Creating and Using a Performance-Based API-LC/MSD Mass Spectral Library with NIST MS Search Software

Application

Liquid Chromatography/Mass Spectrometry

Authors

C. Kai Meng and Paul Zavitsanos
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

Currently, due to the different tune requirements, instrument parameters, and hardware differences, universal liquid chromatography/mass spectrometry libraries do not exist as they do for gas chromatography/mass spectrometry electron impact systems. However, using performance-based tune criteria for instruments, consistent and reproducible spectra can be obtained. This is the concept for creating universal searchable liquid chromatography/mass spectrometry libraries.

This application note shows step-by-step procedures for tuning the liquid chromatography/mass selective detector (LC/MSD) to performance-based criteria, for creating a spectral library, and for searching the library. A mass spectral library developed under the performance-based conditions can be used on any LC/MSD tuned to performance-based criteria.

Introduction

As liquid chromatography/mass spectrometry (LC/MS) becomes easier to use and readily available in analytical labs in all industries, the need for searchable mass spectral libraries for data interpretation becomes more apparent. Currently, due to differences in tune requirements, instrument parameters, and hardware, universal LC/MS libraries do not exist as they do for gas chromatography/mass spectrometry (GC/MS) electron impact (EI) systems. LC/MS libraries created from different vendor systems are not transferable. Libraries created from the same system may not be usable after system service.

Using performance-based conditions for different liquid chromatography/mass selective detector (LC/MSD) systems, consistent and reproducible spectra can be obtained with or without collision-induced-dissociation (CID). These spectra can be searched against a library created under the same performance-based criteria but perhaps from a different LC/MSD system [1].

The performance-based conditions are standard conditions that are defined by instrument performance (ion ratios of the tune compounds) rather than just specific instrument settings from Autotune. The instrument is first tuned to fixed ion

optic voltages and then set to the fragmentor voltage to meet the standardized ion-ratio criteria of the tune compounds. Such performance-based results should be comparable from one instrument to another, whether of the same type or from different manufacturers.

To search universal mass spectra libraries with high confirmation confidence, the performance-based conditions include low-, mid-, and high-fragmentation energies. The instrument must be set to certain ion ratios using the tune mixture for each energy level. The three energy levels yield varying degrees of fragmentation of the tune compounds or analytes. These fragmentor voltages are also the CID energies. Three CID libraries (low-, mid-, and high-energy) are created from analyzing standards at three different energy levels. The actual sample analysis thus involves the low-, mid-, and high-fragmentation energies (that is, three separate injections) to obtain the molecular weight and the structural information of the peaks of interest. The analyst can then search the low-, mid-, and high-energy libraries respectively to identify the peaks with confirmation from different energy-level libraries. It is not necessary to make three libraries. All the library entries can be put together in one library.

This application note talks about using the National Institute of Science and Technology (NIST) MS search software to create and use a performance-based mass spectral library.

Experimental

The Agilent ES Tune Mix (part no. G2421A) was chosen as the tune mixture. This mix includes a series of fluorinated compounds and is designed to fragment across a wide range of fragmentation voltages, providing a variety of masses to use at different energy levels. The tune mix was diluted 1:1 using a mixture of 50% acetonitrile and 50% water for flow injection analysis (FIA). FIA is an established process for fast, multiple analyses that bypasses the chromatographic column and injects the sample directly into the detector. The time between injections is usually 0.5–1 minute.

Procedure for tuning the 1100 LC/MSD to performance-based criteria

1. Autotune the 1100 LC/MSD (see Table 1).

Table 1. The Conditions Used to Tune 1100 LC/MSD to Designated Tune Criteria

1100 LC/MSD	G1946D (SL)
Mode	ESI Positive
FIA volume	5 µL
Flow rate	200 µL/min
Mobile phase	50%:50% water:acetonitrile
Scan range	50–800 Da
Gain	1.0
Threshold	150
Stepsize	0.1
Peak width	0.12 min
Drying gas temperature	350 °C
Drying gas flow	10 L/min
Nebulizer pressure	30 psi
Capillary voltage	3500 V
Fragmentor voltage	Varied

2. Prepare the tuning mix in a 2-mL sample vial by diluting the ES tuning mix 1:1 in mobile phase (1:1 water:acetonitrile) for FIA.
3. Run FIA series (from the Method menu) across all fragmentor ranges for the desired CID energy levels (see Figures 1 and 2). The voltage is stepped at 5- or 10-V increments over the range shown below. A portion of a typical FIA analysis while varying the fragmentor voltage from 50 to 400 V appears in Figure 3. The mass spectra should be stored in “Condensed” mode as noted in Figure 2.

1100 LC/MSD	G1946A and G1946B MSD	G1946C and G1946D MSD
Low energy	70–110 V	80–180 V
Mid energy	130–170 V	210–310 V
High energy	200–240 V	320–400 V

