Qualitative Identification of an Unknown Pesticide Using GC-MS and Online Resources

Grant N. Holder,* Steven J. Breiner, David G. Farrar, David M. Gooden, and Laurel L. McClure

A. R. Smith Department of Chemistry, Appalachian State University, Boone, NC 28607, holdergn@appstate.edu

Abstract: A senior-level instrumental analysis experiment is described in which students examine fruit for pesticide residues. This experiment involves fundamental instruction in sample preparation, the use of gas chromatography (GC) to resolve and deduce important components of a commercial pesticide preparation by matching against a certified standard, and unambiguous identification of the active pesticide by using mass spectrometry in conjunction with public data available on the Internet.

Introduction

The gas chromatograph is often one of the first instruments students are exposed to at the undergraduate level. Simple GC units are often employed in the sophomore-level organic laboratory for such well-known examples as to separate mixtures of hydrocarbons or to monitor the progress of a reaction such as methanolysis of diethyl malonate [1]. For the senior student with an interest in analytical methods, an example with more realistic applicability is necessary. Therefore, we have developed an interesting experiment that makes use of a food product that has been adulterated with an amount of pesticide whose identity is unknown to the student. This is complicated by the fact that the pesticide is not available to the student in pure form, but just as a component of the commercial preparation, replete with all the inert ingredients found therein. Thus, the student needs to identify the unknown in an environment rife with interferences—literally hundreds of compounds present in large excess that mask or otherwise interfere with the analysis. Such a matrix is commonly seen by those performing environmental analysis but represents a challenge for the student accustomed to analyzing either pure compounds or simple mixtures doped by a small amount of pure unknown.

Because analyzing for a particular agent amidst myriad background components present in large excess is difficult even for the most experienced analyst and the most well-equipped laboratory, some accommodations to student frustration are made. The pesticide residue is one of a dozen possibilities that are presented as components of a certified standard. Students began the analysis by obtaining gas chromatograms of their certified standard with electron capture detection (ECD), beginning with a preliminary temperature program provided by the instructors for this relatively uncomplicated sample. A chromatogram of a representative sample of the contaminated fruit was compared with one from a blank of untreated fruit, to help identify peaks associated with the species of interest. The students were encouraged to play with the temperature program to obtain acceptable separations of the components they identified as contaminants. It should be noted that the identities of the components of the certified standard were *not* made available to the students. Standard, untreated fruit sample, and contaminated fruit sample were then analyzed by GC with mass spectrometric detection (MS). Each component has a representative mass spectrum, which can be analyzed to obtain the molecular formula. Armed with this information, students accessed the National Institute of Standards and Technology (NIST) Website, where a vast library of information on pesticides and similar compounds, including their mass spectra, is stored. Comparison with these spectra on the basis of molecular formula usually yielded the correct identification.

Such an experiment has educational value in that, while not exactly a real-world exercise (a limited number of components in relatively large concentrations comprise the unknown species), it is as close as undergraduates usually get to a really complex problem in sampling and analysis. For an advanced class, quantitation is possible, because the concentration of the pesticide of interest in the certified standard is known. However, students by this point in their chemical education have had much experience with quantitative analysis; this represents an exciting and fun experiment, with current societal relevance, that can, at the discretion of the instructor, be made as difficult or as simple as needed.

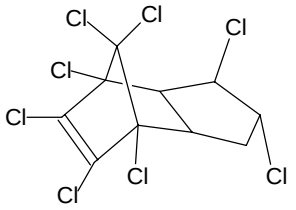
This experiment can be done in a single three-hour laboratory period, depending on the number of students. Each GC run takes about half an hour, and the sample preparation time takes about forty-five minutes to one hour.

Pedagogically, this experiment meshes well with the end of the mass spectrometry presentation. In our program, this is approximately two-thirds through the semester, after sampling, chromatography, and the majority of spectroscopic techniques have been reviewed. The students in our instrumental analysis course have, by this point in the semester, already performed chromatographic experiments and have, therefore, developed some expertise in chromatographic separations. Our focus in this experiment on structural elucidation, with its secondary reinforcement of chromatographic principles, well suits the advanced placement in our course sequence. However, if one wished to convert this to a quantitative experiment, it can be performed before the semester break. For such use, the instructor may wish either to provide students with a definitive temperature program or to give them an additional laboratory period to experiment with chromatographic optimization. The procedure is versatile enough to allow for many satisfying convolutions.

