Multi-Technique Spectral Searching in the KnowItAll® Spectroscopy Software

Gregory Banik, Ph.D.,[1] and Karl Nedwed[2]
[1] Bio-Rad Laboratories, Inc., Informatics Division, Philadelphia, PA, USA
[2] Bio-Rad Laboratories, Inc., Informatics Division, Graz, Austria

Spectroscopy 280029

Abstract

The use of spectral search software and reference databases in NMR, MS, and IR is a longstanding technique in unknown identification, compound verification, and structure elucidation. Typically, a score or hit quality index (HQI) is calculated to describe the correlation between a spectrum of the unknown compound and spectra of known compounds in reference databases. A new system for multi-technique spectral searching is described that utilizes multi-dimensional analysis of several hit lists resulting from spectral similarity searches performed simultaneously in reference databases for multiple complementary analytical techniques. The multi-dimensional approach permits the optimization of chemical similarity based on several analytical techniques to maximize the chemical knowledge obtained on the unknown compound.

Introduction

A variety of complementary spectroscopic and chromatographic techniques (Figure 1) are applied in an attempt to answer one of two fundamental issues: compound verification and unknown identification. The former is encountered in quality control environments; the latter in such fields as compound synthesis or natural product identification.

Technique	Information From This Technique
IR & Raman	Which functional groups are present?
NMR	How are carbon and hydrogen atoms bonded?
MS	What is the mass of the molecule and which groups of atoms are present?
UV-Visible	What is the electronic system of the molecule like?
Chromatography	How many different chemicals make up a sample and what is their relative abundance?

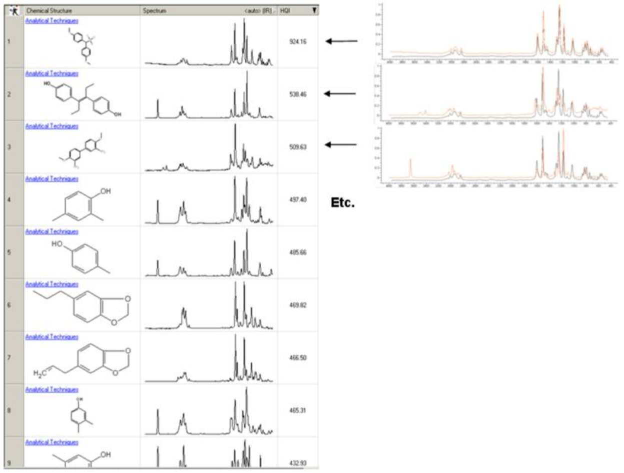
Fig. 1. Information Obtained from Analytical Techniques

Compound verification and unknown identification progressed significantly with the advent of computer-searchable electronic databases of reference spectra.

Algorithmic comparison of the unknown spectra to those in reference databases could provide strong evidence for the compound's identity, or at least clues that could lead to an ultimate identification or confirmation. In dealing with the search results of reference spectra and their associated chemical structures in a software searching environment, three concepts have become traditional to the point of being dogmatic:

1. **Hit Lists** – A list of similar spectra called a hit list is generated by the software program and displayed in a tabular fashion.
2. **Hit Quality Index** – A hit quality index (HQI) value is associated with each reference spectrum and is a numerical measure of the closeness of fit between the unknown spectrum and each reference spectrum. The higher the HQI value, the closer the unknown spectrum is to the reference spectrum.
3. **Rank Ordering** – The hits are sorted so that those with the highest HQI value are at the top of the list and those with the lowest HQI are at the bottom of the list.

These three concepts are illustrated in Figure 2, which shows a rank-ordered hit list from an IR spectral search.



