Automated Screening of GC-TOFMS Chromatograms with Specific Detection for Chlorine, Bromine, and Sulfur Containing Compounds

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1. Introduction

The use of automated searching of mass spectra for specific patterns allows the GC-TOFMS system to be used as a selective detection system for numerous types of compounds. When applied to the full chromatogram, this automatic search capability may be used to identify chlorinated, brominated and sulfur containing contaminants such as pesticides in foods or environmental samples. With the use of GCxGC-TOFMS, trace components with specific functionality can be identified in complex mixtures. This work demonstrates the use of the GC-TOFMS system as a specific detector with the ability to provide mass spectral identification of compounds containing specific groups.

In some types of analysis, the objective is to screen for compounds with some specific characteristic. For example, the objective of an analysis could be identification of chlorinated or brominated compounds in the environment in the search for pollutants or in assessing a terrorist threat. Sulfur containing compounds are not common in the environment and often have biological significance. Given that many pesticides contain sulfur, specific detection of sulfur containing compounds can help focus the examination of a GC/MS peak table for likely compounds.

The manual search of a large peak table is time consuming, particularly for GCxGC-TOFMS chromatograms of complex environmental samples. If one relies on library search results, compounds of interest may be missed because they either are not in the library or interfering signals have prevented correct identification by library matching of spectra. Automated searching for distinctive patterns related to the functionality of interest can identify the presence of functionality independent of library searching.

In the work shown here, compounds containing chlorine, bromine and sulfur are automatically identified, based on the isotope distributions of these A+2 atoms, which can be identified in the molecular ion in the mass spectrum.

Spectral Selection

Automated selection of spectra is accomplished by the use of the Classification and Scripting features available in ChromaTOF®. The scripting feature provides the facility to use VBScript programming to establish rules for filtering spectral data. Scripts are used in conjunction with the ChromaTOF classification feature to classify compounds according to the spectral features identified with the scripts.

Identification of compounds occurs in two steps:

1. Identification of the parent ion.
2. Identification of the expected isotopic or fragmentation pattern.

The parent ion is identified by examining the spectrum starting from the highest mass and testing for a mass signal that is significantly above the background in the spectrum. Typically, a chlorinated or brominated compound will have a fairly strong parent ion or large fragment showing the isotope pattern for the halogen atoms present. Finding either the parent ion or a halogen containing fragment is an adequate target for identifying halogen containing compounds. The first mass found with an abundance above 10% of the base peak is tested to see if it is part of the halogen isotope cluster. If not, the compound is marked as non-halogenated and the examination of the spectrum ends.

The relative abundance ratios for compounds containing two atoms of chlorine, or one atom of bromine is a sufficient filter to reduce false hits to an acceptable level. Monochloro compounds include the identification of a significant m-35 (loss of chlorine) peak as well as the m/m+2 abundance ratio.

Because the m+2 abundance for sulfur is only 4.4% relative to that for the corresponding m and the parent ion may be weaker than the ion sought for halogen containing molecules, a different approach must be taken for locating the parent ion. In this case, abundances obtained for the highest masses in the spectrum are used to estimate the noise in the background of the spectrum. As the spectrum is further examined, any abundance above six standard deviation units greater than the mean noise is deemed to be part of the spectrum and is tested to see if it meets the criteria to be the type of parent ion sought. As successive masses are examined, any signal not part of the spectrum is included in computing the noise mean and standard deviation.

2. Experimental Conditions

Chromatography

The work shown in this paper is the result of retrospective data analysis. It should be noted that TOF-MS data is particularly useful in this respect, as the mass range acquired does not affect sensitivity. With the capability of acquiring data at maximum sensitivity across a wide mass range, it is practical to acquire data in anticipation of retrospective data analysis, with the intention of the searching for compounds that were not of interest at the time the data were acquired. The chromatograms shown were acquired on various column sets and varying chromatographic conditions. However, in all cases, thermal modulation was used to provide sharp second dimension peaks and TOF-MS was used to provide acquisition of full spectra at high sensitivity and at high acquisition rate. While GCxGC with thermal modulation provides a significant increase in peak capacity, the samples were still sufficiently complex that spectral deconvolution was required to obtain peak identity. The LECO Pegasus® 4D was used in all of this work.

