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Extending the Limits of Mass Spectrometry

Creation of Accurate Mass Library for NIST Database Search

Novel MS calibration has been shown to enable accurate mass and elemental composition determination on quadrupole GC/MS systems for either molecular or fragment ions. This capability, which is normally only available from high resolution mass spectrometers, can now be combined with NIST library search to facilitate high confident compound identification as demonstrated previously. With a constraint of either mass tolerance or elemental composition, NIST library search can significantly reduce its possible candidate list to a group isomers defined by the specified elemental composition. In the NIST library, the majority of GC/MS spectra has molecular ions and can certainly benefit from the restricted search as they carry the information of exact mass and elemental composition so the constraints can apply during the search. On the other hand, a significant portion of the library spectra do not have molecular ions and could not be used for the search with the constraints. Here we propose a new approach to performing high mass accuracy GC/MS library search. Instead of utilizing only integer mass accuracy for both molecular and fragment ions in the current NIST library, a new library will be created to include high mass accuracy GC/MS spectra achieved through the novel MS calibration. This new approach will not only expand the applicability of the restricted search to the compounds without molecular ions, but will also enhance search performance for the molecular ions containing spectra by adding high mass accuracy of all the fragment ions for further confirmation. Served as a brief tutorial, this article will document procedures for generating an accurate mass library and performing accurate mass library search. A great example will also be presented.

Both Semi-Volatile Organic Compound (SVOC) samples and Perfluorotributylamine (PFTBA) were acquired with an Agilent 5973N GC(6890)/MS system in a raw (profile) scanning mode. The PFTBA spectra acquired for 20 seconds at the end of GC/MS separation were used to build a calibration which was then applied to the sample spectra to obtain high mass accuracy GC/MS spectra by MassWorks. Two data files acquired under different GC conditions were selected for feasibility studies. The spectra from one data file were used to create an accurate mass library, while those from another data file were employed to perform an accurate mass library search using NIST MS Search.

The procedure for creating an accurate mass library consists of three main steps: exporting accurate mass spectra from MassWorks to NIST MS Search, editing the exported spectra to include all necessary and relevant chemical information, and saving the spectra in it. Below is an example of the entire process, illustrated with screenshots.

To export spectra from MassWorks to NIST MS Search is straightforward. It is done by right clicking in a spectrum window of MassWorks to have a pop-up menu show up (Figure 1). By selecting “Library Search” from the menu, as marked with the red oval circle, a regular NIST library search will be performed. As a part of the search process, the accurate mass spectra are transferred from MassWorks to NIST MS Search. Make sure the compound highlighted in blue inside the lower left spectral list window in Figure 2 is the correct one before proceeding further. The next step, spectral editing, is a more involved process. Note that there are two rows in this screenshot. The top row is for the accurate mass spectra imported from MassWorks, while the bottom row is for the original integer mass spectra in the NIST library. In each row, the left is a spectral list window, listing all candidates from a given search; the middle is a spectra window, displaying the spectra highlighted at the left; and the right is an information window, indicating name, exact mass, elemental composition, etc., of a selected compound.

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The purpose of editing the spectra is to establish a complete record for the spectra, to be included in the accurate mass library. This can be done by copying available information for the same compound which already exists in the library marked with a box in red to the Spectrum Information window of the new spectra. Since there are many manual operations needed to edit the spectra, an efficient sequence is recommended, as follows:

1. Copy all the spectrum information from the library compound – usually displayed in the pane at the lower-right (using ctrl+A and ctrl+C) and then paste (ctrl+V) it to a Notepad instance, as shown in Figure 3. This is a time-saving operation to avoid multiple switches from the "Lib. Search" tab to the "Librarian" tab for copy/paste operations. Once all the information is copied into Notepad, selective information can be copied from the Notepad to its individual space in the Spectrum Information window of the accurate mass spectra without need to go back to the "Lib. Search" tab for copying.
2. Bring up the Spectrum Information window. This can be done by clicking "Librarian" and "Names" tab (marked with a red oval), highlighting the top compound on the list, then clicking the "ed" toolbar button (pointed at with a black arrow in Figure 4) to have the spectrum information window appear (Figure 5).
3. Selectively copy information from Notepad and paste it to the Spectrum Information window, updating the contents of the fields in the following manner:
    1) Paste the "Name" of the compound.
    2) Paste the "CAS Number".
    3) Click "Attach Struct" and "From Main" (Figure 6a) to get molecular structure.
    4) Click "From structure" to get "Formula" and "Mol. Weight".
    5) Paste the "Other Names".
    6) Write customized message into "Comments".
    7) Click "Replace" (Figure 5), then "OK" (Figure 6b) to ignore the warning message and to update all the information copied/pasted into the spectrum window.

To save the spectra into an accurate mass library as the last step is very simple. As all the editing is done, the accurate mass spectra now have complete records including name, formula, structure, etc., (as shown in Figure 7) and reappear on the top of spectral list window with a new updated name. To create the library (the first time only), highlight the updated spectra, click the "Create Library" toolbar button (pointed at with a red arrow in Figure 7), and provide a name for the library to be created - for example, "AM_test" (as shown in Figure 8a). The final operation is to click the "Add to library" toolbar button (pointed at with a black arrow in Figure 7), select the created library, for example "AM_test", from the pop-up window (Figure 8b), and click OK to complete.

Successful creation of an accurate mass library allows high mass accuracy GC/MS spectra achieved through MassWorks to be searched for high confidence compound identification. Here is a quick example. Before the search, the accurate mass library created needs to be in included for search through "Library Search Options". Under the main menu item "Option" of NIST MS Search, select "Library Search Options", click the "Libraries" tab, select "am_test", and then click ">> Add >>" (Figure 9). The operation to perform accurate mass NIST library search is the same as regular NIST library search, as shown in Figure 2. In the current search, two libraries were used. One is NIST main library and another is an accurate mass library that was just created, called "am_test". Search results for the test spectra of a SVOA compound are shown in Figure 10. Although this compound was found in both

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libraries, the spectra matched the best with the spectra in the accurate mass library, achieving the top hit with the best match values and probabilities. In addition, high mass accuracy and spectral accuracy for molecular ions (inserts of Fig 10) and high mass accuracy for all fragments provide valuable confirmation.

