

## Using NIST Search with Agilent MassHunter Qualitative Analysis Software

James Little, Eastman Chemical Company, Jan. 26, 2012

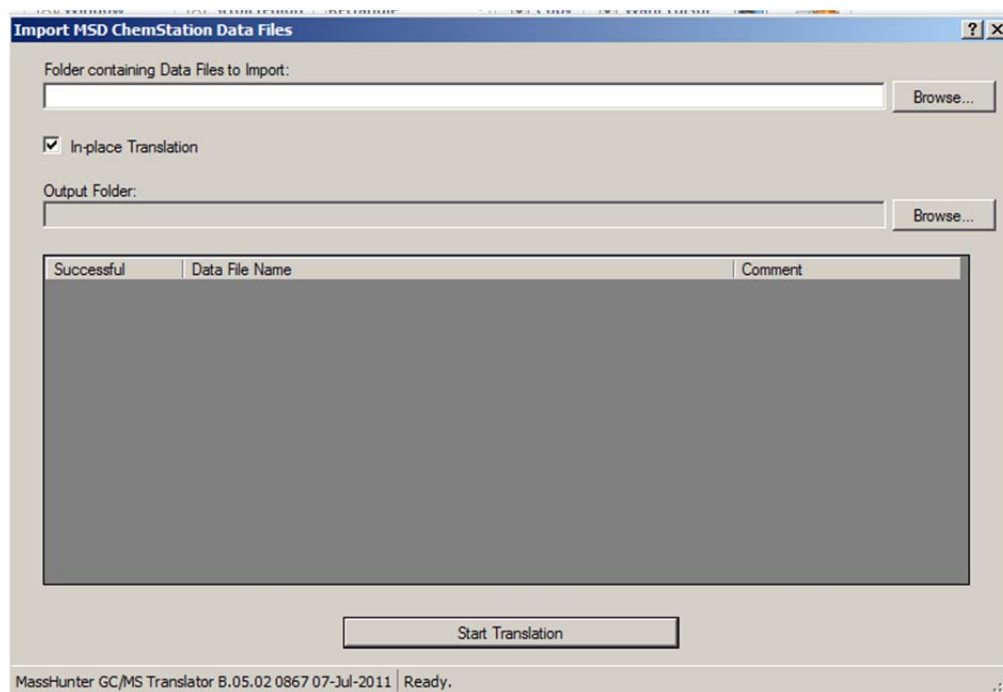
These screen shots were taken from B.05.00, but the instructions will likely be useful for earlier versions of the software. After loading the qualitative analysis software, I did a standard install of the NIST Version 2.0 g build May 19, 2011. The NIST software worked in conjunction with the Agilent software with no setup.

A license must be purchased for the NIST software and associated EI and MS/MS databases. I believe the NIST software can be purchased from Agilent as Part No. G1033A or from Scientific Instrument Services as Part No. 741010.

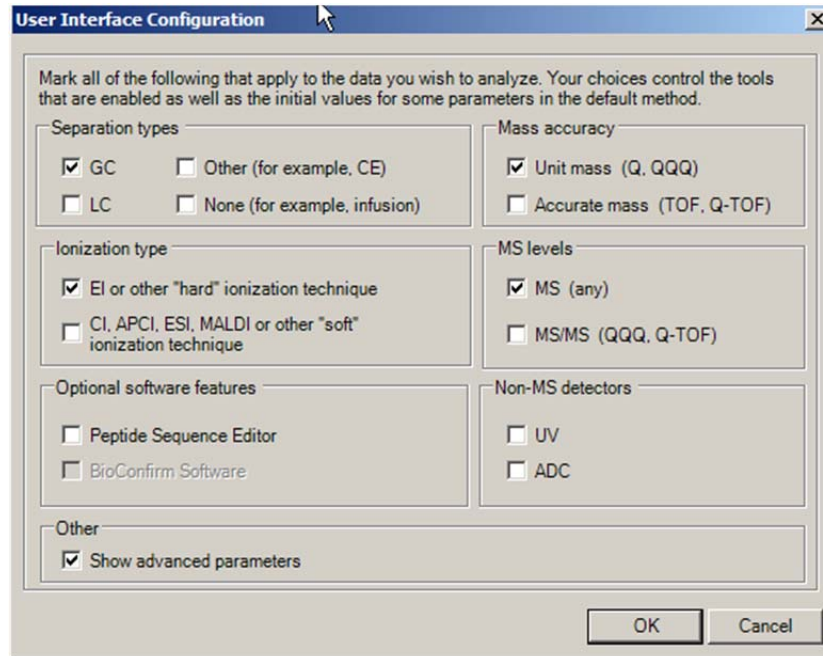
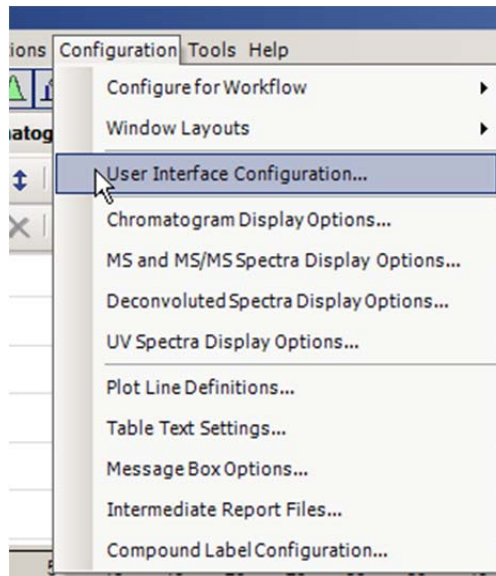
Users can create their own reference databases with the software. Eastman has written macros to combine and distribute our own corporate proprietary databases nightly. These capabilities are shown on my website at:

