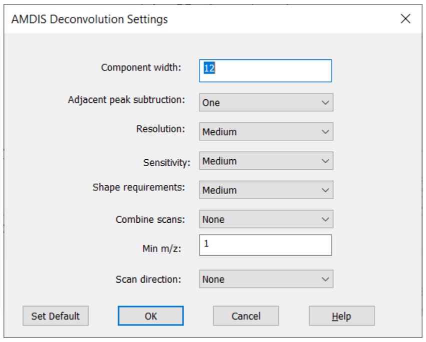
The position of the O represents the retention time (spectrum number, horizontal position) and the relative chromatographic abundance (vertical position.) ***Nota Bene***: the position of O representing a component when using **Tandem Spectra** in the **Chromatogram** tab view is not defined in the same way as **EI** data.

When O is selected on the **RTIC Chromatogram** window, the Hit is highlighted in the **Hit List** window. If a Hit is highlighted in the **Hit List** window the component is selected on the **RTIC chromatogram** window turning the color of O from clear or yellow to green.

The **Hit List** can be filtered based on several items in a dropdown list box just above the **Chromatogram** tab view's **RTIC Chromatogram** and **Plot/Tabular** windows (*Figure 13*). Selecting **Reload on Starup** results in the contents of the **Chromatogram** tab view, at the time of MS Search's closing, being restored when the program is restarted. This may delay the startup of MS Search. The **Highlight Score** selection will result in the circle identifying a component symbol being filled (yellow by default) if the **Score** is equal to or greater than the specified number in the dropdown list box to the left. The **Filter** setting determines the items in the displayed **Hit List** and designated on the Chromatogram based on the Score. Only spectra that have a Score equal to or greater than the specified number in the dropdown list box to the left will appear in the **Hit List** in the **Hit List** window. The next filter is **Max2Med** with a dropdown list box. This is the ratio of maximum to median ion intensity in spectrum – low values indicate low signal strength. The last selection on the **Filter Bar** is the **Merge Duplicate ID**. The **None** option in this list box turns the process off. The other values have to do with the differences in the retention time of two identical identities (explained in more detail later in this manual).

By default, the **Hit List** window is initially sorted by spectrum number in increasing order (first column right of the **Component Number** column labeled **Scan**); however, the **Hit List** can be sorted by any desired column header by selecting its title. The first selection results in sorting by decreasing value. A second selection results in a sort by increasing value. The sorted column will contain a ▼ (decreasing) or ▲ (increasing) preceding the title. Often it is desirable to sort by **Abund.Rel.** This allows examination of result in order of decreasing abundance by progressing down the **Hit List**.

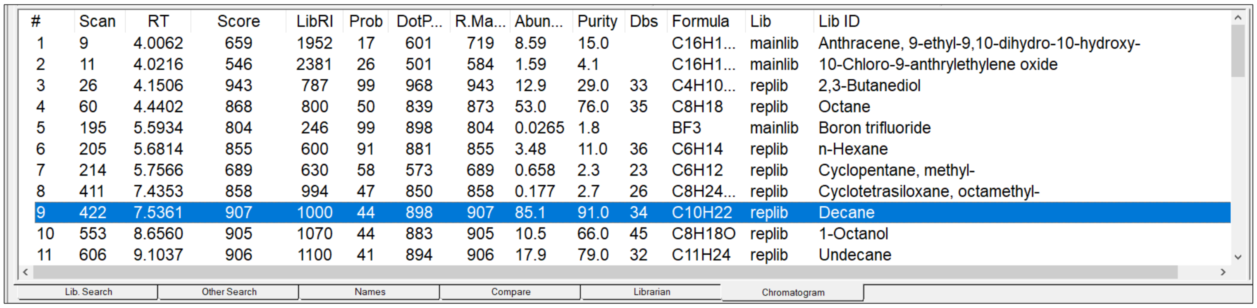
The number of Hits displayed for the same spectrum number is controlled in the **AMDIS Deconvolution Settings** dialog box (displayed by selecting the by the **AMDIS Deconvolution Settings** button in the **Select LowRes Input/Calibration Files** dialog box) and adjusting the number of **Adjacent peaks subtraction**. If **None** is selected, a single Hit will be displayed for any spectrum; if **One** (default) is selected, up to two Hits can be displayed; and, if **Two** is selected, up to three Hits can be displayed for a single spectrum. More details about this setting can be found in the **AMDIS Help File**, 6.3.3 **Deconv.** (Deconvolution) tab. To get to this selection of the **AMDIS Help File**, select **Settings** from the **Analyze** menu (selected from the AMDIS Main Menu). Then select the **Deconv.** tab followed by selecting the **Help** button on the dialog box.


AMDIS Deconvolution Settings

Component width: 12
Adjacent peak subtruction: One
Resolution: Medium
Sensitivity: Medium
Shape requirements: Medium
Combine scans: None
Min m/z: 1
Scan direction: None

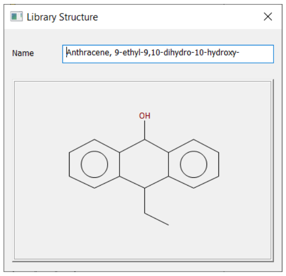
Combine scans: is a setting that is ***NEW*** to the employment of AMDIS in the **Chromatogram** tab view. This is to improve results from GC/MS instruments that acquire spectra faster than the elution of the chromatographic peak. The AMDIS algorithm works best when the number spectra for a chromatographic peak is between 5 and 10. This is ~2 spectra sec^{-1}. If the acquisition rate is too fast, AMDIS will produce multiple identifications of the same compound appearing close to one another. If the rate is too slow small chromatographic peaks can be missed. The peak width can be determined by expanding a single sharp peak and subtracting the time from beginning from that at the ending of the full width at half-maximum (FWHM) of the peak., e.g., value is 2.6 sec. The acquisition rate is obtained by multiplying the **RT** value for the last Hit in **Hit List** of the **Chromatogram** tab view by 60 to have the time in seconds and divide this by the number of reported spectra for the last Hit, e.g., (RT of 30.1571 min (60) / spec no. (13662) = 0.132 sec spec^{-1}. Divide this number of sec spec^{-1} into the number of seconds for the FWHM of the chromatographic peak, e.g., 2.4 / 0.132 = 18 spectra (FWHM) per chromatographic peak. If the acquisition rate is <0.5 enter a factor in the **Combine scans** field to result in a rate of ~0.5 sec. spec^{-1}. For this example, that value would be 3; however, for this example, these settings can be ignored.

Scan direction: All commercial transmission quadruple mass spectrometers scan from low *m/z* value to high except for the Agilent Technologies instrument. It scans from high *m/z* value to low. This will impact spectral skewing, which is a factor in deconvolution. Time-of-flight and quadrupole ion traps are pulse instrument and have no scan direction.



#	Scan	RT	Score	LibRI	Prob	DotP...	R.Ma...	Abun...	Purity	Dbs	Formula	Lib	Lib ID
1	9	4.0062	659	1952	17	601	719	8.59	15.0		C16H1...	mainlib	Anthracene, 9-ethyl-9,10-dihydro-10-hydroxy-
2	11	4.0216	546	2381	26	501	584	1.59	4.1		C16H1...	mainlib	10-Chloro-9-anthrylethylene oxide
3	26	4.1506	943	787	99	968	943	12.9	29.0	33	C4H10...	replib	2,3-Butanediol
4	60	4.4402	868	800	50	839	873	53.0	76.0	35	C8H18	replib	Octane
5	195	5.5934	804	246	99	898	804	0.0265	1.8		BF3	mainlib	Boron trifluoride
6	205	5.6814	855	600	91	881	855	3.48	11.0	36	C6H14	replib	n-Hexane
7	214	5.7566	689	630	58	573	689	0.658	2.3	23	C6H12	replib	Cyclopentane, methyl-
8	411	7.4353	858	994	47	850	858	0.177	2.7	26	C8H24...	replib	Cyclotetrasiloxane, octamethyl-
9	422	7.5361	907	1000	44	898	907	85.1	91.0	34	C10H22	replib	Decane
10	553	8.6560	905	1070	44	883	905	10.5	66.0	45	C8H18O	replib	1-Octanol
11	606	9.1037	906	1100	41	894	906	17.9	79.0	32	C11H24	replib	Undecane

Figure 14. Hit List window in Chromatogram tab view after Processing EI Data File in this example.



Name: Anthracene, 9-ethyl-9,10-dihydro-10-hydroxy-

In addition to the **Structure** window on the lower left of the **Chromatogram** tab view, the structure associated with the library spectrum of a Hit can be displayed in a floating window. To display this window, select **Show Structure** from the **View** menu, displayed by selecting **View** on the Main Menu. This **Structure** window can be dismissed by selecting the ☒ button in the upper right corner or deselecting the choice in the **View** menu. The **Show Structure** option on the **View** menu has been added to all tab views in v.4.0 of MS Search. In the **Chromatogram** tab view, this floating window can be used instead of the fixed **Structure** window allowing for expansion of the width of the **Hit List** window. This allows the fixed **Structure** to be dismissed by sliding the vertical diver bar between it and the **Hit List** window to the right. This allows for more space for the **Hit List** window, if required.

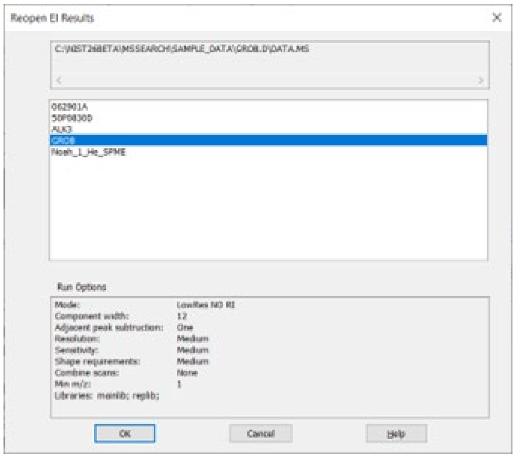
The MS Search Title Bar (NIST MS Search 4.0 (All mode) - [GROB, nHits=66]) is displayed at the top of **Chromatogram** tab.

As Filters are adjusted in the Filter Bar, the **nHits=** value can change. This can be informative.

The results of all data files processed in the **Chromatogram** tab view are automatically saved. Examination of the file menu shows selections to **Reopen** and **Delete** the results of processed files.

When the **File\Reopen** selection is made the dialog box on the left is displayed showing all the conditions of the process including the searched libraries (shown left).

The **Hit List** window **Chromatography Properties** dialog box has all the initial selections for the column headers as shown in *Figure 15*. There are 11 different selections when the program is used in the normal mode. There are an additional 10 when **Include Expert Results** in the **Chromatogram Properties** dialog box is selected. The following is list available when the expanded mode is selected along with definitions (those only available when the **Expert mode** is selected are designated with an *):

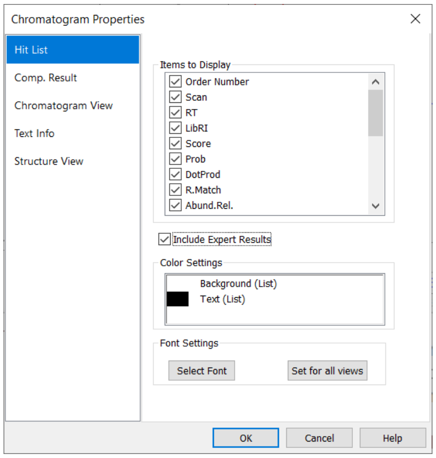


C:\NIST26BETA\MSSEARCH\SAMPLE_DATA\GROB.D\DATA.MS

062901A
50P08305
ALK3
GROB
Noah_1_He_SPME

Mode:	LowRes NO RI
Component width:	12
Adjacent peak subtraction:	One
Resolution:	Medium
Sensitivity:	Medium
Shape requirements:	Medium
Combine scans:	None
Min m/z:	1
Libraries: mainlib; replib;	

Order Number – Position in Current Hit List
***Scan** – spectrum number
RT – Retention time for compound in minutes
***LibRI** – statistical SSNP **Experimental RI** median value if present. If not, the **AI predicted** SSNP retention index (n-alkane scale) value is used.
Score – NIST Identity Score
Prob – Probability of hit being correct assuming correct ID is in library
Dot Prod – Cosine Similarity (Unweighted)
R.Match – Score not including query peak not in library spectrum
Abund.Rel. – Percent abundance relative to largest abundance
***#mpeaks** – number of peaks matching between the library and sample
***Purity** – Percent total signal in fragmentation zone due to contam. ions
***Max2Med** – Ratio of max to median intensity in spectrum – low values indicate low signal strength
***Width** (scan) – No. of spectra that constitute the component
***nSpec** – Number of duplicate IDs within selected narrow RT range
Other DBs – Compound Ubiquity Index (# DBs comp. is present in)
Formula – Chemical formula of library compound
***AbUncertPct** – Percent of abundance from uncertain peaks
***SumAB** – Sum of peak abundances with base peak = 1
Library – Name of the library containing the Hit
***#peaks** – Total number of peaks in the spectrum including uncertain peaks



☑ Order Number
☑ Scan
☑ RT
☑ LibRI
☑ Score
☑ Prob
☑ DotProd
☑ R.Match
☑ Abund.Rel.

☑ Include Expert Results

Figure 15. Nota Bene: Some items may not appear as selectable Hist List header options depending on the type of analysis performed (Analysis with Calibration File, Analysis with No RI, Analysis w/RI Estimation).

The deconvoluted spectrum (the spectrum pointing up in the Head-to-Tail display of the **Sample/Lib Match Spectrum** window) may have dashed peaks. These peaks represent ions that may not belong to the rest of the spectrum. They are called *uncertain peaks.* These *uncertain peaks* are used in the determining of the closeness of the match between the library and sample spectrum if they match library spectrum peaks (**Score**, **Dot Product**, **R.Match**) in the **Chromatogram** tab view's deconvolution and search. The *uncertain peaks* are igored if the don't match the library spectrum peaks.

The deconvoluted spectrum can be sent to MS Search's **Spec List** window (**Lib. Search/Librarian** tab views) by selecting (highlighting) one or more components in the **Chromatogram** tab view **Hit List** window, then with the Mouse pointer in the **Hit List** window, click the Right Mouse button, and select **Send to** from the **RMB** menu (*Figure 16*). This action does NOT bring the **Lib. Search/Librarian** tab view into focus. The **RTIC Chromatogram** window's **RMB** menu also has a **Send To** selection; only a single spectrum at a time can be sent. If **Library Seach** is selected from these two **RMB** menus, the deconvoluted spectrum/spectra is/are sent to the **Spec List** and searched using the settings and selected libraries in the **Library Search Options** dialog box (displayed by selecting the **Library Search Options** ( ) button on the **Button Bar** of the **Lib. Search** tab view or selecting **Library Search Options** from **Options** on the Main Menu). The **Lib. Search** tab view will be brought into focus. If the deconvoluted spectrum is already in the **Spec List** window, when the **Lib. Search** tab view comes into focus, the existing entry will be highlighted and searched. When spectra with *uncertain peaks* are sent to the **Spec List** by the **Library Search** command of the **RMB** menu, the *uncertain peaks* will also be sent. Whether or not *uncertain peaks* are used in the search is controlled by a selection in the **Search** page of the **Library Search Options** dialog box. When the display of ***m/z*-Intensity List** is selected in the **Unknown Text Info** page of the **Library Search Properties** dialog box, a **B** (background) or **N** (noise) designation will appear to right the intensity value for the uncertain peaks as shown in the figure above. **L** is another type of uncertain AMDIS peak (*Figure 17*). These are due to overlapping peak uncertainty between neighboring deconvoluted components.

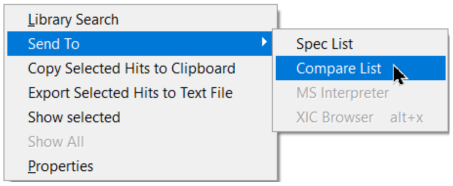
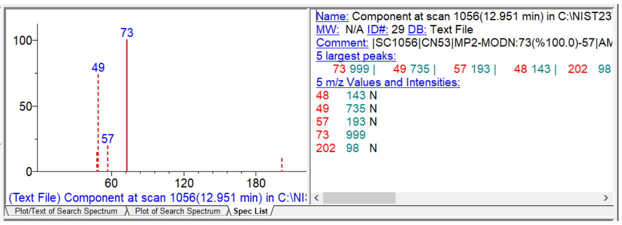


Figure 16. RMB menu displayed in Hit List window of Chromatogram tab view.



Name: Component at scan 1056(12.951 min) in C:\NIST23
MW: N/A ID#: 29 DB: Text File
Comment: |SC1056|CN53|MP2-MODN:73(%100.0)-57|AM
5 largest peaks:
73 999 | 49 735 | 57 193 | 48 143 | 202 98
5 m/z Values and Intensities:
48 143 N
49 735 N
57 193 N
73 999
202 98 N

Figure 17. Spec List Plot/Meta Data windows of Lib. Search tab view of spectrum sent from Chromatogram tab view Hit List window.

The spectrum from the **Meta Data Lib Match** or **Tabular Data for Deconvoluted Spectrum** window can be **Library Search**ed or **Sent to** the **Spec List** of the **Lib. Search** tab view or the **Compare List** of the **Compare** tab view using their respective **RMB** menus. These functions cannot be performed from either of the two **Plot** windows.

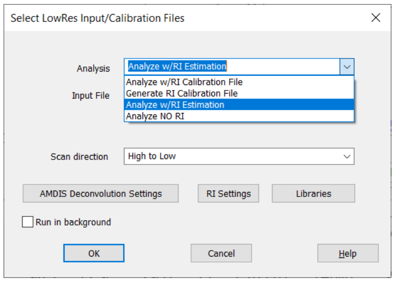
> ***Nota Bene:*** When a selection in the **Chromatogram** window or a selection or selections in the **Hit List** window of the **Chromatogram** tab view are sent to the **Compare List** of the **Compare** tab view using the **RMB** menu, the only thing sent is *deconvoluted* spectra. If the **Compare** tab view is formatted with a top spectrum window, a **Compare** window, and a window containing spectra to compare with the top window's spectrum, the **Compare** tab view will be cleared, and the spectra/spectrum will appear in individual windows. If subsequent spectra are sent from the **Chromatogram** tab view, these spectra will be prepended to the top of the display. The default number of spectra in the display is 3 but can be change in the Properties dialog box displayed with **RMB** menu in the **Compare** tab view. ***Caution:*** If another file is processed in the **Chromatogram** tab view and deconvoluted spectra sent to the **Compare List** of the **Compare** tab view, these spectra will be prepended to the list of spectra that was created from the previous file. It is best to bring the **Compare** tab view into focus and use the **RMB** menu to **Clear** this tab view before processing a new file.

If a group of spectra have been highlighted in the **Hit List** window of the **Chromatogram** tab view, they can be exclusively viewed using the **RMB** menu's **Show Selected** option. The excluded spectra can be redisplayed using the **RMB** menu's **Show All** option. The **Hit List** window's **RMB** menu options to **Export Selected Hits to Text File** or **Copy Selected Hits to Clipboard** are *New* in v.4.0. These options are also present on the **RTIC chromatogram** window's **RMB** menu. These options have also been added to the **RMB** menus of various windows of other tab views.

The three vertical and one horizontal splitter bars in the **Chromatogram** tab view can be adjusted. The **Change Splitter Orientation** found on the **RMB** menu in some of the other tab views is not available in the **Chromatogram** tab view.

Various properties such as font type, size and style can be adjusted using the **Properties** selection of the **RMB** menu. The thickness of the lines that constitute the mass spectral peaks along with other items can also be changed using the **Properties** dialog box displayed as a selection from the **RMB** menu called from any of the windows for this tab view. This dialog box is unique to the window from which it is called.

The **RTIC Chromatogram** window (upper left of the **Chromatogram** tab view) can be zoomed multiple times. A complete zoom-out to the original display is accomplished by placing the Mouse pointer on the window and double clicking the left Mouse button. This zoom out feature for all graphics display has been an undocumented feature in MS Search since NIST 08 and maybe before. The zoom can also be canceled from a selection on the **RMB menu** (**Undo Zoom**, grayed when the display is not expanded).



Analysis: Analyze w/RI Estimation

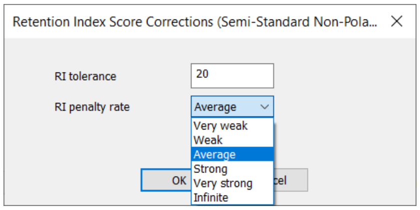
Scan direction: High to Low

☐ Run in background

Figure 18. Dialog box displayed by selecting File/Open EI Input File from Main Menu.

When MS Search is exited and reopened, the contents of the **Chromatogram** tab view are not displayed unless the **Reload on Startup** checkbox (☑) has been selected. If MS Search is closed with this box selected while viewing a populated **Chromatogram** tab, when the program is restarted the display in the **Chromatogram** tab view will be what was being viewed when the program was closed. MS Search has always opened to the tab view that was present when the program was closed. Selecting this setting may increase the time needed for the program to open.

One of the **Chromatogram** tab view **EI Analysis** types is **Analysis w/RI Estimation** (*Figure 19*). If this search is performed, the **Hit List** window will have a column labeled **EstRI** (Estimated Retention Index value) containing estimated semi-standard non-polar RI values. Display of this column is not controlled with the **Properties** dialog box. Its presence is a function of the **Search Type** selected in the **Select Input/Calibration Files** dialog box displayed by selecting the RI appropriate item from the **Analysis** dropdown list box. This column's display cannot be turned on and off in the **Hit List** page of the **Properties** dialog box.



RI tolerance: 20

RI penalty rate: Average

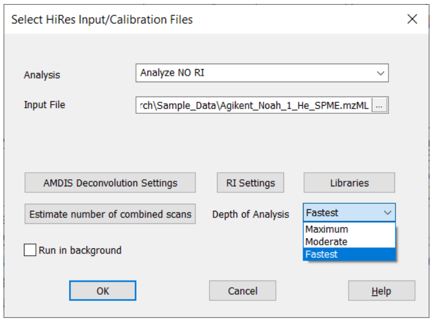
Figure 19. RI Score Correction dialog box displayed by selecting RI Settings button.

It is also possible to select **Analysis w/RI Calibration File**. This requires the generation of a Calibration File. It is also possible to set the **RI tolerance** and **RI penalty rate** in the Retention Index Score Corrections (Semi-Standard Non-Polar) dialog box displayed by selecting the **RI Settings** button on the **Select LowRes Input/Calibration Files** dialog box (*Figure 22*). The default setting for the **RI penalty rate** is **Average**. Descriptions of the other options can be found in the Help File, displayed by selecting the **Help** button in the **Select LowRes Input/Calibration Files** dialog box.

> ***Nota Bene***: Over the last few releases of the NIST\EPA\NIH Electron Ionization Mass Spectral Library, increasing emphasis has been place of the use of the retention index (RI). A great deal of effort was put into the collection of RI values on Standard Non-Polar (e.g. DB-1), Semi-Standard Non-Polar (e.g. DB-5), and Standard Polar (e.g. DB-Wax) packed and capillary columns. At the same time the RI values were harvested, the details of the GC Methods were recorded. This effort constitutes the NIST GC Method/Retention Index Database. Now, when NIST measures an EI mass spectrum, it always measures the compound's retention index on a 250 µm x 15 m (film thickness 0.25 µm) SSNP column. The details of this method are included with the spectrum's record in the Library. RI has been a usable constraint for several MS Search's releases. Each day, NIST evaluators run an *n*-alkane standard before measuring spectra. With the addition of **Chromatogram** tab view in v.4.0, RI has become a bigger part of using MS Search in the identification of unknowns. Its use is encouraged. Its value is significant.
>
> ***Nota Bene***: Analysts should always remember, they, not mass spectral libraries or software, identify unknowns.

The ***NEW*** **Chromatogram** tab view is a very powerful tool for dealing with nominal *m/z* value, accurate *m/z* value GC/MS data files, and LC-MS/MS (DDA) data files. This tool offers a great deal of information to provide a high assurance that a substance has been correctly identified. Sometimes, the amount of support information provided can be overwhelming and requires deep digging to get the highest level of confidence the identity is correct, the time necessary to learning the value of all the information coming from the **Chromatogram** tab view is well worth the eventual reward. Combined with the increase in depth and breadth of the expanded NIST\EPA\NIH Electron Ionization (EI), NIST 23 and the NIST 26 Tandem Libraries, MS Search, v.4.0 along with MS Interpreter, and the 2017 version of the Hybrid Search is a quintessential tool for organic mass spectrometry.

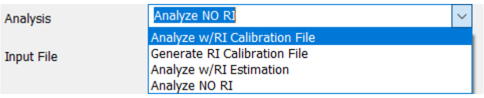
Processing EI HiRes EI Input Files in Chromatogram tab view



Analysis: Analyze NO RI
Input File: ·ch\Sample_Data\Agikent_Noah_1_He_SPME.mzML
Depth of Analysis: Fastest
☐ Run in background

Figure 20. Dialog box displayed when Analyze HiRes is selected from the File menu of the Chromatogram tab view.

As of the release of NIST 26 and MS Search v.4.0 there are a number of GC/MS instruments that measure accurate *m/z* values for ions formed in an EI ion source. The methods of processing an integer EI GC/MS file (**Analyze EI Input File** from the **File** menu) and a HiRes GC/MS Data (**Analyze EI HiRes Input File**) are very similar to one another. There are some differences, when **Analyze HiRes Input File** is selected from the **File** menu, the **Select HiRes Input/Calibration Files** dialog box is different than the one displayed when **Analyze EI Input File** is selected (*Figure 20*). Analyze menu displayed from the top dropdown list box is the same.



Analysis: Analyze NO RI

The **AMDIS Deconvolution Settings**, **RI Settings**, and **Libraries** buttons display the same dialog boxes as displayed when these buttons are selected in the **Select LowRes Input /Calibration Files** dialog box. The **Estimate number of combined scans** button is grayed until an **Input File** is selected. Once selected, it is best to select this button. Accurate *m/z* instruments such as Q-TOFs and Orbitraps acquired data more rapidly than the transmission quadrupole. This fact often results in multiple IDs for a single chromatographic peak. This can result in confusion. If multiple spectra (scans) are added together, this can be avoided.

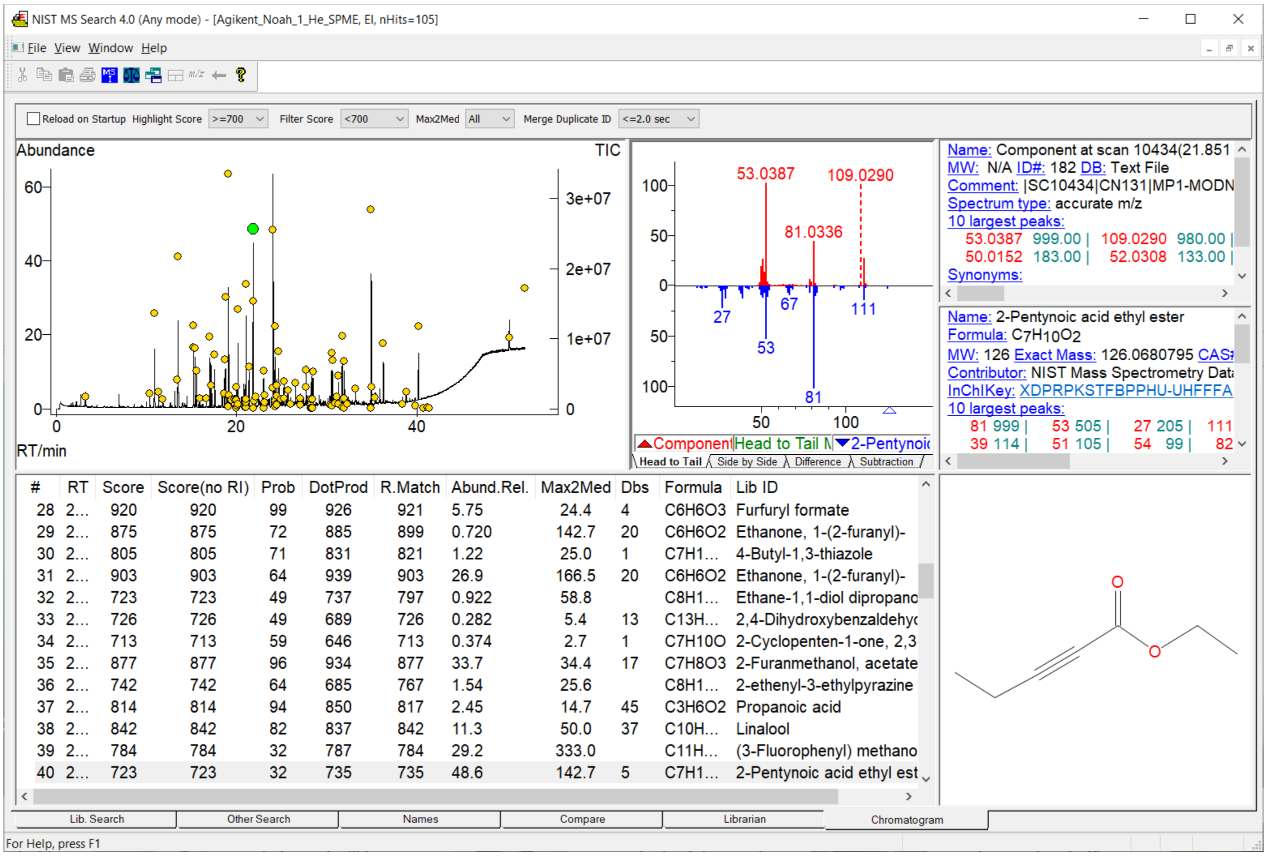
Once selected, a brief period passes (be patient, this can take some time depending on the file size) and then a dialog box with **Yes** and **No** buttons is displayed with the statement as to the **Estimated number of combined scans is X. Accept?** Selecting the **Yes** button causes the deconvolution process to use the program's estimate. If **No** is selected, the number for this setting in the **AMDIS Deconvolution Settings** dialog box will be used. The **Depth of Analysis** selection in the dropdown list box determines how many components are allowed. This is a sensitivity setting. One experiment showed the same data file resulted in 49 components when set to **Fastest**, 196 when set to **Moderate**, and 235 when set to **Maximum**. The increase in processing time is exponential.

Currently, there are no accurate *m/z* EI NIST libraries provided by NIST. The searches are against the integer NIST EI libraries (mainlib and replib) unless others have been specified in the Libraries dialog box displayed by selecting the **Libraries** button in the **Select HiRes Input/Calibration Files** dialog box. The **Hit List** columns available for the processing of both HiRes and LowRes EI files are the same. There is a single command on the **File** menu to recall previously processed EI data files (HiRes or LowRes) The **Filter Bars** for the two modes are the same**.** When the file is selected in the **Reopen EI Results** dialog box the top line of the **Run Options** window is labeled **Mode:** and specifies whether the file is LowRes or HiRes.

Before processing an accurate *m/z* EI GC/MS data file, the previous section on processing integer *m/z* value data should be carefully reviewed. Once the data file is processed, the **Chromatogram** tab view will appear as shown in *Figure 19,* on the next page. The only major difference is labeling of the bar graph display of the deconvoluted component. They are labeled to four decimal places. The rules for sending and using uncertain peaks in the **Lib. Search** tab view are the same for the HiRes and LowRes data.

Analyzing w/RI Calibration File

The above two examples do not include the use of the components' retention Index (RI). The **Chromatogram** tab view data processing is designed to facilitate identification using RI Experimental values in the searched NIST Libraries when a semi-standard non-polar (SSNP) GC column is used. If an Experimental value is not available for the mainlib or replib spectrum, the AI estimated RI value is used. Using the same column used for the analysis and the same chromatographic conditions, perform an analysis of a sequential linear hydrocarbon mix; it is good practice to acquire this data file every day before samples are analyzed. The existing calibration file should be evaluated using the newly acquired data file. It is important to create a new calibration file each time the chromatographic conditions are changed. If the calibration data file is in a specific folder and the calibration file has that folder's name, when a new calibration file is created, it will overwrite the current file name.



☐ Reload on Startup   Highlight Score >=700   Filter Score <700   Max2Med All   Merge Duplicate ID <=2.0 sec

#	RT	Score	Score(no RI)	Prob	DotProd	R.Match	Abund.Rel.	Max2Med	Dbs	Formula	Lib ID
28	2...	920	920	99	926	921	5.75	24.4	4	C6H6O3	Furfuryl formate
29	2...	875	875	72	885	899	0.720	142.7	20	C6H6O2	Ethanone, 1-(2-furanyl)-
30	2...	805	805	71	831	821	1.22	25.0	1	C7H1...	4-Butyl-1,3-thiazole
31	2...	903	903	64	939	903	26.9	166.5	20	C6H6O2	Ethanone, 1-(2-furanyl)-
32	2...	723	723	49	737	797	0.922	58.8		C8H1...	Ethane-1,1-diol dipropano
33	2...	726	726	49	689	726	0.282	5.4	13	C13H...	2,4-Dihydroxybenzaldehy
34	2...	713	713	59	646	713	0.374	2.7	1	C7H10O	2-Cyclopenten-1-one, 2,3
35	2...	877	877	96	934	877	33.7	34.4	17	C7H8O3	2-Furanmethanol, acetate
36	2...	742	742	64	685	767	1.54	25.6		C8H1...	2-ethenyl-3-ethylpyrazine
37	2...	814	814	94	850	817	2.45	14.7	45	C3H6O2	Propanoic acid
38	2...	842	842	82	837	842	11.3	50.0	37	C10H...	Linalool
39	2...	784	784	32	787	784	29.2	333.0		C11H...	(3-Fluorophenyl) methano
40	2...	723	723	32	735	735	48.6	142.7	5	C7H1...	2-Pentynoic acid ethyl est

Name: Component at scan 10434(21.851
MW: N/A ID#: 182 DB: Text File
Comment: |SC10434|CN131|MP1-MODN
Spectrum type: accurate m/z
10 largest peaks:
53.0387 999.00 | 109.0290 980.00 |
50.0152 183.00 | 52.0308 133.00 |
Synonyms:

Name: 2-Pentynoic acid ethyl ester
Formula: $C_7H_{10}O_2$
MW: 126 Exact Mass: 126.0680795 CAS#
Contributor: NIST Mass Spectrometry Data
InChIKey: XDPRPKSTFBPPHU-UHFFFA
10 largest peaks:
81 999 | 53 505 | 27 205 | 111
39 114 | 51 105 | 54 99 | 82

Figure 21. Chromatogram tab view with populated windows after a HiRes EI data file has been processed.

An effective way to check the validity of the calibration file is to perform an **Analysis w/RI Calibration File** using the just created calibration file and the data file used to create it. The resulting Hit List should show all the *n*-alkanes. If branched compounds are identified, then manual evaluation of the file is required.

It should be noted that problems may exist above RI values of 3000 when the calibration is generated by MS Search. The calibration file (*.CAL) is a simple text file and can be easily opened with Windows® Notepad. The calibration file will have the name of the folder it is in, e.g., if the calibration data file is located on the \NIST26\MSSearch\SAMPLE DATA\ folder, the calibration file, after creation, will be in that folder and have the name SAMPLE DATA.CAL.

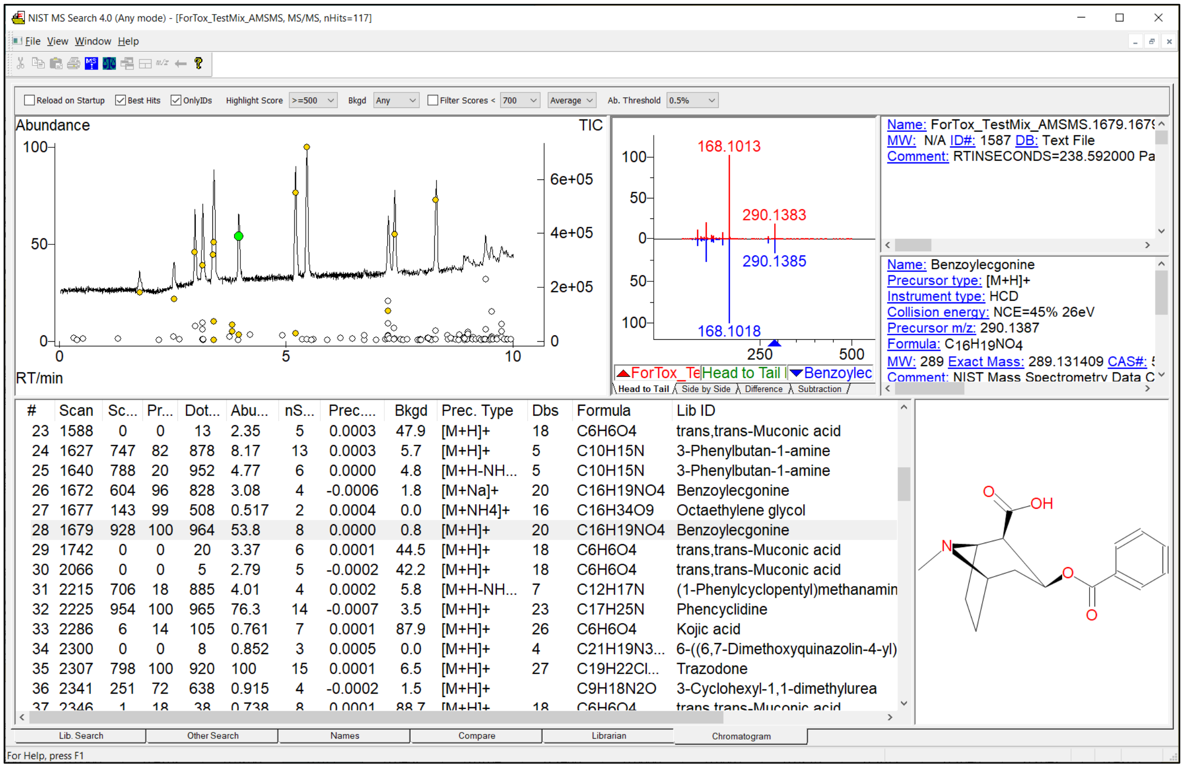
If it is desirable to use RI values above 3000, it may be necessary to open the CAL file in Notepad and edit it. If the file is edited, make sure the format is maintained with only one space been each of the columns.

It is important to keep in mind that the acquisition rate can affect the calibration file, especially when measuring integer GC/MS data with a transmission quadrupole instrument. The faster the acquisition rate, the less time is spent measuring the ion current of any individual *m/z* value.

The calibration file is generated from the **Select LowRes Input/Calibration File** or **Select HiRes Input/Calibration File** dialog box. Once the calibration data file is selected from the **Select Data File** (LowRes) or **Open** (HiRes) dialog box and the Open button is selected, the dialog box closes leaving the Select dialog box open. Select **Ok** button. The dialog box closes, the data are processed, and an **Information** dialog box is displayed that reads **Calibration File Created** and an **OK** button; selecting the **OK** button closes this **Information** dialog box and the program is ready to process a data file.

> ***Nota Bene:*** Unlike LowRes (GC/MS EI) data files only generic mzML, CDF, and mzData files are support for processing in the **Chromatogram** tab view. Support for some instrument manufacturers native file formats for LowRes (**Analyze EI Input File**) is a carryover from AMDIS.

Processing LC-MS/MS Files in the Chromatogram tab view



☐ Reload on Startup ☑ Best Hits ☑ OnlyIDs Highlight Score >=500 Bkgd Any ☐ Filter Scores < 700 Average Ab. Threshold 0.5%

Name: ForTox_TestMix_AMSMS.1679.167
MW: N/A ID#: 1587 DB: Text File
Comment: RTINSECONDS=238.592000 Pa

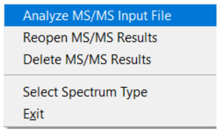
Name: Benzoylecgonine
Precursor type: [M+H]+
Instrument type: HCD
Collision energy: NCE=45% 26eV
Precursor m/z: 290.1387
Formula: $C_{16}H_{19}NO_4$
MW: 289 Exact Mass: 289.131409 CAS#:
Comment: NIST Mass Spectrometry Data C

#	Scan	Sc...	Pr...	Dot...	Abu...	nS...	Prec....	Bkgd	Prec. Type	Dbs	Formula	Lib ID
23	1588	0	0	13	2.35	5	0.0003	47.9	[M+H]+	18	C6H6O4	trans,trans-Muconic acid
24	1627	747	82	878	8.17	13	0.0003	5.7	[M+H]+	5	C10H15N	3-Phenylbutan-1-amine
25	1640	788	20	952	4.77	6	0.0000	4.8	[M+H-NH...	5	C10H15N	3-Phenylbutan-1-amine
26	1672	604	96	828	3.08	4	-0.0006	1.8	[M+Na]+	20	C16H19NO4	Benzoylecgonine
27	1677	143	99	508	0.517	2	0.0004	0.0	[M+NH4]+	16	C16H34O9	Octaethylene glycol
28	1679	928	100	964	53.8	8	0.0000	0.8	[M+H]+	20	C16H19NO4	Benzoylecgonine
29	1742	0	0	20	3.37	6	0.0001	44.5	[M+H]+	18	C6H6O4	trans,trans-Muconic acid
30	2066	0	0	5	2.79	5	-0.0002	42.2	[M+H]+	18	C6H6O4	trans,trans-Muconic acid
31	2215	706	18	885	4.01	4	0.0002	5.8	[M+H-NH...	7	C12H17N	(1-Phenylcyclopentyl)methanamin
32	2225	954	100	965	76.3	14	-0.0007	3.5	[M+H]+	23	C17H25N	Phencyclidine
33	2286	6	14	105	0.761	7	0.0001	87.9	[M+H]+	26	C6H6O4	Kojic acid
34	2300	0	0	8	0.852	3	0.0005	0.0	[M+H]+	4	C21H19N3...	6-((6,7-Dimethoxyquinazolin-4-yl)
35	2307	798	100	920	100	15	0.0001	6.5	[M+H]+	27	C19H22Cl...	Trazodone
36	2341	251	72	638	0.915	4	-0.0002	1.5	[M+H]+		C9H18N2O	3-Cyclohexyl-1,1-dimethylurea
37	2346	1	18	38	0.738	8	0.0001	88.7	[M+H]+	18	C6H6O4	trans,trans-Muconic acid

Figure 22. Chromatogram tab view with a processed LC-MS/MS data file.

[*In this manual, **Selecting** means to place the Mouse pointer on an object and to click the left Mouse button once.*]

For LC-MS/MS (DDA) chromatogram files, the **Chromatogram** tab view enables searching of all MS2 spectra against the NIST Tandem Libraries and other such libraries using updated NIST MS Search algorithms. This routine will also allow for component deconvolution and performing the *NIST17 Hybrid Search*, which has proved very effective in the aid of the identity of unknowns when it is believed there are no spectra for these compounds in the library. In addition, processing of MS1 data is done by the *XICanalyzer* program[5] which identifies the chromatographic peak for each spectrum which can be viewed with the *XIC Browser*, callable from the **Hit List** or **RTIC Chromatogram** window with the **RMB** menu **Send To** selection. Each MS2 spectrum can be further analyzed using the tools provided with MS Search, including the **Hybrid Search**, which is an aid to identifying compounds that do not have spectra in the searched libraries. Extracted MS2 spectra can be saved to user-libraries using the MS Search **Librarian** tab view.



File menu when Spectrum Type is Tandem

The layout of the **Chromatogram** tab view when being used for LC-MS/MS (DDA) data is the same as when it is being used for GC/MS data, except for the **Filter Bar**. The two modes have different filters. See *Figure 8* for a definition of the layout and windows in the **Chromatogram** tab view. When the selected **Spectrum Type** is **Tandem**, the **File** menu has a different appearance as shown on the left.

Another difference between the **Chromatogram** tab view used with LC-MS/MS (DDA) and EI (GC/MS) data is the information that can be displayed in the **Hit List**. Like the **Chromatogram** tab view **Hit List** columns for EI (GC/MS), the available **Hit List** columns for LC-MS/MS (DDA) are divided into two categories: **Default** and **Expert**. In the following list of definitions, those only available when the **Include Expert Results** checkbox is selected are marked with an *:

Order Number – Position in current **Hit List**
Scan – Spectrum Sequence number in chromatogram (only needed to compare to original data)
RT – Retention time for compound (minutes)

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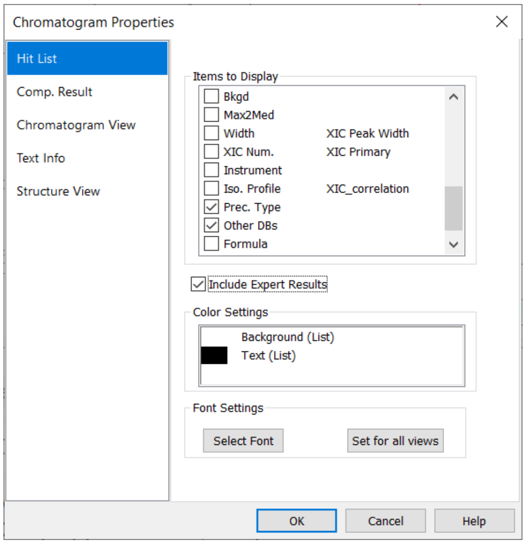
[5] Guanghui Wang, Zheng Zhang, Yi Liu, Meghan C. Burke, Sergey L. Sheetlin, and Stephen E. Stein, “An XIC-Centric Strategy for Improved Identification and Quantification in Proteomic Data Analyses



☐ Bkgd
☐ Max2Med
☐ Width XIC Peak Width
☐ XIC Num. XIC Primary
☐ Instrument
☐ Iso. Profile XIC_correlation
☑ Prec. Type
☑ Other DBs
☐ Formula

☑ Include Expert Results

Figure 23. RMB menu display of Hit List page of Chromatogram Properties dialog box.

Score – NIST Identity Score – excludes large losses and dimers

Score (Unfiltered) – Score of any top library ID, including all in-source spectra (including large losses)

DotProd – Cosine Similarity (Unweighted)

Prob – Probability of hit being correct assuming correct ID is in library

***R.Match** – Reverse Match score ignores query peak not matching library peak

***#mpeaks** – Number of query peaks matching library peaks

Prec.Shift – Using MS1 abundances, shifting of *m/z* used for fragmentation needed to match principal ion

Abund.Rel – Percent abundance relative to largest abundance

***nSpec –** Number of MS2 spectra acquired for a component (XIC) peak

Prec. *m/z* – Precursor *m/z* of MS/MS spectrum

***Charge** – Charge of ion consistent with isotope spacing

***dPPM** – Difference in *m/z* between measured and library spectrum

***Purity** – Percentage of total signal in fragmentation zone due to contaminant ions

***Bkgd** – Background percent provides measure of relative intensity of precursor ion for perceiving background ions eluting over long periods

***Max2Med** – Ratio of maximum to median ion intensity in spectrum – low values indicate low signal strength

***Width (XIC Peak Width)** – Width of component peak (XIC) in seconds

***XIC Number (XIC Primary)** – Sequence number of component XIC peak

***Instrument** – Type of fragmentation for library spectrum

***Iso.Profile (XIC_correlation)** – Degree of matching isotope peaks in Component peak (XIC)

Prec.Type – Type of precursor for library spectrum – e.g., [M+H-H_2O] where for protonated ion with water loss from precursor ion

Other DBs – Compound Ubiquity Index (DBs) - the number of external libraries in which the compound is present

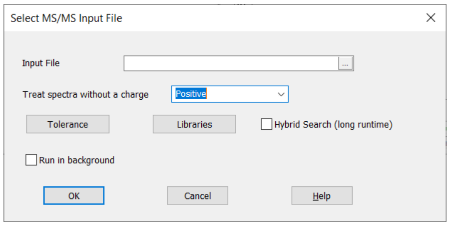
***Formula** – Chemical formula of the library match

***Mobility (1/K))** – Ion mobility (1/k0) when reported by instrument

***SumAb** –Total abundance of query spectrum with base peak assigned value of 1

Library – Name of Library containing the spectrum

***#peaks** – number of peaks in the deconvoluted component.

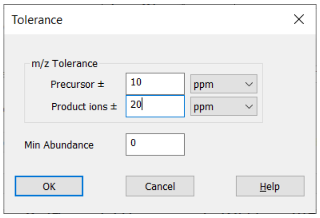


Treat spectra without a charge: Positive

☐ Hybrid Search (long runtime)

☐ Run in background

The **Tandem Spectrum Type** of the **Chromatogram** tab view has a number of features requiring a deep understanding; however, it can be used simply to provide identifications of unknowns in an LC-MS/MS (DDA) chromatogram. This example illustrates that process.



Precursor ± 10 ppm

Product ions ± 20 ppm

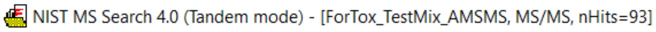
Min Abundance 0

Nota Bene: See **Nota Bene** *page 24* about **Run in background** checkbox.

Nota Bene: See **The Program Chromatogram tab view** *page 58* for information on **Hybrid Search**.

From the **File** menu select **Analyze MS/MS Input File**. This results in the display of the **Select MS/MS Input File** dialog box (above left). Select the **Libraries** button. If the hr_nist_msms library is not in the **Include Libs:** window (lower window), highlight it in the upper window and use the **>>Add>>** button to add it. Then select the **OK** button to close the **Libraries** dialog box. There is button labeled **Tolerance** in the **Select MS/MS Input File** dialog box. Selecting this button results in **Tolerance** dialog box being displayed allowing the input of *m/z* tolerance for the data. If actual values are not available use 20 for the Precursor and 40 for the Product when analyzing Q-TOF data and 10 and 20, respectively, when analyzing Orbitrap data. The data being analyzed in this example is from a Q-TOF. For this example, leave the **Min Abundance** at 0. Select the **OK** button to close the dialog box.

After establishing the proper mass accuracy values and marking sure the correct library will be used, select the button (labeled **…**) on the right side of the **Input File** entry field. This results in the display of a file **Open** dialog box. The only file types supported by the **Analyze MS/MS Input File** selection from the **File** menu are **mzML Files (*.mzML)**. Most instrument manufacturers have a function to out files in the generic format or there are third party programs which can be used. For this example, navigate and find the file named **ForTox_TestMix_AMSMS.mzML**; highlight it and select the **Open** button. This closes the file **Open** dialog box and the **Select MS/MS Input File** dialog box is now visible. Select the **OK** button in this dialog box. The data file will have components deconvoluted and each component searched as a function of the process will be processed. When complete all the windows of the **Chromatogram** tab view will be populated.

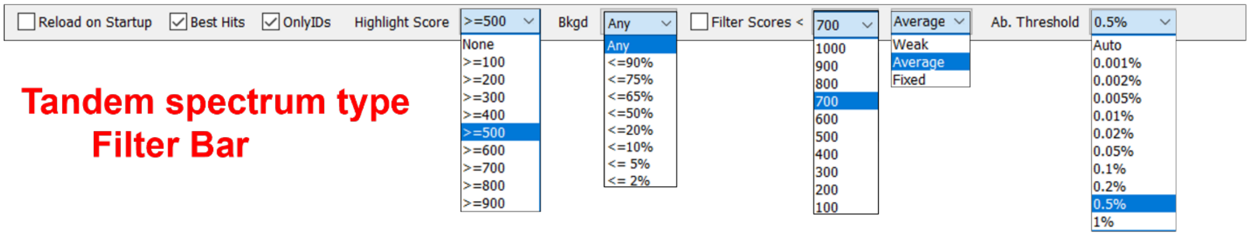


NIST MS Search 4.0 (Tandem mode) - [ForTox_TestMix_AMSMS, MS/MS, nHits=93]

Check the **Filter Bar** to make sure the settings are the same as shown below. This should result in the **Title Bar** at the top of the display showing:

where **nHits = 93**, shows number of components identified.

With the **Filter Bar** set as described above, the upper left window will contain a reconstructed total ion current (**RTIC**) **chromatogram** and a number of clear circles (○). Each ○ represents a found component (Hit). The first ○ on the left is larger than the others and colored (green, by default). This corresponds to the first item in the **Hit List** window (lower left) which is highlighted.



☐ Reload on Startup ☑ Best Hits ☑ OnlyIDs Highlight Score >=500 Bkgd Any ☐ Filter Scores < 700 Average Ab. Threshold 0.5%

Figure 24. Filter Bar for Chromatogram tab view with the Tandem Spectrum type selected.

> ***Nota Bene:*** In the **RTIC Chromatogram** window, the vertical position of the ○ representing a component (if the **Best Hits** option is selected) is the MS1 area of the XIC associated with the identification. If the **Best Hits** option is not selected, the ○ represents an MS2, with the intensity being that of the MS2 spectrum. In both cases, the vertical position is relative to the most abundant in the chromatogram. The **RTIC chromatogram's** peak-heights are the sums of all MS1 abundances for each MS2 spectrum.

As stated in the MS Search Help file, with Best Hits selected, the Y-axis abundance of each ID is the area of its XIC (extracted ion chromatogram) of the MS1 intensity of its precursor ion and isotopes. When not selected, the abundance is that of its MS2 spectrum. In neither case does the Y-abundance exactly match its TIC abundance (if there is one) on the right axis. The TIC sums all MS1 electrospray ions associated with an LC-separated component. It therefore depends on peak width and abundances of all ions associated with the component. These *in-source* ions may often be observed as well-defined co-eluting columns of XIC identifications.

The first checkbox in the **Filter Bar** is labeled **Reload on Startup**. Unless selected, the currently viewed results will not be redisplayed when MS Search is closed and then reopened. The results can be restored in such a case by use of Main Menu selection **File**/**Reopen MS/MS Results**. This results in the display of the **Reopen MS/MS Results** dialog box. Highlight the name of the file to be restored and select the **OK** button and the previous results obtained by processing that particular file will be displayed.

The second checkbox (selected above) is the **Best Hits** label. The highest scoring identification of any chromatographic **XICs** will be the only designated components highlighted. This results in more reliable quantitative results and can reduce background ions or repeated identifications of chromatographic peaks that are multiply sampled. The number of duplicated IDs for a component can be shown in the **nSpec** column of the **Hit List** window (only accessible when the **Include Expert Results** checkbox is selected in the **Hit List** page of the **Chromatogram Properties** dialog box).

To the right of **Best Hits** label is a checkbox next to the **Only IDs** label. Select this. This allows only the display of spectra that match a NIST Tandem Library spectrum regardless of score. Unidentified spectra are given names beginning with *NoID_<precursor m/z>*). Selecting/deselecting this checkbox will result in the **nHits** value in the **Title Bar** changing.

Following the **Only IDs** checkbox is the **Highlight Score** label to the left of a dropdown list box. Some of the ○s in the **RTIC chromatogram** window are now filled (yellow, by default) and some are clear. Only the ones with an identity Score of 500 or greater are filled, the remainder are clear, i.e., the only components represented by filled ○s are the ones that have probably been identified. If the value is changed, the **nHits** value in the **Title Bar** WILL NOT change.

Following the **Highlight Score** dropdown list box in the **Filter Bar** is the **Bkgd** label followed by another dropdown list box. The background is the maximum percent of signal allowed for the appearance in the **Hit List**. This can be important in eliminating the influence of background ion sampling. In the example the setting is Any. The value can be changed by selecting the dropdown arrow on the right side of the list box to the right of the **Bkgd** label. Changing the value will result in the **nHits** value in the **Title Bar** changing.

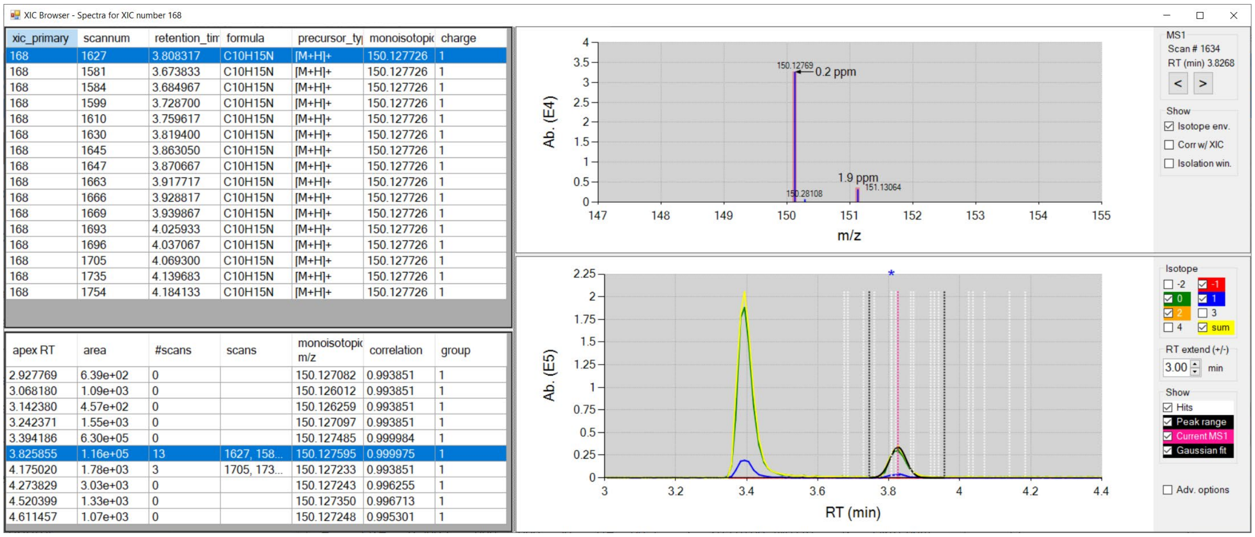
The penultimate selection on the **Filter Bar** is **Filter Scores**. Selecting the checkbox to the left of this label allows the filtering of lower quality spectra at three levels. These are selected from the dropdown list box that becomes active when the checkbox is selected. There are three options available (**Weak**, **Average** and **Fixed**). The **Fixed** option allows the elimination of all IDs below a specified **Score**. The use of the **Non-Filtered Score** is based on four different quality factors (bkgd, purity, max2min, and isotope analysis), all of which may be optionally viewed in the **Hit List**. The value for the **Fixed** selection is entered through a dropdown list box to the left of dropdown list box used to specify the level of filtering. This box becomes active when the checkbox next the **Filter Score** label is selected but only pertains to the **Fixed** selection. In this example, the **Filter Scores** checkbox is deselected and the values in the dropdown list boxes are not relevant. Selecting this checkbox and setting values in the two dropdown lists will result in changes in the **nHits=** value in the **Title Bar**.

The last filter is the absolute threshold (**Ab. Threshold** to the left of a dropdown list box) setting. The values run from 0.001 to 1.0%, with an Auto setting which will set the value based on the data. Changes to the setting will cause the **nHit =** value in the **Title Bar** to change. The setting is 0.5% for this example.

After making sure the values set in the **Filter Bar** are set to those shown above and **nHit = 117** is displayed in the **Title Bar**, sort the **Hit List** by **Score**. This is done by placing the Mouse pointer on the **Score** column header in the **Hit List** and clicking the left Mouse button. A ▼ symbol preceding the column title will appear indicating the sort is from high to low.

> ***Nota Bene*:** The **Hit List** can be sorted by any column. Place the Mouse pointer on the column title and click the left Mouse button. If the column is not sorted, this will sort it in descending order, and a ▼ symbol will precede the title. A second click of the left Mouse button will reverse the direction of the sort (increasing order), and a ▲ symbol will precede the title.

The **Lib ID** (primary name in library) for the first Hit will read **Phencyclidine**, with a **Score** of 954. Scroll down the **Hit List** and compare the butterfly plots in the **Component/Library Match Plot** window. When a **Plot** window match is observed that is not good, put the Mouse pointer on the ○ in the **RTIC chromatogram** window or leave it on the entry in the **Hit List** window and click the Right Mouse button to display the **RMB** menu. Selecting the **Send To** menu option **XIC Browser** will open what appears to be a new program; the XIC Browser which can provide information about the component that may aid in confirming the identification. For this example, select item 11 (3-phenylbutan-1-amine, Score 747). The component spectrum can be sent to the **Spec List** of the **Lib. Search/Librarian** tab view, which will not bring either into focus, or searched using the current **Library Search Options**, which does bring the **Lib. Search** tab view into focus. If the components shown are a subset of a **Hit List**, the **Show All** selection restores the view from which the subset was selected.



Scan # 1634
RT (min) 3.8268
3.00 min

xic_primary	scannum	retention_tim	formula	precursor_ty	monoisotopic	charge
168	1627	3.808317	C10H15N	[M+H]+	150.127726	1
168	1581	3.673833	C10H15N	[M+H]+	150.127726	1
168	1584	3.684967	C10H15N	[M+H]+	150.127726	1
168	1599	3.728700	C10H15N	[M+H]+	150.127726	1
168	1610	3.759617	C10H15N	[M+H]+	150.127726	1
168	1630	3.819400	C10H15N	[M+H]+	150.127726	1
168	1645	3.863050	C10H15N	[M+H]+	150.127726	1
168	1647	3.870667	C10H15N	[M+H]+	150.127726	1
168	1663	3.917717	C10H15N	[M+H]+	150.127726	1
168	1666	3.928817	C10H15N	[M+H]+	150.127726	1
168	1669	3.939867	C10H15N	[M+H]+	150.127726	1
168	1693	4.025933	C10H15N	[M+H]+	150.127726	1
168	1696	4.037067	C10H15N	[M+H]+	150.127726	1
168	1705	4.069300	C10H15N	[M+H]+	150.127726	1
168	1735	4.139683	C10H15N	[M+H]+	150.127726	1
168	1754	4.184133	C10H15N	[M+H]+	150.127726	1

apex RT	area	#scans	scans	monoisotopic m/z	correlation	group
2.927769	6.39e+02	0		150.127082	0.993851	1
3.068180	1.09e+03	0		150.126012	0.993851	1
3.142380	4.57e+02	0		150.126259	0.993851	1
3.242371	1.55e+03	0		150.127097	0.993851	1
3.394186	6.30e+05	0		150.127485	0.999984	1
3.825855	1.16e+05	13	1627, 158...	150.127595	0.999975	1
4.175020	1.78e+03	3	1705, 173...	150.127233	0.993851	1
4.273829	3.03e+03	0		150.127243	0.996255	1
4.520399	1.33e+03	0		150.127350	0.996713	1
4.611457	1.07e+03	0		150.127248	0.995301	1

Figure 25. XIC Browser.

Searching of Imported Spectra (Lib. Search tab view)

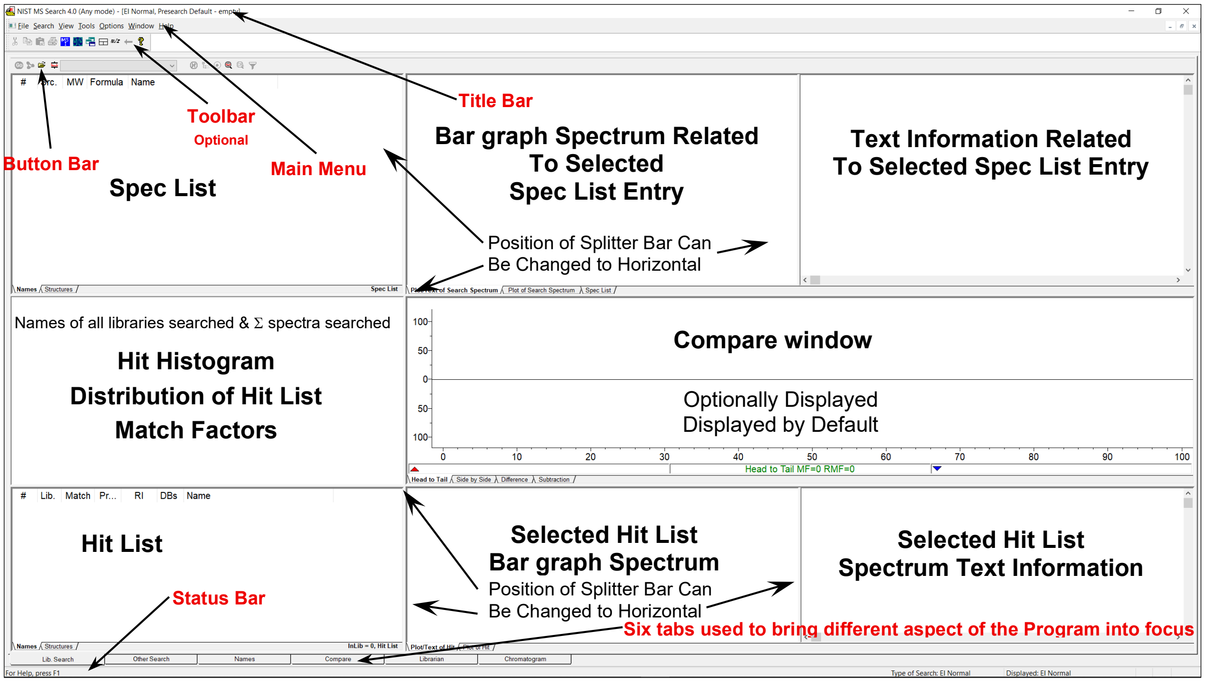


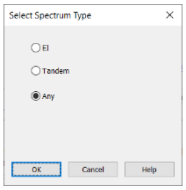
Figure 26. NIST Mass Spectral Search Program Initial View.

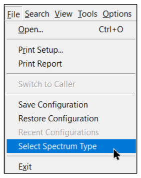
[*In this manual* ***Selecting*** *means to place the Mouse pointer an object and click the left Mouse button once.*]

The **NIST Mass Spectral Search Program v.4.0** is multifaceted. It has capabilities far beyond the comparison of a measured spectrum of an unidentified compound against NIST Mass Spectral Libraries, or those of a third-parties. MS Search works with different types of spectra [Electron Ionization (EI), Product-ion (LC-MS and LC-MS/MS)], both integer and accurate *m/z* values. Above (*Figure 26)* is the view when the Program is started for the first time after running **SETUP.EXE** from one of the two **NIST 26 Installations** (EI or Tandem).

The Program opens in the **Lib. Search** tab view (six tabs on the bottom of the MS Search Program Window, each with a different function). Spectra of individual compounds are listed in the **Spec List** (upper left window). These compounds are shown in the **Names** display (the left-hand tab at the bottom of the **Spec List** window). The window can be changed to the **Structure** display (if structures are associated with the spectra) by selecting the **Structure** tab at the bottom of the window. If no structure is associated with the spectrum and the **Formula** (elemental composition) field is populated, that will be displayed. If this field is not populated the space will be blank in the **Spec List** display.

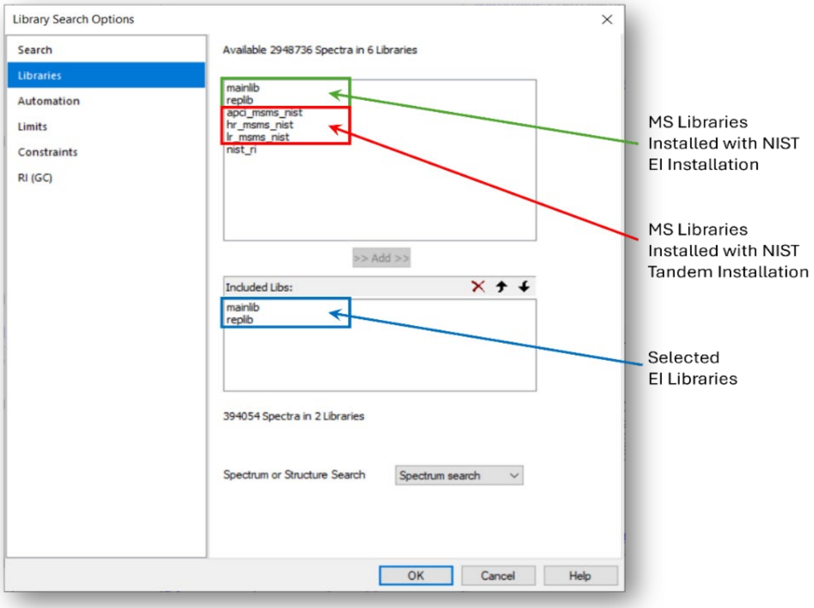
If either the **NIST 26 EI** or the **NIST 26 Tandem** installation was performed, the **Spectrum Type** based on the type of mass spectral data (**EI** or **Tandem**) being worked with is selected by default. This **Select Spectrum Type** on the **File** menu is only needed if both types of data are being used.





If using both types of spectra, from the **File** menu (upper left of the display on the Main Menu Bar) choose **Select Spectrum Type** (next to last selection in the **File** menu shown on the right). Selecting this will result in the display of a dialog box shown on the left. The three options are **EI**, **Tandem**, and **Any**. Whatever is selected will affect what options will be offered when the Program is being used. The **Spectrum** type is determined by selectiong the radio button next to its name.

If the **NIST EI Installation** is performed, three libraries will be avaiable: **mainlib**, **replib**, and **nist_ri**. If the **NIST Tandem Installation** is performed, three libraries are available: **apci_msms_nist**, **hr_nist_msms**, **lr_nist_msms**. Libraies to be searched are selected from the **Libraries** page of the **Library Ssearch Options** dialog box (displayed by selecting **Library Search Options** from the Main Menu's **Options** menu or by clicking on the **Library Search Options** button on the **Lib. Search** tab view's **Button Bar**, just above the **Spec List**. See *Figure 26*. If both the EI and Tandem mass spectral data have been installed, the appropriate libraries for the selected **Spectrum Type** should be selected before performing searches. It should be remembered that whatever libraries are in the **Included Libs:** window of the **Libraries** page (*Figure 27*) of the **Library Search Options** dialog box when the **Septum Type** is selected will be the libraries used for the search until the list is manually changed or changed by restoring an **INI** file. Changing settings or adding user-libraries, may be a reason to save configuration **INI** files. Saving and restoring **INI** files is discussed later.



Available 2948736 Spectra in 6 Libraries

mainlib
replib
apci_msms_nist
hr_msms_nist
lr_msms_nist
nist_ri

Included Libs:

mainlib
replib

394054 Spectra in 2 Libraries

Spectrum or Structure Search: Spectrum search

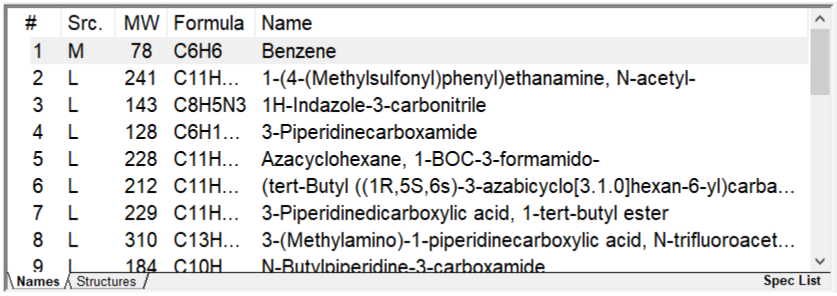
MS Libraries
Installed with NIST
EI Installation

MS Libraries
Installed with NIST
Tandem Installation

Selected
EI Libraries

Figure 27. Libraries page view of Library Search Options dialog box after both EI and Tandem Libraries Installed.

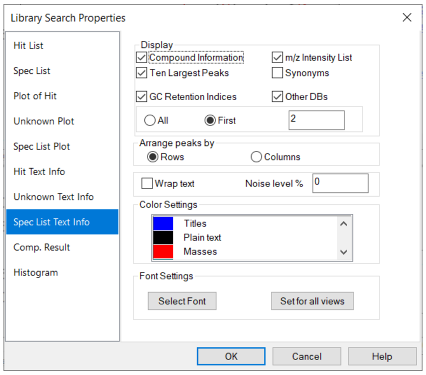
First Action in the Lib. Search tab view



#	Src.	MW	Formula	Name
1	M	78	C6H6	Benzene
2	L	241	C11H...	1-(4-(Methylsulfonyl)phenyl)ethanamine, N-acetyl-
3	L	143	C8H5N3	1H-Indazole-3-carbonitrile
4	L	128	C6H1...	3-Piperidinecarboxamide
5	L	228	C11H...	Azacyclohexane, 1-BOC-3-formamido-
6	L	212	C11H...	(tert-Butyl ((1R,5S,6s)-3-azabicyclo[3.1.0]hexan-6-yl)carba...
7	L	229	C11H...	3-Piperidinedicarboxylic acid, 1-tert-butyl ester
8	L	310	C13H...	3-(Methylamino)-1-piperidinecarboxylic acid, N-trifluoroacet...
9	L	184	C10H...	N-Butylpiperidine-3-carboxamide

A good place to start is with a single spectrum in the **Spec List** (upper left of the **Lib. Search** tab view); when the program is installed, different spectra will appear in the **Spec List** depending on the **Spectrum Type** installation. The following pertains to the **EI Spectrum Type**. The **Spec List** allows for the display of multiple columns (left-to-right) containing position **#** of entry, Source (**Src.**) information related to each selection of the spectrum source [**E**dit, **M**ainlib, **R**eplib, **A** imported from TXT (MSP or MSPEC) file (which could include 3rd party applications), **aa** user-library short name], **MW** (Nominal Mass), **Formula** (element composition), and **Name** (primary library name).

Select the name of one of the compounds (Cocaine) in the **Spec List** window. Selecting the entry in the **Spec List** will cause it to be highlighted. The bar graph spectrum along with the structure (if associated with the spectrum and the ☐ **Structure on Plot** option has been selected in the **Spec List Plot** page of the **Library Search Options** dialog box) of the compound will appear in the left part of the of the window to the right of the **Spec List**. If the spectrum has a **Formula** associated and no structure, the **Formula** will appear in the window. If there is no structure nor Formula, only the spectrum plot will be displayed.



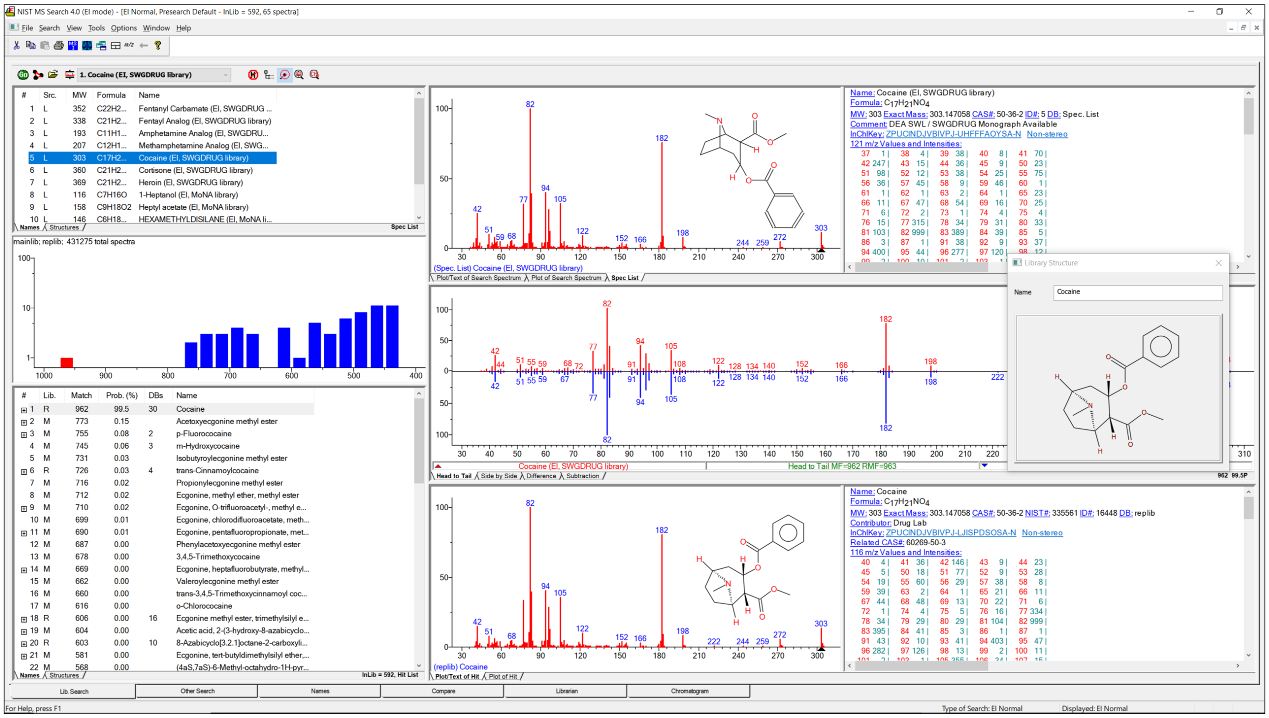
Display
☑ Compound Information ☑ m/z Intensity List
☑ Ten Largest Peaks ☐ Synonyms
☑ GC Retention Indices ☑ Other DBs
○ All ● First 2
Arrange peaks by
● Rows ○ Columns
☐ Wrap text Noise level % 0
Color Settings
🟦 Titles
⬛ Plain text
🟥 Masses
Font Settings

Figure 28. Spec List page of Library Search Properties dialog box.

This window has the **Spec List** tab (bottom of the window on right side) selected. The right side of this window contains **Text Information** (metadata) pertaining to the compound. The top line of the **Text Information** window contains the **Name** (mainlib name or primary name given to spectrum). If the entry was from a spectrum exported by AMDIS or the data analysis software of an instrument, the name would be related to the spectrum, such as the data file name, the spectrum number, and other items the exporting source has determined is relevant. The **Spec List** entries are from the NIST Libraries; therefore, the next line is the **Formula** (elemental composition), if associated with the spectrum. The third line has **MW** (nominal mass), if associated with the spectrum; **Exact Mass** (calculated value from the monoisotopic masses of compound's elemental composition), if entered a **CAS#** (Chemical Abstracts Services registry number); and, if provided with the spectrum, the **NIST#** (a unique number assigned to all spectra provided by NIST and only associated with spectra in a NIST Library). If a non-NIST spectrum is assigned a CAS# but has no associated structure, and there is an NIST spectrum with that **CAS#**, the NIST structure will be displayed on the plot of that spectrum. This third line of text in the **Spec List Text Information** window will always include the **ID#** (position in List or Library) and **DB:** (name of spectrum source). The next line contains the **Comments** (misc. information) or the **Contributor** (source of the spectra in the NIST Libraries). If a structure is associated with the spectrum the next line contains the **InChIKey**, (a unique text string that describes the structure). The following lines contain **Related CAS#** (NIST Library Spectra only), **10 largest peaks:**, and **Synonyms** (a list of other names for the compound when available, which are displayed as a column (default) or changed to a wrapped display by making a change in the **Library Search Properties** dialog box's **Spec List Text Info's** page. The **Library Search Properties** dialog box is displayed using the Right Mouse Button menu (**RMB**) with the Mouse pointer in the **Spec List Text Information** or **Plot** window. **Synonyms** not only contain chemical names, but there are also trade and commercial names. **Other DBs** are non-mass spectral databases where the compound is listed. The **Other DBs** field is unique to NIST spectra. **Retention Index** information (EI spectra only) is also displayed. The **InChIKey** is a hot link which will result in a Google Search for the compound in the default Internet Browser. Prior to NIST 23, this linked to PubChem. Other information can appear on subsequent lines with the number of ***m/z* Values and Intensities** pairs on the penultimate. The last lines contain a list of the individual *m/z* value and intensity pairs; the specific format is described later. The fields available in the **Spec List Text Information** window are also available in the **Hit List Text Information** window. A new feature in NIST 26 is the **Show Structure** floating window with the structure of the selected Hit, initiated from Main Menu's **View** menu.

Nota Bene about Chemical Abstract Services (CAS) registry numbers and the NIST\EPA\NIH Electron Ionization (EI) Mass Spectral Library: A significant number of spectra in this library have not been assigned CAS#s. This does not mean these compounds do not have CAS#s assigned by the Chemical Abstracts Services. It means, for various reasons NIST has not assigned them.

Nota Bene about **Show Structure** selection from **View** menu in the **Lib. Search** tab view: If an entry is selected from the **Spec List** window, the structure, even if associated will not be displayed. The last selected entry in the **Hit List** will continue to be displayed until a new selection is made in the **Hit List**.



#	Src.	MW	Formula	Name
1	L	352	C22H2...	Fentanyl Carbamate (EI, SWGDRUG ...
2	L	338	C21H2...	Fentayl Analog (EI, SWGDRUG library)
3	L	193	C11H1...	Amphetamine Analog (EI, SWGDRU...
4	L	207	C12H1...	Methamphetamine Analog (EI, SWG...
5	L	303	C17H2...	Cocaine (EI, SWGDRUG library)
6	L	360	C21H2...	Cortisone (EI, SWGDRUG library)
7	L	369	C21H2...	Heroin (EI, SWGDRUG library)
8	L	116	C7H16O	1-Heptanol (EI, MoNA library)
9	L	158	C9H18O2	Heptyl acetate (EI, MoNA library)
10	L	146	C6H18...	HEXAMETHYLDISILANE (EI, MoNA li...