Chlordane

The use of technical chlordane for control of termites and agricultural pests was discontinued by 1988 in the United States, most European countries, and Japan, though its consumption in other countries continues. The environmental occurrence of chlordane and its metabolites has been well-studied; chlordane is even present in specimens from remote areas such as the Antarctic and is now considered a possible human carcinogen [2–7]. Research interest in the compound has focused on toxic environmental effects, specifically the significantly changed isomer composition present in biological samples [8, 9].

Technical chlordane is a complex mixture, the analysis of which is complicated by the fact that the commercial pesticide consists of at least 11 major components and up to 120 total components [10–12]. However, the two major components that are useful for qualitative identification are *trans*-chlordane (**1**) and *cis*-chlordane (**2**) which, according to various sources, each constitute 10–20% (w/w) of the total mixture [13–15].



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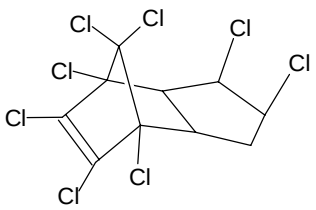

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Other components, similar chlorinated compounds, such as heptachlor, *trans*-nonachlor, and α-, β-, and γ- isomers of chlordene, are present but do not interfere with the identification of **1** and **2**. The eight chlorine atoms on the chlordane molecule lead to very low detection limits on the ECD, but can lead to a difficult identification for students using mass spectrometry alone.

The purposes of this laboratory are (a) to prepare representative samples of food both uncontaminated and contaminated by unknown pesticide, (b) to resolve by GC/ECD the components of the contaminated food sample to the extent necessary so that identification of unknown pesticide can be made, (c) to resolve the components of a standard mixture of chlorinated pesticides by gas chromatography and to obtain a mass spectrum of the target pesticide, (d) to use the mass spectrum to determine the molecular formula of the contaminant, and (e) to unambiguously identify the unknown pesticide by comparison with data available from the NIST Website.

Experimental

Materials. Twenty white seedless grapes were purchased and sorted into two groups of ten each. One group was contaminated with 50 μL of technical grade chlordane, a portion of which was injected into each grape. The technical grade chlordane was a commercial sample stored in our archives. The second group was used as a control. Each group of ten was separately blended to a smooth, semisolid mass.

Each mass was removed from the blender and divided into approximate thirds to allow for volumes of pulp that were easy to handle. The blender was carefully cleaned after each homogenization to prevent cross contamination of samples. Each resulting division was placed into an Erlenmeyer flask with 50 mL of hexanes (Fisher Chem. Co.) and the flask shaken vigorously for several minutes, followed by decantation of the hexanes. Any residual water present was drained away from the decantate. The extracts from the contaminated grapes were combined, as were the extracts from the uncontaminated control. In a concession to the limited time available during the laboratory period, students were not asked to perform multiple extractions of the residual pulp—operations that would have been necessary were only trace quantities of pesticide present.

Extracts of contaminated samples were then combined, as were those for uncontaminated samples, after which each combined extract was concentrated on a rotary evaporator to a volume of approximately 5 mL. (The procedure can be repeated, as desired, with water and/or methanol in place of, or in addition to, the hexanes.) We have found the hexanes extraction method to be most satisfactory in terms of chromatographic resolution.

