

**NIST/EPA/NIH Mass Spectral Library (NIST 05) and  
NIST Mass Spectral Search Program (Version 2.0d)**

**For Use with Microsoft® Windows  
User's Guide**

**Scientific Instrument Services, Inc. Version  
SEE REVISION NOTICE**

---

**The NIST Mass Spectrometry Data Center**

Stephen E. Stein, Director

*Evaluators:*

Anzor Mikaia, Principal  
Vladimir Zaikin (EI)  
Damo Zhu (EI)  
Boris Milman (MS/MS)  
Valeri Babishok (RI)  
Igor Zenkevich (RI)

*Programmers:*

Peter Linstrom  
Yuri Mirokhin  
Dmitrii Tchekhovskoi

*Documentation and Help:*

W. Gary Mallard  
O. David Sparkman  
Joan A. Sparkman

June 2005

**U.S. Department of Commerce**

Technology Administration  
National Institute of Standards and Technology  
Standard Reference Data Program  
Gaithersburg, MD 20899

---

The National Institute of Standards and Technology (NIST) uses its best efforts to deliver a high-quality copy of the Database and to verify that the data contained therein have been selected on the basis of sound scientific judgment. However, NIST makes no warranties to that effect; and NIST shall not be liable for any damage that may result from errors or omissions in the Database.

---

**REVISION NOTICE: Scientific Instrument Services, Inc. has modified and expanded the original documentation provided by the National Institute of Standards and Technology to better serve its customers. Therefore, the contents of this manual may have significant differences from the original documentation provided by NIST.**

---

© 1987, 1988, 1990, 1992, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2005 copyright by the U.S. Secretary of Commerce on behalf of the United States of America. All rights reserved.

No part of this Database may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the distributor. Portions of this program are also copyrighted by Microsoft Corp., XVT Software, Inc., and © 1984–1996 FairCom Corporation.



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 necessary the best available for the purpose.

Microsoft is a registered trademark of Microsoft Corporation, and Windows is a trademark of Microsoft Corporation. ISIS is a trademark of MDL Information Systems, Inc. "FairCom" and "c-tree Plus" are trademarks of FairCom Corporation and are registered in the United States and other countries. All Rights Reserved.

# CONTENTS

INSTALLATION	1
INTRODUCTION	3
Version 2.0d New Features	3
Tab Displays	5
Retention Indices	6
MSMS Tab and the NIST MS/MS Library	8
Lib. Search Tab	10
Button Bar	11
Title Bar, Tool Bar, and Status Bar	13
Neutral Loss Display (Important New Feature in V.2.0d)	15
Spectral Analysis Utilities	16
AMDIS	16
MS Interpreter	16
EXAMPLE SEARCHES	17
User Spectrum Search	17
The Lib. Search Options Dialog Box	19
Automations	21
Limits and Constraints	22
Any Peaks Search	23
Name Search	24
BASIS FOR INTERPRETATION OF RESULTS (User Search)	25
SUBSTRUCTURE INFORMATION	26
USE WITH INSTRUMENT DATA SYSTEMS	28
USE WITH THIRD-PARTY DRAWING PROGRAMS	30
USER LIBRARIES	31
NIST Text Format of Individual Spectra	32
APPENDIX 1: Important Info on Creating AUTOIMP Files	33
APPENDIX 2: Use with ChemStation Software	33
Additional Information Regarding Modes Other Than Enhanced Quantitation	34
APPENDIX 3: Copy Other Libs to NIST Format	37
APPENDIX 4: Use with ThermoQuest XCALIBUR	39
APPENDIX 5: Search Algorithms	41
APPENDIX 6: Support Contacts	43



---

## INSTALLATION

The NIST Installation Program will install both the NIST MS Search Program and AMDIS. If you already have these programs installed and are upgrading, this installation will use the current directories or you can select another directory.

### INSTALLATION of NIST/EPA/NIH Database in ChemStation Format

Users **MUST** copy the **\NIST05.L** folder from the CD ROM to the \DATABASE folder on the computer that has the ChemStation software installed before following the 10237 installation instructions. This Database will be accessed in the normal way specified by your mass spectral data system's software (see instructions below on making the NIST Database the Default PBM Search Library).

### INSTALLATION of – NIST/EPA/NIH Database in GCQ/Saturn Format

Users **MUST** copy the **\NIST05.LBR** file from the CD ROM to the \LBR folder in the directory that contains your data system software before following the 10237 installation instructions. This Database will be accessed in the normal way specified by your mass spectral data system's software.

### INSTALLATION of NIST/EPA/NIH Database in the TurboMass/Mass Lynx Format

Users **MUST** copy the folder **\TurboMass** from the CD ROM to the directory reserved for libraries in your data system software before following the 10237 installation instructions. This Database will be accessed in the normal way specified by your mass spectral data system's software.

**INSTALLATION of The NIST MS Search Program** is installed from a CD ROM. Place the CD ROM in the CD-ROM drive.

The default installation directories have been changed from the installation of previous versions of the NIST MS Search Program and/or AMDIS. New installations should use the new defaults: \nist05\mssearch\ for the NIST MS Search Program and the NIST/EPA/NIH Database, and \nist02\amdis32\ for AMDIS.

If you are upgrading from a previous version, it is recommended that you retain your original install directories.

## Windows 95/98/Me, Windows 2000, Windows NT, Windows XP

- Select the **Start** button on the lower left of your screen. Then select **Run** from the **Start** menu.
- Assuming your CD-ROM drive is D, type D:\SETUP.EXE and select **OK**. If your CD-ROM drive is a device other than D, replace D with the appropriate drive letter.
- Follow the instructions presented by the SETUP program.
- In upgrading from previous Windows versions, 1.6-1.7, of the NIST MS Search Program, v.2.0 will be added to the previous version's directory unless the install directory is changed from NISTMS. Previous versions of the Program will remain usable unless the contents of the Database directory is replaced.

## Windows 3.1 Installation

The NIST MS Search Program V.2.0 is not compatible with Windows 3.11. You may use the NIST05 MS Library with MS Search v.1.7 under Windows 3.1. However, not all data will be retrieved or displayed properly.

Copy the contents of the CD that contains the directory \NIST2v17 to your computer. You must go to the director \NIST2v17\MSSEARCH and make ALL files non READ ONLY. Start the Program from Program Manager, select **File** from the Menu Bar and then select **Run** from the **File** menu. Then enter C:\NIST2v17\MSSEARCH\NIST\$.EXE. A dialog box will appear with a path. Change D:\ to C:\ in the path. You will have to create an icon for the NIST MS Search Program, Isotope Calculator and HP2NIST.

## Instructions to Make the NIST/EPA/NIH Mass Spectral and Structures Databases the Defaults for the ChemStation PBM Search

When the ChemStation Data Analysis software is installed, DEMO.L is the default mass spectral database and DEMO.SDB is the default structures database. To use the NIST/EPA/NIH Mass Spectral and Structures Databases as the defaults, you must change the current defaults.

### Setting Default Mass Spectral Database

With the Data Analysis program active, select **Spectrum** from the Main Menu Bar. Then click on Select Library in the **Spectrum** menu. The first entry in the displayed **Library Search Parameters** dialog box is C:\DATABASE\DEMO.L (assuming that the \DATABASE folder is on the C:\ drive). This is highlighted. Press the ? key. This will result in the **Browse for Folder** dialog box opening. Highlight the \NIST02.L folder and click on the **OK** button. The first line of the **Library Search Parameters** dialog box now reads C:\DATABASE\NIST02.L. Click on the **OK** button. This will set the NIST/EPA/NIH Mass Spectral Database as the default for this session. To have this Database remain as the default for future sessions, you must save the current Method.

Select **Method** from the Main Menu Bar. Select **Save Method** from the **Method** menu. This will cause the **Save Method as** dialog box to be displayed. The entry in the text input area should read DEFAULT.M. As you will create new methods from this DEFAULT.M method, click on the **OK** button. This will save the DEFAULT.M method with the NIST/EPA/NIH Mass Spectral Database as the default database for ChemStation searches.

### Setting Default Structures Database

After the default mass spectral database has been set, select **View** from the Main Menu Bar and select **Parametric Retrieval** from the **View** menu. This will result in the Parametric Retrieval Mode screen replacing the chromatogram/spectrum display. The **Search Parameters** dialog box will also be displayed. Click on the **Cancel** button in this dialog box. Select **Structures** from Parametric Retrieval Mode's Main Menu Bar. Click on **Select Structure Database** from the **Structures** menu. The **Chemical Structures Databases** dialog box will be displayed. Click on the **Add New File** button. This will cause the **Chemical Structures Database Select** dialog box to be displayed. Highlight the MOLSTRUC folder and click on the **Open** button. Highlight STRUCT05.SDB and click on the **Open** button. This will close the **Chemical Structures Database Select** dialog box and add a line to the Chemical Structures Databases. Highlight the first line in this dialog box, which is the DEMO.SDB structure database and click on the **Remove From List** button. This leaves only the STRUCT05.SDB entry in the dialog box. Click the **OK** button.

The STRUCT05.SDB structures database will remain the default structures database until other structures databases are added or another database replaces STRUCT05.SDB. If you are upgrading from a previous version the NIST/EPA/NIH Mass Spectral Database such as NBS75K.L, NIST02.L, etc. there may be an entry in the **Chemical Structures Database** dialog box with the name MOLSTRU.SDB. Remove this structures database when you add the STRUCT05.SDB database. STRUCT05.SDB replaces MOLSTRUC.SDB or another SDB file.

## Instructions: Interfacing the NIST Mass Spectral Search Program for Structure Export to Other Program; e.g., ChemSite Pro

When Version 2 of the NIST Mass Spectral (MS) Search Program saves a structure to the Windows Clipboard using the Right Mouse button (RMB) menu's command "Copy Structure to Clipboard", at the same time a file in MOL File format with the name Clipboard.MOL is written to the NIST MS Search Program's folder (by default, \NIST02\MSSearch). ChemSite Pro can either use the structure as it appears on the Windows Clipboard or this file for structure importation into ChemSite Pro.

In order to send a structure from the NIST MS Search Program to ChemSite Pro, you have to create a file in the NIST MS Search Program's directory with the name AUTOIMP.STR. This file can be created with Note Pad. It is a text file that contains the following:

```
"ChemSite" "C:\MolSuite\ChemSite\ChemSite.exe" "%1"
```

This assumes that ChemSite Pro is in the ChemSite folder, which is in the MolSuite folder on the C:\ drive. If this is not the location of ChemSite Pro, then it will be necessary to modify the text between the middle set of " ". THIS TASK HAS TO BE DONE ONLY ONCE!

### DANGER! DANGER! WARNING WILL ROBERTSON! DANGER!

When you create a file with Notepad using some later versions of Windows, an extension of TXT is applied to the file regardless of however you enter the file name. If you select **Save** from the **File** menu of Notepad and enter the name AUTOIMP.TXT, the file will be saved with the name AUTOIMP.STR.TXT. These versions of Windows also have the *check box* next to "Hide extensions for know file types" in the View tab of the **Folder Options** dialog box (displayed by selecting **Tools/Folder Options** from the Main Menu Bar of the Windows Explorer) selected. This means when you look at the listing AUTOIMP.STR in the Windows Explorer, the TXT extension will not be displayed. If you highlight AUTOIMP.STR, click the RMB, and select "Rename" from the displayed menu, you will see the TXT extension. The .TXT characters must be removed so that the file name reads AUTOIMP.STR in order for this file to function with the NIST MS Search Program and ChemSite.

You will also need to make sure the file CSWSUITE.INI is in the \Windows or \WINNT folder. This file will be put in the \Molesuite\Chemsite\ folder when ChemSite Pro is installed. You can copy this file to the \Windows or \WINNT folder.

After you have created the AUTOIMP.STR file in the NIST MS Search Program's folder, you can send a structure from the Windows Clipboard at the same time you call the ChemSite Pro program into focus from within the NIST MS Search Program.

Save a displayed structure in the NIST MS Search Program by placing the Mouse pointer on a section containing a structure. Click the RMB and select "Copy Structure to Clipboard" from the RMB's displayed menu. Click the RMB a second time to redisplay the RMB's menu. This time, select "Send to...". A "Send to" menu will be displayed. The bottom item will be ChemSite. Select **ChemSite**. This will bring the ChemSite Pro program into focus (start it if it is not already running), and the structure saved to the Windows Clipboard will appear.

If you copy a structure to the Windows Clipboard in ChemSite Pro (Copy MDL Mol File from the Main Menu Bar's **Edit** menu), then select **Calculate** from the Main Menu Bar, and then select **NIST Mass Spectral Search**, the NIST MS Search Program will be brought into focus with the **Structure** dialog box open and containing the structure that appeared in ChemSite Pro. This structure can then be put into the List area of the Lib. Search tab by clicking with the LMB the **OK** button on the **Structure** dialog box.

---

## INTRODUCTION

The National Institute of Standards and Technology (NIST) introduced a version of its popular search software used with the NIST/U.S. Environmental Protection Agency (EPA)/National Institutes of Health (NIH) Mass Spectral Database for Microsoft Windows in 1995. At that time, the NIST Mass Spectral Search Program for Windows (the Program) would allow for various types of comparisons of an acquired or unknown spectrum with the NIST/EPA/NIH Database, its Library of Replicate Spectra, and user libraries. The NIST/EPA/NIH Database was also searchable by multiple names that were associated with various spectra. This **Incremental name** search, which first appeared in a DOS version of the Program, was what analysts had been looking for since commercial libraries became available with mass spectrometry data systems; "how to retrieve a spectrum of a compound without having to search an acquired spectrum against the Library." Another unique feature of the Program's spectral comparison algorithms, even in the DOS version, was that the spectra selected from the library during the presearch had all of their peaks compared with all of the peaks in the sample spectrum. All other commercial library search systems at that time still used condensed spectra.

Other features retained in v.2.0 of the Program that were in the original DOS and Windows versions are the ability to retrieve spectra using **Molecular weight**, **Formula**, **Chemical Abstract Services registry numbers (CAS rn)**, and **Library Id number**. All these search methods, along with the versatile **Any Peaks** search, are available for use with the NIST/EPA/NIH MS Library and user libraries. In addition, multiple constraints can be added to searches that would limit the searched population. The first Windows version introduced the ability to search multiple libraries simultaneously.

