

The Creation and Unknown Search of Accurate Mass Library with GC/MS Quadrupole Systems

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Overview

➤ The procedures of creating accurate mass library with single quadrupole GC/MS data are described, including exporting accurate mass spectra from MassWorks to NIST MS Search and editing/saving the spectra into an accurate mass library.

➤ High confident NIST library search is demonstrated with high mass accuracy library search.

Introduction

Although elemental composition determination with unit mass resolution GC/MS enabled by unique instrument line shape calibration technology has enhanced confidence of unknown identification through NIST library search, additional confirmation is always desirable and often is required in the rapid identification of releases of hazardous or toxic chemicals, or forensic materials. In this paper, we will report a new approach to building high mass accuracy library with GC/MS quadrupole systems to further improve the performance of compound identification.

GC/MS is the tool of choice for the rapid identification of well-characterized Volatile Organic Compounds (VOCs) and Semivolatile Organic Compounds (SVOCs) with standard methods. However, for non-target compounds or true unknowns, a significant uncertainty can exist when relying only on conventional GC/MS library search routines for identification. Miss-identification can lead to an expensive, and/or potentially dangerous or fatal incorrect response by first responders and law enforcement. Uncertainties or delays in confident identification can also contribute to high costs and consume valuable public resources not to mention disastrous consequences. Prior work in this laboratory has demonstrated that elemental composition determination of molecular ion or main fragment ions can be achieved to facilitate compound identification with NIST library search. Our current effort is to take advantages of accurate mass measurements on all ions and create high mass accuracy library with quadrupole GC/MS systems. This was initiated by building a high mass accuracy library with a small set of VOCs for evaluation. First, high mass accuracy spectra were obtained through the unique instrument line shape calibration implemented by commercially available software. Second, multiple spectra were exported and saved into the Spec List file in NIST MS Search program by launching conventional NIST library search within the software. Third, individual spectra were edited to include relevant information such as compound name, structure, and CAS #. Fourth, a customized library was created and the entire accurate mass spectra were added into the library. Our preliminary results do demonstrate high mass accuracy advantages in the NIST library search. For the first time, accurate mass measurements of all the ions acquired from GC/MS quadrupole systems can be directly compared with those in a high mass accuracy GC/MS library for the purpose of compound identification.

Methods

➤ **Data Acquisition:** All data were acquired in an Agilent 5973N GC(6890)/MS system in a raw (profile) scanning mode and followed by NIST MS Database Search and elemental composition determination by MassWorks. A total of 122 SVOC compounds were investigated. About 0.1 mg of each compound were injected and separated by GC/MS according to EPA methods. At the end of run, PFTBA spectra were acquired for 20 seconds to built calibration by MassWorks. The system can hold the calibration for days if not weeks before recalibration

➤ **MassWorks processing:** MassWorks calibrates both the mass position and the mass spectral peak shape function, a key for achieving high mass accuracy. When applied to raw mass spectral data, the raw mass spectrum can be transformed into its calibrated version with mass spectral peaks located at accurate mass positions. Furthermore, the mass spectral peak shape would also be transformed in the same process to a mathematically definable function, a key for achieving high spectral accuracy and CLIPS formula ID.

➤ **NIST MS Database Search:** Through MassWorks software, the profile spectra were automatically converted into centroid spectra for NIST MS Database Search.

Results and Discussion

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

To export spectra from MassWorks to NIST MS Search is straightforward. It is done by right-click in a MassWorks spectrum window to have a pop-up menu showed up (Fig1). From the menu, by selecting "Library Search" as marked with oval circle in red 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. It is important to make sure the correct compound is found and highlighted in blue inside lower left spectral list window in Fig 2 before proceeding further. The next step, spectral editing is more involved process. Note that there are two rows in this screenshot. The top row is for the accurate mass spectra imported from MassWorks,