#	Lib.	Match	Prob. (%)	DBs	Name
⊞ 1	R	962	99.5	30	Cocaine
⊞ 2	M	773	0.15		Acetoxyecgonine methyl ester
⊞ 3	M	755	0.08	2	p-Fluorococaine
4	M	745	0.06	3	m-Hydroxycocaine
5	M	731	0.03		Isobutyroylecgonine methyl ester
⊞ 6	R	726	0.03	4	trans-Cinnamoylcocaine
7	M	716	0.02		Propionylecgonine methyl ester
8	M	712	0.02		Ecgonine, methyl ether, methyl ester
⊞ 9	M	710	0.02		Ecgonine, O-trifluoroacetyl-, methyl e...
10	M	699	0.01		Ecgonine, chlorodifluoroacetate, meth...
⊞ 11	M	690	0.01		Ecgonine, pentafluoropropionate, met...
12	M	687	0.00		Phenylacetoxyecgonine methyl ester
13	M	678	0.00		3,4,5-Trimethoxycocaine
⊞ 14	M	669	0.00		Ecgonine, heptafluorobutyrate, methyl...
15	M	662	0.00		Valeroylecgonine methyl ester
16	M	660	0.00		trans-3,4,5-Trimethoxycinnamoyl coc...
17	M	616	0.00		o-Chlorococaine
⊞ 18	R	606	0.00	16	Ecgonine methyl ester, trimethylsilyl e...
⊞ 19	M	604	0.00		Acetic acid, 2-(3-hydroxy-8-azabicyclo...
⊞ 20	R	603	0.00	10	8-Azabicyclo[3.2.1]octane-2-carboxyli...
⊞ 21	M	581	0.00		Ecgonine, tert-butyldimethylsilyl ether,...
22	M	568	0.00		(4aS,7aS)-6-Methyl-octahydro-1H-pyr...

Name: Cocaine (EI, SWGDRUG library)
Formula: $C_{17}H_{21}NO_4$
MW: 303 Exact Mass: 303.147058 CAS#: 50-36-2 ID#: 5 DB: Spec. List
Comment: DEA SWL / SWGDRUG Monograph Available
InChIKey: ZPUCINDJVBIVPJ-UHFFFAOYSA-N Non-stereo
121 m/z Values and Intensities:

```
    37   1 |  38   4 |  39  38 |  40   8 |  41  70 |
    42 247 |  43  15 |  44  36 |  45   9 |  50  23 |
    51  98 |  52  12 |  53  38 |  54  25 |  55  75 |
    56  36 |  57  45 |  58   9 |  59  46 |  60   1 |
    61   1 |  62   1 |  63   2 |  64   1 |  65  23 |
    66  11 |  67  47 |  68  54 |  69  16 |  70  25 |
    71   6 |  72   2 |  73   1 |  74   4 |  75   4 |
    76  15 |  77 315 |  78  34 |  79  31 |  80  33 |
    81 103 |  82 999 |  83 389 |  84  39 |  85   5 |
    86   3 |  87   1 |  91  38 |  92   9 |  93  37 |
    94 400 |  95  44 |  96 277 |  97 120 |
```

Name: Cocaine
Formula: $C_{17}H_{21}NO_4$
MW: 303 Exact Mass: 303.147058 CAS#: 50-36-2 NIST#: 335561 ID#: 16448 DB: replib
Contributor: Drug Lab
InChIKey: ZPUCINDJVBIVPJ-LJISPDSOSA-N Non-stereo
Related CAS#: 60269-50-3
116 m/z Values and Intensities:

```
    40   4 |  41  36 |  42 146 |  43   9 |  44  23 |
    45   5 |  50  18 |  51  77 |  52   9 |  53  28 |
    54  19 |  55  60 |  56  29 |  57  38 |  58   8 |
    59  39 |  63   2 |  64   1 |  65  21 |  66  11 |
    67  44 |  68  48 |  69  13 |  70  22 |  71   6 |
    72   1 |  74   4 |  75   5 |  76  16 |  77 334 |
    78  34 |  79  29 |  80  29 |  81 104 |  82 999 |
    83 395 |  84  41 |  85   3 |  86   1 |  87   1 |
    91  43 |  92  10 |  93  41 |  94 403 |  95  47 |
    96 282 |  97 126 |  98  13 |  99   2 | 100  11 |
```

Figure 29. Fully Populated Lib. Search tab View Resulting from an EI Normal Identity Search of the Spectrum.

For spectra from the NIST/EPA/NIH EI Library, below the header **Experimental RI median ±deviation (#data),** is a list for up to three different column types (**Semi-standard non-polar** DB-5 **Standard non-polar** DB-1, and **Polar** DB-WAX) if there is experimental RI data in the **NIST GC Method/Retention Index Database**. An example is: **Semi-standard non-polar: 2198±2 (9)** which means the deviation from the average of 9 values is ±2. Below this is the header **Estimated non-polar retention index (n-alkane scale)**, The value has a **Confidence interval** (explained later).

> ***Nota Bene:*** Some non-NIST mass spectral libraries will have a field labeled **Experimental RI median ±deviation (#data)**. The information under that heading is usually something like *Unspecified xxx±N/a (1)*. This is usually an estimated value that has been inserted and not a measured value.

The **Estimated non-polar retention index (n-alkane scale)** label follows. A value will appear for any spectrum with a structure in the NIST Library, third-party and user-libraries. Beginning with NIST 20, a new type of estimated Retention Index was also displayed. This is the **AI predicted non-polar retention index (n-alkane scale)**, where **AI** is an abbreviation for **Artificial Intelligence**. This is unique to the NIST/EPA/NIH EI Mass Spectral Library. This value has proven to have a better comparison to the measured values and will be discussed later in this manual. The is a value for semi-standard non-polar (**SSNP**) columns (**DB-5**). Following that is the header **Retention Index**, under which (by default) the first two GC Method/RI listings from the nist_ri database appear. This gives not only the RI value but also the GC method and a citation to the source of the method from which the RI value and the GC Method were taken. The number of citations displayed is adjustable on the **Hit Text Info** page of the **Library Search Properties** dialog box. These citations can be searched for keywords using the **Find** selection on the **RMB** menu displayed in the **Hit List Text Information** window. Display of the RI (experimental and estimated) can be dismissed by a de-selection of the **GC Retention Indices** checkbox on the **Hit Text Info** page in the **Library Search Properties** dialog box.

On the right side of this **Text Information** windows for the **Hist List** and the **Spec List** displays is a *scroll bar*. Pulling the *scroll bar* up or down reveals information not currently visible in the window.

Search the Spectrum

First, verify that the settings are correct. Select the button (Library Search Options). This will display the **Library Search Options** dialog box. If the **Search** page is not selected, select it. Make sure the radio button next to the **Identity** label is selected and the label in the dropdown list box under the label reads **EI Normal**. Next, select the **Libraries** page. Make sure the **mainlib** and **replib** are the only entries in the lower (**Include Libs:**) window. If other names are present, highlight them and click on the × in the **Title Bar**. If the **mainlib** and **replib** are not in the lower window, highlight them in the upper window and use the **>>Add>>** button to select them. Once these settings are verified, select the dialog box's **OK** button to close the dialog box. There are several ways to initiate the spectrum search:

1. Put the Mouse pointer on the compound name in the **Spec List** and double click the left Mouse button.
2. With the Mouse pointer on the **Go** button (Go), click the left mouse button to search the highlighted spectrum.
3. With the spectrum highlighted in the **Spec List,** and the **Mouse** pointer in the **Spec List**, **Spec List Plot**, or **Spec List Text info** window, click the Right Mouse button and select **Library Search** from the **RMB** menu.

This will fully populate the **Lib. Search tab** windows as shown in *Figure 29*. The lower left window is the **Hit List**. To the right of that is a split window containing the graphic display of the first **Hit List** spectrum (left) and the **Tabular information** (metadata) for that compound (right). As different entries in the **Hit List** window are highlighted, the display in the two windows of the **Hit List** compound window will change.

The center-right window is a graphic display comparing the selected **Hit List** spectrum (lower portion) with that of the searched spectrum. The display of this window can be turned on and off by selecting **View** from the Main Menu and then selecting/deselecting **Compare pane**. The display in the **Compare pane** (window) also changes as different items in the **Hit List** are selected.

The **Compare** window has four different display options: **Head to Tail**, **Side by Side**, **Difference**, and **Subtraction**. The different displays are selected by selecting the labeled tab at the bottom of the **Compare** window and clicking the left Mouse button. Each of these displays will be explained in more detail later in this manual.

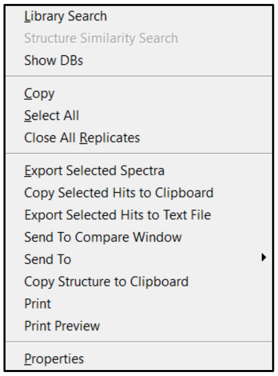
The **Hit List** has four numeric values starting with the third column: **Match**, **Reverse Match**, **PSS.Match** (introduced in v.3.0 with NIST 23), and **Prob. %**. The **Match Factor** (1 – 999) is calculated based on a comparison of all peaks (*m/z* values and relative intensities) in the searched spectrum and the library spectrum. The **Reverse Match Factor** is a match factor calculated disregarding any peaks in the sample spectrum that are not in the **Hit List** spectrum. The **Partial Spectrum Search Match** is a Match Factor calculated disregarding any peaks in the library spectrum not in the sample spectrum. The **Prob%** is a value indicating whether the searched spectrum has been identified. This value is used in conjunction with the **InLib = xxx** value shown in the **Title Bar** of the **Lib. Search tab** view. Understanding these two values is explained in the section on search algorithms and they are only displayed when a **Normal EI Identity** search is performed when the **Spectrum Search Options Method** is set to **Full Spectrum Search (Score)**. Before exploring the various Library Search options, review the *Example Searches* section on page *102*.

By default, the **Hit List** is sorted from high to low **March Factor**. The higher the value of the **Match Factor**, the more likely the Hit spectrum and the searched spectrum are due to the same compound. As a general guide, **900** or greater is an **excellent match**; **800–900**, a **good match**; **700–800**, a **fair match**. **Less than 600** is a **very poor match**. However, spectra of unidentified compounds with many peaks will tend to yield lower **Match Factors** than similar spectra with fewer peaks. The **Prob. (%)** value and the value for the search (**InLib**, located in the lower right corner of the **Hit List** window and on the **Title Bar** of the **Lib. Search** tab) require more explanation; however, the lower the **% Prob.**, the greater the number of similar spectra in the **Hit List**. When searching the spectrum of *o*-xylene, the **% Prob.** for the various isomers and ethylbenzene will be low because of the similarity of the four spectra for the four different compounds. The confidence level for a match is described later in the manual. The **Reverse Match Factor** is a good indicator of a spectrum representing more than a single compound. If a high value (higher than that of the MF) of a Hit that is seen lower in the **Hit List** and is associated with a somewhat low MF, that Hit should be considered as possible one of more than a single compound represented by the searched spectrum. The difference between the searched spectrum and the Hit can be shown in the **Compare window** by selecting the **Subtraction** tab. The RMB menu selection Library Search can then be used to search results.

The **Hit List** window can be sorted by any column in either ascending or descending order. Place the Mouse pointer on the column header title and click the left Mouse button once. A ▼ symbol will appear in the header showing that the sort is in descending order. Click the left Mouse pointer and the direction of the sort reverses showing a ▲ symbol to indicate an ascending sort. The header used for the sort will have a ▼ or ▲ symbol on the left side of the column depending on the direction of the sort. Selected lines in a **Hit List** or **Spec List** can be copied into the Windows Clipboard in tab-separated text format, by highlighting the desired lines and then pressing the **Ctrl+K** keyboard keys. Selected lines of the **Hit List** or **Spec List** can be copied to a Text File or to the Windows Clipboard using items on the **RMB** menu with the Mouse pointer in the **Hit List** or **Spec List**. Selected spectra in the Hit List can be exported to an MSP or MSPEC file with a selection on the **RMB** menu.

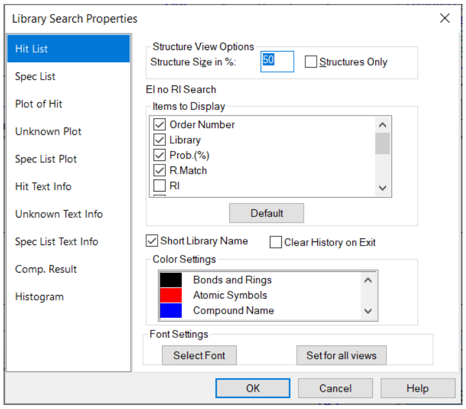
Customize Appearance of the Lib. Search tab view

Put the Mouse pointer on the **Hit List** window, lower left of the **Lib. Search** tab view, click the Right Mouse button to display the **RMB** menu (shown on left below). Selecting **Properties** from this menu results in the display of the **Library Search Properties** dialog box (shown on the right). The first task is to change the **Font** to something that better fits the display being used. The default Font is *Microsoft Sans Serif*, *Regular* style, Size *10 pt.*



RMB menu displayed with Pointer in Hit List.

Select the **Select Font** button of the **Library Search Properties** dialog box. This opens the **Font** dialog box. Select the desired **Font** (e.g., Arial), **Font Style** (e.g., Regular), and **Size** (e.g.,12). Then select the **OK** button. The **Font** dialog box closes and returns to the display of the **Library Search Properties** dialog box which was still displayed but underneath the display of the **Font** dialog box.



Structure Size in %: 50
☐ Structures Only

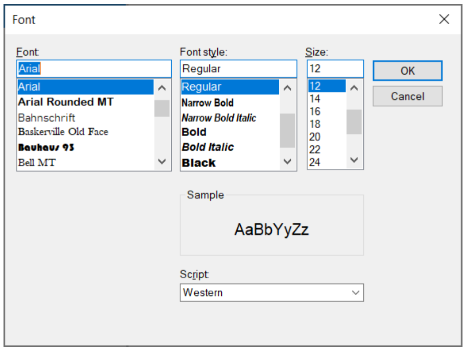
Items to Display
☑ Order Number
☑ Library
☑ Prob.(%)
☑ R.Match
☐ RI

☑ Short Library Name
☐ Clear History on Exit

Bonds and Rings
Atomic Symbols
Compound Name

Library Search Properties dialog box as displayed by selecting Properties with Pointer in Hit List window.

The next step to consider is the setting of all the windows of this tab view to the same font just selected. This is done by selecting the **Set for all views** button (just to the right of the **Select Font** button). This causes a confirmation dialog box to be displayed with a message and **Yes** and **No** buttons. To change the font in all the windows of this tab display, select the **Yes** button. This action closes the confirmation dialog box and returns the view to that of the **Library Search Properties** dialog box. Selecting the **OK** button of the **Library Search Properties** dialog box causes dialog box to close. The **Font** changes are now visible in all of this tab view's windows.



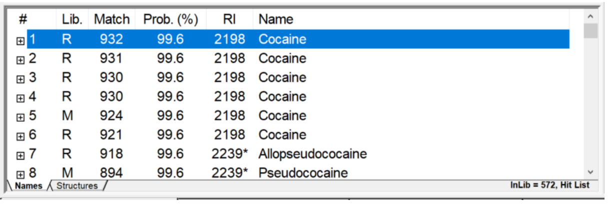
Font: Arial
Font style: Regular
Size: 12
Script: Western

Font dialog box displayed by selecting the Select Font button on the Library Search Properties dialog box.

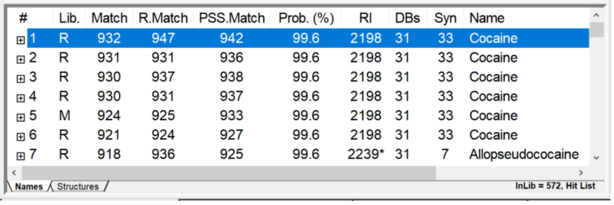
When EI spectra of unidentified substances are searched, there are several columns it may be desirable to add to the **Hit List** window. The default columns are: **#** (order number; the ⊞ next to the numeral means there are replicate spectra for this compound); **Lib** (abbreviated to two characters with **Short Library Name** checkbox selected); **Match** (Match Factor, always displayed); **Prob%** (if these is a spectrum in the library for the unidentified compound, this is the probability that it has been identified); and, **Name** (the primary compound name assigned to the library spectrum). Other columns that may be useful in determining if the Hit is correct, are the **R.Match** (a Reverse Match Factor calculated after disregarding any peaks in the searched spectrum that are not in the library spectrum; this is useful when the searched spectrum is of more than a single compound), and the Experimental Retention Index value from the **NIST GC Method/RI Database**, when available. If experimental RI data are not available when the **Semi-Standard Non-Polar** column type has been selected, the **Artificial Intelligence** estimated **RI** value is displayed. If another column type has been selected, and there is not an **Experimental Value**, the field will have a dash (-). These and other columns can be displayed based on selections in the **Library Search Parameters** dialog box's **Hit List** page. The selection window (which has a scroll bar) is under the label **Items to Display**. In addition to these two additional column titles, MS Search also can display the columns for **No. Synonyms** (the numerical value for the number of synonyms associated with the Hit List compound) and **No. Other Dbs** (the numerical value for the number of non-mass spectral databases that the Hit List compound is in). The higher these two numbers, the more common the compound. More common compounds may have a higher probability of being the correct identification. This list of available displays has been changing over the last few releases and should be carefully explored.

Several of the selectable items are specific to a certain type of search. These search types are listed to the right of the selectable item. The columns that are displayed by default can be specific for the type of search being performed. The Column headers for the **Identity Normal EI** search are different than those for a **Similarity EI Hybrid** search. This will be explained in more detail when reviewing specific types of searches (see *Example Searches* on page *102*).

Once all additional column headers have been selected, select the **OK** button of the **Hit List** page of the **Library Search Properties** dialog box. The desktop now has a different appearance. Most notable is the **Hit List** window. See below, a before and after example of changes to the Hit List window.



#	Lib.	Match	Prob. (%)	RI	Name
⊞ 1	R	932	99.6	2198	Cocaine
⊞ 2	R	931	99.6	2198	Cocaine
⊞ 3	R	930	99.6	2198	Cocaine
⊞ 4	R	930	99.6	2198	Cocaine
⊞ 5	M	924	99.6	2198	Cocaine
⊞ 6	R	921	99.6	2198	Cocaine
⊞ 7	R	918	99.6	2239*	Allopseudococaine
⊞ 8	M	894	99.6	2239*	Pseudococaine



#	Lib.	Match	R.Match	PSS.Match	Prob. (%)	RI	DBs	Syn	Name
⊞ 1	R	932	947	942	99.6	2198	31	33	Cocaine
⊞ 2	R	931	931	936	99.6	2198	31	33	Cocaine
⊞ 3	R	930	937	938	99.6	2198	31	33	Cocaine
⊞ 4	R	930	931	937	99.6	2198	31	33	Cocaine
⊞ 5	M	924	925	933	99.6	2198	31	33	Cocaine
⊞ 6	R	921	924	927	99.6	2198	31	33	Cocaine
⊞ 7	R	918	936	925	99.6	2239*	31	7	Allopseudococaine

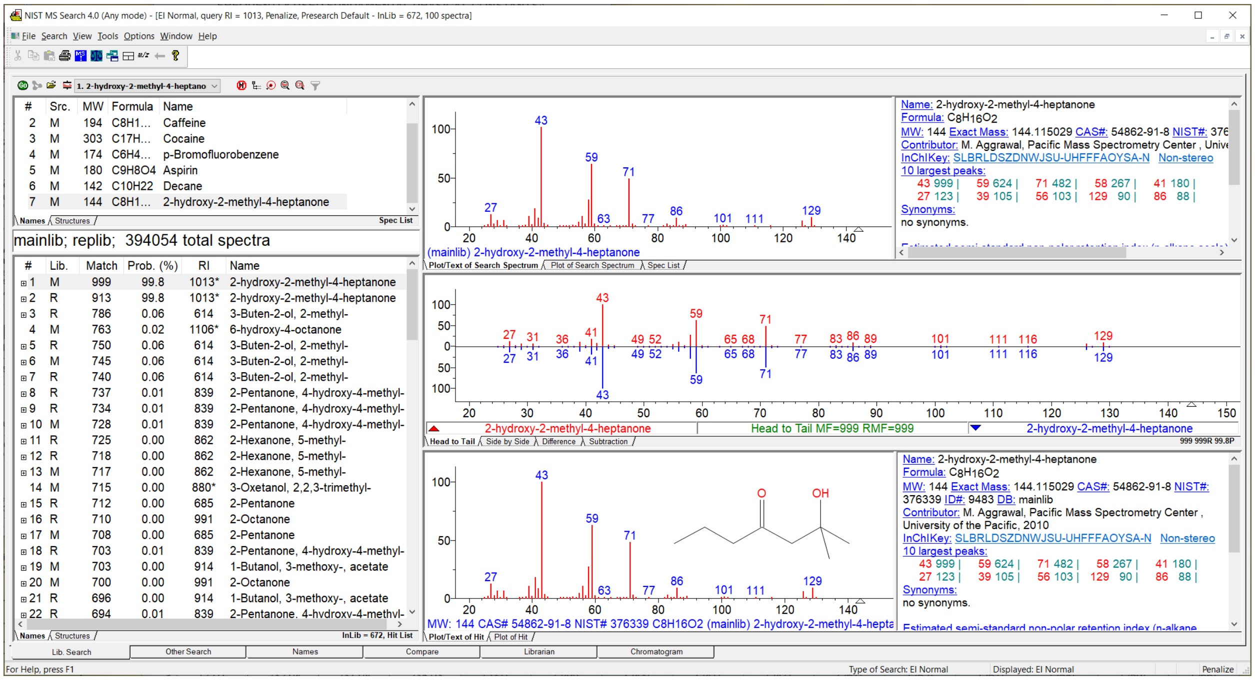
The next step is to adjust the column widths in the **Hit List** and **Spec List** windows. Place the Mouse pointer between two of the column titles on the **Hit List** window **Title Bar**. The pointer changes shape. Holding down the left Mouse button and dragging to the left or right results in a change in column widths. The results are shown in the two views of the **Hit List** above. The position of any column in the **Hit List** can be changed by putting the Mouse pointer on the Column header while holding down the left Mouse button and dragging the pointer to the desired new location. An important thing to remember is that many of these changes can be reversed by selecting the **Set default layout** button (🗗) on the **Tool Bar**. Items such as font and thickness of peaks in bar graph spectra will not be restored.

Now try a different configuration. Put the Mouse pointer in either the top or bottom window on the right side of the **Lib. Search** tab view and display the **RMB** menu. From the displayed **RMB** menu, select **Change Splitter Orientation**. Next, select **View** on the Main Menu **Bar** at the top of the display. Deselect (use Mouse pointer to remove the ✓ mark) the **Compare** window and **Status Bar** (the text information at the bottom of the Program's desktop).

Not all **RMB** menus are the same. Display the **RMB** menu for each of the windows and each of the two panes of the two **Spectrum/Text information** and **Plot** windows (right side of the display, top and bottom). Multiple configurations and the **Change Splitter Orientation RMB** menu selection are not avaialbale for the **Chromatogram** and **Compare** tab views. There is a **Change Change Layout** button (⊟) in the **Button Bar** of the **Lib. Search**, **Other Search**, **Names**, and **Librarian** tab views. This will cycle through multiple layouts for thse tab views.

Splitter Bars

Each tab view has sliderbars, which allow changing the size and shape of the various windows for that tab view. Put the Mouse pointer on one of the bars (verticle or horzional) that consititues a window. If the Mouse pointer image changes, this is a splitter bar. The changed pointer will have either a horinontial or verticle orentation, which tells you the direction it can be moved. In the defalut configurations, there is a verticle silder bar in the **Lib. Search**, **Other Search**, **Names**, and **Librarian** tab views, that seprates the two halves. *Figure 30* is an example of where the splitter bar separating the **Hit List** window and the **Histogram** window was moved to only show the names of the libraries and total number of spectra searched in the Histogram window.. This allows for more of the 100 Hits to be displayed in the Hit List window. The **Fonts** were changed. The width of the mass spectral peaks were changed and the **Rention Index** column header was added to the **Hit List**.



NIST MS Search 4.0 (Any mode) - [EI Normal, query RI = 1013, Penalize, Presearch Default - InLib = 672, 100 spectra]

#	Src.	MW	Formula	Name
2	M	194	C8H1...	Caffeine
3	M	303	C17H...	Cocaine
4	M	174	C6H4...	p-Bromofluorobenzene
5	M	180	C9H8O4	Aspirin
6	M	142	C10H22	Decane
7	M	144	C8H1...	2-hydroxy-2-methyl-4-heptanone

mainlib; replib; 394054 total spectra

#	Lib.	Match	Prob. (%)	RI	Name
⊞ 1	M	999	99.8	1013*	2-hydroxy-2-methyl-4-heptanone
⊞ 2	R	913	99.8	1013*	2-hydroxy-2-methyl-4-heptanone
⊞ 3	R	786	0.06	614	3-Buten-2-ol, 2-methyl-
4	M	763	0.02	1106*	6-hydroxy-4-octanone
⊞ 5	R	750	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 6	M	745	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 7	R	740	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 8	R	737	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 9	R	734	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 10	M	728	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 11	R	725	0.00	862	2-Hexanone, 5-methyl-
⊞ 12	R	718	0.00	862	2-Hexanone, 5-methyl-
⊞ 13	M	717	0.00	862	2-Hexanone, 5-methyl-
14	M	715	0.00	880*	3-Oxetanol, 2,2,3-trimethyl-
⊞ 15	R	712	0.00	685	2-Pentanone
⊞ 16	R	710	0.00	991	2-Octanone
⊞ 17	M	708	0.00	685	2-Pentanone
⊞ 18	R	703	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 19	M	703	0.00	914	1-Butanol, 3-methoxy-, acetate
⊞ 20	M	700	0.00	991	2-Octanone
⊞ 21	R	696	0.00	914	1-Butanol, 3-methoxy-, acetate
⊞ 22	R	694	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-

Name: 2-hydroxy-2-methyl-4-heptanone
Formula: $C_8H_{16}O_2$
MW: 144 Exact Mass: 144.115029 CAS#: 54862-91-8 NIST#: 376
Contributor: M. Aggrawal, Pacific Mass Spectrometry Center , Unive
InChIKey: SLBRLDSZDNWJSU-UHFFFAOYSA-N Non-stereo
10 largest peaks:
43 999 | 59 624 | 71 482 | 58 267 | 41 180 |
27 123 | 39 105 | 56 103 | 129 90 | 86 88 |
Synonyms:
no synonyms.

(mainlib) 2-hydroxy-2-methyl-4-heptanone

Head to Tail MF=999 RMF=999

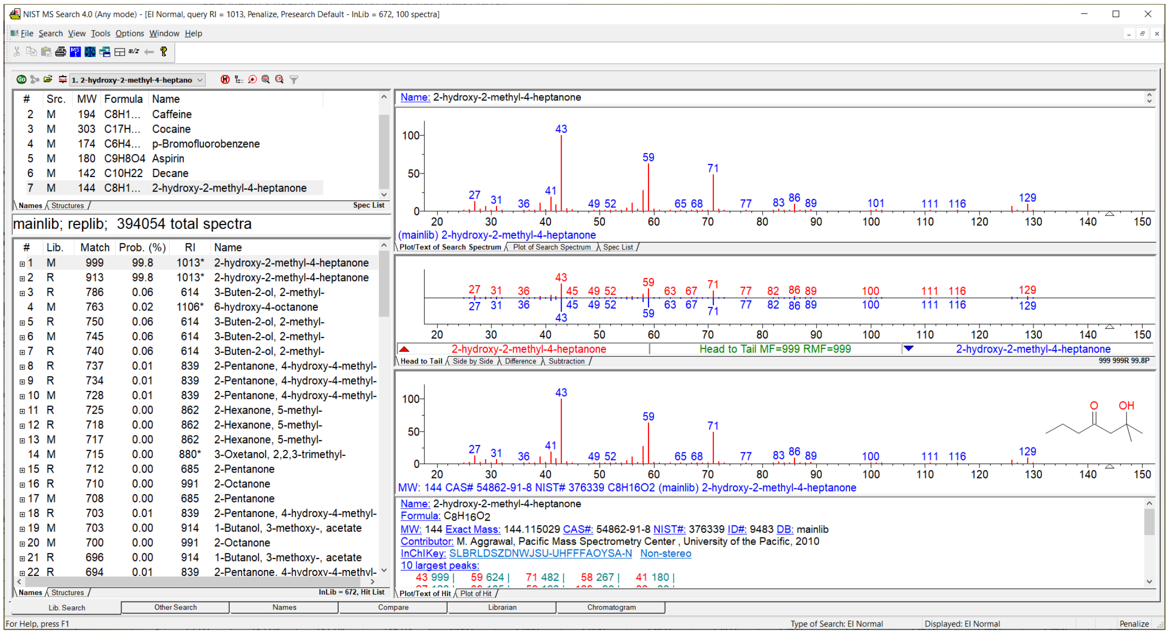
999 999R 99.8P

Name: 2-hydroxy-2-methyl-4-heptanone
Formula: $C_8H_{16}O_2$
MW: 144 Exact Mass: 144.115029 CAS#: 54862-91-8 NIST#: 376339 ID#: 9483 DB: mainlib
Contributor: M. Aggrawal, Pacific Mass Spectrometry Center , University of the Pacific, 2010
InChIKey: SLBRLDSZDNWJSU-UHFFFAOYSA-N Non-stereo
10 largest peaks:
43 999 | 59 624 | 71 482 | 58 267 | 41 180 |
27 123 | 39 105 | 56 103 | 129 90 | 86 88 |
Synonyms:
no synonyms.
Estimated semi-standard non-polar retention index (n-alkane

MW: 144 CAS#: 54862-91-8 NIST# 376339 C8H16O2 (mainlib) 2-hydroxy-2-methyl-4-hepta

For Help, press F1
Type of Search: EI Normal
Displayed: EI Normal
Penalize

Figure 30. Lib. Search tab view after, changing Fonts, adjusting Column widths, adding Hit List Column headers, and splitter bars positions.



NIST MS Search 4.0 (Any mode) - [EI Normal, query RI = 1013, Penalize, Presearch Default - InLib = 672, 100 spectra]

#	Src.	MW	Formula	Name
2	M	194	C8H1...	Caffeine
3	M	303	C17H...	Cocaine
4	M	174	C6H4...	p-Bromofluorobenzene
5	M	180	C9H8O4	Aspirin
6	M	142	C10H22	Decane
7	M	144	C8H1...	2-hydroxy-2-methyl-4-heptanone

Name: 2-hydroxy-2-methyl-4-heptanone

(mainlib) 2-hydroxy-2-methyl-4-heptanone

mainlib; replib; 394054 total spectra

#	Lib.	Match	Prob. (%)	RI	Name
⊞ 1	M	999	99.8	1013*	2-hydroxy-2-methyl-4-heptanone
⊞ 2	R	913	99.8	1013*	2-hydroxy-2-methyl-4-heptanone
⊞ 3	R	786	0.06	614	3-Buten-2-ol, 2-methyl-
4	M	763	0.02	1106*	6-hydroxy-4-octanone
⊞ 5	R	750	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 6	M	745	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 7	R	740	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 8	R	737	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 9	R	734	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 10	M	728	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 11	R	725	0.00	862	2-Hexanone, 5-methyl-
⊞ 12	R	718	0.00	862	2-Hexanone, 5-methyl-
⊞ 13	M	717	0.00	862	2-Hexanone, 5-methyl-
14	M	715	0.00	880*	3-Oxetanol, 2,2,3-trimethyl-
⊞ 15	R	712	0.00	685	2-Pentanone
⊞ 16	R	710	0.00	991	2-Octanone
⊞ 17	M	708	0.00	685	2-Pentanone
⊞ 18	R	703	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 19	M	703	0.00	914	1-Butanol, 3-methoxy-, acetate
⊞ 20	M	700	0.00	991	2-Octanone
⊞ 21	R	696	0.00	914	1-Butanol, 3-methoxy-, acetate
⊞ 22	R	694	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-

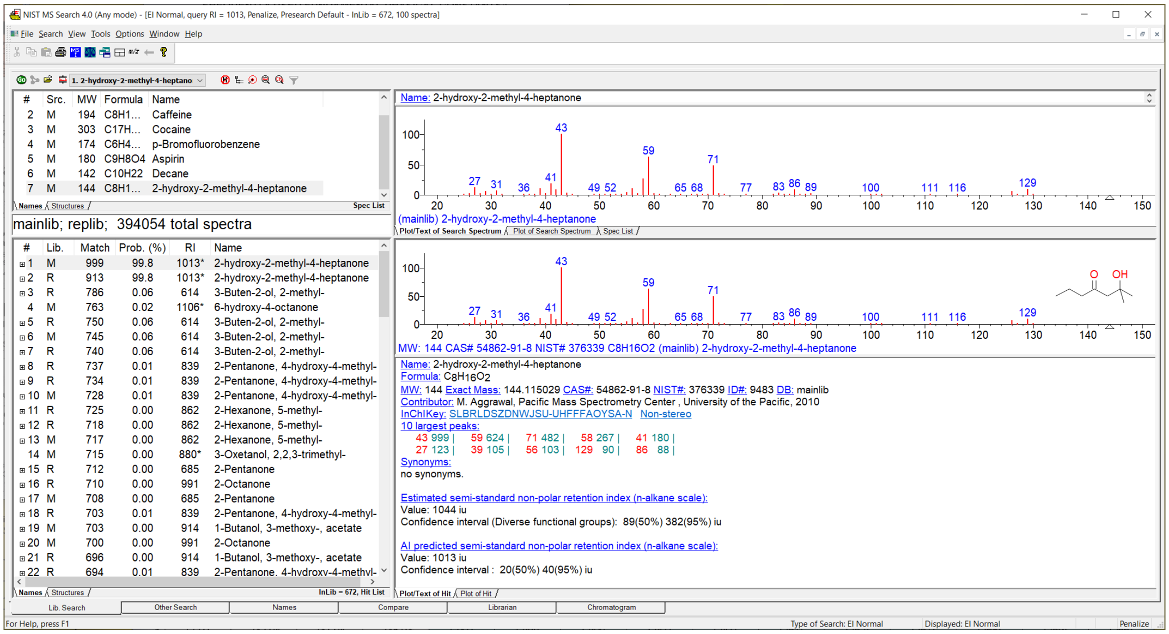
MW: 144 CAS# 54862-91-8 NIST# 376339 C8H16O2 (mainlib) 2-hydroxy-2-methyl-4-heptanone

Name: 2-hydroxy-2-methyl-4-heptanone
Formula: $C_8H_{16}O_2$
MW: 144 Exact Mass: 144.115029 CAS#: 54862-91-8 NIST#: 376339 ID#: 9483 DB: mainlib
Contributor: M. Aggrawal, Pacific Mass Spectrometry Center , University of the Pacific, 2010
InChIKey: SLBRLDSZDNWJSU-UHFFFAOYSA-N Non-stereo
10 largest peaks:
43 999 | 59 624 | 71 482 | 58 267 | 41 180 |

Figure 31. Lib. Search tab view after changing splitter orientation and adding Hit List columns.

Figure 32 is the same search results after selecting **Change Splitter Orientation** from the **RMB** menu and moving horizontal splitter bars to no longer display the **Text Information** window for the searched spectrum. *Figure 32* shows the same display as *Figure 31* with the **Compare** window closed by use of the splitter bars and the **Toolbar** and **Status Bar** options being deselected from the **View** menu. The **Toolbar** contains many useful buttons, and it is recommended that it always be displayed.

The display Title Bar for the **Lib. Search** tab view will contain the type of **Search** performed and the **Presearch** used, the **InLib** value for the search, and the number of Hits found. The maximum of Hits is 100 if the **Presearch** is set to **Default** or **Fast**. If the **Presearch** is set to **Off**, there can be as many as 400 Hits.



NIST MS Search 4.0 (Any mode) - [EI Normal, query RI = 1013, Penalize, Presearch Default - InLib = 672, 100 spectra]

#	Src.	MW	Formula	Name
2	M	194	C8H1...	Caffeine
3	M	303	C17H...	Cocaine
4	M	174	C6H4...	p-Bromofluorobenzene
5	M	180	C9H8O4	Aspirin
6	M	142	C10H22	Decane
7	M	144	C8H1...	2-hydroxy-2-methyl-4-heptanone

Name: 2-hydroxy-2-methyl-4-heptanone

(mainlib) 2-hydroxy-2-methyl-4-heptanone

mainlib; replib; 394054 total spectra

#	Lib.	Match	Prob. (%)	RI	Name
⊞ 1	M	999	99.8	1013*	2-hydroxy-2-methyl-4-heptanone
⊞ 2	R	913	99.8	1013*	2-hydroxy-2-methyl-4-heptanone
⊞ 3	R	786	0.06	614	3-Buten-2-ol, 2-methyl-
4	M	763	0.02	1106*	6-hydroxy-4-octanone
⊞ 5	R	750	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 6	M	745	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 7	R	740	0.06	614	3-Buten-2-ol, 2-methyl-
⊞ 8	R	737	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 9	R	734	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 10	M	728	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 11	R	725	0.00	862	2-Hexanone, 5-methyl-
⊞ 12	R	718	0.00	862	2-Hexanone, 5-methyl-
⊞ 13	M	717	0.00	862	2-Hexanone, 5-methyl-
14	M	715	0.00	880*	3-Oxetanol, 2,2,3-trimethyl-
⊞ 15	R	712	0.00	685	2-Pentanone
⊞ 16	R	710	0.00	991	2-Octanone
⊞ 17	M	708	0.00	685	2-Pentanone
⊞ 18	R	703	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-
⊞ 19	M	703	0.00	914	1-Butanol, 3-methoxy-, acetate
⊞ 20	M	700	0.00	991	2-Octanone
⊞ 21	R	696	0.00	914	1-Butanol, 3-methoxy-, acetate
⊞ 22	R	694	0.01	839	2-Pentanone, 4-hydroxy-4-methyl-

MW: 144 CAS# 54862-91-8 NIST# 376339 C8H16O2 (mainlib) 2-hydroxy-2-methyl-4-heptanone

Name: 2-hydroxy-2-methyl-4-heptanone
Formula: $C_8H_{16}O_2$
MW: 144 Exact Mass: 144.115029 CAS#: 54862-91-8 NIST#: 376339 ID#: 9483 DB: mainlib
Contributor: M. Aggrawal, Pacific Mass Spectrometry Center , University of the Pacific, 2010
InChIKey: SLBRLDSZDNWJSU-UHFFFAOYSA-N Non-stereo
10 largest peaks:
43 999 | 59 624 | 71 482 | 58 267 | 41 180 |
27 123 | 39 105 | 56 103 | 129 90 | 86 88 |
Synonyms:
no synonyms.

Estimated semi-standard non-polar retention index (n-alkane scale):
Value: 1044 iu
Confidence interval (Diverse functional groups): 89(50%) 382(95%) iu

AI predicted semi-standard non-polar retention index (n-alkane scale):
Value: 1013 iu
Confidence interval : 20(50%) 40(95%) iu

Figure 32. Lib. Search tab view after closing display of Compare window and Changing the Splitter Bar orientation.

Selecting the ⊟ button in the **Toolbar** (✂ ⎘ 📋 🖶 MS ⚖ ⊟ m/z ← ?) will cycle through three different configurations of the **Lib. Search**, **Other Search**, **Names**, and **Librarian** tab views. The vertical bars separating the two sides of the various windows can be moved to provide more convenient views. The horizontal bars on each side of the vertical bar can be repositioned such as that shown above, on the left, where **Histogram** window has been minimized to allow for more **Hit List** information to be displayed.

Zooming Graphic Windows

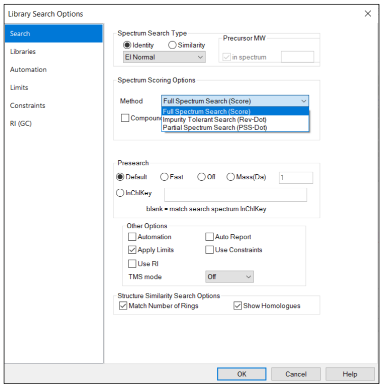
Any spectrum plot display in any of the six-tab views can be Zoomed by placing the Mouse pointer in the window containing the display, holding down the Left Mouse button, and dragging the pointer to form a box around what is to be magnified. The pointer will turn into a magnifying glass 🔍 icon. An un-zoom is accomplished by selecting **Zoom Out** from the **RMB** menu displayed with the Mouse pointer in a **Plot** window. Another way to zoom out is to place the Mouse pointer in a zoomed Plot window and double click the left Mouse button. In tab views with multiple graphics windows like the **Lib. Search** and **Compare** tab views. All graphic windows of spectra will be zoomed and un-zoomed together.

NOTE on Behavior of Dialog boxes

The NIST MS Search Programs uses several interactive dialog boxes. Some of these have multiple pages as seen in the **Library Search Options** dialog box. In order to cause changes in these pages, the dialog box must be closed by selecting the **OK** button. All changes in all pages will be in effect when the dialog box is closed by selecting the **OK** button. The next time the dialog box is displayed, the page visible at the time of the exit using the **OK** button will be displayed. If the **Cancel** button or the ☒ at the upper right corner of the dialog box is used, the page display when the dialog box was opened will be displayed when reopened.

More on the Spectrum Search

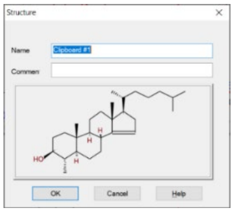
The default **Search** for the **EI Spectrum Type** is the **Identity\EI Normal** search of the **mainlib** and **replib** libraries. The default search for the **NIST 26 Tandem Library** installation is the **Identity\MS/MS** search of the **hr_nist_msms** and **lr_msms-nist** libraries. When a **Spectrum Type** is selected, the currently selected libraries DO NOT automatically change. Information on other ways of comparing an EI mass spectrum of an unidentified compound and those in the NIST\EPA\NIH and other libraries like the *Wiley Registry of Mass Spectra Data* and using the NIST Product-ion libraries of LC-MS/MS data are explained in more depth later in this manual.



Spectrum Search Type: ● Identity ○ Similarity
EI 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) 1
○ InChIKey
blank = match search spectrum InChIKey
Other Options
☐ Automation ☐ Auto Report
☑ Apply Limits ☐ Use Constraints
☐ Use RI
TMS mode: Off
Structure Similarity Search Options
☑ Match Number of Rings ☑ Show Homologues

The **Library Search Options** dialog box's top page is labeled **Search**. The most often used **Spectrum Search Type** for EI (GC/MS) data is **Identify/EI Normal**. There is a dropdown list box under the heading **Spectrum Search Options** near the top of the page. By default, the **Full Spectrum Search (Score)** is selected. The second option is **Impurity Tolerant Search (Rev-Dot)**. This is what was called **Reverse Search** in previous versions of MS Search. When this is selected, the **Hit List** is automatically sorted by the **Reverse Match Factor**. There is a third option (**Partial Spectrum Search (PSS-DOT)**) which is explained elsewhere in this manual but results in the **Hit List** being sorted by the **PSS.Match** column. In this section is a checkbox next to **Compound Ubiquity Correction** label. This is a ***NEW*** feature in v.4.0 of MS Search used to enhance the **Match Factor** of more common compounds, when selected. If the Hit has a high **Compound Ubiquity Index** (**CUI**), its **Match Factor** will be increased. When dealing with an unknown spectrum and two have almost identical Match Factors, the one with the higher CUI is the more likely candidate. As used in MS Search, the CUI is a citation index that reports the number of 58 diverse chemical collections containing the compound. This is described in the literature[6].

Another important feature of the **Lib. Search** tab view is the ability to perform either a **structure similarity search** or an **exact structure search**. Both structures and spectra can be pasted from the Windows Clipboard into the **Spec List** window. After copying a structure in a MOL file format or a spectrum as a text file in the MSP/MSPEC format onto the Windows Clipboard, the **RMB** menu, with the Mouse pointer in the **Spec List** window, is used to copy either to the **Spec List** window. The appropriate selections are: **Insert Clipboard Structure** or **Insert Clipboard Spectrum**.



Name: Clipboard #1
Commen:

Structures associated with spectrum-displays can be put onto the **Windows Clipboard** by using the **RMB** menu and selecting **Copy Structure to Clipboard** when the Mouse pointer is on either a text or graphic view of a spectrum or the **Hit List** or **Spec List**. Structures can also be put on to the **Windows Clipboard** from most third-party structure drawing programs using the copy command in these programs. With the structure on the **Clipboard** and the Mouse pointer in the **Spec List** window, display the **RMB** menu and select **Insert Clipboard**

---

[6] Deborah F. McGlynn, Lindsay D. Yee, H. Martin Garraffo, Lewis Y. Geer, Tytus D. Mak, Yuri A. Mirokhin, Dmitrii V. Tchekhovskoi, Coty N. Jen, Allen H. Goldstein, Anthony J. Kearsley, and Stephen E. Stein* " New Library-Based Methods for Nontargeted Compound Identification by GC-EI-MS" *J A Soc. Mass Spectrom,* Vol 36/Issue 2, 389 – 299

Structure. This results in the displayed dialog box shown on the previous page. After making the desired edits, select the **OK** button to add the structure to the **Spec List**. To perform a **structure similarity search**, highlight the entry in the **Spec List** and then click on the button.

The libraries to be searched are specified in the page labeled **Libraries** in the **Library Search Options** dialog box. (See right side of *Figure 33*.)

The libraries used for a **structure similarity search** are specified separate from those specified for a spectrum search, even though they can be the same libraries. If no libraries are selected for the spectrum similarity searches, all sections associated with this task will be grayed. If a structure is associated with a spectrum in the **Spec List**, selecting the button will perform a **Structure Similarity Search**. To perform an **exact structure search**, copy the **InChIKey** shown for the structure in the **Text Information** window to the Windows Clipboard. Select the **Library Search Options** button ( ). Then paste **InChIKey** to the text entry box next to the **InChIKey** label in the **Search** page of the **Library Search Options** dialog box in the Presearch area. Select the radio button to the left of the **InChIKey** label. Select the **OK button** in the dialog box and then select the Go button in the **Button Bar**, with any spectrum highlighted. Unlike the **Structure Similarity Search**, this uses the libraries associated with a spectrum search.

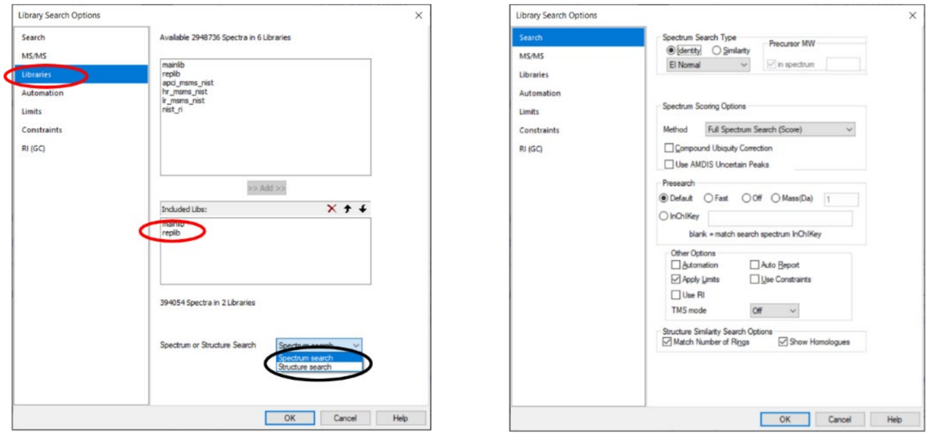
When a structure is put into the **Spec List**, it is assigned an **InChIKey**, which is displayed in the **Text Information** window of the selected **Spec List** entry.

There are four **Presearch** selections on the **Search** page of the **Library Search Options** dialog box, each with a radio button. Just under this is a radio button to the left of the **InChIKey** label. Selecting this will cause whatever **Presearch** radio button is selected to be deselected. If the field to the right of the **InChIKey** label is left blank, the **InChIKey** of the structure of the selected spectrum in the **Spec List** will be searched. The spectrum will not. This is a search for a matching structure in the libraries being searched, which were selected for the spectrum search of the page labeled **Libraries**. This is the **exact structure search**. This only works for **Spec List** entries of spectra with structures and for structures only. If a structure similarity search is attempted on a spectrum with no structure, no Hits will be found.

The **RMB** menu for all windows in all tab views have a **Library Search** selection except for the two **Plot** windows in the **Chromatogram** tab view. These two windows and the **Compare** windows in the **Lib. Search** and **Compare** tab views are the only windows in MS Search that do not have **Structure Search** as a selection on the RMB.

CAUTION

It is easy to create problems when settings are made in the **Library Search Options** dialog box's **Search** page for a specific type of operation. It is a good idea to check the setting in this dialog box frequently to avoid errors in results. This is especially true when the **Use Constraints** box is checked.



Available 2948736 Spectra in 6 Libraries

mainlib
replib
apci_msms_nist
hr_msms_nist
lr_msms_nist
nist_ri

Included Libs:

mainlib
replib

394054 Spectra in 2 Libraries

Spectrum or Structure Search: Spectrum search

Spectrum search
Structure search

Spectrum Search Type
● Identity ○ Similarity
EI Normal

Precursor MW
☑ in spectrum

Spectrum Scoring Options
Method: Full Spectrum Search (Score)
☐ Compound Ubiquity Correction
☐ Use AMDIS Uncertain Peaks

Presearch
● Default ○ Fast ○ Off ○ Mass(Da) 1
○ InChIKey
blank = match search spectrum InChIKey

Other Options
☐ Automation ☐ Auto Report
☑ Apply Limits ☐ Use Constraints
☐ Use RI
TMS mode: Off

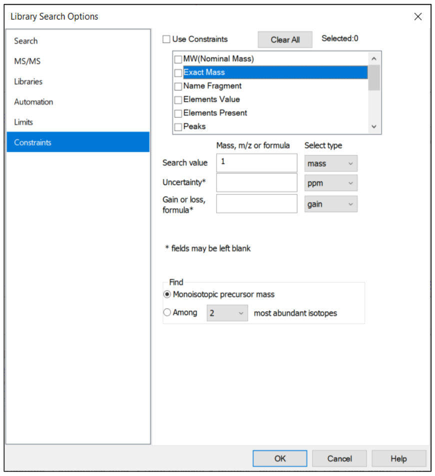
Structure Similarity Search Options
☑ Match Number of Rings ☑ Show Homologues

Figure 33. Libraries (left) and Search (right) pages in the Library Search Properties dialog box for the Any spectrum type. The RI (GC) page not available for Tandem spectrum type: MS/MS page not available for EI.

Presearch

Searching large numbers of spectra can take some time. One of the ways is to prepare a list of good candidates and then search only those spectra, peak by peak (*m/z* value and relative intensity). MS Search has two ways of performing a presearch. One is **Default** and has proved very effective with few missed identifications. The other is the **Fast** way which reduces the presearch time but can have a higher error rate. It is recommended to always use the **Default Presearch**. The third radio button is **Off**. This will perform a peak-by-peak search of all spectra in all selected libraries. This can be time consuming. The **Default** and **Fast** Presearch results in a maximum of 100 Hits. The **Off Presearch** will give a maximum of 400 Hits. The last **Presearch** option is **Mass(Da)**. This will only search spectra that have the nominal mass entered in the box following the label. This presearch is only selectable for the **EI** spectrum type.

Constraints



☐ Use Constraints Selected:0

☐ MW(Nominal Mass)
☐ Exact Mass
☐ Name Fragment
☐ Elements Value
☐ Elements Present
☐ Peaks

Mass, m/z or formula / Select type
Search value: 1 / mass
Uncertainty*: / ppm
Gain or loss, formula*: / gain

* fields may be left blank

Find
● Monoisotopic precursor mass
○ Among 2 most abundant isotopes

Figure 34.Library Search Options Constraint page Exact Mass Selected.

In the lower part of the **Search** page of the **Library Search Options** dialog box is the ☐ **Use Constrains** selection. The **Hit List** of any library search (search of a sample spectrum against spectra in various mass spectral libraries) can be limited to exclude spectra that have various properties, **Constraints**. These constraints are set on the **Constrains** page of the **Library Search Options** dialog box. This same **Constraints** page is the second page on the **Sequential Search** dialog box displayed in the **Other Search** tab view. When MS Search is being used with **EI** spectra, these constraints are **MW(Nominal Mass)**, **Exact Mass**, **Name Fragment**, **Elements Value**, **Elements Present**, **Peaks**, and **Tages in Comments**. When searching **Tandem** data, there are additional constraints: **MS/MS Charge**, **Peptide Number of Residues**, **MS/MS Instrument Type**, and **MS/MS Precursor Type**. The **EI** constraints are explained in the following **Other Search** tab view section under the **Sequential Search** heading. The additional **Tandem** constraints are explained later in this manual.

The constraints are listed in a window, with a scroll bar on the right side, near the top of the page. Each has a checkbox (☐) to the left of the constraint label. When a check is put in one of these boxes, the checkbox next to the **Use Constraints** label (at the top of the page) is automatically selected, as is the checkbox next to the **Use Constraints** label on the lower part of **Search** page. Unchecking either, automatically unchecks the other. The unchecking of either of these **Use Constraints** checkboxes does not uncheck the box next to the individual constraints in the window with the scroll bar. Before setting any constraint, it is best to select the **Clear All** button at the top of the **Constraints** page. The **Clear All** button deselects all the individual constraints and the two **Use Constraints** checkboxes; however, the settings within these individual constraint pages remain. If an individual constraint is subsequently selected, make sure the settings are those desired for the new search. If the **Use Constraints** checkbox is reselected and there are selected individual constraints, those selected constraints will be used. Next to the **Clear All** button is a label, **Selected X**, where X is the number of selected constraints.

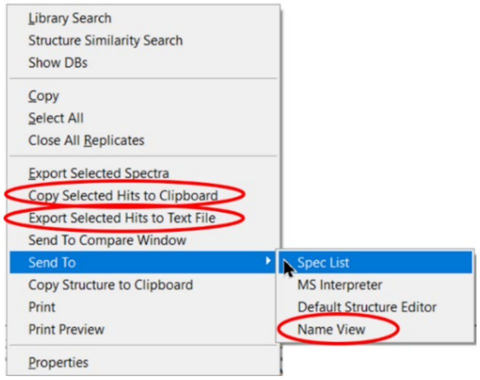
Selecting the checkbox next to the label for any individual constraint in the scroll-down list window will result in highlighting the constraint's name and an appearance change of the lower part of the **Constraints** page to be relevant to the selected constraint. The individual constraint's page can be brought into focus without selecting it by selecting the title rather than the checkbox. Even if information is changed in the new **Constraints**' page, the selection will not be used until the checkbox next to its label is selected.

Order for Search Results

By default, the Library Search **Hit List** is ordered starting with the best Hit regardless of what library the Hit is from. There is no need to be concerned about the order of libraries in the **Includer Libs:** window of the **Libraries** page of the **Library Search Options** dialog box. The window does have ↑ ↓ buttons used to move the position of the library. These are not necessary for a Library Search. In the case of the **Other Search** tab view, the order can be important. Libraries are searched in the order they appear in the list. Some of the **Other Search** options, limit the number of Hits that are displayed. If that number is exceeded before all the libraries are searched, then Hits may not be observed for the remainer of that library and subsequent libraries.

Other important Information with respect to dealing with the Lib. Search tab view

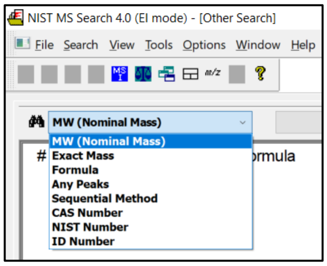
Another important new feature of v.4.0 is additions to the **RMB** menu. When the Mouse pointer is in a **Hit List** window of any tab view the **RMB** menu will appear as shown here. It is now possible to copy selected lines from any **Hit List** (except that in the **Names** tab view) to the Window Clipboard from the **RMB** menu. It is also possible to create a text file that can be pasted into Excel. In addition, a selected spectrum can be sent to the **Names** tab view (which also brings it into focus). There have been other undocumented features added to v.4.0. Previous users should explore all the Main Menus of all tab views as well the **RMB** menu of all windows of all tab views.



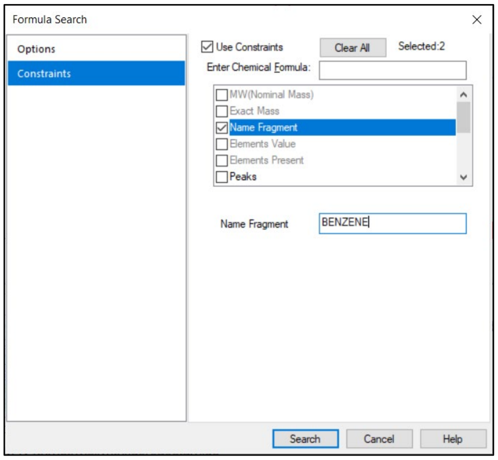
Other Search tab view

The NIST mass spectral data, third-party libraries of mass spectra, and user-libraries can be searched using several other methods besides submitting an unidentified mass spectrum. These are the **Other Searches**, carried out in the **Other Search** tab view. Just like the **Lib. Search** tab view, this view can be customized in the same way through **Properties** dialog boxes displayed by selecting **Properties** from the **RMB** menu when the Mouse pointer is in any of the three windows. The **RMB** menu command, **Change Splitter Orientation**, and the **Change Layout** and **Set Default Layou**t buttons are also available in this tab view.

The other ways to search mass spectral libraries (selected by a drop-down list box to the right of the button) are by: **MW** (Nominal Mass); **Exact Mass**; **Formula** (elemental composition); **MS/MS/ Precursor *m/z*** (only available when the **Spectrum Type** is **Tandem** or **Any**), **Any Peaks** (explained in detail later in this manual); **Sequential Method** (combining different search criteria to create a focused search); **CAS** (Chemical Abstracts Services) **Number** (registry number); **NIST Number** (a unique identifier number given each spectrum in the NIST archive); and **ID Number** (the position of a spectrum in the library being searched). The **MW**, **Exact Mass**, **MS/MS Precursor *m/z***, **Formula**, **Any Peaks**, **Sequential**, and **CAS Number** searches allow the simultaneous use of multiple libraries. MS Search will allow for up to 127 libraries. The **NIST Number** search is only used with libraries distributed by NIST. The **NIST Number** search can search all NIST Libraries at once. The **ID Number** search can only search a single library at a time. The **MW**, **Exact Mass**, **MS/MS Precursor *m/z***, **Formula**, **Any Peaks** and **Sequential** searches can be restricted with respect to several other properties. These are viewed by selecting the **Constraints** page in the ***Type* Search** dialog box for an individual search. Some, but not necessarily all, constraints are available for each of these search types. Each search type has its own dialog box named ***Type* Search**, e.g., **MW (Nominal Mass) Search**. The Constraints page for each of these search types is the same; however, unavailable constraints are grayed in the scrollbar list box. For example, **MW**, **Exact Mass**, **Element Values**, and **Elements Presen**t are not available for a **Formula** search.



Types of Other Searches when Spectrum Type is EI.



☑ Use Constraints

Enter Chemical Formula:

☑ Name Fragment

Name Fragment BENZENE

Formula Search Dialog Box.

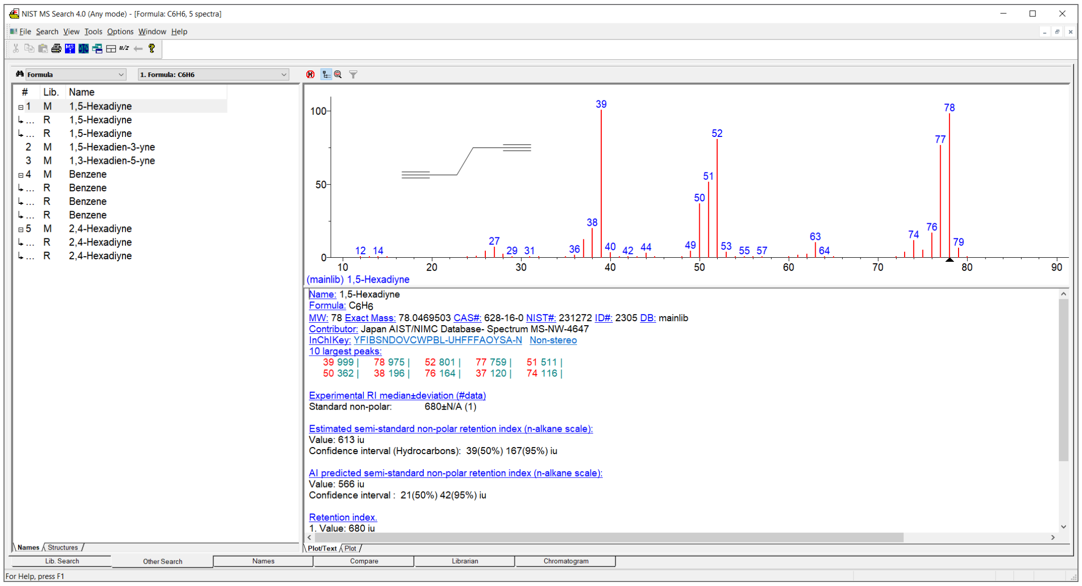
An easy way to experience the use of the **Other Search** tab view is to perform a **Formula** search. After having made any desired appearance changes using the **Properties** dialog box displayed from any window (**Hit List** window [vertical list on left of the **Other Search** tab display]; **Spectrum** window [top right window], or **Text Information** window [lower right window]) select the down arrow button on the right of the list box just above the **Hit List** window. This causes a dropdown menu to be displayed with up to nine items. Select the **Formula** option. This results in the two-page **Formula Search** dialog box being displayed. The **Constrains** page is displayed by default. At the upper left is a checkbox next to the **Use Constraints** label. Make sure this is unchecked. For safety, click on the button labeled **Clear All**. All searches can be constrained. **Constraints** are explained in detail under **Sequential Search**. This first use will not involve **Constraints**. Select the **Options** page. This allows the selection of the libraries to be searched. For this example, the only entry in the **Include Libraries** window should be *mainlib*.

> ***Nota Bene:*** Sometimes the *replib* (NIST EI library of spectra which are in the *mainlib* but are from other sources) is not selectable for searches conducted in the **Other Searches** tab view. To view these spectra, select the **Replicates On/Off** button (⊞) in the **Button Bar** or for an individual compound, select the ⊞ on the left of the # in the **Hit List**. This keeps the *replib* spectra with the *mainlib* spectra.

There is a text entry box just to the right of the **Enter Chemical Formula** label in both the **Options** and the **Constraints** pages of this dialog box. Enter a simple formula, e.g., C6H6. Select the **Search** button at the bottom of the dialog box. The three windows of this tab view (**Hit List**, **Plot**, and **Text Information** are populated (*Figure 35*).

The most powerful of these **Other Searches** is the **Sequential Method Search**. This search allows all libraries in the NIST MS Search format to be searched in multiple ways. It may be necessary to re-index older user libraries and third-party libraries. This is done with the **Tools** menu on the Main Menu bar.

An undocumented feature is the ability to search for a specific InChIKey using the **Sequential Search**. Put the InChIKey in the input area of the **Name Fragment**. All other selections should be cleared first by clicking on the **Clear All**.



#	Lib.	Name
⊟ 1	M	1,5-Hexadiyne
↳ ...	R	1,5-Hexadiyne
↳ ...	R	1,5-Hexadiyne
2	M	1,5-Hexadien-3-yne
3	M	1,3-Hexadien-5-yne
⊟ 4	M	Benzene
↳ ...	R	Benzene
↳ ...	R	Benzene
↳ ...	R	Benzene
⊟ 5	M	2,4-Hexadiyne
↳ ...	R	2,4-Hexadiyne
↳ ...	R	2,4-Hexadiyne

Name: 1,5-Hexadiyne
Formula: C_6H_6
MW: 78 Exact Mass: 78.0469503 CAS#: 628-16-0 NIST#: 231272 ID#: 2305 DB: mainlib
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-4647
InChIKey: YFIBSNDOVCWPBL-UHFFFAOYSA-N Non-stereo
10 largest peaks:
39 999 | 78 975 | 52 801 | 77 759 | 51 511 |
50 362 | 38 196 | 76 164 | 37 120 | 74 116 |

Experimental RI median±deviation (#data)
Standard non-polar: 680±N/A (1)

Estimated semi-standard non-polar retention index (n-alkane scale):
Value: 613 iu
Confidence interval (Hydrocarbons): 39(50%) 167(95%) iu

AI predicted semi-standard non-polar retention index (n-alkane scale):
Value: 566 iu
Confidence interval : 21(50%) 42(95%) iu

Retention index.
1. Value: 680 iu

Figure 35. Appearance of the Other Search tab After the Formula Search has been Executed.

Other DBs in the Hit List and Text Information windows

It has long been understood that when two Hits in a search of an unknown spectrum against mass spectral libraries have close Match Factors, if one is found in several non-mass spectra databases and the other is not, the one with a presence in other (non-mass spectral) databases is the more likely candidate to be the mass spectrum of the unknown. This is also true for the number of Synonyms of the two Hits and when one of the Hits has replicates in the NIST\EPA\NIH EI Library and the other one does not.

After the release of NIST 14, NIST started looking to other non-mass spectral databases than the nine listed in the **Other Databases** constraints tab (no longer present). The **Other Databases List** has been expanded to seven Classes: Wikipedia, Contaminants, Drugs, Environmental, Food, General, and Metabolite. The specific category is listed in the **Text Information** Window for an individual Hit under the Heading **Other DBs**. Under each of these categories are the names of the actual database where the compounds are found. **Hit List**, when the **DBs** column header is displayed in the **Other Search** and **Lib. Search** tab views, will have a number that is the total of other non-mass spectral databses where the compound is found. In the *Other_DBs.pdf* file (found in the NIST26\MSSearch folder). a total of 59 Generic Titles has hot-links to the corresponding Internet sites. Hold the <ctrl> key and click on the link to display this file. The source Excel spreadsheet is also included (Other_DBs.XLSX). Information can then be checked in these non-mass spectral databases about individual compounds. **Other DBs** information is only available for NIST provided libraries. The **Other DBs** field will not appear with metadata of user-libraries produced by MS Search or from third parties.

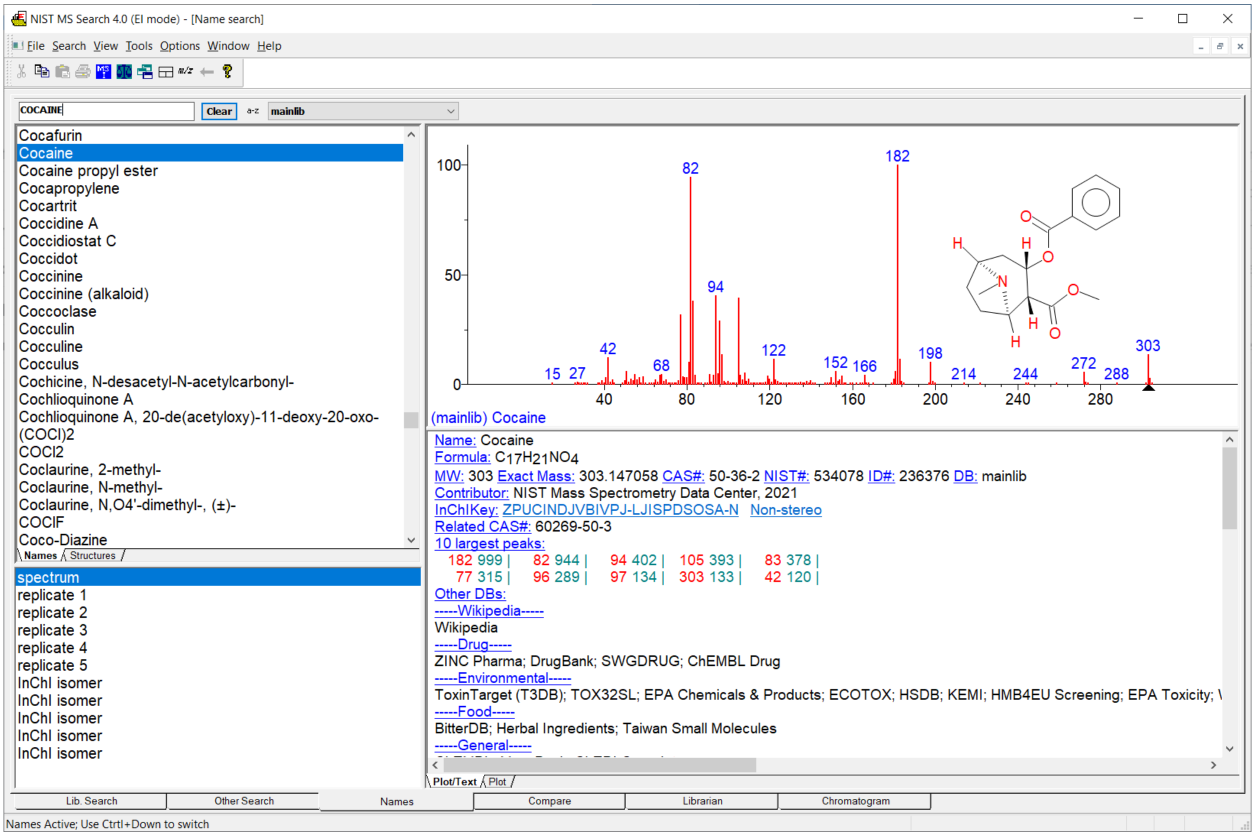
Name: Di-n-octyl phthalate
Formula: $C_{24}H_{38}O_4$
MW: 390 Exact Mass: 390.27701 CAS#: 117-84-0 NIST#: 233289 ID#: 193417 DB: mainlib
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW- 521
InChIKey: MQIUGAXCHLFZKX-UHFFFAOYSA-N Non-stereo
Other DBs:
-----Contaminants-----
Thermo Additives; Plastic Contaminants
-----Environmental-----
ToxinTarget (T3DB); TOX32SL; EPA Chemicals & Products; TSCA; ECOTOX; HSDB; KEMI; HMB4EU Screening; EPA Toxicity; TSCA; Norman EI; Endocrine Distruptors; Dust; Reach EU; Norman Water; EU Suspected Contaminants
-----Food-----
NutriChem; Taiwan Small Molecules; EU Food Safety; FoodDB; Food Packaging
-----General-----
MassBank; ChEBI Complete
-----Metabolite-----
Metab Workbench; HMDB; Blood Exposome; Skin Sensitivity

Figure 36. The Text Information window display in the Name Search Tabe where the other DB has been selected for display in the Text Info page of the Name Search Properties dialog box.

Search Results vs Order of List of Libraries

Unlike the **Library Search** tab view **Hit List**, the **Other Search** tab view **Hit List** is ordered according to the order of the libraries in the **Options/Libraries** page windows of the ***Type* Search** dialog box. The **MW (Nominal Mass)** search is limited to a maximum of 2,000 Hit. The **Any Peaks Search** and **Sequential Search** are both limited to 6,000 Hits. If the maximum number of Hits is reached before all libraries are searched, it is possible that some Hits from libraries lower on the list will not be reported. To make sure all Hits are accounted for; it may be necessary to search a few libraries or only a single library at one time.

Names tab view



Name: Cocaine
Formula: $C_{17}H_{21}NO_4$
MW: 303 Exact Mass: 303.147058 CAS#: 50-36-2 NIST#: 534078 ID#: 236376 DB: mainlib
Contributor: NIST Mass Spectrometry Data Center, 2021
InChIKey: ZPUCINDJVBIVPJ-LJISPDSOSA-N Non-stereo
Related CAS#: 60269-50-3
10 largest peaks:
182 999 | 82 944 | 94 402 | 105 393 | 83 378 |
77 315 | 96 289 | 97 134 | 303 133 | 42 120 |
Other DBs:
-----Wikipedia-----
Wikipedia
-----Drug-----
ZINC Pharma; DrugBank; SWGDRUG; ChEMBL Drug
-----Environmental-----
ToxinTarget (T3DB); TOX32SL; EPA Chemicals & Products; ECOTOX; HSDB; KEMI; HMB4EU Screening; EPA Toxicity;
-----Food-----
BitterDB; Herbal Ingredients; Taiwan Small Molecules
-----General-----

Figure 37. Names Tab View for a NIST Library in MS Search v.4.0.

One of the major changes to v.3.0 of the NIST Mass Spectra Search Program was the **Incremental Name Search** when using the NIST provided libraries. The purpose of the **Names** tab view is to search any single library and retrieve the mass spectrum and metadata of an individual compound using the primary name or any associated synonym. This new variation of the **Names Search** is only with NIST provided Libraries (NIST\EPA\NIH EI and NIST Tandem). The previous **Name Search** still works with other types of libraries and is automatically invoked when one of those libraries is selected. It has always been possible to display spectra from the NIST replicate library (**replib**) of EI spectra along with derivatives of a precursor, diastereomers, or isotopic labeled variants; however, this has been tedious and sometimes confusing in previous versions. Details of the **Names** tab view are explained later in the manual and should be reviewed in detail. This just gives an overview and a brief explanation of the **Incremental Name Search** as it pertains to NIST-provided Libraries. The display for NIST-provided libraries is shown in *Figure 37*.

The default settings for the **Increment Name Search** require that names with numeric and special character prefixes be entered into a Text Entry box just above the Left upper window. It is not possible to type Greek alphabet character such as β, σ, or γ. They need to be spelled out, e.g., β-pinene would be typed as "betapinene". If β-pinene is pasted into the text entry field, it will appear as "BETAPINENE". Another important issue is characters such as -, ', etc., are not recognized and are not allowed in the text entry field. Previously, the text entry field allowed only 16 characters. Version 3.0 of MS Search expanded this to 256 characters. All names (the mainlib name and all synonyms) are displayed in ascending alphabetical order as the name is entered. No matter whether characters are entered as uppercase or lowercase, the echo in the Text Entry box will be uppercase.

More generic searches can be performed by selecting the a-z button to the right of the name text entry box. When this button is selected, numerical and special characters are not accepted when typed. A good example would be if "2pentaone" is typed, the 2 will not appear in the text entry field and the list will contain all the isomers of pentanone (2- and 3-). If this button is not selected, the 2 does appear and all compounds that begin with 2pentanone would be listed.

The default behavior of the **Names** tab view is to update the displayed mass spectral data as each character is typed (all windows in the tab view). Displaying the **Name Search Properties** dialog box, with the **RMB** menu, selecting the **Incremental** page in the dialog box, and deselecting the checkbox next to the **Automatically display selected spectrum** label will change the behavior. The changed behavior is that after the name is entered in the text field, it will be necessary to put the Mouse pointer on the desired name in the upper **Hit List** window and double click the left Mouse button. The behavior and appearance of the windows that make up the **Names** tab view, can be customized by use of various selections of pages in this dialog box.

When searching an NIST Library, the left side of the **Names** tab view is made up of an upper and lower window. Compound names are displayed in the upper window. These are all the names associated with a given spectrum, e.g., the primary name (**mainlib** name) or synonyms. Links to a selected spectrum appear in the lower window. If a synonym is entered, the **mainlib** spectrum for that compound will be displayed with the plot of the spectrum and in the Name: field of the **Plot** and **Text information** windows. An example would be to enter the name "SKELLYSOLVE", and the displayed **Plot** window name would be "Pentane" and the **Name:** field would have Pentane in the **Text Information** window. "Skellysolve A" would appear in the list of **Synonyms** in the **Text Information** window, depending on the settings in the **Text Info.** page of the **Name Search Properties** dialog box.

Compounds in the upper list can be displayed as names in a text form or structures by selecting the appropriate tab (**Names** or **Structure**) at the bottom of the window.

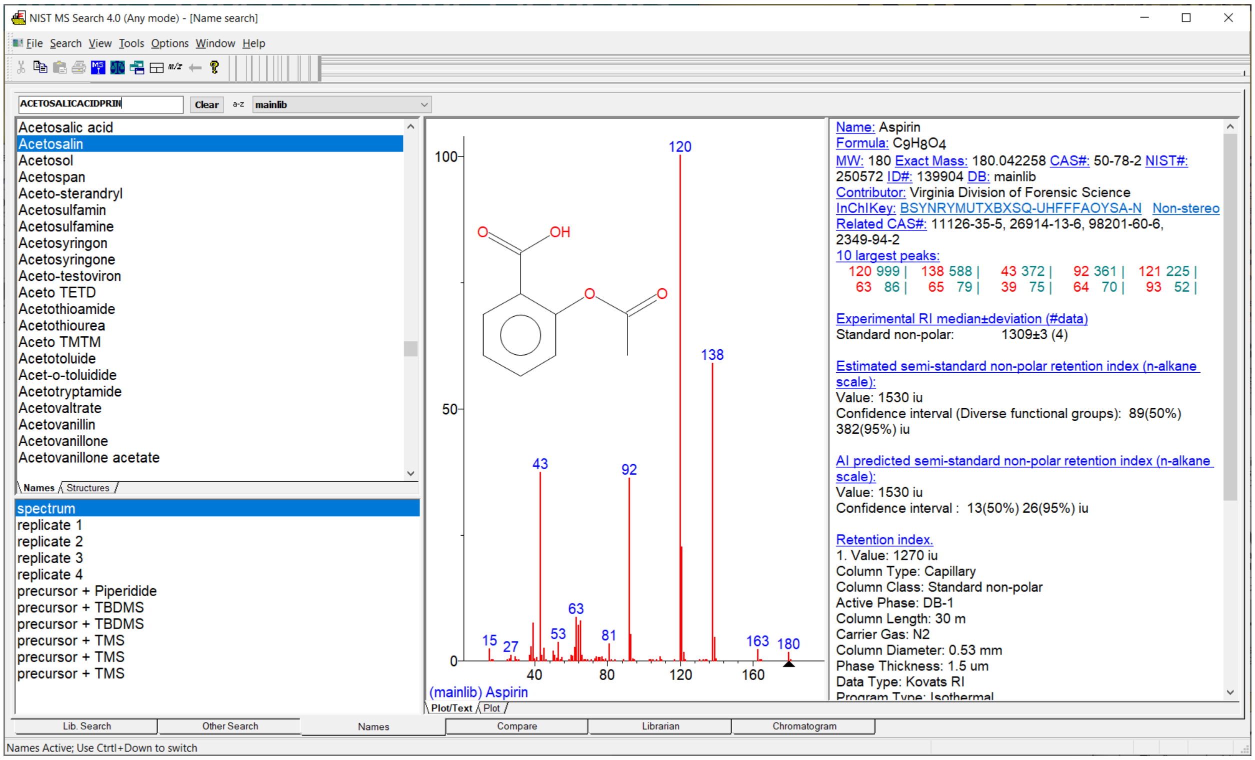
The lower window functions in different ways depending on the NIST Library Type being searched (EI or Tandem). If the EI Library is being searched, the lower window will contain all spectra with the highlighted spectrum in the upper window. The first entry in the list has the name **spectrum** and is highlighted. The displays in the **Plot** and **Text Information** windows are the same as those for the highlight spectrum in the upper window. This is followed by a list of one or more **replicates** (spectra of the same compound but from a different source, which are found in the **replib**) if there are multiple spectra for the compound. As each entry in the lower list is highlighted, the displays in the windows on the right-side will change. The highlight in the left-side upper window remains in its original position.

Select the **Clear** button to the right of the Text Entry field on the far left of the display. This will clear the Text Entry field. A good example for the EI Library is to enter NAPHTHALENE in the Text Entry field, after making sure the **mainlib** appears in the dropdown list box to the right of the a-z button. If something else is listed, select the down arrow on the right side and select **mainlib** from the displayed list. By default, **mainlib** is the first item in the list. This compound has a **mainlib** spectrum (**spectrum**) and four **replib** spectra (**replicate 1**, **replicate 2**, **replicate 3**, and **replicate 4**). Following that are four listings, each with the name **InChI isomer, isotope**. These are spectra of different isotope variants of deuterated naphthalene (1 D atom, 2 D atoms and a **mainlib** and **replib** spectrum of a perdeuterated variant).

Another good EI example to enter is ACETOSALICYLICACID. The name in the **Hit List** (top left window) is Acetosalic Acid. The name under the spectrum plot (middle window) and in the **Name** field of the **Text Info** window (far right) is Aspirin (which is the mainlib name). Acetosalic acid is a synonym for this compound. The position of the horizontal splitter bar has been changed to vertical using the **Change Splitter Bar Orientation** selection on the **RMB** menu displayed with the Mouse pointer in the **Plot** or **Text Info** window. This compound has a **mainlib** spectrum and four replicate spectra (in the **replib**). In addition, this left lower window has 16 labels that begin with "**precursor +**" followed by the name of the derivatizing agent(s) and, the number of individual agents added to the precursor molecule if >1. Both **mainlib** and **replicate** spectra for any compound will be listed.

This view of the **Text Information** window in the **Names** tab view (*Figure 38*) allows for a clear observation of Information pertaining to the Retention Index (RI) of the compound. There is experimental RI data for more than a third of the compounds in the NIST 26 EI Library. These come from the measurement of spectra by NIST and/or the literature. When experimental data are available, these are presented for as many as three different types of GC columns. The number following the column type under the title **Experimental RI median±deviation (#data)** is an average for the measures of the RI value with its deviation and the number of measurements that make up this average. The two predicted RI values are also shown. The display of Synonyms and Other Databases has been deselected.