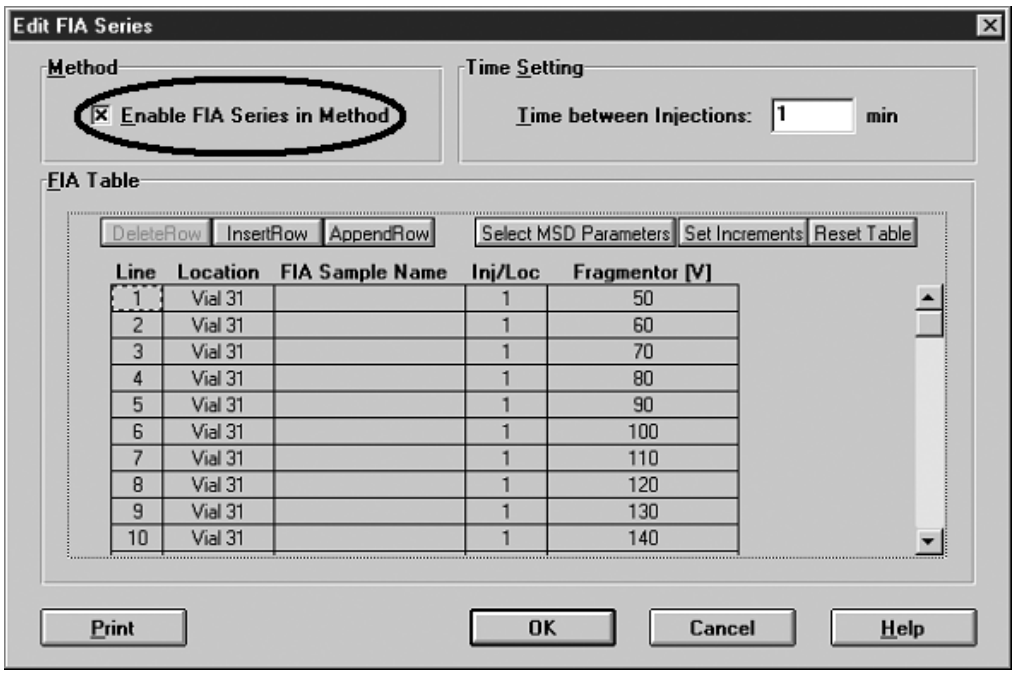

Figure 1. Creating an FIA series in a method.

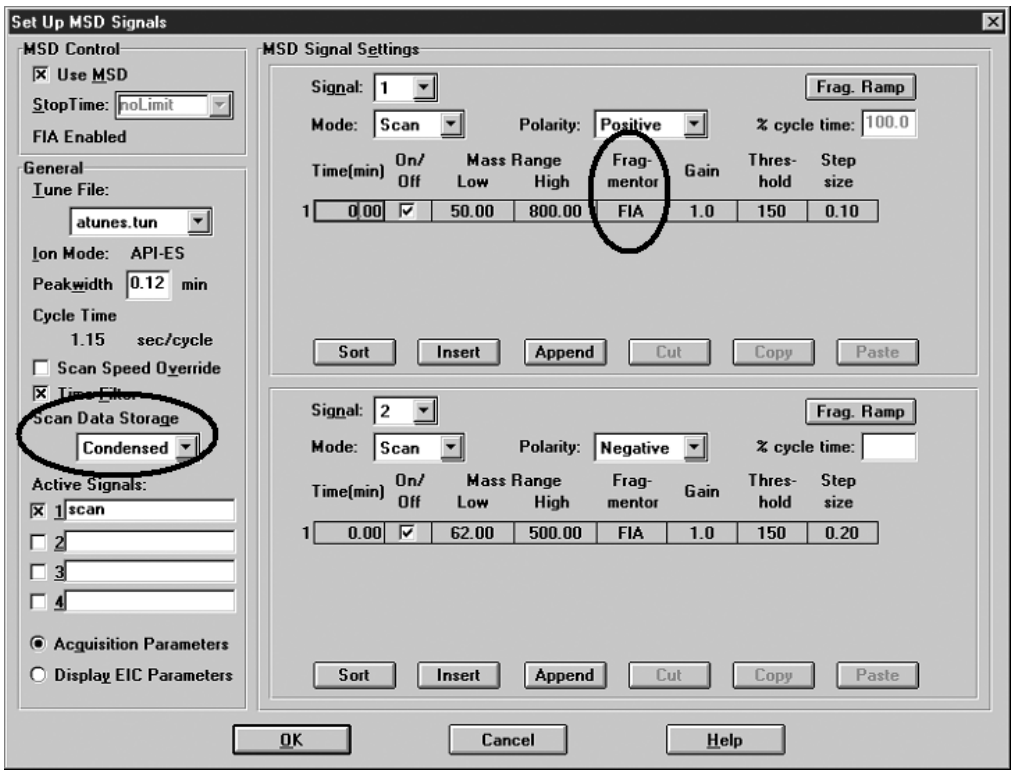

Figure 2. Set up MSD data acquisition parameters with the Fragmentor set to "FIA" and the data storage set to "Condensed".

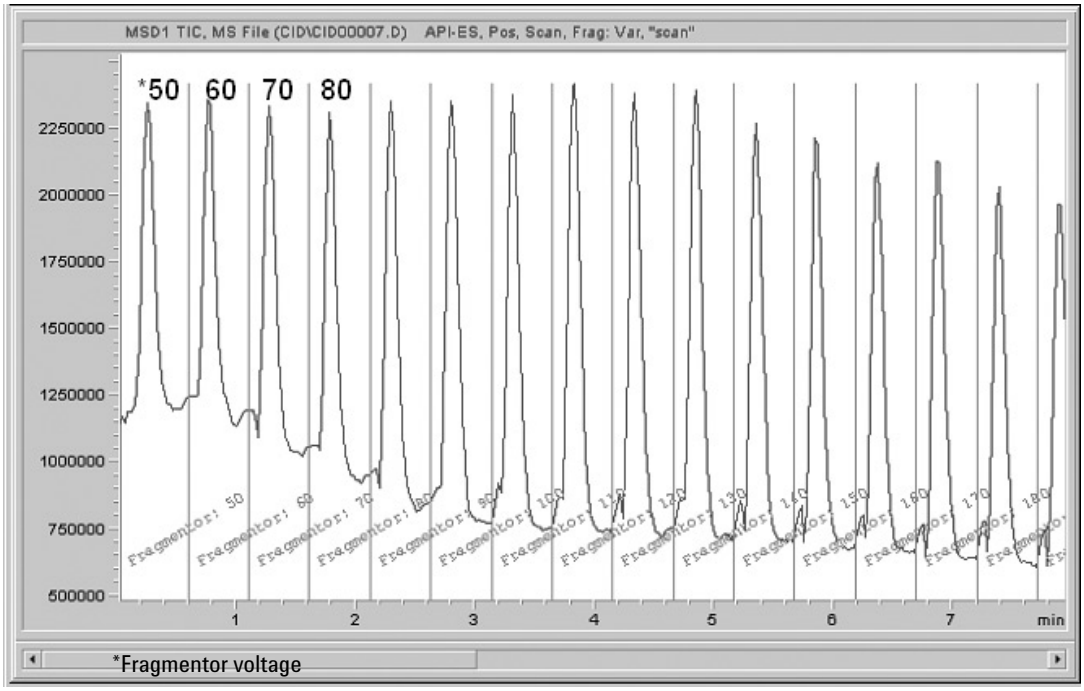

Figure 3. A portion of a typical FIA when varying the fragmentor voltage from 50 to 400 V.