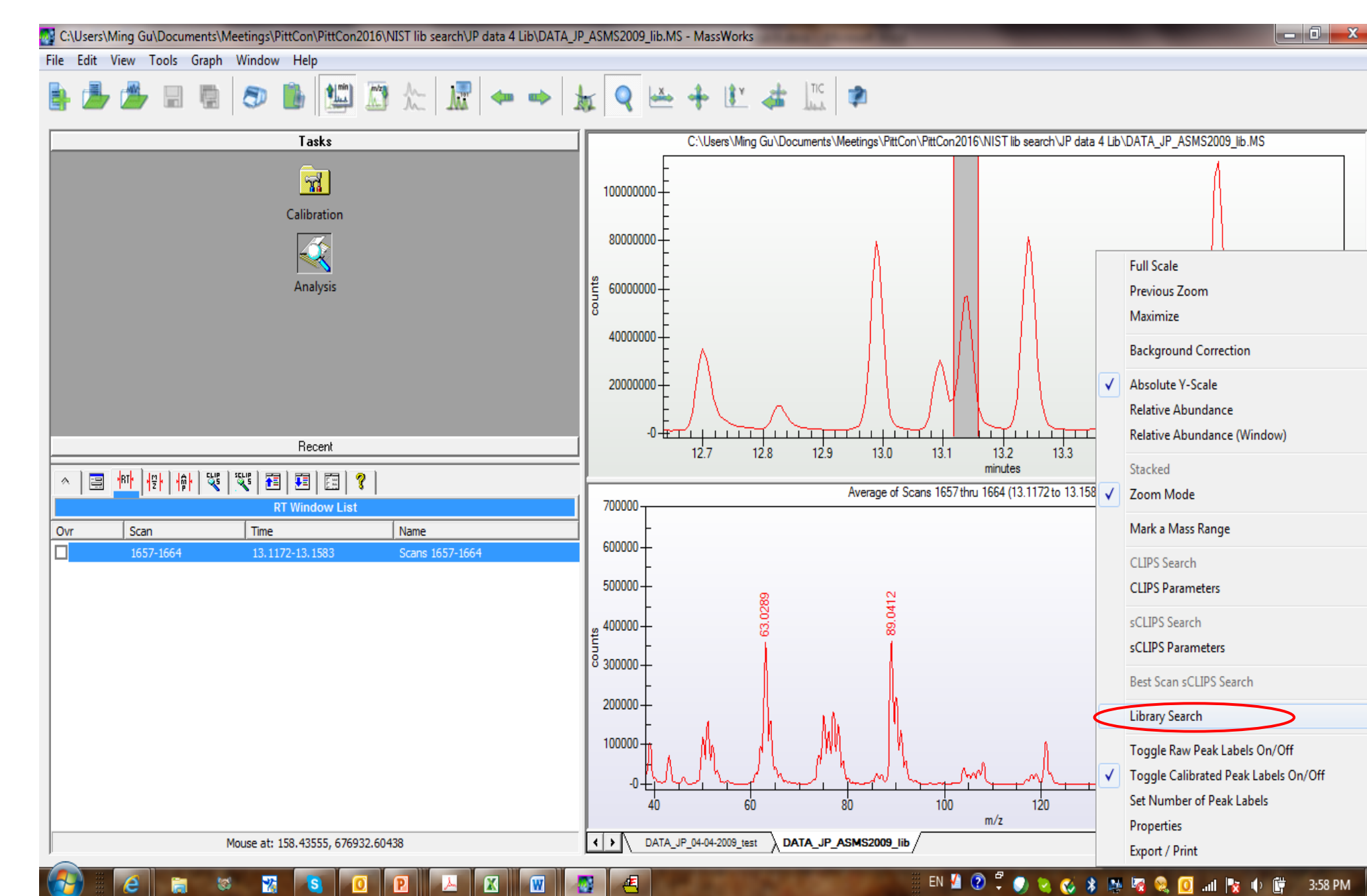


Fig 1. Launch NIST Library Search from MassWorks

while the bottom row is for original integer mass spectra in the NIST library. In each row, the left is spectral list window, listing all candidates from a given search; the middle is spectra window, displaying the spectra highlighted in the left; and the right is information window, indicating name, exact mass, elemental composition, etc. of a selected compound. The purpose to edit the spectra is to establish a complete record for the spectra to be included in the accurate mass library database. This can be done by copying available information of the same compound already existed in the library marked with a box in red to the spectrum window of the new spectra. It is important to point out the CAS number of a compound needs to be copied first in order to take advantage of the capability to retrieve information through the interface (Fig 3). In other words, once CAS number is copied in, the structure, formula, molecular weight of a compound can be calculated and included in the record of accurate mass spectra by clicking a few clicks.