All GC Methods for all four of the RI values used in the composite are listed under the heading **Retention index**. Each method can be brought into focus using the scroll bar on the right side of the **Text Info** window. If the Synonyms were displayed, it would be seen this compound has 157.



Name: Aspirin
Formula: $C_9H_8O_4$
MW: 180 Exact Mass: 180.042258 CAS#: 50-78-2 NIST#: 250572 ID#: 139904 DB: mainlib
Contributor: Virginia Division of Forensic Science
InChIKey: BSYNRYMUTXBXSQ-UHFFFAOYSA-N Non-stereo
Related CAS#: 11126-35-5, 26914-13-6, 98201-60-6, 2349-94-2
10 largest peaks:
120 999 | 138 588 | 43 372 | 92 361 | 121 225 |
63 86 | 65 79 | 39 75 | 64 70 | 93 52 |

Experimental RI median±deviation (#data)
Standard non-polar: 1309±3 (4)

Estimated semi-standard non-polar retention index (n-alkane scale):
Value: 1530 iu
Confidence interval (Diverse functional groups): 89(50%) 382(95%) iu

AI predicted semi-standard non-polar retention index (n-alkane scale):
Value: 1530 iu
Confidence interval : 13(50%) 26(95%) iu

Retention index.
1. Value: 1270 iu
Column Type: Capillary
Column Class: Standard non-polar
Active Phase: DB-1
Column Length: 30 m
Carrier Gas: N2
Column Diameter: 0.53 mm
Phase Thickness: 1.5 um
Data Type: Kovats RI
Program Type: Isothermal

Figure 38. Names Tab View for the search of SALICYLICACID in the NIST/EPA/NIH Electron Ionization Library. The display has been altered from the default by selecting Change Splitter Orientation from the RMB menu with the Mouse pointer in the Spectrum window and hiding the display of Synonyms and Other DBs.

Below the Experimental data is a label that reads: **Estimated non-polar retention index (n-alkane scale):** This is a value that is calculated based on the structure associated with the spectrum. There is a value for this field regardless of the spectrum being in an NIST Library or a user-library such as the *Wiley Registry of Mass Spectral Data* or a user-library created with MS Search. The only requirement is that the spectrum has an associated structure.

Below this estimated value is a label that reads **AI predicted non-polar retention index (n-alkane scale):** This is a value that pertains only to spectra in the NIST/EPA/NIH EI Mass Spectral Library and is calculated based on the structure and measured RI values of spectra in the current library and the NIST archive. This value has been found to be closer to experimentally measured values than the RI values calculated based on structure alone.

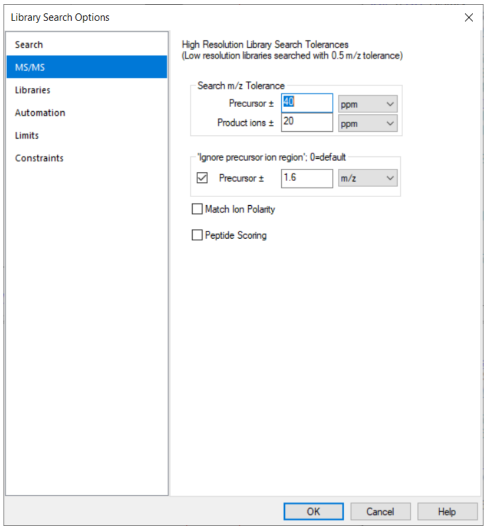
Below the AI predicted RI value is a heading entitled **Retention Index**. Below this heading are the details of the GC method when the various RI values were measured. By default, only the first two methods are listed. The number of methods listed or specifying all methos be listed can be set in the **Text Information** windows Properties dialog box displayed from the **RMB** menu with the Mouse pointer in the window.

If <…> appears after the last listed GC Method/RI value, additional GC Methods/RI sets can be displayed.

Instructions for Spectrum and Name searches of the NIST Tandem Libraries follow.

Search by Spectrum for the NIST Tandem Libraries

Product-ion mass spectra obtained by MS/MS are referred to as MS/MS spectra in MS Search. There are three libraries of product-ion mass spectra provided in the Tandem Installation. These are named **apci_msms_nist** (precursor ions formed by atmospheric pressure chemical ionization) and, **hr_nist_msms** and **lr_nist_msms** (precursor ions formed by electrospray ionization, ESI). There are two possible ways to search a measured ESI product-ion mass spectrum against either or both **hr** and/or **lr** libraries when the spectrum has a known precursor ion: **Identity MSMS** or **Similarity MSMS Hybrid**. These two searches are explained later in the manual. These same two searches can be used with the apci_msms_nist library. These libraries can also be used in the identification of accurate mass spectra produced by in-source CAD, which are spectra without a specified precursor ion: **Identity HiRes NoPrecursor**. In addition to using the NIST provided product-ion libraries, user-libraries can be built using product-ion spectra obtained from in-source CAD techniques associated with APCI. DART, DESI, and product-ion spectra obtained by MS/MS. Instructions on building these user-libraries are provided later in this manual. There are also third-party libraries of product-ion mass spectra produced by ESI, like the MoNA (MassBank of North America, *https://mona.fiehnlab.ucdavis.edu/*) LC-MS/MS library, which can be used with MS Search when properly formatted.


Library Search Options

High Resolution Library Search Tolerances
(Low resolution libraries searched with 0.5 m/z tolerance)

Search m/z Tolerance
Precursor ± 40 ppm
Product ions ± 20 ppm

'Ignore precursor ion region'; 0=default
☑ Precursor ± 1.6 m/z

☐ Match Ion Polarity

☐ Peptide Scoring

Figure 39. MS/MS tab in Library Search Options dialog box.

Some searches require settings in the **MS/MS** page of the **Library Search Options** dialog box (*Figure 39*).

MS/MS, **MS/MS Hybrid**, and **HiRes NoPrecursor** options:
Search *m/z* Tolerance:
Precursor: ± 0.015-100,000 ppm or 6x10^{-5} – 500 *m/z* units (for MS/MS search only)
Product-ion: ± 0.015-100,000 ppm or 6x10^{-5} – 500 *m/z* units.
Note: For Low Resolution library (LR_*) product-ions tolerance is fixed at unit mass resolution.

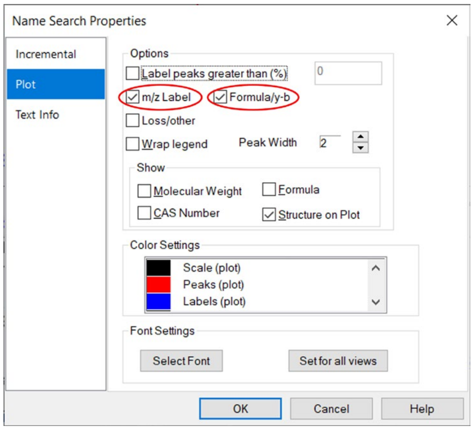
If a library name begins with LR_ (Low Resolution), for its spectra the entered tolerance T is replaced with max(T, 0.45 *m/z*) for all product-ion peaks unless peptide scoring is selected, e.g., if T=4500 ppm, tolerance will be increased to 0.45 *m/z* for *m/z* < 100.

When searching with high mass accuracy spectra, the NIST low resolution library (lr_nist_msms) is searched using wide *m/z* tolerances (unit mass resolution). This ensures that compounds with only low-resolution spectra can be found when searching with high mass accuracy spectra. If searching with low resolution spectra, simply specify a wide *m/z* product-ion tolerance for searching any library.

If the \MSSearch folder is examined for folders and the Tandem Library has been installed, two folders containing accurate *m/z* spectra will be observed (**hr_nist_msms** and **hr_nist_msms#2**). The need for these two files is due to programming limitation. When using v.4.0 of MS Search, you will only see the name **hr_nist_msms**. The two files are combined within the software.

Name Search of NIST Tandem Libraries

When the list of selectable libraries is viewed in the dropdown list box of the **Names** tab view, there is only one listing for the two ESI product-ion mass spectral libraries provided by NIST (**hr/lr NIST Tandem**). This listing contains all the names (primary compound name and synonyms) for the **hr_nist_msms** and **lr_msms-nist** libraries. All names are searched at once. The top window on the left side is a list of all names in the two libraries. The lower window is a list of all spectra associated with that name. Type the name Cocaine in the text entry box on the far left. If the a-z button right side of the Clear button has a blue background, numerals and symbols will not be excepted. To enter numbers and symbols the button must have a white background. Type “2HYDROXY”. The highlighted name will be 2-Hydroxy-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one.


Name Search Properties

Options
☐ Label peaks greater than (%) 0
☑ m/z Label ☑ Formula/y-b
☐ Loss/other
☐ Wrap legend Peak Width 2

Show
☐ Molecular Weight ☐ Formula
☐ CAS Number ☑ Structure on Plot

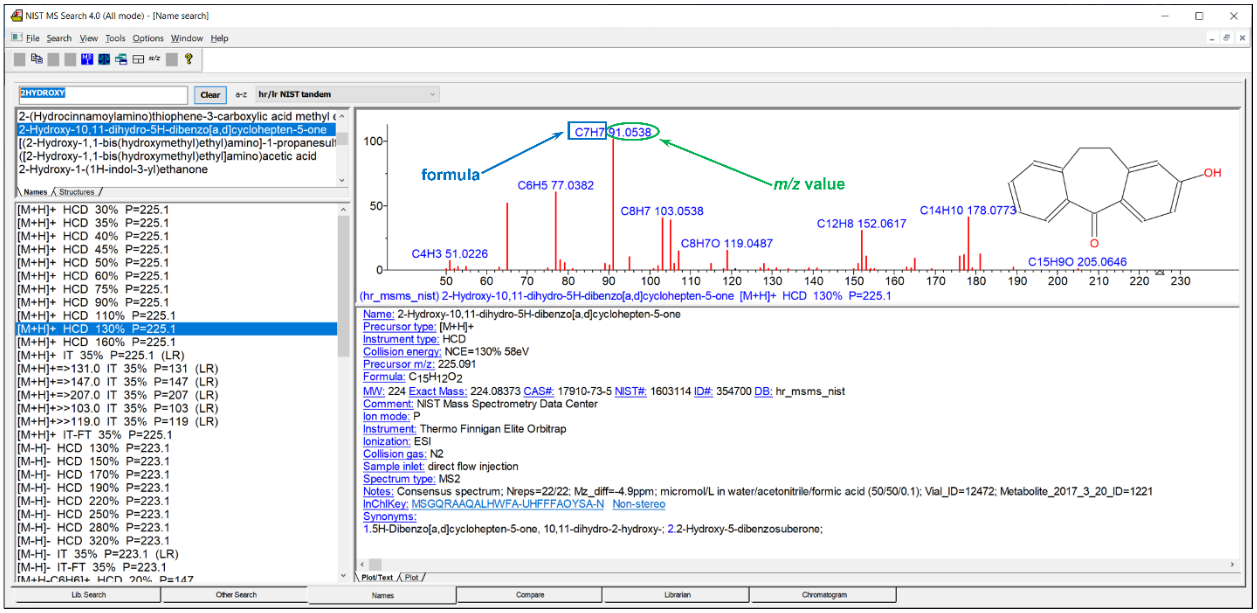
Color Settings
■ Scale (plot)
🟥 Peaks (plot)
🟦 Labels (plot)

Font Settings

***Figure 40**. Settings to display m/z values and formula*

The **Plot** window will show the spectrum is in the **hr_nist_msms library**. The first 11 entries in the lower window will be [M+H]+ HCD xx% P=225.1. This says that a protonated molecule with an *m/z* value of *m/z* ~226.1 (*m/z* 225.091) was analyzed in a higher energy collision cell (HCD, Thermo Scientific Elite Orbitrap Elite) at xx% (collision energy). The **Text Information** window will show the **Collision_energy**: field with 30% 13eV (actual energy). Each of the next 10 items in the list on the lower left of the **Names** tab view will represent different collision energies. The next item has (LR) at the end. This means the spectrum is in the NIST low resolution Tandem Library (**lr_nist_msms**). The IT means the analysis was done using a quadrupole ion trap instrument. The next five items have the form **[M+H]++>xxx.x IT 35% P=131 (LR)**. These are MS^3 spectra where the precursor ion (*m/z* 131 for the first item) is an MS^2 fragment from the precursor of the compound (*m/z* 225.091). The 35% is related to the NEC.

The MS^3 IT spectra are followed by spectra measured on an Orbitrap at different collision energies using the [M – H]$^-$ ion as the precursor ion. There are Orbitrap positive-ion spectra where the precursor ion is due to the loss of a C_6H_6 molecule from the protonated molecule. These spectra are followed by other precursor formed by various losses from the pronated or deprotonated molecule.



2HYDROXY

hr/lr NIST tandem

```
2-(Hydrocinnamoylamino)thiophene-3-carboxylic acid methyl e
2-Hydroxy-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one
[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]-1-propanesulf
([2-Hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)acetic acid
2-Hydroxy-1-(1H-indol-3-yl)ethanone
```

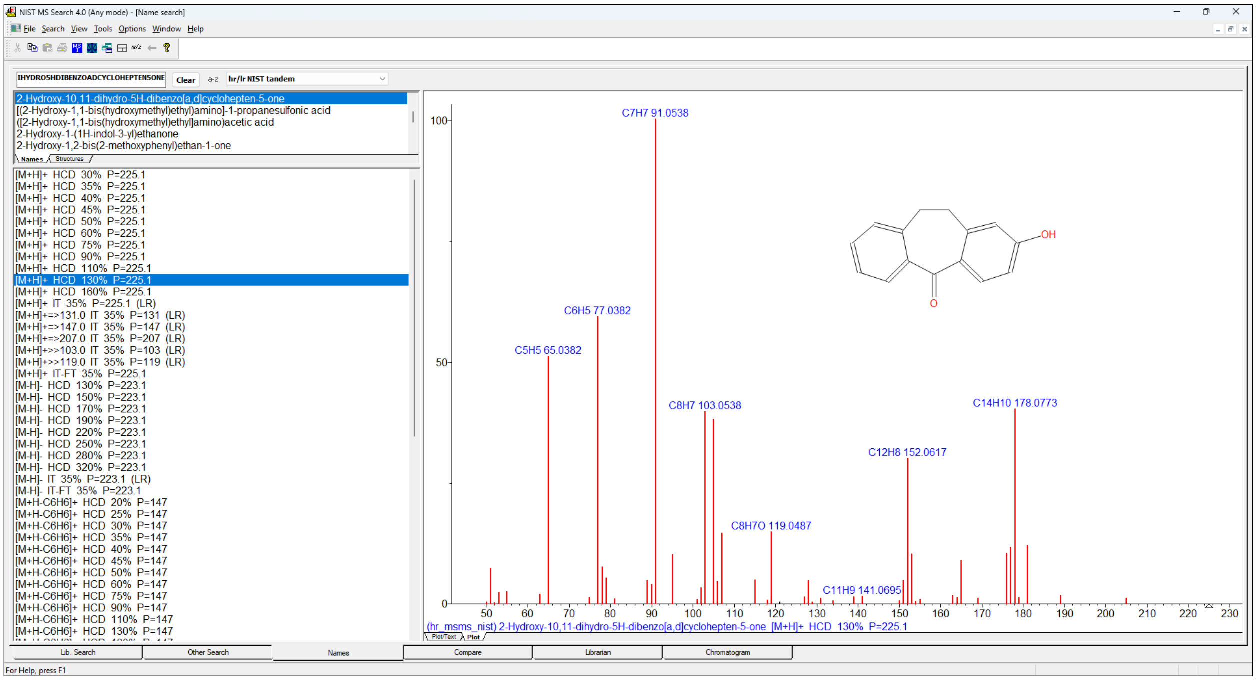
```
[M+H]+  HCD  30%  P=225.1
[M+H]+  HCD  35%  P=225.1
[M+H]+  HCD  40%  P=225.1
[M+H]+  HCD  45%  P=225.1
[M+H]+  HCD  50%  P=225.1
[M+H]+  HCD  60%  P=225.1
[M+H]+  HCD  75%  P=225.1
[M+H]+  HCD  90%  P=225.1
[M+H]+  HCD  110%  P=225.1
[M+H]+  HCD  130%  P=225.1
[M+H]+  HCD  160%  P=225.1
[M+H]+  IT  35%  P=225.1  (LR)
[M+H]+=>131.0  IT  35%  P=131  (LR)
[M+H]+=>147.0  IT  35%  P=147  (LR)
[M+H]+=>207.0  IT  35%  P=207  (LR)
[M+H]+>>103.0  IT  35%  P=103  (LR)
[M+H]+>>119.0  IT  35%  P=119  (LR)
[M+H]+  IT-FT  35%  P=225.1
[M-H]-  HCD  130%  P=223.1
[M-H]-  HCD  150%  P=223.1
[M-H]-  HCD  170%  P=223.1
[M-H]-  HCD  190%  P=223.1
[M-H]-  HCD  220%  P=223.1
[M-H]-  HCD  250%  P=223.1
[M-H]-  HCD  280%  P=223.1
[M-H]-  HCD  320%  P=223.1
[M-H]-  IT  35%  P=223.1  (LR)
[M-H]-  IT-FT  35%  P=223.1
[M+H-C6H6]+  HCD  20%  P=147
```

(hr_msms_nist) 2-Hydroxy-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one [M+H]+ HCD 130% P=225.1

Name: 2-Hydroxy-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one
Precursor type: [M+H]+
Instrument type: HCD
Collision energy: NCE=130% 58eV
Precursor m/z: 225.091
Formula: $C_{15}H_{12}O_2$
MW: 224 Exact Mass: 224.08373 CAS#: 17910-73-5 NIST#: 1603114 ID#: 354700 DB: hr_msms_nist
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
Notes: Consensus spectrum; Nreps=22/22; Mz_diff=-4.9ppm; micromol/L in water/acetonitrile/formic acid (50/50/0.1); Vial_ID=12472; Metabolite_2017_3_20_ID=1221
InChIKey: MSGQRAAQALHWFA-UHFFFAOYSA-N Non-stereo
Synonyms:
1.5H-Dibenzo[a,d]cyclohepten-5-one, 10,11-dihydro-2-hydroxy-; 2.2-Hydroxy-5-dibenzosuberone;

Figure 41. Names tab view for one of the spectra associated with 2-hydroxy-5-dibenzosuberone

The **Plot** window for the Tandem Libraries has the option of displaying the *m/z* values and/or chemical formula of the labeled ions. Using the tabs at bottom of right side windows, the display can be changed between the **Plot** and **Text** information windows or just the **Plot** window by selecting either the **Plot/Text** or **Plot** tab, *Figure 41*.



IHYDROSHDIBENZOADCYCLOHEPTENSONE

hr/lr NIST tandem

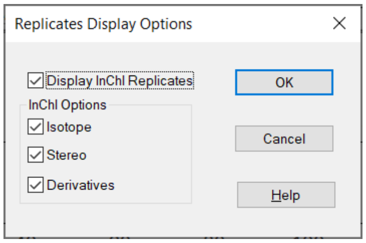
```
2-Hydroxy-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one
[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]-1-propanesulfonic acid
([2-Hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)acetic acid
2-Hydroxy-1-(1H-indol-3-yl)ethanone
2-Hydroxy-1,2-bis(2-methoxyphenyl)ethan-1-one
```

```
[M+H]+  HCD  30%  P=225.1
[M+H]+  HCD  35%  P=225.1
[M+H]+  HCD  40%  P=225.1
[M+H]+  HCD  45%  P=225.1
[M+H]+  HCD  50%  P=225.1
[M+H]+  HCD  60%  P=225.1
[M+H]+  HCD  75%  P=225.1
[M+H]+  HCD  90%  P=225.1
[M+H]+  HCD  110%  P=225.1
[M+H]+  HCD  130%  P=225.1
[M+H]+  HCD  160%  P=225.1
[M+H]+  IT  35%  P=225.1  (LR)
[M+H]+=>131.0  IT  35%  P=131  (LR)
[M+H]+=>147.0  IT  35%  P=147  (LR)
[M+H]+=>207.0  IT  35%  P=207  (LR)
[M+H]+>>103.0  IT  35%  P=103  (LR)
[M+H]+>>119.0  IT  35%  P=119  (LR)
[M+H]+  IT-FT  35%  P=225.1
[M-H]-  HCD  130%  P=223.1
[M-H]-  HCD  150%  P=223.1
[M-H]-  HCD  170%  P=223.1
[M-H]-  HCD  190%  P=223.1
[M-H]-  HCD  220%  P=223.1
[M-H]-  HCD  250%  P=223.1
[M-H]-  HCD  280%  P=223.1
[M-H]-  HCD  320%  P=223.1
[M-H]-  IT  35%  P=223.1  (LR)
[M-H]-  IT-FT  35%  P=223.1
[M+H-C6H6]+  HCD  20%  P=147
[M+H-C6H6]+  HCD  25%  P=147
[M+H-C6H6]+  HCD  30%  P=147
[M+H-C6H6]+  HCD  35%  P=147
[M+H-C6H6]+  HCD  40%  P=147
[M+H-C6H6]+  HCD  45%  P=147
[M+H-C6H6]+  HCD  50%  P=147
[M+H-C6H6]+  HCD  60%  P=147
[M+H-C6H6]+  HCD  75%  P=147
[M+H-C6H6]+  HCD  90%  P=147
[M+H-C6H6]+  HCD  110%  P=147
[M+H-C6H6]+  HCD  130%  P=147
```

(hr_msms_nist) 2-Hydroxy-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one [M+H]+ HCD 130% P=225.1

Figure 42. Names tab view for one of the spectra associated with 2-hydroxy-5-dibenzosuberone in the Plot only view

The **Replicates/Related** selection on the **Options** menu displayed by selecting **Options** on the Main Menu (third item) has no effect on the **Names** tab view searches. This selection primarily impacts EI Library searches and some searches performed in the **Other Searches** tab view of NIST libraries and NIST user-libraries. The functionality is described in more detail later in this manual or by selecting Help in the Replicates Display Options dialog box.


Replicates Display Options

☑ Display InChI Replicates

InChI Options

☑ Isotope

☑ Stereo

☑ Derivatives

Compare tab view

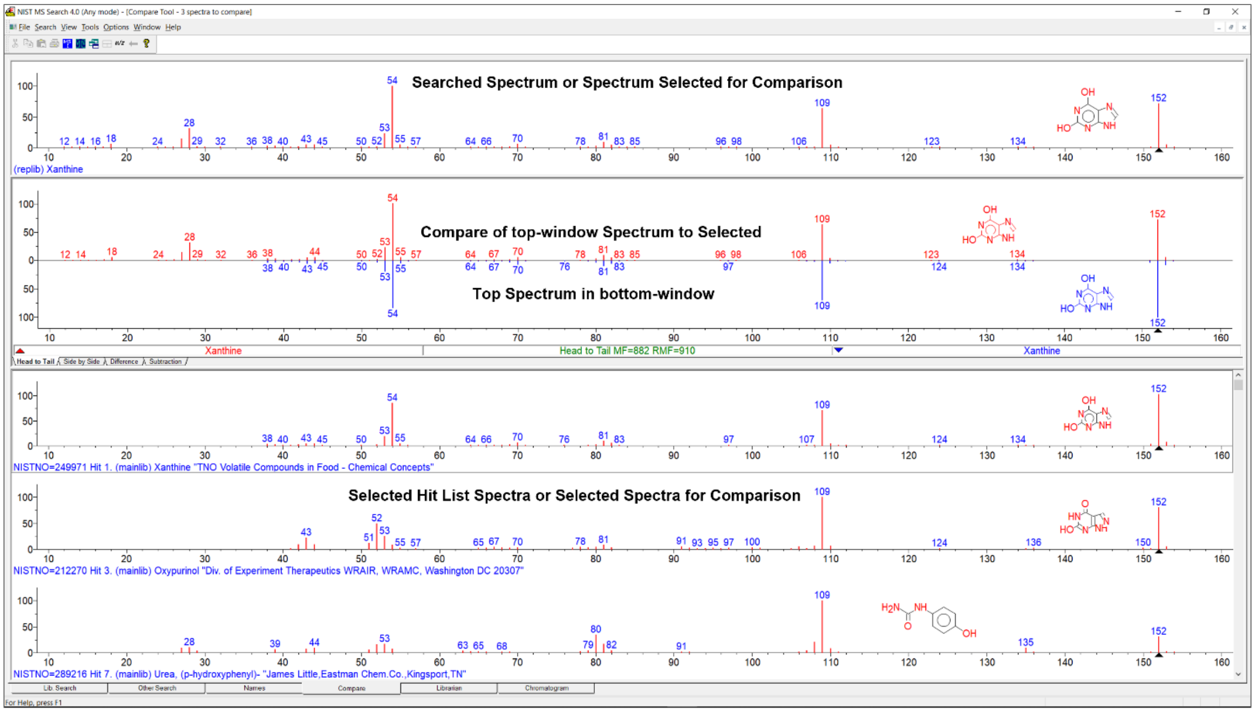
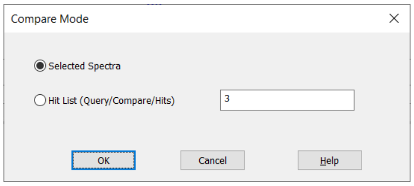


Figure 43. Compare tab view.


Compare Mode

● Selected Spectra

○ Hit List (Query/Compare/Hits) 3

The **Compare** tab view has two modes of operation, selectable from a dialog box displayed by selecting **Select Compare Mode** from the **Options** selection of the Main Menu in the **Compare** tab view (shown on left). If **Selected Spectra** is selected in the dialog box, the **Compare** tab view clears. When a search is executed in the **Lib. Search** tab view and the **Compare** tab view is brought into focus, the **Compare** tab view will remain blank. Select the **Lib. Search** tab view and highlight one or more spectra in the **Hit List** window, issue the **RMB** menu command with the Mouse pointer in the **Hit List** window, and select **Send To\Compare List**. The focus automatically switches to the **Compare** tab view which has the three sections (queried spectrum, **Compare** window, and a list of three of the selected spectra). If more than three spectra were selected, the scroll bar at the right of the lower display can be used to bring them into focus one at a time. If a subsequent search is executed and the focus is switched to the **Compare** tab view, the display will be the same as resulted from the previous **Send To\Compare List** command. Switch the focus back to the **Lib. Search** tab view, highlight one or more spectra in the just generated **Hit List**, and issue the **RMB** menu **Send To\Compare List** command. The focus is automatically changed to the **Compare** tab view, and the display is of the required spectrum, **Compare** window, and the spectra selected at the time of the **Send To\Compare List** command (regardless of the number selected, a maximum of three at a time will be sent).

If **Hit List (Query/Compare/Hits)** is selected in the dialog box, when a search is executed in the **Lib. Search** tab view and the **Compare** tab view is brought into focus, manually, the top spectrum will be that of the queried spectrum, the middle window is the comparison of the required spectrum, and the selected Hit (first one by default), and the lower potion shows the number of spectra corresponding to the number set in the **Compare** tab view's Properties dialog box for display, The first x Hits will be the number of spectra sent to lower section, where x is he number set in the **Compare Mode dialog** box. If the focus is returned to the **Lib. Search** tab view, spectra selected in the Hit List window, and the **Send To\Compare List** command issued, the **Compare** tab view will automatically come into focus with the number of spectra set in the Properties dialog box visible, and all selected spectra in the lower section, visible via the scroll bar.

When sending spectra to the **Compare** tab view from the **Lib. Search** tab view, if they originate in the **Spec List** and the **Spec List** tab is selected in the upper multiple function **Text/Plot** window, the existing contents will be cleared, and subsequent sends will be prepended with those of the sent spectra.

In addition to the functions of the **Compare** tab view described above, spectra (with or without structures) can be pasted into the top or bottom windows to perform manual comparisons. Details of how to do this are described later in this manual.

The Librarian tab view

The **Librarian** tab view is used for building and maintaining user-libraries. It is also used to associate structures with measured spectra for analysis in *MS Interpreter.* The details of how each part of this tab view works will be described later in this manual.

Other Stuff

There are detailed instructions provided for other features of the NIST MS Search Program in this manual. Make sure they are reviewed, especially the different types of searches and **Constraints**. In addition, there is important information in the Program's **Help** file (**Help\Help Topics**). Select the **Index** tab on the **NIST MS Search 4.0 Help** file displayed by selecting **Help Topics** from the **Help** menu (Main Menu), enter *General Points* in the text entry box, and click on the **Display** button. This provides descriptions of **Exact** and **Accurate** mass, **MW** (molecular weight), and defines **Short Cut** keys such as the ability to copy the Hit List to the Clipboard as a TSV file searches and **Constraints** (see *Constraints* on page *46*).

Guide to the Program

The **NIST Mass Spectral Search Program** uses tab displays selectable at the bottom of the Program's desktop. The tabs are: **Lib. Search**, **Other Search**, **Names**, **Compare**, **Librarian**, and **Chromatogram**

Selecting a tab results in the display of a different tab view (Desktop Display). All tab displays have movable divider/slider bars (splitters), some of which can be re-orientated, as well as tabs associated with various windows within a tab view. Selecting **Window/Tile Vertically** or **Window/Tile Horizontally** from the Main Menu displays all the tab views on a single screen. Each tab view window has its own set of window control buttons (minimize maximize/restore close). If one of the minimized buttons is selected, the window is no longer visible and a symbol for that tab view window appears at the bottom of the Desktop, just above the tabs for each tab view

Selecting the maximize button on the symbol will restore that tab view of full screen. Selecting the restore button on the symbol at the bottom of the display for that tab view, will bring the tab view back to its original tab view position. If several of the tab views are closed, the remaining open tab views can be titled by selecting one of the two **Tile** options from the Main Menu's **Windows** menu. Some **Title Bars** will have the name of the tab view displayed (Librarian, Name, Compare Tool). With any tab view in full screen display, selecting any tab at the bottom of the program will bring that tab view into full screen focus.

The Individual Program tab views:

Lib. Search (*Figure 23*) used for all comparisons of spectra and/or structures to one or more libraries (maximum of 127 libraries).

Other Search, **Exact Mass**, **MS/MS Precursor *m/z*, Formula** (elemental composition), **Any Peaks**, **CAS Number**, **NIST Number**, and **ID Number**, all of which can be constrained. There is a wild card search (**Sequential Method**) which allows users to create a specific multiple-criteria search. These are described individually later in this manual.

Names (Incremental Name Search) allows a name to be typed and have the program match the name. This search uses all names and synonyms in the specified library. It has unique features when searching NIST provided libraries.

Compare for easy comparison between spectra. It is, by default, automatically refreshed with the latest spectral search of the library. Selected spectra can also be input for comparisons.

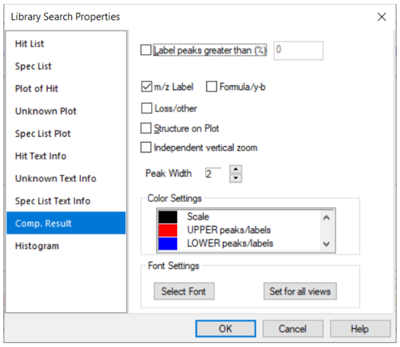
Librarian keeps a history of the recent spectral searches. This tab view can also be used for import, editing, and user library construction. In some cases, the **Librarian** tab is referred to as the Spec List. The left-side vertical window in the **Lib. Search tab** is also called the **Spec List**.

NEW in v.4.0 **Chromatogram** allows for the opening of chromatographic files (GC/MS or LC-MS/MS), finding individual components and identifying them against the library spectra (***NEW*** in NIST 26)

Alternate layouts can be selected using the **Change layout** button in the **Tool Bar** which is just under the **Main Menu**. A Tool Tip (description of what an item does) is displayed when the Mouse pointer is placed over a **Tool Bar** button. There is also a **Tool Bar** button to restore the default layout (**Set default layout**). Alternatively, **Default Layout** and **Change Layout** in the **Window** menu (selected from the Main Menu) may be used.

There is a **Right Mouse Button (RMB)** menu that can be displayed from any window of any tab view. The function of the menu items will be explained throughout this manual. In every tab view, properties of the individual windows can be controlled by accessing the **Properties** dialog box from the **RMB** menu (last selection). These dialog boxes will have multiple pages. Each tab view has its own multiple page **Properties** dialog box like the one shown for the **Lib. Search** tab view's **Comp. Results** page (*Figure 56*). The displayed page is dependent on the window where the Mouse pointer is displayed when the **RMB** menu is displayed. The pages on these dialog boxes control the information displayed in the tab view's individual windows. The contents are self-explanatory. There is a **Help** button that will display more detail.

Individual pages of the various **Properties** dialog boxes are displayed by selecting the page name in the left column of the dialog box.



☐ Label peaks greater than (%) 0

☑ m/z Label ☐ Formula/y-b

☐ Loss/other

☐ Structure on Plot

☐ Independent vertical zoom

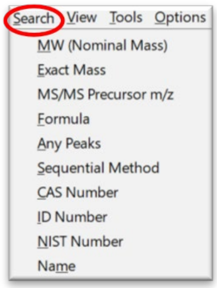
Peak Width 2

Figure 44. Library Search Properties dialog box, Compare window page

All tab views have the same Main Menu except for the **Chromatogram** tab view. File Search View Tools Options Window Help They also have the same **Toolbar** . Some items will be grayed when not available. The various menu selections and **Toolbar** buttons available for each of these tabs are described in more detail throughout the manual. The display of the **Toolbar** can be turned on and off by selecting selecting/deselecting **Toolbar** from the **View** menu of the Main Menu. The **Button Bar** (**Lib. Search**, **Other, Search**, **Names**, and **Librarian** tab views) is below the **Toolbar** and is always displayed. It is composed of several buttons, each having its own Tool Tip. The **Lib. Search**, **Other Search**, and **Names** tab view have one or more dropdown list boxes, The **Chromatogram** tab view has a **Filter Bar** in place of the **Button Bar**. The **Filter Bar** for the **Tandem** and **EI** spectrum types are different.

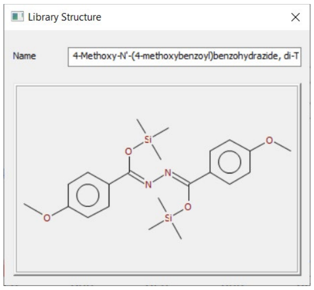
The **File** menu has **Save Configuration** and **Restore Configuration** selections which allow all the various tab display configurations to be saved to a specific file name and then recalled. Multiple users or those using both **Tandem** and **EI** spectrum types may have different ways of configuring each tab view that is best for them or specific tasks. Each user can restore a specific set of display options. This does not include data in the **Search History**. There is no change in the **Search History** or **Spec List** resulting from a **Restore Configuration**. Different configurations for different types of searches can be convenient. Selected libraries and search constraints are also saved.

The **File** menu has a **Select Spectrum Type** option (*New* in NIST 23) which allows the type of spectrum (**EI**, **Tandem**, or **Any**) currently worked with to be specified. This results in some options of the Program being hidden depending on the Spectrum Type. These are options that do not pertain to the search of the selected **Spectrum Type**. The **Any** selection results in the display of all options.



The **Search** selection on the Main Menu allows for quick selection of non-spectral type search. Depending on the choice, the selection will transfer you to the correct tab view and bring up the options for that search.

The **View** option on the Main Menu (*Figure 63*) allows the on/off display of the **Tool Bar** and/or the **Status Bar** (bottom portion of which contains the **For Help press F1** message on the left). There is an option to turn off the display of the **Compare Pane** window in the **Lib. Search** and **Compare** tab views in the **View** menu. ***NEW*** to v.4.0 of MS Search is the selection from the **View** menu, **Show Structure** (*Figure 45*). This results in the display of a floating fixed-size window with the structure and the name of the compound whose spectrum is being displayed. As different spectra are selected from a **Hit List**, the display is updated. Sometimes, when using MS Search with a small display, the automatic sizing of structure in displayed spectral plots will distort the structure and lead to an incorrect conclusion such as a bond repenting an attached free methyl as being attached to an N atom and forming a five-membered ring. This *new* feature will help to avoid that problem.



Name 4-Methoxy-N'-(4-methoxybenzoyl)benzohydrazide, di-T

Figure 45. Floating structure window.

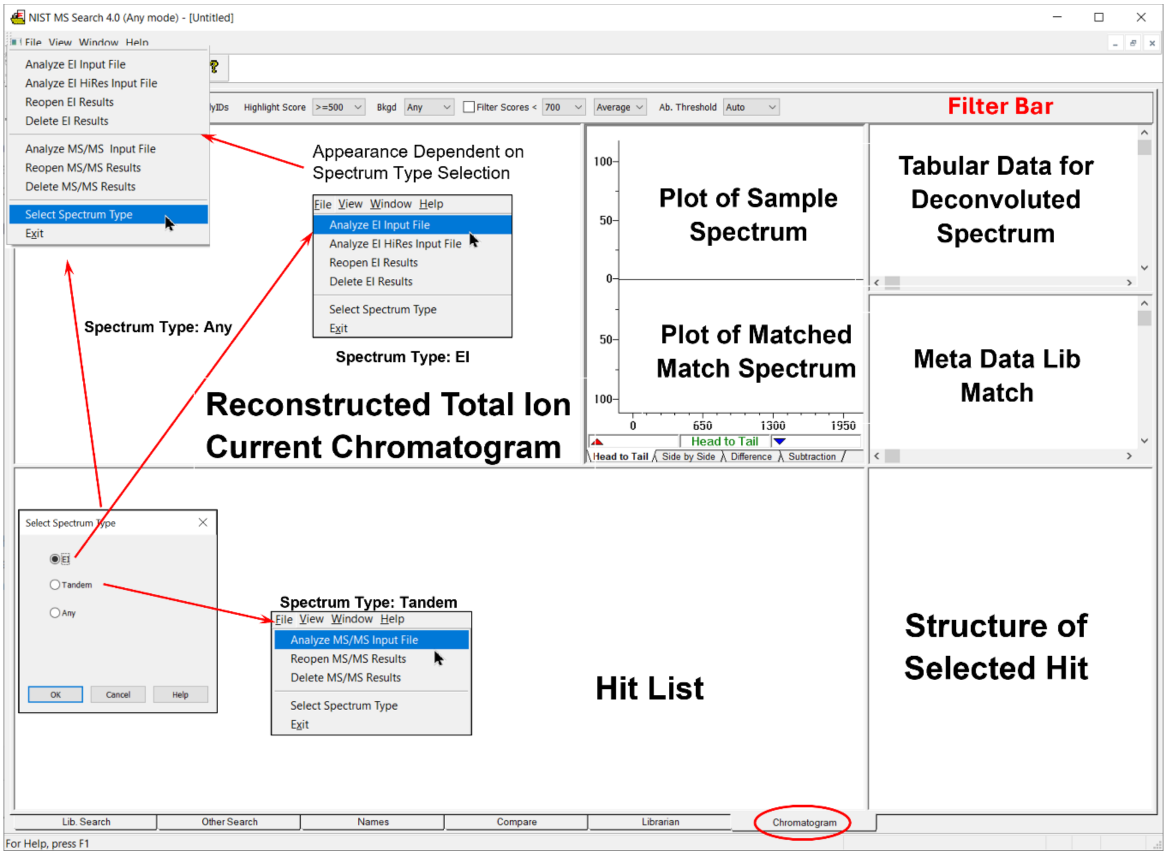
> ***Nota Bene*** about **Show Structure** selection from **View** menu in the **Lib. Search** tab view: If an entry is selected from the **Spec List** window, the structure, even if associated will not be displayed. The last selected entry in the **Hit List** will continue to be displayed until a new selection is made in the **Hit List** of **Lib. Search** tab view. In the **Librarian** tab view, as spectra are selected, structures will be displayed in the floating window.
>
> Another important note about structures is fact that when there is no structure associated with a spectrum and the **Formula** (elemental composition) field is populated, the elemental composition will appear in the floating **Spectrum** window or be displayed with the spectrum (when **Structure on Plot** is selected in an appropriate **Properties** dialog box) *in lieu of* a structure. If the **Formula** field of the **Text Information** window is not populated, there will be no display.
>
> In the **Names** tab view (of NIST Libraries) the structure associated with the just selected spectrum of either the upper or lower window of the **Hit List** will be displayed.

The **View** menu also allows the display of **Best matching only** ON and OFF and is used to display the **Derivative Precursor** dialog box in the **Lib. Search** tab view. The Best matching also has a button ( ) in the **Button Bar**. When this is turned ON, the Hit List will only contain the spectrum with the highest Match Factor when several spectra with the same InChIKey were found.

Tab views can be brought into focus by selecting the tab name. It is also possible to switch between tab views using the hot keys **Ctrl-Tab** (left-to-right) and **Ctrl-Shift-Tab** (right-to-left)

Many of the controls for the appearance of the windows' layout for individual tab views windows are similar to one another. These are covered in detail for the **Lib. Search** tab view but apply for most of the tab views.

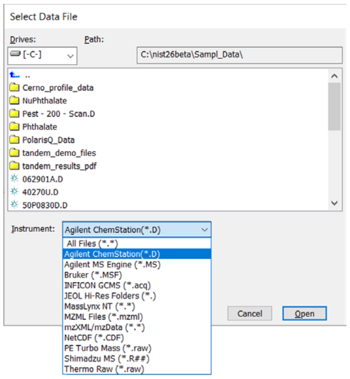
The Chromatogram tab view


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

Highlight Score >=500
Bkgd Any
☐ Filter Scores < 700
Average
Ab. Threshold Auto

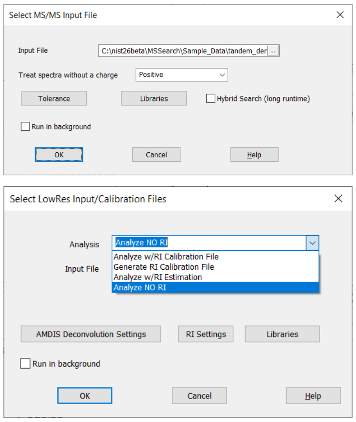
Figure 46. Unpopulated Chromatogram tab view. Specifying a Spectrum Type Changes the Appearance

The **Chromatogram** tab view allows chromatographic files (GC/MS and LC-MS/MS) to be processed. Processing a chromatographic file results in the selection of individual components in the file and provides a library search match against either EI or Tandem mass spectral libraries. Different routines are used for the two types of chromatographic data. Depending on the spectrum type selection, the **File** menu will have different selections. For Tandem files, selecting **Analyze MS/MS Input File** from the **File** menu displays the **Select MS/MS Input File** dialog box. For GC/MS (EI) files, Selecting **Analyze EI Input File** from the **File** menu displays the **Select LowRes/Calibration Files** dialog box. In addition, when the search type is **EI** the **File** menu has a selection which is used to process accurate *m/z* EI GC/MS data files (**Analyze EI HiRes Input Files**, which displays the **Select HiRes Input/Calibration Files** dialog box, not shown) These dialog boxes allow for the preparation of the various processes involved with each specific type of data and are used to execute the analyses. As can be seen from *Figure 47*, LowRes GC/MS files from multiple instrument manufacturers can be selected as well as netCDF files, which most manufacturers instruments data analysis software will produce. Tandem spectrum type allows Raw Files (specific to the Thermo Fisher instruments, *.raw), mzML Files (*.mzML, most manufacturers' tandem MS/MS instruments data analysis systems all for the output of the file type), and tdf Files (specific to the Bruker tims TOF instruments *.tdf). HiRes EI file types that can be processed are Raw Files (*.raw, specific to Thermo Fisher GC Orbitrap), mzData (*.mzData), mzML Files (*.mzML), and CDF Files (*.CDF).


Select Data File

Drives: [-C-]
Path: C:\nist26beta\Sampl_Data\
Instrument: Agilent ChemStation(*.D)

Figure 47. LowRes File selection dialog box.

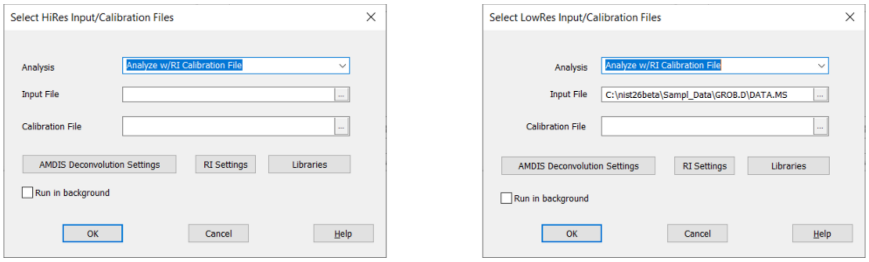

Select MS/MS Input File

Input File C:\nist26beta\MSSearch\Sample_Data\tandem_der
Treat spectra without a charge Positive
☐ Hybrid Search (long runtime)
☐ Run in background

Select LowRes Input/Calibration Files

Analysis Analyze NO RI
Input File
☐ Run in background

Figure 48. Analysis settings dialog box for MS/MS (top) & LowRes EI chroma files.



Select HiRes Input/Calibration Files:

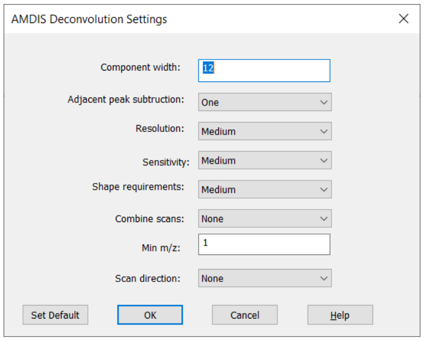
Analysis: Analyze w/RI Calibration File
Input File:
Calibration File:
☐ Run in background

Select LowRes Input/Calibration Files:

Analysis: Analyze w/RI Calibration File
Input File: C:\nist26beta\Sampl_Data\GROB.D\DATA.MS
Calibration File:
☐ Run in background

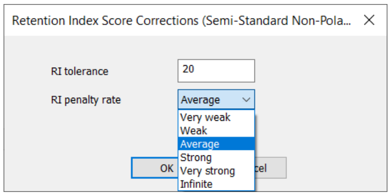
Figure 49. The Select Input/Calibration Files dialog box for High and Low Resolution GC/MS Data

As can be seen from *Figure 49*, the **Select LowRes/Calibration Files** and **Select HiRes Input/Calibration Files** dialog boxes are identical except for the *Titles*. Both have an **AMDIS Deconvolution Settings**, **RI Settings**, and **Libraries buttons**. The Analysis types for both are the same. Selecting the **AMDIS** button displays a dialog box with settings specific to the deconvolution of GC/MS data.



Component width: 12
Adjacent peak subtruction: One
Resolution: Medium
Sensitivity: Medium
Shape requirements: Medium
Combine scans: None
Min m/z: 1
Scan direction: None

Selecting the **RI Settings** button results in the display of a dialog box that allows the setting of the variation in the sample's measured RI value and that of the library match, which is specified in a more qualitative way.



RI tolerance: 20
RI penalty rate: Average

The **Libraries** button causes the Libraries selection page to be displayed to allow the specification of the libraries to be used in the data analysis process. A maximum of 127 libraries can be used. The Hits are based on the Score, not the order in which the libraries are searched.

After selecting a file, and the **Open** button is selected, the **File** dialog box closes, and the file is processed. After a GC/MS file is selected and the **Open** button is selected, the **Select Data File** dialog box closes, leaving the **Select Raw/Calibration Files** box dialog displayed. When the **OK** button is selected in this dialog box, the file is processed.

If the **File\Open** selection on the Main Menu is chosen with the tab view being other than **Lib. Search**, when the file is selected by highlining the name and clicking on the **Open** button, the focus will change to the **Lib. Search** tab view and display the import dialog box allowing the selection of the spectra to be imported. If **Automation** checkbox in the **Lib. Search** tab view's **Library Search Options** dialog box's **Search** page has been selected, the spectrum/spectra will be searched. If spectrum/spectra is/are sent to the **Spec List** (will be sent to both **Librarian** and **Lib. Search** tab views) using the **RMB**, the spectrum, unlike those that are imported through the **Choose file for spectra/structure import** dialog box will not be searched.

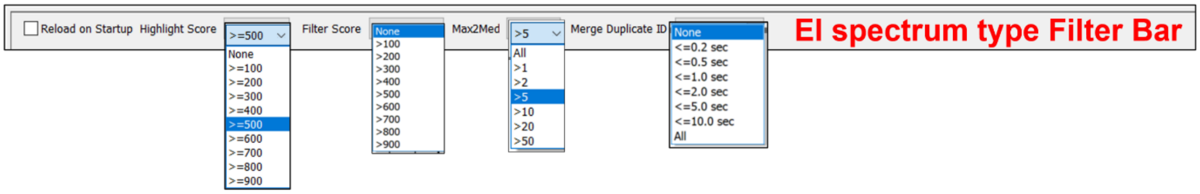
When **Library Search** is selected from the **RMB** menu with the Mouse pointer in the **Hit List** window of the **Chromatogram** tab view, all highlighted spectra will be sent to the two **Spec Lists** and searched depending on whether the **Automation** checkbox in the **Library Search Options Seach's Search** page has been selected.

> ***Nota Bene:*** If multiple spectra are searched at one time, the result for each search is available through the **History List** dropdown list box, which is in the middle of the **Button Bar** just above the **Spec List** window and below the **Tool Bar** (if displayed) in the **Lib. Search** tab view. Select the ∨ button on the right side of the dropdown list box and then select the desired search. The display changes to the results of that search, including **Plot/Text of Search Spectrum** tab view of the split window next to the **Spec List** window (in default view). The chosen **History** number will not necessarily correspond to that spectrum's number in the **Spec List**. If the spectrum searched is in the **History List** and has been deleted from the **Spec List** window, it will be displayed in the **Plot/Text of Search Spectrum** tab view of the split window when the spectrum is recalled from the **History List**. When a previously searched spectrum is recalled, the display of the **Spec List** window DOES NOT CHANGE. This behavior can be confusing. Care must be taken in drawing conclusion for the resulting display.

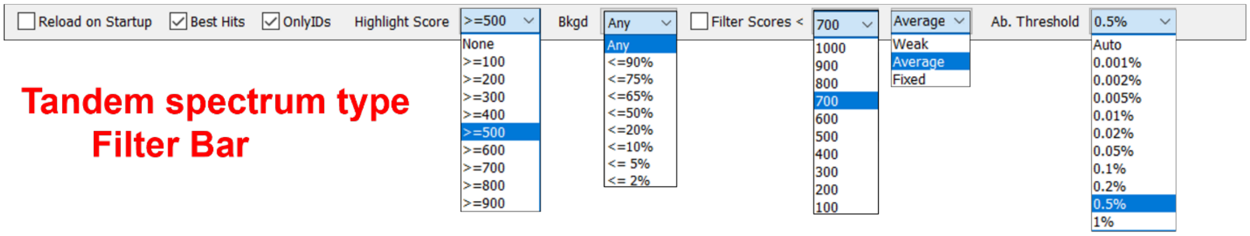
Details of each window and the processes are explained beginning on *page 22* of this manual. Other details are provided in the MS Search Program's Help file.

Filter Bars and the Title Bar for the Chromatogram tab view

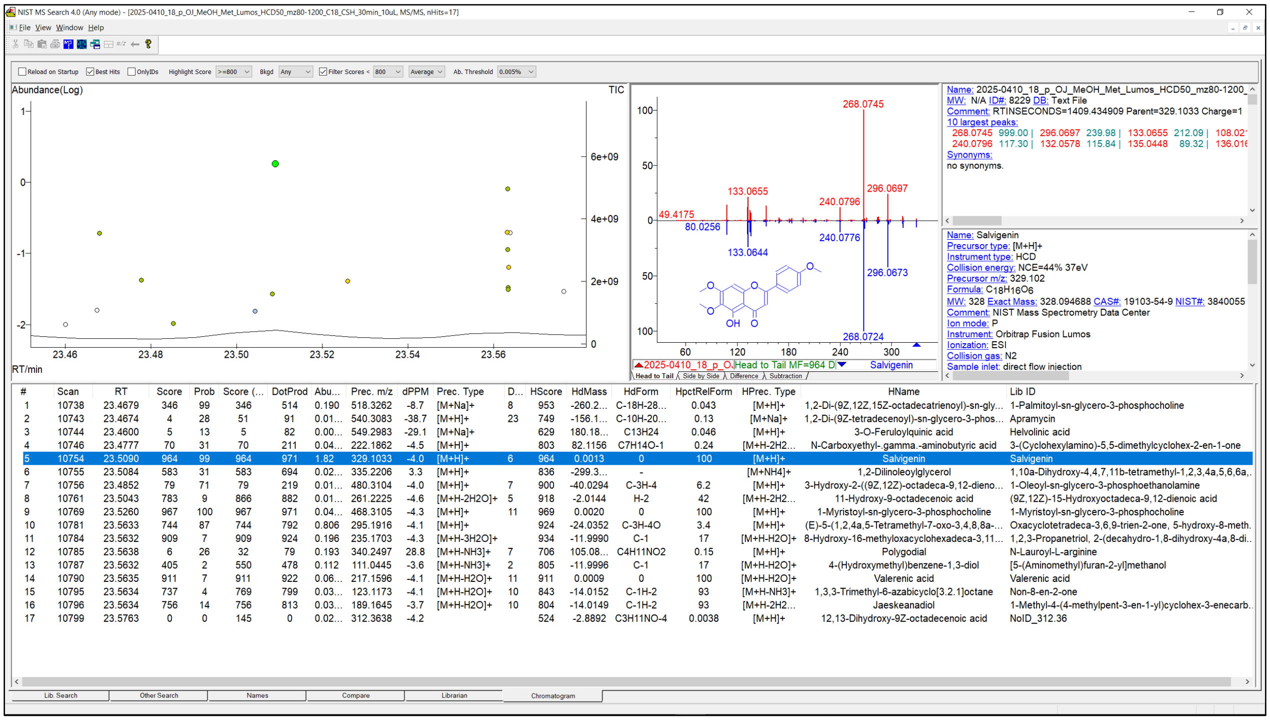
The Title Bar contains the name and version number of the program, the Spectrum Mode (EI, Tandem, Any), the name of the data file processed, the type of processing (MS/MS {for both Hybrid and non-Hybrid} modes, and EI {for both HiRes & LowRes}), and **nHits**. The value for **n** is a function of both the settings in **Filter Bar** and the zoomed region of the **Chromatogram** window.



Filter Bar for LowRes and HiRes EI Data.



Filter Bar Regular MS/MS Search and Hybrid Search if Tandem Data Files.



NIST MS Search 4.0 (Any mode) - [2025-0410_18_p_OJ_MeOH_Met_Lumos_HCD50_mz80-1200_C18_CSH_30min_10uL, MS/MS, nHits=17]

Name: 2025-0410_18_p_OJ_MeOH_Met_Lumos_HCD50_mz80-1200_
MW: N/A ID#: 8229 DB: Text File
Comment: RTINSECONDS=1409.434909 Parent=329.1033 Charge=1
10 largest peaks:
268.0745 999.00 | 296.0697 239.98 | 133.0655 212.09 | 108.02
240.0796 117.30 | 132.0578 115.84 | 135.0448 89.32 | 136.01
Synonyms:
no synonyms.

Name: Salvigenin
Precursor type: [M+H]+
Instrument type: HCD
Collision energy: NCE=44% 37eV
Precursor m/z: 329.102
Formula: $C_{18}H_{16}O_6$
MW: 328 Exact Mass: 328.094688 CAS#: 19103-54-9 NIST#: 3840055
Comment: NIST Mass Spectrometry Data Center
Ion mode: P
Instrument: Orbitrap Fusion Lumos
Ionization: ESI
Collision gas: N2
Sample inlet: direct flow injection

#	Scan	RT	Score	Prob	Score (...	DotProd	Abu...	Prec. m/z	dPPM	Prec. Type	D...	HScore	HdMass	HdForm	HpctRelForm	HPrec. Type	HName	Lib ID
1	10738	23.4679	346	99	346	514	0.190	518.3262	-8.7	[M+Na]+	8	953	-260.2...	C-18H-28...	0.043	[M+H]+	1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-sn-gly...	1-Palmitoyl-sn-glycero-3-phosphocholine
2	10743	23.4674	4	28	51	91	0.01...	540.3083	-38.7	[M+H]+	23	749	-156.1...	C-10H-20...	0.13	[M+Na]+	1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phos...	Apramycin
3	10744	23.4600	5	13	5	82	0.00...	549.2983	-29.1	[M+Na]+		629	180.18...	C13H24	0.046	[M+H]+	3-O-Feruloylquinic acid	Helvolinic acid
4	10746	23.4777	70	31	70	211	0.04...	222.1862	-4.5	[M+H]+		803	82.1156	C7H14O-1	0.24	[M+H-2H2...	N-Carboxyethyl-.gamma.-aminobutyric acid	3-(Cyclohexylamino)-5,5-dimethylcyclohex-2-en-1-one
5	10754	23.5090	964	99	964	971	1.82	329.1033	-4.0	[M+H]+	6	964	0.0013	0	100	[M+H]+	Salvigenin	Salvigenin
6	10755	23.5084	583	31	583	694	0.02...	335.2206	3.3	[M+H]+		836	-299.3...	-		[M+NH4]+	1,2-Dilinoleoylglycerol	1,10a-Dihydroxy-4,4,7,11b-tetramethyl-1,2,3,4a,5,6,6a,.
7	10756	23.4852	79	71	79	219	0.01...	480.3104	-4.0	[M+H]+	7	900	-40.0294	C-3H-4	6.2	[M+H]+	3-Hydroxy-2-((9Z,12Z)-octadeca-9,12-dieno...	1-Oleoyl-sn-glycero-3-phosphoethanolamine
8	10761	23.5043	783	9	866	882	0.01...	261.2225	-4.6	[M+H-2H2O]+	5	918	-2.0144	H-2	42	[M+H-2H2...	11-Hydroxy-9-octadecenoic acid	(9Z,12Z)-15-Hydroxyoctadeca-9,12-dienoic acid
9	10769	23.5260	967	100	967	971	0.04...	468.3105	-4.3	[M+H]+	11	969	0.0020	0	100	[M+H]+	1-Myristoyl-sn-glycero-3-phosphocholine	1-Myristoyl-sn-glycero-3-phosphocholine
10	10781	23.5633	744	87	744	792	0.806	295.1916	-4.1	[M+H]+		924	-24.0352	C-3H-4O	3.4	[M+H]+	(E)-5-(1,2,4a,5-Tetramethyl-7-oxo-3,4,8,8a-...	Oxacyclotetradeca-3,6,9-trien-2-one, 5-hydroxy-8-meth.
11	10784	23.5632	909	7	909	924	0.196	235.1703	-4.3	[M+H-3H2O]+		934	-11.9990	C-1	17	[M+H-H2O]+	8-Hydroxy-16-methyloxacyclohexadeca-3,11...	1,2,3-Propanetriol, 2-(decahydro-1,8-dihydroxy-4a,8-di..
12	10785	23.5638	6	26	32	79	0.193	340.2497	28.8	[M+H-NH3]+	7	706	105.08...	C4H11NO2	0.15	[M+H]+	Polygodial	N-Lauroyl-L-arginine
13	10787	23.5632	405	2	550	478	0.112	111.0445	-3.6	[M+H-NH3]+	2	805	-11.9996	C-1	17	[M+H-H2O]+	4-(Hydroxymethyl)benzene-1,3-diol	[5-(Aminomethyl)furan-2-yl]methanol
14	10790	23.5635	911	7	911	922	0.06...	217.1596	-4.1	[M+H-H2O]+	11	911	0.0009	0	100	[M+H-H2O]+	Valerenic acid	Valerenic acid
15	10795	23.5634	737	4	769	799	0.03...	123.1173	-4.1	[M+H-H2O]+	10	843	-14.0152	C-1H-2	93	[M+H-NH3]+	1,3,3-Trimethyl-6-azabicyclo[3.2.1]octane	Non-8-en-2-one
16	10796	23.5634	756	14	756	813	0.03...	189.1645	-3.7	[M+H-H2O]+	10	804	-14.0149	C-1H-2	93	[M+H-2H2...	Jaeskeanadiol	1-Methyl-4-(4-methylpent-3-en-1-yl)cyclohex-3-enecarb..
17	10799	23.5763	0	0	145	0	0.02...	312.3638	-4.2			524	-2.8892	C3H11NO-4	0.0038	[M+H]+	12,13-Dihydroxy-9Z-octadecenoic acid	NoID_312.36

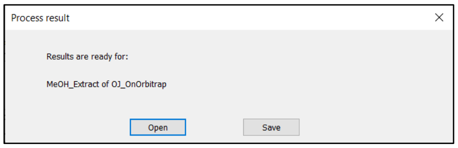
Desktop display of an expanded region of the Chromatogram tab view resulting from Enhanced Analysis MS/MS Hybrid routine with a selected component which has a Highlight Score >=800 and a Filter Score <800 (See MS/MS Chromatogram Window in on-line Help file (Search) while displaying Chromatogram tab view. The sample was commercial orange juice extracted with methanol, separation with 2.1mm C18 column (m/z range 80 to 1200) on Thermo Fisher Lumos Orbitrap mass spectrometer (10 µL sample, analysis time 30 min).

Processing an LC-MS/MS (DDA) Data file using the NIST Hybrid Search

When processing LC-MS/MS data files, either a conventional Identity Search can be carried out or an advanced search involving the conventional search and a Hybrid Search can be performed.

Before initiating the Hybrid option of an **Analyze MS/MS Input File**, it is a good idea to move the slider bar between the **Hit List** and **Structure** windows of the **Chromatogram** tab view all the way to the right so that the entire lower part of the **Chromatogram** tab view is available for the **Hit List**. Several column headers beginning with **H** will appear in the **Hit List** as result of the operation which are selected by default but only appear in **Hit List** window when the Hybrid search is selected. The structure for Hits can be viewed using the **Show Structures** selection from the **View** menu of the Main Menu. This provides the floating **Structure** window. The hybrid Search function should be used with the hr_nist_msms Library

To initiate the Hybrid Search of deconvoluted components, select the **Hybrid Search (long run time)** check box (☐) in the **Select MS/MS Input File** dialog box displayed by selecting **Analyze MS/MS Input File** from the **File** menu displayed by selecting **File** on the Main Menu. Depending on the size of the data file, this can involve a long run time (~55 min. for the file shown below. It is suggested that this procedure always be run in the background (select ☐ **Run in background** in the dialog box). If the **Chromatogram** tab view has the contents of a previous run displayed, that will remain until MS Search is instructed to display the results of the just completed process. During this process, the label **Running Background** will appear in **RED** in the **Filter Bar** of the **Chromatogram** tab view. Other features of MS Search can be used during this time. Once the file has been processed, the dialog box at the left will be displayed regardless of what tab view is currently displayed. If the Open selection is chosen on the dialog box, the display can take place in more than 30 to 60 sec. During the processing, the selections **Analyze EI Input File**, **Analyze HiRes EI Input File**, and **Analyze MS/MS Input Files** will be grayed (unavailable) on the **File** menu displayed by selecting **File** on the Main Menu.



Results are ready for:

MeOH_Extract of OJ_OnOrbitrap

As seen in *Figure 50*, if the search results are displayed with a linear abundance axis, distinguishing components and their types can be difficult; therefore, it advantageous to show the abundance axis using a log scale (*Figure 51*)

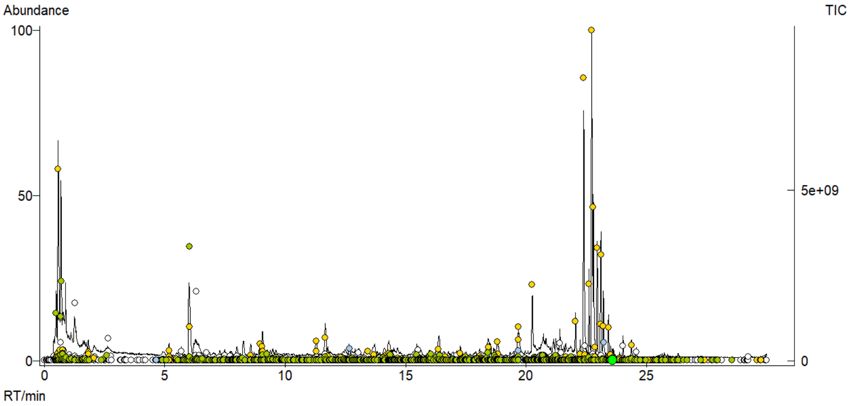


Figure 50. Processed LC-MS/MS data file using combination of Hybrid/Conventional Tandem Library Search with a linear abundance axis.

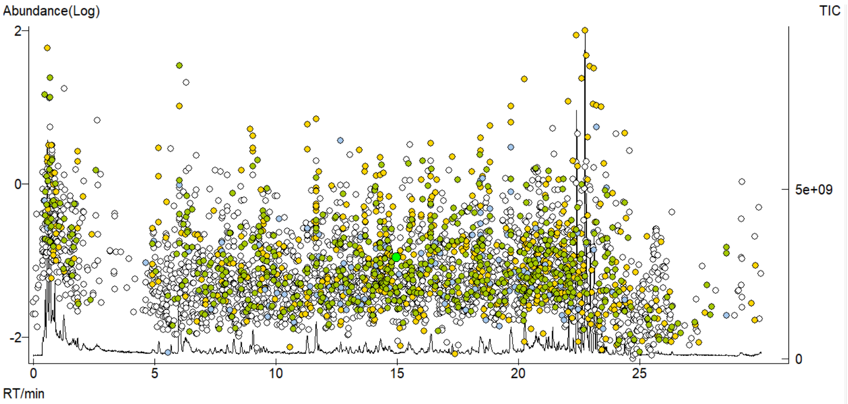
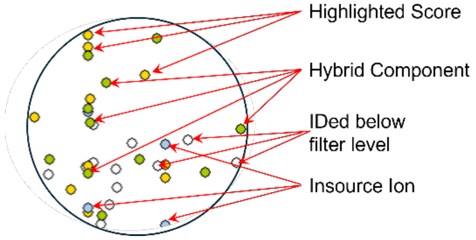
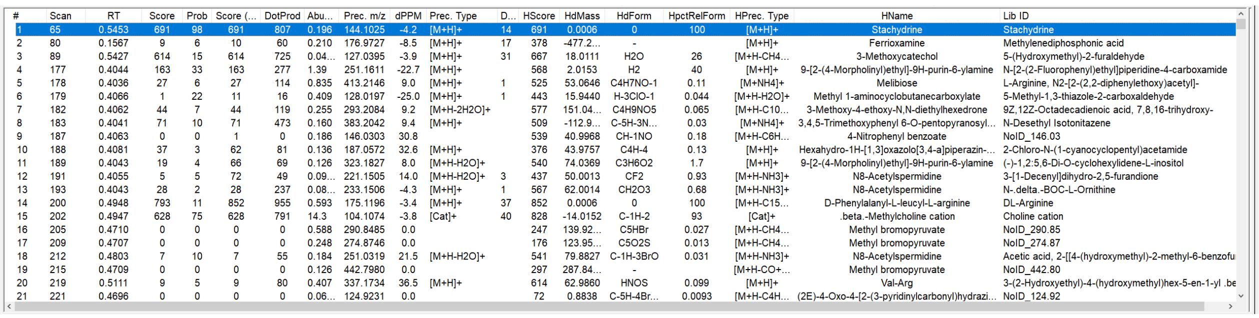


Figure 51. Processed LC-MS/MS data file using combination of Hybrid/Conventional Tandem Library Search with a log abundance axis. Individual components are much easier to Identify.



Identified components are indicated by individual circles (○). Colors are used to identify the classification of the component (●, selected; ●, Highlighted; ●, Insource; ●, Hybrid; and ○, < highlight value) When the **Chromatogram** window is expanded the value of **n** in the **Title Bar** is reduced to the number of components visible in the window and the **Hit List** window now only contains the Hits for the components shown in the **Chromatogram** window. See *Figure 51*.



#	Scan	RT	Score	Prob	Score (...	DotProd	Abu...	Prec. m/z	dPPM	Prec. Type	D...	HScore	HdMass	HdForm	HpctRelForm	HPrec. Type	HName	Lib ID
1	65	0.5453	691	98	691	807	0.196	144.1025	-4.2	[M+H]+	14	691	0.0006	0	100	[M+H]+	Stachydrine	Stachydrine
2	80	0.1567	9	6	10	60	0.210	176.9727	-8.5	[M+H]+	17	378	-477.2...	-		[M+H]+	Ferrioxamine	Methylenediphosphonic acid
3	89	0.5427	614	15	614	725	0.04...	127.0395	-3.9	[M+H]+	31	667	18.0111	H2O	26	[M+H-CH4...	3-Methoxycatechol	5-(Hydroxymethyl)-2-furaldehyde
4	177	0.4044	163	33	163	277	1.39	251.1611	-22.7	[M+H]+		568	2.0153	H2	40	[M+H]+	9-[2-(4-Morpholinyl)ethyl]-9H-purin-6-ylamine	N-[2-(2-Fluorophenyl)ethyl]piperidine-4-carboxamide
5	178	0.4036	27	6	27	114	0.835	413.2146	9.0	[M+H]+	1	525	53.0646	C4H7NO-1	0.11	[M+NH4]+	Melibiose	L-Arginine, N2-[2-(2,2-diphenylethoxy)acetyl]-
6	179	0.4066	1	22	11	16	0.409	128.0197	-25.0	[M+H]+	1	443	15.9440	H-3ClO-1	0.044	[M+H-H2O]+	Methyl 1-aminocyclobutanecarboxylate	5-Methyl-1,3-thiazole-2-carboxaldehyde
7	182	0.4062	44	7	44	119	0.255	293.2084	9.2	[M+H-2H2O]+		577	151.04...	C4H9NO5	0.065	[M+H-C10...	3-Methoxy-4-ethoxy-N,N-diethylhexedrone	9Z,12Z-Octadecadienoic acid, 7,8,16-trihydroxy-
8	183	0.4041	71	10	71	473	0.160	383.2042	9.4	[M+H]+		509	-112.9...	C-5H-3N...	0.03	[M+NH4]+	3,4,5-Trimethoxyphenyl 6-O-pentopyranosyl...	N-Desethyl Isotonitazene
9	187	0.4063	0	0	1	0	0.186	146.0303	30.8			539	40.9968	CH-1NO	0.18	[M+H-C6H...	4-Nitrophenyl benzoate	NoID_146.03
10	188	0.4081	37	3	62	81	0.136	187.0572	32.6	[M+H]+		376	43.9757	C4H-4	0.13	[M+H]+	Hexahydro-1H-[1,3]oxazolo[3,4-a]piperazin-...	2-Chloro-N-(1-cyanocyclopentyl)acetamide
11	189	0.4043	19	4	66	69	0.126	323.1827	8.0	[M+H-H2O]+		540	74.0369	C3H6O2	1.7	[M+H]+	9-[2-(4-Morpholinyl)ethyl]-9H-purin-6-ylamine	(-)-1,2:5,6-Di-O-cyclohexylidene-L-inositol
12	191	0.4055	5	5	72	49	0.09...	221.1505	14.0	[M+H-H2O]+	3	437	50.0013	CF2	0.93	[M+H-NH3]+	N8-Acetylspermidine	3-[1-Decenyl]dihydro-2,5-furandione
13	193	0.4043	28	2	28	237	0.08...	233.1506	-4.3	[M+H]+	1	567	62.0014	CH2O3	0.68	[M+H-NH3]+	N8-Acetylspermidine	N-.delta.-BOC-L-Ornithine
14	200	0.4948	793	11	852	955	0.593	175.1196	-3.4	[M+H]+	37	852	0.0006	0	100	[M+H-C15...	D-Phenylalanyl-L-leucyl-L-arginine	DL-Arginine
15	202	0.4947	628	75	628	791	14.3	104.1074	-3.8	[Cat]+	40	828	-14.0152	C-1H-2	93	[Cat]+	.beta.-Methylcholine cation	Choline cation
16	205	0.4710	0	0	0	0	0.588	290.8485	0.0			247	139.92...	C5HBr	0.027	[M+H-CH4...	Methyl bromopyruvate	NoID_290.85
17	209	0.4707	0	0	0	0	0.248	274.8746	0.0			176	123.95...	C5O2S	0.013	[M+H-CH4...	Methyl bromopyruvate	NoID_274.87
18	212	0.4803	7	10	7	55	0.184	251.0319	21.5	[M+H-H2O]+		541	79.8827	C-1H-3BrO	0.031	[M+H-NH3]+	N8-Acetylspermidine	Acetic acid, 2-[[4-(hydroxymethyl)-2-methyl-6-benzofu
19	215	0.4709	0	0	0	0	0.126	442.7980	0.0			297	287.84...	-		[M+H-CO+...	Methyl bromopyruvate	NoID_442.80
20	219	0.5111	9	5	9	80	0.407	337.1734	36.5	[M+H]+		614	62.9860	HNOS	0.099	[M+H]+	Val-Arg	3-(2-Hydroxyethyl)-4-(hydroxymethyl)hex-5-en-1-yl .be
21	221	0.4696	0	0	0	0	0.06...	124.9231	0.0			72	0.8838	C-5H-4Br...	0.0093	[M+H-C4H...	(2E)-4-Oxo-4-[2-(3-pyridinylcarbonyl)hydrazi...	NoID_124.92

Figure 52. Hit List for Hybrid Search as displayed as a result of processing data using the Analyze MS/MS Input File.

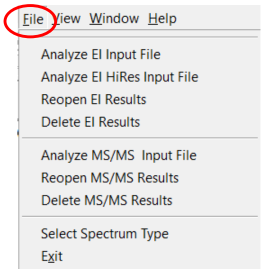
As can be seen in *Figure 52,* the **Hit List** for the Hybrid Search in **Chromatogram** tab view is very complex, as is the **Hit List** for the Hybrid Search in **Lib Search** tab view.

Column Header Names Related to the Hybrid Search in LC-MS/MS Chromatogram Processing
HdMass – Delta Mass from best matching hybrid search identification
HScore – Best Hybrid Score
HdForm – Chemical formula corresponding to HdMass
HpctRelForm – Percent frequency of HdForm relative to most common -CH2- (100%)
HPrec.Type – Precursor Type of library spectrum with highest hybrid score
HName – Name of compound with best hybrid match

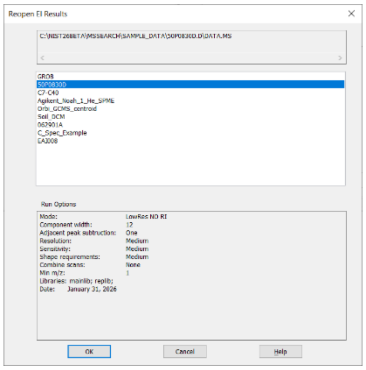
Evaluation of Search results, especially when using the Hybrid Search in the **Chromatogram** tab view, can be facilitated by repositioning columns. This is accomplished by putting the Mouse pointer on the column header title and dragging it the right or left. Sizes of the column can also be adjusted. A good place to get a better understanding of using the Hybrid search in the **Chromatogram** tab view is in the On-Help file. Select **Help** from the Main Menu. From the **Help** menu, select Help Topics to display the **Help** dialog box. If the left panel is not displayed, Select the **Show** button in the button bar of the dialog box (first button on left with panel not displayed). This will display the panel. Select the **Search** tab, in the **Keywords** text entry box type **MSMS**, and then select the **List Topics** button to the right and under the text entry box. Then select **MS/MS Chromatogram Window**. The display will have the details of the Chromatogram tab view's

If the **Lib ID** and the **HName** are the same in the **Hit List**, this is a good indicator that the component has been identified as a good match. The **Score**, **Hscore**, **DotProd**, and **Scored (Unfiltered)** will be high (above 850) and close in value. If the **HScore** is high and **Score** is low, them the **HName** and **HdForm** should be explored as possibilities leading to Hybrid search ID.

Important Note About processed data in the Chromatogram tab view



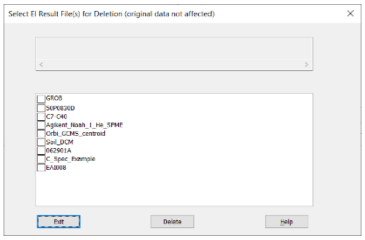
When MS Search is exited, the current results in the **Chromatogram** tab view of a processed data file are not reloaded when the program is restarted unless **Reload on Startup** ☐ is selected in the **Filter Bar**. The results of each processed data file (GC/MS and LC-MS/MS) are stored in perpetuity until they are purposely removed whether this check box is selected or not. It is also possible to redisplay a previously proceeded data file (before closing MS Search or during a current processing session). Both the **Reopen** and **Remove** commands are accomplished with selections on the **File** menu of the Main Menu in the Chromatogram tab view using the **Reopen** and **Delete** selections for **EI Results** and **MS/MS Results**. If these actions are carried out during the processing of a data file, when the process is complete the Process result dialog box shown on *page 61* will be displayed.



C:\NIST26BETA\MSSEARCH\SAMPLE_DATA\50P0830D.D\DATA.MS

GROB
50P0830D
C7-C40
Agilent_Noah_1_He_SPME
Orbi_GCMS_centroid
Soil_DCM
062901A
C_Spec_Example
EA1008

Mode: LowRes NO RI
Component width: 12
Adjacent peak subtraction: One
Resolution: Medium
Sensitivity: Medium
Shape requirements: Medium
Combine scans: None
Min m/z: 1
Libraries: mainlib; replib;
Date: January 31, 2026



☐ GROB
☐ 50P0830D
☐ C7-C40
☐ Agilent_Noah_1_He_SPME
☐ Orbi_GCMS_centroid
☐ Soil_DCM
☐ 062901A
☐ C_Spec_Example
☐ EA1008

The Restore and Delete actions are done using the **File** menu. is done from the **File** menu regardless of the **Spectrum type** but commands are specific for the **Spectrum type**. Just above, to the left is the **File** menu displayed when the **Spectrum type** is set to **Any**. The dialog box on the left is displayed if one of the **Restore Results** options is selected. The upper window of the dialog box contains a list of the names of processed data files. The lower window contains a list of options used in the processing of the data files. Be prepared to edit the columns displayed in the **Reopen**ed file's **Hit List**. after a file is selected for **Reopen**ing. The dialog box on the right is displayed when one of the **Delete Results** selections is made from the **File** menu. Select the check box (☐) next to the file to be deleted and then select the **Delete** button. If the Exit button is selected, without first selecting the **Delete** button, the dialog box will close, and the file will NOT be deleted.

Cautionary Statement About the Use of MS Search

The NIST Mass Spectral Search Program is not *maintenance free*. By default, the results of each spectrum search performed in the **Lib Search** tab view, each search performed in the **Other Search** tab view, and the results of processed file in the **Chromatogram** tab view are automatically stored when the program is exited. This can result in the unnecessary use of valuable computer storage. The program can be configured to automatically delete the **Lib Search** and **Other Search** tab view's **Histories** when the program is exited (settings in the **Hit List** and/or **Spec List** pages of the **Library Search Options** or the **Hit List** page of **Search (Other) Properties** dialog boxes of the **Lib Search** and **Other Search** tab views, respectively). These histories can also be deleted using the **Clear history** button (Ⓗ) in the button bar of either tab view.

The Chromatogram tab view CANNOT be configured to be automatically deleted, and proactive actions must be taken by the operator to control the possible deleterious results of such storage.

Lib. Search tab view

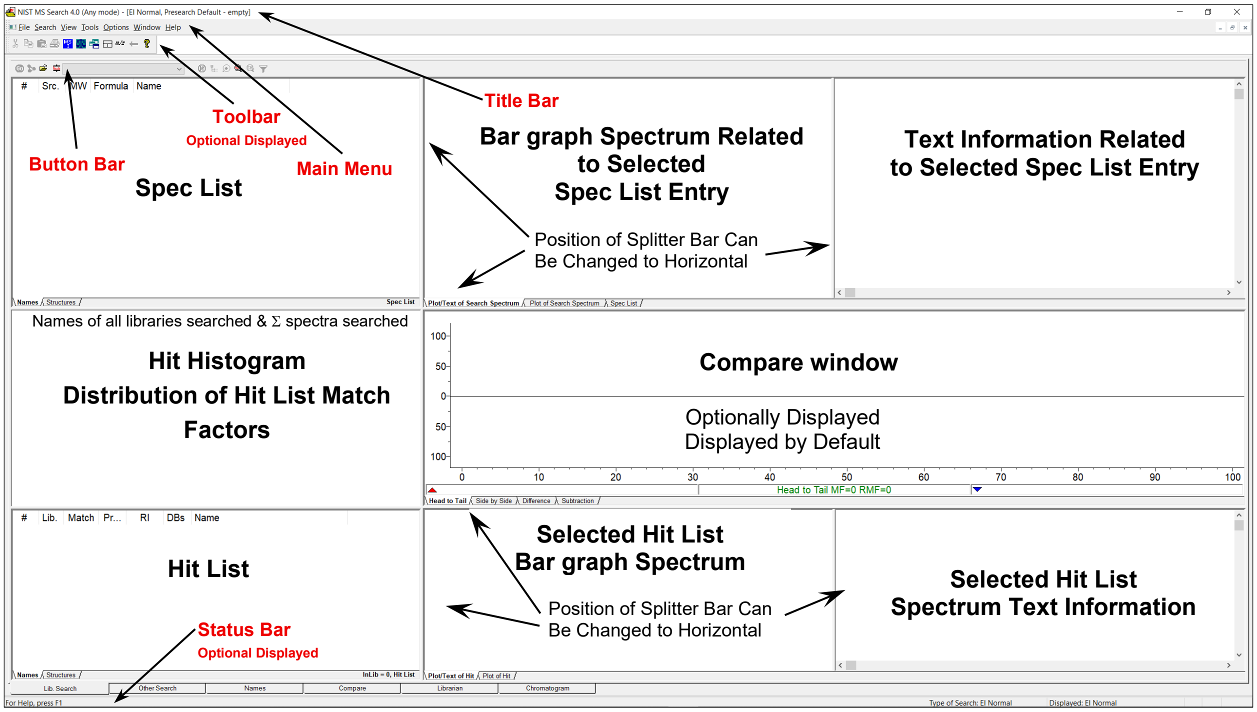
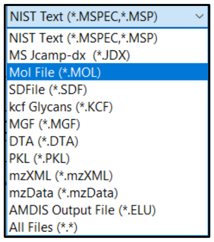


Figure 53. Lib. Search tab view.