Since the introduction of the first Windows version of the Program, other features have been added: 1) mass defect correction of *m/z* values in imported spectra, 2) the ability to associate structures as MOL files with user library spectra, 3) the ability to have synonyms associated with spectra in user libraries, 4) the ability to use the **Incremental name** search with user libraries, 5) the ability to evaluate a Hit List from a Neutral Loss or Hybrid Neutral loss "Similarity" search to determine the probability of the presence and absence of various structures, 6) the ability to estimate the molecular weight of an unknown along with the ability to determine the number of atoms of chlorine and/or bromine atoms that may be present in an analyte that produced a mass spectrum, 7) the addition of the mass spectral interpretation tools **Isoform** and **MS Interpreter**; and, most recently, an MS/MS Search, the ability to estimate retention indexes for most of compounds in the Mass Spectral Library, and the ability to view the spectra and other information in the MS/MS Library that is a part of NIST 05. Since the introduction of the first Windows version of the Program, three new versions of the NIST/EPA/NIH Mass Spectral Library (NIST 98, NIST 02 and NIST 05) have been introduced.

### New Features in Version 2.0d of the MS Search Program

All the features in the previous versions of the NIST MS Search Program for Windows **Version 2.0** have been retained. Several new features are present in addition to those previously introduced. A new tab (**MS/MS**) has been added to allow the viewing of the NIST MS/MS Library. This Library contains 5,191 spectra of 1,943 different ions (1,671 positive and 341 negative ions). A range of instruments is represented, including ion trap and triple quadrupole mass spectrometers. These spectra, along with an increased number of fields relevant to MS/MS data, are provided in a set of files separate from the Main and Replicates sets of files that comprise the Library of EI spectra. Spectra have been provided by contributors, measured at NIST, and extracted from the literature. In view of the relatively small size of the library, it is intended primarily to serve as a "starter set". It also documents spectrum variations between instrument classes under different conditions. The preparation of the MS/MS Library revealed that at sufficiently high signal-to-noise measurement conditions, modern instruments are capable of providing very reproducible "library-searchable" spectra. Although collision energy can be an important variable, spectra vary in an understandable way depending on the compound and instrument type as well as collision-energy conditions.

Another new feature is the ability to view the **Retention Index** data that are contained in a new RI Database. This new addition, containing 121,112 Kovats Retention Index values for 25,893 compounds on nonpolar columns, 12,433 of which are compounds represented in the Main EI Library (mainlib), is fully annotated, including literature source and measurement conditions. For most of the compounds that have chemical structures the retention indices are also automatically estimated using a group approach. Provided the text pane view has been set to display **Retention Indices**, these data will be displayed with the results of any search. The `nist_ri` library can be searched by itself using any of the Program's Search routines other than the Unknown Spectrum Search. In addition to the experimental data, for a large subset of the mass spectral database, estimated RI are provided along with uncertainty estimates.

Changes were made to the Program to allow importation of spectra from data files that have long file and path names. There were also modifications to the Agilent Technologies ChemStation Macros to allow for long file and path names as well as search data on devices that the operator does not have write-access.

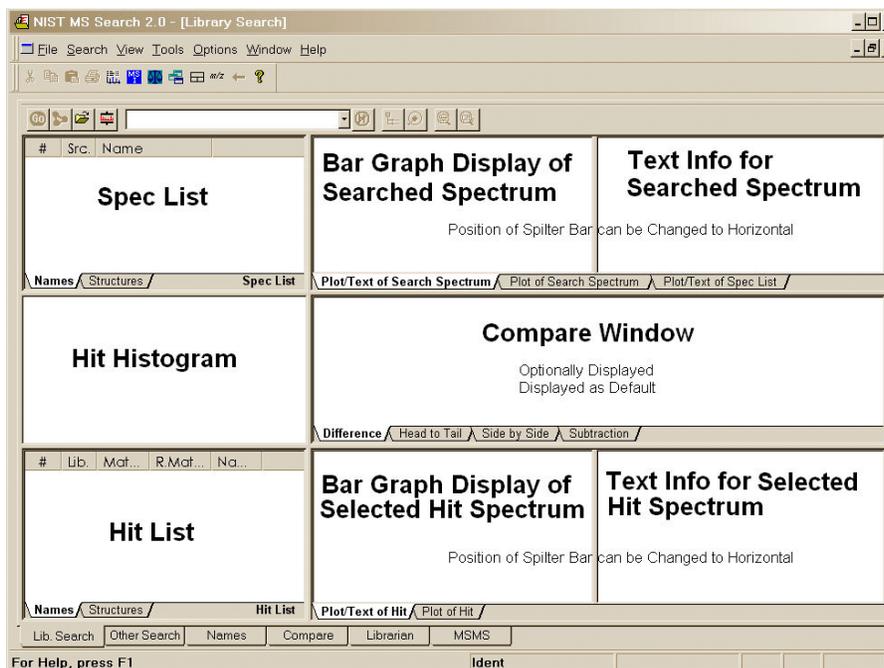
The 2005 version of the NIST/EPA/NIH Mass Spectral Library (**NIST 05**) contains 190,825 spectra of 163,198 different chemical compounds. This is an increase of 16K new compounds (~11%) and 21K spectra overall. The

binary format has not changed from the 1998 version (**NIST 98**), although several new files have been added mainly to associate equivalent compounds and link individual compounds to the Retention Index Database.

Contents of the NIST/EPA/NIH (NIST 05) Mass Spectral Library		
Library Name	Library Description	Library Contents
mainlib	Main EI MS library	163,198 spectra, 12,433 have RI
replib	Replicate to mainlib EI library	27,627 spectra
nist_ri	Retention Index Library	13,295 compounds, with no spectra
nist_msms	MS/MS Library	5,191 spectra of 1,671 positive and 341 negative ions
	Retention Index Database (located in mainlib)	121,112 Kovats RI values for 25,728 compounds

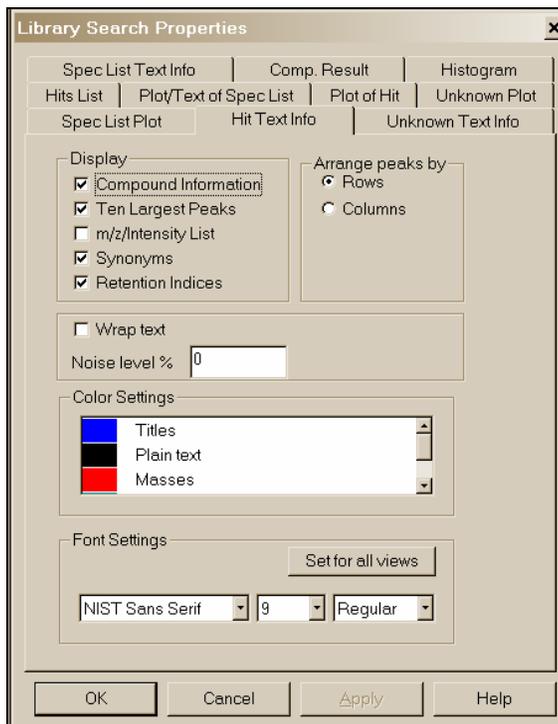
## Tab Displays

**Version 2.0** of the Program uses tab displays. Selecting a tab results in the display of a different Window. Each Window serves a different function, and each Window has a number of panes or sections ranging from 5 for the Lib. Search tab to 3 for the Other (searches), Name (incremental name search), Librarian, and MSMS. The panes that are used for spectral displays also have tabs that allow for different types of displays; e.g., a bar-graph spectrum or a bar-graph and text display of the spectrum. These two displays are separated by a splitter bar. The splitter bars allow for the adjustment of the amount of the display designated for the bar-graph spectrum and the text information. The panes on the left side of each Window are reserved for a list of spectra. The pane on the top left of the Lib. Search tab is used for the Spec List, and the pane on the lower left of this Window is used for the Hit List. The pane on the left of the Other and Names tabs is used for the Hit List, and the pane on the Librarian and MSMS tabs is a list of spectra that can be displayed. All of these lists can be displayed as text row or as graphic structures by selecting the Names or Structures tab at the bottom of the pane.



**Figure 1. Lib. Search tab.**

Placing the Mouse Pointer in one of these panes and clicking the Right Mouse button once will result in the display of a Right Mouse button menu. The last item on this menu is **Properties**. Selecting **Properties** will result in the display of a dialog box. This dialog box has tabs at the top that 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, and fonts used for alpha-numeric characters can also be set. Each pane can have its own customized set of attributes. This customized display can be saved using the **File/Save Configuration** command from the **Main Menu Bar**. Different saved configurations can be recalled by using the **File/Restore Configuration** command from the **Main Menu Bar**.



**Figure 2. Properties dialog box displayed by selecting Properties on the Right Mouse Button Menu displayed when the mouse pointer is on one of the panes of the LibSearch tab.**

### Retention Indices

One of the items on the Hit Text Info and Spec List Text Info tabs is **Retention Indices**. Having a check in the box next to **Retention Index** will cause the experimental data for the retention index to be displayed if present. In addition, for many compounds in the database an **Estimated Kovats Retention Index (RI)** will be displayed based on the structure associated with the spectrum. These values are reported in *index units (iu)* with a confidence interval, e.g. "Confidence interval (Ketones): 57(50%) 246(95%) iu" reported for acetone. This is saying that 50% of the time the given value falls within 57 iu of the estimated value and 95% of the time the given value falls within 246 iu of the estimated value. This is based on an analysis of the all data for the class of the compound given; i.e. in this example, ketones. If there are literature data in the NIST/EPA/NIH Library for Kovats Indices of a compound whose spectrum is displayed, these data will be in the Text Information of the spectrum. Compounds for which the retention indices are present in the RI database and no mass spectral data is present in the EI database are located in the **nist\_ri** Library. By selecting **nist\_ri** Library in a list of libraries to be searched, a Structure Similarity Search, any of the Other search routines or the Name search can be used to retrieve information for these compounds. The **nist\_ri** Library contains 13,295 compounds. There are literature data for other 12,433 compounds that are in the **mainlib** Library. A total of 121,112 Kovats Retention Index values for 25,728 compounds on nonpolar columns are present in the NIST 05 MS Library.

The first tab (lower left of the Program's display), **Lib.Search**, is used with a search of a spectrum or structure against mass spectral libraries: a user library or the NIST 05 libraries (Figure 1). The spectrum and structure searches can be done against multiple libraries (maximum of 16), and the selected libraries can be different from those selected for the various searches found in the Other Search tab.

The second tab display, **Other Search**, is used to perform a **Formula, CAS registry number, Molecular weight, Sequential, Any peaks, ID number, or NIST number** search. The **NIST number** search can only be done on the NIST main library (**mainlib**) and its replicate library (**replib**). **NIST numbers** establish correspondence between spectra in different releases of the NIST/EPA/NIH MS Library. The **ID number** search can only be done on one library at a time (Figure 2.) The other searches in this group can search multiple libraries (maximum of 16), and you can have different sets of libraries for each different type of search.

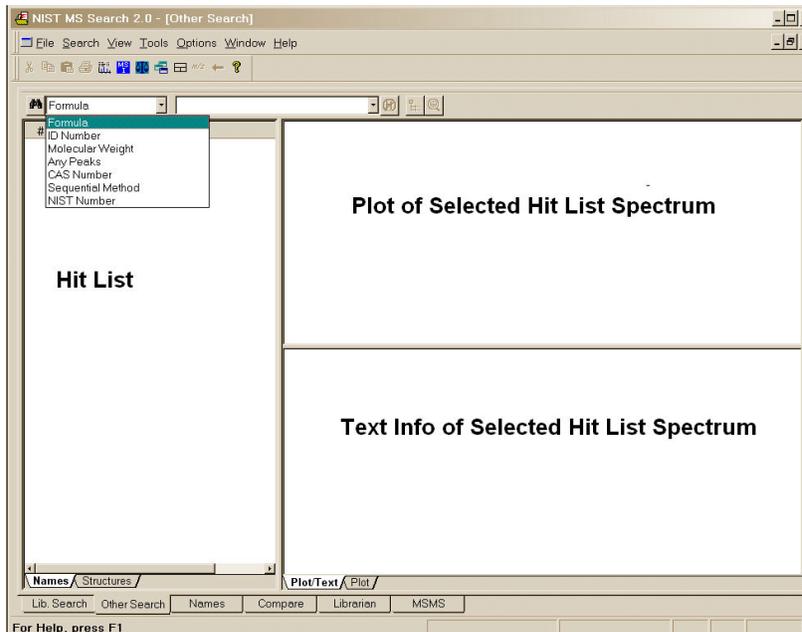


Figure 3. Other Search tab.

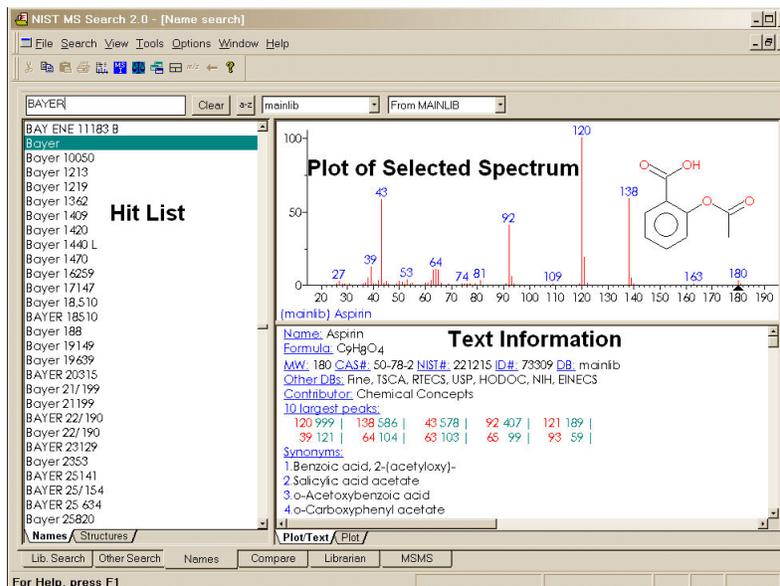


Figure 4. Incremental Name Search tab.