Data Analysis

Peak deconvolution and classification were obtained with ChromaTOF software. Automated spectral selection was performed with the scripting feature, available in ChromaTOF. The specific limits set for detecting ion ratios in the parent ion isotope clusters are shown elsewhere.[1]

Results

Application of the automatic filters to previously acquired chromatograms resulted in the identification of compounds both previously identified and, to this point, unidentified and the samples.

Chlorine and Bromine Compounds in Environmental Samples

Retrospective analysis of chromatograms obtained for the quantititation of PCB's in fish[2] provides additional information on the contamination of the waters from which the fish were obtained. The sample preparation for these samples involved acid hydrolysis and isolation of the non-polar compounds in the resulting reaction mixture. The peak table from the chromatogram was filtered with scripts designed to locate chlorinated or brominated compounds. A typical chromatogram of a fish extract is shown above (Figure 1). The chromatogram is dominated by column bleed, resulting in a peak table with over 3000 peaks. Filtering the peak table for chromatographic peaks showing spectral characteristics indicating the presence of chlorine (1 to 7 chlorine atoms in the molecule) or bromine (up to 3 bromine atoms in the molecule), the number of peaks displayed is reduced to about 90 (Figure 2). The peaks included PCB's (which were the target of the original analysis) and other chlorinated compounds, spectra and structures of which are shown in Figure 3. In some cases multiple isomers were identified. These are not included in the table. Examination of chromatograms obtained from samples other than those shown in Figures 1 and 2 in the study revealed additional compounds (Figure 4).

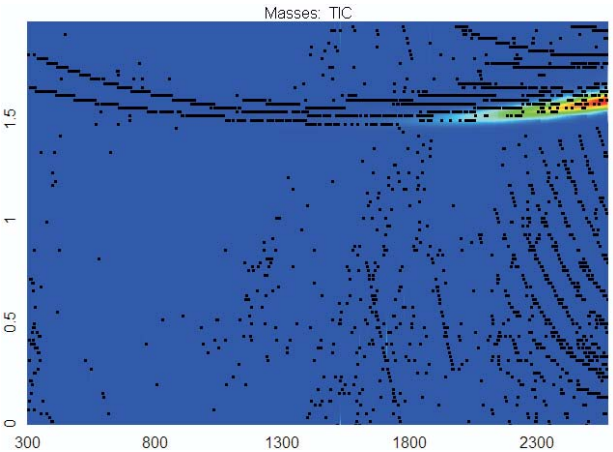

Figure 1. GCxGC Chromatogram of Fish extract. The chromatogram, used for PCB analysis contains many other compounds and is dominated by column bleed.

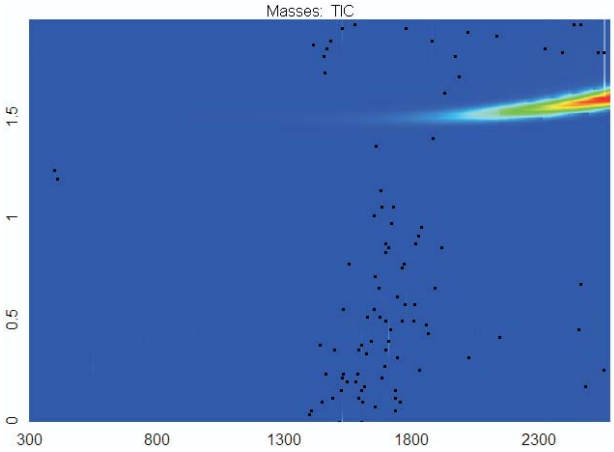

Figure 2. The same chromatogram as shown in Figure 1, but with the peak table filtered for those peaks showing the spectral characteristics of the presence of chlorine or bromine.