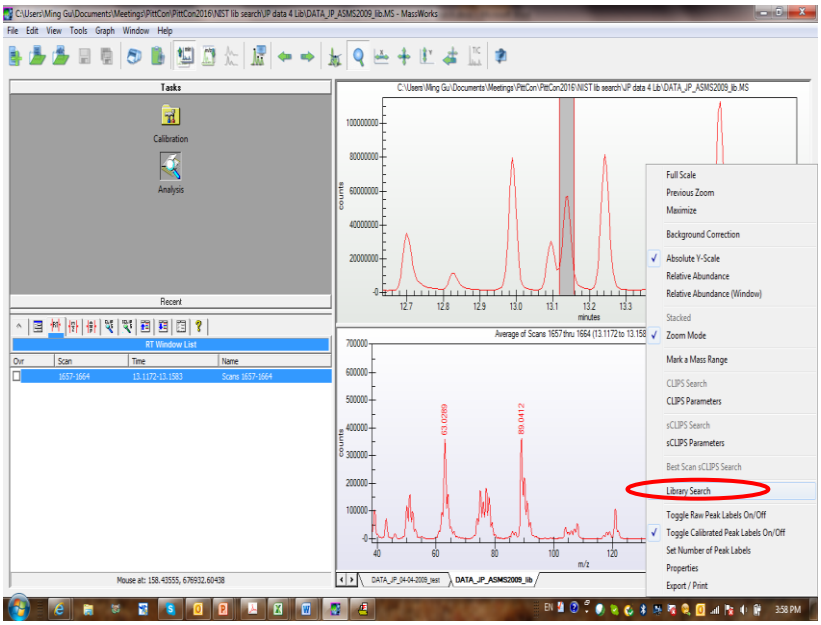

Figure 1. Launch conventional NIST library search from MassWorks

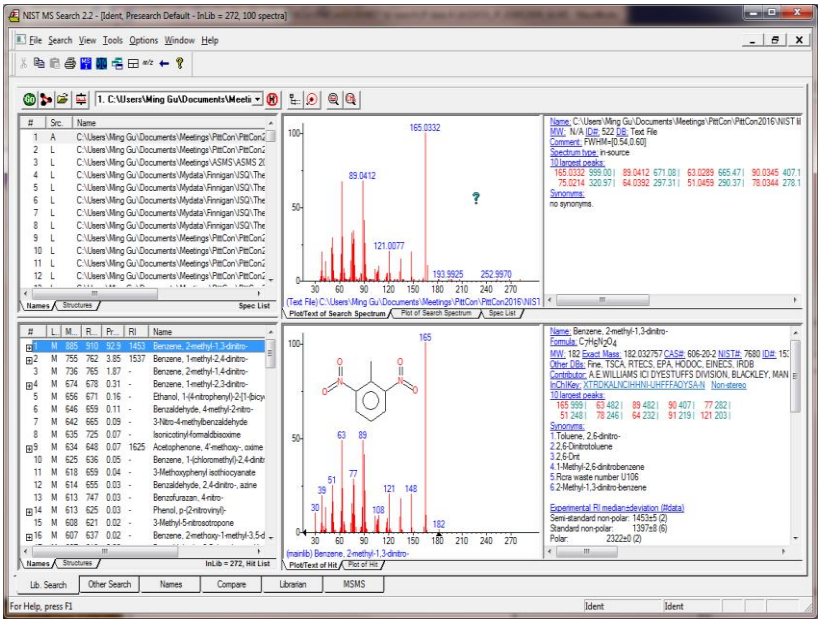

Figure 2. NIST Library search results with corrected compound identified and highlighted in blue.

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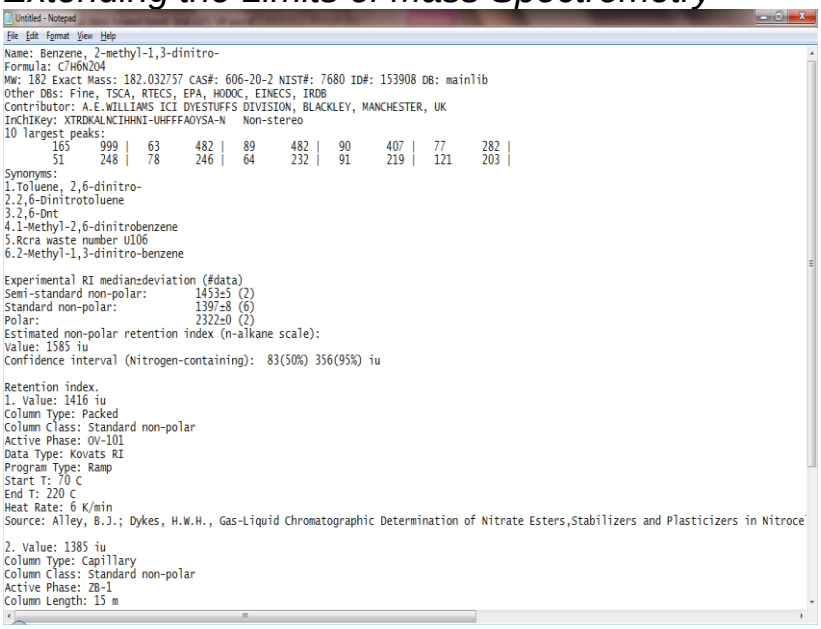

```
Name: Benzene, 2-methyl-1,3-dinitro-
Formula: C7H6N2O4
MW: 182 Exact Mass: 182.032757 CAS#: 606-20-2 NIST#: 7680 ID#: 153908 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, EINECS, IRDB
Contributor: A.E.WILLIAMS ICI DYESTUFFS DIVISION, BLACKLEY, MANCHESTER, UK
InChIKey: XTRDKALNCIHHNI-UHFFFAOYSA-N   Non-stereo
10 largest peaks:
        165     999 |   63      482 |   89      482 |   90      407 |   77      282 |
         51     248 |   78      246 |   64      232 |   91      219 |  121      203 |
Synonyms:
1.Toluene, 2,6-dinitro-
2.2,6-Dinitrotoluene
3.2,6-Dnt
4.1-Methyl-2,6-dinitrobenzene
5.Rcra waste number U106
6.2-Methyl-1,3-dinitro-benzene

Experimental RI median±deviation (#data)
Semi-standard non-polar:        1453±5 (2)
Standard non-polar:             1397±8 (6)
Polar:                          2322±0 (2)
Estimated non-polar retention index (n-alkane scale):
Value: 1585 iu
Confidence interval (Nitrogen-containing):  83(50%) 356(95%) iu

Retention index.
1. Value: 1416 iu
Column Type: Packed
Column Class: Standard non-polar
Active Phase: OV-101
Data Type: Kovats RI
Program Type: Ramp
Start T: 70 C
End T: 220 C
Heat Rate: 6 K/min
Source: Alley, B.J.; Dykes, H.W.H., Gas-Liquid Chromatographic Determination of Nitrate Esters,Stabilizers and Plasticizers in Nitroce

2. Value: 1385 iu
Column Type: Capillary
Column Class: Standard non-polar
Active Phase: ZB-1
Column Length: 15 m
```