The **Lib. Search** tab view is where a search of an individual spectrum or individual structure against mass spectral libraries is performed: user-libraries, and/or the **NIST 26 Libraries**. The spectrum and structure searches can be done against multiple libraries (maximum of 127), and the selected libraries can be different from those selected for the various searches found in the **Other Search** tab view. Spectra can be imported into the **Spec List** window from linked mass spectral data system software, text files containing one or more spectra in the NIST format (some other text file formats can also be used), from spectra on the Windows Clipboard as text files in the NIST format, or of components (product-ion or EI) found in the **Chromatogram** tab view.

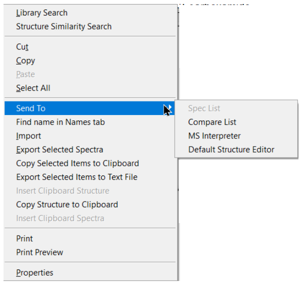
> ***Nota Bene:*** The NIST mass spectral file format is simply a text file that can be formed or edited using any text editor, such as *Notepad*. The layout of the file has specific requirements. These are separate for EI and Tandem spectra, specifically described in the **Librarian** section of this manual.



Recognized NIST mass spectral file extensions.

Files in NIST text formats are imported by selecting **File\Open** from the Main Menu. This results in the display of the **Choose file for spectra/structure import** dialog box being displayed. On the lower right portion of the dialog box, above the **Open** and **Cancel** buttons is a dropdown list box with the extension of most of the text files recognized by MS Search. The one that is missing is the *.TXT file. The *.TXT files will appear in the dialog box when the **All Files (*.*)** option. is selected. If emailing an NIST text spectrum, it is best to use the TXT extension or send as a ZIP or 7z file.

The **Spec List** window is at the top left of the **Lib. Search** tab view. The **Hit Histogram** window is directly below the **Spec List** window, showing the distribution of Hits according to the **Match Factor** values, the names of searched libraries, and the total number of spectra searched. This **Histogram** window can be displayed with a linear or log vertical axis. The text at the top of this window is the names of the libraries searched, and the total number of spectra searched. The **Hit List** window is on the lower left directly below the **Histogram** window. It is important to note that in addition to the Hit position in the list, name of the library containing the Hit spectrum, a value for the match, and the primary name for each Hit, several other values may be displayed using settings available in the **Hit List** page of the **Library Search Properties** dialog box, which is display using the **RMB** menu. Hits in the **Hit List** window are listed according to descending **Match Factor** values (by default), regardless of which of the searched libraries they come from.



RMB menu displayed from the Spec List window.

On the right side of the single movable vertical splitter bar, going from the top to the bottom, are three separate windows. The top window is the **Search Spectrum/Spec List Spectrum** window which has three separate views. Each is brought into focus by selecting one of the three tabs at the bottom of the window. The first tab display is the **Plot/Text of Searched Spectrum** window. It is divided into two parts with a vertical splitter bar (Plot and Text Information). After a search, this view is automatically defaulted. The second display is that of the **Plot of Searched Spectrum**. This view only comes into focus when the tab is selected and shows just the bar graph spectrum of the last searched spectrum, no Text Information. The third view, **Spec List**, is automatically selected when an entry in the **Spec List** window is selected (highlighted). This view is divided with a vertical splitter bar. One side is the bar graph plot, and the other is the text information of the currently selected spectrum in the **Spec List** window.

The middle window on the right side of the **Lib. Search** tab view is the **Compare** window. By selecting **View** from the Main Menu, the display of the **Compare** window can be dismissed; it may also be minimized using the horizontal splitter bar just above the **Plot/Text of Hit** windows. This window displays the plot of the currently searched spectrum and the selected Hit from the **Hit List**. There are four tabs: **Head to Tail** (search, red and Hit blue, default), **Side by Side** (*ibid*), **Difference** (left over by subtracting Hit from searched (green, by default)/leftover by subtracting searched from Hit blue, by default), and **Subtraction** (remaining when Hit is subtracted from search, green by default). The **Subtraction** is helpful when the searched spectrum may represent more than a single compound. The spectrum displayed when the **Subtraction** tab is selected can be searched using the **RMB** menu when the Mouse pointer is in the **Compare** window. Peaks colors for all tab views can be changed in the Compare page of the Library Search Properties dialog box when displayed from the RMB menu with the Mouse pointer in the **Compare** window.

The bottom window is for the plot and text information (**Plot/Text of Hit**) or just the plot (**Plot of Hit**) of the selected entry in **Hit List** window. These views are selectable by tabs at the bottom of this window. There is no automatic switching between the two views. The view remains until changed back by selecting the appropriate tab.

If the Mouse pointer is in the **Search Spectrum/Spec List Spectrum** window or the **Hit List Spectrum** window, the **RMB** menu option **Change Splitter Orientation** can be used to change the orientation (back and forth between vertical and horizontal) of the splitter bar between the plot of and text information for the spectrum. In all cases the **RMB** menu can be used to access the appropriate page in the **Properties** dialog box for each window within any tab view.

Using the horizontal splitter bars between the three windows on the left side of the **Lib. Search** tab view allows the vertical size of these windows to be adjusted. This can be used to maximize the **Hit List** window's vertical space to view a larger number of Hits without resorting to the scroll bar on the right of the window. The vertical splitter bar on the right side of the **Hit List** window of the **Lib. Search**, **Other Search,** and **Names** tab views can be used to increase the **Hit List** window's width. The lefthand vertical window in the **Librarian** tab view contains the spectra listed in the **Spec List** window of the **Lib. Search** tab view. It is important to know that the **Spec List** window in the **Lib. Search** tab view and the **Spec List** window in the **Librarian** tab view is the SAME LIST. Spectra may be copied to and deleted from this **Spec List** window in either the **Lib. Search** or **Librarian** tab view. Actions taken in the **Spec List** window of either of these tab views are reflected in the other. The **Hit Lists** in the **Lib. Search**, **Other Search**, and **Chromatogram** tab views are read-only. If a spectrum is edited in the **Librarian** tab view, the edits will appear when that spectrum is viewed from the

Lib. Search tab view. The **Spec List** window in **Lib. Search** and **Librarian** tab views and the **Hit List** window in the **Lib. Search** and **Other Search** tab views can be displayed as text or as graphic structures by selecting the **Names** or **Structures** tab at the bottom of the window. By default, limited text information is included with the structure display. This can be turned off using the **Properties** dialog box displayed using the **RMB** menu. If the structure display is selected for the Hit Lis or Spec List window and the spectrum has no associated structure, but the Formula field for the spectrum is populated, the element composition will appear in lieu of the structure. If the **Formula** is not populated, the block in the **List** that would normally display the structure/elemental composition will be blank.

Shown in the figure on the previous page, on the right, is the **RMB** menu displayed when the Mouse pointer is in the **Spec List** window of the **Lib. Search** tab view. The **RMB** menus for all windows in all tab views are similar. Some items are self-explanatory, like **Export Selected** and **Import**, which open **File** dialog boxes. When a spectrum or group of spectra are exported to an existing file, the option to **Overwrite** or **Append** that file is offered. The items on the **Send To** submenu are self-explanatory. The **Default Structure Editor** is only on the menu if there is a default structure editor set up on the computer that has the NIST MS Search Program installed. Both structures (in the MOL file format) and spectra (in the MSP/MSPEC text format) can be imported from the Windows Clipboard. Structures, but not spectra can be copied from MS Search to the Windows Clipboard. One very important feature of the **Spec List** window (in the **Lib. Search** and **Librarian** tab views) and the **Hit List** window (in the **Lib. Search** and **Other Search** tab views) **RMB** menus is the ability to print the contents or view a preview of the print of the contents of either the **Hit Lists** or the **Spec List** windows. The purpose of the **Properties** selection of these **RMB** menus is discussed throughout this manual.

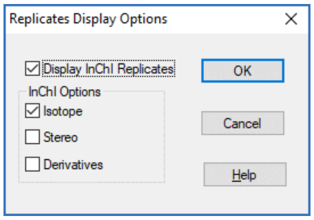
Each **Hit List** window (except the one in the **Names** tab view) and the **Spec List** window can be sorted by ascending or descending order by clicking on the column name in the **Title Bar** at the top of the window. The **Hit List** windows in the **Lib. Search** and the **Other Search** tab views have optionally displayed columns. Three of these show the number of Synonyms, the number of Other Databases (non-mass spectral DBs) containing the compound and the Retention Index. The **Hit List** window (except for the one shown window in the **Names** tab view) can also be sorted by these columns (or other columns) by pointing to the column header and clicking the left Mouse button. The position of the columns on the **Hit Lists** can be changed by putting the Mouse pointer on the column title to be moved, holding down the left Mouse button and dragging the pointer to the desired position and then releasing the Mouse button. To restore to the previous position, the process must be repeated.

Creating individual configurations to match a Library Search type or for individual user's preferences is why the **Save Configurations** and **Restore Configurations** item was placed on the **File** menu. Additional information on items in the **Properties** dialog boxes, displayed by selecting **Properties** from the **RMB** menu, can be found by selecting the **Help** button in the **Properties** dialog box**.**

Selected lines in a **Hit List** or **Spec List** can be copied into the Windows Clipboard in tab-separated text format, by highlighting the desired lines and then pressing the **Ctrl+K** keyboard keys. This only copies the contents of **Hit List** or **Spec List** window, not the entire spectrum.

New to v.4.0 are the **RMB** menu selections **Copy Select Hits to Clipboard** and **Export Select Spectra to Text File** displayed when the Mouse pointer is in any **Hit List** or **Spec List** window (**Lib. Search**, **Other Search**, **Librarian**, or **Chromatogram** tab views). Items to be copied, that are heighted, are then exported to a text file and sent to the Windows Clipboard.

Also new to v.4.0 is when a spectrum is sent to the **Spec List** using the **RMB** menu's **Send To\Spec List** selection and that identical spectrum is already on the **Spec List**, of the tab view being sent to (**Lib. Search**/**Librarian**), that spectrum will be highlighted if that tab view is switched to right after sending. In previous version of MS Search, such circumstances resulted in the display of an error message. If multiple (more than 1) spectra are sent an error message will be displayed.


Replicates Display Options

☑ Display InChI Replicates

InChI Options

☑ Isotope

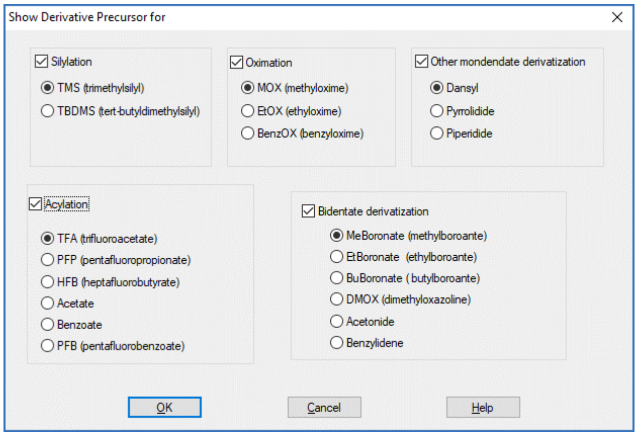
☐ Stereo

☐ Derivatives

In some cases, the **Hit Lists** will also have a ⊞ symbol in front of the Hit number. This symbol means that there are replicate spectra for that Hit. Selecting the ⊞ symbol will cause a list of the replicates to appear below the Hit. Some of the names of the replicates will be preceded by **R**, and some by **m** or **r**. If an **R** precedes the name, the spectrum is of the same compound as the Hit (has same InChIKey) and is in the **NIST replib Library**. If the name is preceded by an **m** or an **r** it means that the replicate is a derivative, isotopic variant, or isomer of the Hit. If **m**, the spectrum is in the NIST **mainlib** and if **r,** the spectrum in the **NIST replib**. The display of spectra of derivatives and isomers as replicates of a compound is controlled through the **Replicates Display Options** dialog box (shown above) selected from **Replicates/Related** on the **Options** menu, displayed from the Main Menu. Details of how this works are found by selecting the **Help** button.

Selecting **View/Derivative Precursor** menu opens the **Show Derivative Precursor for** dialog box (Figure 46). Selecting derivative type(s) affects **Lib. Search** tab view's **Hit List** window's structure display. The derivative precursors and the derivatizing agents are shown as separate item instead of the structure of the derivative. Only **mainlib** and **replib** hits are affected. Deselecting displayed derivative type(s) immediately changes structure display back to derivative. See MS

Search help topic "Show Derivative Precursor" for more details. Only one subtype per derivative type is allowed. Multiple derivative types can be selected.



Show Derivative Precursor for

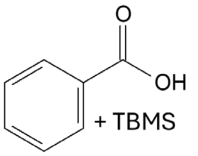
☑ Silylation
● TMS (trimethylsilyl)
○ TBDMS (tert-butyldimethylsilyl)

☑ Oximation
● MOX (methyloxime)
○ EtOX (ethyloxime)
○ BenzOX (benzyloxime)

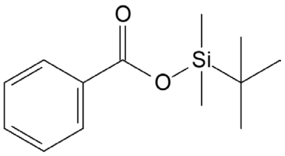
☑ Other mondendate derivatization
● Dansyl
○ Pyrrolidide
○ Piperidide

☑ Acylation
● TFA (trifluoroacetate)
○ PFP (pentafluoropropionate)
○ HFB (heptafluorobutyrate)
○ Acetate
○ Benzoate
○ PFB (pentafluorobenzoate)

☑ Bidentate derivatization
● MeBoronate (methylboroante)
○ EtBoronate (ethylboroante)
○ BuBoronate (butylboroante)
○ DMOX (dimethyloxazoline)
○ Acetonide
○ Benzylidene



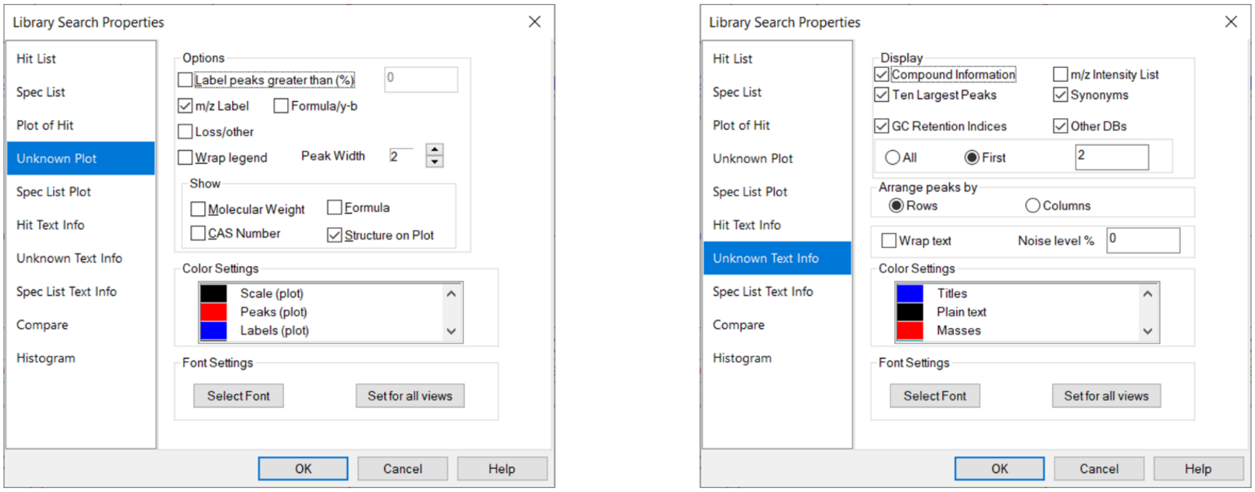
Alternate Display



Normal Display

Figure 54. Select any one of the five Derivative type checkboxes; then select the subcategory radio button.

In the **Lib. Search** tab view, the upper right window with a vertical splitter bar separating the **Plot** and **Text Info** windows for the **Spec List/Searched** spectrum has settable attributes that are found in the **Properties** dialog box described below. Displayed in the lower left corner of this **Plot** window is the origin of the spectrum (source name) and the primary name associated with the spectrum (also referred to as the mainlib name). The origin of the spectrum can be the “name of the library” from which the spectrum is from; “Text File” for spectra imported from outside sources; or, when an existing **Spec List** spectrum is displayed after closing and re-starting the MS Search, “Spec List”. Optionally, preceding the (source name), the Molecular Weight (nominal mass) of the compound, CAS registry, and NIST numbers (if the spectrum has one, if not only the CAS#), and Formula (elemental composition) can be displayed provided if these fields are populated in the spectrum’s record. This legend display can be forced to wrap so that all characters will be visible. Also, optional, a structure (if associated with the spectrum) can be displayed on the spectrum, if associated with the spectrum. The structure will be replaced by the elemental composition if the **Formula** field is populated, and no structure is associated with the spectrum. Labeling of the peaks is either by default or set on the **Spec List Plot** page of the **Library Search Properties** dialog box, which has a **Label peaks greater than (%)** option followed by a *text entry box* allowing for the input of a number. If the spectrum is to be displayed without peak labeling, enter 100 in this box or deselect the ***m/z* Label** option. It is also possible to use this page of this **Library Search Properties** dialog box to change the colors of the mass spectral peaks, scale, labels, bonds and rings in structures, atomic symbols in structures, and the spectrum background. Selecting the **Set default layout** button on the **Tool Bar** will restore colors to the original values. It is also possible to adjust the thickness of the mass spectral peaks. This value is not restored when the **Set default layout** button is selected. The **Spec List Plot** and **Unknown Plot** pages have the same selections; however, they are independent.



Library Search Properties

Options
☐ Label peaks greater than (%) 0
☑ m/z Label ☐ Formula/y-b
☐ Loss/other
☐ Wrap legend Peak Width 2
Show
☐ Molecular Weight ☐ Formula
☐ CAS Number ☑ Structure on Plot
Color Settings
Scale (plot)
Peaks (plot)
Labels (plot)

Library Search Properties

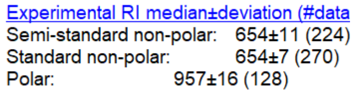
Display
☑ Compound Information ☐ m/z Intensity List
☑ Ten Largest Peaks ☑ Synonyms
☑ GC Retention Indices ☑ Other DBs
○ All ● First 2
Arrange peaks by
● Rows ○ Columns
☐ Wrap text Noise level % 0
Color Settings
Titles
Plain text
Masses

Figure 55. Spec List/Unknown Plot page and Unknown Text info page of the Library Search Properties dialog box displayed in the Lib. Search tab view.

The **Text Info** side of this window, by default, is to the right of the portion displaying the plot of the spectrum. This orientation can be changed so that the **Text Info** window is just above the **Plot** presentation by using the **RMB** menu selection **Change Splitter Orientation**. In the other tab views where both the plot of the spectrum and the text information are displayed, these two windows are oriented, by default, with the **Plot** window above the **Text Info** window. Again, this orientation can be changed using the appropriate selection on the **RMB** menu when the Mouse pointer is on either of the two windows. The **Unknown Text Info** page of the **Library Search Properties** has options with respect to what text data are displayed. These options are identical to those on the **Hit Text Info** page.

The **Hit Text Info** page (**Plot/Text** tab in some tab views) in the Plot of Hit pages of the **Library Search Properties** dialog box have the same selectable display options in the form of checkboxes as the **Unknown Text Info** and **Unknown Plot /Spec List Plot** pages. The mainlib name is the only thing displayed on the first line and is included as part of the display of the **Compound Information**. The second line of is reserved for the **Formula** (elemental composition), which is included in the display of the **Compound Information**. The third line contains the following **Compound Information** components: **MW** (nominal mass); **Exact Mass** (if there is an elemental composition associated with the spectrum); **CAS** registry number, if in the record; **NIST** number (only NIST spectra, sequential number assigned to spectra in the NIST Archive); **ID** number (position in the library containing the spectrum); and name of the library containing the spectrum. The fourth line also contains information about the compound/spectrum: **Contributor** (NIST Library)/**Comments** (user libraries). The fifth line is the compound's **InChIKey** (if a structure is associated with the spectrum), and the sixth line contains **Related CAS** registry numbers, if any (NIST distributed libraries only). All of the above is only displayed when the **Compound Information** checkbox in the **Text Info** page is selected.

For the most part the other checkboxes under the section labeled **Display** of the **Hit Text Info**, **Unknown Text Info**, and **Spec List Info** pages of the **Library Search Properties** dialog box are self-explanatory, except for the one next to the **GC Retention Indices** label. If there is a cheek in this box, following list of **Synonyms** in the **Text Information** window will be the label **Experimental RI median±deviation (#data)**, if the spectrum is from the NIST EI library and that **CASrn** has entries in the GC Metod/RI database. Under this label are up to three lines; one each for **Semi-standard non-polar**, **Standard non-polar**, and **Polar** GC columns. Each line is followed by a number representing the median RI value of all the enters in the GC Method/RI database for the compound measured on that type of column. The number following the ± symbol is the deviation. The last number, in parentheses, is the number of measurements in the GC Method/RI database.

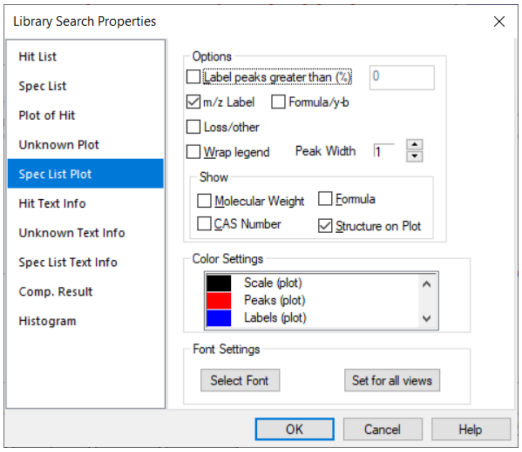


Experimental RI median±deviation (#data
Semi-standard non-polar: 654±11 (224)
Standard non-polar: 654±7 (270)
Polar: 957±16 (128)

Experimental data from the NIST GC Method/RI Database for Benzene (NIST# 114388).

The next label under the experimental RI data is **Estimated semi-standard non-polar retention index (n-alkane scale)**. This value will be displayed for any spectrum with a structure, regardless of it being an NIST EI spectrum or not. This value is rule-based and may not be displayed for some compounds like organophosphate, even if the compound is in the NIST EI Library. The next label is **AI predicted semi-standard non-polar retention index (n-alkane scale)**. This value is exclusive to spectra in the NIST EI Library. There is a value for every compound in the NIST EI Library. This value has been found to be in better agreement with the experimental RI values than those determined using the rule-based method.

The next labeled field is **Retention Index**. If there are records containing literature GC method/Retention Index data, these records will be displayed following this labeled field. The number of displayed GC Method/Retention Indexes can be controlled. Just under the **GC Retention Indices** and **Other DBs** checkboxes in the **Library Search Properties** dialog box are two radio buttons: **All** and **First**. If **All** is selected, all retention indices associated with that compound will be displayed, one after another. If **First** is selected, only the number entered in the text box to the right will be displayed. By default, only the first two records are displayed. If there are more than the specified number of records, <…> will appear just below the last line of the bottom record. The records are string-searchable using the **Find** selection on the **RMB** menu when displayed in a **Text Info** window. All text is searched, which means GC column types, active phases, author names, or text in the article titles can be searched. Only the records displayed are searched.



Library Search Properties

Options

☐ Label peaks greater than (%) 0

☑ m/z Label ☐ Formula/y-b

☐ Loss/other

☐ Wrap legend Peak Width 1

Show

☐ Molecular Weight ☐ Formula

☐ CAS Number ☑ Structure on Plot

Color Settings

Scale (plot)

Peaks (plot)

Labels (plot)

Font Settings

Figure 56. Properties dialog box displayed by selecting Properties on the RMB menu displayed when the Mouse pointer is on one of the windows of the Lib. Search tab.

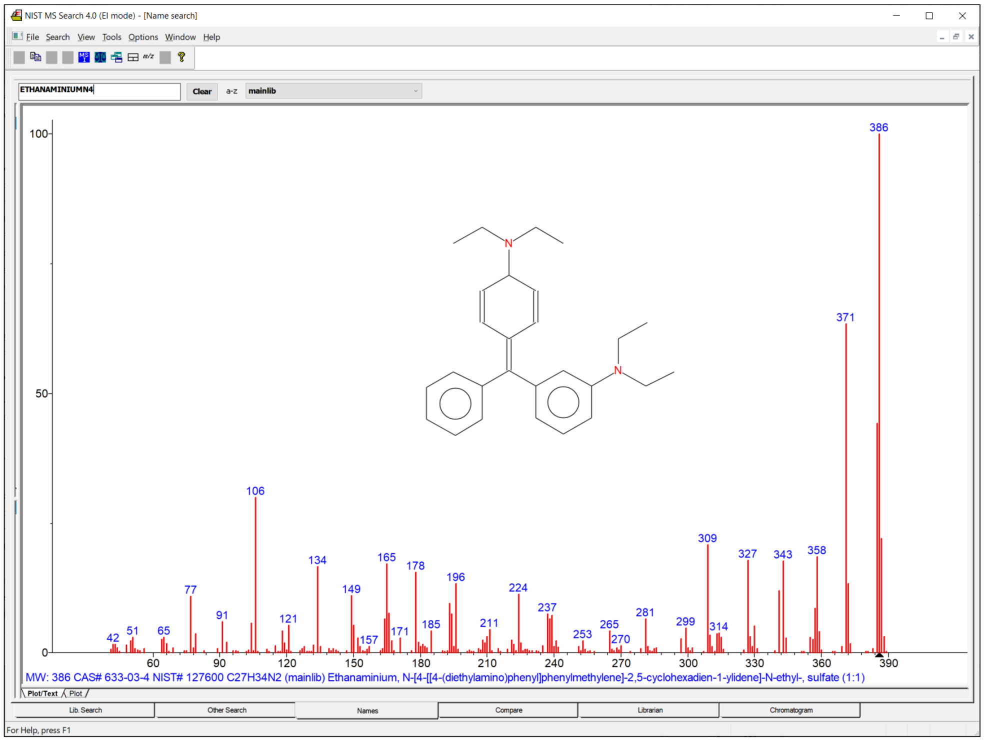
It should also be noted that even though the checkbox for **GC Retention Index** in the **Text Info** tab of the **Properties** dialog box has been selected, if an NIST Tandem Library is being viewed, the GC Method/Retention Index information will not be displayed.

The **RMB** menu selection **Properties** will result in the display of a multiple-page dialog box (*Figure 56*).

The page names (left side) are self-explanatory. This allows customization of the appearance of the display and control of what is displayed such as a structure on a spectrum, the 10 most intense peaks in the spectrum in the text display, etc. Colors of structures, mass spectral peaks and axes, etc., and fonts used for alphanumeric characters can also be set. Windows in the various tab view can have their own customized set of attributes. These customized displays can be saved using the **File**/**Save Configuration** command from the Main Menu. Different saved configurations can be recalled by using the **File**/**Restore Configuration** command from the Main Menu. Separate customizations can be established for each of the six separate tab views. This includes selected libraries to be searched.

The **GC Methods/RI Database** is also available separate from NIST 26 with its own Search program, which allows for more extensive use of this **Database** (ability to search RI ranges). Distributors are allowed to provide this program at no additional charge when the NIST 26 EI installation is purchased. Purchasers may have to remind their distributor.

Examples of the possible content of the **Plot of Hit** window and **Hit Text Info** window are shown below in *Figure 57* and *Figure 58* respectively (next page). These figures were prepared by adjusting the splitter bars to produce full desktop displays of the **Plot** and **Text Information** windows of the **Names** tab view.



NIST MS Search 4.0 (EI mode) - [Name search]

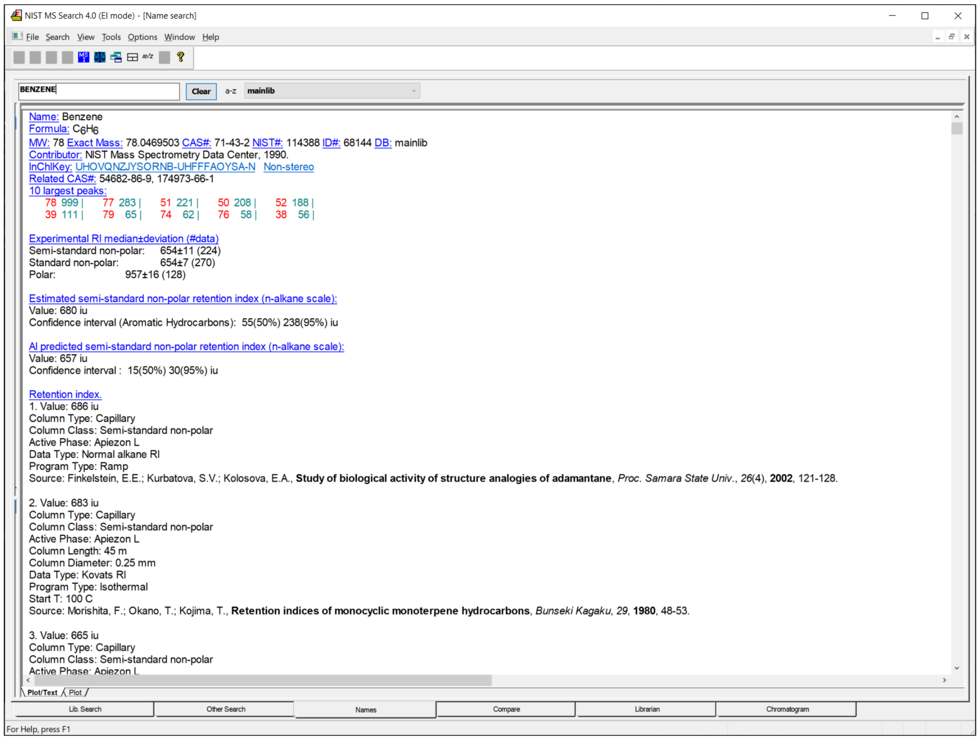
ETHANAMINIUMN4

mainlib

MW: 386 CAS# 633-03-4 NIST# 127600 C27H34N2 (mainlib) Ethanaminium, N-[4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, sulfate (1:1)

For Help, press F1

Figure 57. The Spectral Plot display of a spectrum in the Name tab with the nominal mass, CAS registry number, elemental composition name of the library containing the spectrum, and the name associated with the spectrum. The display is wrapped, the structure is shown, and the peaks are not labeled.



Name: Benzene
Formula: C_6H_6
MW: 78 Exact Mass: 78.0469503 CAS#: 71-43-2 NIST#: 114388 ID#: 68144 DB: mainlib
Contributor: NIST Mass Spectrometry Data Center, 1990.
InChIKey: UHOVQNZJYSORNB-UHFFFAOYSA-N Non-stereo
Related CAS#: 54682-86-9, 174973-66-1
10 largest peaks:
78 999 | 77 283 | 51 221 | 50 208 | 52 188 |
39 111 | 79 65 | 74 62 | 76 58 | 38 56 |

Experimental RI median±deviation (#data)
Semi-standard non-polar: 654±11 (224)
Standard non-polar: 654±7 (270)
Polar: 957±16 (128)

Estimated semi-standard non-polar retention index (n-alkane scale):
Value: 680 iu
Confidence interval (Aromatic Hydrocarbons): 55(50%) 238(95%) iu

AI predicted semi-standard non-polar retention index (n-alkane scale):
Value: 657 iu
Confidence interval : 15(50%) 30(95%) iu

Retention index.
1. Value: 686 iu
Column Type: Capillary
Column Class: Semi-standard non-polar
Active Phase: Apiezon L
Data Type: Normal alkane RI
Program Type: Ramp
Source: Finkelstein, E.E.; Kurbatova, S.V.; Kolosova, E.A., **Study of biological activity of structure analogies of adamantane**, *Proc. Samara State Univ.*, *26*(4), **2002**, 121-128.

2. Value: 683 iu
Column Type: Capillary
Column Class: Semi-standard non-polar
Active Phase: Apiezon L
Column Length: 45 m
Column Diameter: 0.25 mm
Data Type: Kovats RI
Program Type: Isothermal
Start T: 100 C
Source: Morishita, F.; Okano, T.; Kojima, T., **Retention indices of monocyclic monoterpene hydrocarbons**, *Bunseki Kagaku*, *29*, **1980**, 48-53.

3. Value: 665 iu
Column Type: Capillary
Column Class: Semi-standard non-polar
Active Phase: Apiezon L

Figure 58. The Text Info display with Compound Information, Synonyms, and Retention Indices as the only selected display options.

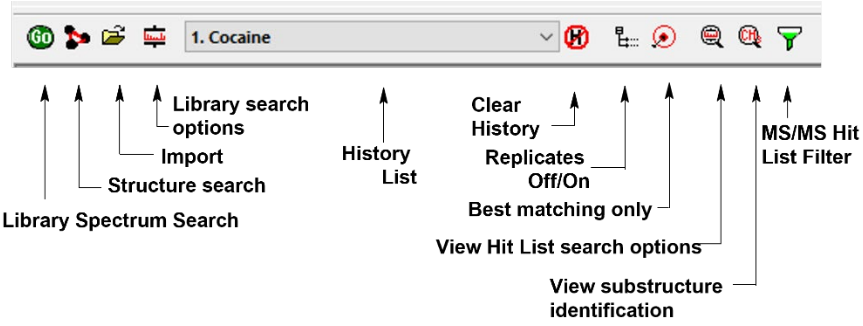


Figure 59. Lib. Search tab view Button Bar.

At the top left of the **Lib. Search** tab view (*Figure 53*) is the **Button Bar** (between **Spec List** window and **Toolbar)**, if displayed. The **Other Search** and **Librarian** tab views also have **Button Bars**. The meaning of each of the buttons in the **Lib. Search** view is shown in *Figure 59*.

The Go button will launch a search of the highlighted spectrum in the **Spec List**. The **Structure Search** button () will perform a structure similarity search of the highlighted structure in the **Spec List**. The **Import** button () is used to import spectra from a text file. The **Library Search Options** button () is used to display the dialog box used in selecting libraries to be searched, search types, and other factors associated with the search of spectra and structures.

The dropdown list box contains the **Search History**. The results of a previous **Lib. Search** can be restored and displayed by highlighting an entry in this list. Each search in the **Lib. Search** History is retained until they are removed with the **Clear History** button (H). **NOTE**: the **Program** can be set to automatically clear the **History List**, when it is exited, by selecting the **Clear History** checkbox in the **Hit List** page of the **Library Search Properties** dialog box. Separately, the **Program** can also be configured to delete the **Spec List** by selecting the checkbox with this same label in the **Spec List** tab of this dialog box; both tabs are displayed by selecting **Properties** from the **RMB** menu with the Mouse pointer in appropriate window. Individual and multiple entries can be deleted from the **Spec List** using the **Microsoft Windows <Delete>** command.

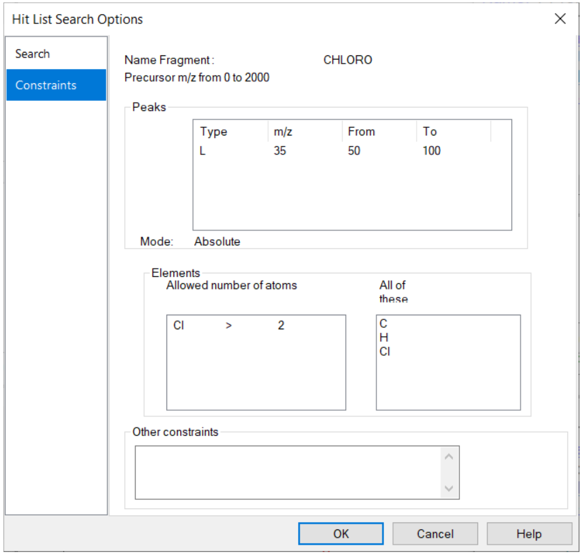
> **NOTE ON RECALLING Hybrid Search Results from HISTORY LIST:** When a **Hybrid Search** is performed, if the **Head to Tail** tab has been selected, the **Compare Pane** window displays the spectrum of the undefined are compound on top (peaks pointing up) and a combination of the hybrid spectrum and the selected Hit spectrum (explained in more detail later in this manual) with peaks pointing down. If the results of such a search are recalled from the **History List** after another type of search has been performed (say an **EI Normal**), in order to reconstitute the **Hybrid Search Compare Pane** display, it will be necessary to select the appropriate **Hybrid Search** under the **Similarity Spectrum Search Type** heading in the **Library Search Options** dialog box. The result can be recalled from the **History List** and then the **Spectrum Search Type** reset; however, this will necessitate selecting a Hit from the recalled **Hit List**.

Replicates are indicated for any compound in any library that has a CASrn that is the same as that in the NIST EI Library. Replicates are indicated by the ⊞ symbol at the beginning of the Hits line in the **Hit List** window. The display of these replicates is turned on by clicking on the symbol. The **Replicates On/Off** button ( ) will toggle the display of Hits of replicate spectra on and off.

The **Best matching only** button ( ) will force the **Hit List** to display only the hit with the highest **Match Factor** for any given set of spectra where the first block of the InChIKey is the same regardless of which searched library it is in. This feature is especially useful when more than one library is being searched. In the case of an MS/MS Search, the best matching spectrum is selected out of Hits that have the same InChIkey or names and charge.

The **View Hit List Search Options** button ( ) will result in the display of a dialog box with two pages if Constraints have been used in the search. The **Search** or **Options** tab (depending on whether the **Hit List Search Options** are for a **Lib. Search,** or an **Other Search** carried out), lists the libraries searched (and type of Search and Search Options for **Library Spectrum Searches** only). The **Constraints** page (if present) lists all different constraints along with values used in the search (*Figure 60* and *Figure 61*).

It should be noted that even though an **EI Library Spectrum Search** is carried out against a list of libraries that does not include **mainlib** and **replib,** these two libraries will be listed as having been included (*Figure 61*) as long as the **Display InChI Replicates** checkbox is selected in the **Replicates Display Options** dialog box displayed by selecting **Replicates/Related** from the **Options** menu in the Main Menu. This will result in the display of the symbol indicating replicates were found when the user-only library was searched. This means that **InChI** search options are available for those spectra in libraries like the Wiley Registry of Mass Spectra and various third-party libraries.



Name Fragment: CHLORO
Precursor m/z from 0 to 2000

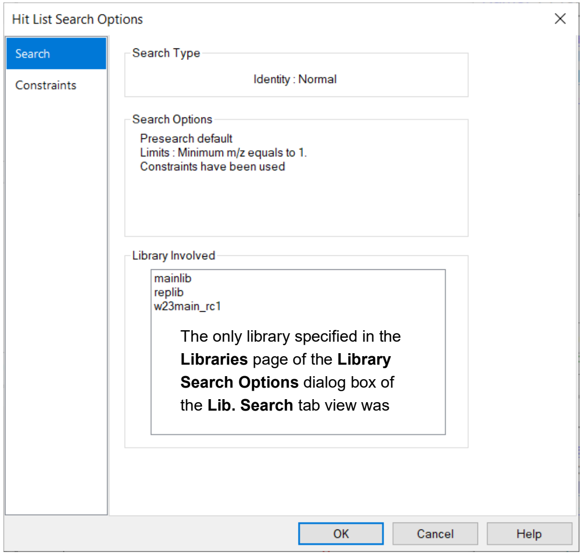
Type	m/z	From	To
L	35	50	100

Mode: Absolute

Allowed number of atoms: Cl > 2

All of these: C, H, Cl

Figure 60. List of Constraints used for a specific search along with their values.



Search Type: Identity : Normal

Search Options:
Presearch default
Limits : Minimum m/z equals to 1.
Constraints have been used

Library Involved:
mainlib
replib
w23main_rc1

The only library specified in the **Libraries** page of the **Library Search Options** dialog box of the **Lib. Search** tab view was

Figure 61. List of libraries used in a Library spectrum Search along with the Search Options.

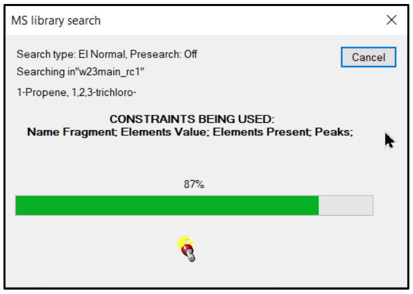
Three other Important Points about the use of Constraints:

1.) When a **Constrained Search** is carried out, the **Search** is performed as if there was no constraint. Only the spectra that were found in the **Search** that fall within the constraint criteria appear in the **Hit List**.

2.) The number of available constraints exceeds the display area; a slider bar on the right side of the **Constraints** window in the **Search** dialog box allows access to all constraints. The **Constraint** window will only allow for the display of six constraint options at a time. Once values have been entered for any of these options, those values will be used in subsequent searches until individually changed or the **Constraint** is deselected. Once an available **Constraint** has been selected (check mark in checkbox entered by putting the Mouse pointer on the checkbox and clicking the left Mouse button), the values previously entered for that constraint will be used when future **Constrained Searches** are performed unless they are changed. If a **Constraint** is selected and is not visible in the Window, an unwanted result may occur. The number of constraints selected is displayed in the **Constraint** page of the various **Search** dialog boxes next to the label **Selected**. A **Clear All** button is on the **Constraints** page of the various **Search** dialog boxes. The **Clear All** button removes the checks in all the various constraints; it DOES NOT change or delete the current values. It is recommended to select the **Clear All** button and reselect any desired **Constraints** before starting a new **Constrained Search**. When values in a **Constraint** are entered, the **Use Constraints** checkbox is automatically selected. Clicking on any individual **Constraints** checkbox in the **Constraints** page causes appearance of the specific **Constraints** page of the **Search** dialog box to change to allow for the entry (or edit) of values for that **Constraint**. The appearance can also be changed to that for a specific **Constraint** by selecting the constraint description in the window.

3.) In the **Other Search** tab view, some **Constraints** are not allowed, and they will be grayed in the **Constraint** list window. An example is when a **Formula** search is being performed the Elements Values and Elements Present Constraints are not allowed. These may have their checkboxes selected, which will also be grayed. It is not possible to de-select these checkboxes; however, if the values in the Constraints are not relevant, the program will prevent them from being de-deselected.

> **Nota Bene:** Any time a Library search is carried out in the **Lib. Search** or **Other Search** tab view, a search status dialog box is sometimes displayed. The figure below is a good example. Sometimes searches are very rapid so that there is no dialog box displayed.

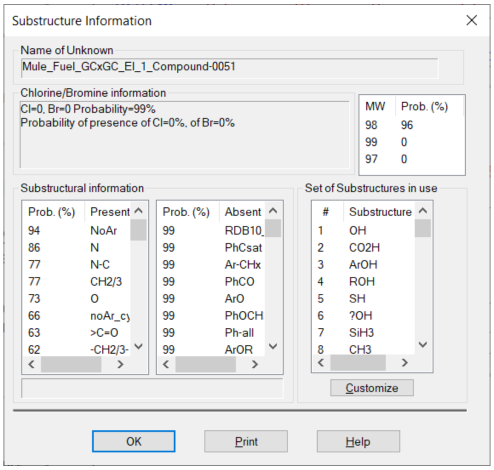


Search type: EI Normal, Presearch: Off
Searching in"w23main_rc1"
1-Propene, 1,2,3-trichloro-

CONSTRAINTS BEING USED:
Name Fragment; Elements Value; Elements Present; Peaks;

87%

The last button on the right, the **View substructure information** button ( ) displays the **Substructure Information** dialog box (shown on right). The **Substructure Information** dialog box contains a list of the probability of the presence and absence of substructures that may be associated with a compound that produced the searched mass spectrum along with additional predictions about the molecular weight of this compound and estimate of the numbers of atoms of chlorine and/or bromine that may be contained. This is especially useful with an **Identity** or **Similarity** search, for either **EI** or **Tandem** spectrum type. An example of the use of **Substructure Information** resulting from an **MS/MS in EI Similarity Search** of the **NIST/EPA/NIH EI Library** is provided later in this manual. All of the different searches are only available when the **Spectrum Type** selected from the **File** menu is **Any**. Otherwise just those specific to EI or Tandem spectrum types are available.



Name of Unknown
Mule_Fuel_GCxGC_EI_1_Compound-0051

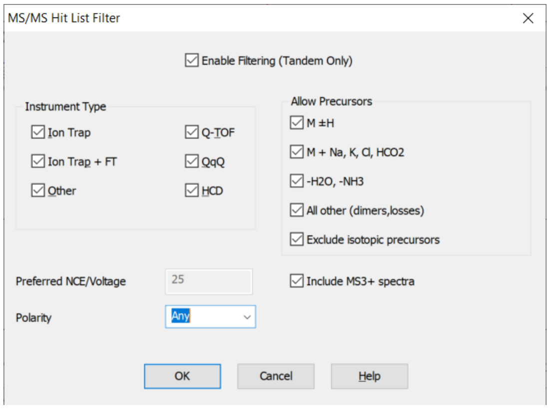
Chlorine/Bromine information
Cl=0, Br=0 Probability=99%
Probability of presence of Cl=0%, of Br=0%

MW	Prob. (%)
98	96
99	0
97	0

Prob. (%)	Present
94	NoAr
86	N
77	N-C
77	CH2/3
73	O
66	noAr_cy
63	>C=O
62	-CH2/3-

Prob. (%)	Absent
99	RDB10_
99	PhCsat
99	Ar-CHx
99	PhCO
99	ArO
99	PhOCH
99	Ph-all
99	ArOR

#	Substructure
1	OH
2	CO2H
3	ArOH
4	ROH
5	SH
6	?OH
7	SiH3
8	CH3



☑ Enable Filtering (Tandem Only)

Instrument Type
☑ Ion Trap
☑ Ion Trap + FT
☑ Other
☑ Q-TOF
☑ QqQ
☑ HCD

Allow Precursors
☑ M ±H
☑ M + Na, K, Cl, HCO2
☑ -H2O, -NH3
☑ All other (dimers,losses)
☑ Exclude isotopic precursors

Preferred NCE/Voltage: 25
☑ Include MS3+ spectra
Polarity: Any

Figure 62. Tandem Hit List Filter Used to Limit Hits Displayed from Library and Other Searches Only Applicable to the Tandem Libraries.

The **Filter** **Button** pertains to searches of the Tandem libraries and is only visible when the spectrum type is **Tandem** or **Any**. *Tandem Mass Spectra Filtering is available for all **Hit Lists*** (**Lib. Search** and **Other Search** tab views). The NIST Tandem Libraries contain spectra acquired over a range of energies, from different precursor types, and fragmentation methods. This means that a search of the **Tandem Libraries** can produce many Hits generated from a single compound, which can be tedious to sort through. To facilitate this task, a filtering process is available that can easily control the types of spectra shown in a **Hit List**. This is invoked by pressing the filter button in the **Button Bar** above the **Hit List** showing results. This is available for both the **Lib. Search** and **Other Search** tab views. The filtering process may be turned on and off with the control at the top (☐ **Enable Filtering (TandemOnly)**) of the **MS/MS Hit List Filter** dialog box (below). When the filter is enabled, the filter button will appear to be pressed. Selecting the **OK** button in the **MS/MS Hit List Filter** dialog box, displayed by selecting the button, the **Hit List** will reflect the selections in this dialog box. These controls match those in the Constraints section; except they do not permanently exclude the display of Hits – all original Hits can be seen by deselecting the 'Enable Filtering' checkbox. This filter has no effect on EI searches.

A good illustration of the **MS/MS Hit List Filter** dialog box is to select the **Other Search** tab view with spectrum type being **Tandem** or **Any**. From the dropdown list box at the upper left of the display, select **Sequential Method**. When the **Sequential Seach** dialog box is displayed, the view will be of the **Constraints** page. Select the **Clear All** button at the top of the dialog box. The ☐ next to the **Use Constraints** label will be deselected. Locate the **Name Fragment** label in the window with the scroll bar just below the **Clear All** button and select it. In the text entry box to the right of the **Name Fragment** label and just below the window with the scroll bar, type C H O L E S T E R O L as a contiguous string. The ☐'s next to the **Use Constraints** and the **Name Fragments** labels should now be selected. Select the link to the **Options** page. Remove any names in the **Included Libs:** window. In the upper window select hr-msms_nist. Then select the **Add** button in the middle of the dialog box. Next, select the **Search** button. The Hit List will have over 500 Hits. Select the button, which results in the **MS/MS Hit List Filter** dialog box. Select the ☐ next to the **Enable Filtering (Tandem Only)**; label in the top/center of the dialog box, then deselect the ☐ next to the -H2O. -NH3, -CO2 label in the column with **Allow Precursors** label (right side of the dialog box), followed by selecting the **Ok** button. This results in a reduction of the number of items in the **Hit List** because the filter is allowing only precursor ions that are [M – H_2O]. None of the previous Hits are lost, they are just not displayed.

Title Bar, Tool Bar, and Status Bar

At the top of the display of each Desktop is a **Title Bar**. This also contains information that pertains to the active tab view. On the left side of the **Title Bar** is the version of the NIST MS Search program and the selected **Spectrum type** (NIST MS Search 4.0 (All mode)). When the **Lib. Search** or **Other Search** tab view is displayed, this **Title Bar** will contain information about the currently displayed search. In the case of a **Lib. Search** tab view this will include the search type used, the Presearch type, **Constrained** (if constraints were used), the **RI** value associated with the searched spectrum if provided, if any type of **Limits** were applied to the search, **Lib. Search = value** and the number of Hits in the **Hit List** window. The maximum of Hits is 100, unless the **Presearch** is set to **Off** in the **Search** page of the L**ibrary Search Options** dialog box. In this case the maximum is 400. In the case of the **Other Search** tab view, the **Title Bar** will contain the type of search that was done (e.g., **Formula, Molecular weight**, etc. and the searched value, i.e., **Formula** (C9H8O4), **MW** (500), whether or not constraints were used, and the number of Hits found as well as the number of Hits displayed when the number of Hits is greater than the display limit. If a TXT file containing CAS registry numbers or NIST number is searched, the **Title Bar** will contain the path and file name.

The **Tool Bar** display (*Figure 63***)** is optional by selecting it from the **View** menu on the Main Menu. It is located between the Main Menu and the **Button Bar**. The functionality of the **Switch to Caller** and **Print Report** buttons are also available on the **File** menu; **MS Interpreter**, and **AMDIS** also may be launched from the **Tools** menu as well as the corresponding buttons on the **Tool Bar**.

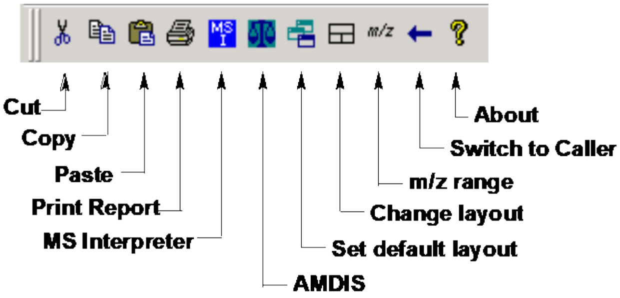
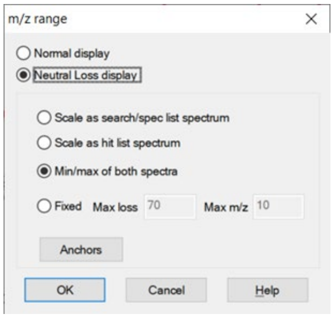


Figure 63. NIST MS Search Tool Bar.

A Tool Tip is associated with each of the buttons with an explanation of the button function. The ▤ button will return all displays on the currently viewed tab to the default values. The ⊟ button toggles through three separate views for the currently displayed layouts.

The ***m/z* range** button displays a dialog box to fix the starting and ending *m/z* values for bar-graph spectra displays, and to choose whether the peaks should be labeled with neutral losses or with their *m/z* value.



m/z range ×

○ Normal display
● Neutral Loss display

○ Scale as search/spec list spectrum
○ Scale as hit list spectrum
● Min/max of both spectra
○ Fixed Max loss 70 Max m/z 10

Figure 64. m/z Range Dialog Box.

An explanation of each of the items in this dialog box can be found by selecting the **Help** button in the dialog box. The **Anchors** dialog box is used when viewing spectra with a neutral loss label. Selecting **Anchors** will result in the display of the **Anchors** dialog box, which is explained by selecting the **Help** button in that dialog box.

The two radio buttons at the top of the ***m/z* range** dialog box have to do with labeling spectra normally or with neutral loss values**. Normal display** is the most common**.**

One of the important tools in deducing a structure from a mass spectrum is to look at the mass spectral peaks as losses from the molecular ion rather than as individual peaks. In this way of viewing the mass spectrum, the peak at *m/z* 269 in methyl stearate becomes the $[M - 29]^+$ peak (loss of an ethyl radical) and the peak at *m/z* 267 becomes the $[M - 31]^+$ peak (loss of a methoxy radical). See *Figure 64*.

The program can display a spectrum with neutral loss labeling of the library and searched spectrum with the first label on the rightmost peak being 0. To activate this display, select **Neutral Loss Display** from the **RMB** menu.

Nota Bene: If you do not know the molecular weight of the search spectrum, the program will attempt to estimate it. This value may be overridden by using the Librarian to edit the spectrum and specifying a molecular weight and then using the edited spectrum to perform the library search.

At the bottom of the tab view display, below the **Program** tabs, is the optionally displayed **Status Bar**. The **Status Bar** display can be turned off by selecting or deselecting **Status Bar** from the **View** menu. When a **Spectrum Search** is being conducted, the left side of the **Status Bar** will have displayed "Comparing XXX Library spectra with submitted spectrum" where XXX is the number of spectra that were found during the Presearch (see *APPENDIX 5: Search Algorithms*). This is followed by a rolling % completion display. Once the search is completed this display is cleared. If **Off** has been selected in the Presearch area of the **Search** tab of the **Library Search Options** dialog box, XXX will represent the total number of spectra in the libraries being searched. As items in the **Tool Bar** are pointed to, the Tool Tip for that item will appear in the **Status Bar**. Depending on the position of the Mouse Pointer, the left side of the **Status Bar** can display **For Help, press F1**.

There are two fields on the right side of the **Status Bar** when the **Lib. Search** tab view is active. The left-most of these two fields will display the search type that is currently selected in the **Library Search Options** dialog box following the **Type of Search:** label. The next information shown following the **Displayed:** label is the type of search responsible for the current display. If a **Neutral Loss** or **Hybrid Similarity Search** has been performed, the number to the left of the **Displayed:** search type is the *m/z* value of the precursor ion.

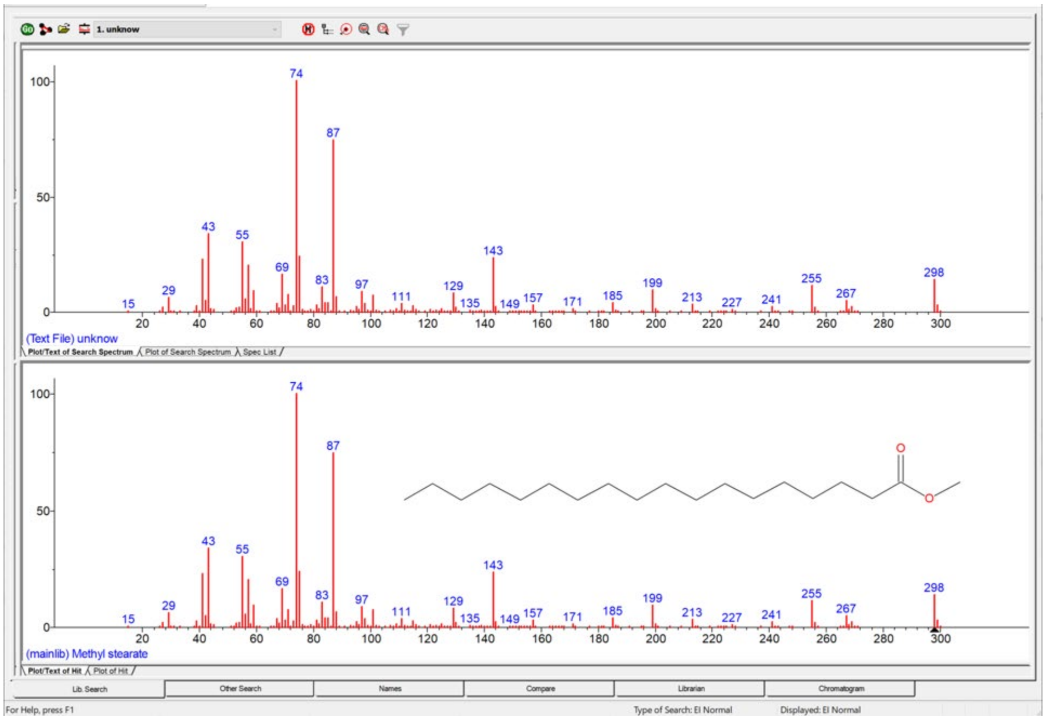


Figure 65. Nominal spectrum labeling where the top spectrum is of an unidentified compound and bottom spectrum is of the first Hit from a search of the NIST/EPA/NIH Mass Spectral Library (NIST 26).

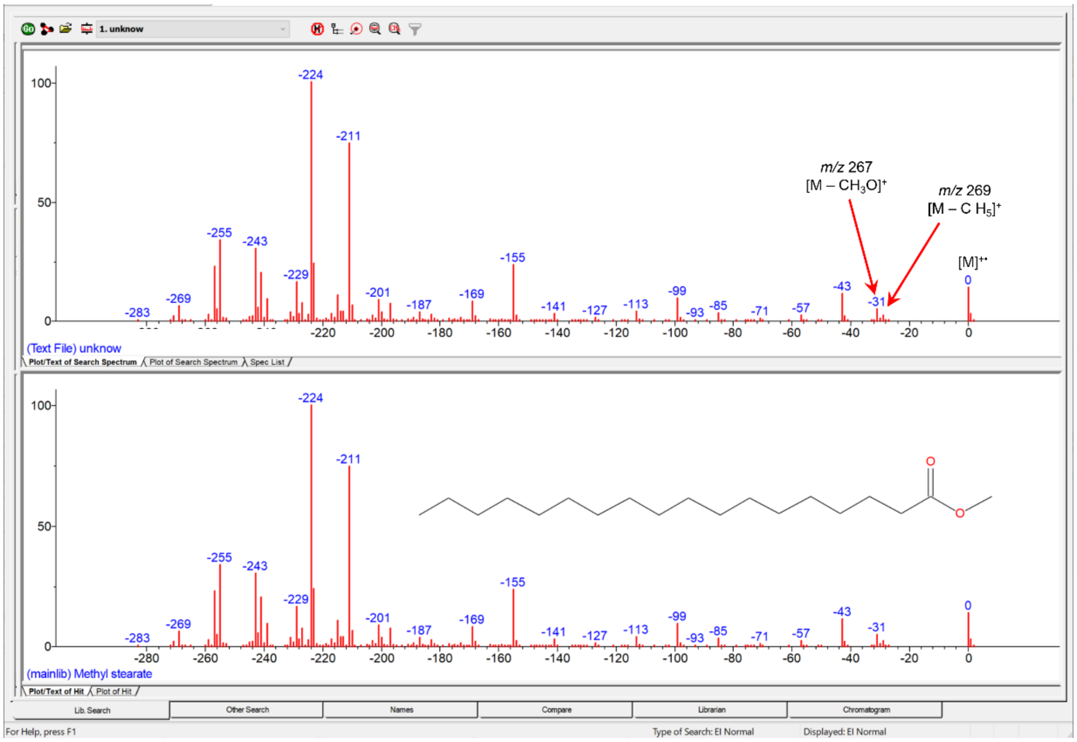


Figure 66. Neutral Loss spectrum labeling where the top spectrum is of an unidentified compound and bottom spectrum is of the first Hit from a search of the NIST/EPA/NIH Mass Spectral Library (NIST 26).

Main Menu

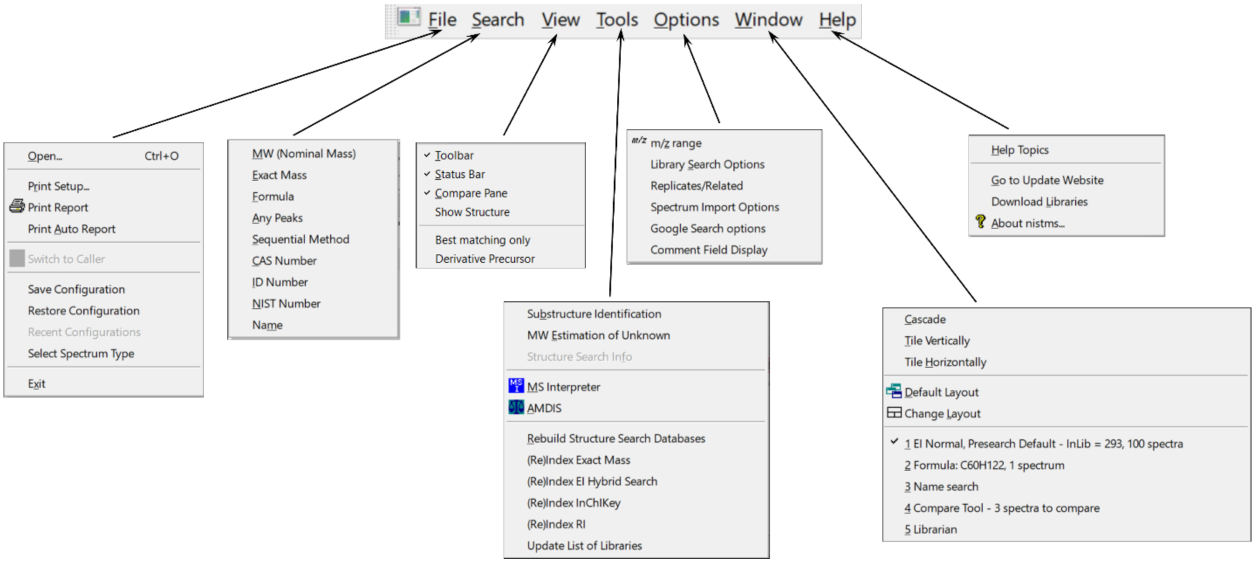
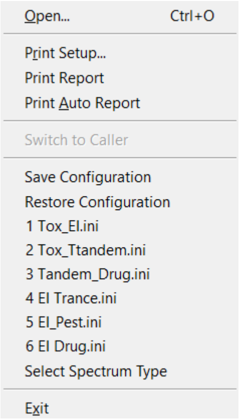


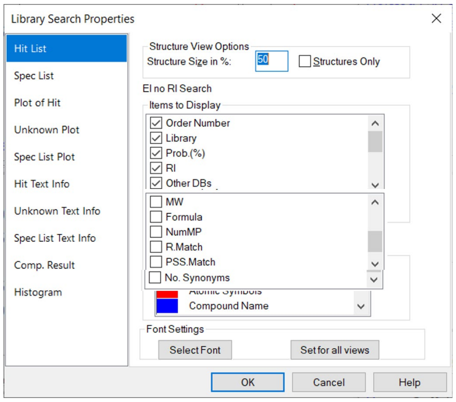
Figure 67. Menu for each selection on the Main Menu of NIST 26.

The above figure was created from the **Lib. Search tab** view. Some items on an individual menu may not be present when the selection is made from these items on the Main Menu in other tab views of the Program.



An important feature of the MS Search is the ability to save configurations. This is true when there are multiple users and/or multiple specific analyses being performed. In *Figure 67* above, the antepenultimate selection of the **File** menu is grayed. It is labeled **Recent Configurations**. When a configuration is saved, this label is replaced by the name of the saved configuration. A maximum of six configurations can appear on the **File** menu: the most recent saved six (each is numbered). More than six configurations can be saved. They will appear in the **Open** dialog box displayed when **Restore Configuration** is selected. If one of the listed **Configuration** is selected, a dialog box appears asking if the current configuration should be saved is displayed, with **Yes** and **No** buttons. If **Yes** button is selected, a **Save As** dialog box is displayed allowing the current configuration to be saved. If **No** is selected, that selected configuration will be restored, overwriting the current configuration. Selected libraries, constraints, and other items in the **Library Search Options** dialog box of the **Lib. Search** tab view are also saved.

The other grayed select on the **File** menu is **Return to Caller**. This selection is only available if MS Search has been started or brought into focus from a command line instruction from other software. This will return the focus to the calling program. There is a button to use to do this in the **Tool Bar**, which is only available under the same circumstances.



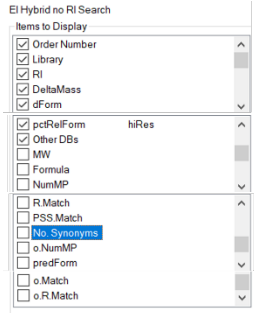
Structure Size in %: 50
☐ Structures Only

EI no RI Search
Items to Display
☑ Order Number
☑ Library
☑ Prob.(%)
☑ RI
☑ Other DBs
☐ MW
☐ Formula
☐ NumMP
☐ R.Match
☐ PSS.Match
☐ No. Synonyms

Figure 68. Altered view of Items to display showing the 11 available for an EI Normal Search.

Hit List window in the Lib. Search tab view

The **Hit List** window in the **Lib. Search** tab view can provide a great deal of information. Some items are specific to the **Tandem** or **EI** spectrum type. Some items are specific to types of searches performed within an individual spectrum type. The display of these information items is selectable through the **Library Seach Properties** dialog box **Hit List** page (*Figure 71*). In the EI Hybrid Hit List window, there are 18 selections. The **EI Hybrid** search **Hit List** selections selections are shown on the right. The same is true for the **Tandem** spectrum type. ***NEW*** to v.4.0 is sets of predefined Hit List items. When a specific search is performed, the appropriate default **Hit List** is displayed. This dialog box includes a Help button to provide defintions for the various column headers.


EI Hybrid no RI Search
Items to Display
☑ Order Number
☑ Library
☑ RI
☑ DeltaMass
☑ dForm
☑ pctRelForm
hiRes
☑ Other DBs
☐ MW
☐ Formula
☐ NumMP
☐ R.Match
☐ PSS.Match
☐ No. Synonyms
☐ o.NumMP
☐ predForm
☐ o.Match
☐ o.R.Match

It has been possible in the past several releases to copy selected items to the Windows Clipboard in a tab-separated text format, by highlighting the desired lines and then pressing the **Ctrl+K** keyboard keys; however, it is worth pointing out again that an important feature added to v.4.0 are the **RMB** menu selections of **Copy Select Hits to Clipboard** and **Export Select Spectra to Text File** displayed when the Mouse pointer is in any **Hit List** or **Spec List** window (**Lib. Search**, **Other Search**, **Librarian**, or **Chromatogram** tab views). Highlighted items will then be copied and exported to a text file or sent to the Windows Clipboard.

The **Hit List** window, like all windows on all tab views can the adjusted in size with the vertical and horizontal splitter bars. The vertical splitter bar adjusts the sizes of all three horizonal windows on the left side of the display to the same width. The widths of the columns in the **Hit List** window are adjustable by placing the Mouse pointer on the dividing line between two columns (the Mouse pointer will change shape (↔)), hold down the left Mouse button and push or pull to the right or left. Pulling to the right increases the size of the column to the left of the pointer. Pushing to left will decrease the width of the column to the left of the pointer. The position of the columns can be changed by placing the Mouse pointer in the center of a column, holding down the left Mouse button and moving to the right or left (desired direction to move the column). When the column to be moved is over the column next to the desired new location, release the Mouse button. The **Hit List** page of the **Library Search Properties** dialog box has a checkbox next to the **Short Library Names** label which limits the name in the **Lib.** column to two digits when selected (default). If not selected, the name of the folder that makes up library is displayed. There is also a checkbox to the left of the **Clear History** label. When selected, the **Search History** list will automatically be deleted when MS Search is closed and restarted. There have been several additions to the available information for all of the Hit List in v.4.0. Review these for information you have always wanted.

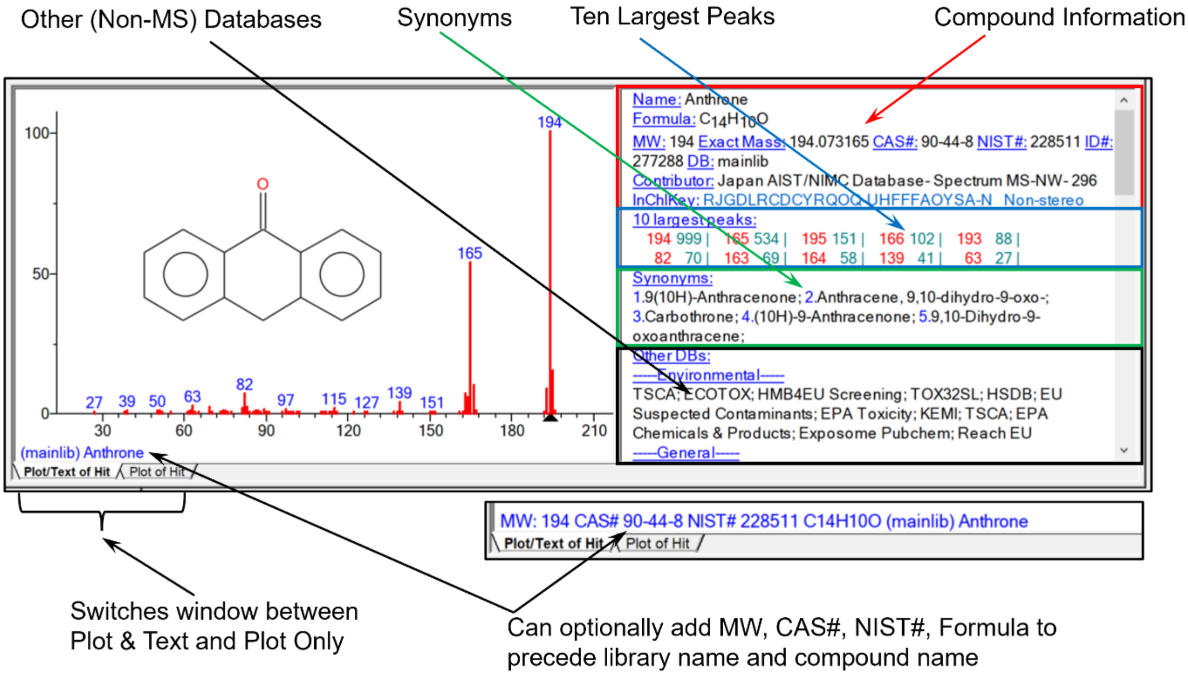
The **Library Spectrum Search** and the **Structure Search** are usually carried out from the **Lib. Search** tab by selecting on the **Library Search** button (Go) or **Structure Search** button (), respectively. Another way of initiating a search is to select a spectrum in any **Hit List** or **Spec List**, **Plot** window or **Text Info** window in any tab view, or in a **Compare** window; display the **RMB** menu and select **Library Search** for a spectrum search or **Structure Similarity Search** for a structure search, if a structure is associated with the spectrum. A third way to initiate a **Spectrum Search** is to double-click the left Mouse button on an entry in the **Spec List**. To search more than one spectrum at once, make a multiple selection in any List window of spectra (**Hit List** or **Spec List**), display the **RMB** menu, and select **Library Search** or **Structure Similarity Search**. Regardless of the tab view displayed, when a search is carried out, the focus will change to the **Lib. Search** tab view. In the default display of the **Lib. Search** tab view, the **Hit List** is on the lower left. With most spectrum searches, the third column (from the left) of the **Hit List** contains the **Match Factor** or **Score** (depending on the spectrum mode and type of search). For a **Structure Similarity Search**, the match column is labeled as **DotProduct**. A **DotProduct** of 1000 for the **Structure Search** does not mean an exact match; it means that the found structure is a homologue of the submitted structure. **Match Factors** and **Scores** for the **Spectrum Search** cannot exceed 999, which is considered an exact match. Just under section controlling the display of structures in the Hit List page of the Library Search Options dialog box a label appears that specify the Search that the current Hits List pertain to. In *Figure 68* on the previous page, it is **EI no RI Search**. To the right of the Structure Size in % numeric entry box is a checkbox followed by the **Structures Only** label. Selecting the checkbox will cause no text to be associated with the **Hit List** when the **Structures** tab at the bottom is selected.

Histogram List window in the Lib. Search tab view

This window has two functions: show a distribution of **Hits** with specific **Match Factors** in either a linear or logarithmic distribution (settable from the **Histogram** page of the **Library Seabeach Properties** dialog box). The default display is with a logarithmic ordinate. The second is to provide a list of the names of all the libraries used for a specific search and the total number of spectra searched. If this text exceeds the width of the window, the text will wrap. This feature is optional; selectable in the **Histogram** page of this dialog box (a checkbox next to the **Show libraries searched** label).

Spec List window in the Lib. Search tab view

Two new selectable columns (from the **Spec List** page of the appropriate Properties dialog box) have been added to the **Spec List** window of the **Lib. Search** and **Librarian** tab views. These are molecular weight, **MW** (nominal mass) and formula, **Formula** (elemental composition). Whatever action is taken with the **Spec List** in the **Lib. Search** tab view will be echoed in the **Spec List** of the **Librarian** tab view. Items can be deleted from the Spec List by highlight and then use the keyboard **Del** key. When items are deleted, there will be gaps in the number sequence. When MS Search is closed and restarted, the Spec List will be renumbered to provide a continuous series. Different layouts are available. The ⊟ button toggles through three separate views for the currently displayed layouts. The button will return all displays on the currently viewed tab to the default values. The **RMB** menu can be used in the **Lib. Search** and **Librarian** tab view to change the splitter bar orientation (**RMB** menu and select **Change Splitter Orientation**).



Name: Anthrone
Formula: $C_{14}H_{10}O$
MW: 194 Exact Mass: 194.073165 CAS#: 90-44-8 NIST#: 228511 ID#: 277288 DB: mainlib
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW- 296
InChIKey: RJGDLRCDCYRQOQ-UHFFFAOYSA-N Non-stereo
10 largest peaks:
194 999 | 165 534 | 195 151 | 166 102 | 193 88 |
82 70 | 163 69 | 164 58 | 139 41 | 63 27 |
Synonyms:
1.9(10H)-Anthracenone; 2.Anthracene, 9,10-dihydro-9-oxo-; 3.Carbothrone; 4.(10H)-9-Anthracenone; 5.9,10-Dihydro-9-oxoanthracene;
Other DBs:
----Environmental----
TSCA; ECOTOX; HMB4EU Screening; TOX32SL; HSDB; EU Suspected Contaminants; EPA Toxicity; KEMI; TSCA; EPA Chemicals & Products; Exposome Pubchem; Reach EU
----General----

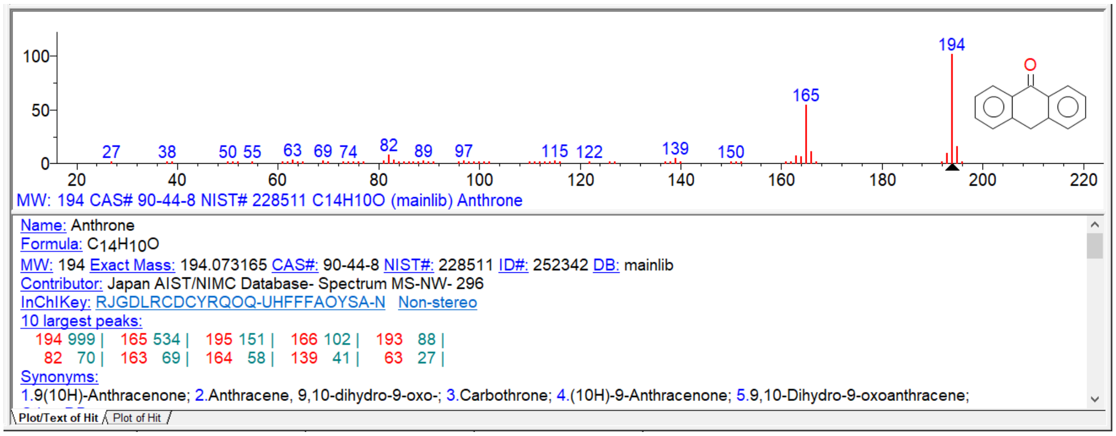
(mainlib) Anthrone

MW: 194 CAS# 90-44-8 NIST# 228511 C14H10O (mainlib) Anthrone

Figure 69. The Plot/Text window showing some of the Optional Displays. In addition to those shown for the Text window, a list of all m/z values/relative intensity pairs and GC Retention information can be displayed with MS Peaks in Rows or Columns and the Text information Wrapped or not.

Plot & Text Information window of Selected Hit in the Lib. Search tab view

The Plot/Text windows in the **Lib. Search** tab view have separate pages in the **Lib. Search Properties** dialog box: **Hit Text Info** and **Plot of Hit**. An important feature of Pages of all Plot windows is the ability to adjust the thickness of the mass spectral peak: list box to the right of **Peak With** label. The **Change Splitter Bar Orientation** selection is only available when the **RMB** menu is displayed in either of the two windows **associated** with a selected **Hit** or with a selected **Spec List** spectrum. The splitter bar between the **Plot** and **Text Info** windows of the Spec List and Hit List selection changes from horizontal to vertical or *vice versa*.

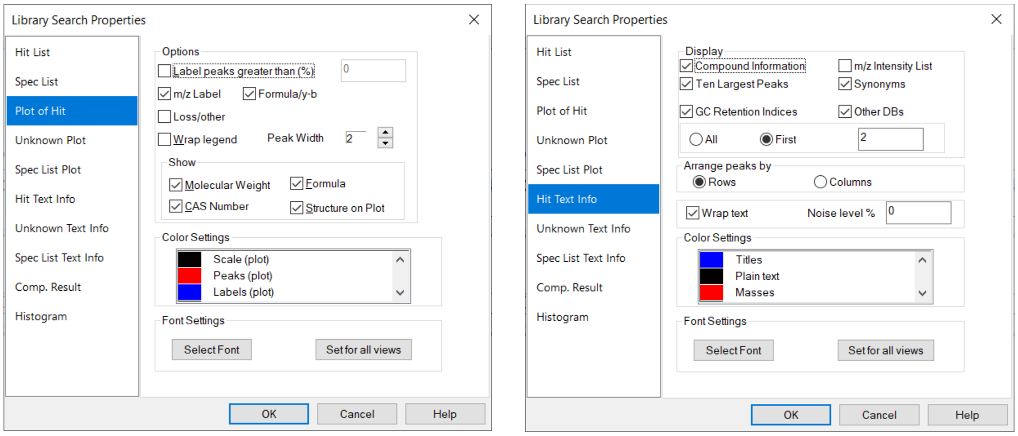


MW: 194 CAS# 90-44-8 NIST# 228511 C14H10O (mainlib) Anthrone

Name: Anthrone
Formula: $C_{14}H_{10}O$
MW: 194 Exact Mass: 194.073165 CAS#: 90-44-8 NIST#: 228511 ID#: 252342 DB: mainlib
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW- 296
InChIKey: RJGDLRCDCYRQOQ-UHFFFAOYSA-N Non-stereo
10 largest peaks:
194 999 | 165 534 | 195 151 | 166 102 | 193 88 |
82 70 | 163 69 | 164 58 | 139 41 | 63 27 |
Synonyms:
1.9(10H)-Anthracenone; 2.Anthracene, 9,10-dihydro-9-oxo-; 3.Carbothrone; 4.(10H)-9-Anthracenone; 5.9,10-Dihydro-9-oxoanthracene;

Figure 70. Plot/Text window of selected Hit at using the Changer Splitter Orientation on RMB menu with Mouse pointer in one of the two windows. By closing the Compare windows using the horizontal splitter bar above the Plot window, the Spec List/Searched Spectrum and the selected Hit List spectrum are on top of one another.

The Plot of Hit and Hit Text Info pages of the Library Search Properties dialog box are shown in *Figure 71*. One important fact about the **Hit Text Info** page is the **GC Retention Indices**. Either **All** or a limited number of complete citations can be displayed. These can be searched using the **RMB** menu's **Find** selection.