Sulfur in Petroleum

Application of a filter for sulfur containing compounds reduced the peak table for a diesel sample[3] from 6600 peaks (Figure 5) to 530 (Figure 6). Of these 530 compounds, 334 were also identified as sulfur containing compounds by library search. Forty-five were identified as something else, however. Of the 151 compounds classified as containing sulfur by the scripts, but not identified by library search, many appeared to be sulfur containing compounds by examination of the spectrum.

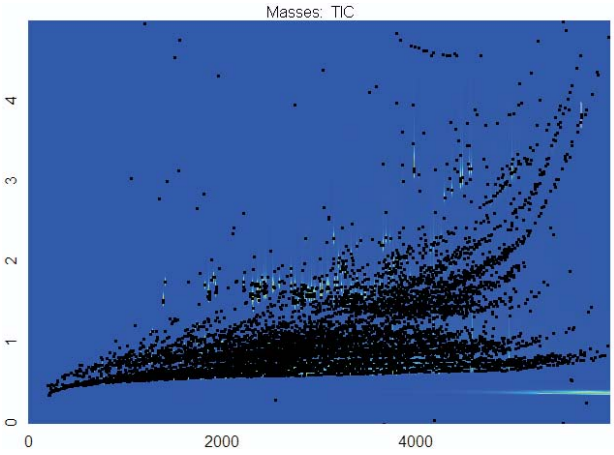

Figure 5. Diesel showing peak markers for all compounds in the peak table.

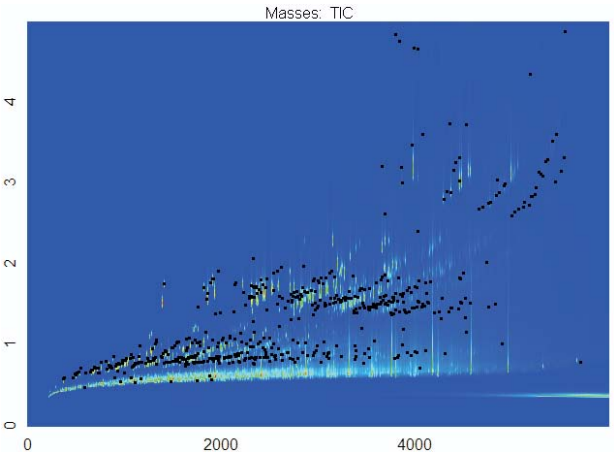

Figure 6. Diesel showing peak markers for compounds showing a parent ion with an m+2 abundance indicating the presence of sulfur.