4. Select a fragmentor voltage that best satisfies the criteria listed in Table 2 for each CID energy (low, mid, or high). Display the FIA in "Extract Ions" mode to help the voltage selection process.

Table 2 Performance-based Criteria for Generating CID Mass Spectra Library

Low-energy fragmentor range 70–110 V (or 80–180 V)	
Ion ratio	**Criterion**
290/586	50%–200%
322/118	<100%
622/118	100%–300%
Mid-energy fragmentor range 130–170 V (or 210–310 V)	
Ion ratio	**Criterion**
540/290	50%–200%
540/260	50%–200%
322/228	<100%
High-energy fragmentor range 200–240 V (or 320–400 V)	
Ion ratio	**Criterion**
790/622	50%–200%
678/622	<150%
540/678	<100%

5. Store the three fragmentor voltages that best meet the low-, mid-, and high-fragmentation criteria in three individual method files. These methods will be used to generate libraries or generate data to be searched in a previously generated library. Typically, variations in fragmentor voltages of 10 V result in little change in the match or search factor. These fragmentor voltages can also be used when the 1100 MSD is switched to electrospray ionization (ESI) negative ion detection.

Procedure for adding LC/MSD spectra into the NIST Mass Spectral Search Program [2, 3]

1. Make sure the NIST search program (G1043A) is installed on the LC/MSD ChemStation PC. If not, load the program onto the PC.
2. Start the LC/MSD ChemStation and Load an ultraviolet (UV) library (Spectra → Library → Open library...).

3. Figure 4 shows that two additional commands and icons are created to link the LC/MSD ChemStation to NIST MS search software.

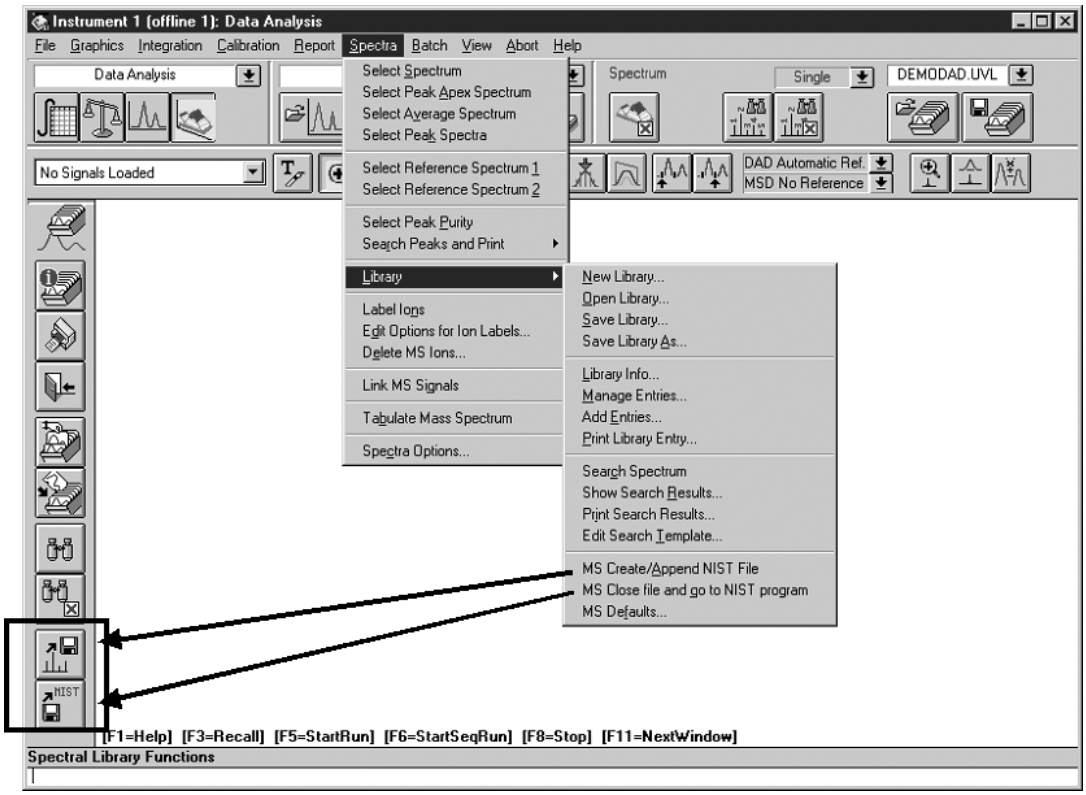

Figure 4. Two additional commands and icons are created to link the LC/MSD ChemStation to the NIST MS search software.

4. Select Spectra → Library → MS Default... to customize. Select "Append" to append (not overwrite) selected spectrum from the ChemStation to the clipboard (see Figure 5).

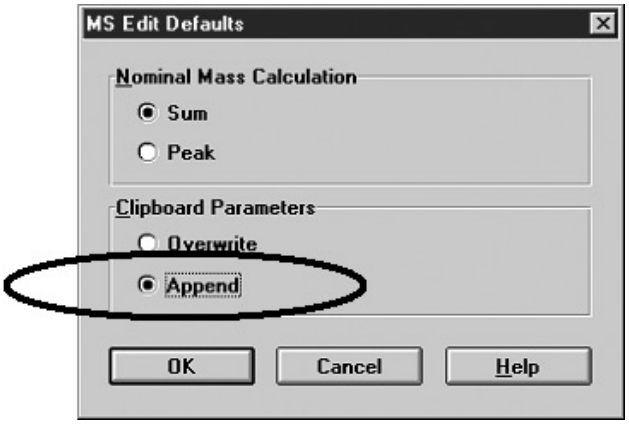

Figure 5. Select "Append" to append (not overwrite) selected spectrum from the ChemStation to the clipboard.

5. Analyze target compounds (standards) by LC/MSD using the three fragmentor voltages determined by the performance-based tune. Perform a quick check with a few standards to make sure the different CID energies can generate both molecular weight and fragmentation information.
6. Generate the apex spectrum of a peak and do a background subtraction to eliminate matrix interference, if any.
7. Verify there is no co-elution by comparing the spectra at the front, the apex, and the tail of the peak.