Library Search Properties

Options

☐ Label peaks greater than (%) 0

☑ m/z Label ☑ Formula/y-b

☐ Loss/other

☐ Wrap legend Peak Width 2

Show

☑ Molecular Weight ☑ Formula

☑ CAS Number ☑ Structure on Plot

Color Settings

■ Scale (plot)
🟥 Peaks (plot)
🟦 Labels (plot)

Font Settings

Library Search Properties

Display

☑ Compound Information ☐ m/z Intensity List

☑ Ten Largest Peaks ☑ Synonyms

☑ GC Retention Indices ☑ Other DBs

○ All ● First 2

Arrange peaks by

● Rows ○ Columns

☑ Wrap text Noise level % 0

Color Settings

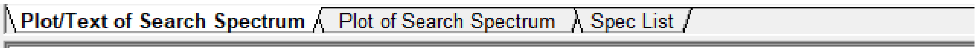
🟦 Titles
■ Plain text
🟥 Masses

Font Settings

Figure 71. Plot of Hit (left) and Hit Text Info. (right) pages of the Library Search Properties dialog box.

Plot & Text Information window of Selected Item in the Spec List window

The window to the right of the **Spec List** of the Lib. Search tab view is similar to the one to the right of the **Hit List**, with one important difference. This window serves three functions:



This window is divided into the **Plot** and **Text Info** windows of an item searched from the **Spec List** or just the **Plot** of the searched item from **Spec List**. There is a third tab labeled **Spec List**, which will be a two-window display (**Plot** and **Text Info.**) of whatever item is currently highlighted in the **Spec List**. Highlighting a **Spec List** item results in the automatic switch to the **Spec List** tab view, located under the window. The spectrum displayed in the **Plot/Text of Seach Spectrum** or **Plot of Spectrum** tab view will be same; however, they may be different from the spectrum display in the **Spec List** tab view. It is important to be aware of this distinction to avoid confusion. There are different **Plot** and **Text Info.** pages in the **Library Search Properties** dialog box for the **Plot/Text of Search Spectrum** and **Spec List** tab views. These pages are labeled **Unknown Plot** and **Unknown Text Information**, and **Spec List Plot** and **Spec List Text Info**, respectively. The **Library Search Properties** dialog box page for the **Plot** window of the **Plot/Text of Search Spectrum** and **Plot of Search Spectrum** are the same: **Unknown Plot**.

Compare window of the Lib. Search tab view

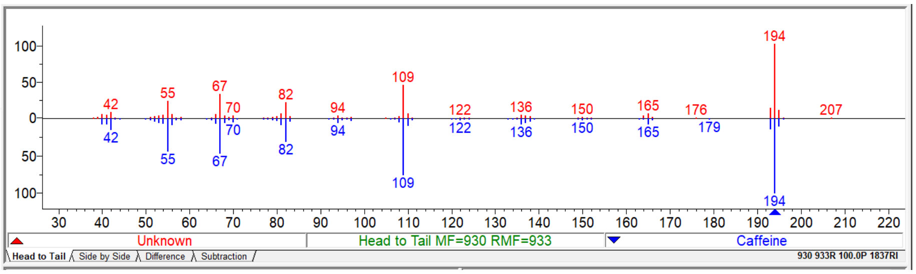


Figure 72. The Compare window of the Lib. Search tab view with the Head to Tail display.

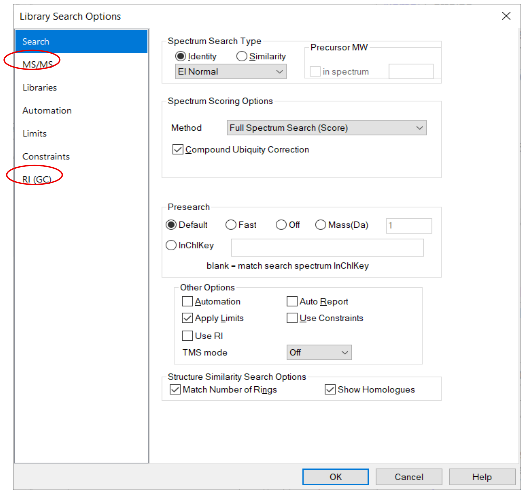
The **Compare** window can be closed by selecting **View** from the Main Menu and deselection **Compare Pane** from the dropdown menu. It can also be made not visual by adjusting the horizontal splitter bars on the top and bottom of the window. If this is done, visibility can be restored by selecting the button in the Buton Bar. If the splitter bar orientation has been changed with the RMB menu, it will be restored to the original position. If the **Compare** window has been made not visible with the **View** menu, the use of the button will not restore it. There are four ways to compare the spectrum in the Plot window above the **Compare** window with the spectrum in the Plot window below. One is the **Subtraction view**. This will be useful if it is suspected that the query spectrum is of more than one compound.

The Compare window is very important when a Hybrid Search is performed. This is explained in the section regarding the Hybrid Search.

Library Search Options dialog box

Search page in the Library Search Options dialog box

This section on the Search page of the Library Search Options box is repeated later in this manual


Library Search Options

Spectrum Search Type: ● Identity ○ Similarity; EI Normal

Precursor MW: ☐ in spectrum

Spectrum Scoring Options: Method: Full Spectrum Search (Score)

☑ Compound Ubiquity Correction

Presearch: ● Default ○ Fast ○ Off ○ Mass(Da) 1

○ InChIKey

blank = match search spectrum InChIKey

Other Options:

☐ Automation ☐ Auto Report

☑ Apply Limits ☐ Use Constraints

☐ Use RI

TMS mode: Off

Structure Similarity Search Options:

☑ Match Number of Rings ☑ Show Homologues

Figure 73. Library Search Options Search page for an Identity EI Normal search in the Any Spectrum Type mode.

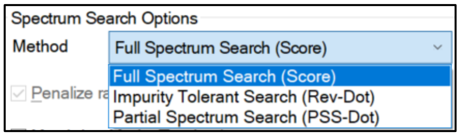
Selecting **Library Search Options** on the **Options** menu from any tab view or the **Library Search Options** button ( ) on the **Button Bar** of the **Lib. Search** tab view will display the multiple-page **Library Search Options** dialog box (*Figure 73*). The appearance and options of the dialog box are dependent on the **Spectrum Type** selected in the dialog box displayed when the **Select Spectrum Type** option is selected in the **File** menu (Main Menu). The **MS/MS** page does not appear if the spectrum type is **EI**, and the **RI (GC)** page does not appear if the spectrum type is **Tandem**.

Different types of spectral comparison routines for choosing the best matching spectra are available. These are **Spectrum Type** specific. The routine to be used for any **Spectrum Type** is selected from the **Library Search Options** dialog box **Search** page.

The two **Spectrum Search Types** on this dialog box are **Identity** and **Similarity**. These different **Spectrum Search Types** and their options are explained in the **Help Screens** for each of the **Spectrum Types** (topic *Spectrum Search Type*).

Generally, if it is believed that there is a spectrum of the unidentified compound (query) in one or more of the libraries being searched, an **Identity Search** is best used (**EI Normal** for **EI** spectrum type when using nominal *m/z* or accurate GC/MS data, and **MS/MS** for **Tandem** spectrum type using spectra with a specified precursor ion or **HiRes NoPrecursor** when using in-source spectra). If it is believed that there is no spectrum of the query in the libraries searched for reasons such as obtaining only low **Match** and **Reverse Match Factors** for an **Identity Search**, a **Similarity Search** may yield better results. Although there are three options for a **Similarity Search** for the **EI** spectrum type, and essentially one selection for the **Tandem** spectrum type, it is recommended that the Hybrid Search be used (for both spectrum types) when it is believed there is no spectrum of the query compound present in the searched libraries. Introduced with v.2.3 of **MS Search**, these are particularly useful in this respect.[1,2]

A feature that first appeared in v.3.0 is the **Spectrum Sorting Options/Method** (name changed from **Spectrum Search Options** in v.3.0) dropdown list box (shown below). There are three selections. This section also has the **Compound Ubiquity Correction** checkbox. This is a new feature in v.4.0 that is used to enhance the **Match Factor** of more common compounds, when selected. V.3.0 saw the retirement of the **Penalize rare compounds** option. This new feature is a much more sophisticated replacement. If the Hit has a high **Compound Ubiquity Index** (**CUI**), its **Match Factor** will be altered. It has been shown that “a CUI of 0 reduces the inherent likelihood of identification relative to a random compound in the library by nearly a factor of 10, whereas a value of 20 increases this likelihood by a factor of 10.” When dealing with an unknown spectrum and two Hits have almost identical **Match Factors**, the one with the higher CUI is the more likely candidate. As used in MS Search, the CUI is a citation index that reports the number of 58 diverse non-mass spectral chemical collections containing the compound. This is described in the literature citation previously referenced on *page 44, Footnote 6*, Details of how this works are found in that citation.


Spectrum Search Options

Method: Full Spectrum Search (Score)

There are a series of options under the label **Spectrum Scoring Options/Method** section. These are selectable through the dropdown list box next to the **Method** label. The first selection in the list is the search type most offen used where all peaks in the sample spectrum and the library spectrum that are in a match are compared.

---

[1] Arun S. Moorthy, , William E. Wallace, Anthony J. Kearsley,‡ Dmitrii V. Tchekhovskoi, and Stephen E. Stein “Combining Fragment-Ion and Neutral-Loss Matching during Mass Spectral Library Searching: A New General Purpose Algorithm Applicable to Illicit Drug Identification” *Anal. Chem*. **2017**, *89*, 13261−13268.

[2] Ivana Blaženović, Tobias Kind, Michael R. Sa, Jian Ji, Arpana Vaniya, Benjamin Wancewicz, Bryan S. Roberts, Hrvoje Torbašinović, Tack Lee, Sajjan S. Mehta, Megan R. Showalter, Hosook Song, Jessica Kwok1, Dieter Jahn, Jayoung Kim, Oliver Fiehn “Structure Annotation of all Mass Spectra in Untargeted Metabolomics” *Anal. Chem*. **2019**, *91* (3) 2155-2162.

The second option results in the display of the **Hit List** according to descending **Reverse Match Factor** (**RMF**). A **RMF** is a **Match Factor** (**MF**) calculated by disregarding any peaks in the sample mass spectrum that are not in the library mass spectrum. The third selection is displaying the **Hit List** in descending order of the **Partial Spectrum Search** (**PSS**) value. This is a **Match Factor** calculated disregarding any peaks in the library spectrum that are not in the sample spectrum. The **PSS** seach was new to v.3.0 of MS Search. The **Impurity Tolerant Search** was selectable as a checkbox for a **Reverse Search** in this dialog box of versions previous to v.3.0 (v2.4 and previous).

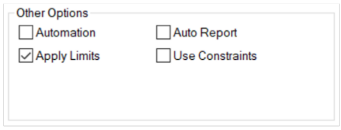
In v.3.0 there was a selection for **Match Ion Mode (Tandem)** when any **Spectrum Mode** other than **EI** is selected. This selection has been moved to the **MS/MS** page (only visible on the **Library Search Options** dialog box when the spectrum type is Tandem).

Following the **Spectrum Search Options** are the **Presearch** selections. These are selectable by clicking on the desired radio button. The **Default** presearch uses a series of presearch screenings to select the spectra which will be compared peak-by-peak in a **Match Factor** (**Score**) calculation. The **Fast** presearch is essentially the same as the Default, but selects a factor of three fewer spectra, on average. The **Off** selection has every spectrum in all the libraries being searched compared with the sample spectrum. This can be time consuming. This will result in a **Hit List** with 400 compounds whereas the other two Presearch options produce a **Hit List** with a maximum of 100 compounds.

The **InChIKey** selection restricts searches to spectra that have the appropriate **InChIKey**; therefore, it is a type of presearch and turns off the other three selections. This can be a full value or a beginning partial value. There will be a spectrum-by-spectrum search of the spectra meeting the **InChIKey** criteria.

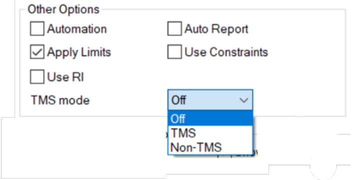
Note the **InChIKey presearch** is used when the structure associated with the spectrum is known. It is a powerful filter that helps with multiple libraries. Existing User- and third-party libraries must be indexed to use this feature. Indexing is accomplished from the **Tools** menu of the Main Menu. Details of the InChIKey presearch are found in the **Help** file.

Other Options section on Search page of Library Search Options dialog box



Other Options
☐ Automation ☐ Auto Report
☑ Apply Limits ☐ Use Constraints

Spectrum Type: Tandem



Other Options
☐ Automation ☐ Auto Report
☑ Apply Limits ☐ Use Constraints
☐ Use RI
TMS mode Off

Spectrum Type: EI

The checkboxes next to the **Automation**, **Apply Limits**, **Use Constraints**, and **Use RI** (only in **EI** and **Any** spectrum mode) options are automatically selected or deselected based on actions taken in the **Automation**, **Limits**, **Constraints**, and **RI (GC)** pages of this dialog box. They can be selected or deselected in this page. If they are not selected, it is a good idea to review the contents of the corresponding page before selecting one of them. This will prevent unwanted or confusing results. The **Automation** checkbox in the **Search** page is turned on an off by the selection/deselection of the **Automatic Search On** checkbox in the **Automation** page. The **Auto Report** checkbox is not automatically selected when the **Automatic Search On** checkbox is selected on the **Automation** page or **Automation** is selected in the **Search** page. If **Auto Report** is selected, whatever settings are in place in the upper section of the **Automation** page will be applied to the report automatically generated at the completions of each library search. The other items are self-explanatory if the page with the appropriate label is made visible. The value in the **Number of Hits to Print** entry box sets the number of Hits that will be in an **Auto Report** when executed from the **File** menu of the Main Menu. The format of these automatically printed reports is dictated by the **Automation** page of the **Library Seach Options** dialog box. This format is different from the one generated when File menu's **Print Report** is executed.

Version 4.0 has two new selections in the **Other Options** section of the **Search** page of the **Library Search Options** dialog box displayed for the **EI** spectrum type. These are **Use RI** and **TMS mode**. The **Use RI** checkbox which can be selected and deselected from the **Search** page is turned on and off by selecting/deselecting the **Use Semi-Standard Non-Polar Colum Retention Index for Scire corrections** checkbox in the **RI (GC)** page.

The **TMS mode** (EI only) selection has three options for compounds that have been derivatized with a trimethylsilyl reagent.: 1) Off - no filter; 2) TMS - for TMS derivatized samples - rejects compounds containing OH and NH with no TMS derivatization; 3) Non-TMS - for non-TMS derivatized runs - rejects all TMS derivatized compounds. This is a highly specific constraint.

The **Search** page of the **Library Search Options** dialog box also has a **Structure Similarity Search Options** selection. This has the **Match Number of Rings** checkbox. When selected, the search of compounds that have rings will be constrained to only spectra produced by compounds with a similar structure and the same number of rings as the spectrum being searched. The **Show Homologues** selection is self-explanatory.

Different Libraries are used for a Structure search and a Spectrum Search. When the **Libraries** page of the **Library Search Options** dialog box is selected, a dropdown list box appears in the lower part. The selections made above are for either **Spectrum searches** or **Structures searches**. It is important to be aware that different libraries may be selected for these two types of searches, especially when working with both at the same time.

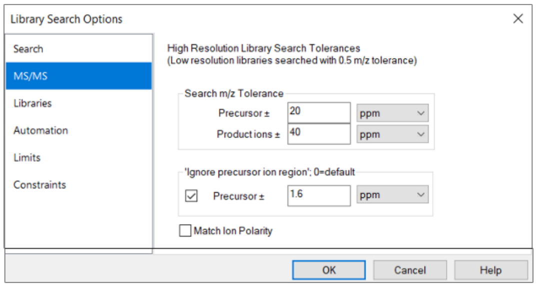
When the **OK** button on any page of the **Library Search Options** dialog box is selected, the dialog box is closed. When this dialog box is next opened, it is opened with the page view present when it was closed. In addition to the **OK** button each page has a **Help** button that opens a contact sensitive Help page. Before initiation, a search of a spectrum or performing a structure similarity search, each page in the **Library Search Options** dialog box should be viewed and understood.

Warning !! If unexpected results are observed for any search, Tandem of EI, the first place to look is the **Search** page of the **Library Search Options** dialog box to see what **Other Options** have been selected. The one that has been the most problematic is the **Use Constraints**. If this checkbox is selected, check the **Constraints** page to see all the settings. Another possible source of error is **Limits** page. The Method chosen in the Spectrum Scoring Options section of the Search page can cause unexpected results.

MS/MS page in Library Search Options dialog box (Tandem Only)

The **MS/MS** page ONLY APPEARS on the **Library Search Options** dialog box when the spectrum type is **Tandem** or **Any**. It permits selection of the *m/z* tolerances for the precursor and product-ions in the **MS/MS** and **In-source HiRes Search**, the option to ignore the precursor ion, and access to a set of options specific to peptides. The values can be entered in **ppm** or as ***m/z*** values. If the checkbox next to the **'Ignore precursor ion region': 0=default** label is selected, mass spectral peaks within a ± interval of the precursor ion are ignored. If 0 (the default) is entered, the larger of precursor and product ions is used.

Precursor ± - precursor *m/z* tolerance range, 0.015-100,000 ppm or 6×10^{-5} - 500 *m/z* units. **Product ions ±** - mass spectral peak tolerance range, 0.015-100,000 ppm or 6×10^{-5} - 500 *m/z* units.



High Resolution Library Search Tolerances
(Low resolution libraries searched with 0.5 m/z tolerance)

Search m/z Tolerance
Precursor ± 20 ppm
Product ions ± 40 ppm

'Ignore precursor ion region'; 0=default
☑ Precursor ± 1.6 ppm

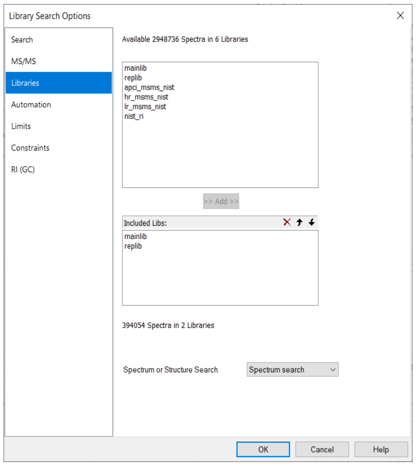
☐ Match Ion Polarity

Nota Bene: For Low Resolution power NIST library (LR_*) product ions tolerance is fixed at unit mass resolution. If a library name begins with LR_ (Low Resolution power), for its spectra the entered tolerance T is replaced with max (T, 0.45 *m/z* units) for all product ion peaks. For example, if T=4500 ppm, tolerance will be increased to 0.45 *m/z* values for ***m/z*** values < 100.

In v.4.0 the **Match Ion Polarity** checkbox was moved from the **Search** page to the **MS/MS** page of this dialog box.

Libraries page in Library Search Options dialog box

The **Libraries** page (*Figure 74*) of this dialog box is used to select the libraries to be included in a **Library Search** of a spectrum. As many as 127 libraries can be searched at the same time. The **EI Installation** includes the **NIST 26 Main Library** (**mainlib**) and the **NIST 26 Replicates Library** (**replib**) and the **NIST GC Method/Retention Index Database** (**nist_ri**). Multiple user-libraries as well as other commercially available libraries from sources other than NIST that are in the **NIST MS Search** user-library format can also be added. The **Tandem Installation** includes three other **NIST 26** Libraries: the **hr_nist_msms**, **lr_nist_msms**, and **apci_msms_nist**. This means that there is space to accommodate an additional 120 user-libraries in either installation. If more mass spectral libraries need to be accommodated, the least used ones can be moved to another folder. To add libraries to be searched, in the **Available Libs** window of the dialog box, highlight the name and click on the **Add** button. To exclude libraries, highlight the name in the **Included Libs** window and then click on the ✕ button above the upper right portion of this window. The order in which libraries are searched can be changed by highlighting the library to be moved in the **Included Libs** window and clicking on the Up or Down arrows in the upper right portion of this window. Each click will move the library one position. The order in which libraries are searched is not important in the search of a spectrum of an unidentified compound



Available 2948736 Spectra in 6 Libraries

mainlib
replib
apci_msms_nist
hr_msms_nist
lr_msms_nist
nist_ri

Included Libs:
mainlib
replib

394054 Spectra in 2 Libraries

Spectrum or Structure Search: Spectrum search

Figure 74. Library Search Options Libraries page.

because the **Hit List** has the 100 Highest **Match Factor Hits** regardless of what library they came from. **Other Searches** also have limited **Hit List**; the order of search may cause **Hits** from libraries lower on the search order not to be included in the **Hit List** if the number of Hits exceeds the limits.

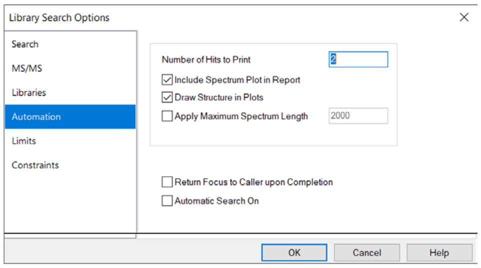
Below the **Available Libs** and **Included Libs** windows is a dropdown list box. This box allows for switching between the lists of libraries to be included in **Spectra** searches or **Structure** searches. These two different types of searches can have different sets of libraries and are specific to whether the search is of a spectrum or a structure.

Automation page in Lib. Search dialog box

The settings in **Automation** page of the **Library Search Options** dialog box have two functions. They are used activate the automatic printing of a report to the MS Searches default printer when the Automatic Search On checkbox is selected, and to set the number of Hits included in the report, whether the quire spectrum should be included in the report, and whether the structure in the Hit spectrum should be included in the report.

When a query spectrum or structure is searched against a library that is imported from a file (using the File/Open command on the Main Menu) or third-party program such as an instrument data system or drawing program, a search can be automatically launched. and/or a report be automatically printed based on the setting in this page. Search and printing can be turned on and off from either the **Search** or **Automations** page of the **Library Search Options** dialog box by the respective selection or deselecting of the appropriate checkbox. *Figure 75* shows the option in the **Automation** page of the **Library Search Options** dialog box.

Automatic Search On on the **Automation** page (*Figure 75*) has the same functionality as the **Automation** checkbox on the **Search** page. Selecting or deselecting this checkbox on either page toggles the checkbox on the other page.



Number of Hits to Print 2
☑ Include Spectrum Plot in Report
☑ Draw Structure in Plots
☐ Apply Maximum Spectrum Length 2000
☐ Return Focus to Caller upon Completion
☐ Automatic Search On

Figure 75. Lib. Search Automation tab.

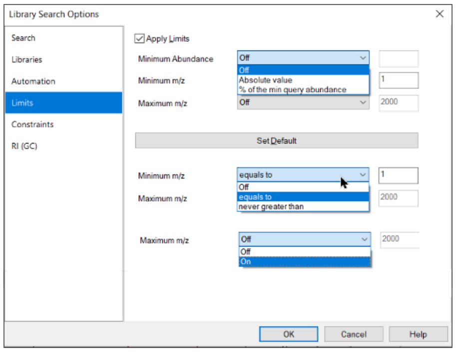
Search results can automatically be printed whether the search is automatically launched. Checkbox **Auto Report** on the **Search** page of the **Library Search Options** dialog box turns on this feature. The **Number of Hits to Print** field is in the **Automation** page (*Figure 75*). In addition, the report in the same format can be printed for **Lib. Search** tab view by selecting **Print Auto Report** from the **File** menu.

The top part of this page of this dialog box pertains to the automated printing of a search result. The format for the **Auto Report** is a sample spectrum at the top of a page followed by the spectra of a specified number of Hits in the **Number of Hits to Print** numeric entry box (2 by default). If the **Include Spectrum Plot in Report** checkbox is selected, there will be three spectra per page. Page one will have the query spectrum at the top followed by the first two Hits. Selecting the **Draw Structures in Plots** checkbox will result in the structure associated with a spectrum being printed in the plotted spectrum.

If the **Apply Maximum Spectrum Length** checkbox is selected, a number can be entered for the maximum m/z value to appear on the spectrum abscissa. If peaks are present at higher m/z values, the abscissa will have a right-pointing arrowhead at its right end. When selected, the **Return Focus to Caller Upon Completion** checkbox will automatically cause a program that brought the **NIST MS Search Program** into focus.

The printer specified by choosing it from the **Print Setup** dialog box displayed by selecting **Print Setup …** from the **File** menu (Main Menu) is the default. Some may want to set the printer as a device that produces a PDF file. If a series of spectra are to be searched, using a PDF printer can be problematic because the system stops to wait for the operator input of a file name. There is commercial software which allows for the automated incrementing of file names from one report to the next or will put all reports into the same file. There are some that or free and others have paid options. One program that does this is Win2PDF™ (*https://www.win2pdf.com/*)

Limits page in the Library Search Options dialog box



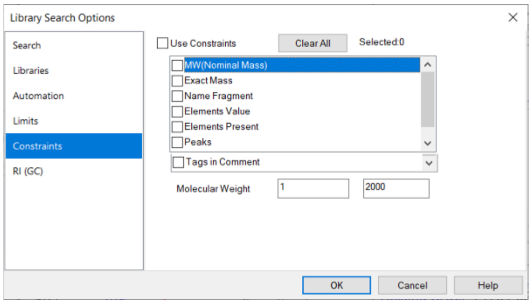
☑ Apply Limits
Minimum Abundance Off
Minimum m/z 1
Maximum m/z Off 2000
Minimum m/z equals to 1
Maximum m/z 2000
Maximum m/z Off 2000

With the **Apply Limits** checkbox deselected, when a **Match Factors** is calculated, the only peaks considered are those above the lowest m/z value where ion current is observed in the library speculum or query spectrum, whichever is the higher. If the lowest m/z value of a library spectrum is high, a short range will be used in the calculation which could result in an erroneous Match Factor. Generally, the spectra in the NIST\EPA\NIH EI Library are complete spectra; however, some contributed spectra start the acquisition at m/z 35 or 50. Previous versions of MS Search had the Apply Limits checkbox deselected, by default. V.4.0 has this attribute selected, by default. The **Minimum m/z** value is set **equal to 1**. The options in the dropdown list box for this selection are **Off**, **equal** to, and **never greater than**. The options for the **Minimum**

Abundance selection are **Off**, **Absolute value**, and **% of the minimum query abundance**. Options for the **Maximum *m/z*** are **On** and **Off.**

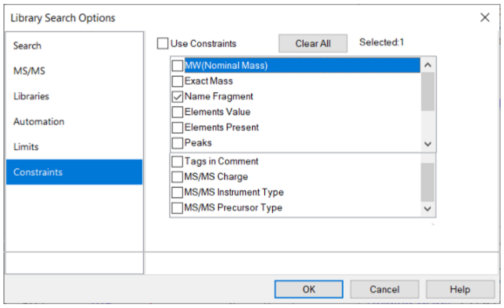
The function of the limits of the starting and ending *m/z* values of a **Library spectrum Search** and the lowest value of a mass spectral peak intensity that will be considered in a search can be set. An explanation of the limits that can be used to constrain a search may be found in the Help file by selecting the **Help** button on the **Limits** tab of the **Library Search Options** dialog box.

Constraints page in the Library Search Options dialog box



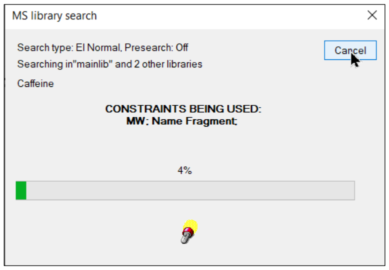
☐ Use Constraints
Selected:0
☐ MW(Nominal Mass)
☐ Exact Mass
☐ Name Fragment
☐ Elements Value
☐ Elements Present
☐ Peaks
☐ Tags in Comment
Molecular Weight 1 2000

EI spectrum type Constraints



☐ Use Constraints
Selected:1
☐ MW(Nominal Mass)
☐ Exact Mass
☑ Name Fragment
☐ Elements Value
☐ Elements Present
☐ Peaks
☐ Tags in Comment
☐ MS/MS Charge
☐ MS/MS Instrument Type
☐ MS/MS Precursor Type

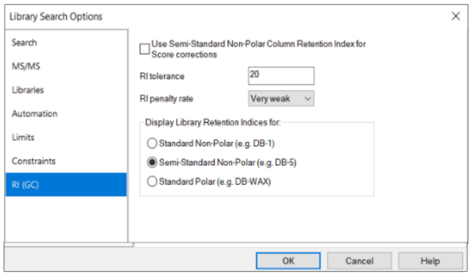
Tandem spectrum type Constraints



Search type: EI Normal, Presearch: Off
Searching in"mainlib" and 2 other libraries
Caffeine
CONSTRAINTS BEING USED:
MW; Name Fragment;
4%

Selecting any checkbox in the list box window with splitter bar of the **Constraint** page will result in queries specific to that individual constraint being displayed in the lower part of this dialog box. (See **Constraints** *page 46* for the **Sequential Search** in the discussion of the **Other Search** tab view.) This will also cause the **Use Constraints** checkbox to be selected. To the right of this checkbox is a **Clear All** button, which deselects all the previously selected constraints. The values enter are still present; however, because the **Use Constraint** checkbox is deselected, these values will not impact the **Hit List** display. To the right of the **Clear All** button is the number of constraints that have been selected. When constraints are used in a search, all the Hits that would have been found if constraints had not been used will still be found; however, only spectra that are within the limits of the constraints will appear in the **Hit List**. This can result in a **Hit List** with less than 100 Hits. The displayed dialog box when a library search is taking place will show that the search is constrained and what constraints are being used.

RI (GC) page in the Library Search Options dialog box



☐ Use Semi-Standard Non-Polar Column Retention Index for Score corrections
RI tolerance 20
RI penalty rate Very weak
Display Library Retention Indices for:
○ Standard Non-Polar (e.g. DB-1)
● Semi-Standard Non-Polar (e.g. DB-5)
○ Standard Polar (e.g. DB-WAX)

The functionality of this page in the **Lib. Search Options** dialog box has undergone significant changes with the release of v.4.0 of MS Search. The lower section, labeled **Display Library Retention Indices for:** allows for the selection of the display of the statistical RI value for any one of the three GC column types in the **NIST GC Method/Retention Index Database**, when **RI** is selected for the display settings in the **Hit List** window of the **Lib. Search** or **Other Search** tab views. If **Semi-Standard Non-Polar (e.g. DB-5)** is selected and there is no value for this column type in the **GC Method/RI Database**, the estimated Artificial Intelligence Retention Index will be displayed.

Selecting or deselecting the checkbox at the top of this page (preceding the **Use Semi-Standard Non-Polar Retention Index for Score correction** label) determines if a measured RI value provided for a SSNP column will be used in conjunction with the SSNP column statistical values in the **GC Method/RI Database** in the calculation of a **Match Factor**. If no statistical data is available for the Hit, the AIRI values will be used in determining the **Match Factor** of the queried spectrum. The displayed **RI** value in the **Hit List** of the **Lib. Search** tab view will be the SSNP statistical value (or AIRI value when not available) regardless of the selection in the **Display Library Retention Indices for:** area.

The selection or deselection of the **Use Semi-Standard Non-Polar Retention Index for Score correction** checkbox has no impact on which of the three column types of RI statistics will be displayed in the **Hit List** of the **Other Search** tab view. If the **Standard Non-Polar (e.g. DB1)** or the **Standard Polar (e.g. DB-WAX)** is selected, and there is no statistical information in the **GC Method/RI Database** for the Hit, this field in the **Hit List** record will be blank. If **Semi-Standard Non-Polar (e.g. DB-5)** is deselected, the statistical information from the **GC Method/RI Database** will be used. If there is no data, the AIRI value will be substituted.

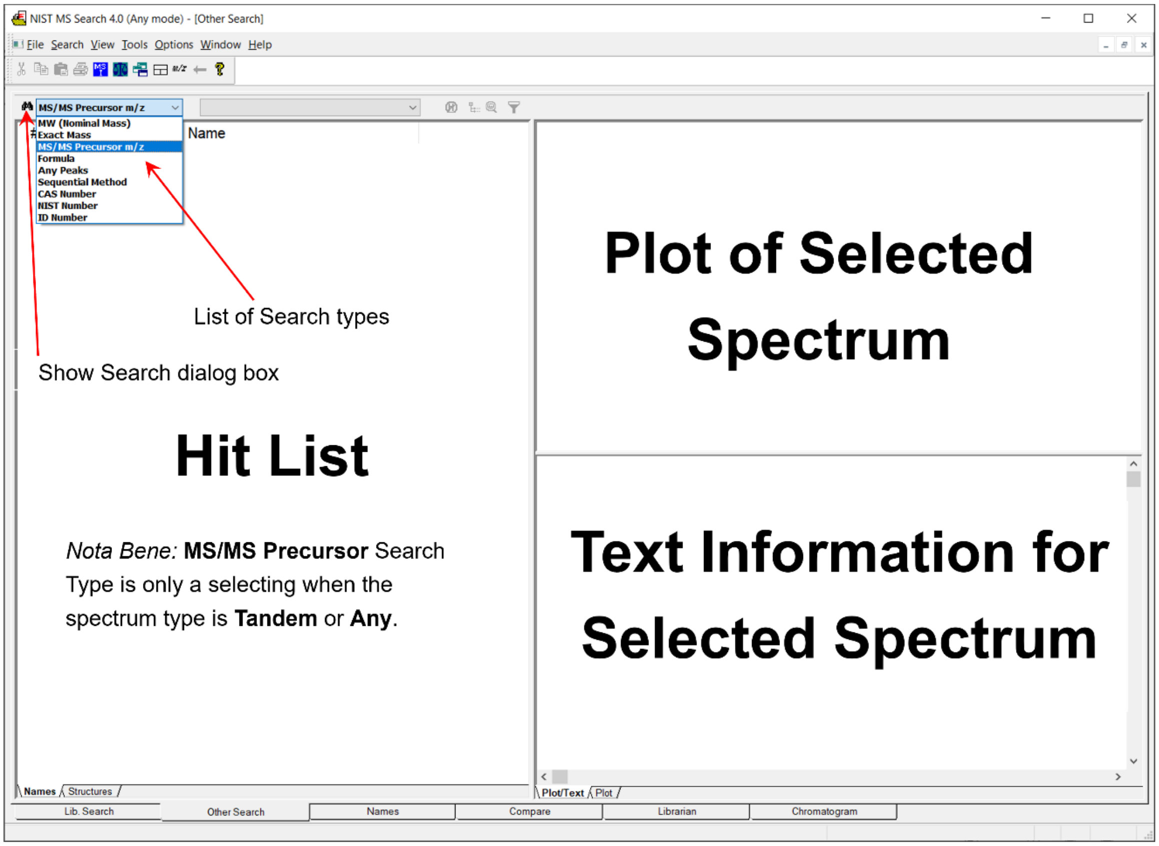
Nota Bene: Settings in this page have no impact on the **LibRI** field of the **Hit List** in the **Chromatogram** tab view.

Other Search tab view

The second tab view, **Other Search** (*Figure 76*), is used to perform a **MW (Nominal Mass)**, **Exact Mass**, **MS/MS Precursor *m/z*** (not available when the **Spectrum Type** is **EI**), **Formula** (elemental composition), **CAS registry number**, **ID Number Search**, **NIST Number** or **Any Peaks**, search when the **EI**, **Tandem**, or **Any** spectrum type is selected. If the **Tandem** or **Any** spectrum type is selected, another search can be performed; the **MS/MS Precursor *m/z*** search. This selection will only appear on the search menu (displayed by selecting the dropdown arrow on the list box just to the right of the button in the **Button Bar**) when the **Tandem** or **Any** spectrum type is selected. These searches are only preformed on mass spectra.

The **Any Peaks Search** allows any NIST or user-library (or multiple libraries) to be queried one mass spectral peak at a time based on the *m/z* value and relative intensity and peak type (**Normal**, **Loss**, **Rank**, **Maxmass**, **Exact Mass**, **AccurateMZ**, or **AccurateLoss**). This search was originally developed for use with the NIH nominal mass library of 12,500 compounds (one spectrum per compound) when its remote access was only available through voice-grade telephone lines, telephone modems, and teletype. This was before the deplanement of data systems. It continued use for integer and accurate *m/z* data has proved very useful. The **Any Peaks Search** was expanded in previous versions to allow for peaks with accurate *m/z* assignments to be searched against the NIST Tandem and user libraries.

The **NIST Number** search can only be carried out on the **NIST/EPA/NIH Main Library** (**mainlib**), the **Replicates Library** (**replib**), the **Tandem Libraries** and the **Retention Index Database** (NIST RI). **NIST Numbers** establish consistency between spectra in different releases of the **NIST MS Libraries** and can be carried out on one or all these libraries at the same time. The **ID Number Search** can only be carried out on one library at a time and can be used with any of the NIST libraries or other libraries such as user-libraries. The **MS/MS Precursor *m/z* Search** works with the NIST Tandem libraries and user-libraries containing Tandem spectra. Many of searches can be performed against multiple libraries at a time (max. of 127), and different sets of libraries can be specified for each different **Searches**.

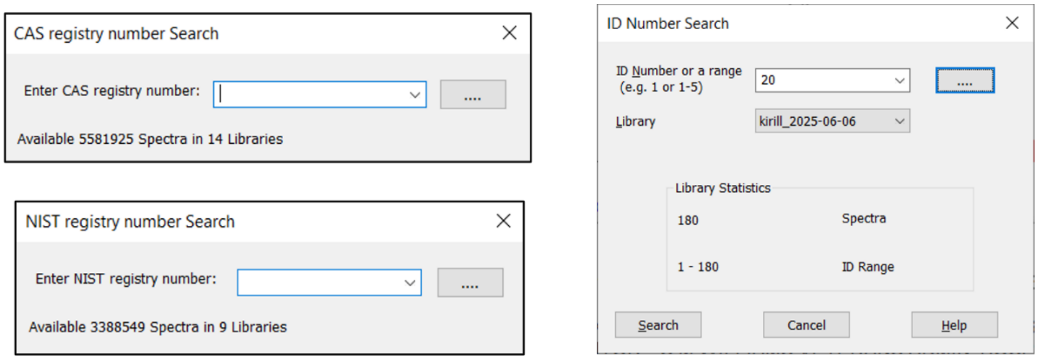


Nota Bene: **MS/MS Precursor** Search Type is only a selecting when the spectrum type is **Tandem** or **Any**.

Figure 76. Other Search tab as viewed for the EI Spectrum type of NIST 26.

Searching List of CAS Registry numbers, NIST Numbers, or ID Numbers

Figure 77 shows the tops of the **CAS rn Search** and **NIST registry number Search** dialog boxes and the **ID number Search** dialog box displayed in the **Other Search** tab view. The Mouse pointer can be placed on the button to the right of the text entry box where the values for these searches are entered. If that button is selected, a file **Open** dialog box is displayed pointing to the folder containing the MS Search Program and looking for a TXT file. If a TXT file containing a list of CAS#, without dashes (-), NIST#, or ID# in a column, it can be selected for the search of the list.



Enter CAS registry number:

Available 5581925 Spectra in 14 Libraries

Enter NIST registry number:

Available 3388549 Spectra in 9 Libraries

ID Number or a range (e.g. 1 or 1-5): 20

Library: kirill_2025-06-06

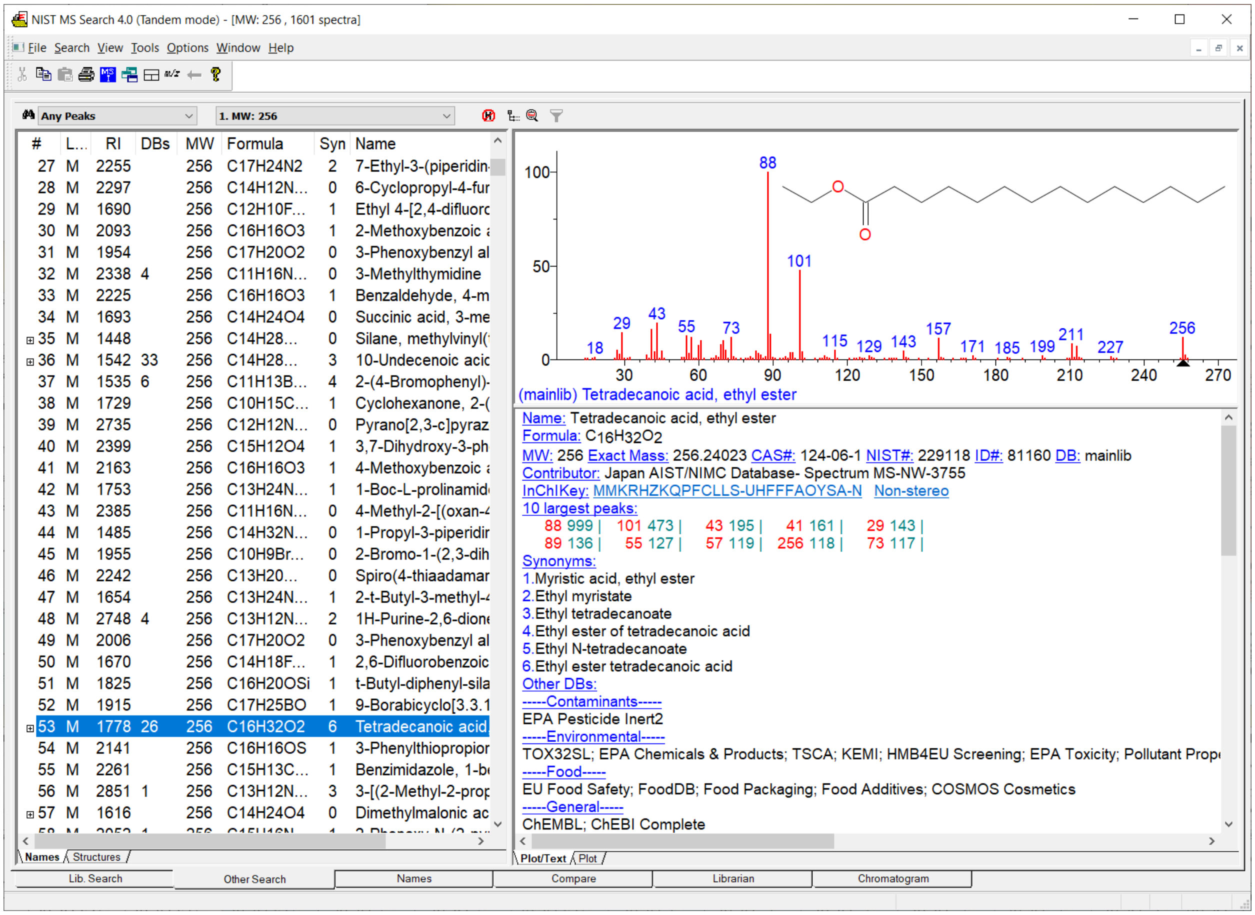
180 Spectra

1 - 180 ID Range

Figure 77. Tops of NIST No. and CASrn Search dialog boxes in the Other Search tab view.

As shown in *Figure 78,* a feature introduced in v.2.2 of **MS Search** (**NIST 14**) was the display of columns showing not only the number of **Synonyms**, the number of **Other** (non-mass spectral) **Databases** in which the **Hit** can be found but also the retention index (**RI**), if available. The **RI** value shown is determined by settings in the **Library Search Options** dialog box, displayed from the **Options** menu of the Main Menu or by selecting the button in the **Lib. Search** tab view. These column headers are available for the **Hit List** of searches performed in **Lib. Search** tab view. The display of these columns is controlled by the **Hit List** page of the **Properties** dialog box displayed by selecting the **Properties** option from the **RMB** menu displayed when the Mouse pointer is in a **Hit List** window of these two tab-views. New in v.4.0 is the possibility to display the **MW** (nominal mass in the case of EI spectra) and **Formula** (elemental composition).

For data when using the Tandem spectrum type, it is possible to add precursor type (**Prec. Type**), instrument type (**Instr. Type**), **Energy**, and modes (**Mods**) fields to the **Hit List**.



#	L...	RI	DBs	MW	Formula	Syn	Name
27	M	2255		256	C17H24N2	2	7-Ethyl-3-(piperidin
28	M	2297		256	C14H12N...	0	6-Cyclopropyl-4-fur
29	M	1690		256	C12H10F...	1	Ethyl 4-[2,4-difluoro
30	M	2093		256	C16H16O3	1	2-Methoxybenzoic
31	M	1954		256	C17H20O2	0	3-Phenoxybenzyl al
32	M	2338	4	256	C11H16N...	0	3-Methylthymidine
33	M	2225		256	C16H16O3	1	Benzaldehyde, 4-m
34	M	1693		256	C14H24O4	0	Succinic acid, 3-me
⊞ 35	M	1448		256	C14H28...	0	Silane, methylvinyl(
⊞ 36	M	1542	33	256	C14H28...	3	10-Undecenoic acid
37	M	1535	6	256	C11H13B...	4	2-(4-Bromophenyl)-
38	M	1729		256	C10H15C...	1	Cyclohexanone, 2-(
39	M	2735		256	C12H12N...	0	Pyrano[2,3-c]pyraz
40	M	2399		256	C15H12O4	1	3,7-Dihydroxy-3-ph
41	M	2163		256	C16H16O3	1	4-Methoxybenzoic
42	M	1753		256	C13H24N...	1	1-Boc-L-prolinamide
43	M	2385		256	C11H16N...	0	4-Methyl-2-[(oxan-4
44	M	1485		256	C14H32N...	0	1-Propyl-3-piperidir
45	M	1955		256	C10H9Br...	0	2-Bromo-1-(2,3-dih
46	M	2242		256	C13H20...	0	Spiro(4-thiaadamar
47	M	1654		256	C13H24N...	1	2-t-Butyl-3-methyl-4
48	M	2748	4	256	C13H12N...	2	1H-Purine-2,6-dione
49	M	2006		256	C17H20O2	0	3-Phenoxybenzyl al
50	M	1670		256	C14H18F...	1	2,6-Difluorobenzoic
51	M	1825		256	C16H20OSi	1	t-Butyl-diphenyl-sila
52	M	1915		256	C17H25BO	1	9-Borabicyclo[3.3.1
⊞ 53	M	1778	26	256	C16H32O2	6	Tetradecanoic acid
54	M	2141		256	C16H16OS	1	3-Phenylthiopropior
55	M	2261		256	C15H13C...	1	Benzimidazole, 1-be
56	M	2851	1	256	C13H12N...	3	3-[(2-Methyl-2-prop
⊞ 57	M	1616		256	C14H24O4	0	Dimethylmalonic ac

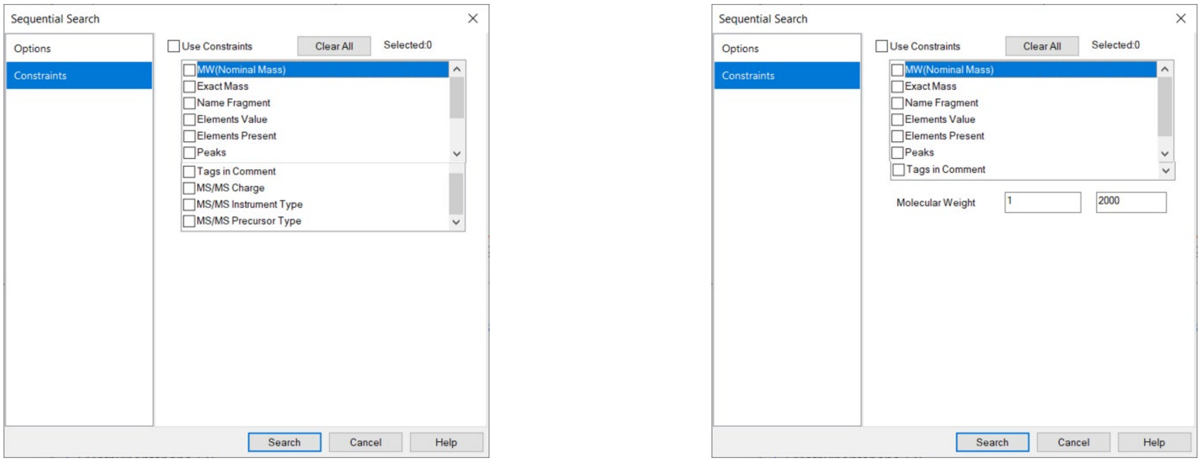
Name: Tetradecanoic acid, ethyl ester
Formula: $C_{16}H_{32}O_2$
MW: 256 Exact Mass: 256.24023 CAS#: 124-06-1 NIST#: 229118 ID#: 81160 DB: mainlib
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-3755
InChIKey: MMKRHZKQPFCLLS-UHFFFAOYSA-N Non-stereo
10 largest peaks:
88 999 | 101 473 | 43 195 | 41 161 | 29 143 |
89 136 | 55 127 | 57 119 | 256 118 | 73 117 |
Synonyms:
1.Myristic acid, ethyl ester
2.Ethyl myristate
3.Ethyl tetradecanoate
4.Ethyl ester of tetradecanoic acid
5.Ethyl N-tetradecanoate
6.Ethyl ester tetradecanoic acid
Other DBs:
-----Contaminants-----
EPA Pesticide Inert2
-----Environmental-----
TOX32SL; EPA Chemicals & Products; TSCA; KEMI; HMB4EU Screening; EPA Toxicity; Pollutant Prop
-----Food-----
EU Food Safety; FoodDB; Food Packaging; Food Additives; COSMOS Cosmetics
-----General-----
ChEMBL; ChEBI Complete

Figure 78. Results of a Formula Search showing the number of Synonyms for the Hits, the number of Other Databases in which the Hits can be found, and the RI value (if present) columns.

A very important search carried out in the **Other Search** tab view is the **Sequential Method Search**. This is a wild card search which allows the user to customize the mass spectral and metadata included in the search. As an example, with the **Sequential Method Search**, the search can be limited to only spectra that comes from a specific contributor.

The dialog box for a **Sequential Search** is different depending on the spectrum type (**EI** or **Tandem**) selected. The available selections are dependent on the selected **Spectrum Type**. The figure below shows what would be seen if the **Any** or **Tandem** (left) and **EI** (right) spectrum types were selected.

The Options page of the Sequential Search dialog box of the Other Search tab view is the same for both spectrum types. It allows the specification of the libraries to be searched and the **Max Num of Hits** (6,000 or less). Libraries are selected in the same way as they are in the **Libraries** page of the **Library Search Options** dialog box of the **Lib. Search** tab view.



Molecular Weight 1 2000

Figure 79. Sequential Search dialog box for the EI Spectrum type (left) and Tandem Spectrum type (right). These selections are the same as the Constraints selection.

Names tab view

The third tab view, **Names**, is used for the NIST **Incremental Name Search** (**Names)**. This view has two different appearances. One is for the search of NIST provided libraries (*Figure 80* and *Figure 81*) and the other is for user-libraries including those provided by third parties such as the *Wiley Registry of Mass Spectral Data* (*Figure 82*).

The **Names** search can only be performed against one mass spectral library at a time. The library to be searched is selected by using the **Names** tab view's dropdown list box next to the a-z button. If the a-z button (far left just below the **Button Bar**) is selected, the only allowed characters in the text entry field are the letters **a–z**. If the a-z button has NOT been selected, numbers as well as letters can be input. Names containing Greek alphabet characters can be pasted into the text entry field. Typed dashes, parentheses [( )] and other punctuation characters will not be accepted. All letters are entered as upper-case regardless of the input. Previous versions of MS Search allowed for a maximum of 18 characters. As of V.3.0, this was expanded to 250 characters.

When an NIST Library is being searched (mainlib, replib, tandem_msms, etc.), the left side of the display is divided into two parts. The upper part is the alphabetical list of names. This is not only the primary name (mainlib name) but also all the synonyms associated with a spectrum.

The **Name** search of both the **NIST/EPA/NIH EI Library**, the **NIST Tandem Mass Spectral Library** and user-libraries use not only the main name but also any names in the synonyms field. As an example, the **mainlib** compound name for acetylsalicylic acid is the common name *Aspirin*. This compound has 157 synonyms, and its spectrum can be displayed by entering any of these names including trade names such as *Bayer*.

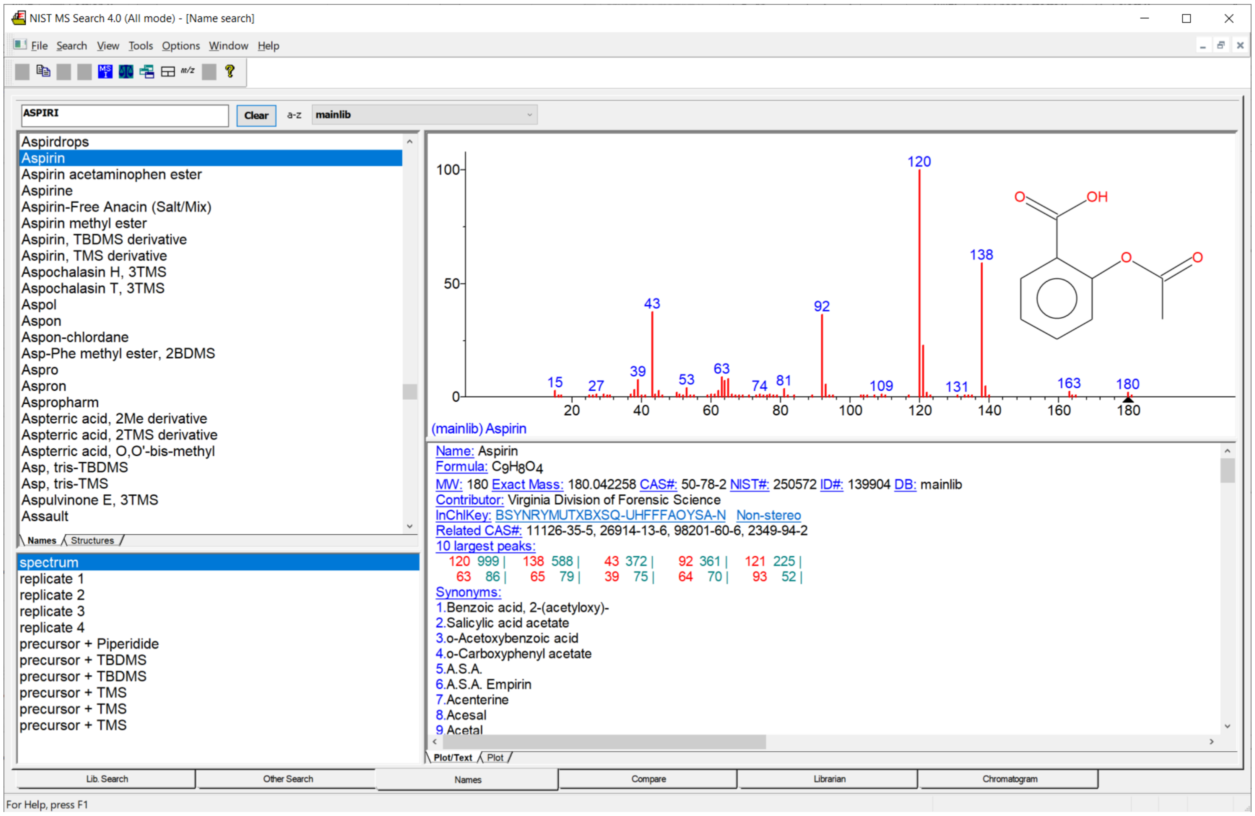
By default, the entered string represents the second name in the **Hit List**. That name is highlighted. Any name in the **Hit List** can be highlighted by placing the Mouse pointer on it and clicking the left Mouse button once. For NIST provided libraries, The upper part of the **Hit List** will continue to appear as before, only the highlighted spectrum is visible in the two windows to the right. The bar graph spectrum for that compound appears in the upper window on the right. This is by default. The display can be changed to three vertical Window, by selecting **Change Splitter Orientation** from the **RMB** menu. Another view can be displayed when clicking on the **Change layout** (⊟) button in the **Button Bar**. The original layout can be restored by clicking on the **Set default layout** ( ) button. The lower window contains the spectrum metadata. Other display options can be found in the **Names Search Properties** 3-page dialog box displayed by selecting **Properties** from the **RMB** menu.

The **Hit List** window is divided into two parts when searching an NIST provided library, The bottom part contains all associated spectra with the selected spectrum in the top part. The first name in the list in the bottom part (**spectrum**) is the display of the highlighted spectrum in the top part of the **Hit List** window. This is followed by a list of one or more **replicate**(s) if there are multiple spectra for the compound. As each entry in the lower list is highlighted, the information in the right-side upper and lower windows will change. The highlight in the left-side upper window remains in its original position.

Following the **replicate**(s) is **structural replicate, isotope**, which in the case of naphthalene are several examples of deuterated naphthalene. Following this in the list is the label (**precursor + #**) followed by the name of a derivatizing agent. These spectra will always be listed regardless of the settings in the **Replicates** dialog box displayed from **Options** on the Main Menu.

If the NIST/EPA/NIH EI Library (mainlib) is being worked with, a good example would be to enter NAPHTHALENE. This compound has one mainlib spectrum (**spectrum**) and three replicate spectra (**replicate 1**, **replicate 2**, and **replicate 3**). Following that are three listings (**structural replicate, isotope**), each representing a different deuterated variant of naphthalene.

For NIST Tandem Libraries, both accurate *m/z* value spectra (high res) and integer *m/z* spectra (low res) are grouped together for the purpose of the **Incremental Name** search (**ht/lr NIST tandem**). Once this Library has been selected, as the name is typed in the text entry box, the extended name will appear in the upper part of the **Hit List**. The lower part of the **Hit List** contains the individual product-ion spectra for the specified precursor ion. The listing begins with the precursor type ([M+H]+, [M-H]-, [M+2H]+, [M+H-C6H5CO2H]+, [M+Na]+, [M-H2O]+, etc.). The precursor type is followed by the instrument type (HCD, IT, QQQ, IT-FT, and QTOF). Next is the percent of Collision Enter value when applicable, and the *m/z* value of precursor ion to a single decimal place; i.e., P= 357.3 for the [**2**M+H]+ of the protonated dimer of 3-methy-5-phenylpentanol (MW 178 Da with an observed precursor-ion at *m/z* 357.2787 when measured using HCD). If the spectrum was measured at other an accurate *m/z* value, the listing will end with (LR). An example of a name search is shown below.



ASPIRI

mainlib

Aspirdrops
Aspirin
Aspirin acetaminophen ester
Aspirine
Aspirin-Free Anacin (Salt/Mix)
Aspirin methyl ester
Aspirin, TBDMS derivative
Aspirin, TMS derivative
Aspochalasin H, 3TMS
Aspochalasin T, 3TMS
Aspol
Aspon
Aspon-chlordane
Asp-Phe methyl ester, 2BDMS
Aspro
Aspron
Aspropharm
Aspterric acid, 2Me derivative
Aspterric acid, 2TMS derivative
Aspterric acid, O,O'-bis-methyl
Asp, tris-TBDMS
Asp, tris-TMS
Aspulvinone E, 3TMS
Assault

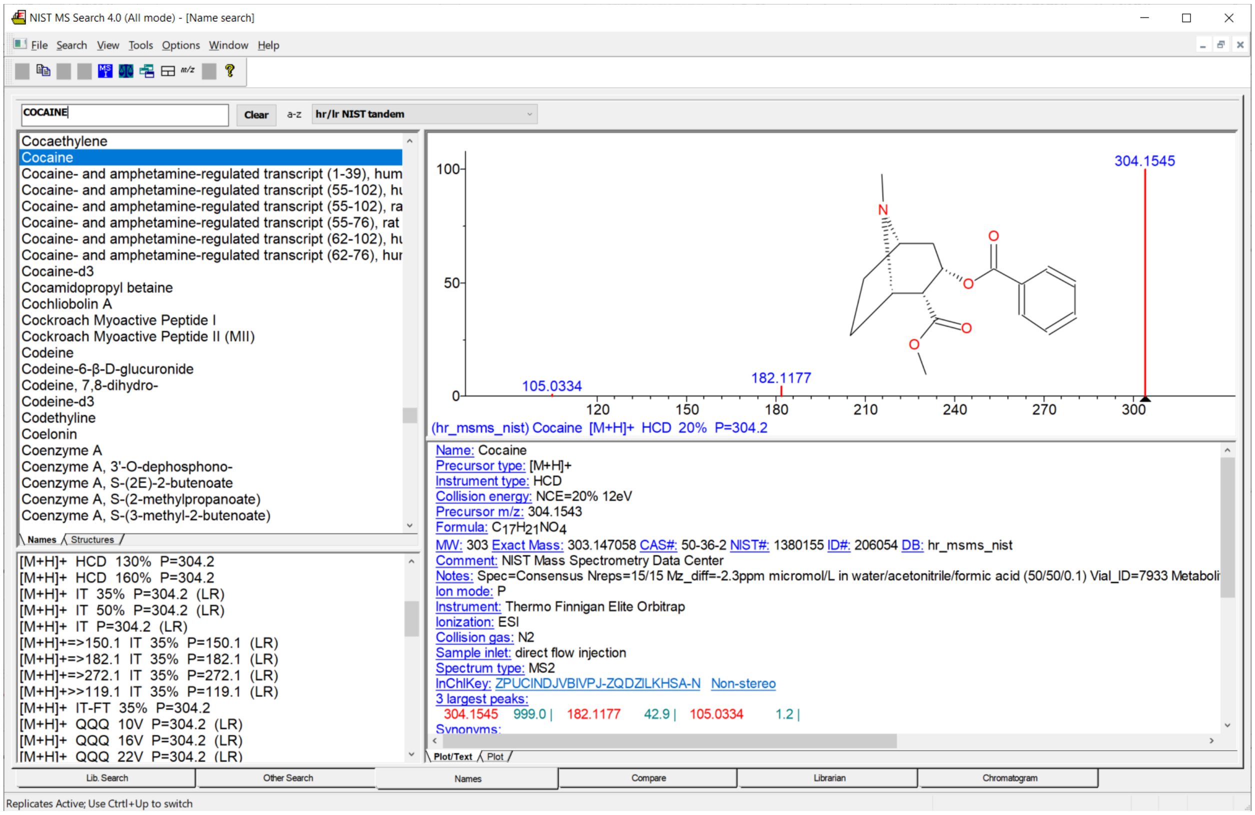
spectrum
replicate 1
replicate 2
replicate 3
replicate 4
precursor + Piperidide
precursor + TBDMS
precursor + TBDMS
precursor + TMS
precursor + TMS
precursor + TMS

(mainlib) Aspirin

Name: Aspirin
Formula: $C_9H_8O_4$
MW: 180 Exact Mass: 180.042258 CAS#: 50-78-2 NIST#: 250572 ID#: 139904 DB: mainlib
Contributor: Virginia Division of Forensic Science
InChIKey: BSYNRYMUTXBXSQ-UHFFFAOYSA-N Non-stereo
Related CAS#: 11126-35-5, 26914-13-6, 98201-60-6, 2349-94-2
10 largest peaks:
120 999 | 138 588 | 43 372 | 92 361 | 121 225 |
63 86 | 65 79 | 39 75 | 64 70 | 93 52 |
Synonyms:
1.Benzoic acid, 2-(acetyloxy)-
2.Salicylic acid acetate
3.o-Acetoxybenzoic acid
4.o-Carboxyphenyl acetate
5.A.S.A.
6.A.S.A. Empirin
7.Acenterine
8.Acesal
9.Acetal

Figure 80. Incremental Name Search Using NIST 26 EI Library.

Nota Bene: The use of synonyms is also supported in user libraries. Thus, if a spectrum is given a synonym “Lab Book 4-PG-22”, then the data can be retrieved by that name using the **Incremental Name** search. Replicate, isotopic variant, and stereoisomeric spectra are not supported in the **Incremental Name** search of third-party and user-libraries.



COCAINE

Cocaethylene
Cocaine
Cocaine- and amphetamine-regulated transcript (1-39), hum
Cocaine- and amphetamine-regulated transcript (55-102), hu
Cocaine- and amphetamine-regulated transcript (55-102), ra
Cocaine- and amphetamine-regulated transcript (55-76), rat
Cocaine- and amphetamine-regulated transcript (62-102), hu
Cocaine- and amphetamine-regulated transcript (62-76), hur
Cocaine-d3
Cocamidopropyl betaine
Cochliobolin A
Cockroach Myoactive Peptide I
Cockroach Myoactive Peptide II (MII)
Codeine
Codeine-6-β-D-glucuronide
Codeine, 7,8-dihydro-
Codeine-d3
Codethyline
Coelonin
Coenzyme A
Coenzyme A, 3'-O-dephosphono-
Coenzyme A, S-(2E)-2-butenoate
Coenzyme A, S-(2-methylpropanoate)
Coenzyme A, S-(3-methyl-2-butenoate)

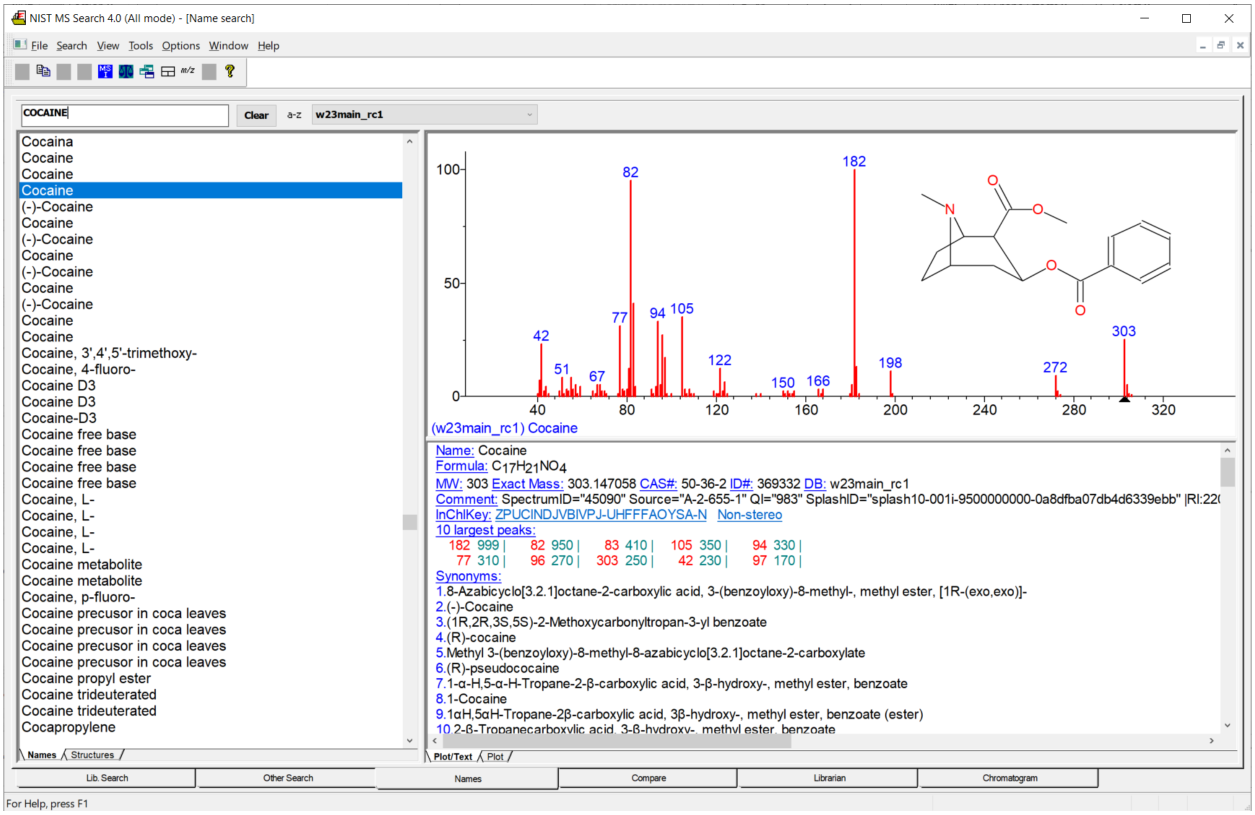
[M+H]+ HCD 130% P=304.2
[M+H]+ HCD 160% P=304.2
[M+H]+ IT 35% P=304.2 (LR)
[M+H]+ IT 50% P=304.2 (LR)
[M+H]+ IT P=304.2 (LR)
[M+H]+=>150.1 IT 35% P=150.1 (LR)
[M+H]+=>182.1 IT 35% P=182.1 (LR)
[M+H]+=>272.1 IT 35% P=272.1 (LR)
[M+H]+>>119.1 IT 35% P=119.1 (LR)
[M+H]+ IT-FT 35% P=304.2
[M+H]+ QQQ 10V P=304.2 (LR)
[M+H]+ QQQ 16V P=304.2 (LR)
[M+H]+ QQQ 22V P=304.2 (LR)

(hr_msms_nist) Cocaine [M+H]+ HCD 20% P=304.2

Name: Cocaine
Precursor type: [M+H]+
Instrument type: HCD
Collision energy: NCE=20% 12eV
Precursor m/z: 304.1543
Formula: $C_{17}H_{21}NO_4$
MW: 303 Exact Mass: 303.147058 CAS#: 50-36-2 NIST#: 1380155 ID#: 206054 DB: hr_msms_nist
Comment: NIST Mass Spectrometry Data Center
Notes: Spec=Consensus Nreps=15/15 Mz_diff=-2.3ppm micromol/L in water/acetonitrile/formic acid (50/50/0.1) Vial_ID=7933 Metaboli
Ion mode: P
Instrument: Thermo Finnigan Elite Orbitrap
Ionization: ESI
Collision gas: N2
Sample inlet: direct flow injection
Spectrum type: MS2
InChIKey: ZPUCINDJVBIVPJ-ZQDZILKHSA-N Non-stereo
3 largest peaks:
304.1545 999.0 | 182.1177 42.9 | 105.0334 1.2 |
Synonyms:

Replicates Active; Use Ctrl+Up to switch

Figure 81. Incremental Name Search Using NIST 26 Tandem Library.



COCAINE

Cocaina
Cocaine
Cocaine
Cocaine
(-)-Cocaine
Cocaine
(-)-Cocaine
Cocaine
(-)-Cocaine
Cocaine
(-)-Cocaine
Cocaine
Cocaine
Cocaine, 3',4',5'-trimethoxy-
Cocaine, 4-fluoro-
Cocaine D3
Cocaine D3
Cocaine-D3
Cocaine free base
Cocaine free base
Cocaine free base
Cocaine free base
Cocaine, L-
Cocaine, L-
Cocaine, L-
Cocaine, L-
Cocaine metabolite
Cocaine metabolite
Cocaine, p-fluoro-
Cocaine precusor in coca leaves
Cocaine precusor in coca leaves
Cocaine precusor in coca leaves
Cocaine precusor in coca leaves
Cocaine propyl ester
Cocaine trideuterated
Cocaine trideuterated
Cocapropylene

(w23main_rc1) Cocaine

Name: Cocaine
Formula: $C_{17}H_{21}NO_4$
MW: 303 Exact Mass: 303.147058 CAS#: 50-36-2 ID#: 369332 DB: w23main_rc1
Comment: SpectrumID="45090" Source="A-2-655-1" QI="983" SplashID="splash10-001i-9500000000-0a8dfba07db4d6339ebb" |RI:22
InChIKey: ZPUCINDJVBIVPJ-UHFFFAOYSA-N Non-stereo
10 largest peaks:
182 999 | 82 950 | 83 410 | 105 350 | 94 330 |
77 310 | 96 270 | 303 250 | 42 230 | 97 170 |
Synonyms:
1.8-Azabicyclo[3.2.1]octane-2-carboxylic acid, 3-(benzoyloxy)-8-methyl-, methyl ester, [1R-(exo,exo)]-
2.(-)-Cocaine
3.(1R,2R,3S,5S)-2-Methoxycarbonyltropan-3-yl benzoate
4.(R)-cocaine
5.Methyl 3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate
6.(R)-pseudococaine
7.1-α-H,5-α-H-Tropane-2-β-carboxylic acid, 3-β-hydroxy-, methyl ester, benzoate
8.1-Cocaine
9.1αH,5αH-Tropane-2β-carboxylic acid, 3β-hydroxy-, methyl ester, benzoate (ester)
10.2-β-Tropanecarboxylic acid, 3-β-hydroxy-, methyl ester, benzoate

For Help, press F1

Figure 82. Incremental Name Search Using a Third-Party EI Library.

The Compare tab view

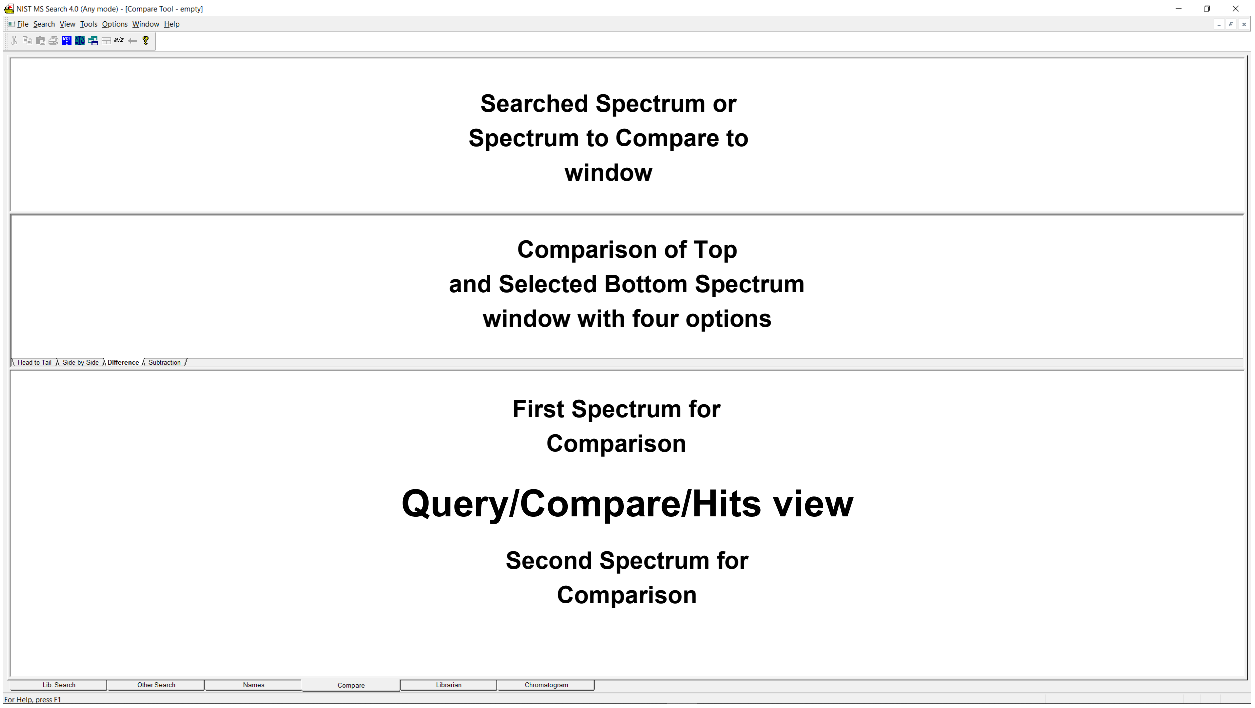
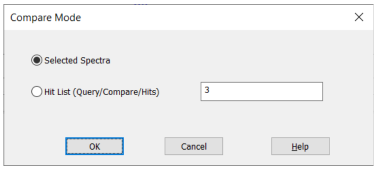


Figure 83. Blank Compare tab view with Hit List (Query/Compare/Hits) option selected.

The fourth tab view, **Compare**, has two modes of operation. These can be alternately chosen by using the **Select Compare Mode** selection from the **Options** menu displayed by selecting **Options** on the Main Menu of the **Compare** tab view. This selection is only available on the **Option** menu of the Main Menu of the **Compare** tab view. The default is **Selected Spectra**. When a selection is chosen in the **Compare Mode** dialog box, the current contents will be deleted. If the **Hit List (Query/Compare/Hits)** option is selected, a blank set of windows will be displayed as shown *Figure 83*.



● Selected Spectra

○ Hit List (Query/Compare/Hits) 3

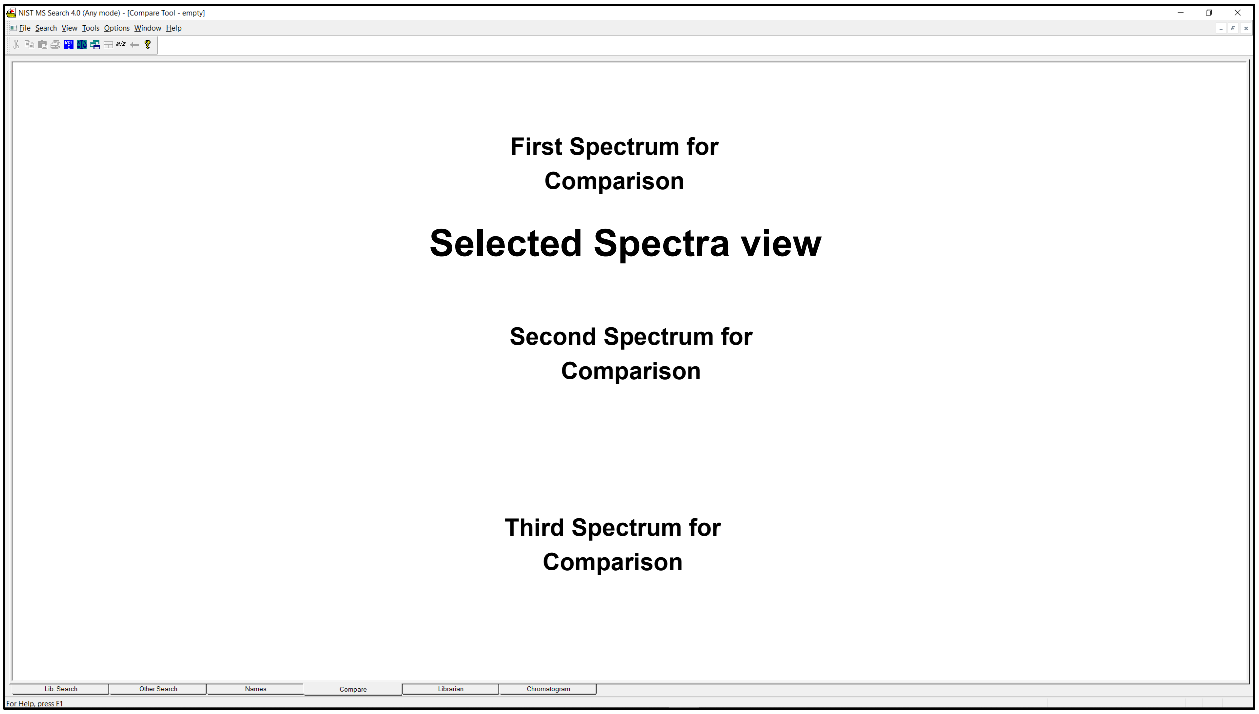
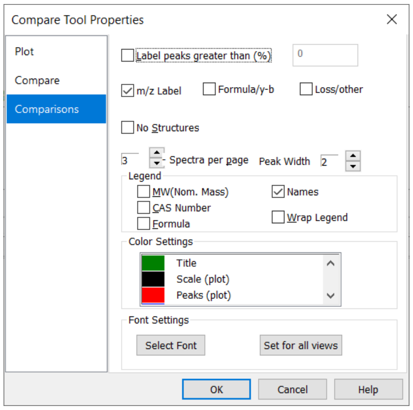


Figure 84. Blank Compare tab view with Selected Spectra option chosen.

If the **Selected Spectra** option is chosen in the **Compare Mode** dialog box, the **Compare** tab view will appear as shown *Figure 84*. Nearly every **RMB** menu in all the windows of all the tab views has a **Send To\Compare List** selection. This action can perform differently depending on where the spectrum/spectra is/are being sent from. The first time MS Search is started, the **Compare** tab view is blank, as shown in *Figure 84*. The **Compare** tab view will retain the contents and configuration present when the program is closed. Therefore, before sending spectra to the Compare tab view, it is best to select the **Compare** tab view and use the **Clear** selection from the **RMB** menu. This would be the time to change the mode in the **Compare Mode** dialog box, if desired.

With the **Compare Mode** set to the default (**Selected Spectra**), after performing a library search in the **Lib. Search** tab view, the **Compare** tab view WILL NOT be brought into focus. Select several spectra for a comparison from the **Hit List** window of the **Lib. Search** tab view. Issue the **RMB** menu **Send To\Compare List** command. The focus will automatedly change to the **Compare** tab view and it will appear as shown in *Figure 83* but populated. The top spectrum is the one just searched. Next, is the **Compare** window with four display options (**Head-to-Tail** (sometimes referred to as a *butterfly* display), **Side-by-Side**, **Difference**, or **Subtraction**). The lower section contains the spectra to be compared with the queried spectrum.



☐ Label peaks greater than (%) 0

☑ m/z Label ☐ Formula/y-b ☐ Loss/other

☐ No Structures

3 Spectra per page Peak Width 2

Legend

☐ MW(Nom. Mass) ☑ Names

☐ CAS Number ☐ Wrap Legend

☐ Formula

Color Settings

🟩 Title

⬛ Scale (plot)

🟥 Peaks (plot)

Font Settings

Select Font Set for all views

Figure 85. Compare Tool Properties dialog box, Compare List page.