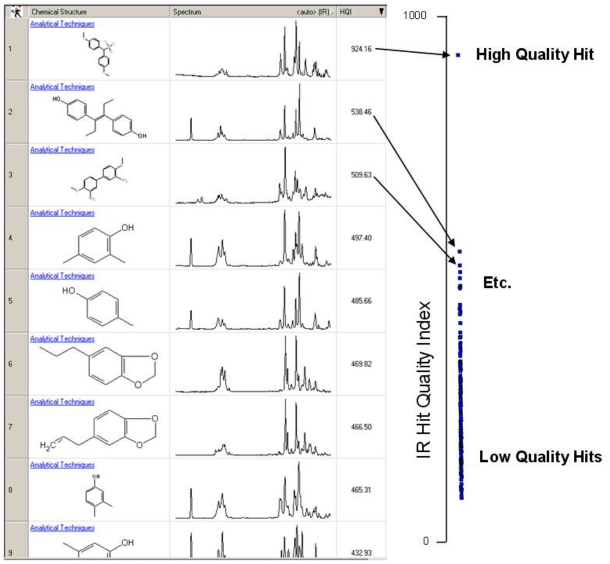
	Chemical Structure	Spectrum	<auto> (IR)	HQI
1	Analytical Techniques			924.16
2	Analytical Techniques			538.46
3	Analytical Techniques			509.63
4	Analytical Techniques			497.40
5	Analytical Techniques			485.66
6	Analytical Techniques			469.82
7	Analytical Techniques			466.50
8	Analytical Techniques			465.31
9	Analytical Techniques			432.93

Fig. 2. Spectral Hit List and Hit Quality Index (HQI)

Multi-Technique Searching – Theory and Practice

Bio-Rad Laboratories, Inc. has introduced a novel and powerful system for searching multiple spectral techniques simultaneously. Using Bio-Rad's KnowItAll Informatics software, the process is very straightforward: multiple spectra from complementary analytical techniques are used as unknowns to search multiple reference databases containing spectra from the complementary analytical techniques. Two powerful innovations, however, transform the traditional hit lists into a dramatic new approach for visualizing spectral search results.

The first innovation is plotting a hit list from a single spectral search as values on a number line. Illustrated in Figure 3, this simple innovation provides value (albeit limited value) in allowing the relative values of all hits in a hit list to be displayed simultaneously. The second and much more significant innovation is plotting multiple hit lists from multiple spectral searches performed in complementary analytical techniques to be plotted as points in a graph. In this innovation, each point in the graph represents a single compound where the hit quality indices for each reference spectrum in each technique are the coordinates in an N-dimensional spectral space (Figure 4). Points (i.e., compounds from the reference spectral databases) that are closer to the origin will have a lower spectral similarity to the unknown than those farthest from the origin.



	Chemical Structure	Spectrum	<auto> (IR)	HQI
1	Analytical Techniques			924.16
2	Analytical Techniques			538.46
3	Analytical Techniques			509.63
4	Analytical Techniques			497.40
5	Analytical Techniques			485.66
6	Analytical Techniques			469.82
7	Analytical Techniques			466.50
8	Analytical Techniques			465.31
9	Analytical Techniques			432.93