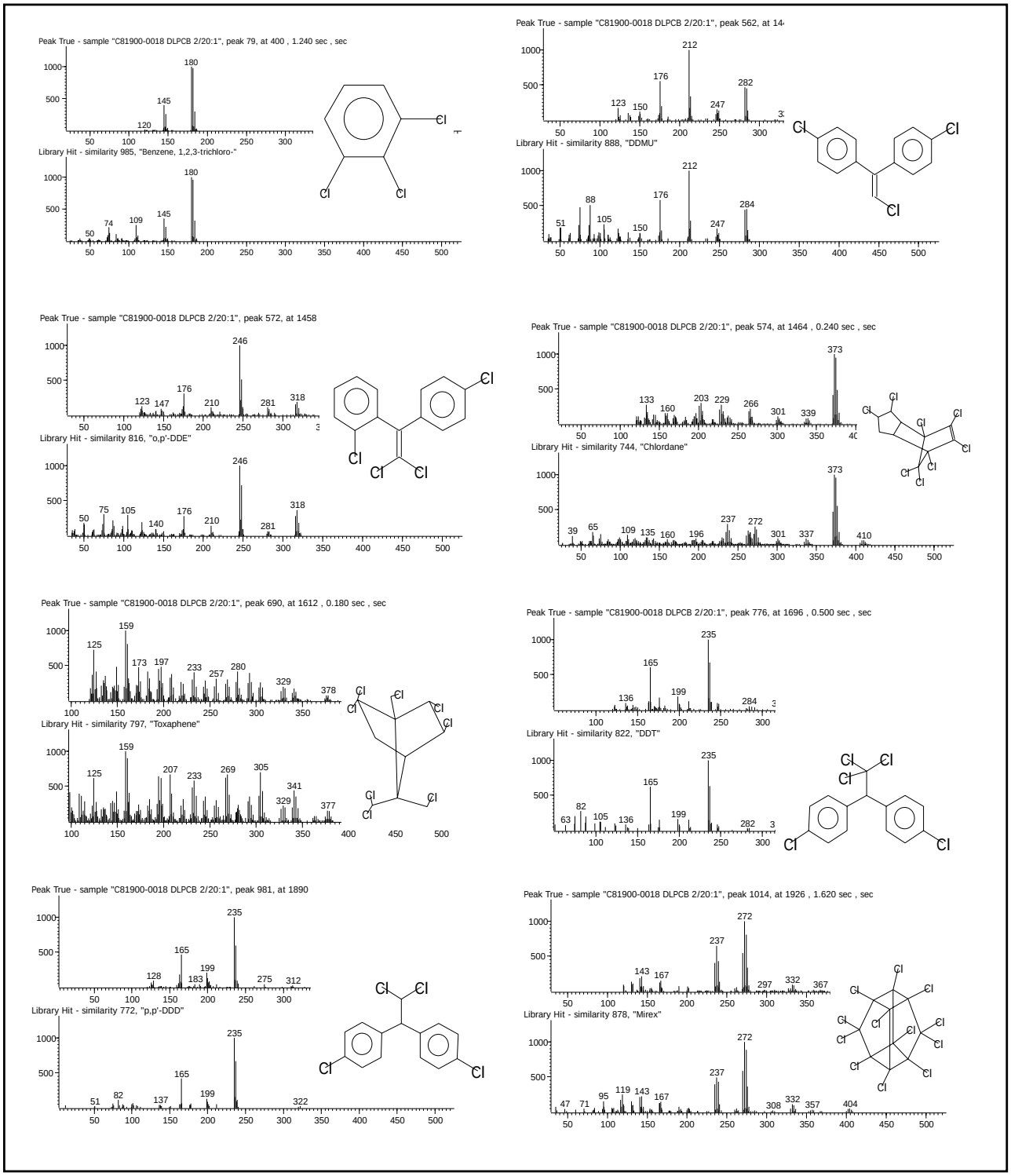

Figure 3. Compounds automatically identified as chlorinated in the chromatogram shown in Figure 1.