8. Press “Create/Append MS/NIST file” to convert the MS spectrum (of peak 1) into NIST format (see Figure 6).

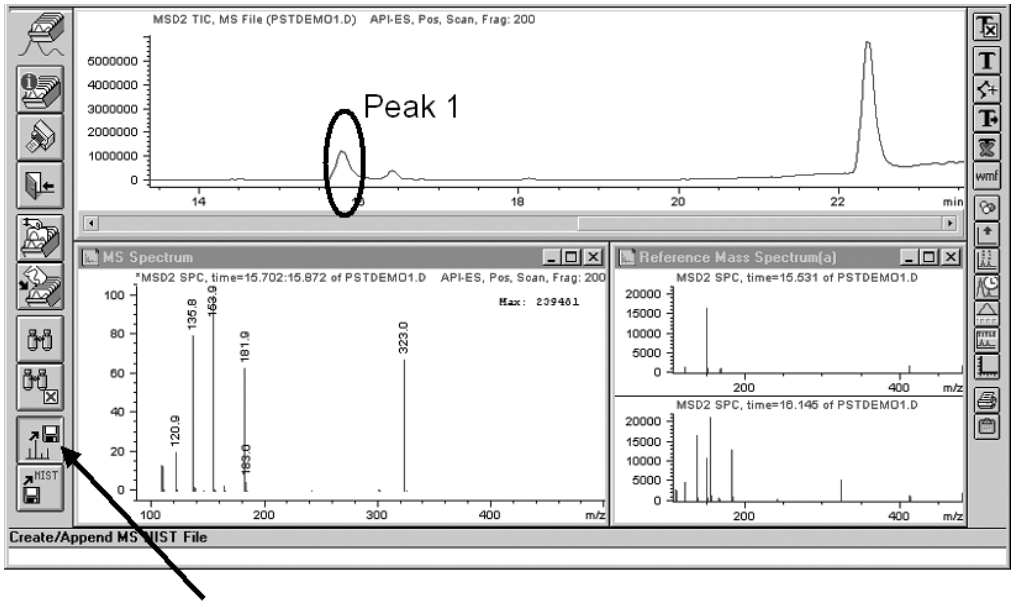

Figure 6. Press "Create/Append MS/NIST file" to convert the MS spectrum (of peak 1) into NIST format.

9. Continue steps 6, 7, and 8 to append other spectra into the clipboard.
10. When all the spectra (in this case, three spectra) are appended to the clipboard, press “Close MS NIST file and go to NIST program” to bring up the NIST program with the three spectra accumulated in the clipboard (see Figure 7).

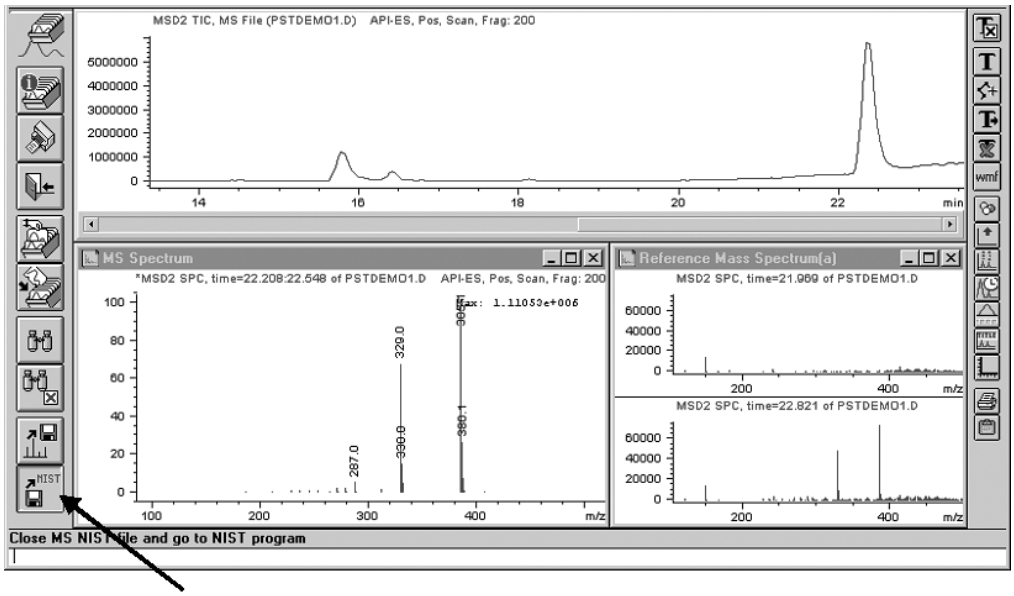

Figure 7. Press "Close MS NIST file and go to NIST program" to bring up the NIST program.

11. Figure 8 is the screen capture of the NIST MS search program with the three spectra from the LC/MSD ChemStation. Your screen may not look exactly the same as the figure.