<http://littlesandsailing.wordpress.com/>

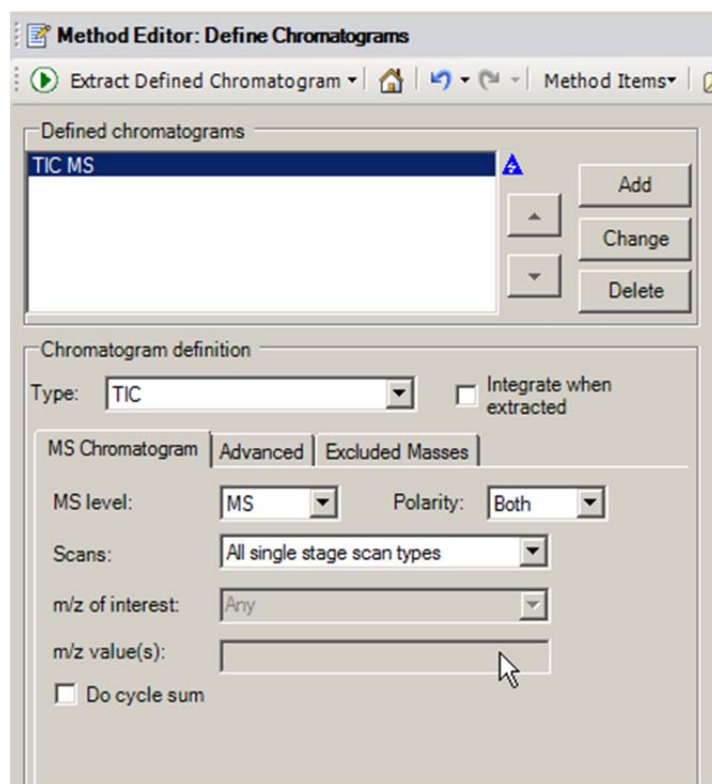
The library can be searched with data from either GC-MS or LC-MS (MS/MS) analyses in either accurate or nominal mass modes. The search below is first demonstrated with the Evaldemo.d file often supplied with the Agilent GC MSD. The file was converted from MSD format to MassHunter format using the GC MSD Translator software delivered with the LC-TOF or QTOF Agilent systems on a supplemental disk.



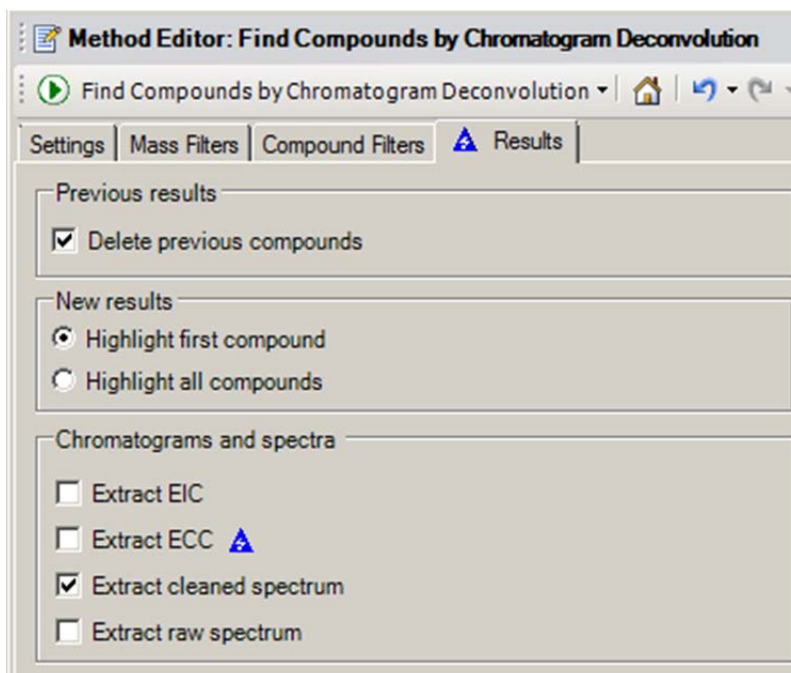
The MassHunter software was configured for processing GC-MS nominal mass data by selecting the configuration option from the MassHunter Qualitative Analysis software:



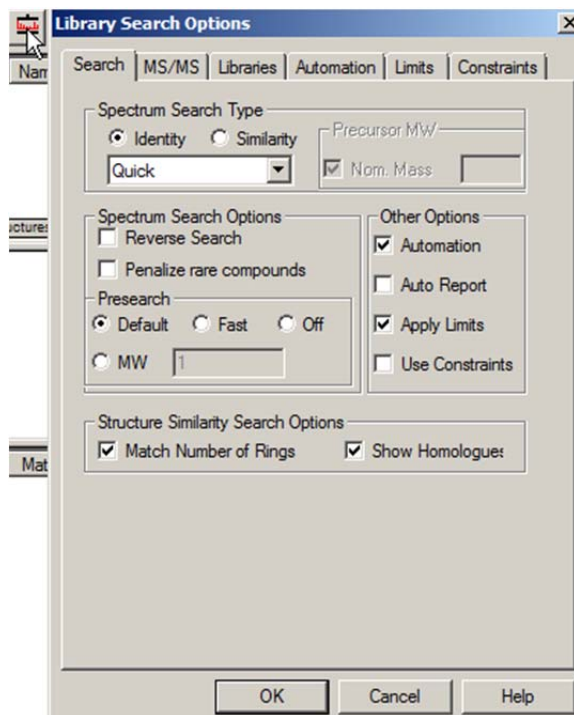
The evaldemo file was first opened using the "Extract Defined Chromatogram" with the options noted below in MassHunter:



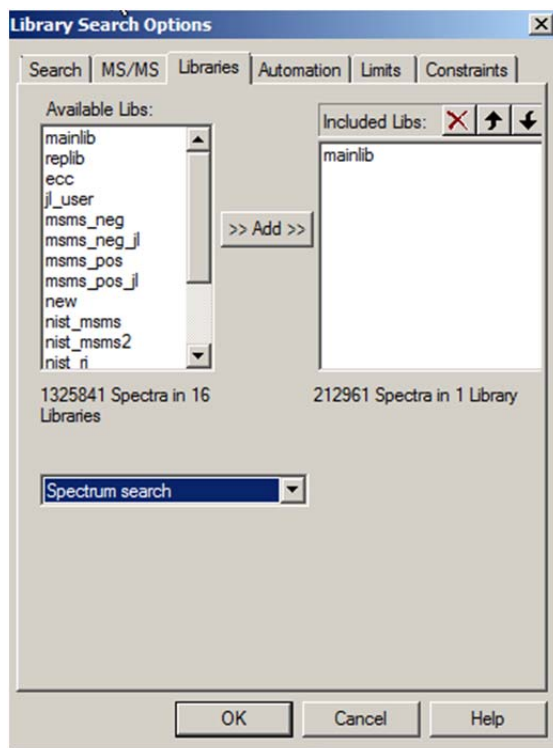
The spectra for components were then obtained automatically using the “Find Compounds by Chromatogram Deconvolution” command in the method editor. The only tab changed from the default settings is noted below:



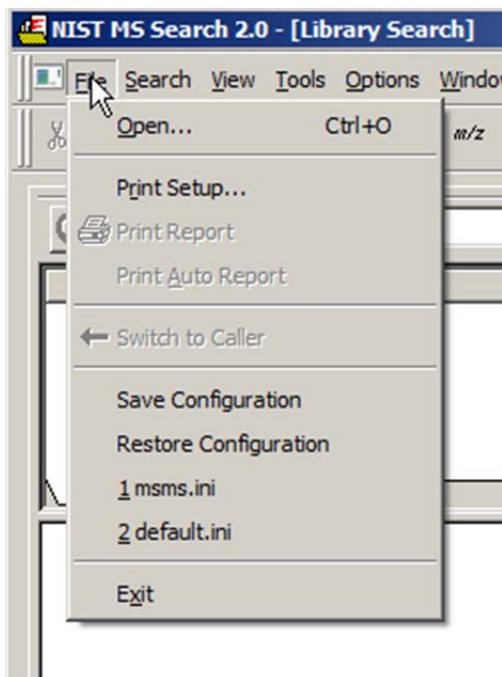
Before sending the spectra of interest to the NIST library, the NIST software was opened and the NIST search parameters were initialized in several different tabs:



We have many libraries including the NIST library (mainlib), the Wiley9, any several others. Below the system was setup to search only the NIST main library by selecting the Libraries tab:



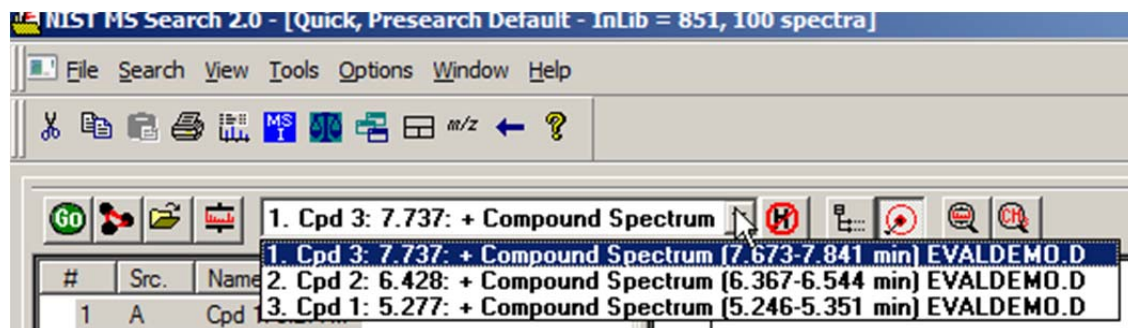
All the NIST settings can be saved for future reference. Almost any window configuration can be changed by “right” clicking on it and selecting the properties tab. My settings were saved for the MS/MS search in the msms.ini file and the EI GC-MS in the default.ini file:



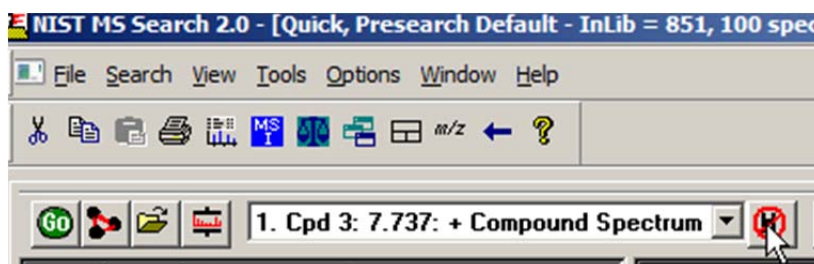
After this initial setup, the spectra can be sent from the MassHunter program to the NIST program for searching. There are several ways to send a spectrum to be searched. One can click on any individual spectrum to select it from the MS spectrum results. After selecting the spectrum, then “right” click within the spectrum box and select the “Search Using NIST MS Program” option.

A menu is displayed to let the user either append or overwrite the spectral entries in the NIST spec list box and the libraries selected are automatically searched and the results displayed. You can also select more than one spectrum by holding either the shift key (spectra next to one another) or ctrl key (spectra not next to one another) while “left” clicking on the spectra of interest in the MassHunter MS spectrum results file. Then “right” click on one of the boxes selected and select the “Search Using NIST MS Program” option to send all the spectra of interest to the NIST program for searching.

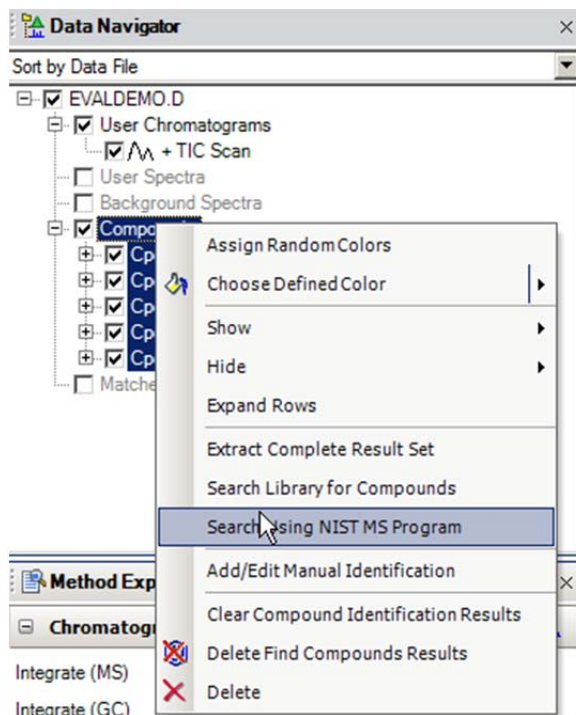
The results for the multiple spectra can be viewed individually by selecting the pull down menu for the history as shown below:



The results in the history can be deleted if desired by selecting the “delete history” icon as shown below:



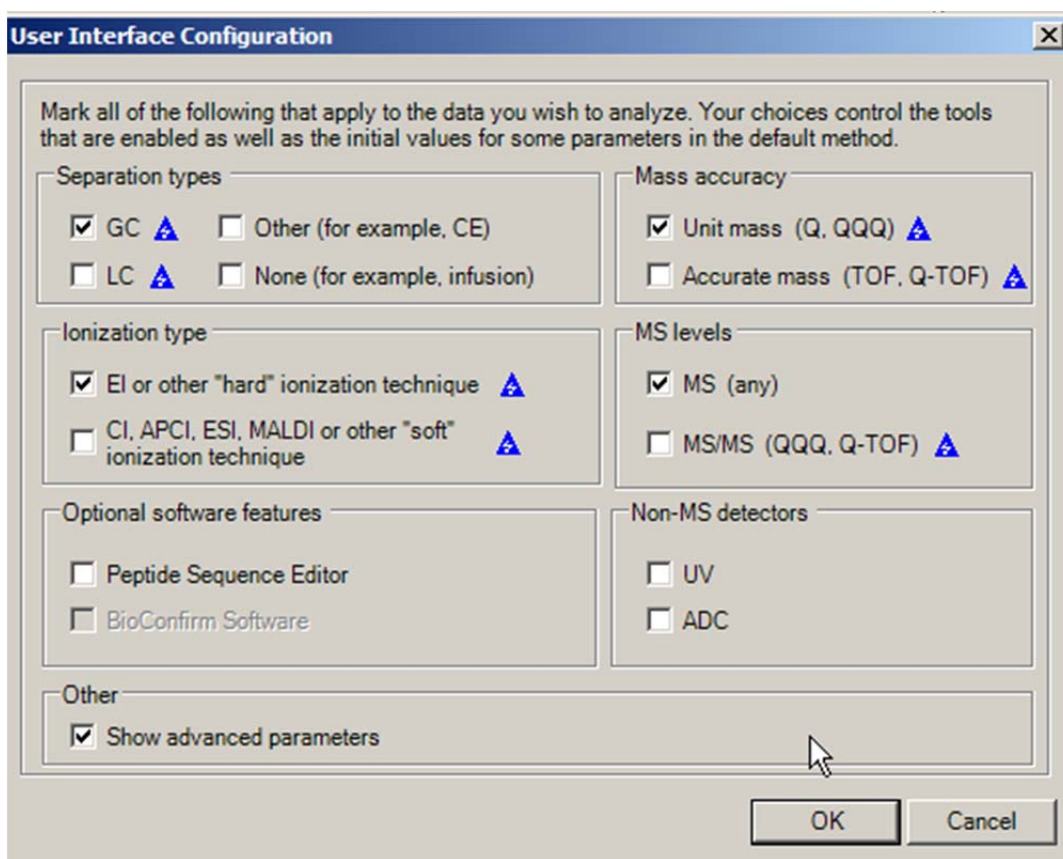
Multiple or single spectra can also be sent to the NIST program for searching by selecting the desired spectra or spectrum from the Data Navigator bar in MassHunter:



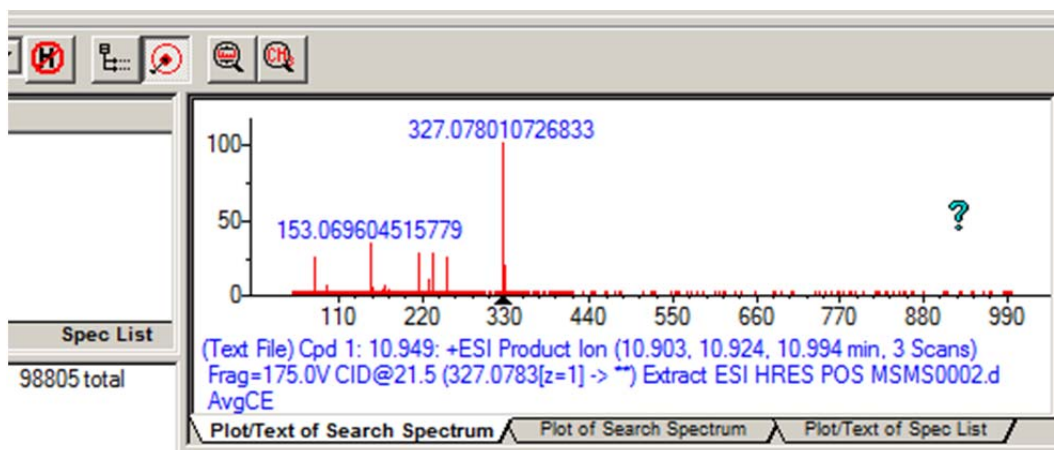
A similar approach is used to process spectra from compounds obtained for MS/MS spectra. Of course several steps must be taken before searching:

1. Change the configuration in MassHunter to settings appropriate for MS/MS data
2. Select the appropriate libraries within the NIST program such as the nist\_msms and possibly other user created libraries
3. Automatically create a compound list with associated spectra using Auto MS/MS, targeted MS/MS, or Molecular Feature functions within the MassHunter Method Explorer/Find Compounds menu
4. Also useful to use MassHunter Method Explorer/Identify Compounds/Generate Formulas option since the spectrum sent to the NIST search will have the molecular formula included in the header for the spectrum within the NIST program