Equipment. Gas chromatography was done on both contaminated and uncontaminated samples. The certified chlorinated pesticide standard against which both were compared, EPA 508/508.1, was purchased from Supelco, Inc. For chromatographic equipment, we used a Hewlett-Packard HP 5880A Series GC equipped with a 5880A Series GC Data Terminal and electron-capture (^{63}Ni) detection, or an HP 5890 GC coupled to an HP 5970 Series Mass Selective Detector, which was held at approximately 2×10^{-5} torr with a Varian turbo pump. The mobile phase was a 5% methane/95% argon mixture for the HP 5880A GC with the ECD detector; the mobile phase for the HP 5890 with the MSD was helium. A Supelco SPB-5 column (diameter, 0.25 mm; film thickness, 0.25 μm; length, 15 m—HP 5880A, 30 m—HP 5890). The injection port temperature was 300 °C in each case. Temperature program for both standard and samples: initial column temperature, 120 °C; increasing at a rate of 30 °C/min to 180 °C; then increasing at a rate of 10 °C/min to 280 °C, with the column held at 280 °C for 15 minutes. This program was sufficient to elute all major components from all samples. The ^{63}Ni detector was held at 350 °C; the GC/MS detector was set to 280 °C. Injection volume was 0.5 μL for the ECD, and 1 μL for the GC/MS.

Results

The strategy of the experiment is as follows:

a) Comparison of ECD chromatograms of the commercial preparation, the pure hexanes extract, the contaminated hexanes extract, and the certified standard allows students to identify the chromatographic peaks that are due to the pesticide.

b) GC/MS analyses of the contaminated hexanes extract and certified standard are used by the students to identify the mass spectrum of the contaminant in each chromatogram.

c) The mass spectra are used to identify molecular ions and common fragments; characteristic isotopic distribution patterns allow for the determination of the molecular formula.

d) Once the molecular formula is assigned, students consult the NIST Website in order to find any mass spectra matching that formula. Experimentally obtained mass spectra are matched with library examples to provide easy identification of chlordane as the contaminant.

Details

(a) The GC/ECD data for the uncontaminated hexanes extract exhibited only a few peaks attributable to natural products from the grape. The contaminated hexanes extract, however, showed several peaks following direct injection onto the column, which were resolved satisfactorily by the approximate temperature program with which the students

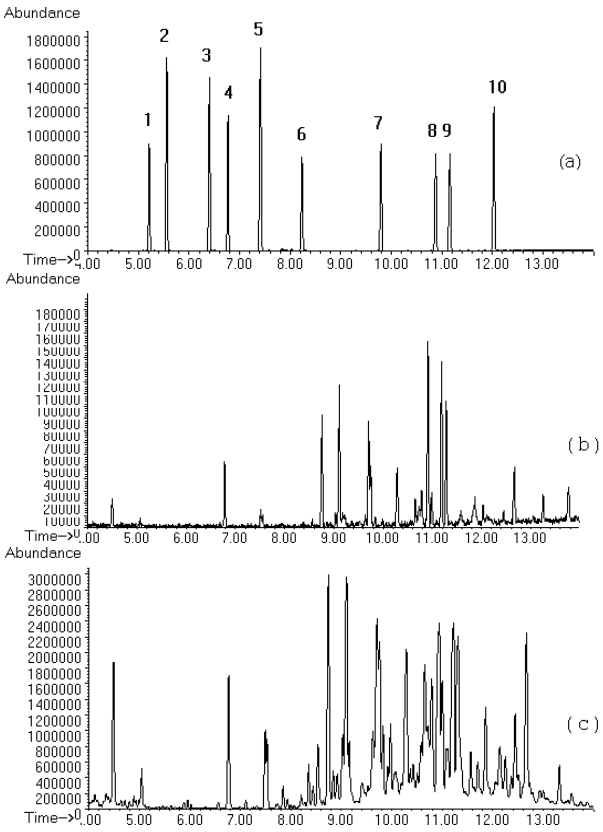

Figure 1. (a) chromatogram of epa 508/508.1 certified standard. Key: etridiazole (1); chloroneb (2); propachlor (3); trifluralin(4); hexachlorobenzene (5); chlorothalonil (6); dacthal (7); γγ-chlordane (8); α-chlordane (9); chlorobenzilate (10). Not shown: permetrhin (cis and trans). (b) chromatogram of hexanes extract of contaminated grape sample. (c) chromatogram of commercial chlordane preparation. All chromatograms obtained under identical conditions, as specified.

were provided; this was then refined by trial and error. The ability of the ECD to detect very small amounts of chlorinated analyte was vividly demonstrated by running comparatively sized samples on the two instruments. For the ECD, injection volumes of 0.5 μL necessitated attenuation of the output signal to keep the chromatogram on scale; the GC/MS required at least twice the sample size for acceptable signal/noise results.