The third tab, **Names**, is used for the NIST signature **Incremental name search (INS)** (Figure 3). The **INS** can only be performed against one database at a time. The database to be searched is selected by use of the “library select” drop-down list box next to the **a-z** button. If the **a-z** button is selected, the only input taken is that of letters **a-z**. If the **a-z** button has not been selected, you can type numbers as well as letters. You can also paste Greek symbols. Typed dashes and other punctuation characters will not be used.

The drop-down list box just to the right of the **a-z** button is used to select the library to be searched. This search only allows the use of one library at a time. The second list box is used to display replicate spectra for the **NIST 05 mainlib** library

The fourth tab, **Compare**, allows the display of a sample spectrum and multiple spectra from the Hit List. Other spectra can also be put into the Compare display (Figure 4). A selected spectrum in the lower part of the Compare tab can be displayed as various types of comparisons with the spectrum in the upper part of the display.

The fifth tab, **Librarian** (not shown), is used to deal with spectra that are in user libraries or that are to be added to user libraries. The layout of this tab is the same as the tab used for the Incremental Name Search. The

Librarian tab 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, synonyms, and structures to be edited, added, or deleted.

Additional information on the **Librarian** tab is explained later in this manual under **User Libraries**.

When a splitter bar divides a pane, by default, its position is horizontal. This position can be changed to vertical by selecting **Change Splitter Orientation** on the Right Mouse button menu displayed when the pointer is in the pane.

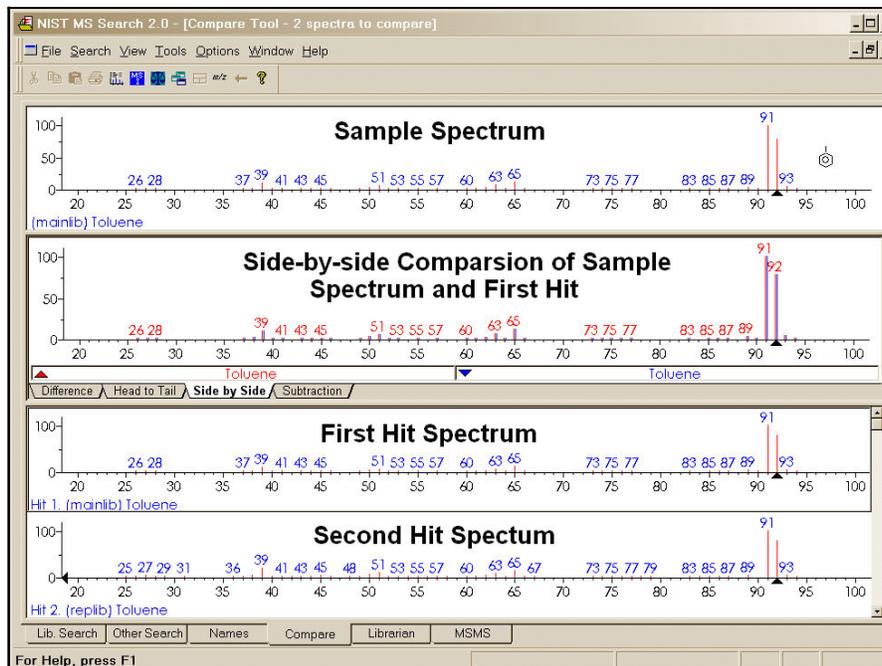


Figure 5. Compare tab.

## The MSMS Tab and The NIST MS/MS Library

The **MSMS** tab is new to Version 2.0d of the Program. Its sole purpose is to view the spectra in the NIST\_MSMS Library. Each time this tab is selected, the three panes of the display are blank with the exception of a single entry in the ion list pane, which is on the left of the tab's display. This entry is the **MSMS Library**. There is a box on the left that has a plus sign (+). Placing the Mouse pointer on this box and clicking expands to the first two levels (**Positive Ions** and **Negative Ions**). There are 1,671 spectra of positive ions and 341 spectra of negative ions. There are plus boxes next to both Positive Ion and Negative Ion. Clicking on one of these plus boxes results in the display of the next level. This is a list of molecular weights of analytes for which a spectrum was obtained by MS/MS. Clicking on the plus box next to one of these molecular weight listings results in the display of a list of elemental compositions that will also have plus boxes next to them. Click on one of the elemental composition check boxes will result in a display of a list of the compound names that have that elemental composition. In most cases, this is only a single compound. Clicking on the plus box next to the compound name will result in the display of a list of the spectra acquired under MS/MS conditions for this compound. If more than one spectrum was acquired, there is a separate entry for each spectrum.

The listing of each spectrum begins with **ms1** or **ms2**. Spectra with **ms1** acquired using a beam-type instrument are the result of fragmentation from in-source collisionally activated dissociation (CAD) [a.k.a. in-source CID]. If the spectra were acquired using a quadrupole ion trap (QIT), the **ms1** is the designation of the spectrum obtained by scanning the ions out of the trap after their accumulation.

For beam-type instruments, the **ms2** designation refers to dissociation that takes place in a collision cell of an isolated precursor ion. For QIT instruments, the **ms2** designation refers to dissociation brought about in the trap after a precursor ion is isolated.

In the case of data acquired using a QIT mass spectrometer, the **ms1** may have a plus box next to it. Clicking on this plus box results in a subsequent spectrum listed as **ms2**, which may also have a plus box. Selection of the plus box next to the **ms2** spectrum can result in an **ms3** spectrum, which is obtained by the isolation of a product ion as a precursor ion for a subsequent iteration of MS/MS.

Nothing follows any **ms1** designation. Clicking on the **ms1** results in the display of the spectrum as seen in Figure 6. The **ms2** designation is followed with the precursor ion *m/z* value for both beam-type and QIT instruments and the collision energy for beam-type instruments.

As can be seen from Figure 6 and Figure 7 on the following page, there is a great deal more information provided in a text format for the spectra in the NIST\_MSMS Library.

The MS/MS Library can be searched with any of the **Other Searches** or the Incremental Name Search. All that is necessary is to include the nist\_msms Library in the **Include Libs** column of the Search dialog box for the **Other Searches** or as the single library to be searched in the **Incremental Name Search**. The Unknown Spectrum Search and Structure Similarity Search are also applicable. However, the user must understand that MS/MS spectra in general are different from EI spectra as well as the fact that structures in the MS/MS Library refer to the neutral form of the precursor ions.

In absence of nist\_msms library the MSMS tab is not displayed.

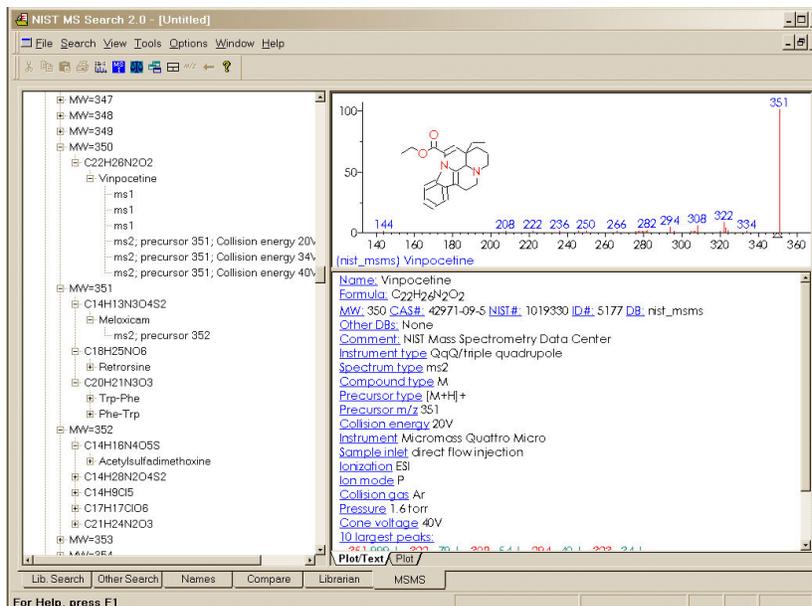


Figure 6. MSMS tab for data acquired using QqQ.

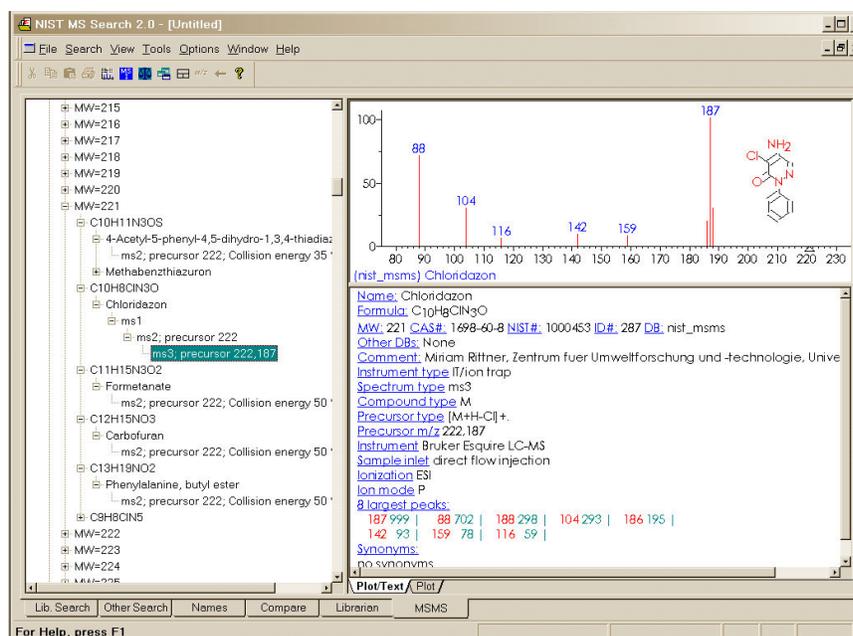


Figure 7. MSMS tab for data acquired using QIT MS<sup>3</sup>.

## The Lib. Search Tab

The Lib. Search tab's display is divided by a vertical splitter bar. All five of the major tab displays have moveable and reorientation divider bars and tabs associated with various windows within a view. In the Lib. Search tab, there are three windows on either side of the splitter bar. On the left side of the splitter bar, the top Window is the **Spec List** (known as the **Clipboard Window** in previous versions of the Program), a **Hit Histogram** in the middle, and a **Hit List** at the bottom. On the right side of the vertical splitter bar are three separate Windows: **Search Spectrum/Spec List Spectrum**, top; **Compare**, middle; and **Hit List Spectrum**, bottom. There are vertical splitter bars associated with these six windows that allow for customization of the display. In addition, by selecting **View** from the Main Menu Bar, you can choose to have the **Compare** Window displayed or not displayed. If the Mouse pointer is in the **Search Spectrum/Spec List Spectrum** Window or the **Hit List Spectrum** Window, the RMB can be clicked to display a menu and select **Change Splitter Orientation** to change the splitter bar between the spectrum plot and text information from vertical to horizontal or back.

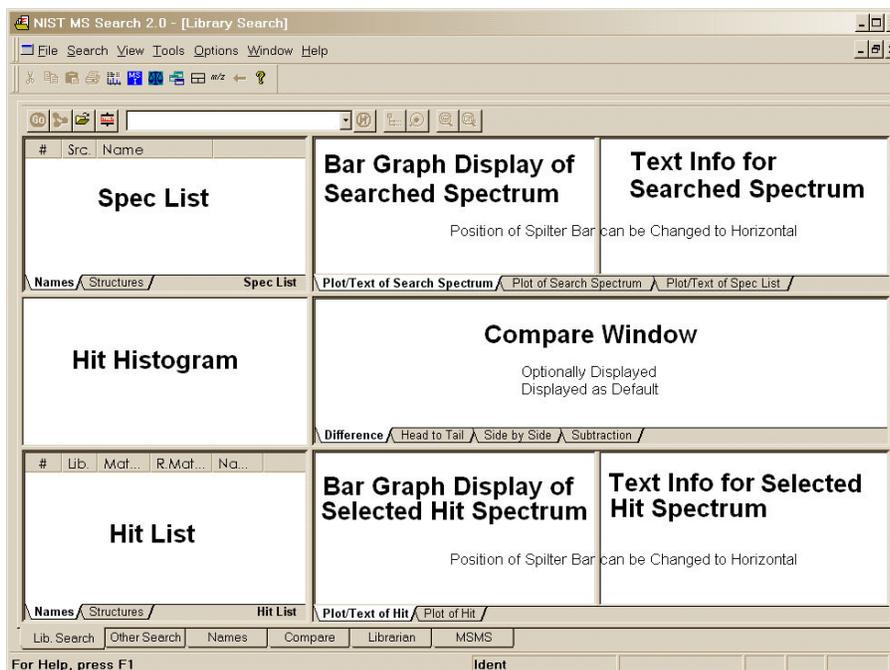
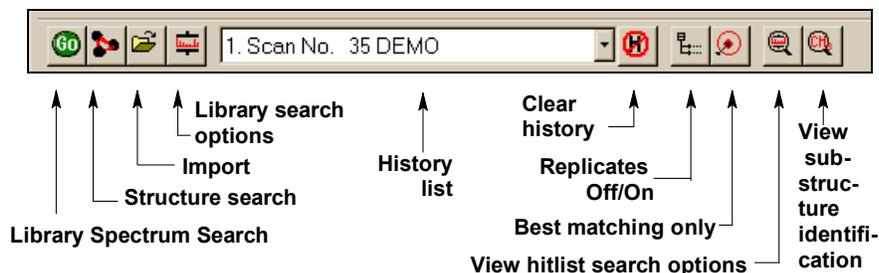


Figure 8. Lib. Search tab.

Just above the Lib. Search tab's window is a button bar.