Fig. 3. 1D Plot of HQI Values from One Spectral Technique

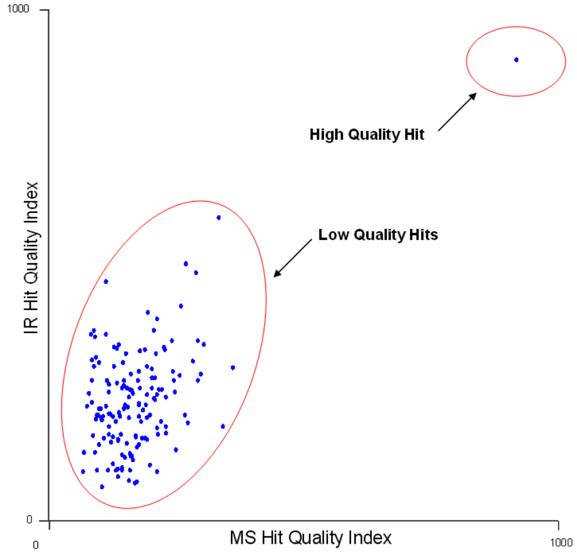


Fig. 4. 2D Plot of HQI Values from Two Spectral Techniques

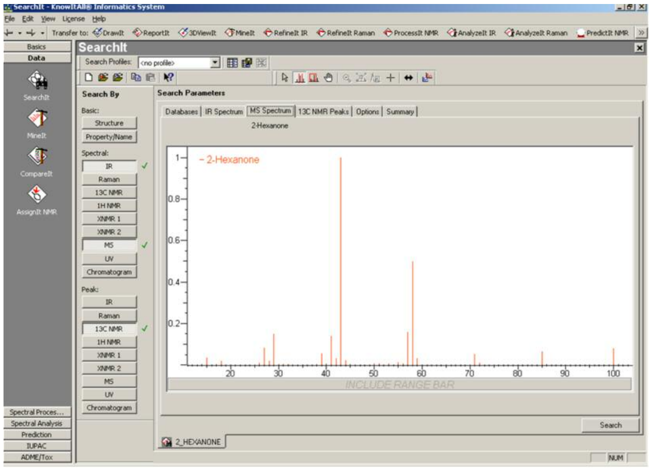
Methods

- **Software:** KnowItAll Informatics System
  - IR Searching - Euclidean Distance Algorithm
  - MS Searching - Modified Finnigan Algorithm
  - ^{13}C NMR - Peak Search Algorithm
- **IR Databases:** Bio-Rad's KnowItAll IR Spectral Library
- **^{13}C NMR Databases:** Bio-Rad's KnowItAll NMR Spectral Library
- **MS Databases:** Bio-Rad's KnowItAll Mass Spectral Library
- **Test Spectra Sets**
  - IR and MS - NIST WebBook (*http://webbook.nist.gov*)
  - ^{13}C NMR - Bio-Rad sample file

Combinations of spectra were searched in the KnowItAll Informatics System (Figure 5). The results from all reference database searches were automatically performed in all spectral techniques simultaneously. The different spectral databases were automatically cross-linked by an exact chemical structure match, and the resulting hit lists were displayed (Figure 6).

Spectra were required to exist for all hits, that is, an IR/MS combined search must return a pair of IR and mass spectra linked by exact structure. This automatic procedure eliminates hits where an IR spectrum exists without the corresponding mass spectrum or vice versa.

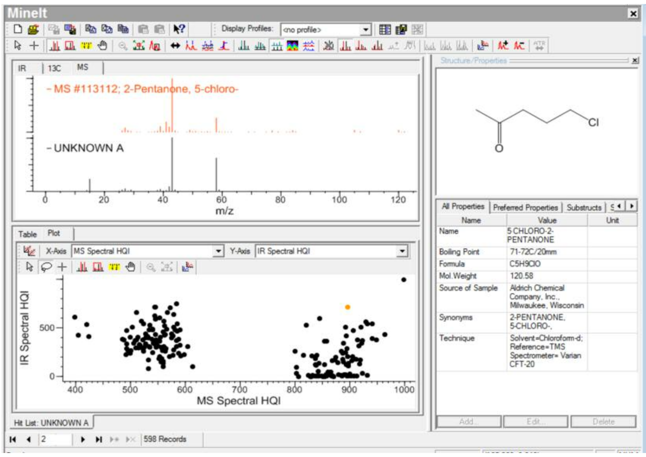
The NIST/EPA/NIH mass spectra reference database was not used as the mass spectra used as test spectra were from the NIST WebBook, and to include them would have biased the study.



Search Profiles: <no profile>

Fig. 5. Defining Multiple Simultaneous Spectral Searches

The top 200 multi-technique hit list was plotted as shown in Figure 6, where an interactive display allows the user to see the corresponding record information associated with data points by clicking on a single data point or lassoing a group of data points.



Display Profiles: <no profile>

X-Axis MS Spectral HQI

Y-Axis IR Spectral HQI

Name	Value	Unit
Name	5-CHLORO-2-PENTANONE	
Boiling Point	71-72C/20mm	
Formula	C5H9ClO	
Mol.Weight	120.58	
Source of Sample	Aldrich Chemical Company, Inc., Milwaukee, Wisconsin	
Synonyms	2-PENTANONE, 5-CHLORO-,	
Technique	Solvent=Chloroform-d; Reference=TMS Spectrometer= Varian CFT-20	

Hit List: UNKNOWN A

2

598 Records

Figure 6 - 2D Spectral HQI Plot (IR/MS)

Results

Example 1 - Cholesteryl Acetate

In this example, the IR and mass spectra of cholesteryl acetate (Figure 7) were searched simultaneously. In this case, exact matches for the test spectra were found in the reference spectra databases for both techniques. The resulting 2D plot of MS vs. IR HQI values (Figure 8) shows that neither IR nor MS alone could clearly identify the compound. Using the two techniques in parallel, however, the system clearly identifies this as cholesteryl acetate. Interestingly, a number of related esters of cholesterol (and cholesterol itself) cluster in the upper right of the graph. There is clearly a high degree of correlation between the spectral space and the chemical space.