As all the edits are done, the accurate mass spectra now have a complete record including name, formula, structure, and etc. as shown in Fig 4 and can be saved into an accurate mass library for search. A successful creation of accurate mass library allows high mass accuracy GC/MS spectra achieved through MassWorks to be searched for high confident compound identification. Here is a quick example. In current search, both NIST main library and newly created accurate mass library were used for search. Search results for the test spectra

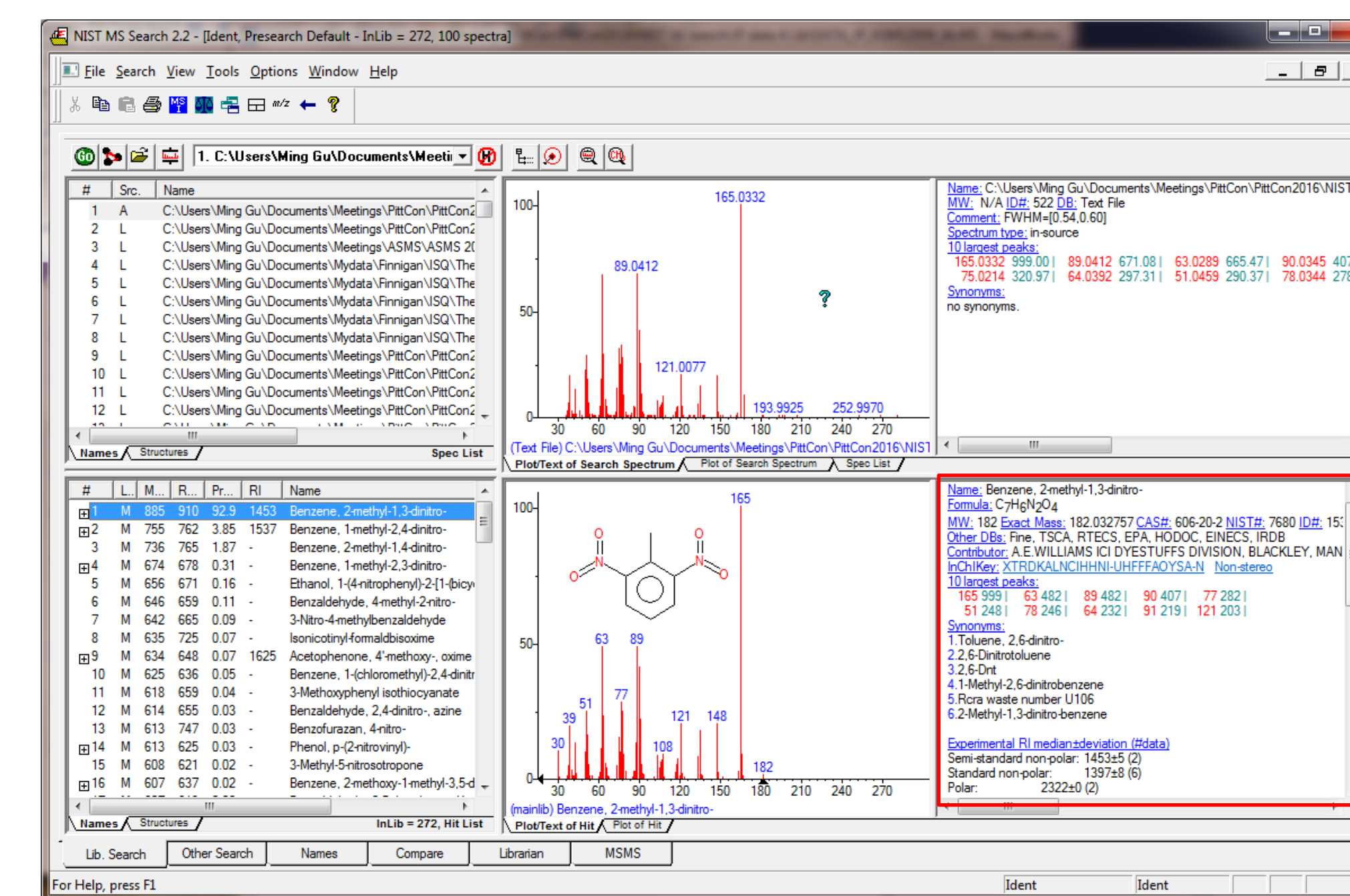