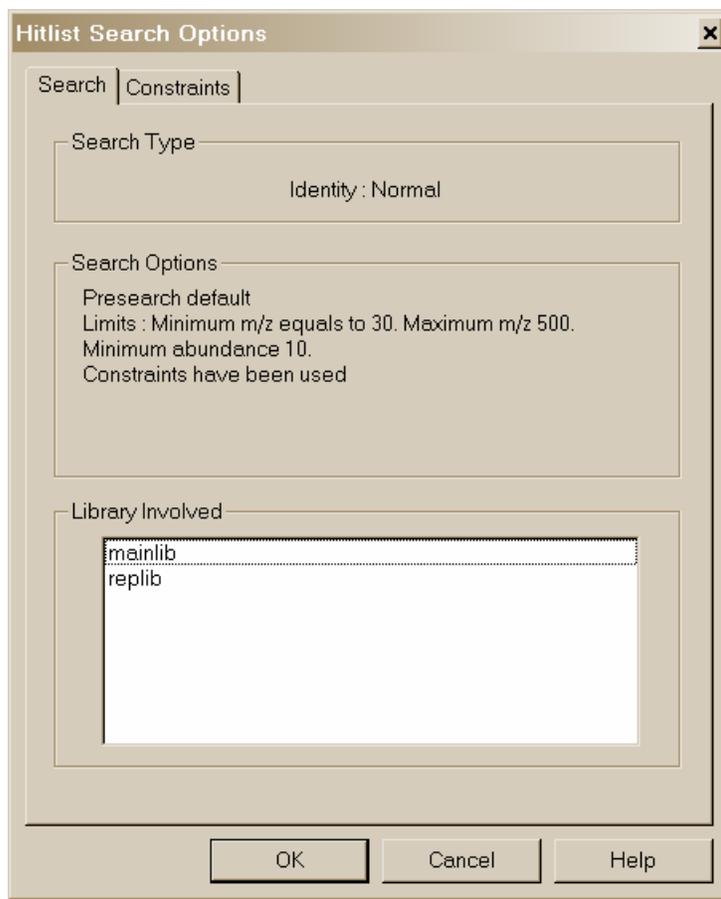
**Figure 9. Lib. Search's button bar.**

The **Go** button will launch a search of the highlighted spectrum in the **Spec List**. The button to the right of the **Go** button will launch a **Structure** search of the highlighted structure in the **Spec List**. The next button to the right (with the file folder icon) is used to import spectra from a text file. The last button, just before the drop-down list box, is used to display the **Library Search Options** dialog box. This dialog box is used in selecting libraries to be searched, search types, and other factors associated with the search of spectra and structures. The drop-down list box contains the Search History. A previous search's display can be restored by highlighting an entry in this list. **NOTE:** the Program can be set to automatically clear the **Hit List** and/or the **Spec List** by selecting the **Clear History on Exit** check box in the Hit List tab and/or Plot/Text of Spec List tab in the **Library Search Properties** dialog box displayed by clicking the right mouse button and selecting **Properties** from the RMB menu. **NOTE:** MS Search v.2.0d uses different from previous versions index files for the **Structure** search in user libraries, therefore these files should be rebuilt using "Rebuild Structure Search Database" from the Tools menu. This should also be done for each new user library connected to the MS Search and every time spectra with structure were added, removed, or edited in a user library because the structure search index files are not updated automatically.

The first button to the right of the "Search History" list box is used to clear the search history (**Clear History** button). The next button (the **Replicate** button) to the right is used to toggle the listing of replicate spectra on and off when the **mainlib** Library of the NIST/EPA/NIH Library is searched.

The button to the right of the **Replicates** button is used to display only hits with the highest Match Factor when more than one hit has the same **CAS rn**. This is the **Best matching only** button (Figure 7). This feature is especially useful when more than one library is being searched, and some of the spectra in these libraries have same **CAS rn**'s associated with them.

The button to the right of the **Best matching only** button is the **View hit list search options** button. Selecting **View hit list search options** button will result in the display of a dialog box that lists the parameters of the currently displayed search including library search, constraints, limits, and type of search (Figure 10).



**Figure 10. List of options used for the currently displayed search results.**

The last button on the left, the **View substructure information** button, displays the **Substructure Information** dialog box. 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 this compound's molecular weight and estimate of the numbers or atoms of chlorine and/or bromine that may be contained. This is especially useful with either a Neutral Loss or a Hybrid Neutral Loss "Similarity" search.

## Title Bar, Tool Bar, and Status Bar

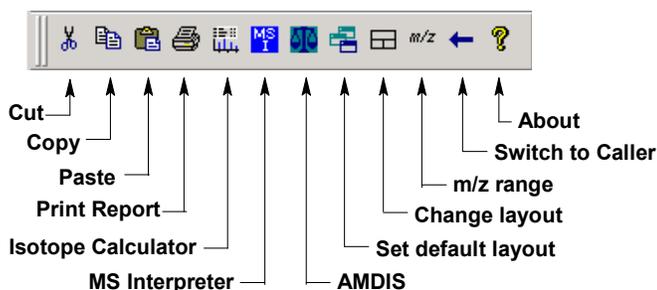
At the top of the display of each of the NIST MS Search Program's tab is a **Title Bar**. The contents of a title bar of the active tab is also displayed in the **Title Bar** of the Program window. When the Lib. Search or Other Search tab is displayed, this **Title Bar** will contain information about the currently displayed search. In the case of a **User spectrum** search, this will include the search type used, whether or not constraints were used, and if any type of limits were applied to the search. In the case of the Other Search tab, the **Title Bar** will contain the type of search that was done (i.e., **Formula**, **Molecular weight**, **Any peaks**, etc.) and whether or not constraints were used. When the Compare tab is displayed, the **Title Bar** contains the number of spectra available for comparison regardless of the number displayed.

At the bottom of Program's display, below the Program's tabs, is the optionally displayed **Status Bar**. The **Status Bar**'s display can be turned off by selecting or deselecting **Status Bar** from the **View** menu. When a **User 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 **Search Algorithms** in Appendix 5). 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 library(ies) being searched.

There are five fields on the right side of the **Status Bar**. The left-most of these five fields will have displayed the selected search type in the "Spectrum Search Type" area of the Search tab of the **Library Search Options** dialog box (Neutral Loss, Hybrid, or Simple if the "Similarity" search is selected; and Quick or Ident depending on whether a Quick or Normal "Identity" search has been selected).

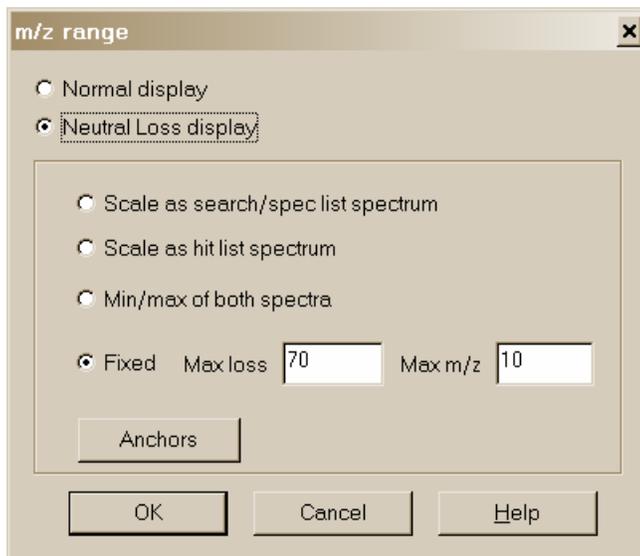
The next four fields to the right of the box indicating the selected search type pertain to the currently displayed search results. The first box to the right contains the type of search that was used in the displayed search results. The next box to the right will have contained "Rev" if the Hit List is sorted according to the Reverse Match value (the Match Factor obtained by ignoring all peaks that are in the sample spectrum but not the library spectrum). To set this mode select Reverse Search *check box* in the Spectrum Search Options section of the Search tab of the **Library Search Options** dialog box. The third box will contain the number used as a precursor value in a Neutral Loss or Hybrid "Similarity" search. If an "Identity" search has been carried out, the fourth box will contain Penalize if the "Penalize rare compounds" *check box* was selected in the Spectrum Search Options section of the Search tab of the **Library Search Options** dialog box.

The **Tool Bar**'s display (Figure 11) is optional by selecting it from the **View** menu. The functionality of its button **Switch to Caller** and **Print Report** is also available on the **File** menu; the Isotope Calculator, MS Interpreter, and AMDIS also may be launched from the **Tools** menu.



**Figure 11. NIST MS Search Toolbar**

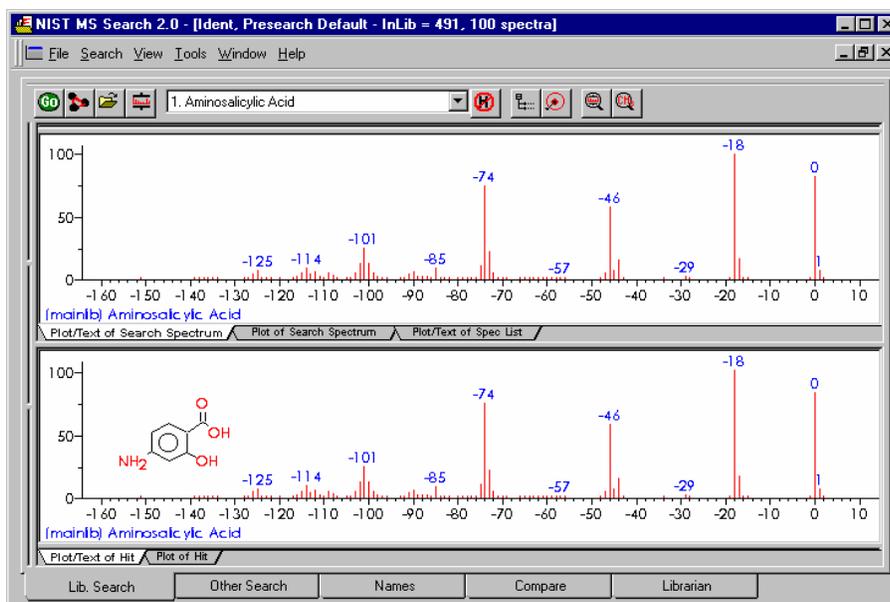
A Tool Tip is associated with each of the buttons with an explanation of the button's function. The  button will return all displays on the currently viewed tab to the default values. The  button toggles through three separate views for the currently displayed Program's tab.



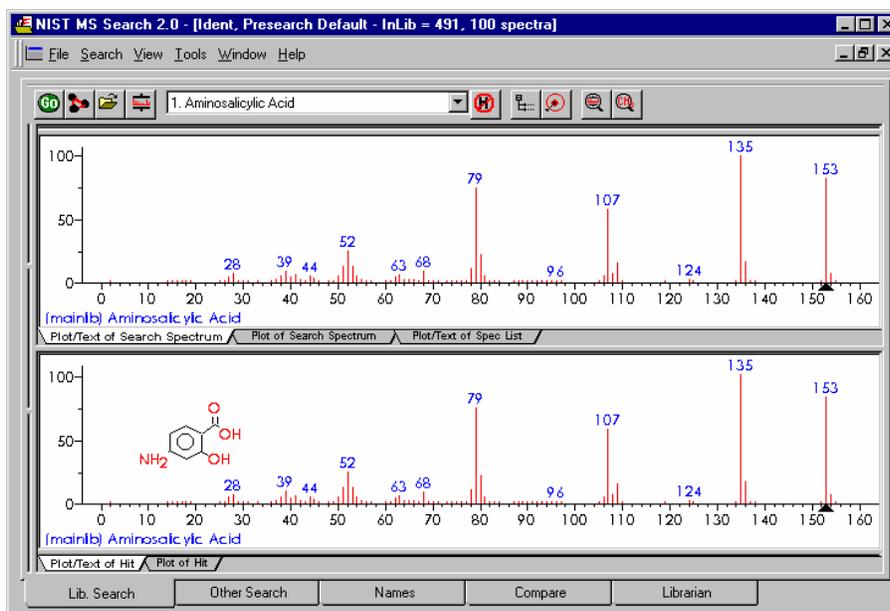
**Figure 12. *m/z* range Spectra Display dialog box.**

The *m/z* range button displays dialog (Figure 12) 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.

An explanation of each of the items in this dialog box can be found by selecting the dialog box's **Help** button. 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.



**Figure 13. Neutral loss labeling of mass spectral peaks. Top: sample spectrum. Bottom: spectrum of first library match.**



**Figure 14. Same display with normal peak labeling.**

### Neutral Loss Display

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).

A feature of the MS Search v.2.0d is the ability to display a spectrum with neutral loss labeling. If a Neutral Loss search is performed, the sample spectrum and the library spectra are labeled as neutral losses with the first label on the right-most peak being zero. Selecting Neutral Loss **anchors** is explained in the Help files (topic  $m/z$  range).

## Spectral Analysis Utilities for NIST 05

### Automated Mass Spectrometry Deconvolution and Identification System (AMDIS)

Included as a separate utility, AMDIS attempts to reconstruct original mass spectra for individual components in arbitrarily complex GC/MS and LC/MS reconstructed total ion current (RTIC) chromatograms; and if a target library is provided, ADMIS can directly identify target compounds. AMDIS is especially useful when an RTIC chromatographic peak represents multiple components. Regardless of each component's concentration, pure mass spectra are deconvoluted for analyses. AMDIS was developed by NIST for the Defense Weapons Agency (Department of Defense) for verifying compliance with a major international treaty (Chemical Weapons Convention) as ratified by the United States Senate in 1997. In order 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 have been tested.

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 Library. AMDIS has a range of other features including the ability to search the entire NIST 05 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.

The instruments that are supported in their native file format are:

- BrukerDALTONICS GC-MS
- Finnigan (GCQ, INCOS, ITDS, ITS-40, ITD-700/800, ITMS)
- ChemStation (GC-MS & LC-MS) and MS Engines
- Inficon
- JEOL
- MassLynx NT Formats
- Micromass
- Perkin-Elmer TurboMass and ITD-700
- Shimadzu MS Files QP5000 and QP5050
- Shrader
- ThermoQuest Xcalibur
- Varian Saturn Files (Both MS and SMS)
- Viking

In addition, the NetCDF format, which is supported by many manufacturers, is supported by AMDIS. Other formats will be added in future releases. Contact NIST for future information.