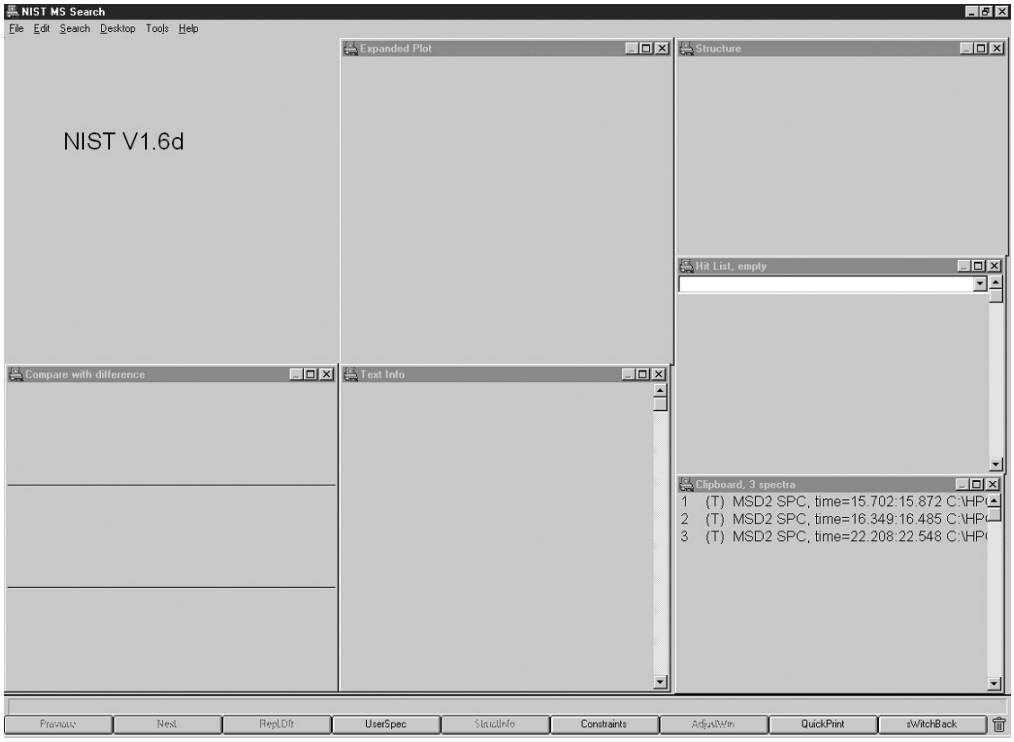

Figure 8. The screen capture of the NIST MS search program with the three spectra from the LC/MSD ChemStation.

Procedure for generating mass spectral library entries using the NIST Mass Spectral Search Program

1. (Continue from Figure 8) Select "Librarian..." command from the Tools menu.
2. As seen in Figure 9, all three entries from the clipboard appear in the “User Library Manager” window. Highlight the first entry, and press “Edit”.

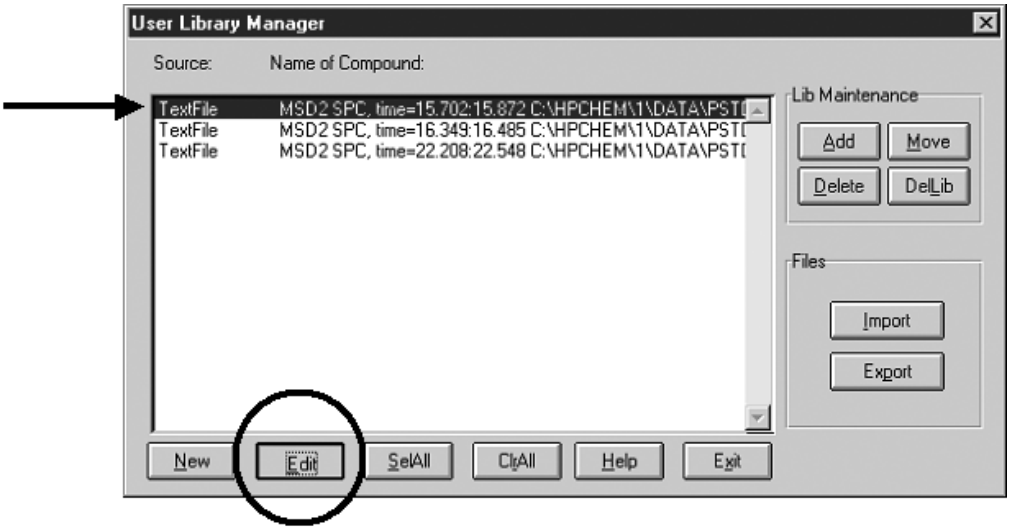

Figure 9. All three entries in the clipboard are shown in the "User Library Manager" screen. Highlight a TextFile entry and press "Edit".

3. Enter the available information for each entry: Name, Chem. Formula, Mol. Weight, Comments, etc. Press “Structure” to attach a structure to the entry, if there is one (see Figure 10). After all the information and structure are entered, press “Save” to complete the edit of the first entry in the library.
4. Repeat the process for the other entries in the library manager.

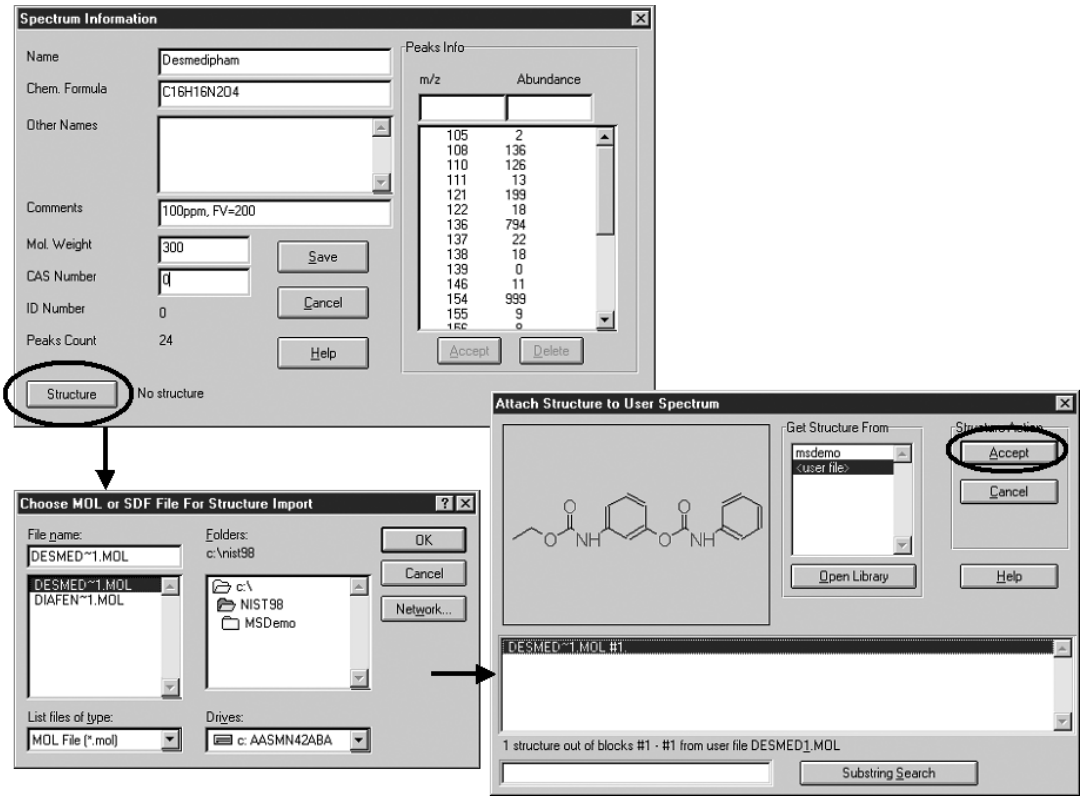

Figure 10. Enter the text and add a structure for the entry.