Fig 2. Accurate Mass and Integer Mass Spectra

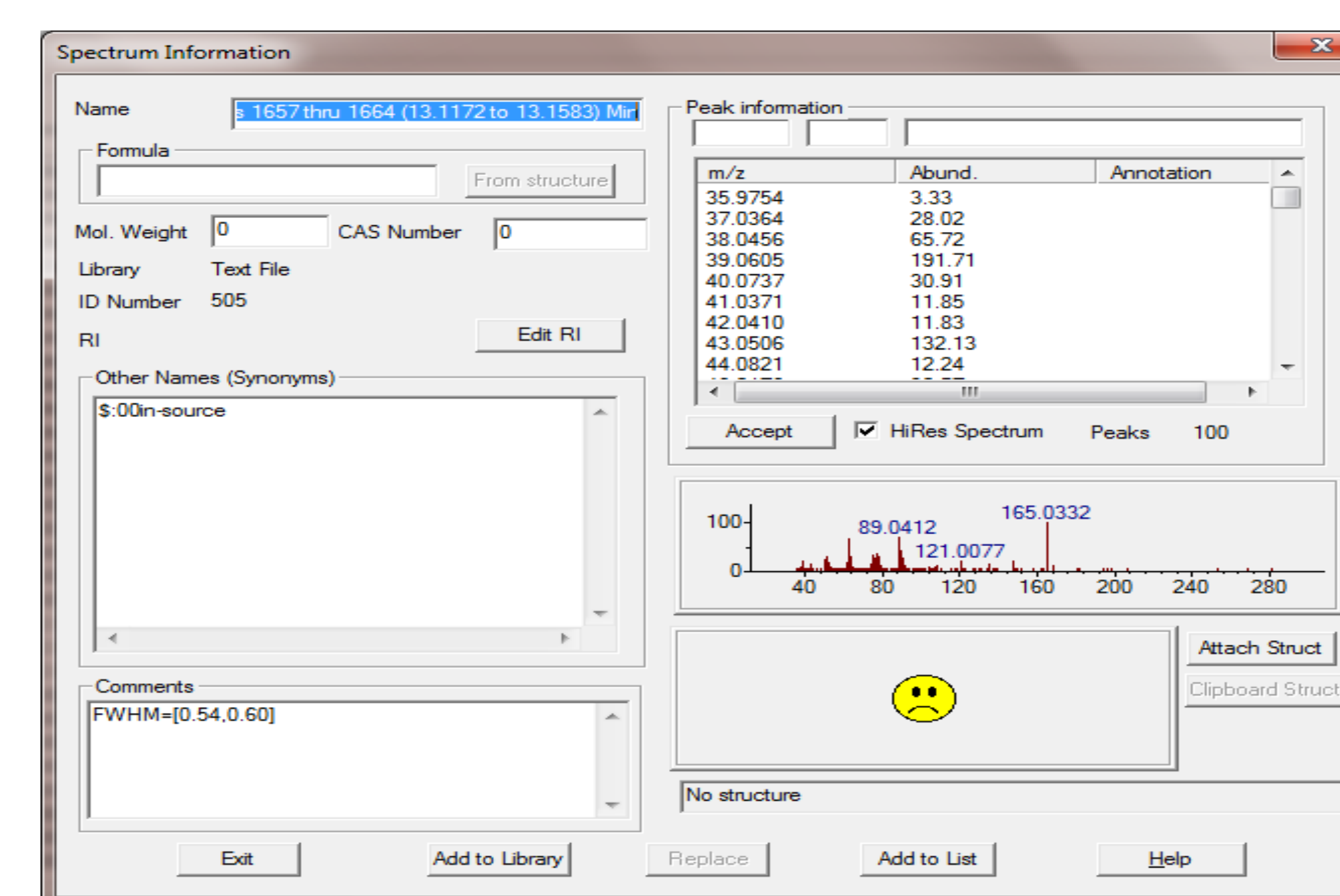


Fig 3. Adding Info into Accurate Mass Spectra

Results and Discussion

are shown in Fig 5. Although this compound was found in the both libraries, the spectra matched the best with the spectra in the accurate mass library to achieve the top hit with the best match values and probabilities. In addition, high mass and spectral accuracy for molecular ions (inserts of Fig 5) and high mass accuracy for all fragments provide valuable further confirmation.