Figure 3. The chemical information from the library compound copied to Notepad

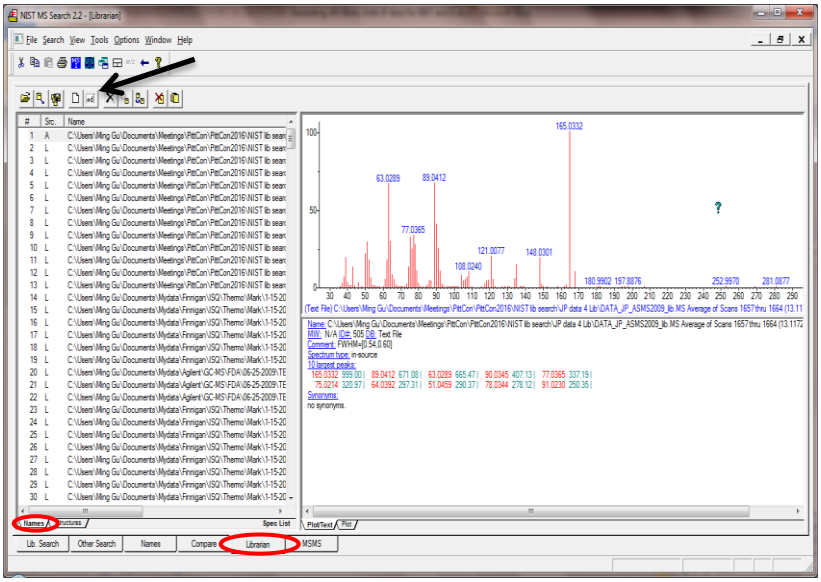

Figure 4. To bring up the Spectrum Information window

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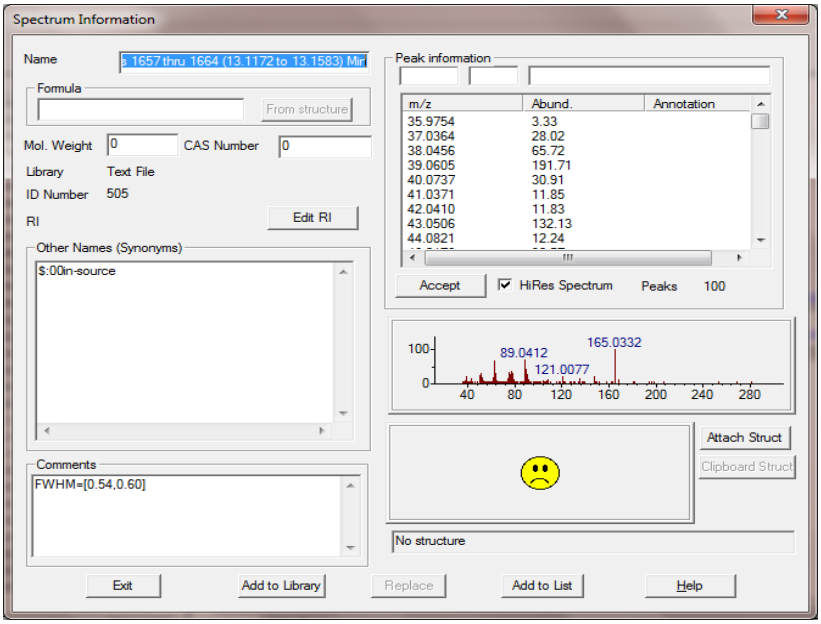

Figure 5. To copy information from Notepad to this Spectrum Information window

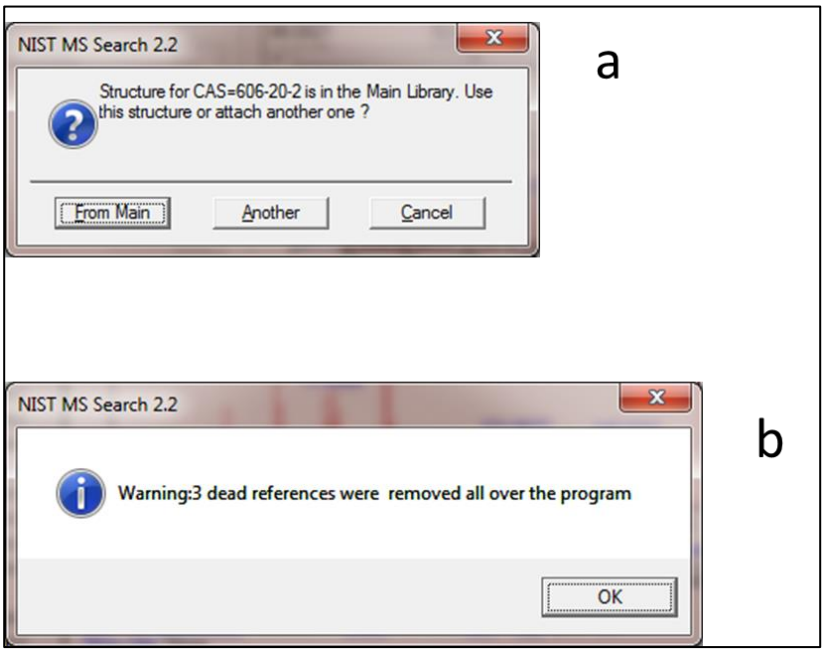

Figure 6. Pop-up message windows during above copy/paste

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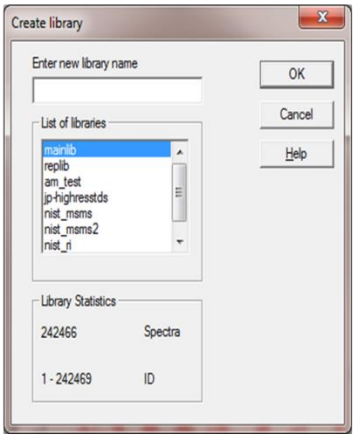
Figure 7. To save the spectra into an accurate mass library