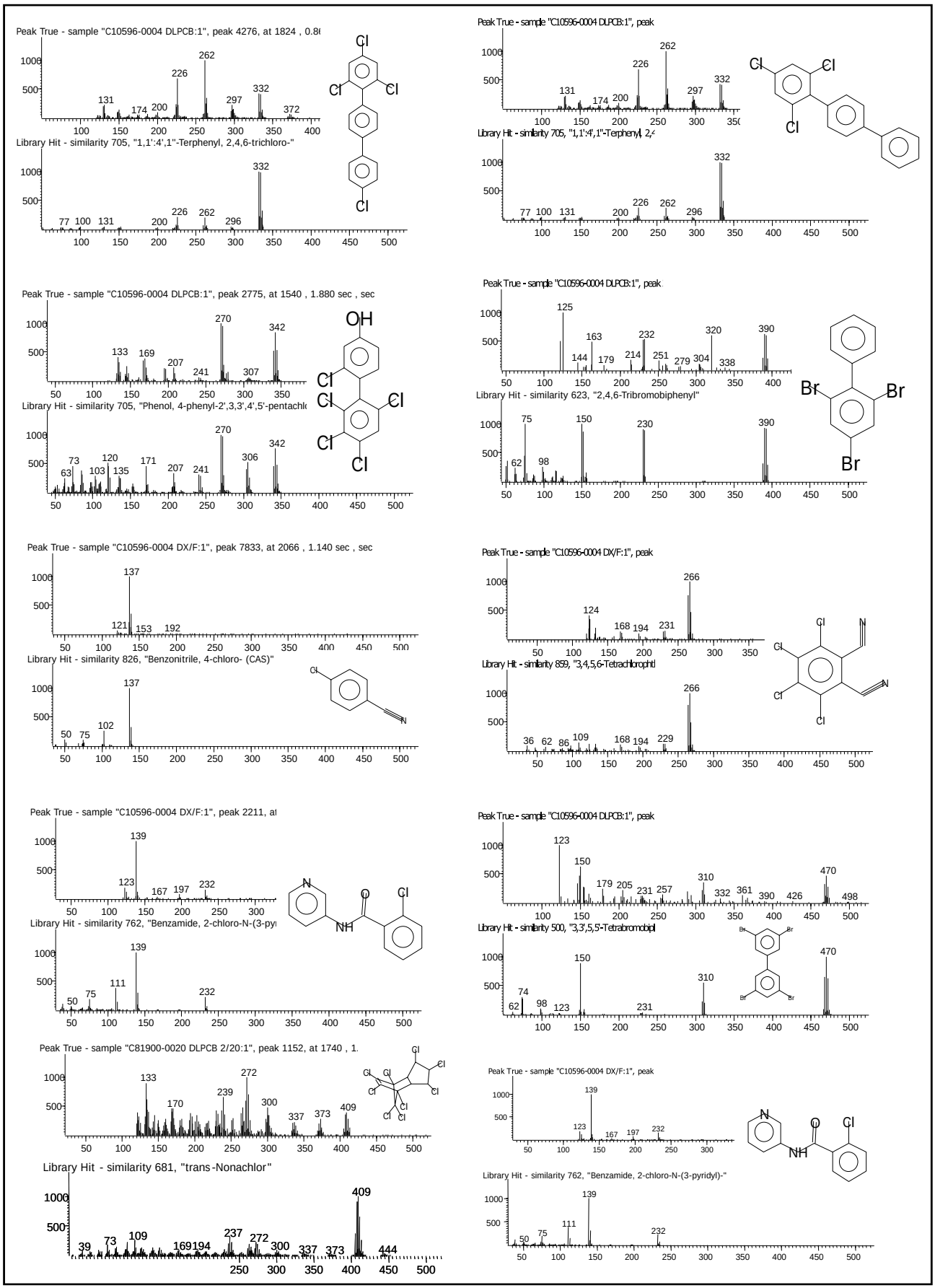

Figure 4. Compounds automatically identified as chlorinated in the other fish samples run in the study. Mass spectral identifications are not confirmed with standards, but spectra are consistent with presence of halogens.

Pesticides in Citrus Oil

Citrus oils are extremely complex mixtures. Contamination of these oils with pesticides is difficult to detect because of the complexity of the mixture and the large number of pesticides possibly present, particularly if there is a question of use of banned pesticides. The utility GCxGC for target analysis in citrus oils has already been demonstrated.[4] Figure 7 shows peak markers in the chromatographic region in which pesticides are typically expected to be found. There are over 3000 identified chromatographic peaks.

Data processing was limited (using the classification features) to the specified area to reduce data processing time and the size of the resulting peak table.

Manual review of the peak table is difficult, at best. The peak table was filtered to identify peaks with spectral patterns typical of compounds containing from one to seven chlorine atoms in the molecule, one to three bromine atoms or a sulfur atom. One hundred forty peaks matched the patterns. Of these 12 peaks were clearly identified by library search as pesticides or degradation products of pesticides. These compounds are marked in Figure 8.

Several of these peaks were readily confirmed by examining the mass spectra and obtaining the identity of the compound, as is demonstrated with the identification of methidathion (Figure 9). In other cases, peaks were identified as compounds potentially containing chlorine or bromine with the ion cluster identifiable, but without a good library match. Figure 10 shows the spectrum for such a chromatographic peak. The ion cluster at m/z 285 appears to be that of a dichloro compound. The chromatographic peak occurs at the expected location for ronnel, the library spectrum of which is shown with the unknown in the figure. (A match for ronel was obtained by searching only masses 280 through 290). Of the 140 peaks identified by the filters, 12 were determined to be known pesticides or degradation products associated with pesticides. The pesticides identified included chloropyrifos, ronnel (fenclorphos), methidathion, dicofol, chlorobenzilate and bromopropylate.

