Gas Chromatography Problem Solving and Troubleshooting

Question

I am getting more than one high-quality mass spectra library match for some unknown compounds in a sample analyzed by gas chromatography–mass spectrometry. How do I know which compound identification is the correct one?

Answer

Compounds cannot be conclusively identified based solely on a mass spectra library match, regardless of the fit quality. Many compounds have remarkably similar mass spectra, thus they cannot be identified based from only a library match. Nearly indistinguishable mass spectra are common for structural or positional isomers (e.g., *o*-, *m*-, and *p*-xylene; methamphetamine and phentermine). It may also occur for nonisomeric compounds. An example of an ambiguous library match is shown in Figure 1. The mass spectrum of the unknown compound (Figure 1A) matches very favorably with the library mass spectrum of 1-dodecanol (Figure 1B), however, a very good library match is also obtained with nonylcyclopropane (Figure 1C). The numerical fit quality was the same for both spectra even though one compound is an alcohol and the other is a hydrocarbon. Conclusive identification cannot be made based only on the mass spectra matches.

Another potential problem with library-based matches is the difference in the analysis conditions used to obtain the library and sample mass spectra. User-built libraries are less prone to this type of problem. Different model mass spectrometers, tuning criteria, and small variations in any of the numerous mass spectrometer parameters may cause small disparities between compound mass spectra. The differences evident in Figures 1B and 1C could be attributed to minor difference between mass spectrometers. The mass spectra in a commercially available library are usually obtained using high-purity compounds. Analyzed samples often contain other compounds originating from the matrix or other sources (i.e., contaminants). These compounds can add to the overall background signal or co-elute with the target analytes, which alters their mass spectra. Obviously, this introduces an additional and significant amount of uncertainty in library matches, especially when using a commercially obtained library.

Even though mass spectral library matches cannot be used as conclusive identification, they can often be used for preliminary or presumptive identifications. Based on the library match results, additional tests can then be completed to obtain a more definitive identification. Using the example shown in Figure 1, a qualitative standard of 1-dodecanol and nonylcyclopropane can be made or obtained. They can be analyzed using the same gas chromatography (GC)–mass spectrometry (MS) conditions as used for the unknown

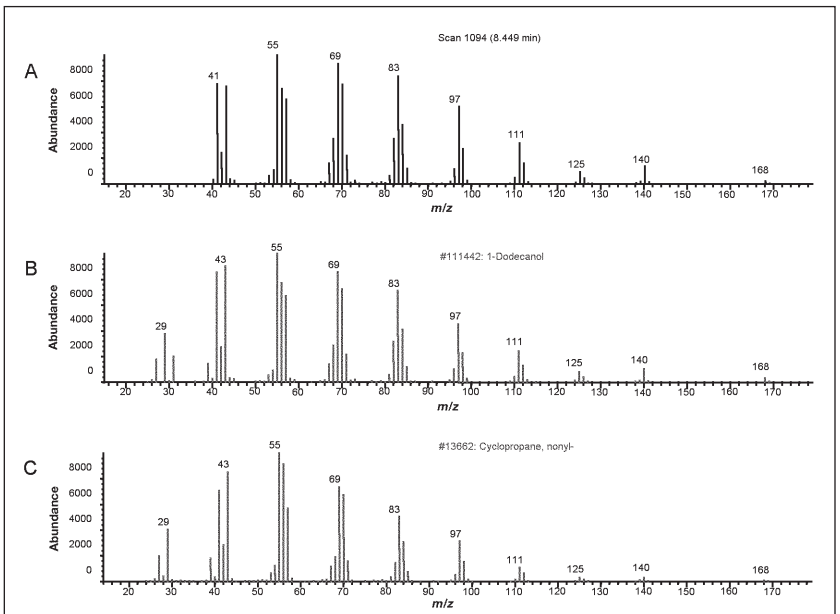

Figure 1. Mass spectra of an (A) unknown compound, (B) library match of 1-dodecanol, and (C) library match of nonylcyclopentane.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to: JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting,* P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

Dean Rood
Associate Editor

sample. In addition to the mass spectral comparison, GC retention time data is now available. By matching the GC retention time and mass spectra, the unknown compound can be identified at a very high confidence level. Using this technique, the unknown compound in Figure 1 was identified as 1-dodecanol, based on mass spectral and GC retention time data.

Building your own mass spectral library is recommended when the list of target analytes is known. Instead of relying on a purchased mass spectrum library, a library is built using the same model mass spectrometer and analysis conditions. More certain and better library matches are obtained with a user-built library. Obviously, if completely unknown compounds are present, a user-built library would not be useful. The library spectra in Figure 1 were generated using a lower mass limit than the unknown compound. A lower limit of *m/z* 40 was used for the unknown sample, thus explaining the lack of masses below *m/z* 40. Building and utilizing a user library would eliminate this problem because the same mass range would be used to obtain the library and sample mass spectra. Positive compound identification is nearly incontestable and highly defendable when a GC retention time and properly generated user library mass spectra match is obtained. Identification of an unknown compound based solely on a mass spectra match from a purchased library is highly suspect, unsound, and indefensible.