### MS Interpreter

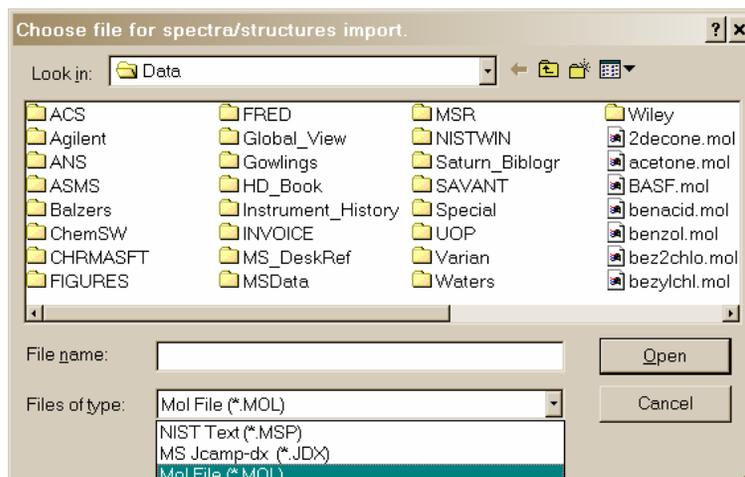
This tool was developed to aid NIST evaluators in their analysis of mass spectra. In one integrated program. It permits a wide range of calculations on a mass spectrum, using a proposed chemical structure if available. Spectra and structures are associated in the library facility of the NIST MS Search Program v.2.0 discussed above, and the program is activated by right-clicking a mass spectrum and selecting "Send To", "MS Interpreter" or from the **Tools** menu. Peaks in the spectrum originating as a logical fragment of the molecule are marked, and corresponding fragments, derived from thermodynamic considerations, may be highlighted. It also allows the analyst to keep track of important neutral losses, both from the parent or a derived ion and to compute readily possible formulas for any peak or neutral loss and isotopic patterns as desired. Observed isotope clusters can be compared to theoretical predictions subject to a number of user-specified constraints. MS Interpreter is a separate program that also has an icon in the **NIST Mass Spectral Database** program group. It also has a simple Help screen that can be called from the program by selecting **Help** on its Menu Bar or by pressing 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 MS Interpreter main window.

## EXAMPLE SEARCHES

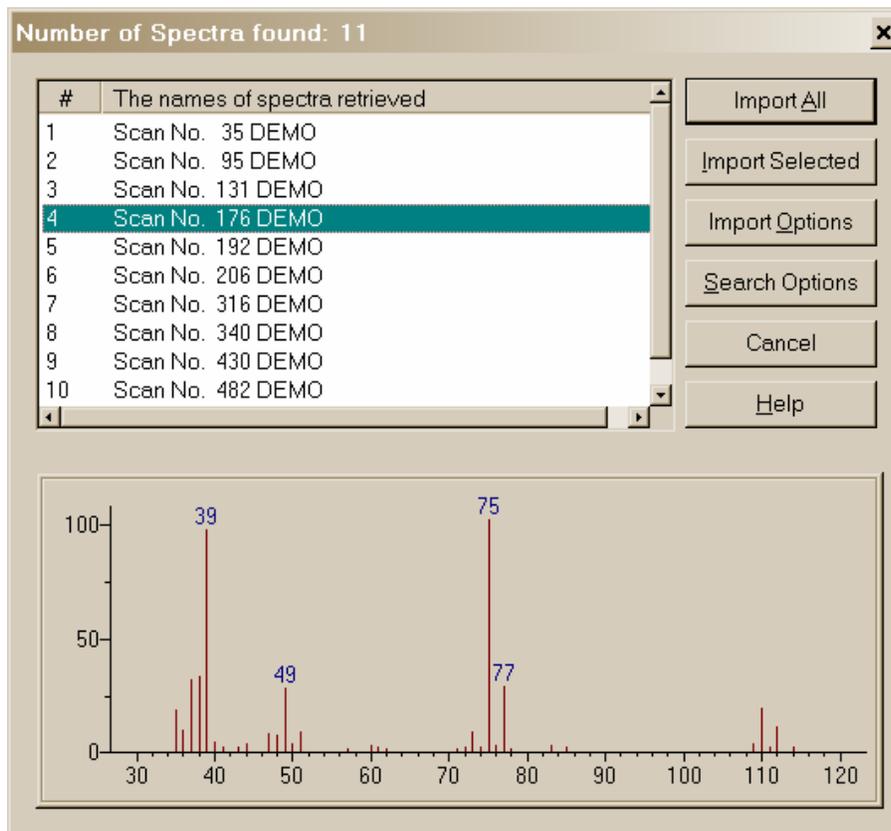
### User Spectrum and Structure Searches

This section describes how to use the NIST Mass Spectral Search Program to show how you can find spectra that are similar to that of an unknown compound. This can be accomplished by importing the spectrum from a third-party mass spectral data analysis program or from a text file. You can also use the Librarian to enter the  $m/z$  and intensity pairs that make up a mass spectrum. The **User spectrum** search and the **Structure** search is usually carried out from the **Lib. Search** tab display by pressing Library Search or Structure Search button. Another way of starting a search is to select and **click with the right mouse button any spectrum** in a Bar Graph pane or Text Info pane in any tab or any List of spectra or Subtraction spectrum in the Compare Window and select the desirable search from the floating menu. To search for more than one spectrum at once make a multiple selection in any List of spectra (Hit List, Spec List, or Other search list except Name Search List), click the list with the right mouse button, and select Library Search or Structure Similarity Search from the floating menu.

Spectra and structures can be imported from files or third-party programs through various types of automations, described later in this section. Spectra or structures can be imported from a file by selecting **File** from the Main Menu Bar and selecting **Open** from the **File** menu (Figure 15). Selecting a file will result in display of the **Structure Import** dialog box or the **Spectrum Import** dialog box. The **Structure Import** dialog box is self-explanatory. The **Spectrum Import** dialog box (Figure 16) is more complex. When a file containing more than one spectrum is selected, one or more selected spectra or all of the spectra can be imported by using either the **Import Selected** or the **Import All** button, respectively. Pressing the **Import Options** button will open the **Spectral Import Options** dialog box (Figure 17). This is where information about the  $m/z$  values of peaks is controlled.

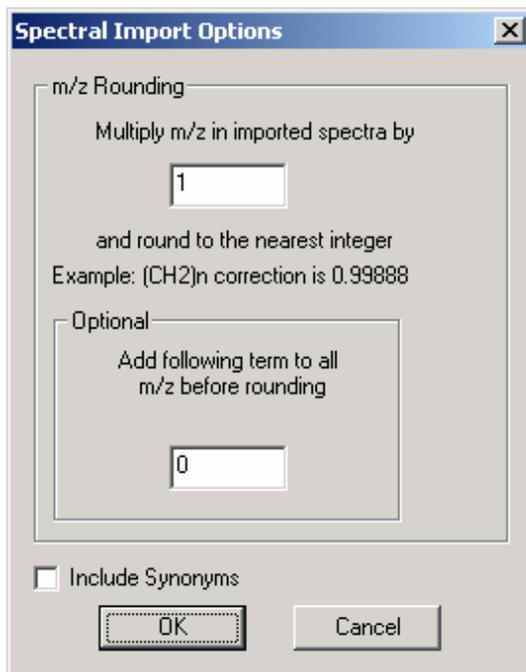


**Figure 15. File Selection dialog box with some of the available file types. MSP and JDJ files are spectra, and MOL files are structures.**



**Figure 16. Spectrum Import dialog box with a single spectrum from a multiple spectrum file highlighted and displayed in a bar-graph format.**

The two fields in the **Spectral Import Options** dialog box are used to make **mass defect** corrections. The difference in the integer mass and the exact mass of an isotope 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  $^1\text{H}$  isotope is 1. The exact mass of this isotope is 1.00725. The  $\text{C}_{50}\text{H}_{100}$  ion has a nominal mass (the sum of integer mass of the most abundant naturally occurring stable isotope of each element times the number of atoms of those elements) of 700,  $(50 \times 12) + (100 \times 1)$ . The integer value for  $\text{C}_{50}\text{H}_{100}$  based on a calculation of the exact mass of each of these elements is 701  $(12.000000 \times 50) + (100 \times 1.007825) = 700.7825$ . The **mass defect** for  $\text{C}_{50}\text{H}_{100}$  is 0.7825, which is equal to about 100 millimass units per 100 mass unit. The NIST MS Search value deals with only integer mass values when searching the NIST05 Library. Spectra are imported with integer mass 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 in order to be properly searched against the NIST05 MS Library or another database that contains only integer  $m/z$  values.



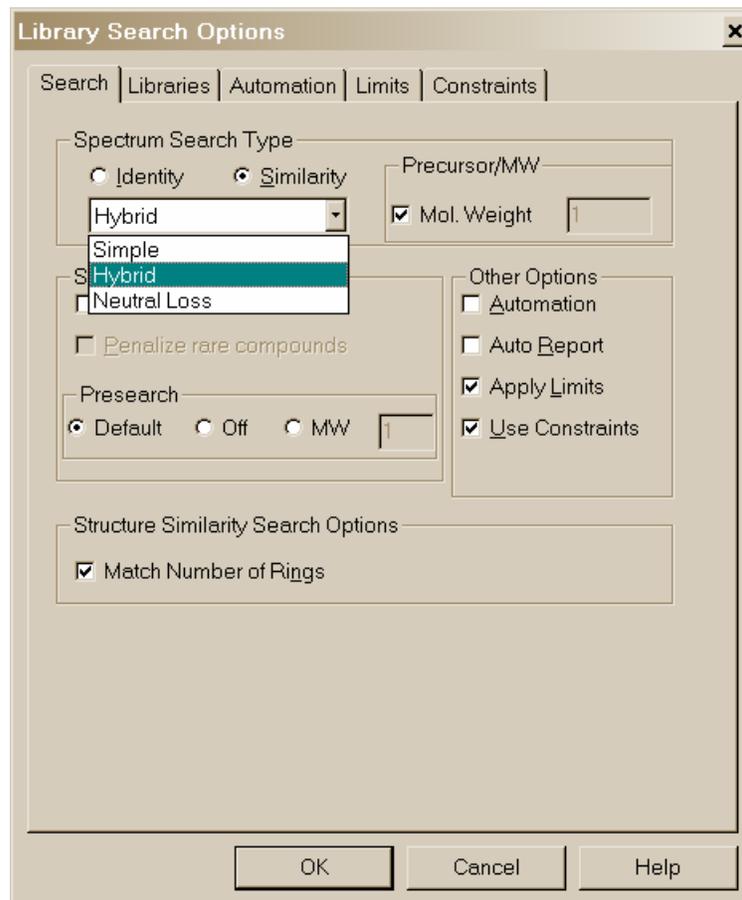
**Figure 17. Spectral Import Options dialog box stating that imported spectra will have only 1 value/integer  $m/z$  and no mass defect correction.**

The first box following the statement “Multiply  $m/z$  in imported spectra by” is where a mass defect factor can be entered. 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 chlorine or bromine (both of which have a significant negative mass defect) a different factor will have to be calculated. In this case, it may be desirable to use the field preceded by “Add following term [value] to all  $m/z$  values before rounding”.

## Library Search Options

Pressing the **Library Search Options** button will display multiple-tab **Library Search Options** dialog box (Figure 18).

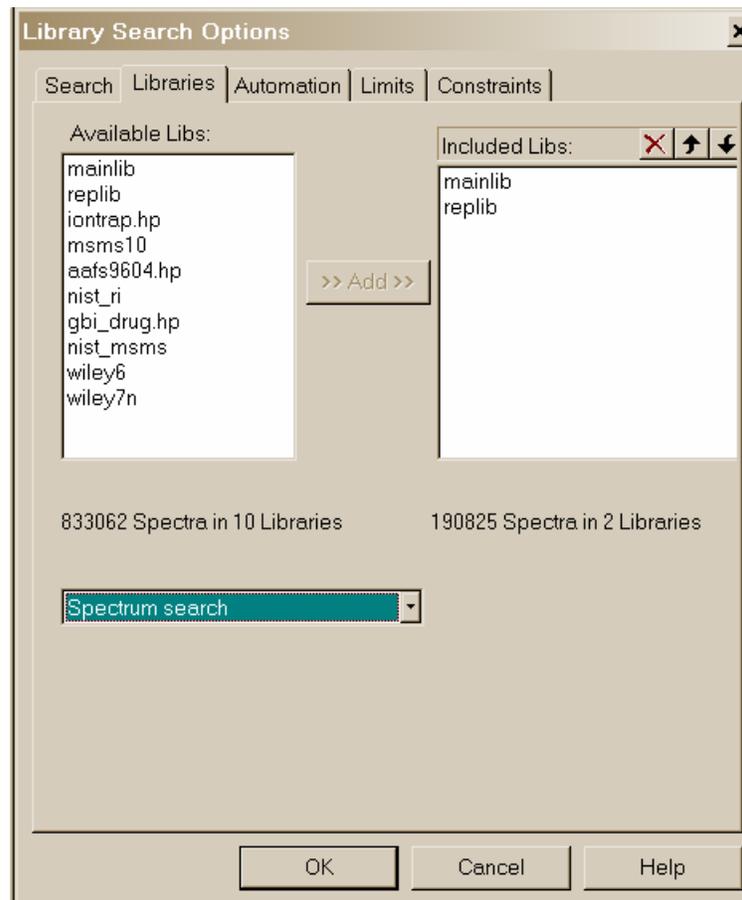
A number of different types of spectral comparison routines for choosing the best matching spectra are available. The routine to be used is selected from the **Library Search Options** dialog box’s Search tab.



**Figure 18. Library Search Options dialog box's Search tab showing options for a "Similarity" search.**

The two search types are "Similarity" (with four options) and "Identity" (with two options). These different searches and their options are explained in the Help Screens (topic Spectrum Search Type).

The Search tab of the **Library Search Options** dialog box also has a **Structure Search constraint** 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.