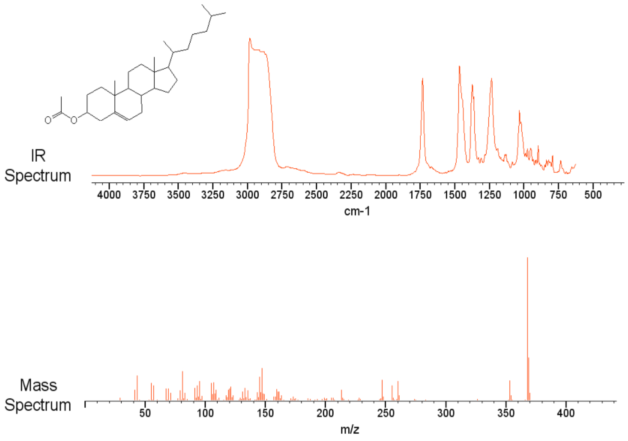


Fig. 7. 2D Spectral HQI Plot (IR/MS)

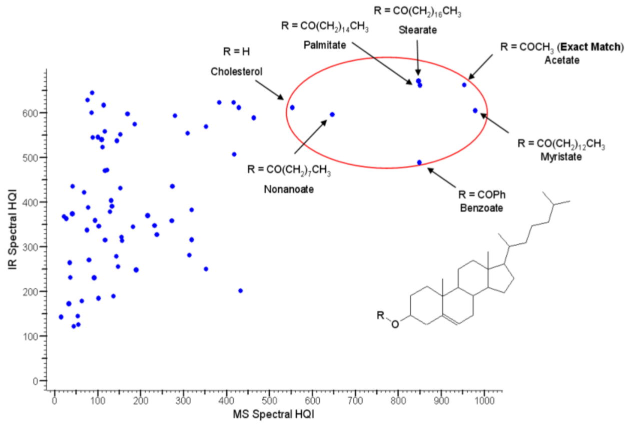


Fig. 8. Example 1 Results: 72 Spectra Pairs (MS/IR)

Example 2 - 1, 1, 4, 4-Tetraphenyl-1, 3-Butadiene

In this example, the IR and mass spectra of 1,1,4,4-tetraphenyl-1,3-butadiene (Figure 9) were searched simultaneously. In this case, exact matches for the test spectra were found in the reference spectra databases for both techniques. The resulting 2D plot of MS vs. IR HQI values (Figure 10) shows that in this case, either IR or MS alone could have identified the compound. Using the two techniques in parallel, however, the system degree of separation between the exact match and the rest of the hit list becomes glaringly obvious.

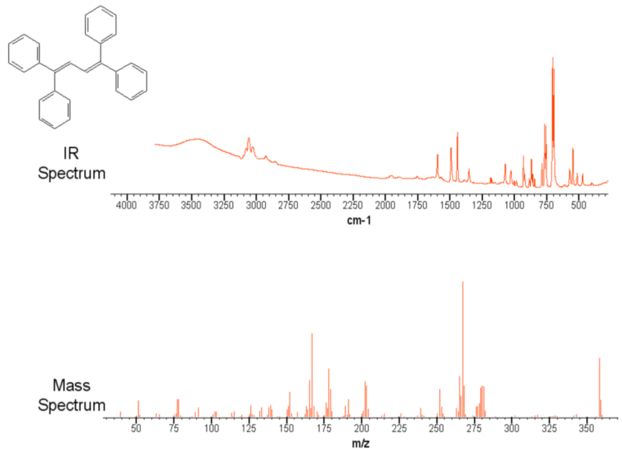


Fig. 9. Example 2: 1, 1, 4, 4-Tetraphenyl-1, 3-butadiene

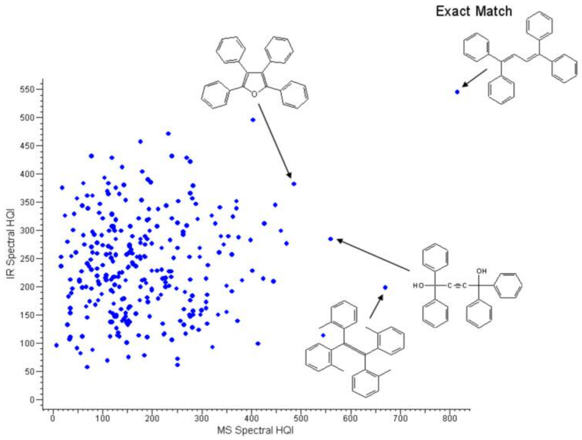


Fig. 10. Example 2 Results: 287 Spectra Pairs (MS/IR)