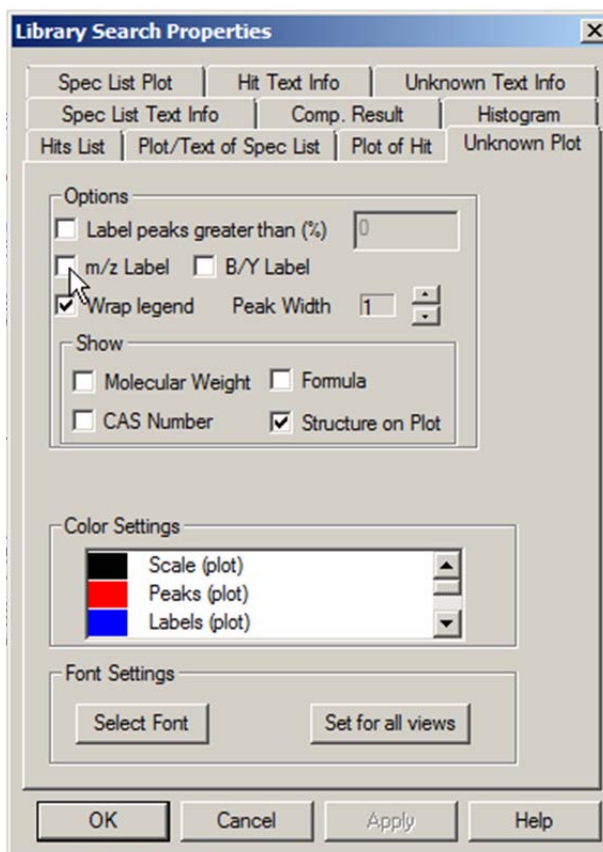
A typical window showing the configuration for MS/MS data is shown below:



As currently programmed, MassHunter exports the spectra with a lot of excess significant figures in the imported spectrum as shown below:



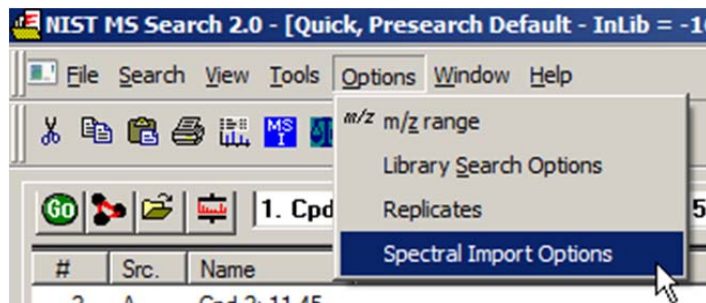
These significant figures do not significantly affect the results from the search. The actual number of significant figures cannot be changed currently, but if they are distracting, the label can be disabled in the NIST unknown display. This is accomplished by “right” clicking on the NIST window then selecting the “Properties” option. Just “unclick” the m/z label option and select “OK.”



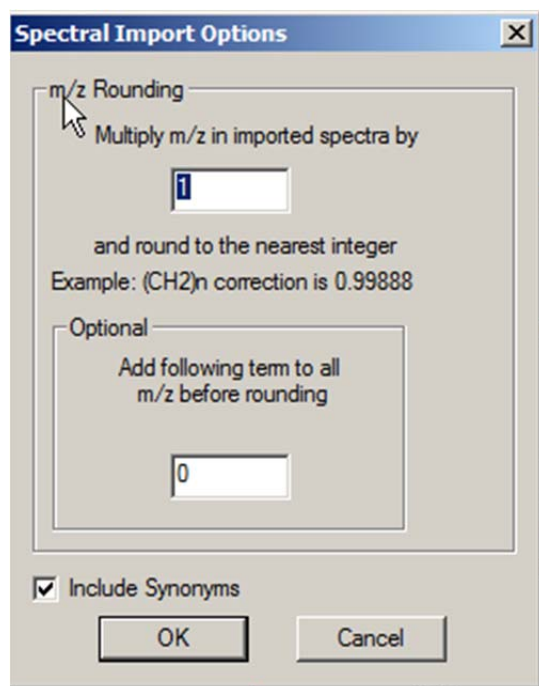
The spectra in the NIST EI MS/MS libraries are entered in at nominal mass. Many higher molecular weight compounds often have enough mass sufficiency (hydrogens) or deficiency (bromine, fluorine, etc.) that the ions in their spectrum cause the observed mass to be increased by one or decreased by

one, respectively. This is of course more of a problem in LC-MS analyses, but can occur in GC-MS analyses.

To correct a user spectrum, one can adjust the observed mass in the imported spectrum to its nominal mass by using a routine in the NIST software. Go to the main menu and select:



The “Multiply m/z in imported spectra by” value will need to be changed. The value of 0.99888 is satisfactory if the mass sufficiency from hydrogens are causing the value to be searched to be rounded up above. A value larger than 1 will be needed to correct for mass deficiency of compounds containing many bromines, chlorines, fluorines, etc.



The “Include Synonyms” should also be checked to import other important information with the spectrum.

Currently, it is not simple to enter spectra into the NIST library from MassHunter in accurate mass mode. The many significant figures imported for the m/z values are retained when spectra are added to a

user's library using the Librarian function in the NIST library. The only way to currently remove them is to first locate the ASCII NIST MSP file created by Agilent. This file SPECTRA.MSP was found at the following location on my computer.