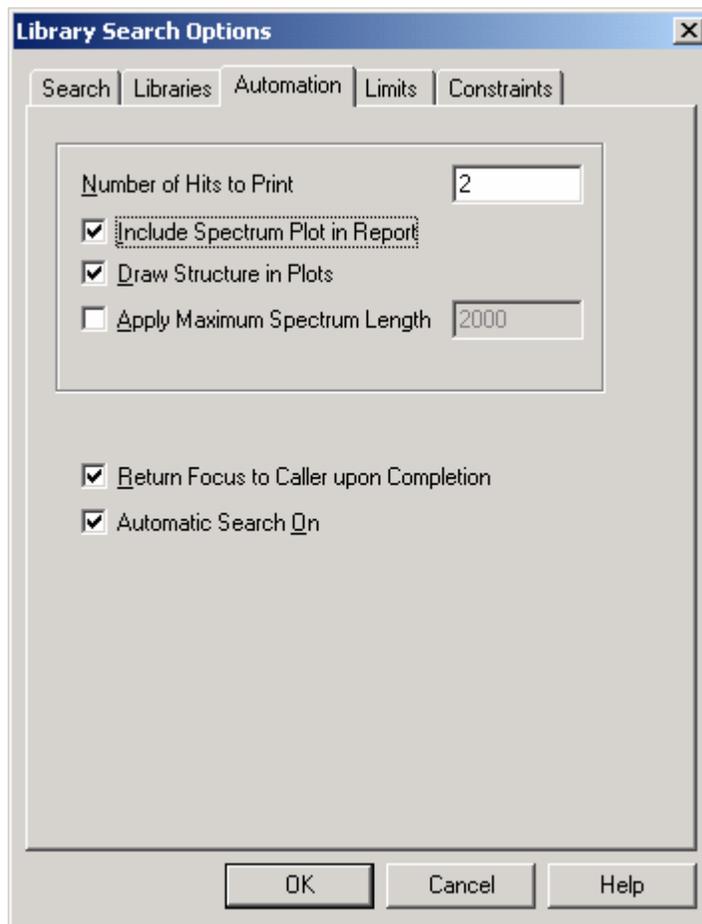
**Figure 19. Library Search Options dialog box's Libraries tab showing selected libraries and available libraries.**

You can use the Libraries tab of this dialog box to select the libraries to be included in the **User spectrum** search. You can have active up to 16 libraries at the same time. This includes the NIST 05 Main Library, **mainlib**, and the NIST 05 Replicates Library, **replib**. By default, two other NIST 05 libraries are also installed, the **nist\_ri** and **nist\_msms**. This means that there is only space to accommodate an additional 12 user libraries. If more MS libraries need to be accommodated, the **nist\_msms** and **nist\_ri** libraries can be moved to another folder. To add libraries to be searched in the "Available Libs" pane of the dialog box, highlight them and click on the **Add** button. To exclude libraries, highlight them in the "Included Libs" pane and then click on the **X** button above the upper right portion of this pane. The order in which libraries are searched can be changed by highlighting the library to be moved in the "Included Libs" pane and clicking on the Up or Down arrows in the upper right portion of this pane. Each click will move the library one position.

Below the "Available Libs" and "Included Libs" panes is a drop-down list box. This box allows for switching between the lists of libraries to be included in **Structure** searches or **Spectra** searches. These two different types of searches can have different sets of libraries.

### Automations

When a **User spectrum** search or a **Structure** search is carried out on a spectrum or a structure that is imported from a file or third-party program like the Agilent Technologies ChemStation or the ACD's structural drawing program ChemSketch, a search can be automatically launched. Automatic searching and/or printing can be turned on or off from the Search tab of the **Library Search Options** dialog box by the respective selection or deselecting of the appropriate *check box* (Figure 18, 19).



**Figure 20. Library Search Options dialog box's Automation tab.**

The **Automation** tab of the **Library Search Options** dialog box is used in conjunction with these automated searches (Figure 20). **Check box Automatic Search On** (Figure 20) has exactly same functionality as **Automation** on Figure 18: it turns on automatic searching.

Search results can automatically be printed whether or not the search is automatically launched. **Check box Auto Report** (Figure 18) turns on this feature. **Number of Hits to Print** field is located in the **Automation** tab (Figure 20). In addition, the report in the same format can be printed for any displayed search result by selecting **Print Auto Report** from the **File** menu.

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

If the "Apply Maximum Spectrum Length" **check box** is selected, a number can be entered for the maximum  $m/z$  value to appear on the spectrum's abscissa. If peaks are present at higher  $m/z$  values, the abscissa will have a right-pointing arrowhead at its right end.

### Limits

The function of the limits of the starting and ending  $m/z$  values of a **User spectrum** search and the lowest value of a mass spectral peak 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

A **User spectrum** search can be constrained by a number of factors such as what (and how many atoms of each) elements must be present in the compounds that produce retrieved spectra, the molecular weight range of hits, whether or not the compounds that produce retrieved spectra are in various other databases, what *m/z* value peaks must be present (and their relative intensity range in retrieved spectra), and what name fragments must be associated with retrieved spectra. These constraints are explained in the Help file, which is displayed by selecting the **Help** button on the Constraints tab of the **Library Search Options** dialog box.

## The Search Menu

The Other Search tab's options are:

Option	Description
<b>Formula</b>	Finds spectra in selected libraries of compounds with specified formula.
<b>ID Number</b>	Finds spectrum (or spectra) in selected library of having entered ID number (or range of ID numbers).
<b>Molecular Weight</b>	Finds spectra in selected libraries of compounds having specified nominal molecular weight.
<b>Any Peaks</b>	Finds spectra in selected libraries based on user-specified peaks.
<b>CAS Number</b>	Finds spectra of the compounds that have the entered CAS registry number.
<b>Sequential Method</b>	Searches selected libraries using a set of constraints provided by the user.
<b>NIST Number</b>	Retrieves a spectrum with the unique NIST Mass Spectral Data Center's Archive identification number (in mainlib and replib only).

These options are also displayed and may be chosen by selecting the drop-down list box on the further most left side of the Other Search tab's display (to the right from the binocular button). Pressing the binocular button starts the currently selected search.

## Search by Any Peaks

The **Any peaks** search became very popular when it was first developed over 20 years ago for use with the on-line Mass Spectral Search System from EPA and NIH. This has evolved into the powerful tool that exists in this version of the NIST Mass Spectral Search Program.

Select **Any peaks** from the Other Search tab's menu. This will display the **Any Peaks Search** dialog box. Click the *m/z* field. The blinking vertical text cursor will be in the "m/z" field. "Normal" will be displayed in "Type" field. *Type 82 and press <Enter>*. This moves the cursor to the "From" field. *Type 70 and press <Enter>*. This moves the cursor to the "To" field. *Type 100 and press <Enter>*. This will enter the peak with its type and intensity range. The cursor is now in the "m/z" field.

*Put the mouse pointer on the ↓ on the right side of the "Type" field and click the left Mouse button. Put the Mouse pointer on "Loss" in the displayed menu and click the left Mouse button again.* This will cause the cursor to reappear in the "m/z" field. "Loss" will be displayed in the "Type" field. The first peak you are entering is an observed peak in the mass spectrum, and it is possible that it is the base peak. The second peak you are entering represents an ion formed by a loss from the molecular ion. *Type 31 and press <Enter>*. *Type 5 and press <Enter>*. *Type 25 and press <Enter>*. The retrieved spectra must have a peak at 31 *m/z* units lower than the molecular weight that exhibits intensity between 5% and 25% of full scale. This reduces the number of spectra in the NIST/EPA/NIH Mass Spectral Main and Replicate Library that match the entered criteria from 1390 for the first peak to 117 that have both peaks.

DAVE: You need to describe the relative intensity. It is especially important for anyone using SIM searches and wanting to understand the rates of false positives. I'm not sure where it goes since you can only use it with the sequential method, but it is important.

The next peak will be a "Maxmass" peak. *Click on the ↓ on the right side of the "Type" field. Click on "Maxmass". Type 303 and press <Enter>*. *Type 1 and press <Enter>*. *Type 50 and press <Enter>*. There will only be seven spectra that contain all three peaks of the designated type and over the designated intensity range.

Select the **Search** button in the **Any Peaks Search** dialog box to copy these 7 spectra into the Hit List.

## Search by Name

One of the most valuable attributes of the NIST Mass Spectral Search Program is to be able to search through hundreds of thousands of names to find mass spectra. When you are given a common name of a compound and want to know if its mass spectrum is in the NIST/EPA/NIH Mass Spectral Library (NIST 05) of approximately 163,000 compounds, you can begin typing the name until it appears in the "Hit List" pane of the Name Search tab. Highlight the desired name, and the display in the **Plot**, **Structure**, **Text Info** and **Hit List** Windows will be refreshed with the appropriate displays 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. You are going to search for the mass spectrum of a plant growth regulator, GIBBERELLIN A9. Begin typing the name. Stop after entering each letter. After you have typed the letter N, you will see that the 13<sup>th</sup> name in the window is your target. You may also paste the name from the Windows Clipboard into the "Name" field by simultaneously pressing keys Shift and Insert.

---

## BASIS FOR INTERPRETATION OF THE LIBRARY SEARCH RESULTS

When you do an "Identity" search with an unknown spectrum (**User spectrum** search), your results will be associated with three numbers for each spectrum shown and one value for the search. These three numbers (which are actually displayed and under your control) are a Match Factor for the unknown and the library spectrum (direct match), a Match Factor for the unknown and the library spectrum ignoring any peaks in the unknown that are not in the library spectrum (reverse search), and a Probability value (Columns Match, R.Match and Prob.) The first two numbers are fairly straightforward. Each is derived from a modified cosine of the angle between the spectra. 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, unknown spectra with many peaks will tend to yield lower Match Factors than similar spectra with fewer peaks. The Probability value and the value for the search (InLib, located in the lower right corner of the Hit List) require more explanation.

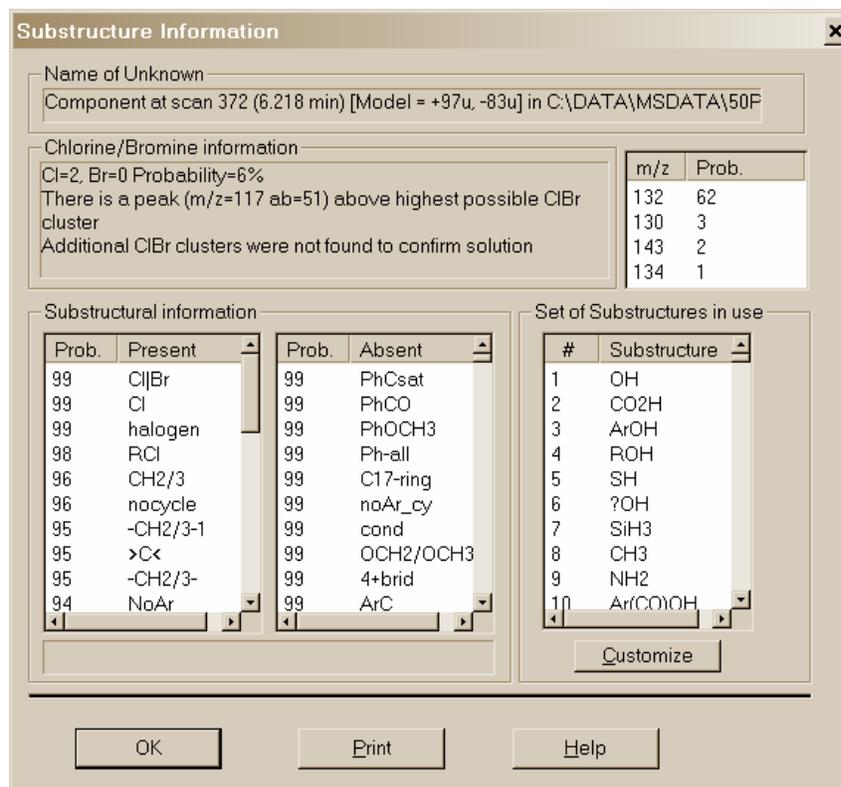
The Probability value for a hit is derived assuming that the compound is in the searched libraries and relies only on the differences between adjacent hits in the hit list. It was derived from an analysis of the results of searching the NIST/EPA/NIH Main Library with a set of replicate spectra (given in the Replicates Library). Since the total probability of the compound being in the searched libraries is assumed to be one, the relative probability of each of the hits requires only the difference values. The other factor (InLib) is a measure of the probability of the compound being in the searched library. This was 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, was parameterized. The parameters were 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 library is large (the number of hits vs. their Match Factors is displayed in Hit Histogram pane located just above the Hit List, see Figure 8).

Like all statistical results, these probability calculations rely on the data sampled. For example, you may find that a compound that has very few similar compounds (or more importantly very few similar mass spectra) will be identified in a more definitive way. Using examples from the Replicates Library and searching the Main Library using a compound like 'folpet', you will find a high probability for the first hit and a high value for InLib. In contrast, if you take replicates of 'cyclohexanol', you will find that not only are the probabilities much lower but also the InLib value is much lower; and, in some cases, the best match 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 them is limited. In many cases, the best that the search can do is to provide you with a class of compounds that have similar mass spectra and, usually, similar structure.

The values of the InLib parameter are meant as guide posts. 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, negative values will occur when there are a large number of 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, reference Stein, S.E. *J. Am. Soc. Mass Spectrom.* **1994**, 5, 316–323.

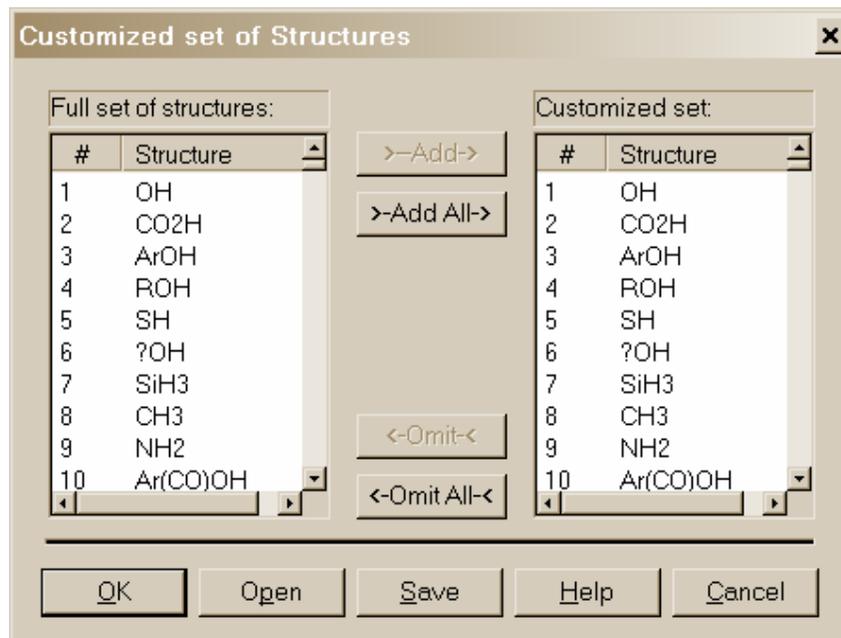
## SUBSTRUCTURE INFORMATION



**Figure 21. Substructure Information dialog box.**

The **Substructure Information** dialog box has the name of the spectrum that was used to create the hit list displayed at the top. There are two panes below this: "Prob. Present" and "Prob. Absent". These two panes 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 you do not understand one of the abbreviations, highlight it by using the Mouse and a detailed explanation appears at the bottom of the Substructural information area. In addition, the dialog box contains an area labeled "Chlorine/Bromine Info", which gives the probabilities and numbers of chlorine and/or bromine atoms present. The results of a molecular weight estimation from the hit list is presented on the right side of the dialog box.