Return the focus to the **Lib. Search** tab view, select a new set of spectra from the **Hit List**, and issue the **Send To\Compare List** command. The focus automatically changes to the **Compare** tab view, and the lower section now contains the just selected spectra for comparison with the queried spectrum, which is still in the top window. The maximum number of comparison spectra visible in the display is set in the **Comparisons** page of the **Compare Tool Properties** dialog box (*Figure 85*). The number of spectra available for comparison can exceed this number and are brought into focus via a scroll bar on the right side of the lower window.

If the **Lib. Search** tab view **Spec List Plot/Text information** window has the **Spec List** tab selected, the spectrum in the top window of the **Compare** tab view may not necessarily be that spectrum. The spectrum displayed in the **Query Spectrum** window of the **Compare** tab view is that which generated the **Hit List**.

When the **Compare** tab view comes into focus, the spectrum in the list of spectra is automatically compared to the spectrum in the **Query Spectrum** window. To compare another spectrum, put the Mouse pointer on the spectrum in the list and click the left Mouse button once. It may be necessary to use the scroll bar on the right side of the compare spectrum area to make the desire spectrum visible.

Selecting **Hit List (Query/Compare/Hits)** in the **Compare Mode** dialog box will cause the **Compare** tab view to be that shown in *Figure 83.* The value in the box to the right of the **Hit List (Query/Compare/Hits)** label is the number of *first* Hits that will be sent to the *spectra for comparison* area. Now, when a search is performed in the **Lib. Search** tab view, these areas in the **Compare** tab view will be populated. The **Compare** tab view WILL NOT come into focus. To show the results, select the **Compare** tab at the bottom of the program's display. Each time a library search is performed, the areas in the **Compare** tab view will be repopulated and the **Compare** tab view will not come into focus until selected. The first X Hits are displayed in the *spectra for comparison* area.

In this mode, the **Lib. Search** tab view can be brought into focus after viewing the **Compare** tab view, one or more spectra selected from the **Hit List** window, and the **RMB** menu **Send To\Compare List** command issued. The **Compare** tab view will automatically come into focus with the additional spectrum/spectra selected from the **Hit List** replacing the existing spectra and appearing in the *spectra for comparison* area of the **Compare** tab view.

Comparing selected spectra with queried spectrum: When the **Compare** tab view is in the configuration shown in *Figure 83*, the top window is a plot of the queried spectrum. The next window is the **Compare** window. This has four different display options: **Head to Tail** (butterfly), **Side by Side**, **Difference**, and **Subtraction**. The different displays are selected by placing the Mouse pointer on the labeled tab at the bottom of the **Compare** window and clicking the left Mouse button. The **Head to Tail** (butterfly) and **Side by Side** displays are self-explanatory. The **Difference** is a butterfly display showing peaks in one color pointing up that are exclusive to the queried spectrum and pointing down that are exclusive to selected spectrum from the *spectra for comparison* area. A third color is used to indicate peaks present in the spectra of both compounds. This can point up or down and their intensity is associated with the spectrum having the highest abundance with lower abundance subtracted. The **Subtraction** display is where all *m/z* values and their intensities in the Hit spectrum are subtracted from the queried spectrum. Searching the defense may identify a compound whose mass spectrum exists in the data with that of the reported Hit.

Spectra can be copied from any source in MS Search and pasted into the **Queried Spectrum** window or the window containing spectra to be compared to the quired spectrum of the **Compare** tab view. If the paste is done to the window containing spectra to be compared, the current **Compare** tab view will be replaced by the spectrum/spectra being pasted. The **Compare** and **Queried Spectrum** windows will no longer be visible. If a single spectrum is pasted to the **Queried Spectrum** window it will replace the contents. If an attempt is made to paste multiple spectra, only the first one will appear.

Put the Mouse pointer on any spectrum display in any **Plot** or **Text Information** window or any **Hits List** or **Spec List** window of any tab view and display the **RMB** menu. Select **Copy** (NOT **Copy Plot**). Bring the **Compare** tab into focus. Put the Mouse pointer on the top of the **Queried Spectrum** window, display the **RMB** menu, and select **Paste**. This will replace the current displayed spectrum. The same actions WILL NOT replace the spectra in the *spectra for comparison* area. The only way to add spectra to the *spectra for comparison* area is to send them from the **Hit List** or **Spec List** using the **RMB** menu **Send To\Compare List** command. If an attempt to Paste spectra to the **Compare** tab view's spectrum compare area is made, the exiting display will be replaced with a queue of spectral plots being sent. The **Send To\Compare List RMB** menu command issued from the **Other Search**, **Names**, **Librarian**, and **Chromatogram** tab views will replace with the selected spectra what is in the **Compare** tab view if it is configured as the **Query/Compare/Hits** view regardless of the selected mode of the **Compare Mode** dialog box. If the contents are that of the **Selected Spectra** view, the sent spectra are prepended to the list. As new spectra are sent, they will be prepended until the **RMB** menu **Clear** command is issued.

By placing the Mouse pointer in either window contaminating text information in the **Chromatogram** tab view, the spectrum (Library Match or Deconvoluted) can be sent to the **Compare** tab view using the **RMB** menu **Send To\Compare List** command. Placing the Mouse pointer on one of the component indicators (○) in the **Chromatogram** window allows that deconvoluted spectrum to be sent using the same **RMB** menu command. One or more selected deconvoluted (only) spectra can be sent to the **Compare** tab view using this same **RMU** menu command from the **Chromatogram** tab view's **Chromatogram** window.

One important consideration in sending spectra from the **Chromatogram** tab view is the situation where spectra have been sent to the **Compare** tab view and then a sequent chromatographic file is processed. If spectra are sent to the **Compare** tab view without first **using the RMB** menu **Clear** command in the **Compare** tab view, the spectra from the new file will prepend to those already present.

Regardless of the **Compare** tab's view (**Query/Compare/Hits** or **Selected Spectra**) or the selected mode of the **Compare Mode** dialog box, spectra from the **Chromatogram** sent using the **RMB** menu **Send To\Compare List** command will overwrite the existing **Query/Compare/Hits** view and appear in the **Selected Spectra** view or prepend the contents of the existing **Selected Spectra** view.

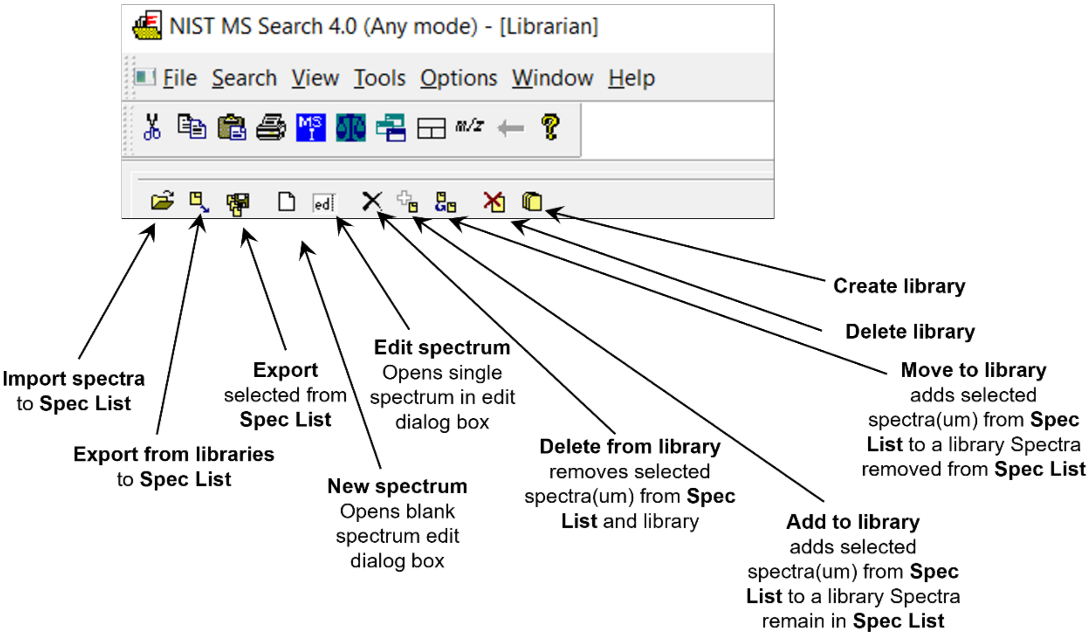
The **Change Layout** button (⊟) on the **Toolbar** is inactive (grayed); however, the **Set default layout** button (⊞) is not. If ⊞ button is selected when the **Compare** tab is in the **Select Spectrum** view, regardless the setting in the **Compare Mode** dialog box, the view will be changed to the **Query/Compare/Hits** view. The Query Spectrum and Compare windows will be blank and the spectra window will be blank. The spectra that were in the Selected Spectra view are now in the lower part of the display

Random spectra can be compared in the **Compare** tab view. Select the **Compare** tab view. If the display is the **Query/Compare/Hits** view, change it to the **Selected Spectrum** view (*Figure 84*) using the **Compare Mode** dialog box displayed by selection **Select Compare Mode** from the **Option** menu of the Main Menu. Select the **Clear** command from the **RMB** menu. Select spectra from various tab views can be copied using the **Copy** command on the **RMB** menu. Bring the **Compare** tab view into focus and then issue the **Paste** command. Alternately, the **Send To\Compare List** command can be used after selecting the spectra. This process can be repeated several times. After all the spectra to be compared to a single spectrum have been collected, select that single spectrum and issue the **RMB** menu **Copy** command. Bring the **Compare** tab view into focus and select the ⊞ button. This will restore **Query/Compare/Hits** view. Place the Mouse pointer in the **Query Spectrum** window and issue the **RMB** menu **Paste** command. Comparisons can then be made by selecting spectra, one at a time, in the *spectra for comparison* area.

There is no **Change Splitter Orientation** on the **RMB** menu when displayed from any of the windows.

.

The Librarian tab view



Import spectra to **Spec List**

Export from libraries to **Spec List**

Export selected from **Spec List**

New spectrum Opens blank spectrum edit dialog box

Edit spectrum Opens single spectrum in edit dialog box

Delete from library removes selected spectra(um) from **Spec List** and library

Add to library adds selected spectra(um) from **Spec List** to a library Spectra remain in **Spec List**

Move to library adds selected spectra(um) from **Spec List** to a library Spectra removed from **Spec List**

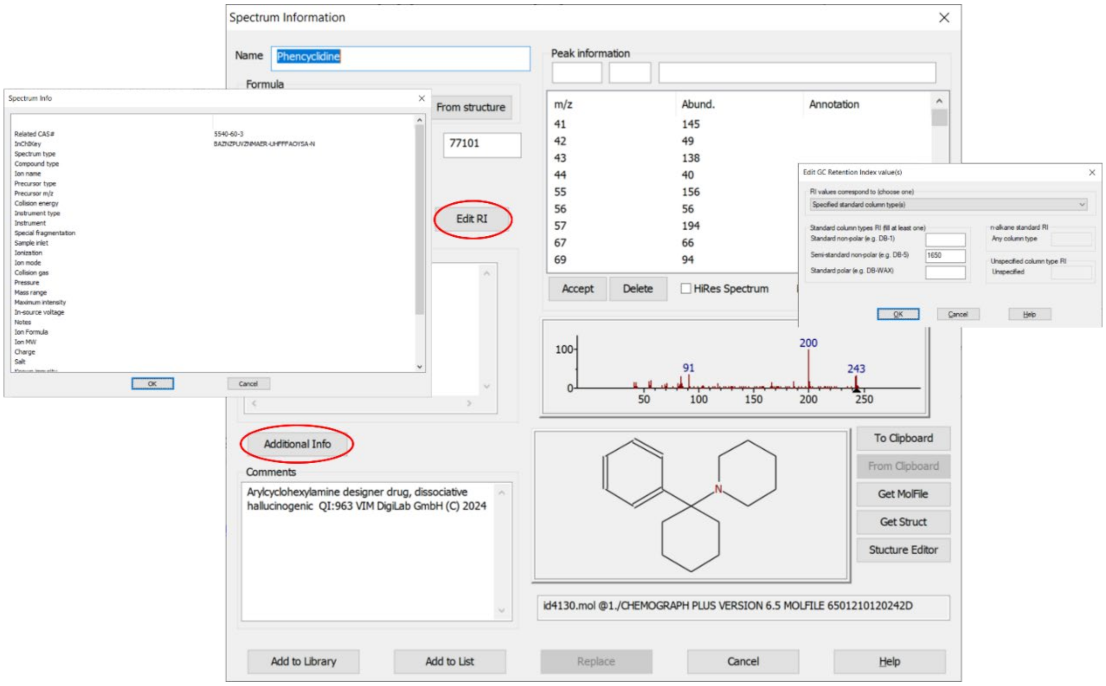
Delete library

Create library

Figure 86. Librarian tab view Button Bar.

The fifth tab view, **Librarian**, is used to deal with spectra that are in user-libraries or that are to be added to user-libraries or have been imported or searched. This tab view is also used to associate structures with measured spectra allowing the comparison of the structure and measured spectrum using the **Mass Spec Interpreter** program. The layout of this tab view is the same as the **Other Search** tab view, with the exception that the far-left window contains the **Spec List** rather than a **Hit List**. This is the same **Spec List** as displayed in the upper left window of the **Lib. Search** tab view.

The **Librarian** tab view is the utility area for mass spectral libraries. This is used to build user libraries, copy spectra to text files for distribution, associate spectra with structures, and add information that may need to be associated with user-libraries.



Name: Phencyclidine

77101

m/z	Abund.	Annotation
41	145	
42	49	
43	138	
44	40	
55	156	
56	56	
57	194	
67	66	
69	94	

Comments: Arylcyclohexylamine designer drug, dissociative hallucinogenic QI:963 VIM DigiLab GmbH (C) 2024

id4130.mol @1./CHEMOGRAPH PLUS VERSION 6.5 MOLFILE 6501210120242D

Figure 87. Spectrum Information dialog box with the Additional Info and Edit RI data dialog boxes overlaid.

The **Librarian** tab view allows for the editing or creating of spectra. This editing not only allows *m/z* intensity pairs to be edited, deleted, or added; but also allows CAS registry numbers, elemental compositions, structures, names, and synonyms to be edited, added, or deleted (structures cannot be deleted once associated with a spectrum; only exchanged).

> ***Nota Bene:*** The process of deleting a structure (not exchanging it for another structure) is somewhat convoluted. The spectrum with the structure must be edited (highlight the spectrum in the **Spec List** of the **Librarian** tab view, and then select the **Edit** button in the **Button Bar**) to assure the CAS# field is not populated. Next, export the spectrum using the **Export** button on the **Button** Bar. The nascent saved spectrum can then be imported, and when viewed, will no longer have a structure. A possible work around is to open a structure drawing program. Select the text tool and with Mouse pointer on the drawing palette, press the <space bar> three or four times. Highlight this area, The drawing program should indicate something is highlighted. Use the program's command to send that which is selected to the Window's Clipboard. Bring MS Search back into focus and highlight the spectrum if it is not already. Select the **Edit** button. On the right side of the **Spectrum Information** dialog box, next to the displayed structure, is a button labeled **From Clipboard**. Select it. The displayed structure will disappear and be replaced with the ☹ icon. Select the **Add to List** button at the bottom of the dialog box and the edited spectrum will appear at the top of the **Spec List**. Select the spectrum to see there is no longer a structure associated. The **Formula** (elemental composition) will be displayed with the spectrum if that field was not depopulated during the editing. If that spectrum is exported to a text file and then imported back into MS Search, a structure will be associated with it, if the export had a populated **CAS#** field, and there is a spectrum with a structure and this **CAS#** in the NIST mainlib.

In addition, detailed text information about an EI or Tandem (integer or accurate *m/z*) spectrum can be added using a predefined set of field titles (the **Additional Info** button on the **Spectrum Information** dialog box). The **Edit** function of the **Librarian** tab view also allows for the addition or editing of the **Retention Index information** for **EI** spectra (the **Edit RI** button). See *Figure 87*.

It should be remembered that all NIST spectra are copyrighted and can be used only on a single computer, accessible by no more than one person at a time. If an NIST spectrum is added to a user-library, even though that spectrum and its Text Information has been edited, the user-library can only be used on the licensed computer. It cannot be shared with another computer.

The **Librarian** tab view can be used to create and add spectra (a single spectrum or a group of spectra) to a text file. As of NIST 23, the default extension for text files is *.MSPEC. Prior versions used the extension *.MSP. This is a Microsoft extension that can cause problems when sending files by email. This file is readable by any text editor such as the Microsoft Notepad program. These text files DO NOT support structures. If you want to share spectra you have measured and associated structures with, they should be put into a user-library and then the user-library, which is a folder, should be put into a ZIP file for sharing.

The position of the splitter bar dividing the **Plot** and **Text Info** windows can be changed by selecting **Change Splitter Orientation** on the **RMB** menu, displayed when the pointer is in either window. There are three alternat configuration available by clicking on the ⊟ button. The ▤ button will return all displays to the default values.

Zoom. Placing the Mouse pointer on a **Plot** window in any of tab views or the Compare window of the **Lib. Search** or **Compare** tab view, holding the left Mouse button down and dragging the Mouse pointer along a diagonal will result in drawing a zoom rectangle. After releasing the Mouse button, a zoomed spectrum will be displayed; the cursor will change to a magnifying glass. To return to the normal display, select **Zoom Out** from the **RMB** menu or double-click with the Mouse pointer in the window. All the windows with spectrum plots in the tab view will be zoom the same.

Additional information on the **Librarian** tab is provided later in this manual under **User-Libraries**.

Chromatogram tab view

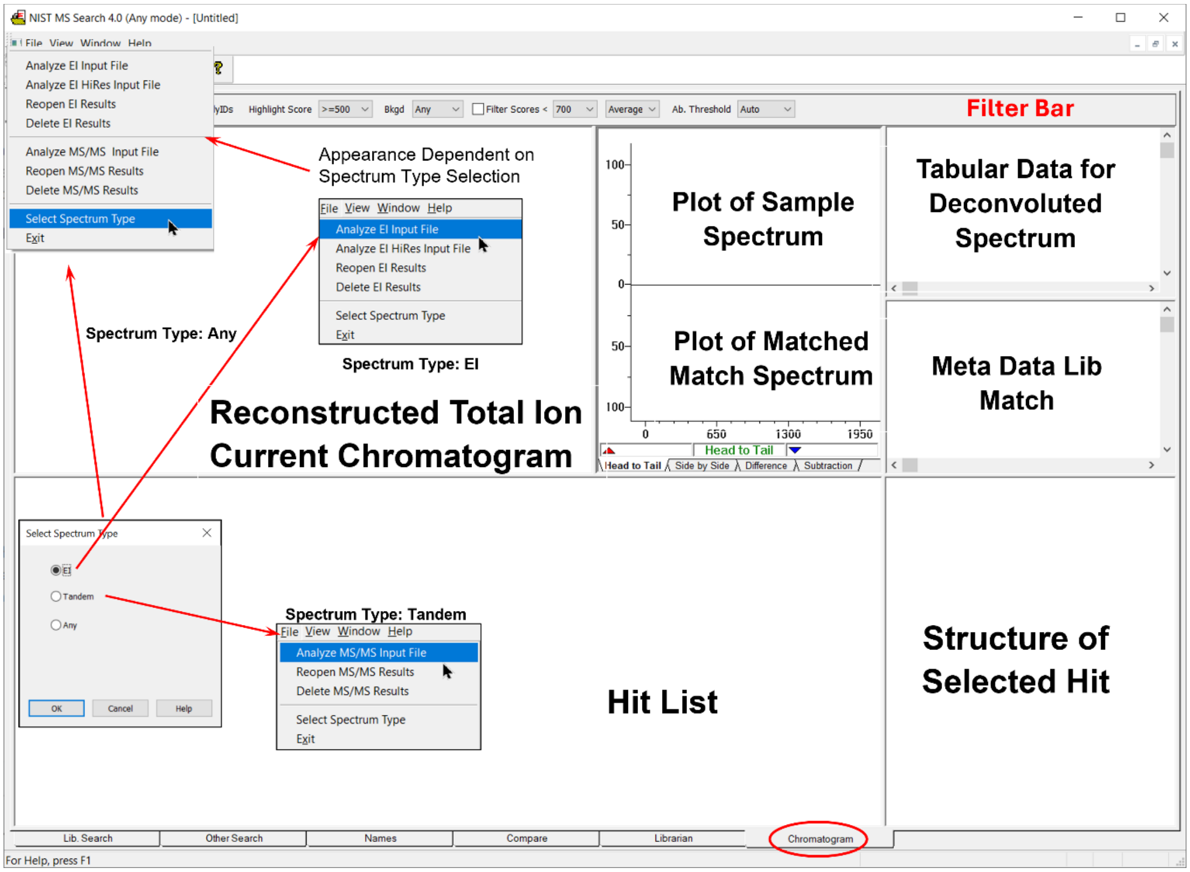
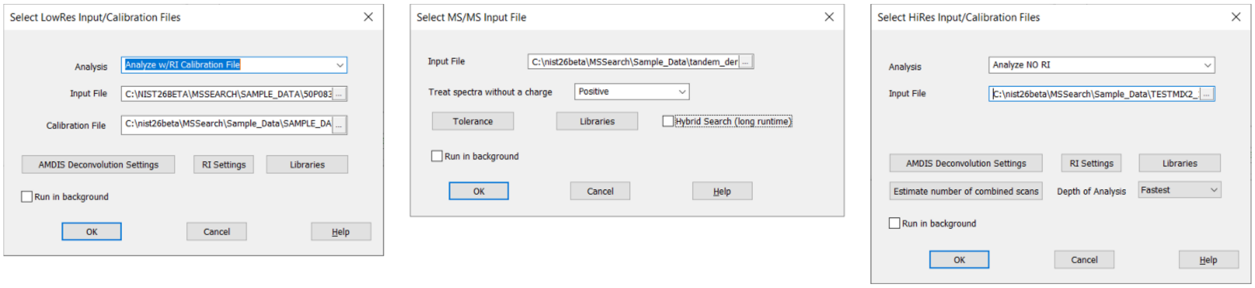


Figure 88. Chromatogram tab view with the Any Spectrum Type selected, the Select Spectrum Type dialog box displayed when Select Spectrum Type is selected from the File menu, and the File menu displayed when EI and Tandem spectrum type have been selected from the Select Spectrum Type dialog box.

The **Chromatogram** tab view is new to v.4.0 of MS Search provided with both the NIST\EPA\NIH Electron Ionization (EI) Mass Spectral Library and the NIST Tandem Library installations of NIST 26. This application requires a 64-bit computer using Microsoft® Windows®. This utility allows library search processing of full data files (GC/MS and LC-MS/MS), both low- and high-resolving power data. For GC/MS data files, the analysis employs an updated version of AMDIS (Automated Mass Spectral Deconvolution and Identification System). AMDIS was introduced with the 1998 version of the NIST\EPA\NIH EI Library (NIST 98). Both nominal *m/z* value and high-resolving power GC/MS data can be processed.

For LC-MS/MS (Dynamic Data Analysis, DDA) chromatogram, all MS2 spectra are searched against the NIST Tandem Library using newly developed software by the NIST Mass Spectrometry Data Center. MS1 data is processed using the *XICanalyzer* program. The *XICanalyzer* program identifies the chromatographic peak for each spectrum, which can then be viewed with the incorporated *XIC Browser* program. Individual MS2 spectra can be further analyzed using multiple tools provided with MS Search which allow for alternate search and display methods, such as the Hybrid Search to identify compounds not in the library. It is possible to save spectra into user-libraries using the tools in the **Librarian** tab view. This new utility (**Chromatogram** tab view) provides advanced spectral filtering to discard low quality, unidentifiable spectra that often make up a large portion of the acquired data.

In the **EI** (or **Any**) **Spectrum Type** mode, the **Open EI Highres Input Files** allows the reading of Thermo Fisher GC-Orbitrap files (*.raw), mzData files (*.msdata), mzML (*.mzML), or netCDF (*.cdf). Integer resolution (lo-res) data files produced by several different manufacturers' instruments or *.CDF files can be read. For LC-MS/MS data (when **Tandem** is selected in the **Select Spectrum Type** dialog box) the file types include the Thermo Fisher Orbitrap® (*.raw) mzML files (*.msML) and the Bruker's tims TOF instruments (*.tdf). Most chromatography/mass spectrometry instrument manufactures have utilities that will copy data from their proprietary format to one supported by the NIST software. If not, msconvert, which is part of ProteoWizard (free download from *https://proteowizard.sourceforge.io/*) can.


Select LowRes Input/Calibration Files
Analysis: Analyze w/RI Calibration File
Input File: C:\NIST26BETA\MSSEARCH\SAMPLE_DATA\50P083
Calibration File: C:\nist26beta\MSSearch\Sample_Data\SAMPLE_DA
☐ Run in background

Select MS/MS Input File
Input File: C:\nist26beta\MSSearch\Sample_Data\tandem_der
Treat spectra without a charge: Positive
☐ Hybrid Search (long runtime)
☐ Run in background

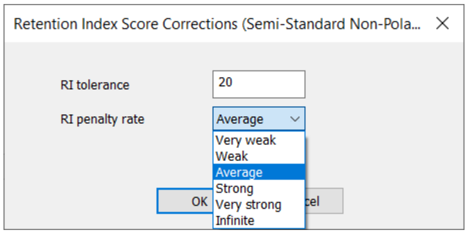
Select HiRes Input/Calibration Files
Analysis: Analyze NO RI
Input File: C:\nist26beta\MSSearch\Sample_Data\TESTMIX2_
Depth of Analysis: Fastest
☐ Run in background

Figure 89. Dialog boxes displayed when one of the three Analyze File types is selected from the File menu of the Chromatogram tab view.

Each chromatogram file type (EI nominal *m/z* GC/MS, HiRes EI GC/MS, LC-MS/MS) will display a multipurpose dialog box. The libraries for the deconvoluted components to be searched against are selected by selecting the **Libraries** button. The **Libraries** dialog box is displayed. This dialog box is universal for all three types of data. It functions the same as the **Libraries** page in the **Library Search Options** dialog box displayed on the **Lib. Search** tab view. There is a section that displays **Available** libraries and one that list the libraries that will be searched (**Included Libs:**). Separate sets of Libraries can be selected for each type data to be processed.

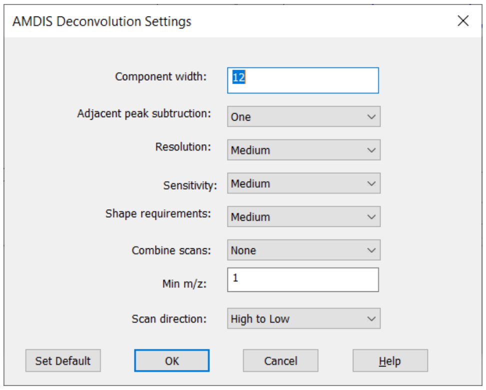
The first input line of the two EI (LowRes and HiRes) dialog box is to specify the type of analysis: Analysis with RI (retention index) Calibration; Generate RI Calibration File; Analyze with RI Estimation; and Analyze NO RI.

If the **Analysis with RI Calibration Analysis** type is chosen, the first step is to generate an RI Calibration File. This EI GC/MS file should reflect the same resolving power as the data (LowRes or HiRes), be acquired using the same GC conditions, and contain data obtained on a mixture of sequential linear hydrocarbons. After the file is selected from the **Select Data File** dialog box and that dialog box closes, the **Select LowRes (or HiRes) Input/Calibration File** dialog box will remain displayed. Now select the OK button on the dialog box and the Calibration file will be generated, at which point the dialog box will close. The next step is to re-display the **Select LowRes (or HiRes) Input/Calibration File** dialog box the File menu on the Main Menu bar and select **Analysis: Analyze w/RI Calibration File**. Then, chose the **Calibration File** by selecting the button with **…** at the end of the entry filed to display the file **Open** dialog box. The Calibration file name will have the extension CAL, be in the same folder that contained the calibration data file and have the name of the folder. Next, choose the **Input File**. This is done in the same way as the Calibration file was chosen using the **Input File** box.

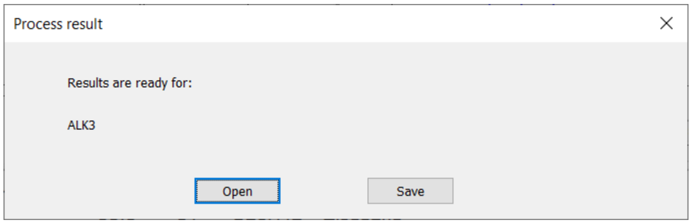

Retention Index Score Corrections (Semi-Standard Non-Pola…
RI tolerance: 20
RI penalty rate: Average

There are several more steps that can be taken before the data file is analyses. Select the **RI Settings** button. This opens the **Retention Index Score Correction (Semi-Standard Non-Polar Column)** dialog box shown on the left. Deciding on the values for this dialog box may require some experimentation. Once set for a series of analyses, this dialog box may not have to be redisplayed.

Another button on the **Select LowRes (or HiRes) Input/Calibration File** dialog box is labeled **AMDIS Deconvolutions Settings** which will display the dialog box shown on the lower right). The **Combine scan** is only relevant to data acquired at high-resolving power. The data acquisition rate of these instruments may result in multiple component-identifications for a single compound. By using a value of 2 or 3, this can be prevented, and it will speed the analysis. Another significant factor is the **Scan direction**. A lot of the data being used is in the netCDF format. Therefore, the scan direction is not known, Most transmission quadrupole mass spectrometers acquire data from low *m/z* to high *m/z* values. All Agilent quadruple instruments acquire from high *m/z* values to low *m/z* values. All Time-of-Flight and other pulsed type instruments, such as the Orbitrap, have no scan direction.


AMDIS Deconvolution Settings
Component width: 12
Adjacent peak subtruction: One
Resolution: Medium
Sensitivity: Medium
Shape requirements: Medium
Combine scans: None
Min m/z: 1
Scan direction: High to Low

The **File** selection on the Main Menu will have an option to reopen a processed data file, depending on the **Spectrum Type** selected (**Tandem** or **EI**). If **Any** has been selected, there are both **Reopen EI Results** and **Reopen MS/MS Results** selections. Processing can be lengthy. This feature can be time saving. The same is true for the **Run in background** ☐ selecting in the **Select Input File** dialog box. Unless the **Reload on startup** ☐ is selected, the processed file visible when MS Search is exited will not be displayed on restart.



After all settings in the **Select Input File** dialog box have been made, select the **Ok** button. If the **Run in background** ☐ has been selected, the dialog box closes, and a **Running Task** label will appear in the **Filter Bar**. The focus may be changed to another tab view and other operations carried out. Once the file processing is complete, the **Process result** dialog box will be displayed. Selecting the **Open** button causes the focus to change to the **Chromatogram** tab view showing the results of the just processed file. Selecting **Save** closes the dialog box and leaves the focus on the current tab view. When the **Chromatogram** tab view is brought back into focus, the contents will be that of the just processed data file.

If the **Run in background** ☐ has NOT been selected, when the **OK** button is selected, the same label will appear in the Task Bar, but the focus cannot be changed to another tab view. A ⌛ icon appears and stays visible until processing is complete, at which point the contents of the **Chromatogram** tab view updates.

Like the other tab views, the **Chromatogram** tab view has splitter bars. One horizontal splitter bar can divide the tab view into two horizontal windows. The top window has two vertical splitter bars. One separates the **Chromatogram** window from the **Plot Deconvoluted/Library Match Compare** window and the two **Deconvoluted/Library Match Text Information** windows, which are divided by a horizontal splitter bar (see *Figure 88*). There is a vertical splitter bar dividing the **Hit List** window and the **Structure** window, which make up the lower part of the display. Processing a chromatogram data file can result in a large amount of information which causes a need for a larger than normal vertical size of the **Hit List** window. This can be accommodated by sliding the vertical splitter bar between the two windows to the right, effectively closing the closing the **Structure** window. The structure of the library spectrum can be displayed using the **Show Structure** selection on **View** menu displayed by selecting **View** from the Main Menu. The **Chromatogram** tab view's Structure window can be made visible using the ▤ (Set default layout) button. This moves all splitter bars to their defaulter positions.

Spectral Analysis Utilities for NIST 26

Automated Mass Spectral Deconvolution and Identification System (AMDIS)

Included as a separate utility with the NIST\EPA\NIH Electron Ionization (EI) Library, **AMDIS** attempts to reconstruct mass spectra for individual components in arbitrarily complex GC/MS chromatograms; and if a target library is provided, **AMDIS** can directly identify target compounds. AMDIS is designed to find components that may be in very low concentrations, not even visible in the reconstructed total ion current (RTIC) chromatogram. **AMDIS** is especially useful when an RTIC chromatographic peak represents multiple components. Regardless of each component concentration, pure mass spectra are deconvoluted for analyses. **AMDIS** was developed by NIST under a contract from the Defense Threat Reduction Agency (DTRA), Department of Defense, for verifying of compliance with a major international treaty (Chemical Weapons Convention) as ratified by the United States Senate in 1997. To meet the rigorous requirements for this purpose, **AMDIS** was tested against more than 30,000 GC/MS data files accumulated by the EPA Contract Laboratory Program without a single false-positive for the target set of known chemical warfare agents. Although this level of reliability may not be required for all laboratories, this shows the degree to which the algorithms were originally tested. The algorithms have undergone modifications and are the backbone for the **Chromatogram** tab view of the NIST MS Search program v.4.0. **AMDIS** can also be used to identify individual non-target components in complex samples. It has been widely used in the flavors and fragrance industry, forensics, the study of natural products, and for the identification of metabolites from EI mass spectra.

AMDIS has been designed to reconstruct “pure component” spectra from complex RTIC chromatograms even when components are present at trace levels. For this purpose, observed chromatographic behavior is used along with a range of noise-reduction methods. **AMDIS** is distributed with specialized libraries (environmental, flavor and fragrance, and drugs and toxins) that were derived from the NIST 02 MS EI Library. **AMDIS** has a range of other features including the ability to search the entire **NIST\EPA\NIH EI MS Library** with any of the spectra extracted from the original data file. It can also employ retention-index windows when identifying target compounds and can make use of internal and external standards maintained in separate libraries. A history list of selected performance standards is also maintained.

AMDIS is installed by default in the c:\NIST26\AMDIS32 folder when the NIST\EPA\NIH EI Library is installation is carried out. This folder contains a file with the name AMDISMan.PDF, which is a program maula. AMDIS has an extensive interactive Help system. The manual can be accessed from an icon in the NIST 26 folder of the Windows Start menu. AMDIS can be started from a selection on the **Tools** menu of MS Search or from a button (⚖) on the **Tools Bar**. There is also an AMDIS Search in the Windows Strat Menu

The instruments that are supported in their native file format are: †

Agilent ChemStation (GC-MS) and MS Engines
Bruker DALTONICS GC-MS (*.MSF)
Finnigan (GCQ, INCOS, ITDS, ITS-40, ITD-700/800, ITMS)
Inficon (*.acq)
JEOL/Shrader (*.lrp)
Kratos Mach3 (*.run)
Micromass (MassLynx, NT Formats) (RAW)
mzXmL/mzData
Perkin-Elmer TurboMass (RAW) and ITD-700 (*.ms)
Shimadzu MS Files QP5000 and QP5050 (*.R##)
Thermo Fisher Xcalibur (RAW)
Varian (MS, SMS, XMS)

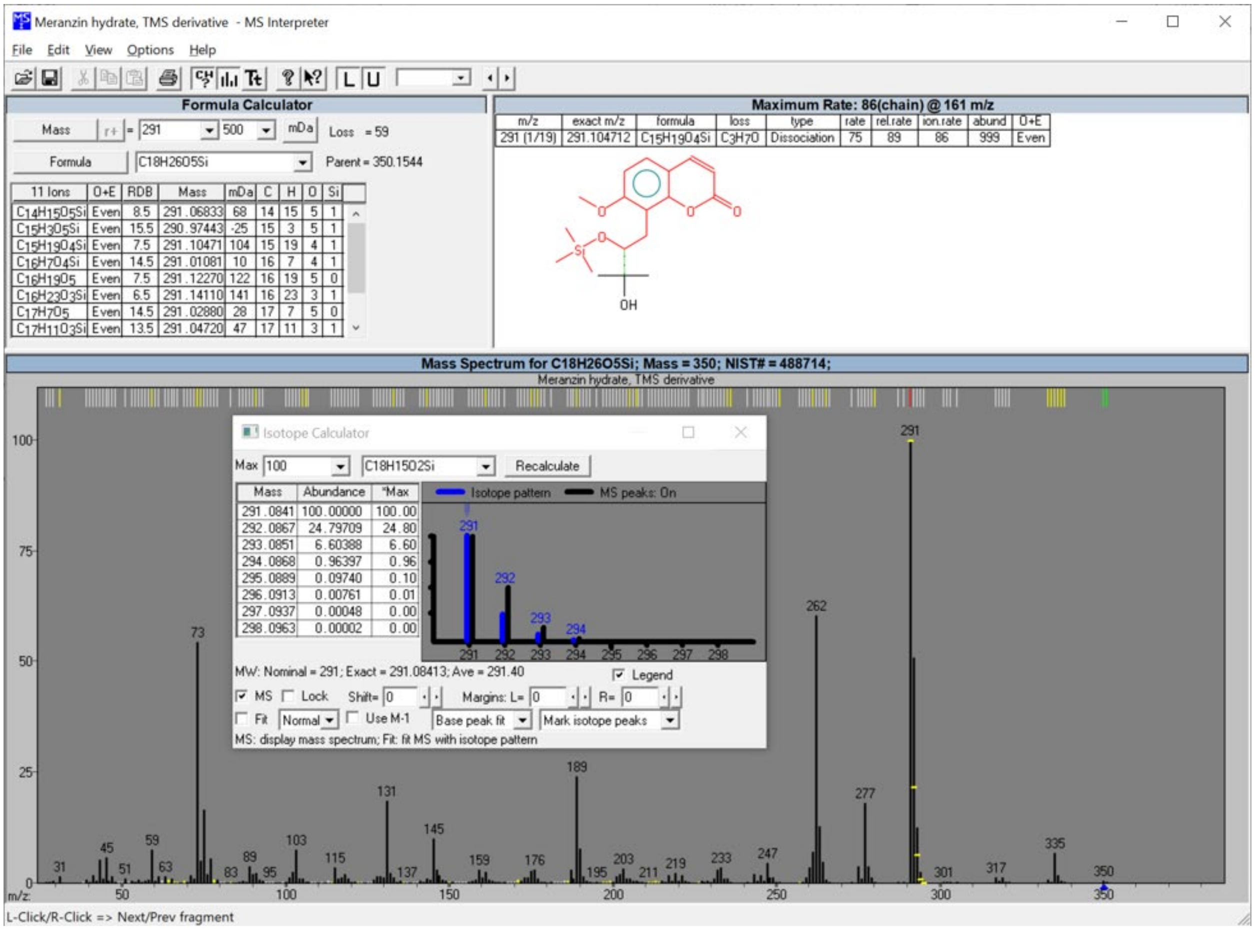
In addition, the NetCDF format, which is supported by many manufacturers, is readable by **AMDIS**. Other formats will be added in future releases. Contact NIST for future information.

A PowerPoint Presentation and a PDF file entitled “Instructions for using AMDIS with NIST MS Search” can be downloaded from *https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:amdis*. Jim Little has AMDIS-use videos on his Website (*https://mzinterpretation.com/*).

† ***The use of specific product and trade names does not indicate an endorsement by the National Institute of Standards and Technology. The specific file formats are supported to ensure that the data is transferred to AMDIS with no information loss. The specific formats and names are covered by the respective copyrights of the individual companies.***

MS Interpreter

This tool was developed to aid NIST evaluators in their analysis of mass spectra. In one integrated program, it enables a variety of calculations on a mass spectrum, using a proposed chemical structure if available. Spectra and structures are associated in the spectral editing component of the **Librarian** tab view of the **MS Search Program** discussed in **USER LIBRARIES** section and **APPENDIX 3** of this manual. **MS Interpreter** is activated for an individual spectrum with the Mouse pointer on a **Plot** or **Text Information** window or by highlighting the name or structure of a compound in a **Hit List** or the **Spec List** and selecting **MS Interpreter** on the **Send To** menu of the **RMB** menu. This action opens **MS Interpreter** with the spectrum and associated structure (*Figure 90*). **MS Interpreter** can also be opened from the **Tools** menu or the button (MS) on the **Tool Bar**. If a spectrum/structure has been opened in **MS Interpreter** and the program is closed and then reopened from either the **Tools** menu or the button on the **Toolbar**, the last contents will be displayed. If **MS Interpreter** is started by either of these two actions just after starting the **Program**, both the **Structure** window and the **Spectrum** window will be empty. Spectra can be imported from MSP files (text files containing spectra in the **NIST MS Search** format), JCAMP-DX, and SDF files. If the folder also contains a *.STB structure file that has the same name as the MSP file, that structure will be associated with a spectrum in **MS Interpreter**. When a structure/spectrum pair is Saved from the **MS Interpreter** File Menu, a single SDF file containing the spectrum and the structure is created. These can be opened in **MS Interpreter** but not **MS Search**.



Mass: 291, 500 mDa; Loss = 59

Formula: C18H26O5Si; Parent = 350.1544

11 Ions	O+E	RDB	Mass	mDa	C	H	O	Si
$C_{14}H_{15}O_5Si$	Even	8.5	291.06833	68	14	15	5	1
$C_{15}H_3O_5Si$	Even	15.5	290.97443	-25	15	3	5	1
$C_{15}H_{19}O_4Si$	Even	7.5	291.10471	104	15	19	4	1
$C_{16}H_7O_4Si$	Even	14.5	291.01081	10	16	7	4	1
$C_{16}H_{19}O_5$	Even	7.5	291.12270	122	16	19	5	0
$C_{16}H_{23}O_3Si$	Even	6.5	291.14110	141	16	23	3	1
$C_{17}H_7O_5$	Even	14.5	291.02880	28	17	7	5	0
$C_{17}H_{11}O_3Si$	Even	13.5	291.04720	47	17	11	3	1

Maximum Rate: 86(chain) @ 161 m/z

m/z	exact m/z	formula	loss	type	rate	rel.rate	ion.rate	abund	O+E
291 (1/19)	291.104712	$C_{15}H_{19}O_4Si$	C_3H_7O	Dissociation	75	89	86	999	Even

Max 100; C18H15O2Si

Mass	Abundance	*Max
291.0841	100.00000	100.00
292.0867	24.79709	24.80
293.0851	6.60388	6.60
294.0868	0.96397	0.96
295.0889	0.09740	0.10
296.0913	0.00761	0.01
297.0937	0.00048	0.00
298.0963	0.00002	0.00

MW: Nominal = 291; Exact = 291.08413; Ave = 291.40

☑ Legend

☑ MS ☐ Lock Shift= 0 Margins: L= 0 R= 0

☐ Fit Normal ☐ Use M-1 Base peak fit Mark isotope peaks

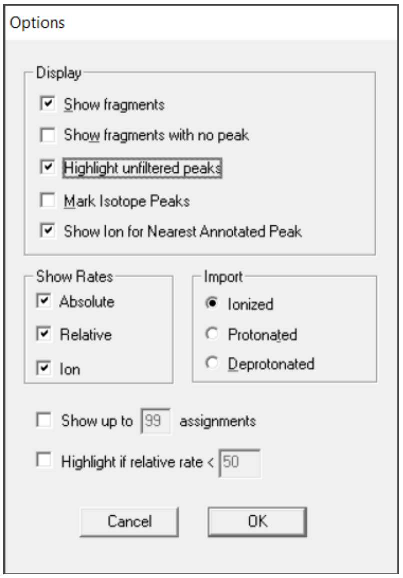
MS: display mass spectrum; Fit: fit MS with isotope pattern

Figure 90. The main Window of MS Interpreter. The black lines indicate the peaks in the mass spectrum that the MS Interpreter can explain. The white lines indicate the ones it cannot. The Insert is the Isotope Calculator.

Peaks in the spectrum originating as a logical fragment of the molecule ($M^{+\bullet}$ or MH^+) are marked at the top of the spectrum display; and corresponding fragments, derived from thermodynamic approximation, may be highlighted by clicking on one of these marks. The program also allows for keeping track of important neutral losses, both from the precursor or a derived ion and to find all possible formulas for any peak or neutral loss. Isotopic patterns for any cluster can be examined separately. These isotope clusters can be compared to theoretical predictions subject to several user-specified constraints. **MS Interpreter** is a separate program that also has an icon in the **NIST Mass Spectral Database** Start Menu folder. It has a detailed Help screen that can be called from the program by selecting **Help** on its

Menu Bar or by pressing the **F1** key. Information about the control currently located under the Mouse pointer and possible user actions is displayed in the **Status Bar** located at the bottom of the program main Window. Placing the Mouse pointer in the mass spectrum display and clicking the right mouse button will result in a somewhat self-explanatory **RMB** menu. Before using the program, explore this **RMB** menu (*Figure 92*).

Selected ions on the plot of the mass spectrum can be sent to **Formula Calculator** and the **Isotope Calculator** using selections on this menu. A specific *m/z* value can be locked as the position from to display calculated losses. In addition, the **Fragment options** dialog box can be displayed (*Figure 91*).



Options

Display

☑ Show fragments

☐ Show fragments with no peak

☑ Highlight unfiltered peaks

☐ Mark Isotope Peaks

☑ Show Ion for Nearest Annotated Peak

Show Rates

☑ Absolute

☑ Relative

☑ Ion

Import

● Ionized

○ Protonated

○ Deprotonated

☐ Show up to 99 assignments

☐ Highlight if relative rate < 50

Figure 91. Fragment options dialog box.

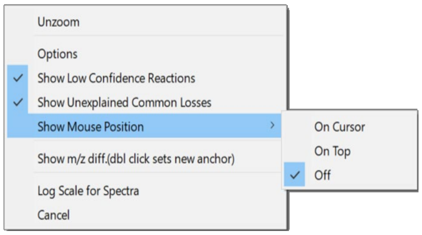


Figure 92. Menu displayed when the Right Mouse button is on the Plot portion of the MSI Program.

More detailed information can be obtained regarding the use of this utility by pressing the **F1** key with the Mouse pointer in one of the **Program** windows.

Exact masses of fragment ions

The Structure view displays not only nominal *m/z* but also exact mass of the predicted fragment ion.

Pasting a chemical structure into the Structure view

- Copy a structure from a structure editor or MS Seach (Molfile format) into Windows Clipboard;
- In MS Interpreter, click the **Structure** window **Title Bar** to activate it;
- Click the **Paste** button (3rd in 2nd group of **Button Bar**) or select from the menu: **Edit/Paste** or press **Ctrl-V**.

Pasting a mass spectrum or a chemical structure into Mass Spectrum view

Contents of a file containing a mass spectrum or a structure or both (SDF file) may be pasted into the MS Interpreter.

Pasting a peak list into the Mass Spectrum view

- Copy a peak list into the Windows Clipboard;
- In MS Interpreter, click **Mass Spectrum** Window to activate it;
- Click **Paste** button or select from the menu: **Edit/Paste**, or press **Ctrl-V**.

NOTES:

- the peak list must be made out of *m/z* and intensity pairs separated by any of the following characters: **space , ; : ( ) [ ] } | Tab**
- each line must contain one or more *m/z* and intensity pairs;
- if at least one line contains an odd number of values, then the program reads only first two numbers (one peak) from each line; a warning is displayed;
- all peaks must be in order of either ascending or descending *m/z*;
- all *m/z* are rounded to the nearest integer by the MS Interpreter;
- there is no need to normalize intensities to 999.
- Structure/spectra pairs can be saved or open from SDF files by MS Interpreter; however, when you try to open these files in MS Search, only the structure will be imported.

Pasting MSP, SDF, MOL, and JDX (JCAMP-DX) files from Windows Explorer

- MSP, SDF, MOL, and JDX files may be copied from Windows Explorer and pasted into the Structure or Mass Spectrum view of MS Interpreter.
- The name of the imported file appears on the MS Interpreter **Title Bar** and in the MRU (most recently used) list in the **File** menu.
- The files of the same type may be dragged from the Windows Explorer and dropped into the same MS Interpreter views. *In all cases, only the first spectrum and/or structure from the first selected file is/are imported.*

Copying calculated isotope envelope table from the Isotope Calculator into Windows Clipboard

- Right-click any line in the Isotope Calculator table
- Select **Copy**.

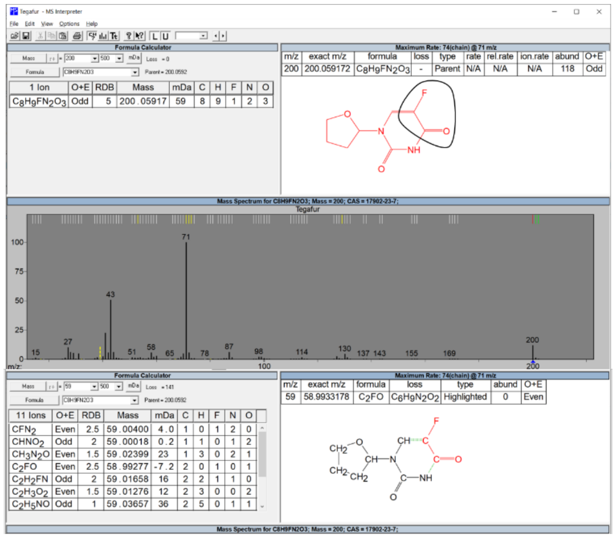
This will copy into the Windows Clipboard a tab-delimited text, which is suitable for pasting into an MS Excel spreadsheet or text editor. The context menu and the text contain words “Default Isotope Table” or the name of the file from which a custom isotope composition was imported (refer to the following section).

Using custom isotope composition table in Isotope Calculator and Formula Calculator

- Open file *MS_Interpreter_isotope_table_test_01.itbl* in a plain text editor. This file is installed in the same folder as MS Interpreter, typically, c:\nist26\mssearch
- Read the **NOTES** in the file
- Edit the file and save it under another name
- From **MS Interpreter** menu, select **File/Import Isotope Table**
- In the **Open Isotope File** dialog box, select the newly created file and click the **Open** button.
- To restore the original table, import file *MS_Interpreter_isotope_table_default.itbl.* The original table is restored automatically after both MS Interpreter and MS Search are shut down.
- Every time a *.itbl file is imported, a *.itbl.log file is created or appended. This file contains the date, time, a full pathname of the .itbl file, and a list of differences (if any) between the current and the imported isotope table or a reason for the failure to import the new table.

Selecting a Portion of the Structure for Mass Calculation (lasso function)

You may drag the mouse around the displayed chemical structure to compute the mass of the selected substructure (highlighted in red). Put the Mouse pointer on a starting point in the Structure window. Hold down the left Mouse button as you drag the pointer around the portion of the structure you want to select until the pointer is back at its starting position (e.g., lasso the portion of the structure you are interested in). When the left Mouse button is released, information is updated and the Structure window shows the selected portion of the structure in red. The mass will also be shown on the spectrum. This need not correspond to an actual peak – predicted or observed (*Figure 93*).



Mass = 200 500 mDa Loss = 0

Formula C8H9FN2O3 Parent = 200.0592

1 Ion	O+E	RDB	Mass	mDa	C	H	F	N	O
$C_8H_9FN_2O_3$	Odd	5	200.05917	59	8	9	1	2	3

m/z	exact m/z	formula	loss	type	rate	rel.rate	ion.rate	abund	O+E
200	200.059172	$C_8H_9FN_2O_3$	-	Parent	N/A	N/A	N/A	118	Odd

Mass = 59 500 mDa Loss = 141

Formula C8H9FN2O3 Parent = 200.0592

11 Ions	O+E	RDB	Mass	mDa	C	H	F	N	O
CFN_2	Even	2.5	59.00400	4.0	1	0	1	2	0
$CHNO_2$	Odd	2	59.00018	0.2	1	1	0	1	2
CH_3N_2O	Even	1.5	59.02399	23	1	3	0	2	1
C_2FO	Even	2.5	58.99277	-7.2	2	0	1	0	1
C_2H_2FN	Odd	2	59.01658	16	2	2	1	1	0
$C_2H_3O_2$	Even	1.5	59.01276	12	2	3	0	0	2
C_2H_5NO	Odd	1	59.03657	36	2	5	0	1	1

m/z	exact m/z	formula	loss	type	abund	O+E
59	58.9933178	C_2FO	$C_6H_9N_2O_2$	Highlighted	0	Even

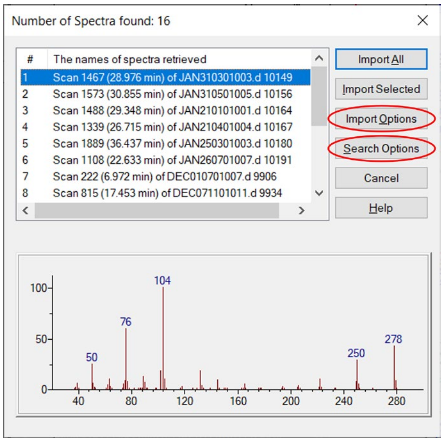
Figure 93. Screen showing the results of Lassoing a part of a structure (top) in the MS Interpreter display and the resulting change in the display bottom. The Formula Calculator is automatically updated.

Example Searches

Library Spectrum and Structure Searches

New in v.3.0 of MS Search was the ability to customize the program to the type of spectra being worked with. In v.4.0 there are three modes which can be selected from the dialog box displayed when the **Select Spectrum Type** option is selected on the **File** menu. The selected mode determines the type of searches available.

EI		Tandem		Any	
Identity	**Similarity**	**Identity**	**Similarity**	**Identity**	**Similarity**
EI Normal	EI Hybrid	MS/MS	MS/MS in-EI	EI Norm	EI Hybrid
HiRes/NoPre	EI Simple	HiRes/NoPre	MS^2 HYB	MS/MS	EI Simple
	EINu Loss			HiRes/No	EINu Loss
					MSMS-in EI
					MS^2 HYB



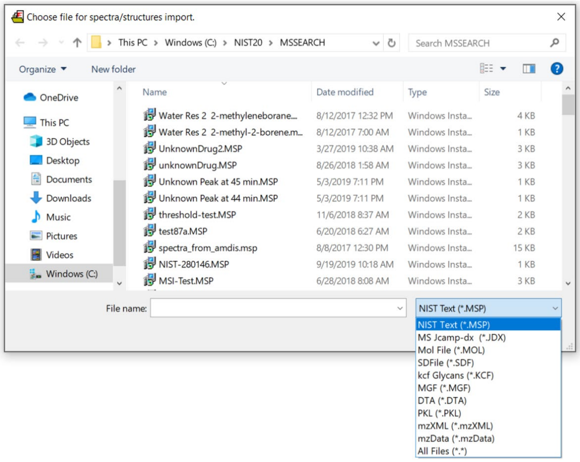
#	The names of spectra retrieved
1	Scan 1467 (28.976 min) of JAN310301003.d 10149
2	Scan 1573 (30.855 min) of JAN310501005.d 10156
3	Scan 1488 (29.348 min) of JAN210101001.d 10164
4	Scan 1339 (26.715 min) of JAN210401004.d 10167
5	Scan 1889 (36.437 min) of JAN250301003.d 10180
6	Scan 1108 (22.633 min) of JAN260701007.d 10191
7	Scan 222 (6.972 min) of DEC010701007.d 9906
8	Scan 815 (17.453 min) of DEC071101011.d 9934

Figure 94. The Library Search Options dialog box when Any is the selected Spectrum Type.

The selections in the **Library Search Options** dialog box (not shown) of the **Lib. Search** tab view will depend on the current mode based on spectrum type. As an example, the **RI (GC)** page on this dialog box is only present for the **EI** and **Any** spectrum types, and the **MS/MS** page is only present for the **Tandem** and **Any** spectrum types. The selections in the dropdown menus under **Identity** and **Similarity Spectrum Search Type** of this dialog box are different for the three spectrum types (as shown in the above table. To select the spectrum type, select **File** in the Main Menu. Select the **Select Spectrum Type** at the bottom of the **File** menu. This will display a dialog box (not shown) with three selections, each having a radio button. Select one, then select the **OK** button at the bottom-left of the dialog box. The spectrum type corresponding to the Library installed (EI or Tandem) is the default.

The selected type of spectrum is shown in the MS Search **Title Bar** at the top of the NIST MS Search 4.0 (Any mode) display.

MS Search can be used to find spectra that are the same or similar to that of an unidentified compound. This can be accomplished by importing the spectrum into the **Spec List** window of the **Lib. Search** tab view from a third-party mass spectral data analysis program, from a text file (*.MSP/MSPEC), or from such a file that is on the Windows Clipboard. Spectra are imported from a file using the dialog box shown in *Figure 94*. This dialog box is displayed after selecting **Open** on **File** of the Main Menu, highlighting a specific file in the displayed **Choose file for spectra/structure import** dialog box, and finally clicking on the **Open** button (*Figure 95*). It should be noted that the dialog used to import spectra has buttons that will display the **Spectra Import Options** or **Library Search Options** dialog box.



Name	Date modified	Type	Size
Water Res 2 2-methyleneborane...	8/12/2017 12:32 PM	Windows Insta...	4 KB
Water Res 2 2-methyl-2-borene.m...	8/12/2017 7:00 AM	Windows Insta...	1 KB
UnknownDrug2.MSP	3/27/2019 10:38 AM	Windows Insta...	3 KB
unknownDrug.MSP	8/26/2018 1:58 AM	Windows Insta...	3 KB
Unknown Peak at 45 min.MSP	5/3/2019 7:11 PM	Windows Insta...	1 KB
Unknown Peak at 44 min.MSP	5/3/2019 7:11 PM	Windows Insta...	1 KB
threshold-test.MSP	11/6/2018 8:37 AM	Windows Insta...	2 KB
test87a.MSP	6/20/2018 6:27 AM	Windows Insta...	2 KB
spectra_from_amdis.msp	8/8/2017 12:30 PM	Windows Insta...	15 KB
NIST-280146.MSP	9/19/2019 10:18 AM	Windows Insta...	1 KB
MSI-Test.MSP	6/28/2018 8:08 AM	Windows Insta...	3 KB

NIST Text (*.MSP)

- 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 (*.*)

Figure 95. File Selection dialog box with some of the available file types. MSP and JDX files are spectra, and MOL files are structures.

Many instrument manufacturers (Agilent, PerkinElmer, Waters, LECO, Thermo Fisher and others) have utilities in their data analysis software which creates, files in the MSP/MSPEC format, starts the **NIST MS Search Program**, or brings it into focus, and sends the spectrum (or a group of spectra) to it which will automatically import them and run the search, if MS Search is so configured. Lacking this link in the manufacturer's GC/MS software, some commercial systems can output a data file in the netCDF format. This file can be imported into **AMDIS** and spectra can be exported for automatic search directly to **MS Search**. **MS Search** can also import individual or groups of spectra in the IDX, SDF, KCF, MGF, DAT, PKL, mzXML, and mzData format. ***NEW*** in v.4.0, whole chromatographic/mass spectral (GC/MS and LC-MS/MS, DDA) data files can be processed in the **Chromatogram** tab view with the automatic searching of spectra (components) identified.

A spectrum can be created using the **Spectrum Information** dialog box displayed in the **Librarian** tab view by selecting the **New** button on the **Button Bar** to enter the *m/z* and intensity pairs that comprise a mass spectrum, along with a structure and metadata. This is described in more detail in the **Librarian** tab view section.

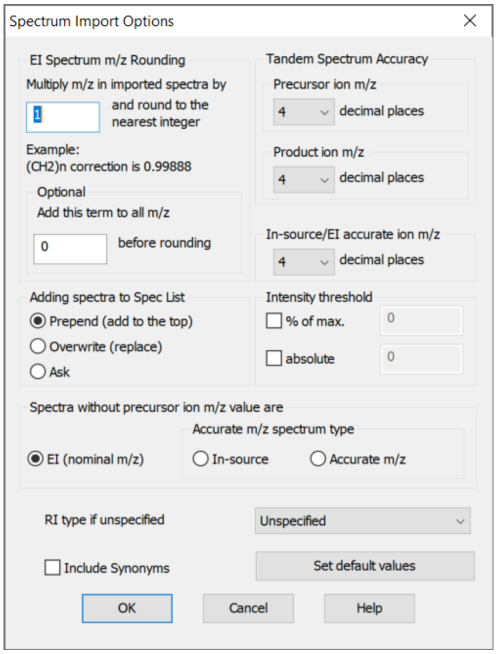
Before attempting to execute a search of a spectrum, the details of the **Library Search Options** and **Spectrum Import Options** dialog boxes should be viewed and understood. Both of these dialog boxes can be displayed by selections from the **Options** menu displayed by selecting **Options** on the Main Menu. The **Library Search Options** dialog box can also be displayed by clicking on the in the **Button Bar** of the **Lib. Search** tab view.

Spectral Import Options dialog box

This dialog box (*Figure 96*) can be displayed by selecting **Spectrum Import Options** from the **Options** menu displayed by selecting **Options** on the Main Menu or the **Import Options** button on the **Number of Spectra found:** dialog box (*Figure 94*) to import spectra after selecting a file name from the **Choose file for spectra/structure import** dialog box (*Figure 95*) displayed when the **File/Open** command is given. The **Spectrum Import Options** dialog box is involved with the import of EI (nominal and accurate *m/z*) and tandem as well as spectra form techniques such as DESI/DART, and in-source CAD. Some sections are specific to a specific type of spectrum (**EI** or **Tandem**). If a section does not pertain to a spectrum type, it should be ignored.

The upper right section of this dialog box, under the heading **Tandem Spectrum Accuracy** pertains only to spectra being imported that have the field header **Precursor *m/z***. This field heading tells MS Search that the spectrum is to be imported with a precession greater than 1. The number of decimal places will be equal to that shown in the text entry box to the left of **decimal places** label. For a tandem quadrupole or a 3D QIT or 2D QIT MS/MS instrument the number should usually be set at 2; because the mass accuracy of these devices is 0.05 *m/z* units. The default number of 4 is satisfactory, the Q-TOF instrument; although larger values can be used, especially for an Orbitrap.

If the value in the file from the instrument has fewer decimal places than specified in the dialog box, the low number of digits will be used. If the spectrum does not have a **Precursor mz:** field header or the value in it is invalid, such as a -1, the spectrum will be imported as an integer value unless one of the two **Accurate *m/z* spectrum type**(s) is selected under the **Spectra without precursor *m/z* valuer:** (lower half of the dialog box) which will cause the radio button next to the **EI (nominal *m/z*)** label to be deselected.



Spectrum Import Options

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

Tandem Spectrum Accuracy
Precursor ion m/z
4 decimal places
Product ion m/z
4 decimal places
In-source/EI accurate ion m/z
4 decimal places

Adding spectra to Spec List
● Prepend (add to the top)
○ Overwrite (replace)
○ Ask

Intensity threshold
☐ % of max. 0
☐ absolute 0

Spectra without precursor ion m/z value are
● EI (nominal m/z)
Accurate m/z spectrum type
○ In-source
○ Accurate m/z

RI type if unspecified: Unspecified
☐ Include Synonyms

Figure 96. Spectral Import Options. Imported spectra integer m/z and no mass defect correction.

The two fields on the upper left of the **Spectral Import Options** dialog box are used to make **mass defect** corrections for integer *m/z* value data, which normally pertains to EI GC/MS data. It may be desirable to use this with LC-in-source CAD/single analyzer instruments when searching the spectra against one of the NIST LC-MS/MS libraries using the **Identity, EI Normal** search.

The difference in the integer nominal mass and the exact mass of a nuclide is the **mass defect**. The most significant effect of mass defect is seen on the integer mass of hydrocarbons due to the **mass defect** of hydrogen. The integer mass of the ^{1}H isotope, which is the nominal mass (most abundant natality occurring nuclide of an elemental) isotope, is 1. The exact mass of this isotope is 1.007825 (monoisotopic mass). The $C_{50}H_{100}$ single-charge positive ion has a nominal mass (the sum of integer mass of the most abundant naturally occurring isotope of each element times the number of atoms of those elements) of 700, (50 C × 12) + (100 H × 1). The integer value for $C_{50}H_{100}$ based on a calculation of the exact mass of each of these elements is 701 (12.000000 × 50) + (100 × 1.007825) = 700.7825 (rounded). The **mass defect** for $C_{50}H_{100}$ is 0.7825, which is equal to about 100 millimass units per 100 mass units. The NIST\EPA\NIH EI Library is based on nominal mass. The **MS Search Program** deals with only integer mass values when searching the **NIST EI MS Library**. Spectra are imported with integer *m/z* values; therefore, spectra recorded with observed values of *m/z*, which are not integers and have not been corrected for **mass defect**, must have a **mass defect** correction applied to be properly searched against the **NIST EI MS Library** or another EI library, which contains only nominal (integer) *m/z* values.

The mass defect factor can be entered in the box **Multiply *m/z* in imported spectra by**. The factor for hydrocarbons based on a general formula of $–(CH_2)_n–$ is 0.99888. When dealing with spectra that have significant mass contribution from multiple atoms of bromine (which has a significant negative mass defect), a different factor should be calculated (1.00087). In this case, it may be desirable to use the field preceded by “Add following term [value] to all *m/z* values before rounding”.

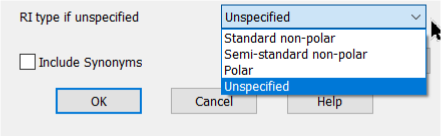
Some GC-MSs have mass defect correction built into the data they store. An example is the Thermo Fisher quadrupole GC/MS. In this case use of **EI Spectrum *m/z* Rounding** section of this dialog box is not necessary; however, if no instrument correction on imported data is available and if compounds with a molecular mass >500 Da are being analyzed, using these settings is advised.

The **Adding spectra to Spec List** section in the **Spectrum Import Options** dialog box allows the permanent specification of the action when importing spectra. Choosing **Prepend** will always add to the top of the current **Spec List**, **Overwrite** will always replace the current **Spec List**. **Ask** was the only option prior to version 2.2 of the search program. Some systems sending spectra to MS Search will specify to prepend or overwrite. If **Ask** is selected, the action specified by the sending software will prevail. The default is to prepend.

Intensity threshold allows the removal of low intensity peaks from the spectrum as it is being imported. This can be done in terms of relative peak intensities – for example 0.1% would keep peaks to 1 part in 1000 of the base-peak. **Absolute** will simply remove data below a user specified threshold. The third selection controls the number of decimal places for ions with no precursor values such as accurate *m/z* EI data and in-source API data.

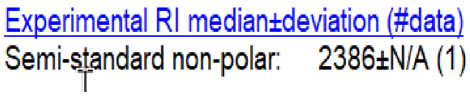
There are three options under the section labeled **Spectra without precursor ion *m/z* value**: **EI (nominal *m/z*)**, **In-source**, and **Accurate *m/z***. The last two options are under the heading **Accurate *m/z* spectrum type**. If **EI** is selected, the data will be imported based as nominal *m/z* values. Some manufacturers’ instruments will report the *m/z* values of ions to the nearest 0.05 value. An example is the Agilent ChemStation Data Analysis software. If the instrument is configured to report these fractional values, they will be imported into **Spec List** as integers if the **EI (nominal *m/z*)** is selected. If it is desirable to have the fractional values imported, **Accurate *m/z*** should be selected in the **Spectra without precursor ion *m/z* value are** area; however, it is recommended to import these spectra as nominal *m/z* values. GC/MS instruments with high *m/z* accuracy data such as the Agilent GC-QTOF, the JEOL AccuTOF Alpha, The Thermo Fisher GC Orbitrap, and LECO Pegasus GC-HRT should have the data imported using the **Accurate *m/z*** setting. These data should be processed using the **EI** spectrum type.

If **Accurate *m/z*** is selected, GC/MS and other accurate data, such as that from DART, will be imported as decimal values. For more details, select the **Help** button in the **Spectrum Import Options** dialog box. Spectra without precursor ion *m/z* values are assumed to be **EI (nominal *m/z*)** data by default but can be changed to be **In-source/EI** with accurate ion *m/z* spectra or **Accurate *m/z*** for accurate GC/MS data. The number of decimal places for In-source/accurate *m/z* is settable under the **HiRes No Precursor Ion *m/z*** label on mid-right side of the dialog box. In the case of LC-MS/MS data with a precursor ion (a specifically labeled header, **Precursor mz:**), the data are assumed to be accurate *m/z* and the section of the dialog box in the upper right under the heading **Tandem Spectrum Accuracy** is applicable for specifying the number of decimal places.


RI type if unspecified: Unspecified

☐ Include Synonyms

Retention Index in input spectra are often not characterized by type of column (SSNP, SNP, POL, ANY or std., or Unspecified). If a particular column type is used and no header tag is input in the text file constituting the spectrum, it may be specified here. If the text file being imported has a **Retention_index:** field header followed by a number, that is not preceded by a column type tag, the column type can be specified by selecting one of the items in the above displayed dropdown menu (lower part of the **Import Options** dialog box). The information appears as shown on the right in the **Text Information** window for the spectrum. If there is a tag associated with the **Retention_index:** field header, that will be the displayed column type, regardless of the setting in this dropdown list box. This is important because as of v.4.0 of MS Search, Match Factors of Hit can only be constrained by RI values based on semi-standard non-polar columns.


Experimental RI median±deviation (#data)

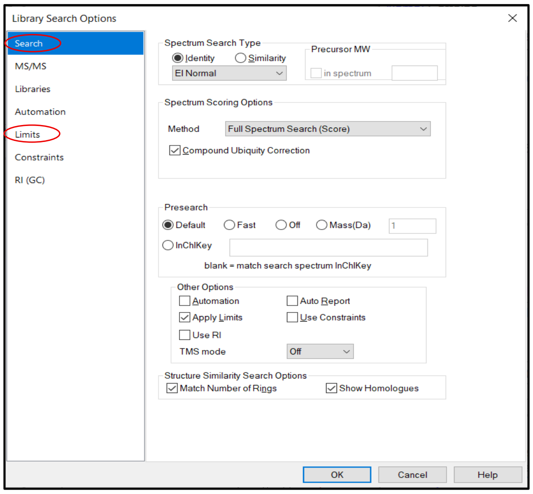
Semi-standard non-polar: 2386±N/A (1)

	GC Column Type	Accepted Tags (case insensitive)
1	Semi-standard non-polar	s, db5, semi, SemiStandardNonPolar, ssnp, SemiStdNP
2	Standard non-polar	n, db1, nonpolar, snp, standard, StandardNonPolar, StdNP
3	Standard polar	p, pol, polar, sp, StandardPolar, stdp, StdP, StdPolar
4	Any (n-alkane standard)	a, all, AllColumnTypes, AnyColumnType, std, Any
5	Unspecified	u, un, uns, unspecified, User

If the tag following the **Retention_index:** field header is Any, the **Text information** window will contain that designated in the **Import Options** dialog box. If the tag is unspecified, the column type will be listed as **Definition**.

The **Include Synonyms** checkbox should be selected if synonyms are included with the imported text files. Most spectra being added to the **Spec List** from files do not have synonyms. However, if the spectrum is from a text file created by a spectrum in an existing library that does have synonyms, it may have synonyms.

Library Search Options dialog box



Spectrum Search Type
● Identity ○ Similarity
EI Normal
Precursor MW
☐ in spectrum

Spectrum Scoring Options
Method: Full Spectrum Search (Score)
☑ Compound Ubiquity Correction

Presearch
● Default ○ Fast ○ Off ○ Mass(Da) 1
○ InChIKey
blank = match search spectrum InChIKey

Other Options
☐ Automation ☐ Auto Report
☑ Apply Limits ☐ Use Constraints
☐ Use RI
TMS mode: Off

Structure Similarity Search Options
☑ Match Number of Rings ☑ Show Homologues

Figure 97. Library Search Options Search page for an Identity EI Normal search in the Any Spectrum Type Mode.

Selecting **Library Search Options** on the **Options** menu, from any tab view, or selecting the **Library Search Options** button (▤) on the **Button Bar** of the **Lib. Search** tab vie will display a multiple-page **Library Search Options** dialog box (*Figure 97*). The appearance and options of the dialog box are dependent on the **Spectrum Type** selected in the dialog box displayed when the **Select Spectrum Type** option is selected in the **File** menu (Main Menu). The **MS/MS** page does not appear if the spectrum type is **EI**, and the **RI (GC)** page does not appear in the spectrum.

Different types of spectral comparison routines for choosing the best matching spectra are available. These are **Spectrum Type** specific. The routine to be used for a **Spectrum Type** is selected from the **Library Search Options** dialog box **Search** page.

The two **Spectrum Search Types** on this dialog box are **Identity** and **Similarity**. These different **Spectrum Search Types** and their options are explained in the **Help Screens** for each of the **Spectrum Types** (topic *Spectrum Search Type*).

It is generally believed that there is a spectrum of the unidentified compound (query) in one or more of the libraries being searched, an **Identity Search** is best used (**EI Normal** for **EI** spectrum type when using nominal *m/z* or accurate GC/MS data, and **MS/MS** for **Tandem** spectrum type using spectra with a specified precursor ion or **HiRes NoPrecursor** when using in-source spectra). If it is believed that there is no spectrum of the query in the libraries searched for reasons such as obtaining only low **Match** and **Reverse Match** factors for an **Identity Search**, a **Similarity Search** may yield better results. Although there are three options for a **Similarity Search** for the **EI** spectrum type, and essentially one selection for the **Tandem** spectrum type, it is recommended that the Hybrid Search be used (for both spectrum types) when it is believed there is no spectrum of the query compound present in the searched libraries. Introduced with v.2.3 of **MS Search**, these are particularly useful in this respect.[3,4]

A feature that first appeared in v.3.0 is the **Spectrum Scoring Options/Method** (name changed from **Spectrum Search Options** in v.3.0) dropdown list box (shown on this page). There are three selections. This section also has the **Compound Ubiquity Correction** checkbox. This is a ***NEW*** feature in v.4.0 that is used to enhance the **Match Factor** of more common compounds, when selected. V.3.0 saw the retirement of the **Penalize rare compounds** option. This new feature is a much more sophisticated replacement. If the Hit has a high **Compound Ubiquity Index** (**CUI**), its **Match Factor** will be altered. It has been shown that "a CUI of 0 reduces the inherent likelihood of identification relative to a random compound in the library by nearly a factor of 10, whereas a value of 20 increases this likelihood by a factor of 10." When dealing with an unknown spectrum and two Hits have almost identical **Match Factors**, the one with the higher CUI is the more likely candidate. As used in MS Search, the CUI is a citation index that reports the number of 58 diverse non-mass spectral chemical collections containing the compound. This is described in the literature previously cited in this manual. Details of how this works are found in a previous citation in the manual.

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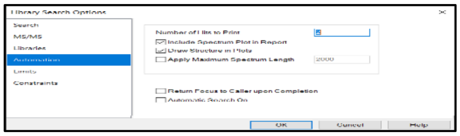
[3] Arun S. Moorthy, , William E. Wallace, Anthony J. Kearsley,‡ Dmitrii V. Tchekhovskoi, and Stephen E. Stein "Combining Fragment-Ion and Neutral-Loss Matching during Mass Spectral Library Searching: A New General Purpose Algorithm Applicable to Illicit Drug Identification" *Anal. Chem*. **2017**, *89*, 13261−13268.

[4] Ivana Blaženović, Tobias Kind, Michael R. Sa, Jian Ji, Arpana Vaniya, Benjamin Wancewicz, Bryan S. Roberts, Hrvoje Torbašinović, Tack Lee, Sajjan S. Mehta, Megan R. Showalter, Hosook Song, Jessica Kwok1, Dieter Jahn, Jayoung Kim, Oliver Fiehn "Structure Annotation of all Mass Spectra in Untargeted Metabolomics" *Anal. Chem*. **2019**, *91* (3) 2155-2162.



Library Search Options

Number of Hits to Print
☑ Include Spectrum Plot in Report
☑ Draw Structure in Plots
☐ Apply Maximum Spectrum Length 2000
☐ Return Focus to Caller upon Completion
☐ Automatic Search On

There are a series of options under the label **Spectrum Scoring Options/Method** section. These are selectable through the dropdown list box next to the **Method** label. The first selection in the list is the search type most often used where all peaks in the sample spectrum and the library spectrum that are in a match are compared. The second option results in the display of the **Hit List** according to descending **Reverse Match Factor** (**RMF**). An **RMF** is a **Match Factor** (**MF**) calculated by disregarding any peaks in the sample mass spectrum that are not in the library mass spectrum. The third selection is displaying the **Hit List** in descending order of the **Partial Spectrum Search** (**PSS**) value. This is a **Match Factor** calculated disregarding any peaks in the library spectrum that are not in the sample spectrum. The **PSS** seach was new to v.3.0 of MS Search. The **Impurity Tolerant Search** was selectable as a checkbox for a **Reverse Search** in this dialog box of versions previous to v.3.0 (v2.4 and previous).

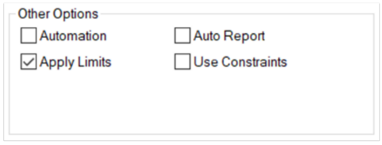
In v.3.0 there was a selection for **Match Ion Mode (Tandem)** when any **Spectrum Mode** other than **EI** is selected. This selection has been moved to the **MS/MS** page (only visible on the **Library Search Options** dialog box when the spectrum type is Tandem).

Following the **Spectrum Search Options** are the **Presearch** selections. These can be chosen by selecting the desired radio button. The **Default** presearch uses a series of presearch screenings to select the spectra which will be compared peak-by-peak in a **Match Factor** (**Score**) calculation. The **Fast** presearch is essentially the same as the **Default**, but selects a factor of three fewer spectra, on average. The **Off** selection has every spectrum in all the libraries being searched compared with the sample spectrum. This can be time consuming. This will result in a **Hit List** with 400 compounds whereas the other two Presearch options produce a **Hit List** with a maximum of 100 compounds.

The **InChIKey** selection restricts searches to spectra that have the appropriate **InChIKey**; therefore, it is a type of presearch and turns off the other three selections. This can be a full value or a beginning partial value. There will be a spectrum-by-spectrum search of the spectra meeting **InChIKey** criteria.

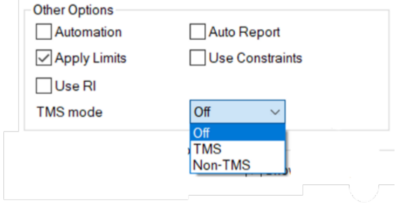
Note the **InChIKey presearch** is used when the structure associated with the spectrum is known. It is a powerful filter that helps with multiple libraries. Existing User and third-party libraries must be indexed to use this feature. Indexing is accomplished from the **Tools** menu of the Main Menu. Details of the InChIKey presearch are found in the **Help** file.

Other Options section on Search page of Library Search Options dialog box



Other Options
☐ Automation
☐ Auto Report
☑ Apply Limits
☐ Use Constraints

Spectrum Type: Tandem



Other Options
☐ Automation
☐ Auto Report
☑ Apply Limits
☐ Use Constraints
☐ Use RI
TMS mode Off

Spectrum Type: EI

The checkboxes next to the **Automation**, **Apply Limits**, **Use Constraints**, and **Use RI** (***NEW v.4.0*** Only in **EI** spectrum mode) options are automatically selected or deselected based on actions taken in the **Automation**, **Limits**, **Constraints**, and **RI (GC)** pages of this dialog box. They can be selected or deselected this page. If they are not selected, it is a good idea to review the contents of the corresponding page before selecting one of them. This will prevent unwanted or confusing results. The **Automation** checkbox in the **Search** page is turned on an off by the selection/deselection of the **Automatic Search On** checkbox in the **Automation** page. The **Auto Report** checkbox is not automatically selected when the **Automatic Search On** checkbox is selected on the **Automation** page or **Automation** is selected on the **Search** page. If Auto Report is selected, whatever settings are in place in the upper section of the **Automation** page will be applied to the report automatically generated at the completions of each library search. The other items are self-explanatory if the tab with the appropriate label is made visible. The value in the **Number of Hits to Print** entry box sets the number of Hits that will be in in an Auto Report when executed from the **File** menu of the Main Menu. The format of these automatically printed reports is dictated by the **Automation** page of the **Library Seach Options** dialog box. This format is different from the one generated when the **Print Report** selection is executed from the **File** menu.

Version 4.0 has two ***NEW*** selections in the **Other Options** section of the **Search** page of the **Library Search Options** dialog box is display for the **EI** spectrum type. These are **Use RI** and **TMS** mode. The **Use RI** checkbox which can be selected and deselected from the **Search** page is turned on and off by selecting/deselecting the **Use Semi-Standard Non-Polar Colum Retention Index for Score corrections**.

The **TMS mode** (EI only) selection has three options for compounds that have been derivatized with a trimethylsilyl reagent.: 1) **Off** - no filter; 2) **TMS** - for TMS derivatized samples - rejects compounds containing OH and NH with no TMS derivatization; 3) **Non-TMS** - for non-TMS derivatized runs - rejects all TMS derivatized compounds. This is a highly specific constraint.