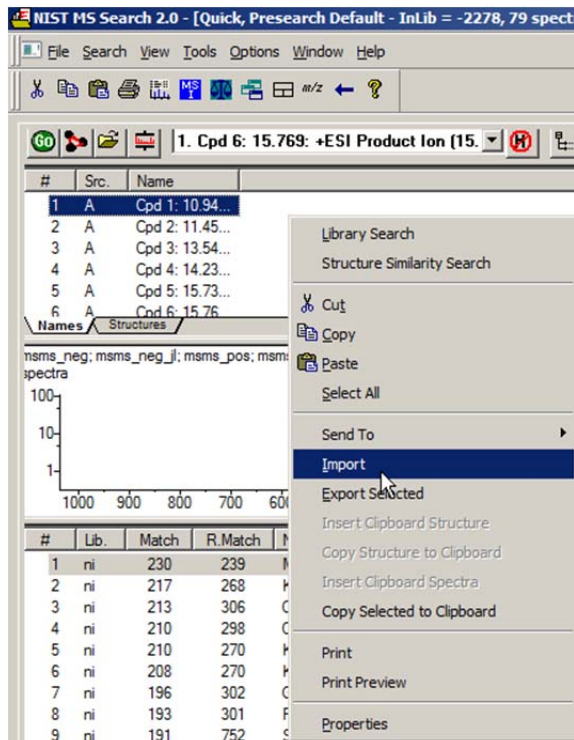
C:\Users\u839430\AppData\Local\Temp

I opened the file and edited one of the lines using the Notepad program. I have to remove the 3<sup>rd</sup> synonym line in the header for the spectrum to be imported. For example in the header below:

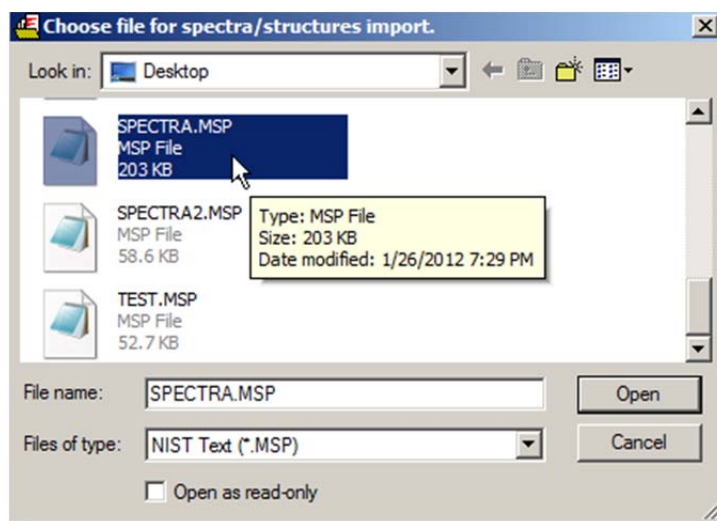
```
name:Cpd 1: 10.949: +ESI Product Ion (10.903, 10.924, 10.994 min, 3 Scans) Frag=175.0V CID@21.5
(327.0783[z=1] -> **) Extract ESI HRES POS MSMS0002.d AvgCE
SYNONYM:$:06Q-TOF MS
SYNONYM:$:00ms2
SYNONYM:$:04327.078338623047
SYNONYM:$:0521.45 V
SYNONYM:$:10ESI
num:1908
50.0158762609922 47.56008
```

The line “SYNONYM:\$:04327.078338623047”, which is the precursor m/z, was removed. This type of line must be removed from every spectrum present in the MSP file. I then saved the file on my desktop as SPECTRA.MSP.

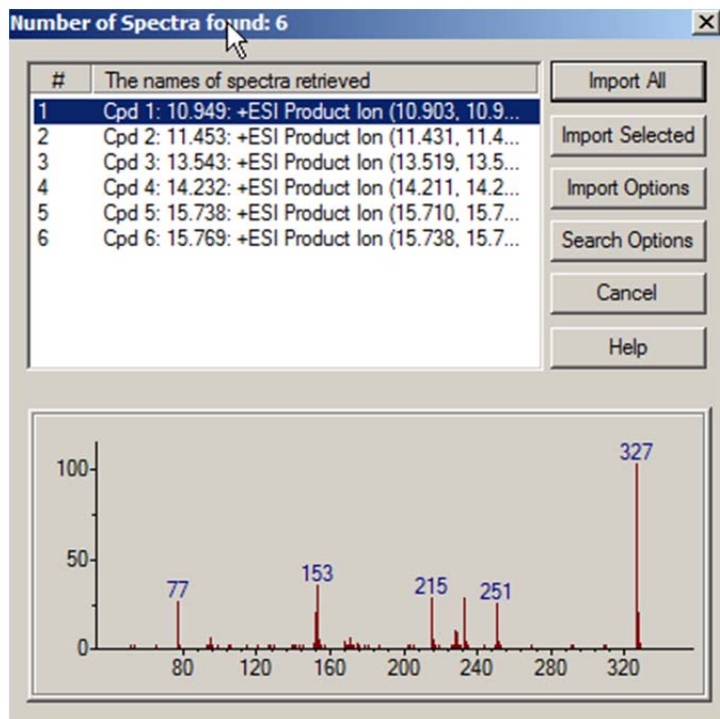
The spectra or spectrum in the SPECTRA.MSP must then be imported into the NIST program. “Right click” in the Spec List window (top one on left) in the NIST program as shown below:



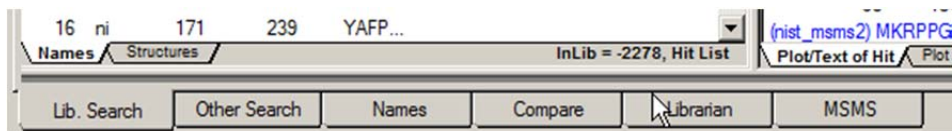
Then select the SPECTRA.MSP and click open.



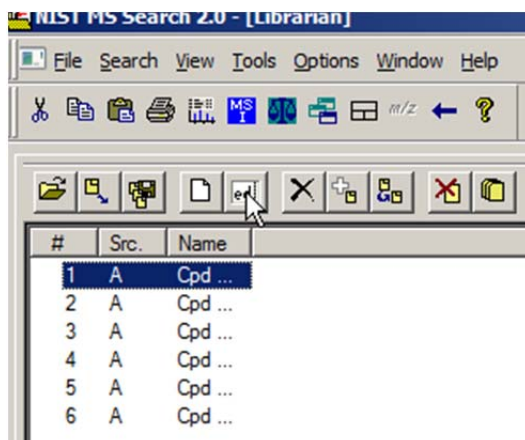
Then import all or import selected spectrum as required to import desired spectral data for library addition:



At this point, the spectrum can be added to the library of interest using the "Librarian" tab at the bottom of the NIST program.



Then select the spectrum of interest to add to the user library:



The desired fields can be edited, the structure attached from the desired drawing program (clipboard structure button), the MW and MF calculated from the inserted structure, comments added, etc.

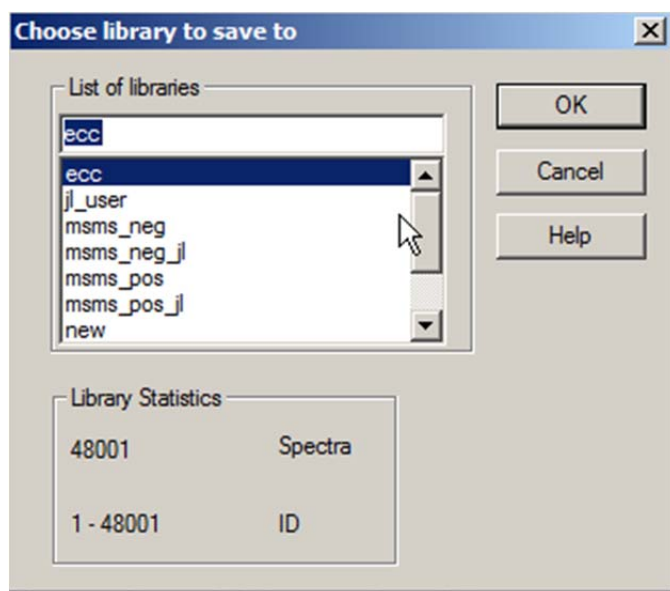
The 'Spectrum Information' dialog box is shown. It contains several fields and sections:

- Name:** triphenyl phosphate
- Formula:** C18H15O4P (with a 'From structure' button)
- Other Names (Synonyms):** \$:06Q-TOF MS, \$:00ms2, \$:0521.45 V, \$:10ESI
- Comments:** found in plastic. Agilent QTOF 6550, J Little
- Mol. Weight:** 326 (ID Number: 49)
- CAS Number:** 0 (Peaks: 68)
- Library:** Text File
- Buttons:** Add to Library, Replace, Add to List, Accept
- Peak information table:**

m/z	Abund.
51	9
53	3
65	2
77	246
78	13
93	10
94	1
95	55
96	4
99	3
105	14
- Mass Spectrum Plot:** Shows relative abundance vs m/z with peaks at 77, 153, 233, and 327.
- Chemical Structure:** A ball-and-stick model of triphenyl phosphate is shown.
- Buttons:** Attach Struct, Clipboard Struct
- Structure field:** Clipboard #2/saw in lcd screen.
- Buttons:** Exit, Help

Often many masses are noted above the molecular ion. These can be removed by first “left clicking” on the first mass to be removed then holding the “shift” key by selecting the last mass in the range to be removed. Not consecutive masses can be selected by using the “ctrl” key to select additional masses. The selected masses are then removed by pushing the “delete” on the computer keyboard.

After all the desired changes have been made, select “Add to Library” to select the library in which to add the spectrum:



If the first entry is being added to a user's library, just type in the name of the new library to be created. After that, additional spectra can be added to the library.