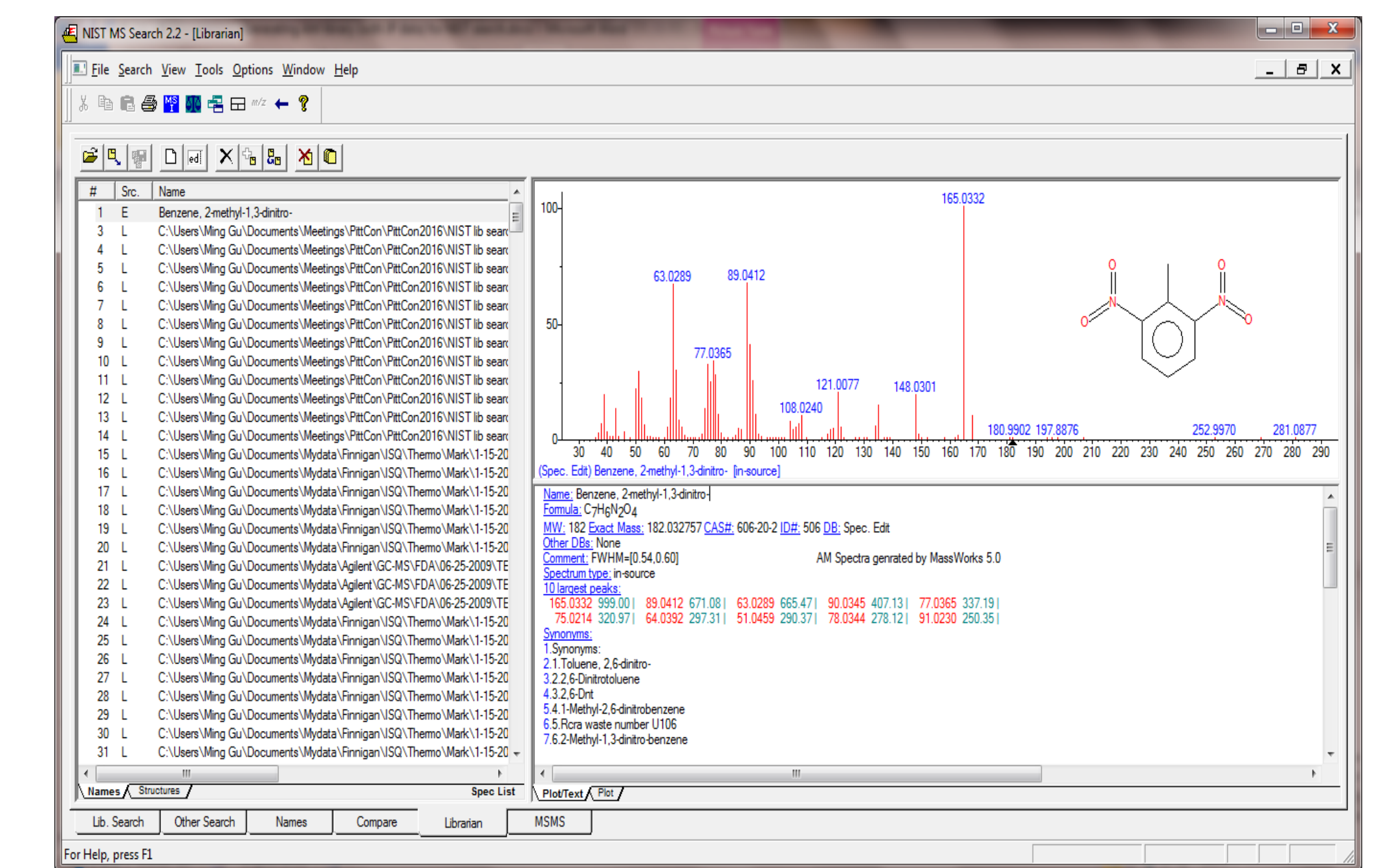


Fig 4. New Accurate Mass Spectra with a Complete Record

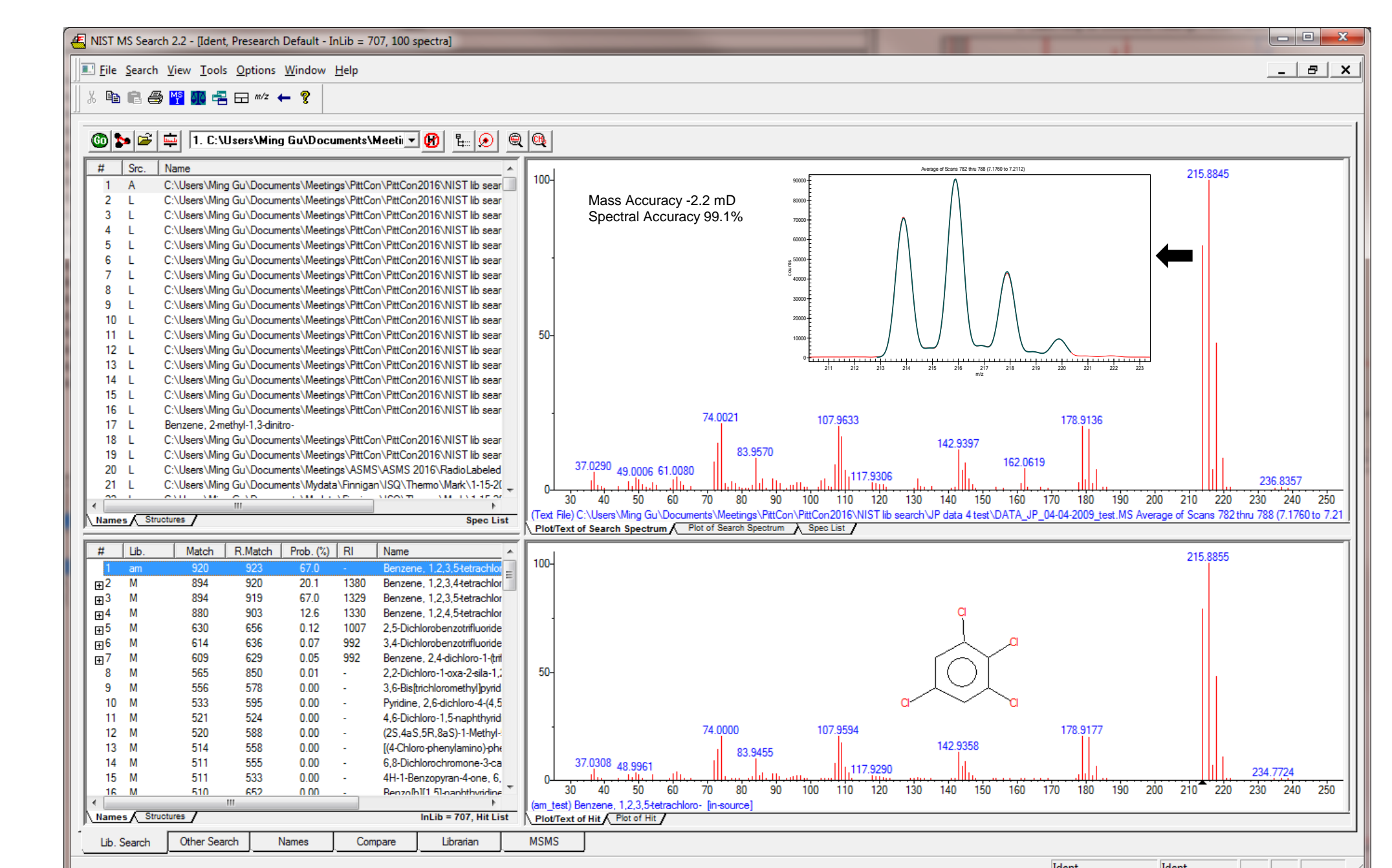


Fig 5. High Mass Accuracy NIST Library Search

Conclusions

➤ The creation of accurate mass GC/MS library from low resolution single quadrupole MS data is feasible.

➤ High confident compound identification can be performed by high mass accuracy NIST library search (high match values) and further confirmation of high mass accuracy of fragment ions, and high mass accuracy and high spectral accuracy of molecular ions if available.

➤ Further work should focus on automation of creating accurate mass library.