The **Search** page of the **Library Search Options** dialog box also has a **Structure Similarity Search Options** selection. This section has the **Match Number of Rings** checkbox. When selected, the search of compounds that have rings will be constrained to only spectra produced by compounds with a similar structure and the same number of rings as the spectrum being searched. The **Show Homologues** selection is self-explanatory.

Different Libraries are used for a **Structure** search and a **Spectrum** Search. When the **Libraries** page of the **Library Search Options** dialog box is selected, a dropdown list box appears in the lower part. The selections made above are for either **Spectrum searches** or **Structures searches**. It is important to be aware that different libraries may be selected for these two types of searches, especially when working with both at the same time. Both pertain only to Structure Similarity Searches. **Structural searches** use different libraries than used by a **Spectrum searches**. See the Library page of this dialog box.

When the **OK** button on any page of the **Library Search Options** dialog box is selected, the dialog box is closed. When this dialog box is next opened, it is opened with the page view present when it was closed. In addition to the **OK** button each page has a **Help** button that opens a contact sensitive Help page. Before initiating a search of a spectrum or performing a structure similarity search, each page in the **Library Search Options** dialog box should be viewed and understood.

Hybrid Search

As stated above, an **Identity** search is used when it is believed that a spectrum of the unidentified compound is in one or more of the searched libraries. A **Similarity** search is carried out when there is a high probability that there is not a spectrum of the unidentified compound in any of the libraries. The most powerful of these **Similarity** searches is the **Hybrid Search** which can be used with integer *m/z* value EI spectra and accurate *m/z* tandem spectra.

> ***Note Bene:*** The **Hybrid Search** (as one of the **Similarity** searches) has been available sinch the inception of the MS Search Program (even back to the DOS Versions of the 1980s); however, with the release of NIST 17 (v.2.3 MS Search, 2017) a radically changed program was introduced. This **Hybrid Search** should not be confused with the previous version.
>
> ***Note Been:*** Any library in the NIST MS Search format, with associated structures, can be used with the **Hybrid Search**. This search is not restricted to only NIST Libraries. Before using a non-NIST EI mass spectra libraries, it is best to subject each library to the **Rebuild Structures Search Database**, **(RE)Index InChIKey**, and **(RE)Index Hybrid Search**, all accessible from the **Tools** selection of the Main Menu. If a user/3rd-party Tandem Library is to be used, it should be processed with the **(RE)Index Exact Mass Too**l. The dialog box for each of these **Tools**' processes has a labeled stating, "Libraries in upper case are probably up to date." Unless first-hand knowledge is available, as in this case, the library should be processed.

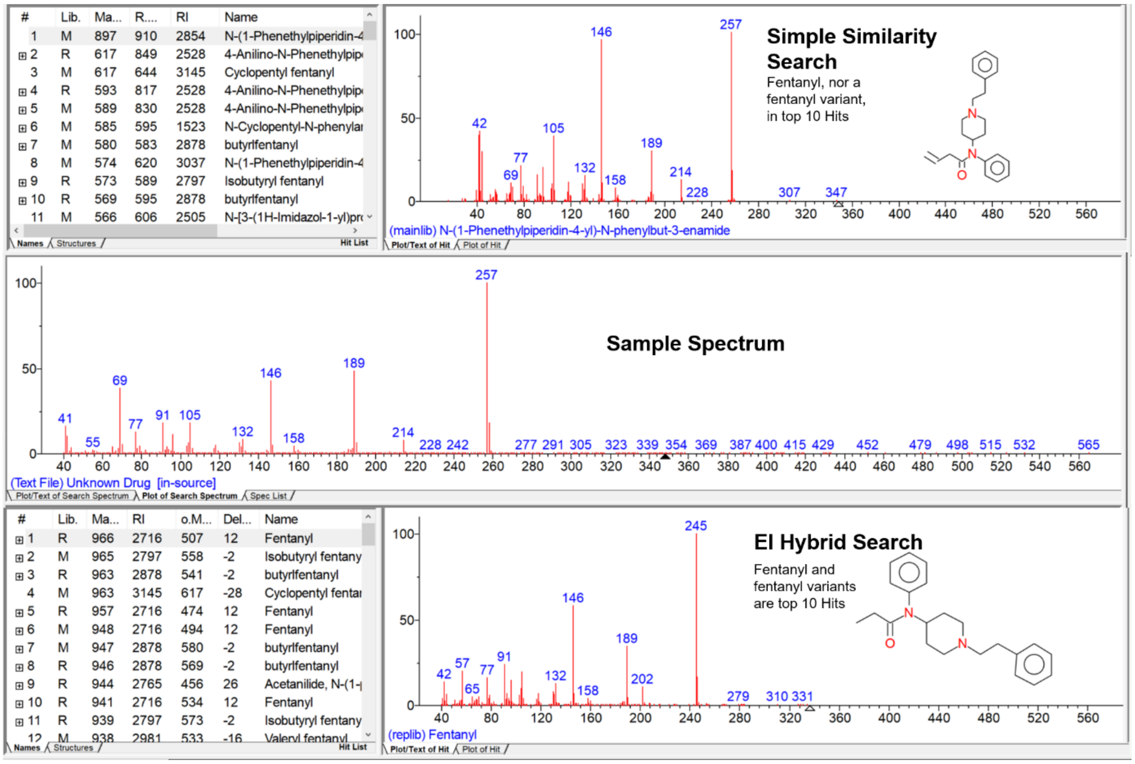
The ***NEW*** addition to the **Hybrid Search** for both EI and Tandem spectra of MS Search v.4.0 is the addition of the columns with the name **dform** (the number of atoms of the most common chemical elements added to or subtracted from the elemental composition of the found library spectrum's structure), **pctRelForm** (relative frequency of occurrence in high scoring hybrid searches), and **preForm** (**dForm** added to the library chemical formula, resulting in the formula of the queried spectrum). Also ***NEW*** to the EI Hybrid Search when using the RI associated with the spectrum as an adjustment factor to the **Match Factor** are the **Hit List** column headers **dRI** (estimated change in RI for **dForm**) and **ddRI** (difference between RI value associated with the queried spectrum and the estimated value). These column headers must be selected in the **Hit List** page of the **Library Search Properties** dialog box displayed from the **RMB** menu in the **Lib. Search** tab view. Even if selected, these column headers only appear when a relevant search is performed.

The following is an example of how valuable the **Hybrid** search is when there is no spectrum for an unidentified compound in any of the searched libraries. Both the **EI Hybrid** search and the **MS/MS Hybrid** search require the use of a precursor-ion value. When performing the **MS/MS Hybrid** search this requirement is easily met because the *m/z* value of the precursor is imported with the spectrum. In the **EI Hybrid** search, the *m/z* value of the molecular ion ($M^{+\bullet}$) is required. If the EI mass spectrum does not exhibit a molecular ion peak (which approximately 20% of the EI spectra in the **NIST/EPA/NIH** EI Library do not), then this value will have to be determined using chemical (CI), field (FI) ionization, photoionization (PI), by derivation, or as an educated guess. CI was used in the following example for *m/z* value of the $M^{+\bullet}$. MS Search will attempt to assign a value for the $M^{+\bullet}$; however, this value should be considered suspect. When performing an **EI Hybrid Search**, a value should be entered in the appropriate area (under the **Precursor MW** label at the top) of the **Search** page of the **Library Search Options** dialog box. The checkbox next to the **in spectrum** label should NOT be selected.

The figure on the next page shows the first **Hit** for a **Similarity EI Simple** search (top); the sample spectrum (middle); and the first **Hit** for a **Similarity EI Hybrid** search (bottom). The Hit List with the first few Hits are also shown.

The **EI Hybrid Search** yields much more compelling results. The top 10 Hits are all fentanyls and the **Hybrid Search Match Factors** are all at levels above 940. The **oM...** column is the **Match Factor** obtained without application of the **Hybrid Search** algorithm. The most valuable additionally displayed column is the **Del…** (**DeltaMass**). This shows the difference between the *m/z* values of corresponding ions and in the sample spectrum and the Hybrid spectrum, allowing for speculation as to the structure difference.

All the top 10 **Hits** being of fentanyl, or a fentanyl variant, is evidence that the unidentified compound is probably a Fentanyl variant that does not have a spectrum in the NIST/EP/NIH Library. fentanyl has an ethyl moiety (29 Da) attached to a carbonyl carbon atom. A cyclopropyl group (41 DA) is 12 Da greater in mass. If the structure for the cyclopropyl group variant is drawn, associated with the spectrum of the unidentified compound, and presented to **MS Interpreter**, the unambiguous identification is confirmed.



#	Lib.	Ma...	R....	RI	Name
1	M	897	910	2854	N-(1-Phenethylpiperidin-4
⊞ 2	R	617	849	2528	4-Anilino-N-Phenethylpip
3	M	617	644	3145	Cyclopentyl fentanyl
⊞ 4	R	593	817	2528	4-Anilino-N-Phenethylpip
⊞ 5	M	589	830	2528	4-Anilino-N-Phenethylpip
⊞ 6	M	585	595	1523	N-Cyclopentyl-N-phenylar
⊞ 7	M	580	583	2878	butyrlfentanyl
8	M	574	620	3037	N-(1-Phenethylpiperidin-4
⊞ 9	R	573	589	2797	Isobutyryl fentanyl
⊞ 10	R	569	595	2878	butyrlfentanyl
11	M	566	606	2505	N-[3-(1H-Imidazol-1-yl)pr

#	Lib.	Ma...	RI	o.M...	Del...	Name
⊞ 1	R	966	2716	507	12	Fentanyl
⊞ 2	M	965	2797	558	-2	Isobutyryl fentany
⊞ 3	R	963	2878	541	-2	butyrlfentanyl
4	M	963	3145	617	-28	Cyclopentyl fentar
⊞ 5	R	957	2716	474	12	Fentanyl
⊞ 6	M	948	2716	494	12	Fentanyl
⊞ 7	M	947	2878	580	-2	butyrlfentanyl
⊞ 8	R	946	2878	569	-2	butyrlfentanyl
⊞ 9	R	944	2765	456	26	Acetanilide, N-(1-
⊞ 10	R	941	2716	534	12	Fentanyl
⊞ 11	R	939	2797	573	-2	Isobutyryl fentany
12	M	938	2981	533	-16	Valeryl fentanyl

Figure 98. EI Hybrid Search of Unidentified Substance searched against the NIST/EPA/NIH EI Library. The top panel shows the results of an EI Simple Similarity Search. The middle panel is the Raw Spectrum. The bottom Panel shows the results of the Hybrid Search.

The first **Hit** of the **EI Simple Similarity Search** is for a fentanyl variant with a relatively good **Match Factor** and **Reverse Match Factor**; however, the difference in the *m/z* values of the two $M^{+\bullet}$ confirms that there is no identification. There are several spectra in the **Hit List** of fentanyl variants, but **Match Factors** (and **R Match Factors**) are low, and the results are definitely unconvincing.

The Delta Mass value is just a number. It can be positive (+) or negative (-). As stated above, for an EI mass spectrum this represents the difference in mass between the compound represented by the sample mass spectrum and the compound whose mass spectrum has been hybridized. In this example it is suggested that the Delta Mass of 12 is the difference between the mass of an ethyl group, Group/Element (1) on the model compound and a cyclopropyl group, Group/Element (2), on the compound that resulted in the sample spectrum. Users of the Hybrid Search have compiled a list of over 500 Delta Mass examples between 0 Da and 150 Da which are contained in *DeltaMass_EI.pdf*. Clicking on this link while holding the <ctrl> key down will open the file. Instructions for using the file are included.

The source DeltaMass_EI.xlsx file is located in the C:\NIST26\MSSearch folder and can be used to add new examples.

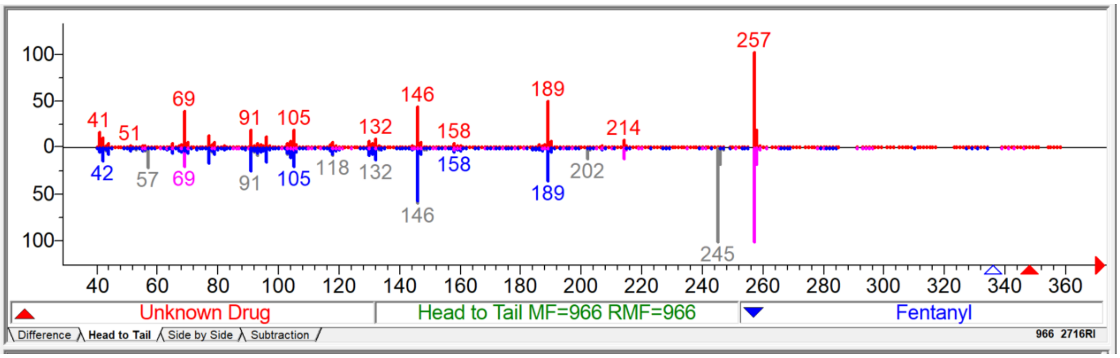
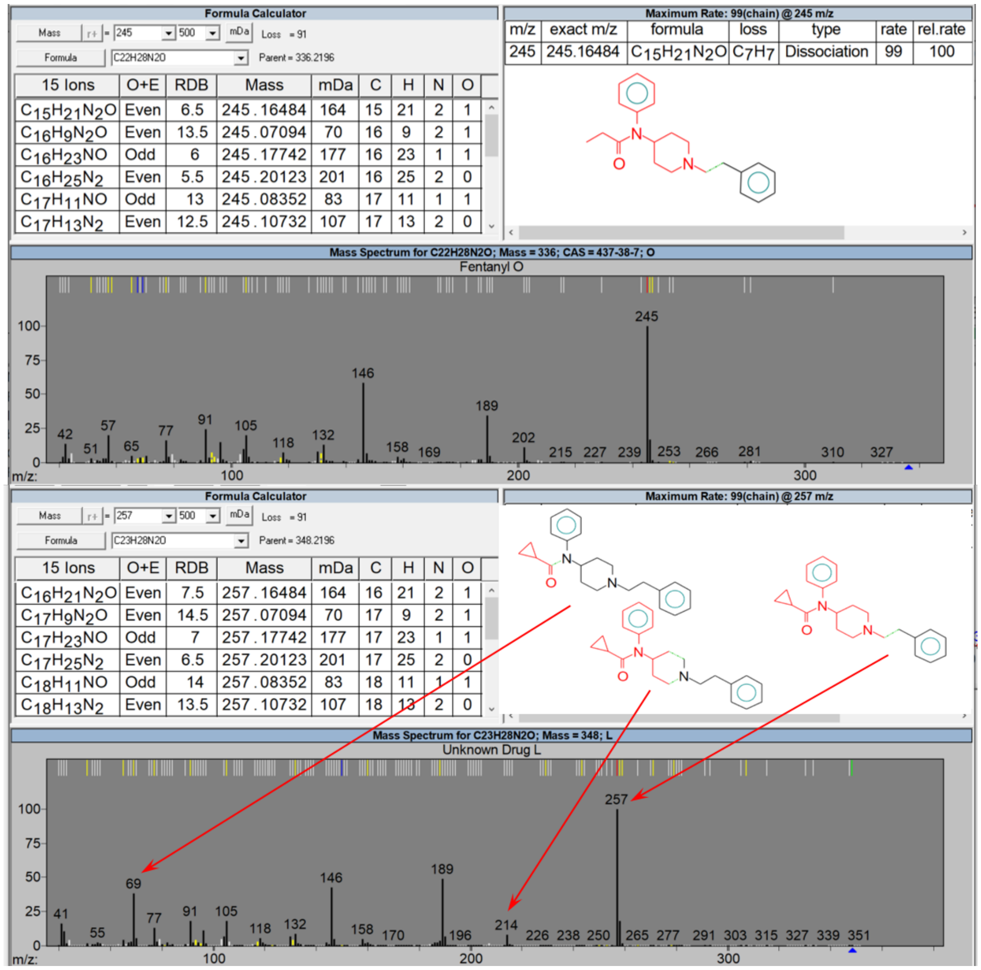


Figure 99. Comparison of Sample Spectrum (top) and Hybrid Spectrum (bottom) blue and pink peaks. The gray peaks on the bottom spectrum belong to the Hit and are not part of the Hybrid. The Delta Mass is represented by the difference between the gray and pink peaks.


Formula Calculator

Mass = 245 | 500 | mDa | Loss = 91

Formula C22H28N2O | Parent = 336.2196

15 Ions	O+E	RDB	Mass	mDa	C	H	N	O
$C_{15}H_{21}N_2O$	Even	6.5	245.16484	164	15	21	2	1
$C_{16}H_9N_2O$	Even	13.5	245.07094	70	16	9	2	1
$C_{16}H_{23}NO$	Odd	6	245.17742	177	16	23	1	1
$C_{16}H_{25}N_2$	Even	5.5	245.20123	201	16	25	2	0
$C_{17}H_{11}NO$	Odd	13	245.08352	83	17	11	1	1
$C_{17}H_{13}N_2$	Even	12.5	245.10732	107	17	13	2	0

Maximum Rate: 99(chain) @ 245 m/z

m/z	exact m/z	formula	loss	type	rate	rel.rate
245	245.16484	$C_{15}H_{21}N_2O$	C_7H_7	Dissociation	99	100

Mass Spectrum for C22H28N2O; Mass = 336; CAS = 437-38-7; O

Fentanyl O

Formula Calculator

Mass = 257 | 500 | mDa | Loss = 91

Formula C23H28N2O | Parent = 348.2196

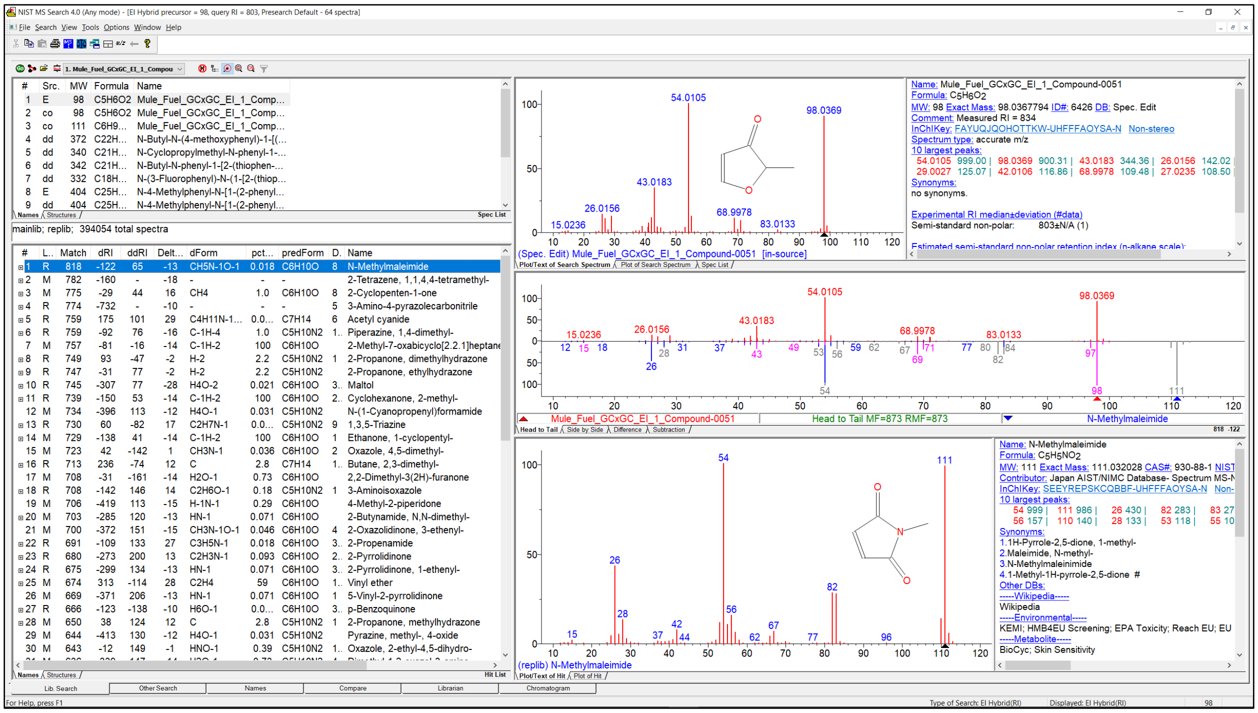
15 Ions	O+E	RDB	Mass	mDa	C	H	N	O
$C_{16}H_{21}N_2O$	Even	7.5	257.16484	164	16	21	2	1
$C_{17}H_9N_2O$	Even	14.5	257.07094	70	17	9	2	1
$C_{17}H_{23}NO$	Odd	7	257.17742	177	17	23	1	1
$C_{17}H_{25}N_2$	Even	6.5	257.20123	201	17	25	2	0
$C_{18}H_{11}NO$	Odd	14	257.08352	83	18	11	1	1
$C_{18}H_{13}N_2$	Even	13.5	257.10732	107	18	13	2	0

Maximum Rate: 99(chain) @ 257 m/z

Mass Spectrum for C23H28N2O; Mass = 348; L

Unknown Drug L

Figure 100. The three structures represent unique fragments of the variant and are the rational for the unambiguous identification.



NIST MS Search 4.0 (Any mode) - [EI Hybrid precursor = 98, query RI = 803, Presearch Default - 64 spectra]

#	Src.	MW	Formula	Name
1	E	98	C5H6O2	Mule_Fuel_GCxGC_EI_1_Comp...
2	co	98	C5H6O2	Mule_Fuel_GCxGC_EI_1_Comp...
3	co	111	C6H9...	Mule_Fuel_GCxGC_EI_1_Comp...
4	dd	372	C22H...	N-Butyl-N-(4-methoxyphenyl)-1-[(...
5	dd	340	C21H...	N-Cyclopropylmethyl-N-phenyl-1-...
6	dd	342	C21H...	N-Butyl-N-phenyl-1-[2-(thiophen-...
7	dd	332	C18H...	N-(3-Fluorophenyl)-N-(1-[2-(thiop...
8	E	404	C25H...	N-4-Methylphenyl-N-[1-(2-phenyl...
9	dd	404	C25H...	N-4-Methylphenyl-N-[1-(2-phenyl...

mainlib; replib; 394054 total spectra

#	L..	Match	dRI	ddRI	Delt...	dForm	pct...	predForm	D.	Name
1	R	818	-122	65	-13	CH5N-1O-1	0.018	C6H10O	8	N-Methylmaleimide
2	M	782	-160	-	-18	-	-	-		2-Tetrazene, 1,1,4,4-tetramethyl-
3	M	775	-29	44	16	CH4	1.0	C6H10O	8	2-Cyclopenten-1-one
4	R	774	-732	-	-10	-	-	-	5	3-Amino-4-pyrazolecarbonitrile
5	R	759	175	101	29	C4H11N-1...	0.0...	C7H14	6	Acetyl cyanide
6	R	759	-92	76	-16	C-1H-4	1.0	C5H10N2	1..	Piperazine, 1,4-dimethyl-
7	M	757	-81	-16	-14	C-1H-2	100	C6H10O		2-Methyl-7-oxabicyclo[2.2.1]heptane
8	R	749	93	-47	-2	H-2	2.2	C5H10N2	1	2-Propanone, dimethylhydrazone
9	R	747	-31	77	-2	H-2	2.2	C5H10N2		2-Propanone, ethylhydrazone
10	R	745	-307	77	-28	H4O-2	0.021	C6H10O	3..	Maltol
11	R	739	-150	53	-14	C-1H-2	100	C6H10O	2..	Cyclohexanone, 2-methyl-
12	M	734	-396	113	-12	H4O-1	0.031	C5H10N2		N-(1-Cyanopropenyl)formamide
13	R	730	60	-82	17	C2H7N-1	0.0...	C5H10N2	9	1,3,5-Triazine
14	M	729	-138	41	-14	C-1H-2	100	C6H10O	1	Ethanone, 1-cyclopentyl-
15	M	723	42	-142	1	CH3N-1	0.036	C6H10O	2	Oxazole, 4,5-dimethyl-
16	R	713	236	-74	12	C	2.8	C7H14	1..	Butane, 2,3-dimethyl-
17	M	708	-31	-161	-14	H2O-1	0.73	C6H10O		2,2-Dimethyl-3(2H)-furanone
18	R	708	-142	146	14	C2H6O-1	0.18	C5H10N2	1	3-Aminoisoxazole
19	M	706	-419	113	-15	H-1N-1	0.29	C6H10O		4-Methyl-2-piperidone
20	M	703	-285	120	-13	HN-1	0.071	C6H10O		2-Butynamide, N,N-dimethyl-
21	M	700	-372	151	-15	CH3N-1O-1	0.046	C6H10O	4	2-Oxazolidinone, 3-ethenyl-
22	R	691	-109	133	27	C3H5N-1	0.018	C6H10O	3..	2-Propenamide
23	R	680	-273	200	13	C2H3N-1	0.093	C6H10O	2..	2-Pyrrolidinone
24	R	675	-299	134	-13	HN-1	0.071	C6H10O	3..	2-Pyrrolidinone, 1-ethenyl-
25	M	674	313	-114	28	C2H4	59	C6H10O	1..	Vinyl ether
26	M	669	-371	206	-13	HN-1	0.071	C6H10O	1	5-Vinyl-2-pyrrolidinone
27	R	666	-123	-138	-10	H6O-1	0.0...	C6H10O	3..	p-Benzoquinone
28	M	650	38	124	12	C	2.8	C5H10N2	1	2-Propanone, methylhydrazone
29	M	644	-413	130	-12	H4O-1	0.031	C5H10N2		Pyrazine, methyl-, 4-oxide
30	M	643	-12	149	-1	HNO-1	0.39	C5H10N2	1..	Oxazole, 2-ethyl-4,5-dihydro-

Name: Mule_Fuel_GCxGC_EI_1_Compound-0051
Formula: $C_5H_6O_2$
MW: 98 Exact Mass: 98.0367794 ID#: 6426 DB: Spec. Edit
Comment: Measured RI = 834
InChIKey: FAYUQJQOHOTTKW-UHFFFAOYSA-N Non-stereo
Spectrum type: accurate m/z
10 largest peaks:
54.0105 999.00 | 98.0369 900.31 | 43.0183 344.36 | 26.0156 142.02 |
29.0027 125.07 | 42.0106 116.86 | 68.9978 109.48 | 27.0235 108.50 |
Synonyms:
no synonyms.

Experimental RI median±deviation (#data)
Semi-standard non-polar: 803±N/A (1)

Estimated semi-standard non-polar retention index (n-alkane scale):

Name: N-Methylmaleimide
Formula: $C_5H_5NO_2$
MW: 111 Exact Mass: 111.032028 CAS#: 930-88-1 NIST
Contributor: Japan AIST/NIMC Database- Spectrum MS-N
InChIKey: SEEYREPSKCQBBF-UHFFFAOYSA-N Non-
10 largest peaks:
54 999 | 111 986 | 26 430 | 82 283 | 83 27
56 157 | 110 140 | 28 133 | 53 118 | 55 10
Synonyms:
1.1H-Pyrrole-2,5-dione, 1-methyl-
2.Maleimide, N-methyl-
3.N-Methylmaleinimide
4.1-Methyl-1H-pyrrole-2,5-dione #
Other DBs:
-----Wikipedia-----
Wikipedia
-----Environmental-----
KEMI; HMB4EU Screening; EPA Toxicity; Reach EU; EU
-----Metabolite-----
BioCyc; Skin Sensitivity

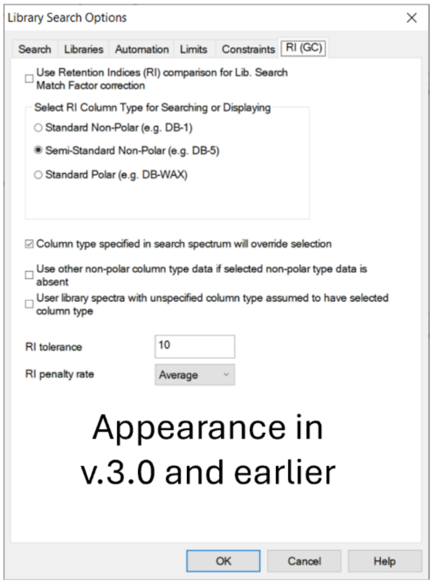
For Help, press F1 | Type of Search: EI Hybrid(RI) | Displayed: EI Hybrid(RI) | 98

Figure 101. Hybrid search of EI accurate m/z value spectra Constrained by Retention Index.

Constraining the **Match Factor** is based on a submitted RI value measured with a Semi-Standard Non-Polar (e.g. DB-5). The spectrum must have a line in the text file that is **Retention Index: SemiStdNP=xxx** (in iu). These will result in display of sections in the **Text Information** window that appears as:

Experimental RI median±deviation (#data)
Semi-standard non-polar: xxxx±N/A (1)

This value is compared against the same SSNP Experimental value in the NIST\EPA\NIH\ Library if present. If there is no Experimental SSNP value for the compound, MS Search will use the **AI predicted semi-standard non-polar retention index (n-alkane scale):** value. Every compound in the NIST\EPA\NIH Library has an AI Retention Index value.



Library Search Options

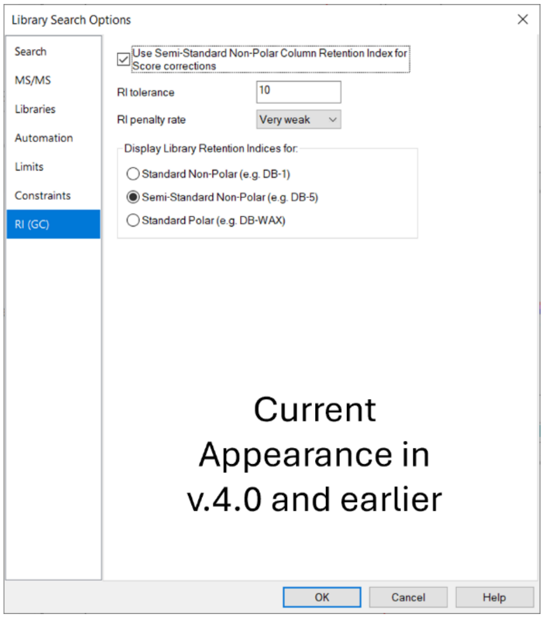
☐ Use Retention Indices (RI) comparison for Lib. Search Match Factor correction

Select RI Column Type for Searching or Displaying
○ Standard Non-Polar (e.g. DB-1)
● Semi-Standard Non-Polar (e.g. DB-5)
○ Standard Polar (e.g. DB-WAX)

☑ Column type specified in search spectrum will override selection
☐ Use other non-polar column type data if selected non-polar type data is absent
☐ User library spectra with unspecified column type assumed to have selected column type

RI tolerance 10
RI penalty rate Average

Appearance in v.3.0 and earlier



Library Search Options

☑ Use Semi-Standard Non-Polar Column Retention Index for Score corrections

RI tolerance 10
RI penalty rate Very weak

Display Library Retention Indices for:
○ Standard Non-Polar (e.g. DB-1)
● Semi-Standard Non-Polar (e.g. DB-5)
○ Standard Polar (e.g. DB-WAX)

Current Appearance in v.4.0 and earlier

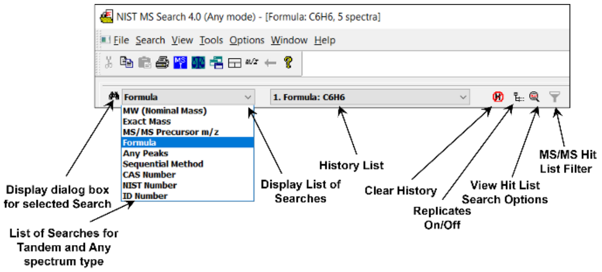
Figure 102. The RI (GC) page in v.4.0 had major appearance and functionality changes

See NOTE ON RECALLING Hybrid Search Results from HISTORY LIST on page 71.

The Search Menu (Select *Search* on Main Menu)

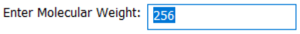
The **Other Search** tab view options are:

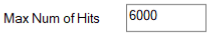
Option	Description	Tab View
MW (Nominal Mass)	Finds spectra in selected libraries of compounds having specified (nominal mass) molecular weight (excludes replib).	**Other Search**
Exact mass	Finds spectra in selected libraries of compounds having an exact mass corresponding to specified accurate mass with a specified variance (excludes replib).	**Other Search**
MS/MS Precursor *m/z*	Searches for Precursor *m/z* using precursor-ion mass, formula, *m/z* values within the uncertainty interval in MS/MS Libraries **NOT LISTED** when EI Spectrum Type is Selected	**Other Search**
Formula (Elemental Composition)	Finds spectra in selected libraries of compounds with specified formula (excludes replib).	**Other Search**
Any Peaks	Finds spectra in selected libraries based on user-specified peaks.	**Other Search**
Sequential Method	Searches selected libraries using a set of constraints provided by the user.	**Other Search**
CAS Number	Finds spectra of the compounds that have the entered CAS registry number.	**Other Search**
NIST Number	Retrieves a spectrum with unique NIST Mass Spectrometry Data Center Archive ID number (in mainlib and replib only).	**Other Search**
ID Number	Finds spectrum (or spectra) in selected library having entered ID number (or range of ID numbers).	**Other Search**
Names	Allows entering a character string to display spectra beginning with name of a compound.	**Names**

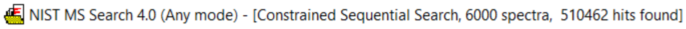


These Search options (except for the **Names** search) are also displayed and may be selected from the dropdown list box at the top left side of the **Other Search** tab view (to the right from the **binocular** button. Selecting the button causes the currently displayed search in the dropdown list box dialog box to overlay the view.

Each **Search** has its own dialog box. The tittle bar of each dialog box is labeled for a specific (**Other) Search**, where **(Other)** is the name of the search. When the **MW (Nominal Mass)**, **Formula**, or **Sequential Method** search is selected, the dialog box opens to the **Constraints** page (*Figure 103*). This basically is the same **Constraints** page displayed from the **Library Search Options** dialog box of the **Lib. Search** tab view. Some items specific to an individual **Other Search** may not be available because they conflict with the specified search. The **MW (Nominal Mass)** and **Formula** searches' dialog boxes have specific fields for entering a nominal mass or elemental composition, respectively. Certain constraints (lower window with scroll bar and names of constraints to the right of checkboxes) are not available and appear grayed. They cannot be selected: **MW (Nominal Mass)** and **Exact Mass** in the **MW (Nominal Mass) Search** dialog box and **Element Values** and **Elements Present** in the **Formula Search** dialog box. These three dialog boxes have two pages: **Options** and **Constraints.** The **Options** page allows for the selection of the libraries to be searched. Each **Other Search** can have its own unique set of libraries to be used. When performing an **MW (Nominal Mass)**, **Exact Madss**, **Formula**, or **CAS Number** search of libraries, the replib is not a selectable library. Spectra in the replib will have these same properties as the corresponding spectrum in the mainlib, and they can be selected for display in the **Hit List** by selecting the ⊞ button to left of the **Hit List #** or by using the button in the **Button Bar** to display all replicates. The **Options** page for the **MW (Nominal Mass)** and **Formula** search will have the same entry field as the **Constraints** page and a **Use Constraints** checkbox.

MW (Nominal Mas) search  Enter Molecular Weight: 256 **Formula** search  Enter Chemical Formula: C6H6

The **Options** page for a **Sequential** search has the Use Constraints checkbox and a  Max Num of Hits 6000 box. The maximum number of Hits for most of the searches performed in this tab view is 6000.
Regardless of the number of Hits specified for a **Sequential** or **Any Peaks Search**, all selected libraries will be searched; however, any Hits found in libraries after the maximum of Hits is reached, will not appear in the Hit List, but the number of Hits in the **Hit List** and the total number of Hit will appear in the MS Search **Title Bar**.


NIST MS Search 4.0 (Any mode) - [Constrained Sequential Search, 6000 spectra, 510462 hits found]

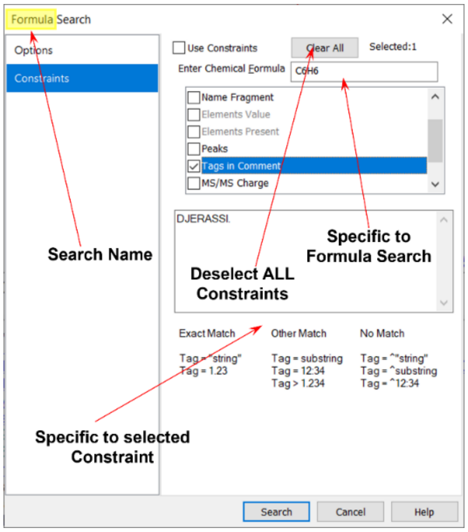
If the conglomerate of the selected libraries for a search that has greater than 6000 potential Hits is attempt an error dialog box with an **OK** button will be display, saying **Warning, too many similar spectra. Search may be incomplete**. When the **OK** button is selected, the first 6,000 spectra will appear in the **Hit List** and only this number in the **Hit List** will appear in the **Other Search** tab view header.

The **CAS Number**, **NIST Number**, and **ID Number** searches have only a single page dialog box. The **Exact Mass**, **MS/MS Precursor**, and **Any Peaks** searches have three-page dialog boxes. The top page has a name related to the search. The other two pages are labeled **Libraries** and **Constraints**. The selection of the libraries is made in the **Libraries** page. When these searches are first selected, they open to the top page with the name of the search rather than the **Constraints** page. The Libraries and Optional pages display the number of spectra in a specific number of libraries above the top window list the available libraries to the left of an **Available** label. Below the Include Libs: window in the lower part of the dialog box is the number of spectra in a specific number of selected libraries.

When a constraint is selected in the **Constraints** window of the **Constraints** page, the **Use Constraints** checkbox will be automatically selected. The **Other Searches** that allow constraints (**MW (Nominal Mass)**, **Exact Mass**, **MS/MS Precursor *m/z***. **Formula**, **Any Peaks**, and **Sequential Method**) will have a corresponding **Use Constraints** checkbox on the **Optional** or **Libraries** page that will mimic the box on the **Constraints** page. All of the **Constraints** pages have a **Clear All** button, which when selected will deselect all the selected constraints, and a **Selected**: label followed by the numeric value of the selected constraints. When a constraint is deselected, it will no longer be a part of a search; however, all the settings will remain. If selected and the settings are not changed, the current setting will be used.

When a constraint is selected, the appearance of **Constraints** page changes to allow entry of specifics for the individual constraint below the **Constraints** window.

As described earlier, text files containing single column list of CAS registry, NIST or ID numbers can be search by entering the name and path of the file in the area normally use for entry of a single number. This file can be selected from an Open dialog box displayed by selecting the  button to the right of the entry field. The files should have the extension TXT and be a simple ASCII file.

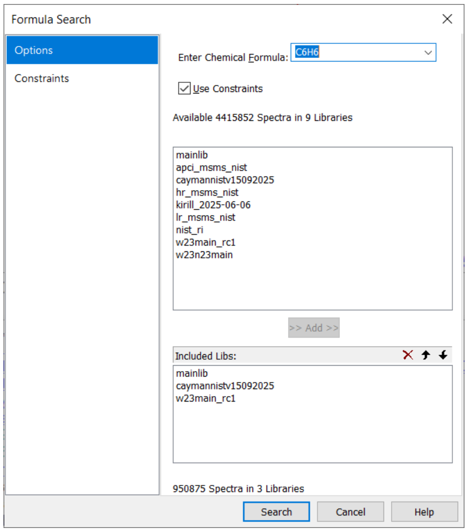


☐ Use Constraints Selected:1
Enter Chemical Formula: C6H6
☐ Name Fragment
☐ Elements Value
☐ Elements Present
☐ Peaks
☑ Tags in Comment
☐ MS/MS Charge

DJERASSI.

Exact Match	Other Match	No Match
Tag = "string"	Tag = substring	Tag = ^"string"
Tag = 1.23	Tag = 12:34	Tag = ^substring
	Tag > 1.234	Tag = ^12:34

Figure 103. Formula Search dialog box with Use Constraints selected and a Tag in Comments entered to yield only spectra contributed by Carl Djerassi.



Enter Chemical Formula: C6H6
☑ Use Constraints
Available 4415852 Spectra in 9 Libraries
mainlib
apci_msms_nist
caymannistv15092025
hr_msms_nist
kirill_2025-06-06
lr_msms_nist
nist_ri
w23main_rc1
w23n23main

Included Libs:
mainlib
caymannistv15092025
w23main_rc1

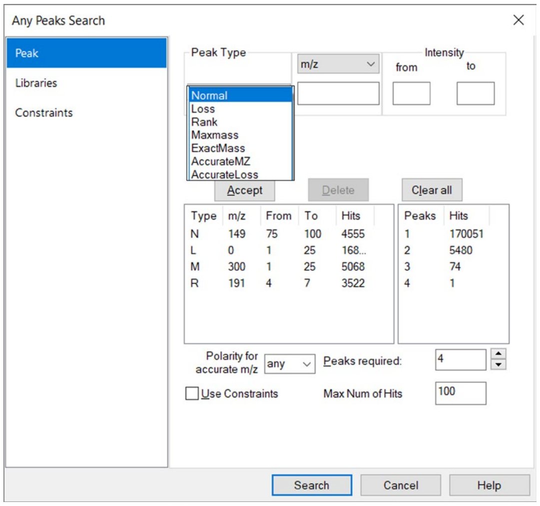
950875 Spectra in 3 Libraries

Figure 104. Options tab of the Sequential Method Search dialog box showing that a maximum of 100 Hits will be displayed.

Any Peaks search in the Other Search

Select **Any Peak** from the **Search** menu or the dropdown list box next to the **binocular** 🔍 button on the **Button Bar**. This will display the **Any Peaks Search** dialog box (*Figure 105*) Select the **Libraries** page. Highlight the mainlib and replib and select the **Add** button. This will show these two libraries in the Includes Libs: window. Select the **Peaks** page.

Select the "*m/z*" entry field (located below the *m/z* dropdown list box). The blinking vertical text cursor will now appear in the ***m/z*** field. **Normal** is displayed in **Type** field. *Enter 149.* This moves the cursor to the **From** field. *Enter 75.* This moves the cursor to the **To** field. *Enter 100.* This will enter the peak with its type, *m/z* value, and intensity range into the window on the lower left. The window on the lower right with column headers of **Peaks** and **Hit** will have 1 in the **Peaks** column and a number based on the selected libraries in the Hits field. The cursor is now back in the ***m/z*** field.



Type	m/z	From	To	Hits
N	149	75	100	4555
L	0	1	25	168...
M	300	1	25	5068
R	191	4	7	3522

Peaks	Hits
1	170051
2	5480
3	74
4	1

Polarity for accurate m/z: any

Peaks required: 4

☐ Use Constraints

Max Num of Hits: 100

Figure 105. Peaks tab of the Any Peaks search dialog box showing the selection of the four different types of peaks and ranges.

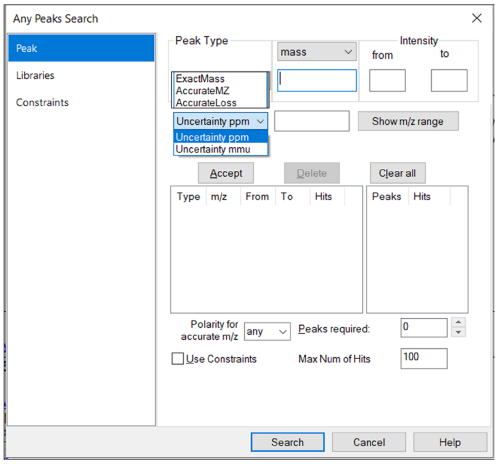
Select the down arrow for the **Peak Type** label to display a dropdown list box. Select **Loss**. This will cause the cursor to reappear in the ***m/z*** field. **Loss** will be displayed in the **Type** field. The first peak entered is an observed peak in the mass spectrum, and it is possible that it is the base peak. This peak is considered to be a *normal mass spectral peak.* The second peak being entered represents an ion formed by a loss from the molecular ion. *Enter 0. Enter 1. Enter 25.* The retrieved spectra must have a peak representing the molecular ion peak that exhibits an intensity between 1% and 25% of full scale. This reduces the number of spectra in the that match the two entered criteria.

In the **Peak Type** dropdown list box select **Maxmass**. This is a peak that has the *m/z* value of the most significant high mass peak in your spectrum. Retrieved spectra will contain a peak at this *m/z* value within the specified abundance range and no significantly larger peaks at higher *m/z* values are present in the spectrum. *Enter* 390 for the ***m/z*** value, 1 for the **from** value, and 25 for the **to** value.

The fourth ion type is **Rank**. Order of peak in relative abundance from the largest (**Rank 1**) to the 16th (**Rank 16**). The base peak is the largest peak in the spectrum. The **Rank** constraint can be used like the classic **8-peak Index** data. The **from** and **to** values are not percent intensities, but a Rank from 1 to 16.

The Any Peaks search can be constrained using the **Constraints** page. The **Any Peaks** search can be used for NIST Libraries and user-libraries like the **Wiley Registry if Mass Spectral Data**.

Any Peaks search for Exact Mass, Accurate *M/Z*, and Accurate Loss



Polarity for accurate m/z: any

Peaks required: 0

☐ Use Constraints

Max Num of Hits: 100

Figure 106. Peaks tab of the Any Peaks search dialog box showing the selection of Accurate/Exact Mass.

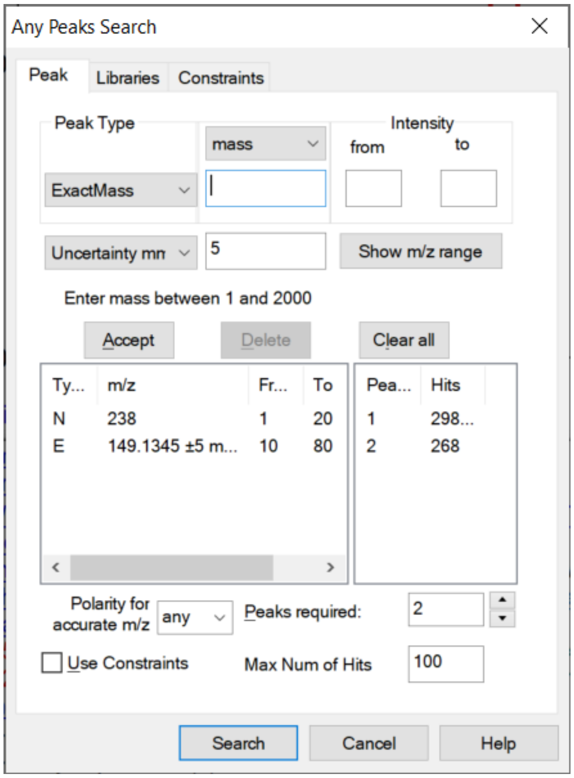
The **Any Peaks** search can also perform searches on **Exact Mass**, **Accurate *M/Z*** (an *m/z* value adjusted for the electron mass (0.000548579909 Da) assuming a singly charged positive ion) or a fragment ion's **Formula** (elemental composition).

However, this search mode only works on the NIST libraries; it will not function on user libraries even if the user library has been indexed by exact mass. The exact mass indexing of the user libraries is done through the Tools/(Re)Index. Exact Mass only indexes the precursor or molecular ion based on the formula. The abundance range is from 0 to 100% of the base peak where 0% means abundance <5 for a base peak intensity of 999. Only monoisotopic peaks may be searched.

Figure 106 shows the **Any Peaks Search** dialog box set to accept an accurate mass value with variance. Remember that the "Exact Mass" **Any Peaks Search** is only valid for monoisotopic peaks (all atoms of all elements in the ion represented by the peak are the most abundant isotopes). Do not try to enter values for peaks that contain higher mass isotope of chlorine (^{37}Cl), bromine (^{80}Br), or other elements. An accurate mass (**Mass**), an *m/z* value (adjusted for electron mass), or an elemental composition (**Formula**) can be entered.

The uncertainty can be in millimass units (mmu) or parts-per-million (ppm). If this field is left blank, an uncertainty of 0.5 less than the last digit entered for the mass is assumed. In this case the uncertainty is always a 0.5 value, e.g., the uncertainty for 120.00 is ±0.005 and 120.000 is ±0.0005.

To enter a chemical formula, select **formula** in the dropdown list box above the first entry field (this is possible only if “Exact Mass” type has been selected.) If the “Uncertainty” field is left blank, the accuracy of searched mass is the same as the accuracy of the mass saved in the library. It is very rare that the exact mass of two different elemental compositions is the same; therefore, there is a high probability that only the spectra where the peak was interpreted with the same elemental composition will be found. Molecular ion peaks in the mass spectrum will give very good results when used for the **Any Peaks Search Exact Mass** option; however, caution must be taken when using the accurate mass of fragment ions. The exact mass values in the **NIST DB** are calculated from the elemental compositions of ions predicted by **MS Interpreter** where peaks are present in the mass spectrum. More than one exact mass may be saved in a library for a peak in a mass spectrum of a compound. For instance, both CO and C_2H_4 may be assigned to the same peak with *m/z* 28. Searching for these two “Formulae” would produce a number of Hits containing both peaks. These Hits correspond to mass spectra where the peak at *m/z* 28 was ambiguously interpreted. The Any Peaks criteria can be used in the **Sequential Method Search** or as a constraint with a **MW (Nominal Mass)**, **Exact Mass**, **Formula Search** or with a **Spectrum Search**; however, the “Exact Mass” Peak type is not available for these searches. When **Peaks** is used as a Constraint, relative intensity can be specified. In this case, the subsequent peaks have intensities relative to the first peak. Thus, if the first peak has an intensity between 50 and 100% of base, the second peak can be specified to have an intensity of between 40 and 200% of the first peak. This is especially important when using SIM (selected ion monitoring) searches and wanting to understand the rates of false positives identifications. Radio buttons appear at the bottom of the **Peaks Constraints** dialog box to switch back and forth between “Absolute” and “Relative” (first Peak is Special!).



Peak Type: ExactMass

mass

Uncertainty mn: 5

Ty...	m/z	Fr...	To
N	238	1	20
E	149.1345 ±5 m...	10	80

Pea...	Hits
1	298...
2	268

Polarity for accurate m/z: any

Peaks required: 2

☐ Use Constraints

Max Num of Hits: 100

Figure 107. Any Peaks Search dialog box, showing an entry of an accurate mass value with variances. Note the position of the splitter bar.

Exact Mass Search

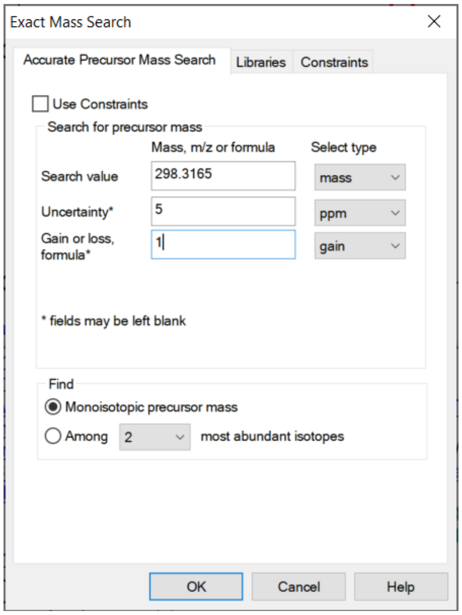
Exact Mass Search can be used directly to search the NIST libraries. In addition, **Exact Mass** (alternately with nominal mass) can be used as a Constraint in **User Spectra Searches** (**Lib. Search** tab), **Any Peaks Search**, and **Sequential Search** (**Other Search** tab).

NOTE – THE EXACT MASS CONSTRAINT IS FOR MOLECULAR OR PRECURSOR-ION MASS

Select **Exact Mass** from the dropdown list box on the upper left of the **Other Search** tab. The three-tab dialog box shown in *Figure 108* will be displayed. The exact mass value to be searched is entered into the "Search value" field on the **Accurate Precursor Mass Search** tab of the dialog box. The "Type" of the value is selected from the dropdown list box next to the "Search value" field. All the fields in the dialog box are explained in detail by selecting the **Help** button in the dialog box. The **Libraries** Page allows the selection of the libraries to be searched. Like the **MW (Nominal Mass) Search** and the **Formula Search**, the **NIST 20 replib** is not available for searching. The **Constraints** tab allows the **Exact Mass Search** to be constrained by name fragment, Tags in the "Comments/ Contributor" field, elements present, and element values. In addition, the formula or the formula for the neutral loss can be entered. In this case, the program will calculate the exact mass and use the uncertainty (if provided).

The **Exact Mass Search** is not restricted to the **NIST/EPA/NIH Libraries**; however, user or other non-NIST libraries must first be indexed by the exact mass. Select **Tools** from the Main Menu and then select **(Re)Index Exact Mass**. This will result in a dialog box being displayed (*Figure 109*). The time to index a library will depend on the size of the library. All **NIST 23 Libraries** are already indexed by the exact mass.

After the desired libraries have been indexed (this must be done only once), an **Exact Mass Search** or another **Search** using **Exact Mass** as a Constraint can be carried out.



☐ Use Constraints

Search for precursor mass

	Mass, m/z or formula	Select type
Search value	298.3165	mass
Uncertainty*	5	ppm
Gain or loss, formula*	1	gain

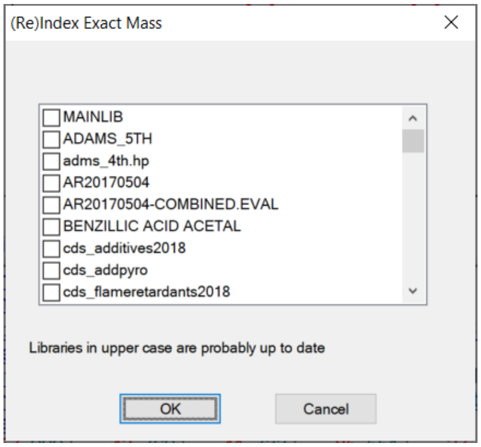
* fields may be left blank

Find

● Monoisotopic precursor mass

○ Among 2 most abundant isotopes

Figure 108. Exact Mass Search dialog box.



☐ MAINLIB
☐ ADAMS_5TH
☐ adms_4th.hp
☐ AR20170504
☐ AR20170504-COMBINED.EVAL
☐ BENZILLIC ACID ACETAL
☐ cds_additives2018
☐ cds_addpyro
☐ cds_flameretardants2018

Libraries in upper case are probably up to date

Figure 109. Dialog box to allow for indexing mass spectral libraries according to exact mass.

Search by Name - Browse

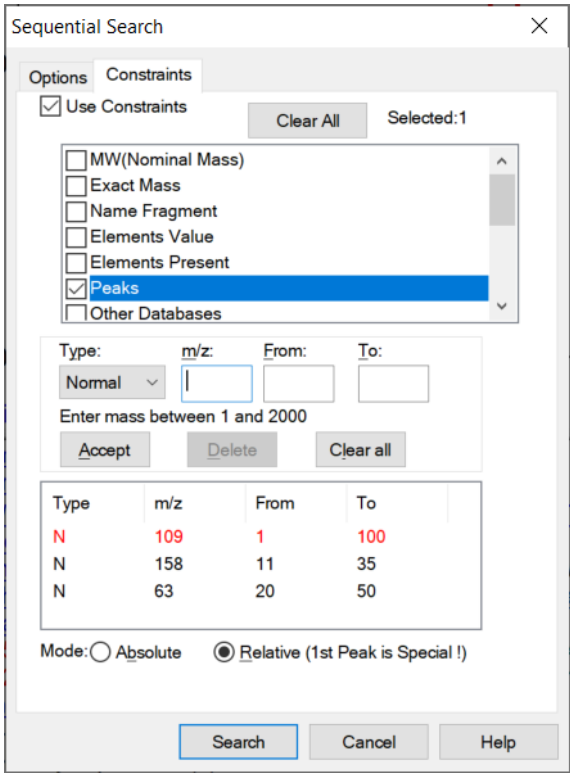
The data in a library can be searched or browsed using the incremental name search (the **Names** tab view). As a name is entered, the program finds the next name that fits all the text typed to that point. The names search works over not only the primary name but all synonyms. This means that common names for chemicals can be quickly searched. Some common compounds have a very large number of synonyms. Once the name is found, highlight the desired name, and the display in the **Plot/Text** and **Plot** Windows will be refreshed with the appropriate display for the compound.

Use the Mouse pointer to place the blinking vertical text cursor in the “Name” field at the top of the **Name Search** tab. To search for the mass spectrum of a plant growth regulator, GIBBERELLIN A9, begin typing the name. Stop after entering each letter. After the letter N has been typed, the 13th name in the **Hit List** window is the target. Names may also be pasted from the Windows Clipboard into the “Name” field by simultaneously pressing keys **Shift** and **Insert** or using the **RMB** menu **Paste** selection.

NOTE: This feature can be used in a user library to give synonyms such as “LabBook05-Pg6” or more simply “B05-P06”, this allows the data to be quickly found by reference to the original experiment.

Sequential Method

This method allows every spectrum in the selected libraries to be subjected to a created search criterion. The criteria that are set in the Constraints section govern the **Hit List** returned. In this search method, it is possible to use not only absolute but also only relative intensities of peaks. This can be an important tool for examining the risk of false positives when using SIM searches.



☑ Use Constraints

Selected:1

☐ MW(Nominal Mass)
☐ Exact Mass
☐ Name Fragment
☐ Elements Value
☐ Elements Present
☑ Peaks
☐ Other Databases

Type: Normal

Enter mass between 1 and 2000

Type	m/z	From	To
N	109	1	100
N	158	11	35
N	63	20	50

Mode: ○ Absolute ● Relative (1st Peak is Special !)

Figure 110. Sequential Search dialog box showing the use of Any Peaks in a Relative mode.

In the example shown in *Figure 110*, the peak at 109 is allowed to be present in a range of 1 to 100% of the base peak. The subsequent peaks (158 and 63) are sought as a fraction of the 109-peak, regardless of its intensity. If the peak with *m/z* 109 is 15% of the intensity of the base peak and the peak with *m/z* 158 is 5% of the intensity of the base peak (i.e., 33% of the 109) it would be accepted. This kind of analysis can give important insights into the risk of false positive matches when using SIM (selected ion monitoring) in GC/MS experiments.

In SIM the entire spectrum is not seen so the relative intensities of the ions observed are the only data that can be extracted from the data file.

NOTE: All the other methods of searching are also present in the Sequential Method. The elements search can also be interesting if the number of compounds in all the libraries that have at least one mercury atom in the molecule needs to be determined. On a modern computer with a large amount of memory these searches do not need to take very long. Typically any sequential search will be faster for subsequent similar searches because much of the data file will be cached in the computer memory.

Basis for Interpretation of the Library Search Results

When an **Identity Search** of an **EI** mass spectrum resulting from an unidentified compound (**Spectrum Library Search**) is performed, the results will be associated with three numbers for each spectrum shown in the **Hit List** (by default) and one value for the search. These three numbers are: 1) a **Match Factor** for the spectrum of the unidentified compound and the library spectrum (direct match of peak *m/z* values and relative intensities), 2) a match factor for the spectrum of the unidentified compound and the library spectrum ignoring any peaks in the spectrum of the unidentified compound that are not in the library spectrum (**Reverse Match**), and 3) a **Probability** value (column headings in the **Lib. Search Hit List: Match**, **R. Match** and **Prob. (%)**, respectively). The first two numbers are straightforward. Each is derived from a modified cosine of the angle between the spectra (normalized dot product). A perfect match results in a value of 999; spectra with no peaks in common result in a value of 0.

As a general guide, **900** or greater is an **excellent match**; **800–900**, a **good match**; **700–800**, a **fair match**. **Less than 600** is a **very poor match**. However, spectrum of unidentified compounds with many peaks will tend to yield lower **Match Factors** than similar spectra with fewer peaks. The **Prob. (%)** value and the value for the search (**InLib**, located in the lower right corner of the **Hit List** window and on the **Title Bar** of the **Lib. Search** tab) require more explanation.

The **Prob. (%)** value for a **Hit** is derived if the compound is represented by a spectrum in the libraries searched. It employs only the differences between adjacent **Hits** in the **Hit List** to get the relative probability that any **Hit** in the **Hit List** is correct. This value is derived from an analysis of the results of searching the **NIST/EPA/NIH Main Library** with a set of replicate spectra (given in the **Replicates Library**). The relative probability of each of the Hits requires only the difference values because the total probability of the compound being in the searched libraries is assumed to be one. The other factor (**InLib**) is a measure of the probability of the compound being in the searched libraries. This value is also derived from the same set of replicate spectral searches. In this case, the correct compound was ignored in the **Hit List**; and the difference between the **Hit List**, with and without the correct compound, is parameterized. The parameters are the maximum value of the match and the largest single difference among the top 20 Hits. If the first **Hit** has a high **Match Factor** (>900) and the next **Hit** has a **Match Factor** of 800 or less, the probability of the compound being correctly identified is very large and the probability of the compound being in the searched library is large (the number of "**Hits** vs. their **Match Factors**" is displayed in the **Hit Histogram** window located just above the **Hit List**, see *Figure 21*).

Like all statistical results, these probability calculations rely on the data sampled. For example, if a compound that has very few similar mass spectra will be identified in a more definitive way (a higher **Probability** and **InLib** value). Using examples from the **Replicates Library** and searching the **Main Library** using a compound like 'folpet', will result in a high probability for the first **Hit** and a high **InLib** value. In contrast, if replicates of 'cyclohexanol' are searched, the results will be much lower **Prob. (%)** and **InLib** values; and, in some cases, the best **Hit** is not even the correct compound. This reflects the fact that there are very few compounds that have mass spectra similar to 'folpet'; but there are a number of compounds that have very similar mass spectra to 'cyclohexanol', and the ability of any search system to distinguish between these similar spectra is limited. In many cases, the best that the search can do is to provide a class of compounds that have similar mass spectra and, usually, similar structures.

The values of the **InLib** parameter are meant as guideposts. Generally, any positive value is acceptable. Values greater than approximately 300 usually mean that the spectrum is nearly unique. Negative values below 200 are generally a warning that the spectrum is not identified. Note that negative values will occur when there are many compounds with similar spectra. In these cases, the difference between the **Match Factors** for different spectra is very small, and the search cannot be assured of providing the correct unique answer. Usually in these cases, especially when **Match Factors** are high, it will provide very good guidance on the structure of the molecule.

For a complete discussion of the methods used in assessing the probabilities, see Stein, S.E. "Estimating Probabilities of Correct Identification from Results of Mass Spectral Library Searches" *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 316 – 323.

Substructure Information

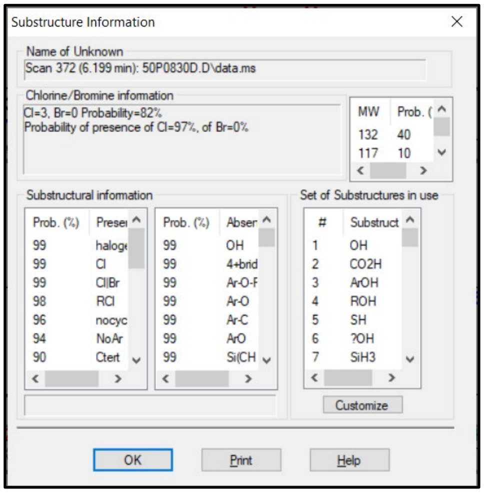
The **Substructure Information** utility is used in the evaluation of a **Hit List** using **the NIST/EPA/NIH Mass Spectral Library** because each spectrum is indexed by the substructures that make up the structure of the compound that resulted in the spectrum. Substructures are functional groups like carbonyls, methoxys, methyl ester moieties, etc.; the number of rings and double bonds; and heteroatoms also are considered to be substructures.

The **Substructure Information** is most useful when the **Hit List** is the result of one of the four **Similarity Searches** (**EI Hybrid**, **EI Simple**, **Neutral Loss**, **MS/MS in EI**, or **MS/MS Hybrid**). This utility is called from the Main Menu's **Tools** menu.

Similarity Searches are carried out when it is believed that a spectrum of the unidentified compound is not in any of the searched libraries. Spectra like the searched spectrum make up the **Hit List**. There is no **Prob** column in the **Hit List** and an **InLib** value is not determined. Not only is the utility useful in determining the structure of an unidentified compound based on its EI mass spectrum, but it has also proved to be very informative in the analysis of collision-induced dissociation (CID) spectra obtained from ions produced by soft ionization LC/MS techniques such as APCI, APPI, and electrospray. This use is described in more detail below.

The **Substructure Information** dialog box (*Figure 111*) has the name of the spectrum that was used to create the **Hit List** displayed at the top. Just below the **Name of Unknown** field in this dialog box is the "Chlorine/Bromine information" field, which gives the probabilities and numbers of atoms of chlorine and/or bromine present (useful regardless of the method by which the spectrum was obtained). The result of molecular weight estimation from the **Hit List** is presented on the right side of the dialog box (useful for EI spectra, especially when no molecular ion peak is observed). There are two windows below this in an area labeled **Substructure information**: **Prob. Present** and **Prob. Absent**. These two windows contain a list of abbreviations for the substructures preceded by a number, which is the percent probability for the presence or absence of the substructure. If one of the abbreviations is not understood, highlight it by using the Mouse and a detailed explanation appears at the bottom of the **Substructural information** area.

Just below the molecular weight information is the **Set of Substructures in use** window with a list box and a **Customize** button. The list box displays all the substructures in the current set.


Substructure Information

Name of Unknown

Scan 372 (6.199 min): 50P0830D.D\data.ms

Chlorine/Bromine information

Cl=3, Br=0 Probability=82%
Probability of presence of Cl=97%, of Br=0%

MW	Prob. (
132	40
117	10

Substructural information

Prob. (%)	Preser
99	haloge
99	Cl
99	Cl|Br
98	RCl
96	nocyc
94	NoAr
90	Ctert

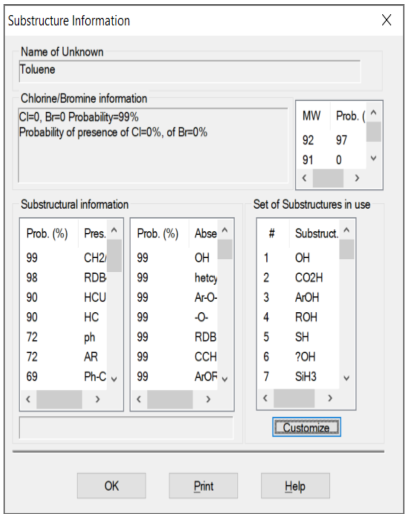
Prob. (%)	Abser
99	OH
99	4+brid
99	Ar-O-F
99	Ar-O
99	Ar-C
99	ArO
99	Si(CH

Set of Substructures in use

#	Substruct
1	OH
2	CO2H
3	ArOH
4	ROH
5	SH
6	?OH
7	SiH3

Figure 111. Compound Identification with Substructure Information of CID Spectrum.

The **Customize** button, when selected, displays the **Customized set of Structures** dialog box (*Figure 112*). his dialog box is used to create a subset of the substructures to be identified. The list of all possible substructures is displayed in the **Full set of structures** window. The list of substructures included in the present file is shown in the **Customized set** window. The first time a customized list is created, both windows will contain the same list. Substructures may be omitted from the **Customized set** window or added from the **Full set of structures** window. Either action occurs on the structures highlighted by Mouse action in the window by selecting the appropriate button **← Omit ←** or **→ Add →**. The usual Windows conventions for selecting multiple items apply. After selecting the first substructure of the list, hold down the **Shift** key while selecting the last substructure. This will select all substructures between the first and last. Multiple noncontiguous substructures can be selected by holding down the **Ctrl** key while selecting each desired substructure.


Substructure Information

Name of Unknown

Toluene

Chlorine/Bromine information

Cl=0, Br=0 Probability=99%
Probability of presence of Cl=0%, of Br=0%

MW	Prob. (
92	97
91	0

Substructural information

Prob. (%)	Pres.
99	CH2/
98	RDB-
90	HCU
90	HC
72	ph
72	AR
69	Ph-C

Prob. (%)	Abse
99	OH
99	hetcy
99	Ar-O-
99	-O-
99	RDB
99	CCH
99	ArOF

Set of Substructures in use

#	Substruct.
1	OH
2	CO2H
3	ArOH
4	ROH
5	SH
6	?OH
7	SiH3

Figure 112. Customized Set of Structures dialog box.

The **Save** and **Open** buttons allow the saving and retrieving of customized substructure sets. A temporary set is created by making modifications to the **Customized set** window and selecting the **OK** button. This dialog box, as well as the **Substructure Information** dialog box, has a **Help** button, which displays a context-sensitive Help screen.

The algorithms used in the substructure identification are described in the Help screen. They are based on developments at NIST (Stein, S.E., "Chemical Substructure Identification by Mass Spectral Library Searching" *J. Am. Soc. Mass Spectrum.* **1995**, *6*, 644–655).

Example Use of Substructure Info in Determining Structure of Unidentified Compounds Using CID

The mass spectrum (*Figure 113*) of a compound with the structure shown inserted in the spectrum was obtained using collision-induced dissociation (CID) on a protonated molecule with *m/z* 248 (**Spectrum Type: Tandem**). An **EI in MS/MS Similarity Search** was performed against the **NIST 26 mainlib** with *m/z* 248 designated as being the precursor ion. This type of search is provided to allow for the use of the vast amount of information in the EI Library with spectra that are produced by LC/MS CID. The **EI in MS/MS Similarly Search** has been optimized for this purpose. The resulting **Hit List** was evaluated using the **Substructure Identification** utility. Based on this evaluation, it was determined that there was a 90% probability that the compound contained one or more atoms of oxygen; a 90% probability that there was a carbonyl group; an 89% probability that there was a heterocyclic ring; and an 88% probability that there was an aromatic ring; but it also revealed a 99% probability that there was not a phenyl carbonyl or a methoxy phenyl group, and a 90% probability that there was not a heterocyclic ring that contained oxygen. Remember, it is just as important to know what is absent as it is to know what is present. The evaluation revealed that there was an 89% probability that there were one or more atoms of nitrogen and an 86% probability that there was a heterocyclic ring with an atom of nitrogen. Continued evaluation of the **Prob. Present** and **Prob. Absent** windows in the **Substructure Identification** dialog box revealed other possible substructures to be present and eliminated others by their high-absence probabilities.

This particular use of the **MS/MS in EI Similarity Search** in conjunction with the **Substructure Identification** has not been extensively tested by NIST; however, anecdotal evidence has shown this to be an effective combination and should be considered when trying to determine the structure of an unidentified compound using CID data. In the example cited above, if the unidentified compound was believed to be a metabolite of some known substances, the information gained from this technique could well result in identification.

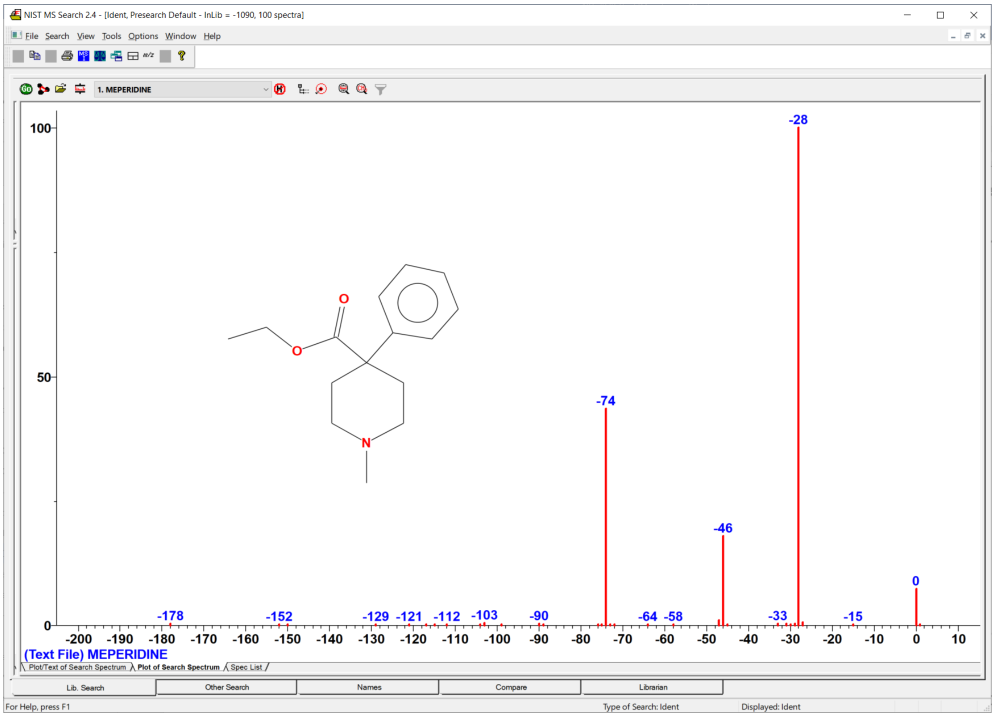


Figure 113. Mass spectrum obtained by CID of ion with m/z 248 and structure.

Use with Instrument Data Systems & Third-Party Data Analysis Software

The features described in the **AUTOMATION** section of this manual were implemented to aid the use of the **NIST MS Search Program** with proprietary mass spectral data systems.

Location of the NIST MS Search Program

It may be necessary to determine the location of the **NIST MS Search Program** files and the location of the **NIST MS Search** working directory. These can be found in the **[NISTMS]** section of the **WIN.INI** file located in the %windir% directory. Below is a typical example of this section:

```
[NISTMS]
Path32=C:\NIST26\MSSEARCH\
WorkDir32=C:\NIST26\MSSEARCH\
Amdis32Path=C:\NIST26\AMDIS32\
AmdisMSPath=C:\NIST26\AMDIS32\
```

The string beyond “Path32=” is the path to **MS Search Program** folder (location of the executable files); the string beyond “WorkDir32=” is the path to the **MS Search Program** working directory (location of the history, Hits, etc.). Two other items refer to AMDIS and its connection to the **MS Search Program**.

Cases have been reported where the connection between **MS Search** and a third-party data analysis system was rendered nonfunctional when this [NISTMS] area of the win.ini file referred to more than one location for various versions of the **NIST MS Search Program**.

Implementation

If the mass spectrometry data system being used is capable of exporting spectra to the NIST format (described in the **User Libraries** section of this manual) and a Windows program can be executed from within the data system, this feature is available. Implementation of this feature has been provided by some manufacturers on their data systems.

The command string used to start or bring into focus the **NIST MS Search Program** for Windows is:

```
C:\NIST26\MSSEARCH\NISTMS$.EXE<space>/instrument
```

assuming the program is in the **C:\NIST26\MSSEARCH** directory; if it is in some other directory, make the appropriate substitution.

Two locator files are required to use the automation features:

1. The first locator file must be in the **MS Search Program** working directory, have the name **AUTOIMP.MSD**, and contain the text string describing the name and location of the second locator file. For example:

   ```
   C:\MYMSDS\FILESPEC.FIL
   ```

   where: C:\MYMSDS is the folder where the file, FILSPEC.FIL, resides.

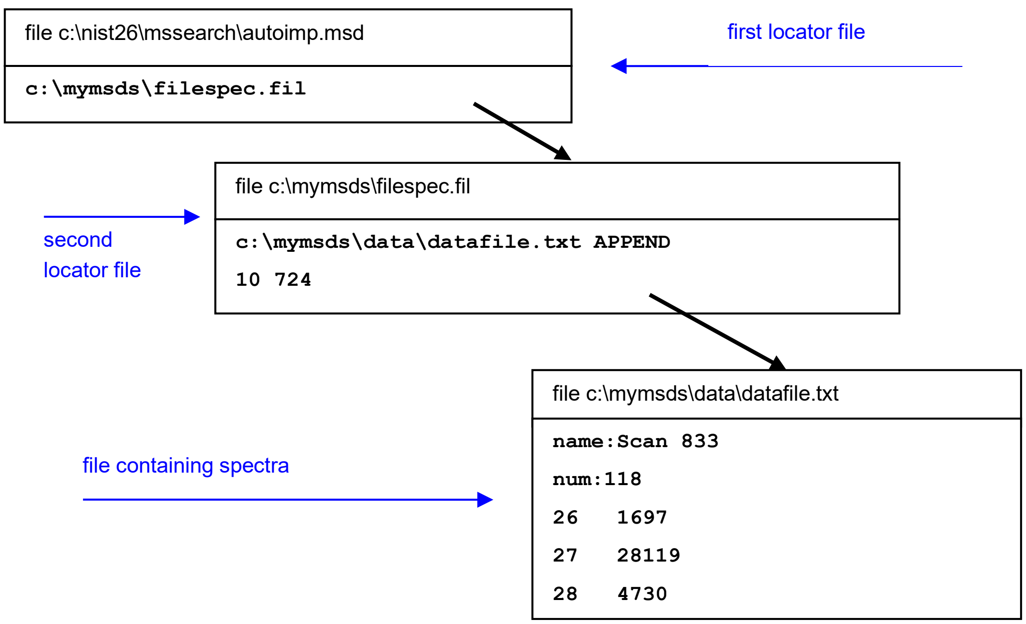
   This file can be created with any text editor.

2. The second locator file is created each time a spectrum or spectra is/are saved to a separate file to be imported by the **MS Search Program**. This second locator file contains the text string that describes the name and location of the text file containing the spectrum (or spectra) in the NIST Text file format described later. It is possible to state whether the spectra currently in the **NIST MS Search Program Spec List** window are to be added to or replaced by the automatic import. An example of the contents of the second locator file is:

   C:\MYMSDS\DATA\DATAFILE.TXT<space>OVERWRITE *or* APPEND

   where: C:\MYMSDS\DATA\DATAFILE.TXT is the full pathname of the file containing the spectra, and one of the words OVERWRITE or APPEND is the instruction as to how the spectra are added to the **Spec List** Window. To enable switching the focus back from the **MS Search Program**, the second line containing **main window handle numbers** of the data system application may be added (see *Figure 114* for details).

   This second locator file is deleted each time the **NIST MS Search Program** is brought into focus and spectra are imported. *Figure 114* illustrates the implementation. The upper part of each box contains a file name; the bottom part is the contents of the file. Black arrows show logical connections between the files.



file c:\nist26\mssearch\autoimp.msd

```
c:\mymsds\filespec.fil
```

file c:\mymsds\filespec.fil

```
c:\mymsds\data\datafile.txt APPEND
10 724
```

file c:\mymsds\data\datafile.txt

```
name:Scan 833
num:118
26   1697
27   28119
28   4730
```

Command line for initiating import of spectra into the MS Search Program:

```
C:\NIST26\MSSEARCH\NISTMS$.EXE /instrument
```

Calculation of the calling program **main window handle numbers**:

hWnd = A02D4(hexadecimal) = HiWord × 2^{16} + LoWord =

10 × 2^{16} + 724 (decimal), that is, HiWord = 10, LoWord = 724

(See the second line in c:\mymsds\filespec.fil; the numbers here are for example only; in each run your application may have a different main window handle.)

Figure 114. The Implementation of Use with a Data System.

NIST MS Search (nistms$.exe) Command Line Options	
Option	**How it works**
/INSTRUMENT	Normal search. Imports and optionally searches spectra (*Figure 114* for details on the implementation), activates **MS Search** Window, activates **switch back** button.
/INSTRUMENT /PAR=2	Background search. Imports and optionally searches spectra (*Figure 114* for details on the implementation), does not activate **MS Search** Window (in fact, it is disabled during the search), writes **Hit Lists** into file SRCRESLT.TXT; when the search is complete creates file SRCREADY.TXT as a signal that SRCRESLT.TXT is ready. Both files are created in the **MS Search** work directory. Number of Hits is determined by the “Number of Hits to Print” in the **Automation** tab (*Figure 75*).
/PAR=4	Start logging. Appends **Hit Lists** of **Spectrum** and **Structure Searches** to the file NISTLOG.TXT located in the **MS Search** work directory. Number of Hits is determined by the “Number of Hits to Print” in the **Automation** tab (*Figure 75*).
/PAR=8	Ends logging. Stops appending **Hit Lists** to the file NISTLOG.TXT.
/MOL=“<full pathname>”	Imports chemical structure from a Molfile or SDfile. If SDfile contains more than one Molfile, only the first one will be imported. See section **USE WITH THIRD-PARTY DRAWING PROGRAMS** for details.

SEE APPENDIX 1 for instructions on the Creation of AUTOIMP.MSD.

Use with Third-Party Drawing Programs

There is a **Structure Search** command. If a structure is in the **Spec List** window of the **Lib. Search** tab or a structure is associated with a spectrum, this structure can be searched to retrieve spectra of all compounds with similar structures by selecting the **Structure Search** button (second button from the left) with the structure highlighted in the **Spec List** window or by use of the Right Mouse button (**RMB**) menu with the Mouse pointer on the spectrum containing a structure.

When the **NIST MS Search Program** saves a structure to the Windows Clipboard using the Right Mouse button menu command **Copy Structure to Clipboard**, the structure is copied to the Windows Clipboard and a file in Molfile format named Clipboard.MOL is written to the **NIST MS Search Program** directory (by default, C:\NIST26\MSSearch). Other programs can either use the structure as it appears on the Windows Clipboard or this file for structure importation.