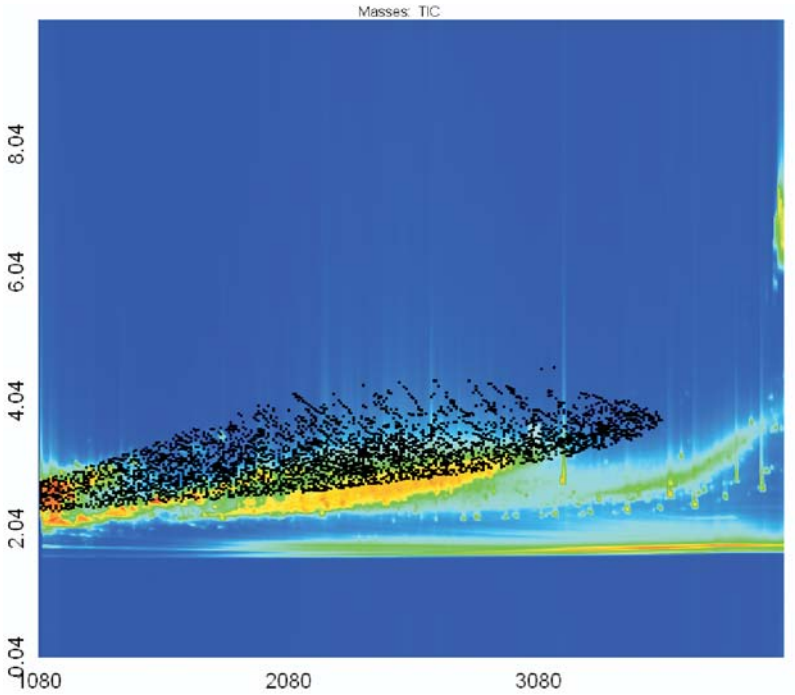

Figure 7. Unspiked orange oil showing peak markers through the region where pesticides may be expected.

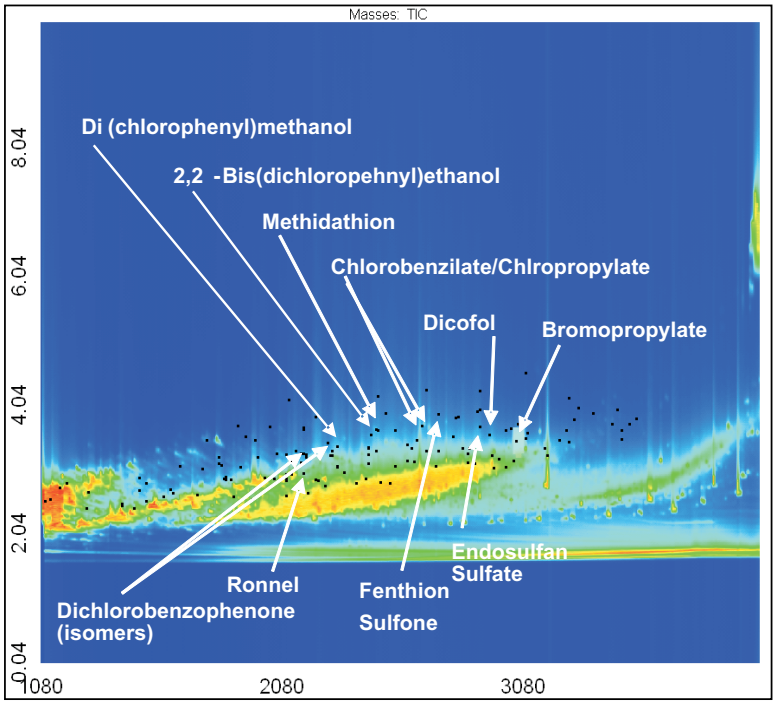

Figure 8. The same Orange Oil showing peak markers for 140 peaks likely to contain chlorine, bromine or sulfur. Identifications are from library search identification.