Example 3 - Phthalic Acid, Butyl Cyclohexyl Ester

In this example, the IR and mass spectra of n-butyl cyclohexyl diester of phthalic acid, (Figure 11) were searched simultaneously. In this case, no exact matches for the test spectra were found in the reference spectra databases. The resulting 2D plot of MS vs. IR HQI values (Figure 12), however, shows an interesting cluster of compounds in the upper right-hand corner of the plot. All of these 11 compounds are phthalic acid diesters. Again, there is clearly a high degree of correlation between the spectral space and the chemical space in this example.

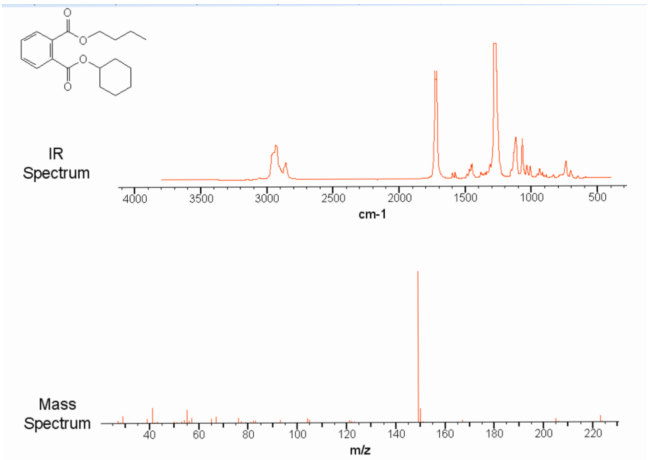


Fig. 11. Example 3: Phthalic Acid, Butyl Cyclohexyl Ester

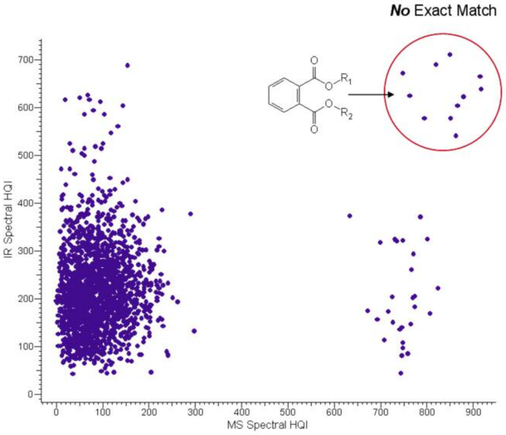


Fig. 12. Example 3 Results: 1,821 Spectra Pairs (MS/IR)

Example 4 - 2-Hexanone

In this example, the 13C NMR, IR and mass spectra of 2-hexanone (Figure 13) were searched simultaneously. In this case, exact matches for the test spectra were found in the reference spectra databases for all three techniques. The resulting 2D plots of 13C NMR vs. IR HQI values (Figure 14), MS vs. 13C NMR HQI values (Figure 15), and MS vs. IR HQI values (Figure 16) show that neither 13C NMR, IR, nor MS alone could clearly identify the compound (Note: the discrete banding in Figures 14 and 15 is an artifact of the NMR peak search algorithm, which matches a discrete number of peaks in the unknown to entries in the database). Using the three techniques in parallel, however, the system clearly identifies this as 2-hexanone. Again, a number of related compounds cluster in the upper right of the graph, likewise indicating a high degree of correlation between the spectral space and the chemical space.