5. When all entries are updated with the compound name, chemical formula, molecular weight, molecular structure, and additional comments, press “SelAll” to highlight all the entries (see Figure 11).

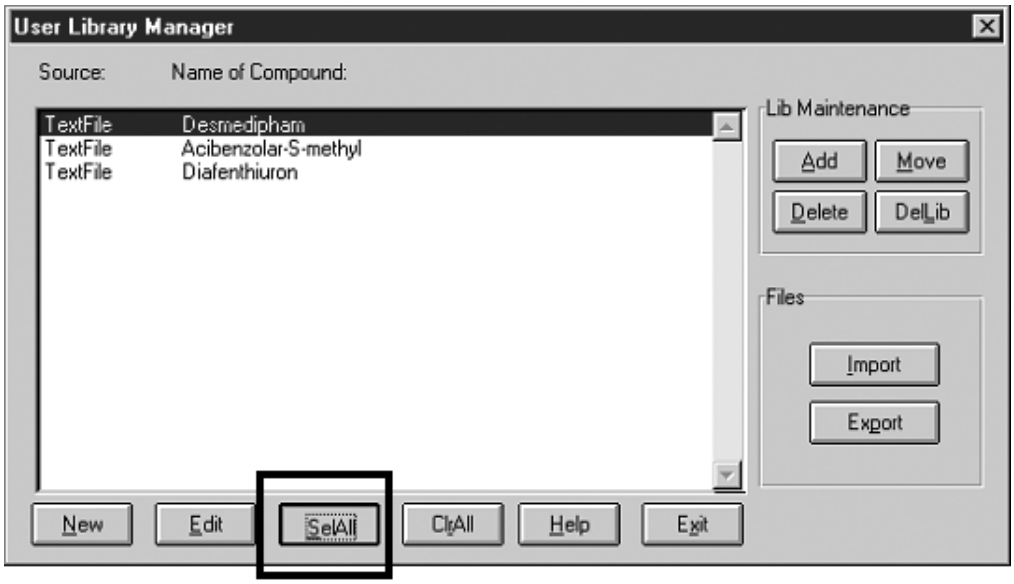

Figure 11. When all the entries are updated, press "SelAll" to highlight all the entries.

6. Press “Add” to add all the entries to an existing library or to a new library. In this example, all the entries were saved to a new library called pest200 (see Figure 12).

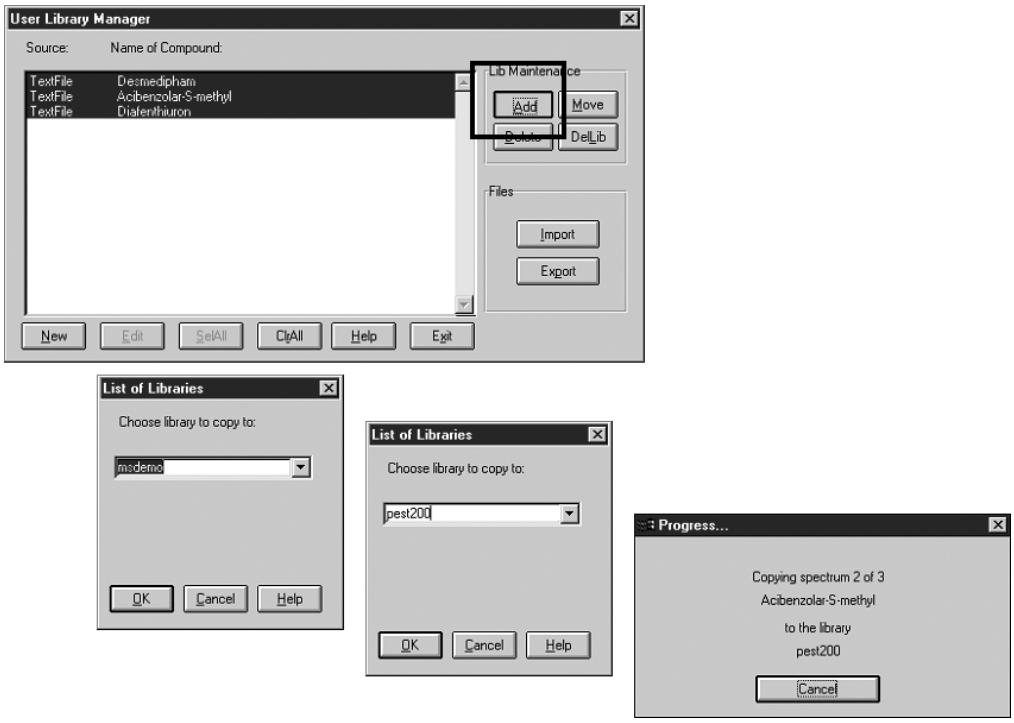

Figure 12. Press "Add" to add all the entries to an existing library or to a new library. In this example, all the entries were saved to a new library called pest200.

In general, store CID spectra in a CID library corresponding to each CID energy. For example Pest_low and Pest_mid are libraries for the low- and mid-CID energies. Storing the spectra in separate libraries for low-, mid-, and high-energies allows the relevant libraries to be searched in a sequential fashion. For example you can choose to search only the mid-energy library if you analyze the unknown using only the mid-CID energy. Of course, all the spectra from the different CID energies can be stored in one library for searching.

Procedure for Searching CID Mass Spectral Libraries Using the NIST Mass Spectral Search Program

1. Analyze unknown(s) using the three performance-based CID energies that you used to build the library. Best results are obtained if all three energies are used (three separate injections) and searched against a library (or three libraries) that contains mass spectra for the three energies.
2. Generate the apex spectrum of a peak and do a background subtraction to eliminate matrix interference, if any.

3. Verify there is no coelution by comparing the spectra at the front, the apex, and the tail of the peak.
4. Press “Create/Append MS/NIST file” to convert the MS spectrum into NIST format (see Figure 6).
5. Continue steps 2–4 to append other spectra to the clipboard.
6. When all the spectra are appended to the clipboard, press “Close MS NIST file and go to NIST program” to bring up the NIST MS search program with the spectra accumulated in the clipboard (see Figure 7).
7. In the NIST MS search program, press “UserSpec” to specify a library (see Figure 13).