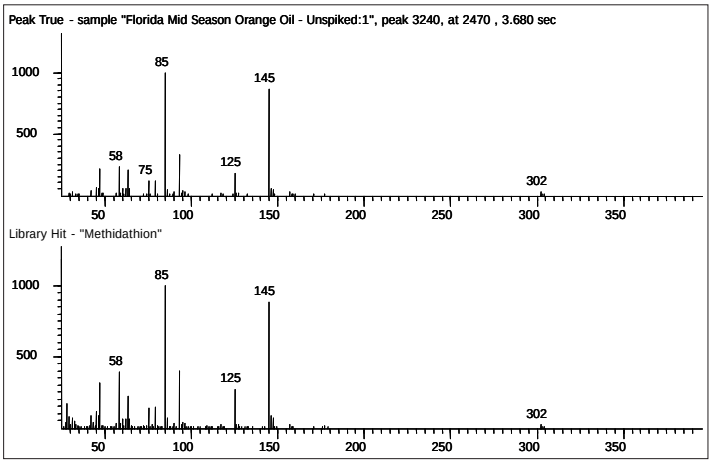

Figure 9. Spectrum of methidation, identified as a sulfur containing compound and easily identified by library search.

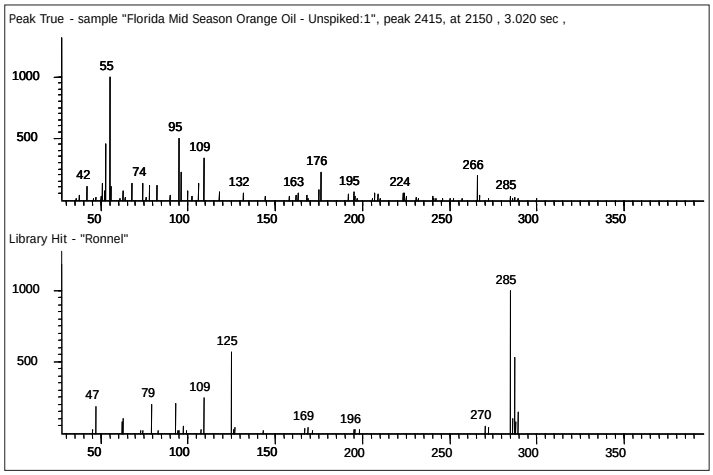

Figure 10. Spectrum showing peak containing ronnel. Identified as a halogen containing compound, match of partial spectrum and retention time in two chromatographic dimensions.

3. Conclusion

Scripting is a powerful tool in identifying compound based on expected spectral characteristics. This tool may be used to identify compounds in mixtures based on class characteristics when there is a suspicion of particular kinds of compounds being present, but individual identities are unknown. As shown here, chlorinated pesticides are easily identified in environmental and agricultural samples, and sulfur-containing compounds are easily identified in a petroleum mixture.

Because the selective detection is provided from a full-mass range spectrum, compound identities are available along with selective detection.

Selective detection using mass spectral data and scripts is a complementary technique to the traditional library searching of a peak table. Each technique offers specific strengths. Used together, one may be used to confirm an identity generated by the other.

4. References

[1]D.C. Hilton, "Automated Screening For Hazardous Components in Complex Mixtures Based on Functional Characteristics Identifiable in GCxGC-TOF-MS Data," Current Trends in Mass Spectrometry, July 2007, 28-34.

[2]Jack Cochran, Frank Dorman, Eric Reiner, Terry Kolic, and Karen MacPhersion, "GCxGC-TOFMS of PCBs," PittCon 2004.

[3]Tincuta Veriotti, "Automated Characterization of a Diesel Sample Using Comprehensive Two-Dimensional GC (GCxGC) and Time-of-Flight Mass Spectrometry (TOF-MS) Detection," LC GC North America, 2004, 22, part 2, Supp, 64.

[4]D.C. Hilton, J. Cochran, T. Veriotti, "Analysis of Pesticide Residues in Citrus Oils by GCxGC-TOF MS with Minimal Sample Preparation," PittCon 2006.

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