Just above the molecular weight information is the "Set of Substructures in use" pane with a list box and a **Customize** button. The list box displays all the substructures in the current set.



**Figure 22. Customized Set of Structures dialog box.**

**Customize:** When this button is selected, the **Customized Set of Structures** dialog box is displayed. This 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” pane. The list of substructures included in the present file is shown in the “Customized set” pane. The first time a customized list is created, both panes will contain the same list.

You may omit substructures from the “Customized set” pane or add from the “Full set of structures” pane. Either action occurs on the structures highlighted by Mouse action in the panes 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.

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” pane 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 Spectrom.* **1995**, 6, 644–655).

---

## USE WITH INSTRUMENT DATA SYSTEMS

The use of the automation features described in the **Automations** section of this manual was implemented to enhance the performance of the NIST MS Search Program when used with proprietary mass spectral data systems.

### Location of the NIST MS Search Program

For the implementation it may be necessary to determine the location of the NIST MS Search program files and the location of the NIST MS Search work directory. These may 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:\NIST05\MSSEARCH\
WorkDir32=C:\NIST05\MSSEARCH\
Amdis32Path=C:\NIST05\AMDIS32\
AmdisMSPath=C:\NIST05\AMDIS32\
```

The string beyond "Path32=" is the path to MS Search program directory; the string beyond "WorkDir32=" is the path to the MS Search work directory. Two other items refer to AMDIS and its connection to the MS Search.

### Implementation

If your data system is capable of exporting spectra to the NIST format (described in the **USER LIBRARIES** section of this manual) and you can call a Windows program from within your data system, this feature is available to you. Implementation of this feature has been provided on some data systems.

The command string used to start or bring into focus the NIST MS Search Program for Windows is:

```
C:\NISTMS\NISTMS$.EXE <space>/instrument
    assuming the program is in the C:\NISTMS 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 same directory as the NIST MS Search Program, have the name AUTOIMP.MSD, and contain the text string describing the name and location of the second locator file. For example:

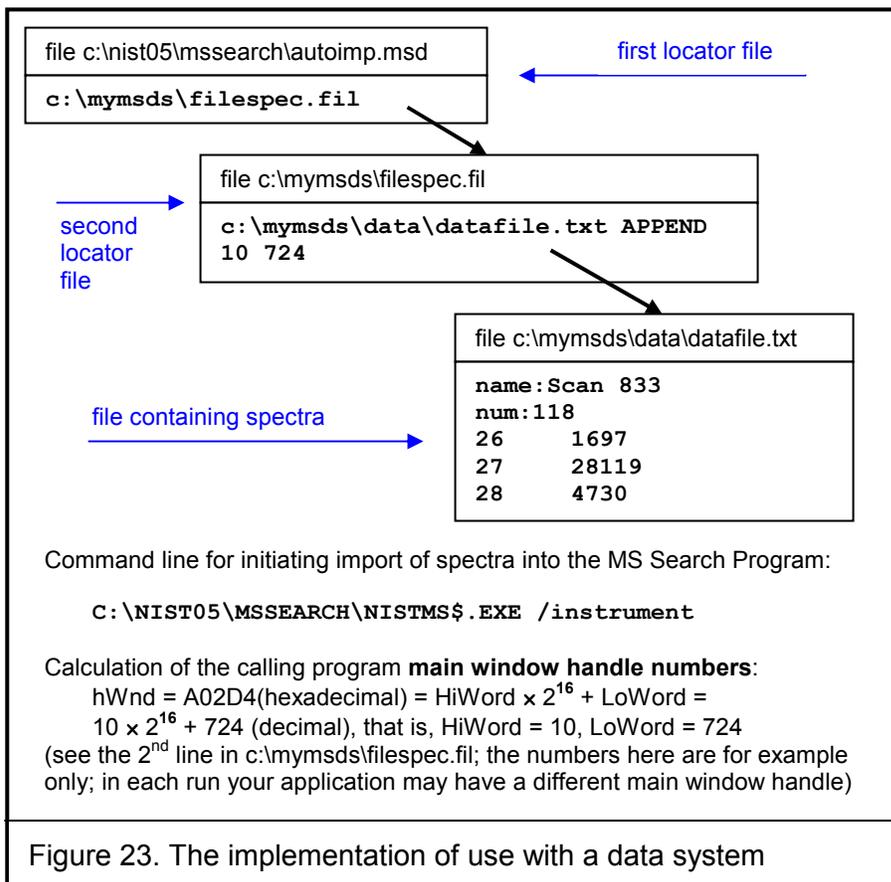
```
C:\MYMSDS\FILESPEC.FIL
    where: C:\MYMSDS is the directory where the file, FILSPEC.FIL, resides.
```

This file can be created with any text editor.

2. The second locator file is written each time you save spectra to a separate file to be imported by the MS Search. 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. You can specify whether the spectra in the NIST MS Search Program's **Spec List** Window are 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
    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 the second line containing main window
    handle numbers of the datasystem application (see Figure 23 for details).
```

This second locator file is deleted each time the NIST MS Search Program is brought into focus and spectra are imported. Figure 23 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.



NIST MS Search (nistms\$.exe) Command Line Options	
Option	How it works
/INSTRUMENT	Normal search. Imports and optionally searches spectra (see Figure 23 for details on the implementation), activates MS Search window, activates switch back button
/INSTRUMENT /PAR=2	Background search. Imports and optionally searches spectra (see Figure 23 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 directory. Number of hits is determined by the "Number of hits to print" in the Automation tab (Figure 20)
/PAR=4	Start logging. Appends hit lists of Spectrum and Structure searches to the file NISTLOG.TXT located in the MS Search directory. Number of hits is determined by the "Number of hits to print" in the Automation tab (Figure 20)
/PAR=8	End 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

Version 2 of the NIST MS Search Program provides more support of structures than previous versions. There is a Structure Search Command. If a structure rather than a spectrum is in the "Spec. List" pane of the Lib. Search tab, this structure can be searched to retrieve spectra of all compounds with similar structures by clicking on the **Structure Search** button (second button from the left) with the structure highlighted in the "Spec. List" pane.



When NIST MS Search v. 2.0 saves a structure to the Windows Clipboard using the right mouse button menu's 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's directory (by default, C:\NIST05\MSSearch). Other programs can either use the structure as it appears on the Windows Clipboard or this file for structure importation.

If an application is associated with files that have extension .MOL then the structure may be sent to that application by selecting in the right mouse button menu "Send To" and "Default Structure Editor".

If a spectrum containing a chemical structure is selected, the RMB menu's 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's 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's ChemSketch starting with the contents of clipboard.mol 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:\NIST05\MSSEARCH\NISTMS$.EXE /MOL=<molfile>
```

assuming the NIST MS Search Program is in the **C:\NIST05\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:\NIST02\MSSEARCH\NISTMS$.EXE /MOL="c:\program files\a.mol"
```

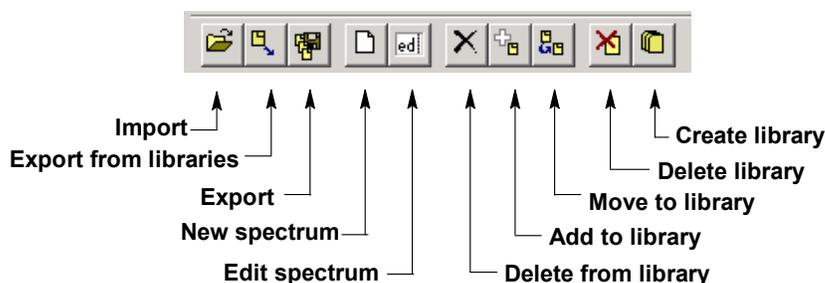
A third-party program can obtain the path to NISTMS\$ from Path32 in the [NISTMS] section of WIN.INI (see **Location of the NIST MS Search Program** in the "USE WITH INSTRUMENT DATA SYSTEMS" section above).

## USER LIBRARIES

One of the primary features of the NIST MS Search Program is the ability to do comparisons of user-generated mass spectra and create user libraries to search against. The Program will allow up to sixteen MS libraries, including libraries from NIST 05 MS Library. The user libraries may be searched in conjunction with the NIST/EPA/NIH Mass Spectral Main and/or Replicates Libraries, or they may be searched by themselves. A maximum of sixteen libraries can be included for search at one time. The Program 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** tab of the **Library Search Options** dialog box displayed (Figure 19).

User libraries are maintained as subdirectories under the MS Search Program's root (work) directory. The name given to the user library is the name of the subdirectory. This subdirectory will contain a number of database files and indexes constituting the user library. These files exist in a proprietary format. The spectra and compound information are put in these files through the **Librarian** tab or by using Lib2NIST library utility.

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. Spectra can also be entered through the Program. This is done by using the **Spectrum Information** dialog box called with the **New spectrum** button in the **Librarian** tab toolbar.



**Figure 24. Librarian Toolbar**

User libraries are easy to create and maintain. You may want to create them using specific sets of spectra from the NIST/EPA/NIH Main or Replicates Library. Any spectrum in the **SpecList** can be added to a user library. Any spectrum in the **Hit List** Window, Other Search list, Names list, Bar Graph window, Text Info window, or Subtraction pane in the Compare window can be copied to the **Spec List** Window by selecting "Send To", "Spec List" from the right mouse button menu. The size of a user library is limited by 65,535 spectra.

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 manufacturers' mass spectrometry data systems. You should check with the manufacturer of your mass spectrometer's data system to see if there is file compatibility.

Instructions on building user libraries are included in the **Librarian** Help screens. These also include the information necessary to add structures to user library spectra.

## NIST Text Format of Individual Spectra

The NIST Text file format is simple. A file can contain as many spectra as you want. Each spectrum must start with the field title "Name:". There must be something in this field in order for the spectrum to be imported (i.e., the datafile name and the scan number of the spectrum's fetus). The next required field title is "Num Peaks:". The contents of this field must be the number of mass/intensity pairs that make up the spectrum. Optional fields with the titles of "Comments:", "Formula:", "MW:", "Synonym:", and "CAS#:" can be between the "Name:" and "Num Peaks:" fields.

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#:" 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 mass/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.

When you create a file 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 **User 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 it is present in the NIST/EPA/NIH Main Library.

**Line 5:**     **CAS: 71-43-2** (Optional)

The actual mass spectral data (number of peaks in the spectrum) must 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 6:**     **Num Peaks: 18** (This prefix and the exact number of mass/intensity pairs is Required.)

**Line 7:** 26 430; 27 340; 28 40; 37 480; 38 611; 39 1411; 49 300; 50 1792;

**Line 8:** 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 masses need not be ordered. The exact spacing and delimiters used for the mass/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 mass intensity pair could be on an individual line. The following characters are accepted as delimiters (except "|"): |space|tab|,|;|:|(|) |[ ] | |

You can give the file any valid file name. However, it is best to use the extension "MSP". This is the recognized default extension in the MS Search Program when you want to import user library spectra.

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.

---

## APPENDIX 1: Important Information on Creating AUTOIMP Files Used in NIST MS Search V.2.0

It may be necessary for you to create AUTOIMP.MSD as the primary locator file used for the importation of spectra from third-party mass spectral data systems and/or AUTOIMP.STR for interaction between the MS Search Program and third-party drawing programs. If you are using Windows 98, Windows 2000, Windows NT, or Windows XP you may create these files using Notepad.

If you use Microsoft Notepad, the default extension is .TXT even though you type AUTOIMP.MSD or AUTOIMP.STR. The default settings for Windows Explorer will not show this .TXT extension. The file will not work unless the .TXT extension is removed. You can rename the file by highlighting the file in Windows Explorer and selecting "Rename" from the **File** menu. When saving the file using "Save As" option make sure the ANSI Encoding is selected.

---

## 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 latest versions of the ChemStation for GC/MS, both the **NIST Output** and **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 05) 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's **Spec List** Window, bringing the MS Search Program into focus. If the appropriate options (including Automation, see Figures 17 and 19) 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 you have an Enhanced Quantitation mode on your 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 **Select Default Search Engine**). Each of these menu options is explained below. If you want to add these options 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 05 MS Library by selecting **ChemStation Menu Modification** from the **NIST Mass Spectral Database** program group.

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 HPCHEMMSEXE or MSDCHEMMSEXE directory for these menu selections to be displayed.

When all the necessary macros have been installed in the ChemStation's MSEXE directory and the modifications have been made to the ChemStation macros, the following options will be available:

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's **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, you can 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 you to select the default library search to be evoked when you double click the RMB on the currently displayed spectrum. The standard PBM search is default until you select another 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 you to copy mass spectral libraries that have been created using the internal utilities of the ChemStation software (including structures) and other mass spectral libraries to user libraries 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 group.

An additional macro is found on the NIST MS Search Program's 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. You must be using G1034C, Version C.3.00.00 or later of ChemStation to utilize these features.

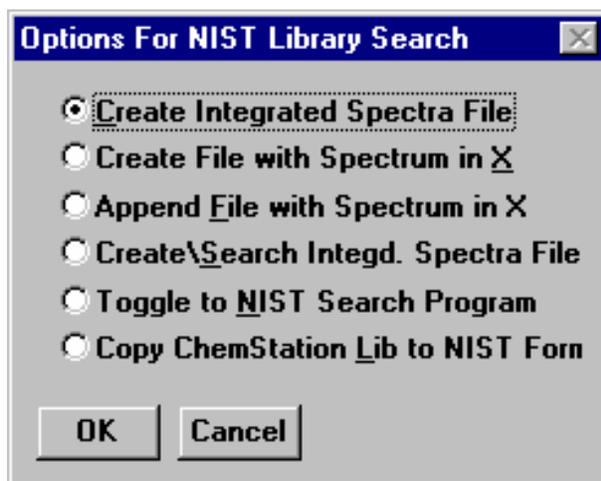
## **Additional Information Regarding Modes Other Than Enhanced Quantitation**

During the installation of the NIST/EPA/NIH MS Library (NIST 05), if you have selected the NIST MS Search Program as your ChemStation default search, each time you double click on a displayed spectrum, the spectrum will be prepended in the NIST MS Search Program's **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). You can change to one of the other above-referenced search methods by selecting the **ChemStation Default Search Mode** icon in the **NIST Mass Spectral Database** program group. YOU DO NOT HAVE TO MODIFY ANY MACROS TO SWITCH FROM NIST MS SEARCH PROGRAM AS THE DEFAULT SEARCH METHOD FOR THE DOUBLE-CLICK FEATURE OF THE ChemStation.

## Agilent\_NIST MACRO (NISTINT.MAC)

When you select **NIST Output** 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. This dialog box consists of six options and **OK** and **Cancel** buttons. Each option has a *radio button* next to it. Use the Mouse pointer and the Left Mouse button (LMB) on the *radio button* to select an option. Selecting the **OK** button results in execution of the option.

Items in the **Options For NIST Library Search** dialog box are selected by putting the Mouse pointer on the appropriate *radio button* and clicking the LMB. Then select the **OK** button to execute the desired option. If any of the first three options is 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's **SpecList** Window.

### Result of selecting **NIST Output** from the **Spectrum** menu

At the same time the HPNIST.TXT 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, you are prompted to **Prepend** or **Overwrite** the current contents. If you select **Prepend**, the new spectra are added to the top of the **Spec List** Window (prepended). If you select **Cancel**, the automatic import will be aborted. The HPNIST.TXT file is not deleted in the case of an abort. If you have selected the options of **Automatic Search On** and **Return Focus to Caller upon Completion** in the **Automation** dialog box, selecting **Cancel** returns the focus to the ChemStation software.

## Menu

The **Create Integrated Spectra File** option runs the ChemStation's 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 you will find background subtracted and averaged spectra. 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's **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's **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. You can return to the ChemStation software by holding down the <Alt> key and pressing the <Tab> key, or you can select the NIST Program's **SwitchBack Desktop Button**. You can then return to the NIST MS Search Program 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.
```