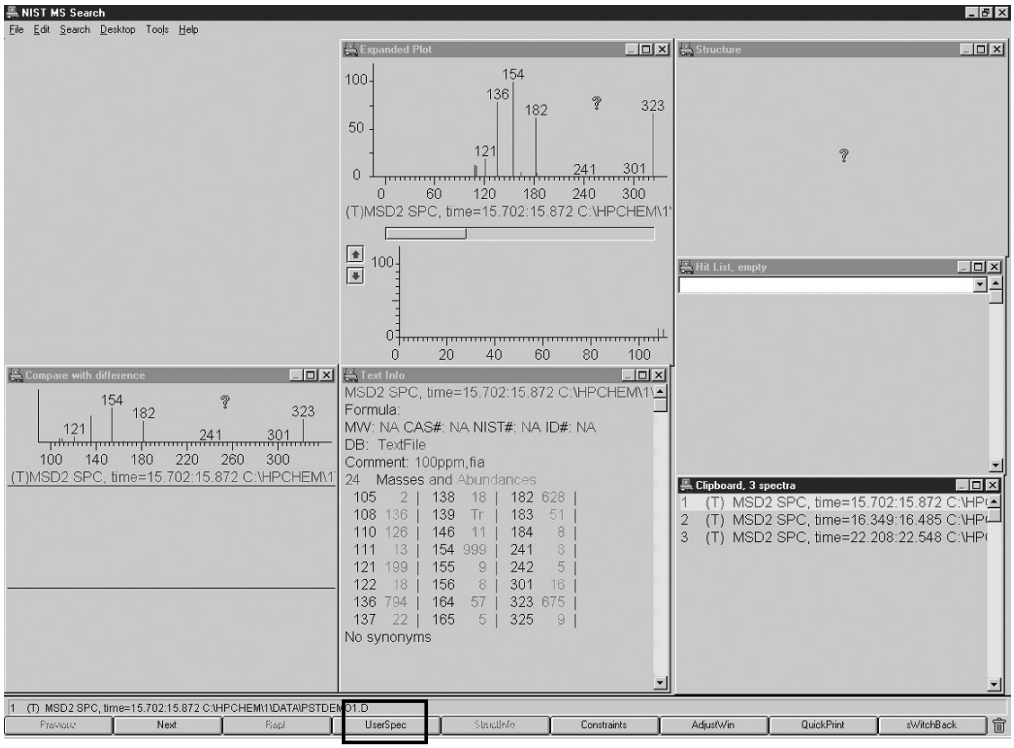

Figure 13. In the NIST MS search program, press "UserSpec" to specify a library.

8. Press “Libs” to add or remove libraries for searching. In this example, pest200 was added to the search list (see Figure 14). If there are more libraries to be searched, add them to the right side of the panel. Press “OK”.

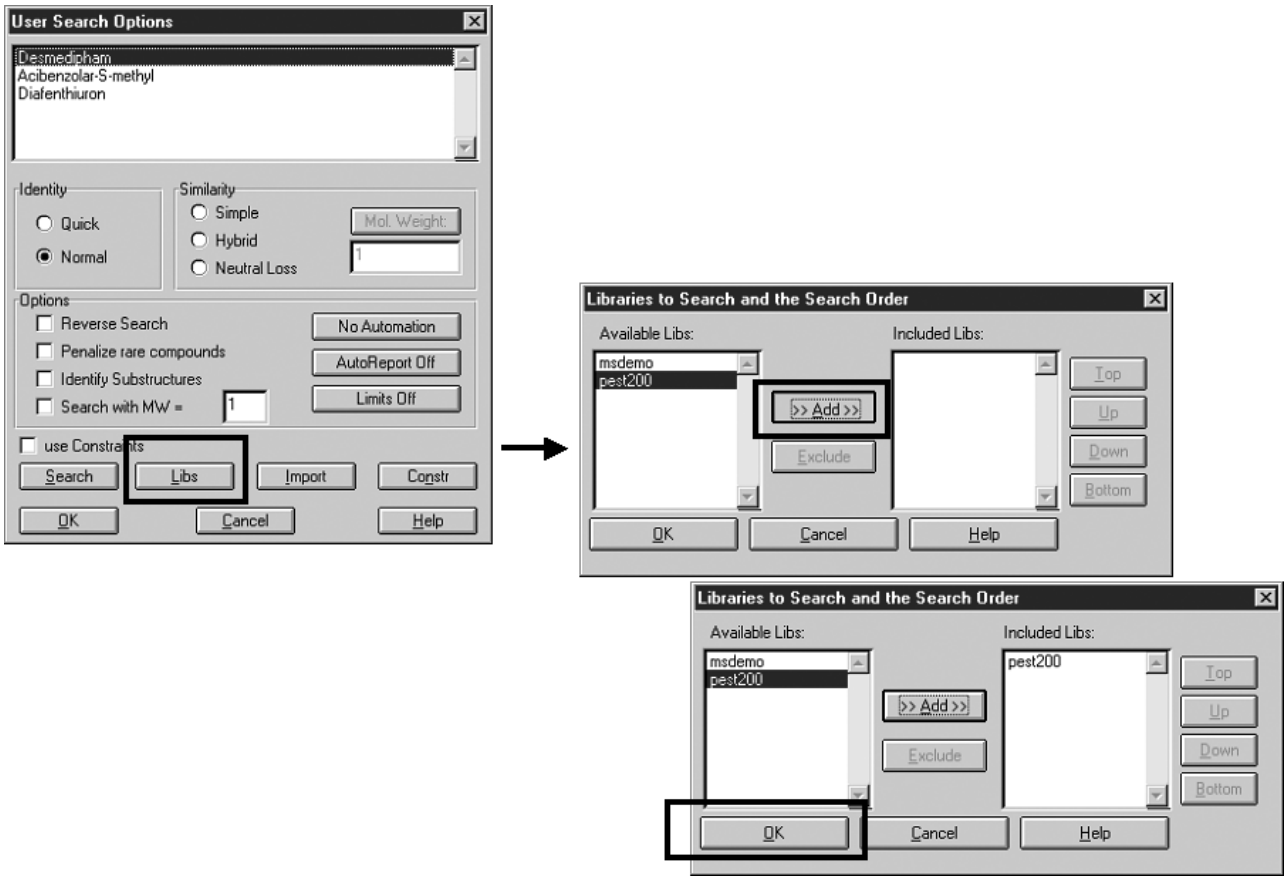

Figure 14. Press "Libs" to add or remove libraries for searching. In this example, pest200 was added to the search list.