Structures saved to the Windows Clipboard may be accepted in the **MS Search Program Librarian Spectrum Information** dialog box if its **Clipboard Struct** button is not grayed. Some drawing programs need to be told how to save a structure in the Windows Clipboard. For example, in ISIS/Draw (MDL Information Systems, Inc.), it is necessary to select **Copy Mol/Rxnfile to the Clipboard** checkbox in **Options/Settings/General** tab of that program.

If an application is associated with files that have a .MOL extension, then the structure may be sent to that application by selecting in the **RMB** menu **Send To** and **Default Structure Editor**.

If a spectrum containing a chemical structure is selected, the **RMB** menu command **Send to** will result in a **Send to** menu that can have up to three additional structure editor items. These **Send to** menu items are a function of the first three lines in a text file **AUTOIMP.STR** located in the **NIST MS Search Program** working directory (the WorkDir32 item in [NISTMS] section of the WIN.INI file). Each line should contain three double-quoted strings separated by spaces:

1) A program name as it appears in the **Send to** menu
2) Program executable and path
3) Program startup parameters. %1 will be replaced by the mol file name.

The following is an example of the contents of an AUTOIMP.STR file:

```
"ChemSite" "C:\MolSuite\ChemSite\ChemSite.exe" "%1"
"ISIS Draw" "D:\ISIS Draw\IDraw32.exe" "%1"
"ACD/ChemSketch" "C:\acd800\chemsk.exe" "%1"
```

The last line of the above example results in ACD lab ChemSketch starting with the contents of clipboard.mol file being imported when ChemSketch opens.

When a program that is to be interactive with the **NIST MS Search Program** is installed, it must UPDATE or CREATE the **AUTOIMP.STR** file, or the **AUTOIMP.STR** file must be edited by the user before the third-party program will appear on and function from the **Send to** menu.

See APPENDIX 1 for instructions on the creation of AUTOIMP.STR.

A third-party program can call the **NIST MS Search Program** by issuing the command:

```
C:\NIST26\MSSEARCH\NISTMS$.EXE /MOL=<molfile>
```

assuming the **NIST MS Search Program** is in the **C:\NIST20\MSSEARCH** directory; if it is in some other directory, make the appropriate substitution (see **Location of the NIST MS Search Program** above)

where <molfile> is a full pathname of the MOL file. If there is a space in the name (e.g., example c:\program files\a.mol), the name should be double quoted:

```
C:\NIST26\MSSEARCH\NISTMS$.EXE /MOL="c:\program files\a .mol"
```

A third-party program can obtain the path to NISTMS$.EXE from Path32 in the [NISTMS] section of WIN.INI (see **Location of the NIST MS Search Program** in the **USE WITH INSTRUMENT DATA SYSTEMS** section above).

User-Libraries

The NIST MS Search program's **Librarian** tab view can create **user-libraries** to search against. These can be libraries of EI or Tandem spectra (integer and accurate *m/z* value). Accurate *m/z* value user-libraries of in-source CAD or open-air desorption ionization spectra, using techniques such as DART or DESI, can also be created within the **Librarian** tab view. Once an integer *m/z* value **user-library** is created, it can be used as is. It can also be updated by adding or editing existing entries using the **Librarian** tab view editing tools. The accurate *m/z* user-libraries are created using the tools in the **Librarian** tab view; however, before they are used, these user-libraries must be reindexed using the **Lib2NIST** program. When an accurate *m/z* spectrum **user-library** is edited in the **Librarian** tab view, it must then be reindexed using the Lib2NIST program. The process of reindexing an accurate *m/z* **user-library** can be found on the NIST website at *https://chemdata.nist.gov*. Up to 127 total libraries can be installed in MS Search. This includes the NIST provided libraries and the **user-libraries**.

Libraries, including the libraries provided with **NIST 26** are searchable at the same time by various search routines in MS Search. EI **user-libraries** may be searched in conjunction with the **NIST/EPA/NIH Mass Spectral Main** (mainlib) and/or **Replicates** (replib) **Libraries**, or they may be searched separately. MS Search allows for the searching of combinations of libraries in any order. The inclusion of libraries and their order of search can be set from the **Libraries** or **Options** tab of the various **Search** dialog boxes (see Figure 96 as an example). When searches of unknown spectra are performed, the search order is not an issue. The **Hit List** is ordered (by default) according to **Match Factor** or **Score** values (unless otherwise specified) regardless of what library the **Hit** is in.

The **user-libraries** are easy to create and maintain. It may be desirable to create them using specific sets of spectra from the **NIST/EPA/NIH Main** or **Replicates Library**. Any spectrum in the **Spec List** can be added to a **user-library**. Any spectrum in the **Hit List** of the **Lib. Search** tab, **Other Search** tab, or **Names** tab, a **Plot** window, **Text Info** window, or **Subtraction** window in the **Compare** window of the **Lib. Search** or the **Compare** tab views can be copied to the **Spec List** window by selecting **Spec List** on the **Send to** menu from the Right Mouse Button (**RMB**) menu. The size of a **user-library** is limited to 65,535 spectra.

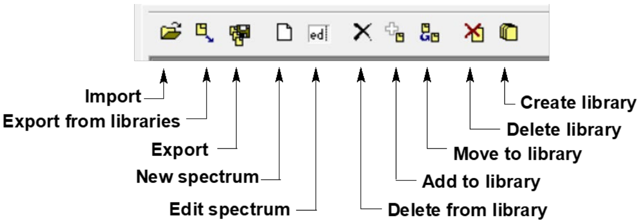
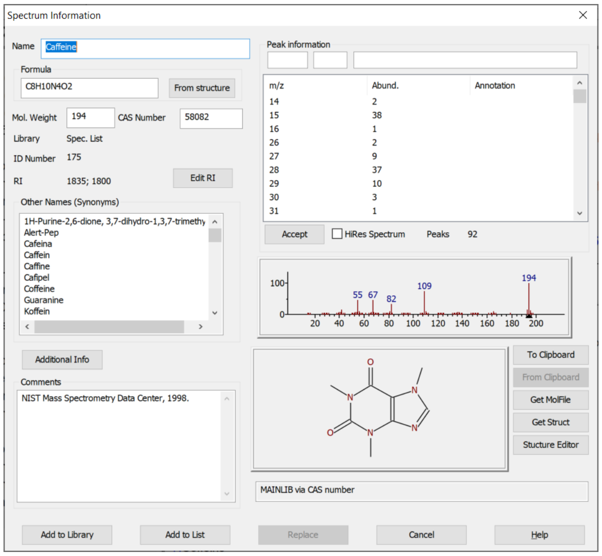


Figure 115. Librarian Button Bar.

The **MS Search Program** can read user-submitted spectra in the JCAMP, U.S. EPA, or NIST Text file formats as well as formats of files submitted by various instrument manufacturer's mass spectrometry data systems.



Name: Caffeine

Formula: C8H10N4O2

Mol. Weight: 194

CAS Number: 58082

Library: Spec. List

ID Number: 175

RI: 1835; 1800

Other Names (Synonyms):
1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethy
Alert-Pep
Cafeina
Caffein
Caffine
Cafipel
Coffeine
Guaranine
Koffein

Comments: NIST Mass Spectrometry Data Center, 1998.

Peak information

m/z	Abund.	Annotation
14	2	
15	38	
16	1	
26	2	
27	9	
28	37	
29	10	
30	3	
31	1	

☐ HiRes Spectrum

Peaks: 92

MAINLIB via CAS number

Figure 116. Spectrum Information dialog box displayed in the Librarian tab view.

User-libraries are maintained as folders in the same folder as the NIST provided libraries (also folders). The name given to the **user-library** is the name of the folder contacting the library. This folder will contain library-files and indices constituting the **user-library**. The spectra and compound information are put into records through the **Librarian** tab or by using **Lib2NIST** along with an msp/mspec or an sdf file.

The spectra and compound information can originate in a text file in the NIST Text file format described in the **NIST TEXT FORMAT of INDIVIDUAL SPECTRA** section of this manual or directly imported from AMDIS. Spectra can be edited by selecting the **Edit Spectrum** button in the **Librarian** tab **Button Bar** (*Figure 115)*. This will display the **Spectrum Information** dialog box (*Figure 116*). This dialog box can be used to add structures to user spectra from the clipboard, add identification information such as CAS numbers or synonyms and to add comments. In addition, the RI value for the spectrum can be either added or edited.

A highlighted spectrum with nothing more than a **Name:**, a Field header titled **Num Paks** on the line following **Name:**, followed by the *m/z*-intensity pairs equaling to the number in the **Num Peaks** field appears in the **Spectrum Information** dialog box when the **ed** button is selected from the **Button Bar**. Usually, the first step to creating a potential entry for a **user-library** is to associate a structure from the Windows Clipboard or an SDF file. If a structure in the Mole file format has been put on the Windows Clipboard, the button on the lower right of the dialog box labeled **From Clipboard** will be available. Selecting this button will cause the structure to appear in the window to the left replacing the ☹ icon. The next step is to enter the **Formula** and **Mol Weight**. This *MUST BE Done* by selecting the **From structure** button next to the text entry input box just below the **Formula** label in the **Spectrum Information** dialog box (top left of *Figure 116*). This is necessary to have the proper indexing for the **user-library**. The **Name** field in the dialog box can be edited and a **CAS Number** added.

Once all editing of the spectrum is completed, if it is a spectrum currently not in a **user-library**, select the button at the bottom of the dialog labeled **Add to List** or **Replace**. If the **Replace** button is selected, the entry in that position of the **Spec List** is replaced and the spectrum will have an '**E**' as the second entry from the left of the line for the spectrum. If the spectrum is from an existing library and has been sent to the **Spec List** by the **RMB** menu **Send To** command, the entry will have the first two letters of the library name of origin following the **Spec List** entry number. When the **Replace** button is selected the edited spectrum replaces what is in the **Spec List** and the spectrum on the library of origin. The edited spectrum replacing a previous spectrum will have the same ID number in the library as did the original spectrum. If the Add to List button is selected, the edited spectrum is prepended to the **Spec List** and assigned Spec List number 1. If the **Add to List** button is selected, the edited spectrum is prepended to the **Spec List** and assigned **Spec List** number 1 and the original spectrum with it Spec List number remains in the List. If the spectrum is from an existing library, it also remains on the **Spec List** in its original position and is unchanged in the **user-library**.

If the Add to Library button is selected, the Chose library to save to dialog box is display with a list of libraries displayed in a window with a slider bar on the right side. All libraries are listed except for those provided by NIST. NIST libraries cannot have spectra added, deleted or edited.

A new spectrum can be created using the **New spectrum** button (🗋) in the **Librarian** tab **Button Bar** (*Figure 115*). A spectrum in the **Spec List** of the **Librarian** tab view that is listed as being in a **user-library** can be deleted by selecting the **Delete from library** button (✕). This results in a dialog box display asking the action to be confirmed. The entry is deleted from the user-library, the **Spec List** of the **Librarian** tab view, and the **Hit List** where it originated from. The **user-library** is reindexed to reflect the number of spectra it contains.

The manufacturer of a specific mass spectrometer's data system should be consulted to see if there is file compatibility. Instructions on building user libraries are included in the **Librarian** tab view's **Spectrum Information** dialog box's Help screen by selecting the **Help** button in the dialog box.

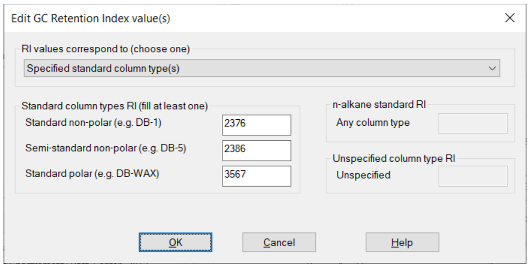
There are four more buttons seen on the **Librarian** tab view's **Button Bar** as seen in *Figure 115*: **Add to library**, **Move to library**, **Delete library**, and **Create library**. If the **Add to library** or **Move to library** button is selected, a new **user-library** name can be entered rather selection from one of the **user-libraries** listed, and that new library will be created. Using the **Delete library** button is a draconian action. Selecting this button results in a warring dialog box asking for confirmation of the action about to be taken. If confirmed, the dialog box closes and the **Choose library to delete** dialog box is displayed. When **OK** button is selected, all spectra associated with the deleted **user-library** are remove from all displays in MS Search and the folder constituting the **user library** is deleted. None of the NIST Libraries can be deleted using this feature. ***WARNING!*** Some third-party mass spectral libraries providers will set their user-libraries to **Real Only**. These libraries will appear in the list of libraries that can be deleted and will be deleted.

When **user-libraries** are created or spectra are added using the **Librarian** tab view, they are indexed for Exact Mass, the Hybrid-17 Search, InChIKey, RI and Derivatives, The structure Search Database is not built/rebuilt. After initially building a new **user-library** or adding a spectrum to an existing user-library, the **Rebuild Structure Search Database** must be run from the **Tools** menu accessible from the Main Menu.

There are two buttons on the **Spectrum Information** dialog box: **Edit RI** and **Additional Info**. A dialog box is displayed after clicking on **Additional Info** button, a **Spectrum Info** dialog box is displayed with 30 Field title label that will appear on a spectrum when content is present for the individual label. Select one of the labels. This will cause area to the right to be highlighted. Select the highlighted area. This results in the highlight area changing to a text entry box. Put the Mouse pointer in this text entry and type a string of any other length. Select another label and repair the process. Once all the desired labels have text entered, select the **OK** button at the bottom of the dialog box. This causes the **Spectrum Info** dialog box to close. The **Spectrum Information** dialog box (edited spectrum) will still be displayed. When the **Spectrum Information** dialog box is closed using one of the various options (**Add to library**, **Add to List**, or **Replace**) and the edited spectrum is displayed, these new Field headers, with their contents, will appear. Most of the Field Headers are related to spectra in Tandem user-libraries; however, it is possible some have relevance to EI spectra.

Selecting the **Edit RI** button results in the display of the **Edit Retention Index value(s)** dialog box being displayed (*Figure 117*). This dialog has a dropdown list box allowing the selection of the column type: **Specified standard column type(s)**; **Any column type (n-alkane standard)**; or **Unspecified column standard**. Selecting a specific column type makes available text entry boxes. RI values from the Chromatogram tab view are Semi-standard non-polar.

Currently the only value that has meaning relative to a spectrum search is **Specified standard column types** (**Semi-standard non-polar (e.g. DB-5)**. Values for the other fields can be added manually. If specific formatting is present in a *.MSP or *.MSPEC file, the **Specified standard column types** (**Standard non-polar (e.g. DB-1)** and **Standard polar (e.g. DB-WAX)** will be populated. This formatting is explained in the next section (**NIST Text Format of Individual Spectra**).



RI values correspond to (choose one): Specified standard column type(s)

Standard non-polar (e.g. DB-1) 2376

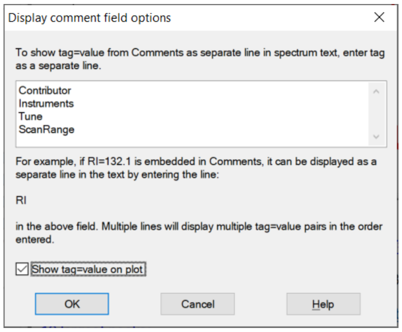
Semi-standard non-polar (e.g. DB-5) 2386

Standard polar (e.g. DB-WAX) 3567

Any column type

Unspecified

Figure 117. Edit GC Retention Index value.



To show tag=value from Comments as separate line in spectrum text, enter tag as a separate line.

Contributor
Instruments
Tune
ScanRange

For example, if RI=132.1 is embedded in Comments, it can be displayed as a separate line in the text by entering the line:

RI

in the above field. Multiple lines will display multiple tag=value pairs in the order entered.

☑ Show tag=value on plot

Figure 118. Display comment field options dialog box.

Non-standard Field headers for the Text Information in **user-library** spectra can be available using the "*Tags* in the "Comments" field" feature of MS Search. A *Tag* is the name of a Field heading that will be displayed with the spectrum in the **Text Information** window **Spec List** or **Hit List** spectrum and can optionally appear in the line below the *Plot* of the spectrum. Text is designated as a *Tag* in the "Comments" field of a user library spectrum if it consists of the Field title (a line of characters without spaces) followed by an = (equals sign). Following the = is the contents of Field. This can be a line of characters without spaces, a number, or a text string enclosed in quotation marks (" "). For example, the following may be entered in the "Comments" field of the **Spectrum Information** dialog box of a **user-library** spectrum:

> Structures were prepared using SoftShell ChemWindow Version 6.0 Contributor="University of the Pacific Mass Spectrometry Facility" Instrument="Agilent 5975 Inert XL MSD w/7890 GC" Tune="Standard Spec w/PFTBA" ScanRange=35-400 GC_column="30 m x 250 µm with 0.3 µm film thickness of BD-5" OvenTemp="50 C 5 min to 250 C @ 5 C/min" Synthesized="Matt Curtis".

As shown in *Figure 119*, the information preceding each equals sign (=) will be displayed as a Field title in the **Text Information** window of the spectrum and can optionally be displayed with the **Plot** window of the spectrum. The information following the equals sign (=) will appear following the Field header. Entry of Field contents (*string*) must conform to the rules above. For this display to appear, the Field titles must be registered in the **Display comment field options** dialog box shown in *Figure 118* (called from the **Comment field options** selection of the **Options** menu. When the *Tags* have been entered in the **Comment field options** dialog box, they will not be displayed in the "Comments" field of the **Text Information** window.

When a search is performed of a user library that contains *Tags* (Field titles), it is possible to constrain that search based on the *Tag* (Field title) and the partial or whole contents of the *Tag* (Field title). More specific details on the use of *Tags* as Constraints are in **MS Search Program** Help file.

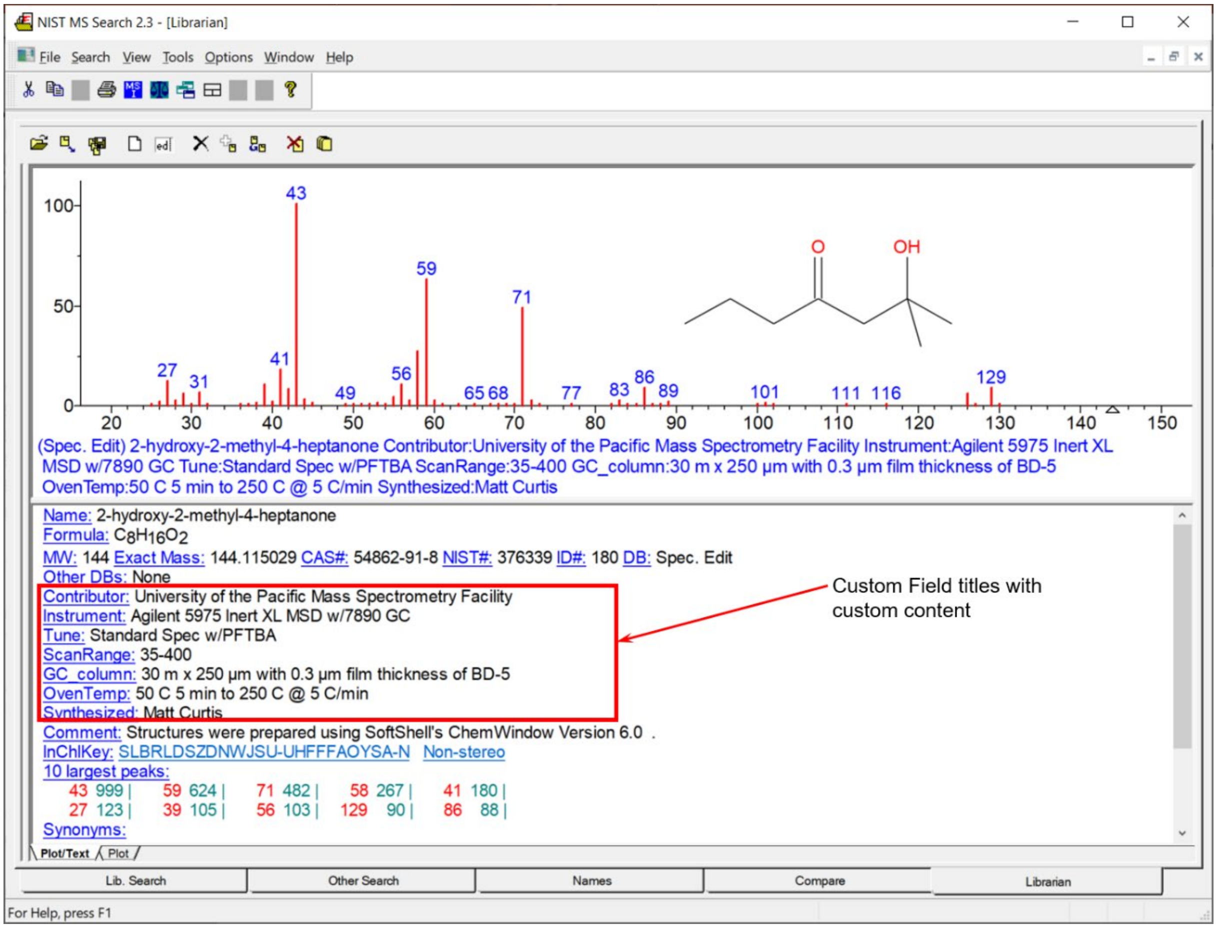
Importance of user-libraries

If mass spectral data is to be shared, the spectrum should have a structure associated with it. The best way to share a single spectrum or multiple spectra is via a user-library. Once the **user-library** has been prepared, use a ZIP program to convert it to a ZIP file and send it.

Warning

Spectra provided in NIST Libraries can put into **user-libraries**. These libraries CANNOT be shared with anyone else. If a **user-library** containing an NIST spectrum is distributed to someone who does not have a license for that version of the library, the violation is the same as if a copy of the library was distributed. Rather than distributing an NIST spectrum, it is better to measure the spectrum and distribute it.

When building a user library, it is very easy to associate a structure with a spectrum by assigning a CAS registry number that is in an NIST Library that has an associated spectrum. If that **user -library** is distributed and the receiver does not have that NIST Library, no structure will be displayed. Copy the structure from the NIST Library to the windows Clipboard and then associate it with the measured spectrum.



(Spec. Edit) 2-hydroxy-2-methyl-4-heptanone Contributor:University of the Pacific Mass Spectrometry Facility Instrument:Agilent 5975 Inert XL MSD w/7890 GC Tune:Standard Spec w/PFTBA ScanRange:35-400 GC_column:30 m x 250 µm with 0.3 µm film thickness of BD-5 OvenTemp:50 C 5 min to 250 C @ 5 C/min Synthesized:Matt Curtis

Name: 2-hydroxy-2-methyl-4-heptanone
Formula: $C_8H_{16}O_2$
MW: 144 Exact Mass: 144.115029 CAS#: 54862-91-8 NIST#: 376339 ID#: 180 DB: Spec. Edit
Other DBs: None
Contributor: University of the Pacific Mass Spectrometry Facility
Instrument: Agilent 5975 Inert XL MSD w/7890 GC
Tune: Standard Spec w/PFTBA
ScanRange: 35-400
GC_column: 30 m x 250 µm with 0.3 µm film thickness of BD-5
OvenTemp: 50 C 5 min to 250 C @ 5 C/min
Synthesized: Matt Curtis
Comment: Structures were prepared using SoftShell's ChemWindow Version 6.0 .
InChIKey: SLBRLDSZDNWJSU-UHFFFAOYSA-N Non-stereo
10 largest peaks:
43 999 | 59 624 | 71 482 | 58 267 | 41 180 |
27 123 | 39 105 | 56 103 | 129 90 | 86 88 |
Synonyms:

Custom Field titles with custom content

Figure 119. Example of custom field titles with custom content added to a user library spectrum using Tags. Graphic is from an earlier version of MS Search.

NIST Text Format of Individual Spectra

The NIST Text file (*msp; *.mspec; *.txt) format is simple. A file can contain as many spectra as desired. Each spectrum must start with the Field title **Name:**. There must be something in this field for the spectrum to be imported (e.g., the data file name and the scan number of the spectrum source). The next required Field title is **Num Peaks:**. The contents of this field must be the number of *m/z value*-intensity pairs that make up the spectrum. Optional fields with the titles of **Comments:**, **Formula:**, **MW:**, **Synon:**, and **CAS#:** can be between the **Name:** and **Num Peaks:** fields. Additional field titles are recognized by MS Search for different types of spectra such as those in Tandem user-libraries. These are described in the MS Search Help file.

When a spectrum is saved to a text file from the **NIST/EPA/NIH Main** or **Replicates Library**, it will also contain fields with the names **NIST#:**, **InChIKey:**, **ExactMass:** and **DB#:**. The field with the name **NIST#:** is on the same line as the **CAS#:** field and separated by a semicolon (;). **DB#** is same as the **ID#** displayed in the **Spectrum Text** Window. The file format does accurate retention index data. This is explained in more detail in the following section. The *m/z* value-intensity list begins on the line following the line with the Field title **Num Peaks:**. The lines of the mass/intensity list have no Field title. Tandem mass spectra contain other filed headers, the one mandatory is **Precursor mz:**.

When a file is created with all the allowable fields, each Field title should be on a separate line. Use the following format. The format for each spectrum in a text file usable by the **Program** should be as follows:

Line 1: **NAME: Molecule** (Required, up to 511 characters)
Line 2: **COMMENT: Run 23, 8/8/88** (Optional, up to 1023 characters)
Line 3: **FORMULA: C6H6** (Optional, up to 23 characters)
Line 4: **MW: 78** (Optional)

If the spectrum submitted to **Library spectrum Search** contains a CAS registry number with its appropriate prefix (**CAS:**), the display of the spectrum in the **Plot**, **Compare**, and **Structure** Windows will have the structure of the compound with that CAS registry number if present in the **NIST/EPA/NIH Main Library**.

Line 5: **CAS: 71-43-2** (Optional)
Line 6: **SYNON: Chemical name synonym** (Optional, may be repeated)

The actual mass spectral data (number of peaks in the spectrum) may begin on the next line. It does not actually matter what line this is, as long as it precedes the line that starts the mass/intensity pair data. The VERY NEXT line and subsequent lines MUST contain the paired mass/intensity values.

Line 7: **Num Peaks: 18** (This prefix and the exact number of mass/intensity pairs is required.)
Line 8: **26 430; 27 340; 28 40; 37 480; 38 611; 39 1411; 49 300; 50 1792;**
Line 9: **51 2052; 52 1962; 63 340; 73 160; 74 480; 75 180;**
…
Line n: **76 721; 77 1401; 78 9806; 79 651;**

The peaks need not be normalized, and the *m/z* values need not be ordered. The exact spacing and delimiters used for the *m/z*-intensity pairs are unimportant. For example, the peaks above could equally be presented as:

(26,430),(27,340),(28,40),(37,480),(38,611),(39,1411),
(49,300),(75,180),(50,1792),(51,2052),(52,1962),(63,340),
(73,160),(74,480),(75,180),(76,721),(77,1401),(78,9806),(79,651)

or each *m/z*-intensity pair could be on an individual line. The following characters are accepted as delimiters (except "|"): `|space|tab|,|;|:|(|)|[|]|}|`

The file can be given any valid file name; however, it is best to use the extension "MSP or MSPEC". These are the recognized default extension in the **MS Search Program** when user-library spectra are to be imported.

Several examples of NIST text format mass spectral files are installed together with the **MS Search Program**, namely SAMPLIB.MSP, UNKNOWN.MSP, and USERDEMO.MSP.

To get a better prospective of file format, use **Export** button from the **Librarian** tab view's **Button Bar** to write one or more spectra to a file from the NIST EI and Tandem Libraries; then, open these files with Windows Notepad.

Import and Export of Retention Indices with a Spectrum

Retention index (RI) format was designed to provide compatibility with existing EI libraries and import of a mass spectrum into **NIST MS Search**.

In general, each RI value must have GC Column Type associated with it. Currently, there are three specific Column Types (1-3 in the table below) and two other (4-5) column types. RI of these two column types can be compared with any selected specific Column Type:

	Column Type	**Accepted Tags (case insensitive)**
1	Semi-standard non-polar	s, db5, semi, SemiStandardNonPolar, ssnp, SemiStdNP
2	Standard non-polar	n, db1, nonpolar, snp, standard, StandardNonPolar, StdNP
3	Standard polar	p, pol, polar, sp, StandardPolar, stdp, StdP, StdPolar
4	Any (n-alkane standard)	a, all, AllColumnTypes, AnyColumnType, std, Any
5	Unspecified	u, un, uns, unspecified, User

When importing or adding a spectrum with RI of Unspecified column type to a library, there is an option of changing RI column type to any of the three specific Column Types or a line with the value for Any column type or the value for the User column type. NIST EI Spectra which have RI values are exported into a single line in the MSP format as shown below.

RI Export with a Spectrum in an MSP or JCAMP file

A compound may have up to three RI values of different specific Column Types :

```
Retention_index: SemiStdNP=654/11/226 StdNP=654/7/286 StdPolar=957/17/131
```

or

```
Retention_index: Any=1000
```

or

```
Retention_index: User=2059
```

Slash-delimited numerical values following **Accepted Tags** are

Experimental RI median/deviation/number of datapoints

In **NIST MS Search** Spectrum Text Information window, RI values of the first example (Benzene) are displayed as:

Experimental RI median±deviation (#data)	
Semi-standard non-polar:	654±11 (226)
Standard non-polar:	654±7 (286)
Polar:	957±17 (131)

Other Unspecified RI values possibly present in the Comment (see the next section) are left unchanged in the exported spectrum.

Single RI value[1], if available, is exported into a JCAMP file as

```
##$RETENTION INDEX=654
```

RI Import with a Spectrum in an MSP or JCAMP file

Legacy MSP file formats allow saving several possibly different RI values of Unspecified column type, which may contradict each other. **NIST MS Search** picks up only one of them according to the priority rules described below.

---

[1] If RI values of multiple specified Column Types are associated with a spectrum, then RI of the first available column type in the following sequence is output into a JCAMP file:

- Semi-Standard Non-Polar
- Standard Non-Polar
- Standard Polar

If the last out of all lines starting with `Retention_index:` or `RI:` or `Synon: $:29` in an MSP file has format described in the previous section with possibly different **Accepted Tags**, then contents of this line are used as input.

Otherwise, there may be up to 4 untagged RI value locations/formats of Unspecified column type. They are described in the following table (*n* is the RI value):

Priority	**Format**	**Location**
Highest	`|RI:`*n*	Last occurrence in comment
	`RI=`*n*	Last occurrence in comment
	`RI:`*n*	Last occurrence in comment
Lowest	`Retention_index:`*n* or `Retention_index=`*n* or `RI:`*n* or `RI=`*n* or `Synon: $:29`*n*	Last occurrence in MSP file

RI value imported from a JCAMP file always has Unspecified column type.

APPENDIX 1: Creating AUTOIMP Files for NIST MS Search

It may be necessary to create AUTOIMP.MSD as the primary locator file used for the importation of spectra or structures from third-party mass spectral data systems and/or AUTOIMP.STR for interaction between the **MS Search Program** and third-party structure drawing programs. These files may be created using Notepad with certain versions of Microsoft Windows.

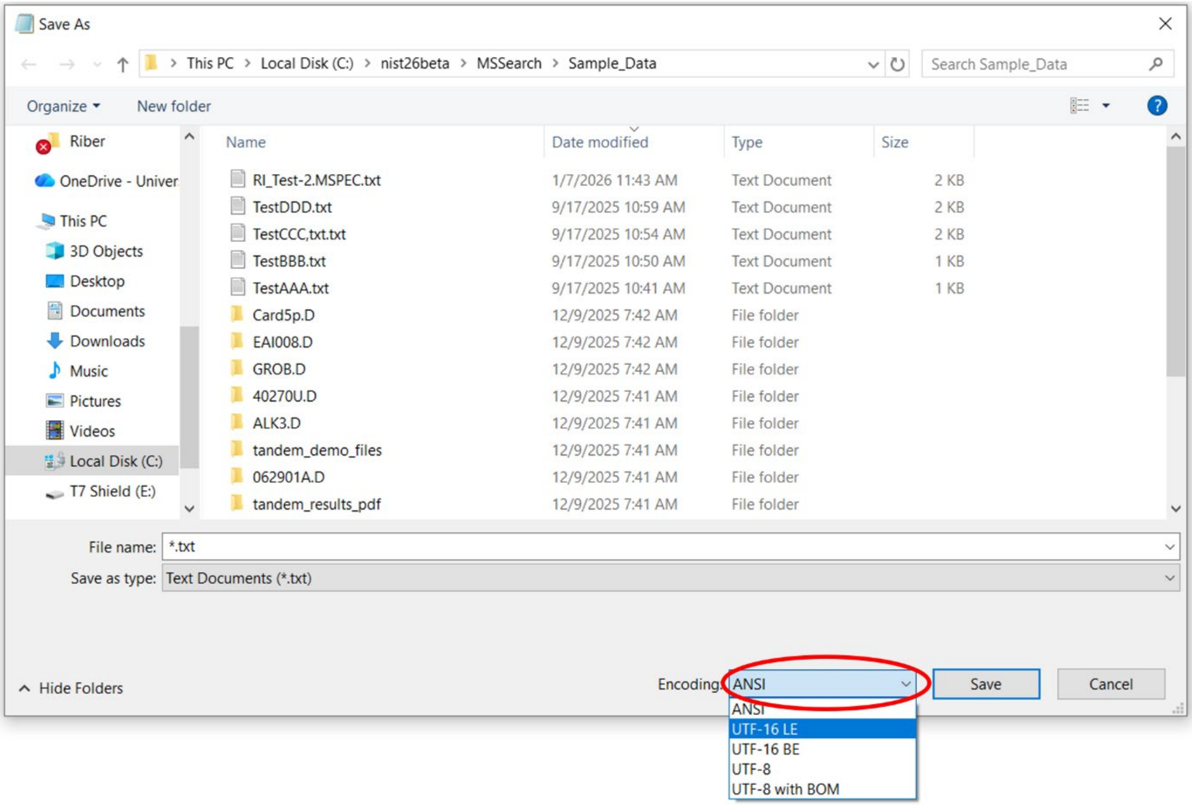
In most cases where MS Search is being installed on a computer containing a data analysis that links with MS Search, the MS Search Installation will create the AUTOIMP.MSD file. Some data analysis systems that link with MS Search are written such that when their software tries to send a spectrum to MS Search, the program looks to where MS Search is installed to determine if there is an AUTOIMP.MSD file in the folder. If there is, the send program will write the secondary locator file in the location specified in the existing AUTOIMP.MSD file. If an AUTOIMP.MSD file does not exist, the sending software will automatically create one in the appropriate MS Search folder.

If Microsoft Notepad is used, the default extension is .TXT (Text Document) even though AUTOIMP.MSD or AUTOIMP.STR has been entered in the “File name:” field. The default settings for Windows File Explorer will not show this .TXT extension. The file will not work unless the .TXT extension is removed. The file can be renamed by highlighting the file in Windows Explorer and selecting **Rename** from the **File** menu.

When saving the file using **Save As** option, this problem may be completely avoided by selecting **All Files** in the **Save as type** dropdown list box (*Figure 120*). In this case, the file will be saved with the extension provided in the “File name:” field (see the **Save As** dialog box shown below).

When saving the file using the Notepad **Save As** option, also make sure the **ANSI** Encoding is selected (in some versions of Windows, the Encoding option is not present).

After **File name**, **Save as type**, and **Encoding** are set correctly, select the **Save** button.



Name	Date modified	Type	Size
RI_Test-2.MSPEC.txt	1/7/2026 11:43 AM	Text Document	2 KB
TestDDD.txt	9/17/2025 10:59 AM	Text Document	2 KB
TestCCC,txt.txt	9/17/2025 10:54 AM	Text Document	2 KB
TestBBB.txt	9/17/2025 10:50 AM	Text Document	1 KB
TestAAA.txt	9/17/2025 10:41 AM	Text Document	1 KB
Card5p.D	12/9/2025 7:42 AM	File folder	
EAI008.D	12/9/2025 7:42 AM	File folder	
GROB.D	12/9/2025 7:42 AM	File folder	
40270U.D	12/9/2025 7:41 AM	File folder	
ALK3.D	12/9/2025 7:41 AM	File folder	
tandem_demo_files	12/9/2025 7:41 AM	File folder	
062901A.D	12/9/2025 7:41 AM	File folder	
tandem_results_pdf	12/9/2025 7:41 AM	File folder	

File name: *.txt
Save as type: Text Documents (*.txt)
Encoding: ANSI

Figure 120. Save As dialog box displayed within Windows Notepad.

APPENDIX 2: Using the NIST MS Search Program with ChemStation

The **NIST MS Search Program** has been supported through several versions of the HP/Agilent Technologies ChemStation. In the recent versions of the ChemStation for GC/MS, both the **NIST Output** and the **AMDIS** options will appear on the **Spectrum** menu of the Data Analysis software when using the Enhanced Quantitation, Standard Quantitation, or Gasoline mode; and on the **Lib.** menu when using the Environmental mode, if the appropriate macros have been installed. The **NIST/EPA/NIH MS Library (NIST 20)** setup will install the two required macros along with additional macros that will allow for greater flexibility with the ChemStation software.

It is possible to use the ChemStation double-click option on a displayed spectrum to send that spectrum to the **NIST MS Search Program Spec List** Window, bringing the **MS Search Program** into focus. If the appropriate options (including **Automation**, see *Figure 73* and *Figure 75*) have been set in the **NIST MS Search Program**, then, after the spectrum is automatically prepended to the **Spec List** Window, it will be searched.

If using the Enhanced Quantitation mode on any version of ChemStation, the setup will automatically add four new options to the **Spectrum** menu (**PBM Quick Search**, **NIST Search**, **Multiple Lib PBM Search**, and **Set Default Search Engine**). Each of these menu options is explained below. If these options are to be added to other modes of the ChemStation software, see the **MENU_MOD** document file on the **NIST MS Search Program** installation CD ROM in the \support.cs directory. This document is also available after installing **NIST 23 MS Library** by selecting **ChemStation Menu Modification** from the **NIST Mass Spectral Database** program folder.

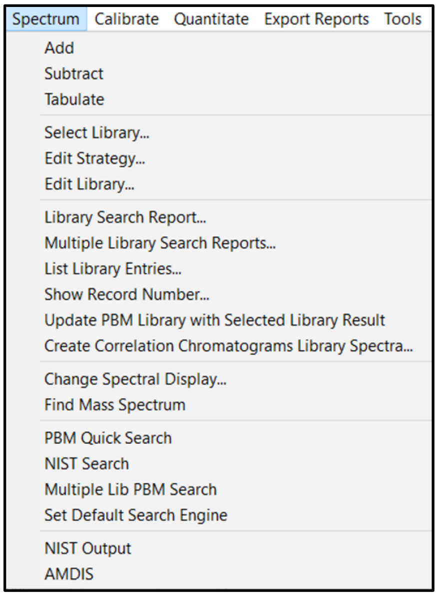


Figure 121. Spectrum Menu as displayed in ChemStation Enhanced data analysis.

Some older versions of **ChemStation** will have **ASCII Output** as opposed to **NIST Output**, the **Spectrum** menu, or the **Lib.** menu found on the Environmental mode. Selecting either the **NIST Output** or **ASCII Output** results in the display of the **Options for NIST Library Search** dialog box with six *radio button* options and **OK** and **Cancel** buttons. The Macro NISTINT.MAC must be installed in the HPCHEM\MSEXE or MSDCHEM\MSEXE directory for these menu selections to be displayed.

When all the necessary macros have been installed in the **ChemStation** MSEXE directory and the modifications have been made to the ChemStation macros, the following options will be available from the ChemStation Spectrum menu (*Figure 121*):

The **PBM Quick Search** (PBMSrch.mac) performs the normal ChemStation PBM search of the currently displayed spectrum.

The **NIST Search** (NISTSrch.mac) passes the currently displayed mass spectrum to the **NIST MS Search Program**. The spectrum will be prepended to the **NIST MS Search Program Spec List** Window. If the **NIST MS Search Program** is configured to search automatically, then the search will be started when the spectrum is passed to the **NIST MS Search Program**. Otherwise, double click on any spectrum in the **Spec List** Window located in the **Lib. Search** tab to start a search.

The **Multiple Lib PBM Search** (LS.mac) performs a **ChemStation PBM Search** using up to three different libraries (all of which have to be specified using the dialog box displayed by selecting the **Select Library** option on the **Search** menu). The top 20 Hits from each library will be displayed in order of decreasing match quality.

Set Default Search Engine (SWITCH.MAC) displays a dialog box that allows the selection of the default library search to be evoked when double clicking the **RMB** menu on the currently displayed spectrum. The **NIST MS Search** is the default until another search type is set as the default.

NIST Output (NISTINT.mac) displays a self-explanatory dialog box that allows for the use of the **NIST MS Search Program** in a number of different ways and the copying of ChemStation user libraries as **NIST MS Search Program** user libraries.

AMDIS (AMDIS.mac) loads AMDIS with the data file currently being viewed with the ChemStation Data Analysis directly into AMDIS for analysis. Once in AMDIS, select **Run** to perform the analysis.

The **Lib2NIST** converter program will be installed along with the **NIST MS Search Program**. This program allows mass spectral libraries that have been created using the internal utilities of the ChemStation software (including structures) and other mass spectral libraries to be copied to the NIST user libraries format that can be employed by the **NIST MS Search Program**. This utility is evoked as an option from the **Options for NIST Library Search** dialog box displayed by selecting the **Copy ChemStation Lib to NIST Format** *radio button* and then selecting the **OK** button or by selecting the **Lib2NIST** icon in the **NIST Mass Spectral Database** program folder.

An additional macro is found on the **NIST MS Search Program** installation disk, **AUTONIST.MAC**. This macro is to be incorporated into automated sequences. It automatically selects spectra based on the autointegration of the current data file and switches to the **NIST MS Search Program** where the spectra are automatically imported into the **Spec List** Window. If the proper settings have been made in the **Program**, these spectra are searched, and the focus returned to the HP MS software. These features can only be utilized with G1034C, Version C.3.00.00 or later of ChemStation.

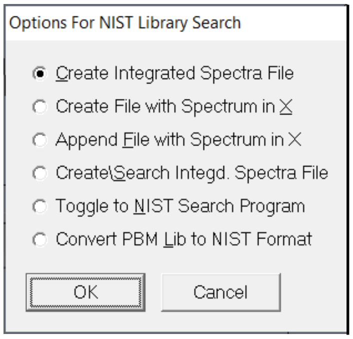
Additional Information Regarding Modes Other Than Enhanced Quantitation

During the installation of the **NIST/EPA/NIH MS Library (NIST 26)**, if the **NIST MS Search Program** has been selected as the default search engine for ChemStation, each time a spectrum is double clicked, the displayed spectrum will be prepended in the **NIST MS Search Program Spec List** Window. The **NIST MS Search Program** will be brought into focus *regardless* of the data analysis mode (Enhanced Quantitation, Standard Quantitation, Gasoline, or Environmental). One of the other above-referenced search methods can be changed to by selecting the **ChemStation Default Search Mode** icon in the **NIST Mass Spectral Database** program folder.

MODIFICATION OF MACROS IS NOT NECESSARY TO SWITCH FROM THE **NIST MS SEARCH PROGRAM** AS THE DEFAULT SEARCH METHOD FOR THE DOUBLE-CLICK FEATURE OF THE ChemStation.

HP_NIST MACRO (NISTINT.MAC)

When **NIST Output** is selected from the **Spectrum** menu of the MS ChemStation or **ASCII Output** from the **Lib** menu of the Environmental software, the **Options For NIST Library Search** dialog box is displayed (*Figure 122*). This dialog box consists of six options and **OK** and **Cancel** buttons. Each option has a *radio button* next to it, used to select an option. Selecting the **OK** button results in execution of the option.



Options For NIST Library Search

● Create Integrated Spectra File
○ Create File with Spectrum in X
○ Append File with Spectrum in X
○ Create\Search Integd. Spectra File
○ Toggle to NIST Search Program
○ Convert PBM Lib to NIST Format

OK Cancel

Figure 122. Result of selecting NIST Output from the Spectrum menu.

In the **Options For NIST Library Search** dialog box, select the desired item and click **OK**. If any of the first three options are executed, the dialog box will be redisplayed.

Each time one of the top four menu selections is executed, the file HPNIST.TXT is created in the ChemStation HPCHEM or MSCHEM directory. This file contains the name and location of the text file that contains the saved spectra in the NIST format. The HPNIST.TXT file is deleted every time the **NIST MS Search Program** is brought into focus, and spectra are automatically imported into the top of the **NIST MS Search Program Spec List** Window.
At the same time, the HPNIST.TXT, the second locator file, is created, a text file named TEMPSPEC.MSP containing one or more spectra is created or appended. The text file is located in the ChemStation directory HPCHEM or MSDCHEM. This text file will remain there until it is overwritten or removed manually.

When the **NIST MS Search Program** comes into focus, the spectra that have been saved in the ChemStation software are automatically copied to the **Spec List** Window. If spectra are already in the **Spec List** Window, a prompt to **Prepend** or **Overwrite** the current contents will be displayed. If **Prepend** is selected, the new spectra are added to the top of the **Spec List** Window (prepended). If **Cancel** is selected, the automatic import will be aborted. However, the HPNIST.TXT file may be deleted even in the case of an abort. If the options of **Automatic Search On** and **Return Focus to Caller upon Completion** have been selected in the **Automation** dialog box, selecting **Cancel** returns the focus to the ChemStation software.

Menu “Options for NIST Library Search”

The **Create Integrated Spectra File** option runs the ChemStation Autointegrate routine on the displayed chromatogram and creates the text file containing the spectra that represent the apex of each chromatographic peak.

The **Create File with Spectrum in X** option creates a text file that contains the spectrum that is in X of the ChemStation Stack display. This is where background-subtracted and averaged spectra will be found. This is the currently displayed spectrum.

The **Append File with Spectrum in X** option appends the spectrum in X of the Stack display to the existing text file. If a text file does not exist, one will be created.

The **Create\Search Integd. Spect. File** option creates a text file that contains spectra representing the tops of each chromatographic peak and either starts or switches to the **NIST MS Search Program** where the spectra are automatically imported into the **Spec List** Window. If **Automatic Search On** in the **NIST MS Search Program Automation** dialog box has been selected, each spectrum will be searched. The results are optionally printed, and the results of each search are stored in the **Hit List** History. If **Return Focus to Caller upon Completion** has been selected in the **Automation** dialog box, then after the last spectrum is searched and printed, the focus will be returned to the ChemStation software. If spectra are already in the **NIST MS Search Program Spec List** Window, the **Append/Overwrite/Cancel** query will be displayed.

The **Toggle to NIST Library Program** option either starts the **NIST MS Search Program** or switches to it if it is already running. The ChemStation software can be redisplayed by holding down the <Alt> key and pressing the <Tab> key, or the **NIST Program Switch To Caller** button can be selected. The **NIST MS Search Program** can be brought back into focus by using the **<Alt><Tab>** command or by selecting the **Toggle** option from the **Options For NIST Library Search** dialog box.

The **Copy ChemStation Lib to NIST Format** option is described in APPENDIX 3.

AUTONIST Macro

This is a non-interactive macro that can be executed using the command line instruction:

```
MACRO "AUTONIST.MAC", go
```

or from within the Method. Every Method directory contains a file DEUSER.MAC. Edit this file to contain the string:

```
MACRO "AUTONIST.MAC", go.
```

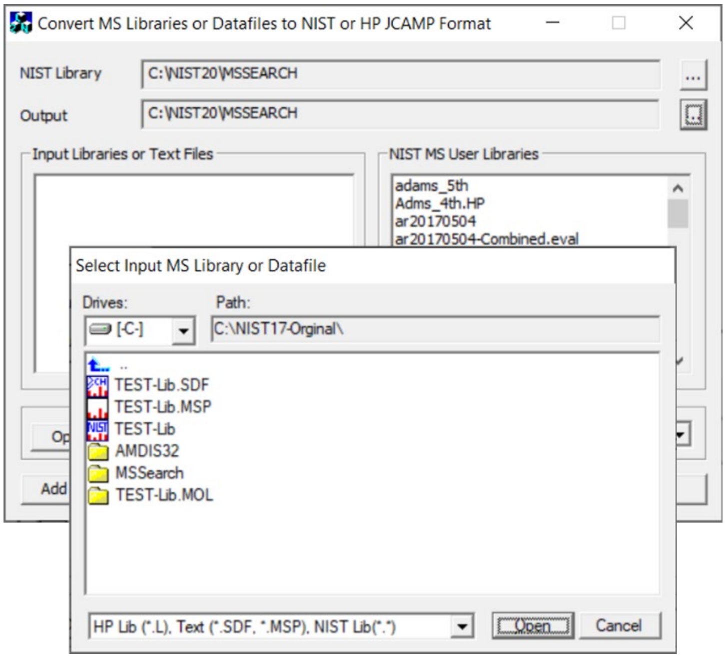
This File is in the \SUPPORT.HP directory of the CD ROM and must be copied to the \HPCHEM\MSEXE directory.

When the Method is run, the AUTONIST Macro will execute. The chromatogram is autointegrated, and the spectra representing the top of each chromatographic peak are stored in a text file. The focus is then switched to the **NIST MS Search Program**. The spectra are imported into the **Program Spec List** Window. If spectra are already in the **Spec List** Window, they will be overwritten.

If **Automatic Search On** and **Return Focus to Caller upon Completion** have been selected in the **NIST MS Search Program Automation** dialog box, the spectra will automatically be searched. After the last spectrum is searched, the focus is returned to the HP MS software. For this macro to function properly, it will be necessary to have selected the above two options in the **Automation** dialog box.

APPENDIX 3: Copy ChemStation and Other Libraries to NIST User Library Format

This is a separate program called by selecting the **Lib2NIST Converter** icon on the **NIST Mass Spectral Database** program folder. This program allows mass spectral libraries in a variety of formats (along with structures or structures in separate files) to be selected and copied to the NIST user library format. For all 32-bit Windows (Windows NT, Windows 98/Me/2000/XP/Vista/Windows 7, 8, 8.1) and Windows 64-bit platforms, the **Lib2NIST** converter program replaces the **HP2NIST** program that was provided with previous versions of the **NIST MS Search Program**. The use of the **HP2NIST** program is described at the end of this section. Another feature of the **Lib2NIST** program is the ability to take user libraries that were developed by using versions 1.5 through 1.7 of the **NIST MS Search Program** and copy them into a format fully compatible with the current version of the **NIST MS Search Program**.



NIST Library C:\NIST20\MSSEARCH

Output C:\NIST20\MSSEARCH

NIST MS User Libraries:

adams_5th
Adms_4th.HP
ar20170504
ar20170504-Combined.eval

Drives: [-C-]

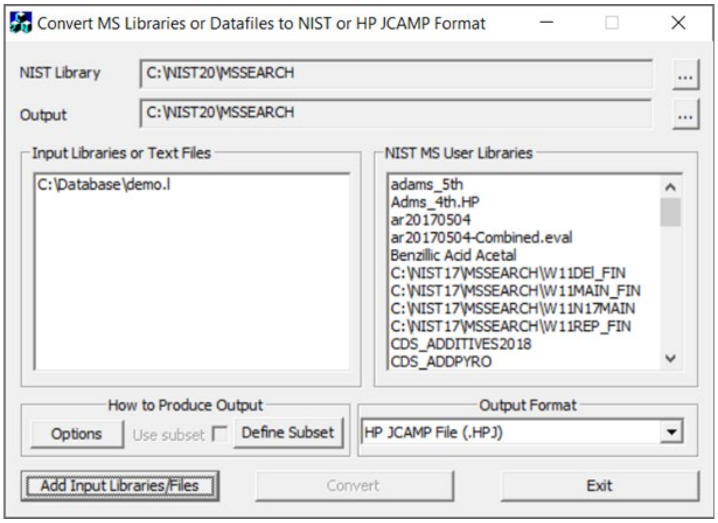
Path: C:\NIST17-Orginal\

..
TEST-Lib.SDF
TEST-Lib.MSP
TEST-Lib
AMDIS32
MSSearch
TEST-Lib.MOL

HP Lib (*.L), Text (*.SDF, *.MSP), NIST Lib(*.*)

Figure 123. Opening display of Lib2NIST program. When started, the Lib2NIST converter program displays a file dialog box overlaid on the program screen.

Select the library to be copied and then click on the **Open** button (*Figure 123*). The dropdown arrow will also allow for selecting from a variety of JCAMP formats. More than a single library can be selected in this dialog box by using the standard Windows multiple file selection techniques.



NIST Library C:\NIST20\MSSEARCH

Output C:\NIST20\MSSEARCH

Input Libraries or Text Files: C:\Database\demo.l

NIST MS User Libraries:

adams_5th
Adms_4th.HP
ar20170504
ar20170504-Combined.eval
Benzilic Acid Acetal
C:\NIST17\MSSEARCH\W11DEI_FIN
C:\NIST17\MSSEARCH\W11MAIN_FIN
C:\NIST17\MSSEARCH\W11N17MAIN
C:\NIST17\MSSEARCH\W11REP_FIN
CDS_ADDITIVES2018
CDS_ADDPYRO

Output Format: HP JCAMP File (.HPJ)

Figure 124. Lib2NIST converter program display.

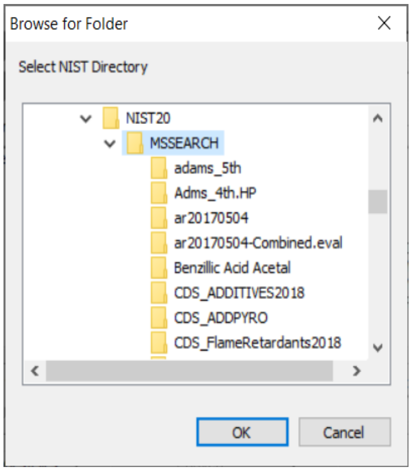


Figure 125. Browse for Folder dialog box.

The selected libraries will now be listed in the **Input Libraries** or **Text Files** window of the **Program** display (*Figure 124*).

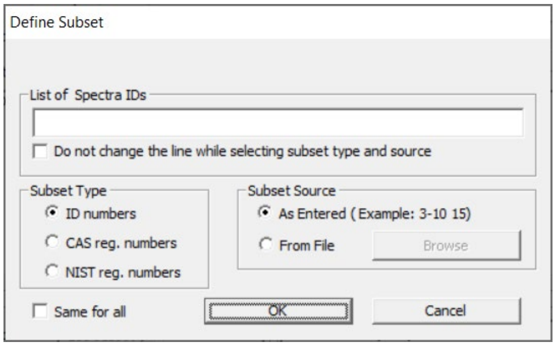
The **NIST Library** and **Output** locations can be changed by selecting the appropriate button located to the right of the two fields. This will result in the display of the **Browse for Folder** dialog box (*Figure 125*).

Select the desired directory (folder) and then click on the **OK** button to change the location.

The **Define Subset** button will display a dialog box that allows for the choice of a range of input spectra ID or CAS numbers. The **Use subset** checkbox is grayed unless an entry has been made in the **Define Subset** dialog box (*Figure 126*).

After making any desired entries in the **Define Subset** dialog box, select the **OK** button to return to the **Program** main display.

The **Options** button on the **Program** display will display the dialog box shown above in *Figure 127*. This allows for the entry of mass defect corrections to be applied to *m/z* values of imported spectra and whether to: 1) include synonyms from the source file, 2) retain the ID numbers (or sequence numbers) from the source library or file[2], 3) calculate molecular weights (nominal mass) from formula, and 4) create or convert MS/MS and/or In-source libraries[3].



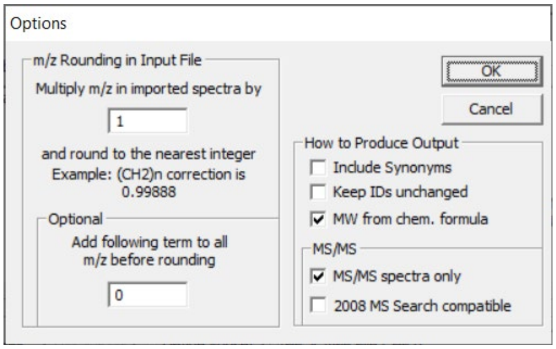
☐ Do not change the line while selecting subset type and source

● ID numbers
○ CAS reg. numbers
○ NIST reg. numbers

● As Entered (Example: 3-10 15)
○ From File

☐ Same for all

Figure 126. Define Subset dialog box.



Multiply m/z in imported spectra by 1

and round to the nearest integer
Example: (CH2)n correction is 0.99888

Add following term to all m/z before rounding 0

☐ Include Synonyms
☐ Keep IDs unchanged
☑ MW from chem. formula

☑ MS/MS spectra only
☐ 2008 MS Search compatible

Figure 127. Options dialog box display.

Select the libraries to be converted in the **Input Libraries or Text Files** window of the **Program** display, make sure the Output Format is selected correctly, and then click on the **Convert** button. The selected libraries will then be converted and placed in the specified output directory. Advanced features of Lib2NIST are explained in the file CMDLINE.pdf installed with the Lib2NIST. Transliteration rules of extended ASCII characters are listed in the ASCII text file HPTRANS.TBL and may be modified in this file by the user.

---

[2] "MS/MS Spectra only" option disables "Keep IDs unchanged". To override this, use command line option /KeepIDs=YES

[3] In most cases, the "2008 MS Search compatible" option should be turned OFF. This option is intended only for saving MS/MS spectra into a MSP text file in a format, which is compatible with old versions of MS Search. MS/MS or In-source HiRes libraries created with this option have obsolete format unsuitable for reliable searching of high resolution spectra.

APPENDIX 4: Using the NIST MS Search Program with Thermo Fisher Scientific Xcalibur Software: (Qual Browser & FreeStyle)

The Thermo Fisher Scientific Xcalibur software uses the NIST MS Dynamic Library (dll) as a library search engine. A copy of the **NIST MS Search Program** and **AMDIS** may be provided with a copy of the Xcalibur software. The **NIST/EPA/NIH Mass Spectral Library** may be optionally provided by Thermo Fisher Scientific. The NIST Tandem Library is installed on all Thermo Fisher MS/MS instruments.

To add the **NIST/EPA/NIH Mass Spectral Libraries** to the Xcalibur software, run the **NIST 26 MS Library** setup. If it locates the following **NIST MS Search Program** and the AMDIS directories:

C:\Program Files\NISTMS\MSSEARCH

C:\Program Files\NISTMS\AMDIS32

then let the setup install **NIST 26** in these directories. The **NIST 26 MS Library** will then be properly installed along with any necessary program updates.

More recent versions of the Xcalibur software detect and use **NIST MS Search** installed in another directory (for example, c:\nist20\mssearch) and the **NIST/EPA/NIH Mass Spectral Library** using means explained in the **USE WITH INSTRUMENT DATA SYSTEMS** section of this manual.

Exporting MS/MS spectra to **NIST MS Search** transfers both the mass spectrum and the precursor *m/z* values.

It should be noted that the Qual Browser of Xcalibur exports only integer *m/z* values NOT accurate *m/z* values. Upon release of NIST 23, the only Thermo Fisher program capable of exporting accurate mass data from Xcalibur acquired spectra is the program *FreeStyle*.

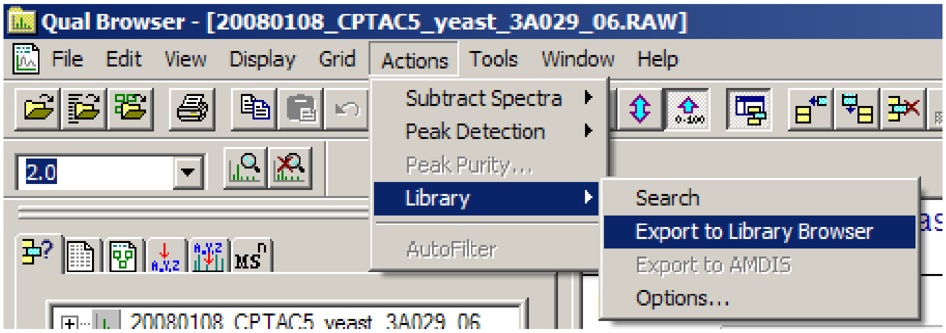


Figure 128. Exporting a Spectrum to NIST MS Search.

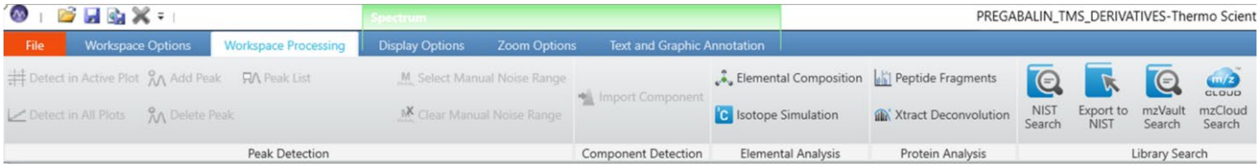


Figure 129. Exporting a Spectrum to NIST MS Search from Xcalibur FreeStyle.

APPENDIX 5: Search Algorithms

There are two general ways that the **NIST MS Search Program** can retrieve library spectra that resemble the submitted spectrum. They are the **Identity Search** and the **Similarity Search**. An **Identity Search** is designed to find exact matches of the compound that produced the submitted spectrum and therefore presumes that the unidentified compound is represented in the reference library. Only experimental variability prevents a perfect match. The **Similarity Search** is optimized to find similar compounds and is intended for use when a compound cannot be identified by the **Identity Search** (it is probably not in the library).

Screening

For the sole purpose of achieving rapid retrieval rates before actually comparing spectra, modern search algorithms first identify a subset of library spectra with important features in common with the spectrum of an unidentified compound. While this can vastly reduce search times, it can also screen out the correct spectrum. When this occurs, the correct retrieval cannot appear in the **Hit List**. Because of unavoidable blind spots in simple algorithms, even a closely matching spectrum can be excluded. This is probably the most serious failure of any mass spectral search system.

A variety of filtering algorithms for Identity searching were tested in an effort to avoid throwing away correct matches without sacrificing performance. These algorithms were tested using the 12,592 spectra that comprised an older version of the **NIST/EPA/NIH Selected Replicates Library**. The best performing algorithm used “a ranked peaks in common” logic similar to that incorporated in existing data systems. This finds library spectra with the largest number of peaks in common with the spectrum of the unidentified compound consistent with a required minimum number of identified spectra. Tests showed this minimum number to be about 50. This procedure retrieved 95% of the matching compounds (5% of matching compounds were lost). By scaling peaks by their *m/z* values, a 98% success rate was achieved at the same search speed. This is the screening logic of the **Quick Identity Search** option. By combining results using several screening criteria, a 99.4% success rate was achieved with a modest reduction in search speed. At this level, virtually all correct matches that were screened out were very dissimilar to the spectrum of the unidentified compound and would have produced low **Match Factors**. Both peak scaling and merging of multiple screening results are used in the **Identity Search** (**EI Normal**). A more detailed description follows.

> **Peak Scaling**: The determination of the largest peaks in a spectrum was made after first multiplying the abundance of each peak by the square of its *m/z* value. The most intense peak in the scaled spectrum of the unidentified compound is compared against the eight most intense peaks in scaled library spectra. The second most intense peak in the scaled spectrum of the unidentified compound is then compared against the nine most intense peaks in the library spectra. This is repeated in decreasing order of intensity of peaks in the scaled spectrum of the unidentified compound until the eighth most intense peaks are compared against the 16 most intense peaks in the scaled library spectra. This is the only screening procedure applied in **Identity Search** (**EI Quick**).
>
> **Merged Subsets**: Since the use of any single set of peak specifications was found to fail for certain classes of spectra, results of multiple sets of peak specifications were merged to reduce this problem. After extensive optimization studies, four separate peak specifications were selected. The first of these specifications is described above for the **Identity Search** (**EI Quick**). The others were:
>
> 1) The fourteen largest peaks in the scaled spectrum of the unidentified compound were matched against the fourteen largest peaks in the scaled library spectra.
>
> 2) The six largest peaks in the original (non-scaled) spectrum were matched against the six largest peaks in the original library spectra.
>
> 3) The five largest peaks along with the “maximum mass” peak in both the unidentified compound spectrum and the library spectrum were matched.

The screening algorithms for the **Similarity Search** are similar to the **Normal Identity Search** except that scaling and maximum mass peaks are not used. When neutral loss peaks are used in the **Hybrid Searches** and **Neutral Loss Search**, neutral loss peaks are used in place of conventional peaks. For neutral loss peaks, abundances in library spectra are required to be within a factor of four of the abundances of corresponding spectra of the unidentified compounds (peak ranking and scaling are not used).

Search

A mass spectrum can be represented as a row vector composed of the ordered peak intensities. It can also be considered to represent a single point in a multidimensional hyperspace defined by the *m/z* variables. Each of the intensities in the row vector represents the value of the coordinate of the spectral point along the individual mass axis in this hyperspace. If two spectra being compared are identical with respect to all the mass intensity pairs, their point representations in this hyperspace will coincide. If these spectra are very similar, their point representations will be close to one another. The **Match Factor**, which provides a sense of spectral similarity, may be regarded as the inverse of distance of the two-point representations when each spectral vector has unit length.

The **Dot-Product** mass spectral search algorithm, which uses the cosine of the angle between the unidentified compound and library spectral vectors, has been optimized by scaling peaks using the square root of their abundance. For the **Identity Search**, peaks were weighted by the square of their *m/z* value, and a second term was added that compares ratios of adjacent peaks in library and the unidentified compound spectra. Its contribution was weighted so that it increases in importance as the proportion of common peaks increases. The only difference in the application of this algorithm to the **Identity Search** and **Similarity Search** is that *m/z* weighting is used only in the former. This defines the search algorithm used for both the **Similarity** and the **Identity Searches** in the **Program**.

For additional information, see Stein, S.E. “Optimization and Testing of Mass Spectral Library Search Algorithms for Compound Identification” *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 859–865.

APPENDIX 6: Syntax for Some Constraints in Searches

Syntax of Name Fragments Constraint

Applies to: all searches
Max. length: 39 characters
First release: 2000

This “Name Constraint” field can contain up to eight name fragments.
The name fragments are searched for in the compound main chemical name and in other names (synonyms).

The following examples explain the conditions when the name constraint is satisfied (spaces around && and !! are optional):

"COCA && INE": at least one name contains both "COCA" and "INE"
"COCA && !! INE": at least one name contains "COCA" and doesn't contain "INE"
"COCA !! INE": at least one name contains "COCA"; no name contains "INE"

Syntax of Tags in Comment Constraint

Applies to: all searches
Max. length: 1023 characters
First release: 2006

General

All information in NIST Peptide libraries is represented by the Tag=value convention. Typically, the “Peptide spectrum comment” field may contain the following items separated by spaces:

Tag=string
Tag="*string*"
Tag=number

Spaces may be inside a *string* only if the string is in double quotes. In older libraries, an unquoted *string* may also have space(s) inside a pair of parentheses ( ). Spaces are not permitted inside any *Tag* or next to the equal sign.

“Comment” fields in older libraries may also contain *Tag* not accompanied by the equal sign and *string.*

All *Tag, string, substring*, and *word* comparisons are described on the next page (**ignore case**).

Entering Tags in Comment Constraint

Items in the “Tags in Comment” constraint field should be separated by spaces and/or linefeed characters. Unlike spectra comments, spaces next to the equal sign (as well as >, <, >=, <=, and **:**) are allowed inside the items.

Spectra returned must satisfy all entered constraints.

Examples

	Item	**Comments of the output spectra must have**
1	"*consensus*"	A Tag or a space-delimited string *consensus*
2	*Parent*	6 letter sequence *parent* anywhere in the Comment
3	*fullname=”r.lecvk.c/2”*	Exactly this Tag=string:: *Fullname=R.LECVK.C/2*
4	*fullname=lec*	String following *Fullname*= contains sequence *LEC*
5	*Mods* **:** *ICAT_cl_hi*	String following *Mods*= contains word[1] *ICAT_cl_hi*
	The following rules apply to strings that are numbers	
6	*Diffmod=6.8*	String following *Diffmod*= begins with number 6.8, for example, *Diffmod=6.8/8* or *Diffmod=6.8*
7	*Diffmod=5.1:6.8*	String following *Diffmod*= begins with number between 5.1 and 6.8, for example, *Diffmod=6.8/8* or *Diffmod=6.8*
8	*Diffmod > 6.8* *Diffmod >= 6.8* *Diffmod < 6.8* *Diffmod <= 6.8*	*Diffmod*= followed by a number greater than 6.8 *Diffmod*= followed by a number not less than 6.8 *Diffmod*= followed by a number less than 6.8 *Diffmod*= followed by a number not greater than 6.8
[1] *word* is a string delimited by any character but a letter, a digit, or an underscore “_”; If *word* contains spaces or equal sign it should be entered in quotes.		

To reverse the meaning of constraints 1–5 (that is, change to “spectra must not have”), enter caret (^) as the first letter:

1	^"*consensus*"	Tag *consensus* is not present
2	*^parent*	Substring *parent* is not present
3	*fullname=*^"*r.lecvk.c/2*"	String tagged *fullname* is not exactly *r.lecvk.c/2*
4	*fullname=^lec*	String tagged *fullname* does not have substring *lec*
5	*Mods* **:** *^ICAT_cl_hi*	String tagged *Mods* does not have word *ICAT_cl_hi*

More formal description

	Item	Comments of the output spectra must have
1	"*Tag*"	Exactly this *Tag* is present in the comment, possibly not followed by the equal sign
2	*Substring*	this substring anywhere in the comment
3a	*Tag*="*string* "	exactly this *string* tagged with this *Tag*=
3b	*Tag*=""	This *Tag* has no *string* or its *string* is “”
4	*Tag*=*substring*	this *substring* in the *string* tagged with this *Tag*=
5a	*Tag* **:** *word*	exactly this *word inside string* tagged with this *Tag*=
5b	*Tag* **:** "*word*"	exactly this *word inside string* tagged with this *Tag*=
6	*Tag*=*number*	tagged string = *number* or *string* begins with *number*
7	*Tag*=*number***:***number*	tagged string *number* inside the given range
8a	*Tag* >= *number*	The number in the string less or equal to *number*
8b	*Tag* > *number*	The number in the string less than the *number*
8c	*Tag* <= *number*	The number in the string greater or equal to *number*
8d	*Tag* < *number*	The number in the string greater than the *number*

NOTES:

- Inside an item, spaces are allowed next to signs =, >, <, >=, <=, or **:**. These signs as well as *Tag* and *string* or *number* or *word* may be located on separate lines.
- If a *Tag* created contains characters = or < or > or **:** then use double quotes to search for such a tag, for example, "<*Tag*>"="*string* ".
- *substring* in rows 2 and 4 may not contain characters < > = **:**.
- To search for a substring that may be interpreted as a number, for example, 1.23e5, use *Tag*==1.12e5 or *Tag*=="1.12e5" instead of *Tag*=1.12e5
- To reverse the meaning of constraints 1–2 (that is, change to “spectra must not have”), insert caret (^) in front of the "*Tag*" or *substring*
- To reverse the meaning of constraints 3–8 (that is, change to “spectra must not have”), insert caret (^) in front of the right-hand side expression.
- To use caret (^) as the first character of a *substring* enter *Tag* == "^*substring*".
- In 3-8, *Tag* may be replaced with *Tag?* In this case the spectrum is accepted not only if the condition(s), described in the above table are satisfied but also if *Tag* is missing.

Example of a multi-item constraint: The following items

Replicate	- Line 2, substring
RT >= 11.2	- Line 8a, number is greater or equal
Sample = "yeast_Nature_CAM"	- Line 3a, Tag=”string”
Datfile = 011599.dat	- Line 4, Tag=substring

fit the following “Comment” field:

Replicate RT=11.220m Sample="yeast_Nature_CAM" Datfile="F011599.dat"

More formal description of reversed constraints

	Item	Comments of the output spectra must have
1a	^"*Tag*"	Exactly this *Tag* is not present in the comment
1b	~"*Tag*"	Some of the tags are not exactly this *Tag*
2a	^*substring*	this *substring* is not anywhere in the comment
2b	~*substring*	this *substring* is not anywhere in the comment
3a	^*Tag*="*string*"	all tags but this *Tag* have exactly this *string*
3b	~*Tag*="*string*"	some tags but not this *Tag* have exactly this *string*
3c	^*Tag*=^"*string*"	all tags but this *Tag* haven’t exactly this *string*
3d	~*Tag*=^"*string*"	some tags but not this *Tag* haven’t exactly this *string*
3e	*Tag*=^"*string*"	this *Tag* has no string or its string differs from *string*
3f	*Tag*=^""	this *Tag* has a non-empty string
3g	**all**=^"*string*"	Each of the tagged strings has not exactly this *string*
3h	**Some**="*string*"	some tagged strings have exactly this *string*

Items 4–8 from the previous section (1.2.2) may be given reversed meaning in the same way as 3a–3f.

Reserved expression all= is same as ^""= and ^=
Reserved expression some= is same as ~""= and ~=

To address a tag named all or some put it in quotes, for example:
"all"=*string* "some"=string

Quoting tags allows searching for exact combination of tags, for example
“Single Tryptic_simple Parent”=358.07 or “Charge=2 Scan”=82

Tag?=^whatever is considered found if either *Tag=^whatever* has been found or *Tag* is not present.

Tags in Comment Constraint for MS/MS Text Information

Text information displayed for **NIST 26 MS/MS Library** mass spectra has special tagged text fields, for example:

```
MSn pathway: [M+H-H2O]+=>311.4
Precursor type: [311.4]+
Spectrum type: MS3
Precursor m/z: 375.2894, 311.4
Instrument type: IT/ion trap
Instrument: Thermo Finnigan Elite Orbitrap
Sample inlet: direct flow injection
Ionization: ESI
Collision gas: He
Collision energy: 35%
Ion mode: P
NOTES: Consensus spectrum; Acetonitrile/Water/Formic acid; Vial_ID=1741
InChIKey: KXGVEGMKQFWNSR-UHFFFAOYSA-N
```

Beginning with **NIST MS Search Program v.2.2**, **Tags in Comment** Constraint allows searching of these tagged fields. Special tags are to be used for this purpose.

To enter such a special tag, type dollar sign ($) immediately followed by the tag displayed in the spectrum text information, omitting spaces. Slashes, dashes, underscores in the tag may be omitted or added. Tags differing only in CAS, CAS#, or CASNO at the end of the tag are equivalent. The special tag and search string are not case sensitive.

The syntax is almost the same as the one described above for **Tags in Comment** Constraint.

Below are several examples.

To search for	**Enter into the Tags in Comment Constraint**
precursor m/z in range* 374.9–375.4	`$Precursor_m/z=374.9:375.4`
Instrument type containing word IT	`$Instrument_type:IT`
the exact *Instrument* name	`$Instrument="Micromass quattro"`
spectra that have any Related CAS# entry	`$Related_CAS`
spectra that have no Collision energy entry	`^$CollisionEnergy`

* **NOTE:** MS^3 spectrum with text information “Precursor m/z: 375.2894, 311.4” also will be found. That is, for MS^n (n>2) spectra, the first precursor m/z (corresponding to MS2) will be found.

List of all acceptable special tags

$aux
$charge
$collision_energy
$collision_gas
$compound_type
$cone_voltage
$inchikey
$in-source_voltage
$instrument
$instrument_type
$ion_formula
$ion_mode
$ion_mw
$ion_name
$ionization
$known_impurity
$mass_range
$maximum_intensity
$msn_pathway
$notes
$peptide_mods
$peptide_sequence
$precursor_m/z
$precursor_type
$pressure
$related_cas#
$retention_index
$ri
$salt
$salt/mix_cas#
$sample_inlet
$special_fragmentation
$spectrum_type

Special tags inside parentheses are considered identical:

($aux $notes) ($in-source_voltage $cone_voltage) ($retention_index $ri)

Some of these tags may be found in other **NIST 26 EI Libraries**, for example, $related_cas#, $inchikey.
See also **MS Search** Help topic “Spectrum Text Information”.

MS/MS Instrument Type Constraint

This feature will only work with **MS/MS Libraries** created using **NIST 23**, or later, or similarly formatted user libraries.

It affects only MS/MS spectra (that is, spectra which have precursor *m/z* value); therefore, it ignores all EI spectra, which means applying this constraint to a search of the EI Library does not affect the **Hit List**.

This constraint has seven options (checkbox*es*) that may be combined to produce a **Hit List** containing such spectra that each spectrum fits one of the selected Instrument Types.

The "Instrument type" field in a MS/MS mass spectrum text information is interpreted in the following way:

Instrument type checkbox	**"Instrument type" field in MS/MS mass spectrum**
Ion Trap	"IT/ion trap" or "QQIT" or "QqLIT"
Q-TOF	"Q-TOF"
Ion Trap + FT	"IT-FT/ion trap with FTMS"
HCD	"HCD"
Triple quadrupole	"QqQ"
Other	any other than the above five instrument types
Unspecified	No instrument type specified

Selecting all seven would select all mass spectra (the same as if the constraint is disabled).

The version of **Lib2NIST** included in **NIST 23** or a later version is needed to produce such a library because the instrument type is saved in the **NIST 23 Tandem** mass spectrum record as a new binary field; therefore, this constraint would not work with the **NIST 08 Tandem Library** or even the **NIST Peptide Libraries** created with the previous version of **Lib2NIST**.

In the case of NIST 11 or NIST 12 MS/MS libraries, Q-TOF checkbox would work as Q-TOF and HCD checkboxes selected together, HCD and Ion Trap + FT would not find any spectra although 737 HCD spectra are present in each of NIST 11 and NIST 12 MS/MS library. However, HCD spectra in these libraries may be found by entering Tags in Comment constraint $Instrument_type=HCD or Name Fragment constraint $:06HCD

See **Read Me Lib2NIST** (Lib2NIST.TXT or Readme_Lib2NIST.txt) for more details on rebuilding MS/MS library.

APPENDIX 7: Contributing EI Mass Spectra to the NIST/EPA/NIH Mass Spectral Library

Users of the **NIST MS Search Program** are invited to submit mass spectra for inclusion in the **NIST/EPA/NIH Mass Spectral Library**. Descriptions of both ideal and minimum acceptable data are provided below. Please provide data that is as close to the ideal as convenient.

Characteristic	**Ideal**	**Minimum Acceptable**
Substance	Pure compounds (single GC component) of confidently known structure that are commercially important occur in the environment, or are a natural product (including analytically useful derivatives)	Any single chemical compound whose identity is confidently known
Method	Acquired by GC/MS at an ionization energy of 70 eV under conditions such that isotope ratios are not distorted and ions above the molecular ion are absent and processed using AMDIS or other deconvolution software	Acquired at an electron ionization of not less than 20 eV under conditions such that isotope ratios are not grossly distorted and significant ions above the molecular ion are absent; significant background subtracted
Requested Information:		
Contributor	Contributor’s name and/or institution (as it is to appear with the spectrum)	Contributor’s name and/or institution (as it is to appear with the spectrum)
Chemical name	Chemical name (preferably in Chemical Abstracts or IUPAC format)	Chemical name (or structure)
Chemical Abstracts Registry Number	Chemical Abstracts Registry Number	-
Chemical structure	Chemical structure (in MOL/SDF file format)	Chemical structure (or name)
Synonyms	Alternative names (trade names, trivial names, etc.	-
Mass spectrum	Mass spectrum (unit *m/z* resolution or better) with all observed nominal masses from no higher than *m/z* 12 to above the molecular ion with intensities of 0.1 % or greater and intensity in electronic format (preferably as an NIST/EPA/NIH user library). Ions at half-mass should be kept in the spectrum and rounded down to the nearest mass.	Mass spectrum (unit mass resolution) with all observed nominal masses beginning no higher than one-third the molecular mass to above the molecular ion with intensities of 2.0%. Ions at half-mass should be kept in the spectrum and rounded down to the nearest mass.
Instrument type	Instrument type (magnetic sector, linear quadrupole, TOF, etc.)	Instrument type (magnetic sector, linear quadrupole, TOF, etc.)
Instrument model	Instrument manufacturer and model name	-
Ion source temperature	Ion source temperature (°C)	-
Inlet	Inlet (GC, probe, etc.) and inlet temperature (of probe, reservoir, etc.)	-
Retention index	Kováts Retention Index (with the commercial name of the GC phase or, if not available, whether the phase is polar or non-polar)	-
Impurities	Note masses not belonging to the compound and their origin, if known.	Note masses not belonging to the compound, if known.

NOTES

APPENDIX 8: Support Contacts

In our continuing commitment to quality, the NIST Mass Spectrometry Data Center is always looking to improve the quality of our Mass Spectral Libraries and programs for accessing them. Comments or questions about the quality of either or other Standard Reference Databases available from the NIST Standard Reference Data Program should be made by contacting:

Mass Spectrometry Data Center
Biomolecular Measurement Division 645.04
National Institute of Standards and Technology
100 Bureau Drive, Mail Stop 8362
Gaithersburg, MD 20899
email: massspec@nist.gov
website: *https://chemdata.nist.gov/*

NIST provides updates and enhancements to the NIST Mass Spectral Search Program, MS Interpreter and AMDIS. They can be downloaded from *https://chemdata.nist.gov/*