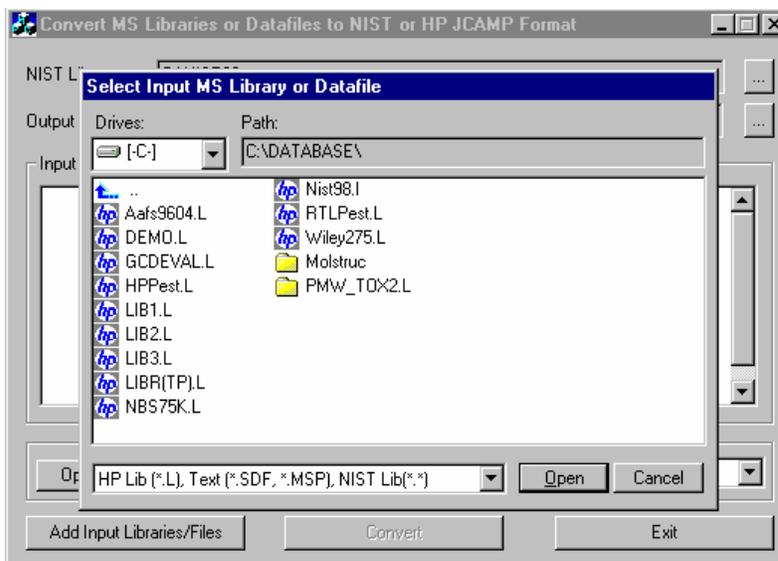
This File is in the \SUPPORT.HP directory of the CD ROM and must be copied to the \HPCHEM\MSEX directory.

When the Method is run, the AUTONIST Macro will execute. The chromatogram is auto-integrated, 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's **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's **Automation** dialog box, the spectra will automatically be searched. After the last spectrum is searched, the focus is returned to the HP MS software. In order for this macro to function properly, it will be necessary to have selected the above two options in the **Automation** dialog box.

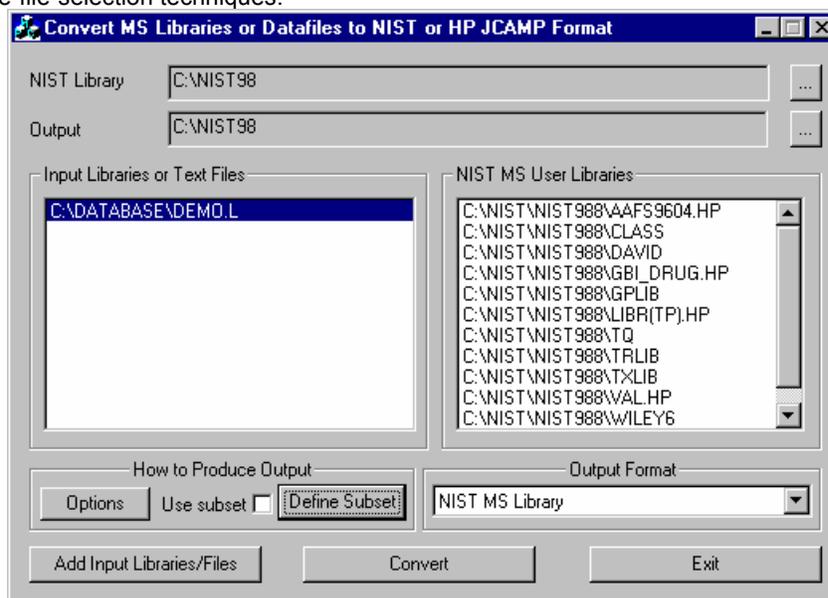
## APPENDIX 3: Copy ChemStation and Other Libraries to NIST User Library Format

This is a separate program called by selecting the **Convert ChemStation Lib to NIST Format** option from the **Options For NIST Library Search** dialog box or by selecting the **Lib2NIST Converter** icon on the **NIST Mass Spectral Database** program group. This program allows you to select mass spectral libraries in a variety of formats (along with structures or structures in separate files) and copy them to the NIST user library format. For all 32-bit Windows (Windows NT, Windows 95, and Windows 98) 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 – 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. When stated, the **Lib2NIST** converter program displays a file dialog box overlaid on the program's screen.



Opening display of Lib2NIST program

Select the library to be copied and then click on the **Open** button. The drop-down arrow will also let you select from a variety of JCAMP formats. More than a single library can be selected in this dialog by using the standard Windows multiple-file-selection techniques.



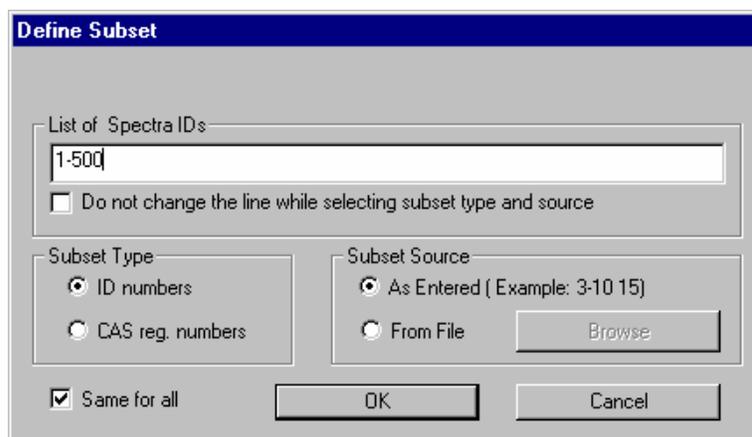
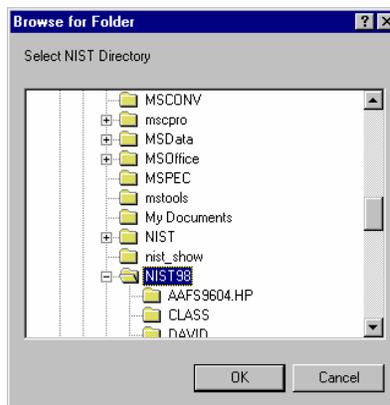
Lib2NIST converter program display

The selected library(ies) will now be listed in the “Input Libraries or Text Files” pane of the program’s display.

The **NIST Library** and **Output** locations can be changed 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.

Select the desired directory (folder) and then click on the **OK** button to change the location.

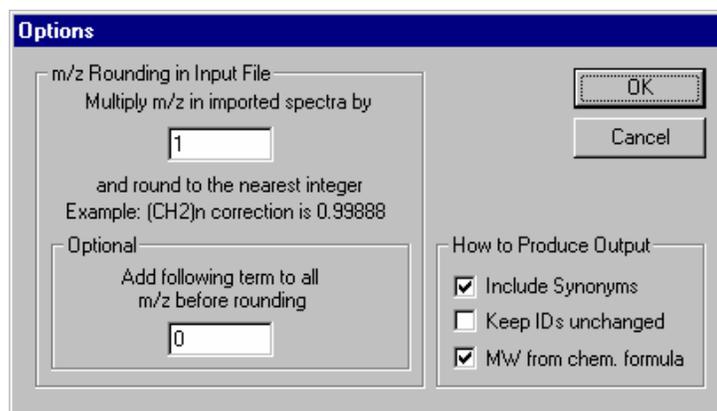
The **Define Subset** button will display a dialog box that allows for the choice of a range of spectral ID or CAS numbers. The “Use subset” *checkbox* is grayed, unless an entry has been made in the **Define Subset** dialog box.



**Define Subset dialog box**

After making any desired entries in the **Define Subset** dialog box, select the **OK** button to return to the Program’s main display.

The **Options** button on the Program’s display will display the dialog box shown below. This allows for the entry of mass defect corrections to be applied to *m/z* values of imported spectra and whether or not to: 1) include synonyms from the source file, 2) calculate molecular weights (nominal mass) from formula, and 3) retain the ID numbers from the source library.



**Options dialog box display**

Select the libraries to be converted in the “Input Libraries or Text Files” pane of the Program’s display 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.rtf 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.

---

## **APPENDIX 4: Using the NIST MS Search Program with ThermoQuest Xcalibur Software**

The ThermoQuest Xcalibur software uses the NIST MS Search Program as its only library search engine. A copy of the NIST MS Search Program and AMDIS is provided with every copy of the Xcalibur software. The NIST/EPA/NIH Mass Spectral Database is an option from ThermoQuest, which is only provided at an additional charge.

To add the NIST/EPA/NIH Mass Spectral Database to the Xcalibur software, run the NIST MS Search Program v.1.7 installation. This will locate the NIST MS Search Program and the AMDIS directories, which are:

C:\Program Files\NISTMS\MSSEARCH\NIST\$.EXE  
C:\Program Files\NISTMS\AMDIS32\AMDIS\_32.EXE

respectively. The Database will then be properly installed along with any necessary program updates.



---

## APPENDIX 5: Search Algorithms

There are two general ways that the NIST MS Search Program can retrieve library spectra that resemble the submitted spectrum. These 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 unknown 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). A great deal of effort has been devoted to optimize both ways of searching.

### 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 unknown spectrum. 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 like "throwing out the baby with the bathwater". 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 were tested using the 12,592 spectra that comprise the NIST/EPA/NIH Selected Replicates Library. The best performing algorithm used a "ranked peaks in common" logic already incorporated in various commercial data systems. This finds library spectra with the largest number of peaks in common with the unknown spectrum, 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 unknown spectrum and would have produced low match factors. Both peak scaling and merging of multiple screening results are used in the Normal "Identity" search. 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 unknown spectrum is compared against the eight most intense peaks in scaled library spectra. The second most intense peak in the scaled unknown spectrum 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 unknown until the eighth most intense peak is compared against the 16 most intense peaks in the scaled library spectra. This is the only screening procedure applied in the Quick "Identity" search.

**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 Quick search. The others were:

- 1) The fourteen largest peaks in the scaled unknown spectrum were matched against the fourteen largest peaks in the scaled library spectra.
- 2) The six largest peaks in the original (nonscaled) 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 unknown and library spectra 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 and Neutral Loss search, up to five neutral loss peaks within 64  $m/z$  of the molecular ion 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 unknown spectra (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 considered. 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.

The dot-product mass spectral search algorithm, which is equal to the cosine of the angle between the unknown 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 unknown spectra. Its contribution was weighted so that it increased in importance as the proportion of common peaks increases. The only difference in the application of this algorithm to the "Identity" 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 "Identity" searches in the NIST Mass Spectral Search 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: 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. If you have comments or questions about the quality of these or other Standard Reference Databases available from the NIST Standard Reference Data Program, please let us know by contacting:

Joan Sauerwein  
National Institute of Standards and Technology  
Standard Reference Data  
100 Bureau Drive, Stop 2310  
Gaithersburg, MD 20899-2310  
Internet: [srdata@nist.gov](mailto:srdata@nist.gov)  
Phone: (301) 975-2208  
FAX #: (301) 926-0416  
Web site: <http://www.nist.gov/srd>

If you have questions or problems pertaining to the mass spectral data or use of this program or just want to make suggestions or contribute mass spectra, contact:

Dr. Stephen Stein  
National Institute of Standards and Technology  
100 Bureau Drive, Stop 8380  
Gaithersburg, MD 20899-8380  
Internet: [stephen.stein@nist.gov](mailto:stephen.stein@nist.gov)  
Phone: (301) 975-2505  
FAX #: (301) 926-4513

or

Dr. Gary Mallard  
National Institute of Standards and Technology  
100 Bureau Drive, Stop 8380  
Gaithersburg, MD 20899-8380  
Internet: [gary.mallard@nist.gov](mailto:gary.mallard@nist.gov)  
Phone: (301) 975-2562  
FAX #: (301) 926-4513

**NIST provides updates and enhancements to the NIST Mass Spectral Search Program and AMDIS. These can be downloaded from <http://chemdata.nist.gov>.**

Upgrades to the NIST/EPA/NIH Mass Spectral Database must be purchased.