9. Double-click on a clipboard entry to search against the pest200 library (see Figure 15). In general, a search factor (in the InLib window of the NIST program) greater than 800 is considered acceptable for identification.

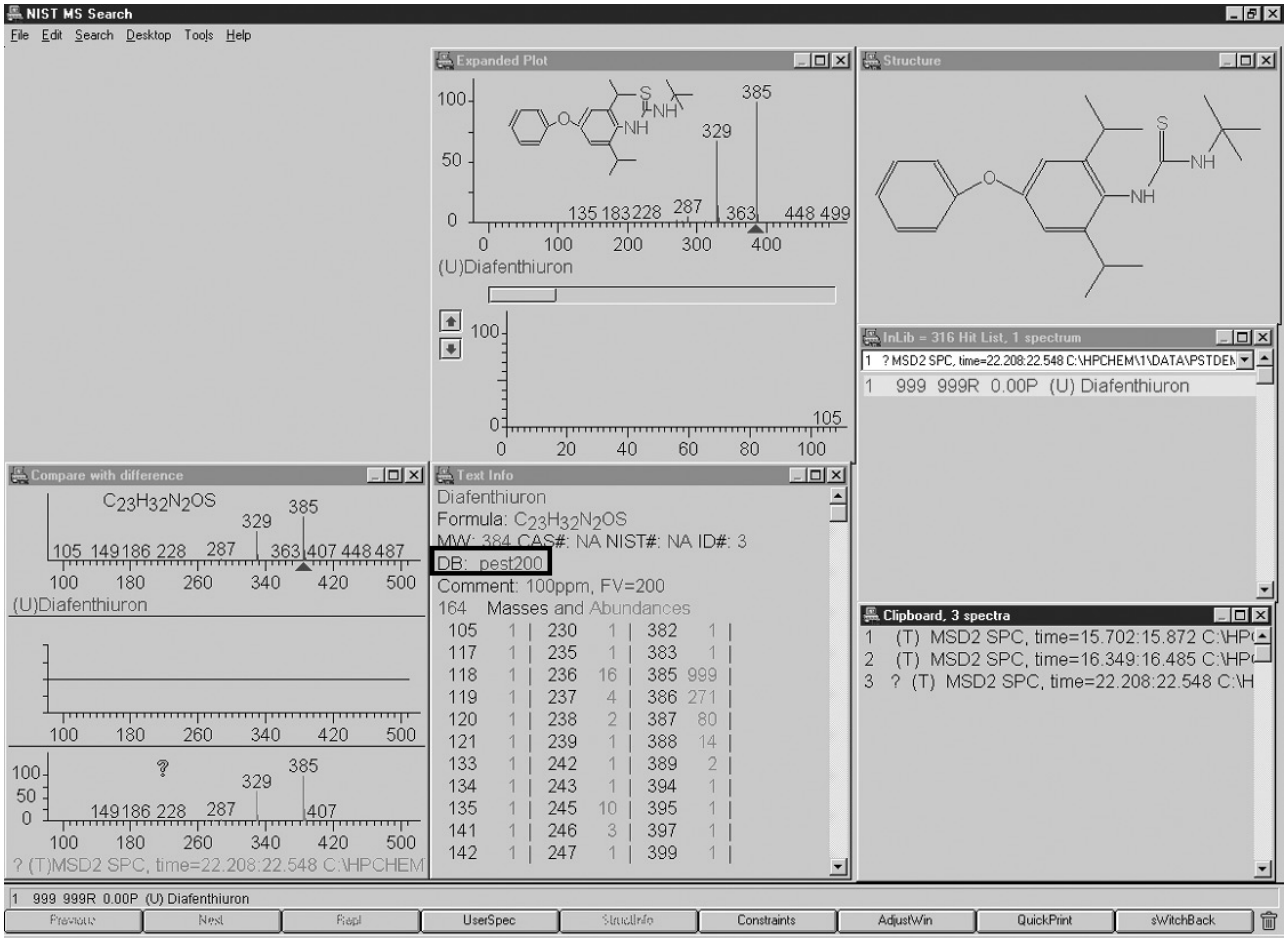

Figure 15. Double-click on a clipboard entry to search against the pest200 library. A reverse search factor greater than 800 is considered acceptable for identification.

Discussion

The solubility of the compounds in the ES Tune Mix solution may change over time. Therefore, a relatively fresh solution should be used for the performance-based tuning to achieve the most consistent results.

Sodiation will change the fragmentation pattern. Therefore, it is important to minimize and monitor the different sodium adducts in the solution and to analyze the samples using all three CID energies.

A pesticide library with over 200 compounds was built on a G1946A LC/MSD Quad system. The library was then copied to a G1946D LC/MSD Quad system. An analysis of some of the target compounds done on the G1946D LC/MSD was successfully searched against the library.

Conclusion

This application note gives step-by-step procedures to create and use a performance-based LC/MSD mass spectral library. The performance-based conditions are standard conditions defined by achieving a set of ion ratios of the tune mixture by adjusting the fragmentor voltage. The instrument is first tuned to fixed ion optic voltages and then set to the fragmentor voltage to meet the standardized ion-ratio criteria of the tune compounds. Therefore, the performance-based results should be comparable from one instrument to another, whether of the same type or from different manufacturers, as is currently true for GC/MS. A mass spectral library developed under the performance-based conditions can be used on any LC/MSD system tuned to performance-based criteria.

References

1. J. M. Hough, C. A. Haney, R. D. Voyksner, and R. Bereman, “Evaluation of Electrospray Transport CID for the Generation of Searchable Libraries,” Analytical Chemistry, 72, 2265-2270, 2000.
2. Sparkman and Agnew, “A New Dimension In Mass Spectral Library Searching,” Agilent Application Note, publication 5965-8424E, December 1999.
3. http://www.nist.gov/srd/nist1a.htm

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