a



b

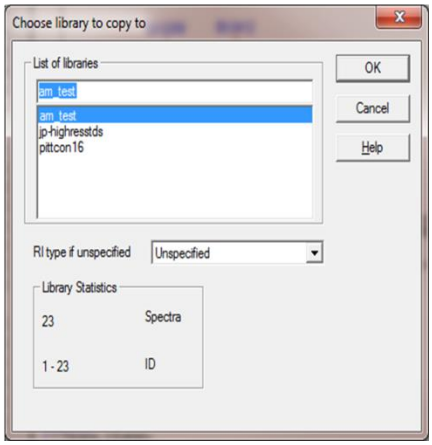


Figure 8. Pop-up message windows during creation of a library

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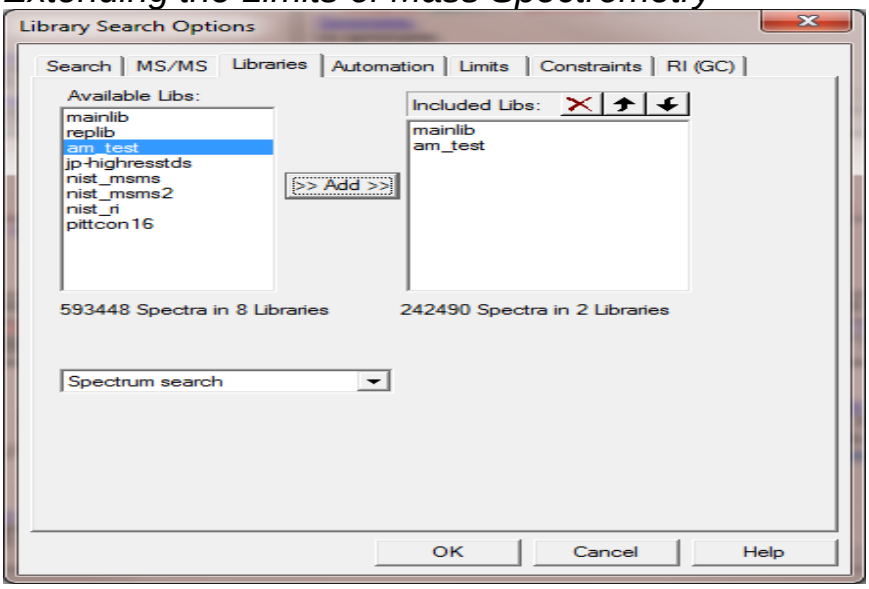

Figure 9. Add newly created accurate mass library for search

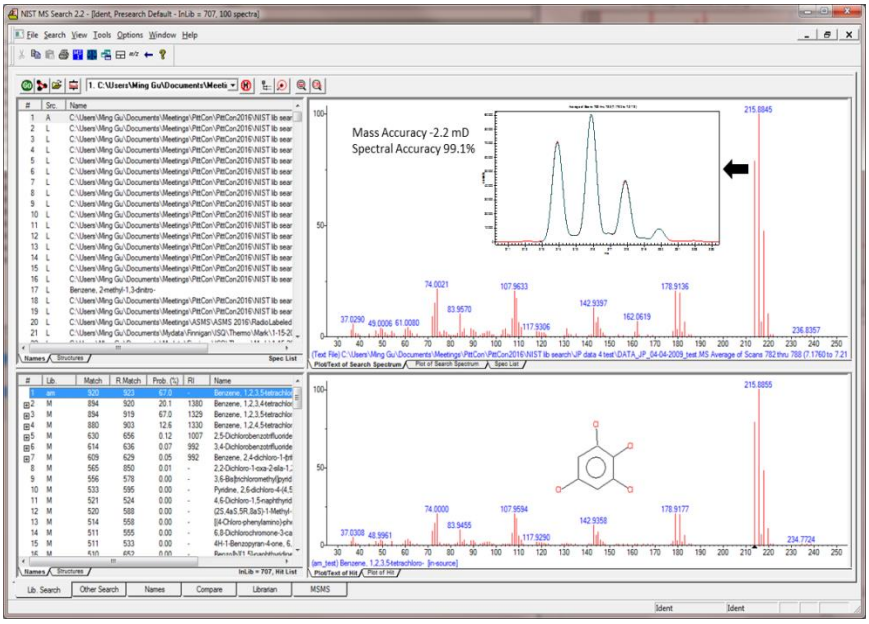

Figure 10. Accurate mass NIST library search results

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