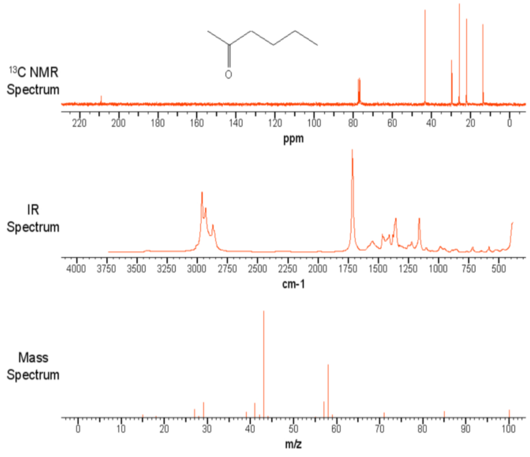


Fig. 13. Example 4: 2-Hexanone

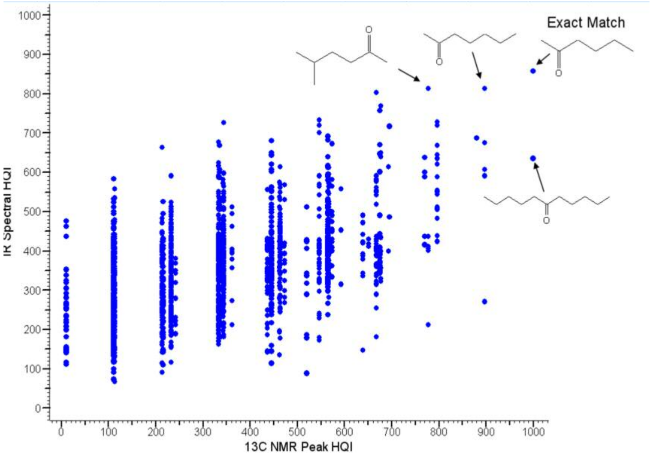


Fig. 14. Example 4 Results: 5,715 Spectra Pairs (NMR/IR)

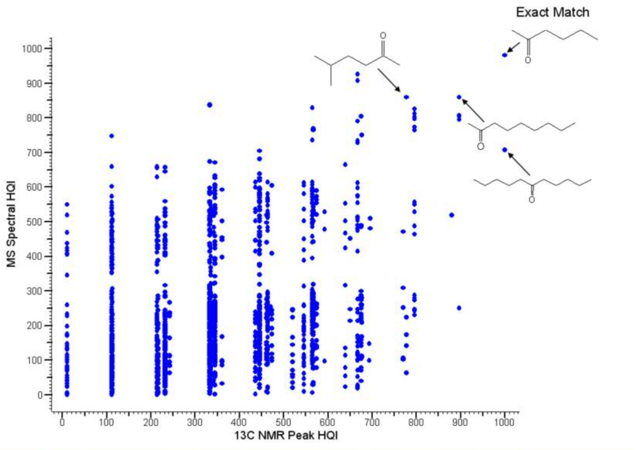


Fig. 15. Example 4 Results: 5,715 Spectra Pairs (NMR/MS)

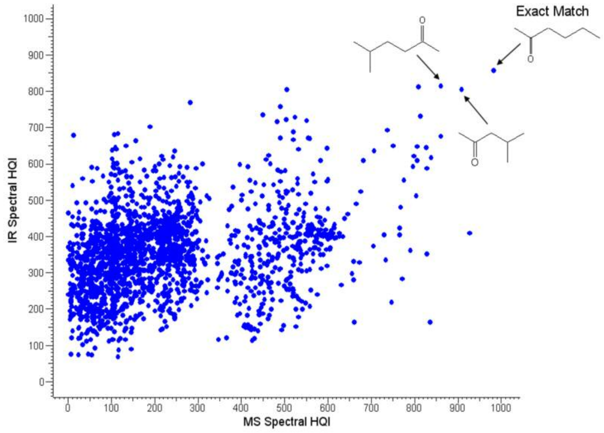


Fig. 16. Example 4 Results: 5,715 Spectra Pairs (MS/IR)

Conclusion

From this study, the following conclusions can be drawn from the fast and easy-to-use system of multi-technique spectral searching:

- Multi-dimensional analysis of multi-technique spectroscopic data is a simple yet incredibly powerful analytical technique for visualizing spectral space.
- The technique allows for efficient visualization of more data than is possible using a traditional spreadsheet approach, making this an effective datamining tool.
- The applicability of the technique in compound verification, unknown identification, and structure elucidation is clear.

Bio-Rad
Laboratories, Inc.

Informatics Division **Website:** www.knowitall.com **Contact Us:** www.knowitall.com/contactus