The GC/ECD analysis of the contaminated hexanes extract was relatively simple, especially compared to that of the commercial preparation injected neat, indicating the large number of water-soluble components in the commercial preparation. Students injected the contaminated sample, the commercial preparation, and the standard. By way of initial cursory analysis, chromatographic peaks common to all three were sought. Only two peaks meet this criterion, at t_R = 10.11 min and t_R = 10.40 min. Students recognized these two peaks as being due to the presence of the unknown pesticide.

(b) Total ion chromatograms were obtained for the contaminated hexanes extract, the commercial preparation, and the EPA 508/508.1 standard. A volume of 1.0 μL of each was injected to begin the qualitative identification of the pesticide. Figures 1(a–c) show the chromatograms of (a) the EPA 508/508.1 certified standard, (b) the contaminated hexanes extract, and (c) the commercial chlordane preparation. Students were not told the identity of the compounds in the certified standard giving rise to peaks 1–10 in Figure 1(a); they were simply told that their unknown contained one or more of these compounds. From these data, it is apparent that the peaks at t_R = 10.86 min and t_R = 11.12 min were consistently present, corresponding to peaks 8 and 9 in Figure 1(a).

Note that the difference in retention times relative to those observed for the GC/ECD experiment reflect the longer SPB-5 column used on the HP 5890; the relative positions of the peaks of interest were so similar that calculations of numerical capacity factors were not necessary for identification purposes. The peaks representing the unknown pesticide were not present in gas chromatograms of the uncontaminated hexanes extract. There was also a match between peak 4 of the standard and a peak in the commercial chlordane preparation, but this compound did not extract into hexanes. There are other fat-soluble components of the commercial chlordane preparation that also appear in Figure 1(b), but these were not present in the standard. Only the peaks at the aforementioned retention times appear in all three chromatograms. At this point, the student should be able to identify these as representing the unknown chlorinated pesticide present in the fruit sample. Retention times did not vary more than 0.07 min run-to-run over the course of the GC/MS experiment.

Data were saved for each student using the proprietary HP Chemstation format (*.d). For maximum efficiency, data manipulation was performed on a PC using a program capable of translating and displaying the data (WSEARCH Mass Spectral Search Software). This is free software (for Microsoft Windows) that displays HP ChemStation and other proprietary data formats. It can be downloaded from http://minyos.its.rmit.edu.au/~rcmfa/search.htm.

(c) The next step in identification is determining the molecular formula of the pesticide. For this, students examine their mass spectral data obtained for each of peaks 8 and 9 (Figure 2). The molecular ion is clearly visible, establishing formula weight as 410. Major losses of Cl are evident in ion clusters centered on *m/z* = 375 (base), 339, and 301. Using WSEARCH, students can expand regions of the spectrum and examine isotope distributions more closely, as well as print out a complete mass–time list.

With molecular weight information obtained, students begin determining the molecular formula by deducing the number of chlorine atoms present; these are the most easily recognizable species present. This value is usually derived by observing the intensity of the ions clustered around the molecular ion; these are, of course, determined by the coefficients of the binomial expansion. As calculation of these values by hand is a tedious task, we use the computer program ISOPRO 3.0 (MS/MS Software) to simulate isotope distribution. This is a shareware program available online at *http://members.aol.com/msmssoft*. Using a very simple interface, students can enter any chemical formula and the isotope distribution around the molecular ion is calculated and displayed on screen. It is a simple matter to enter Cl_n, where $n \geq 2$, and examine the associated envelope. Within a few minutes, students can observe that the distribution pattern around *m/z* = 410 is characteristic of the presence of 8 chlorine atoms. The match is almost exact.

The number of carbon atoms is obtained by examining the (M+1)/(M) ratio. Taking into account the number of carbon atoms in the molecule, this ratio should be 0.1158; the experimentally obtained value is 0.1154. This corresponds to a carbon count of 10.

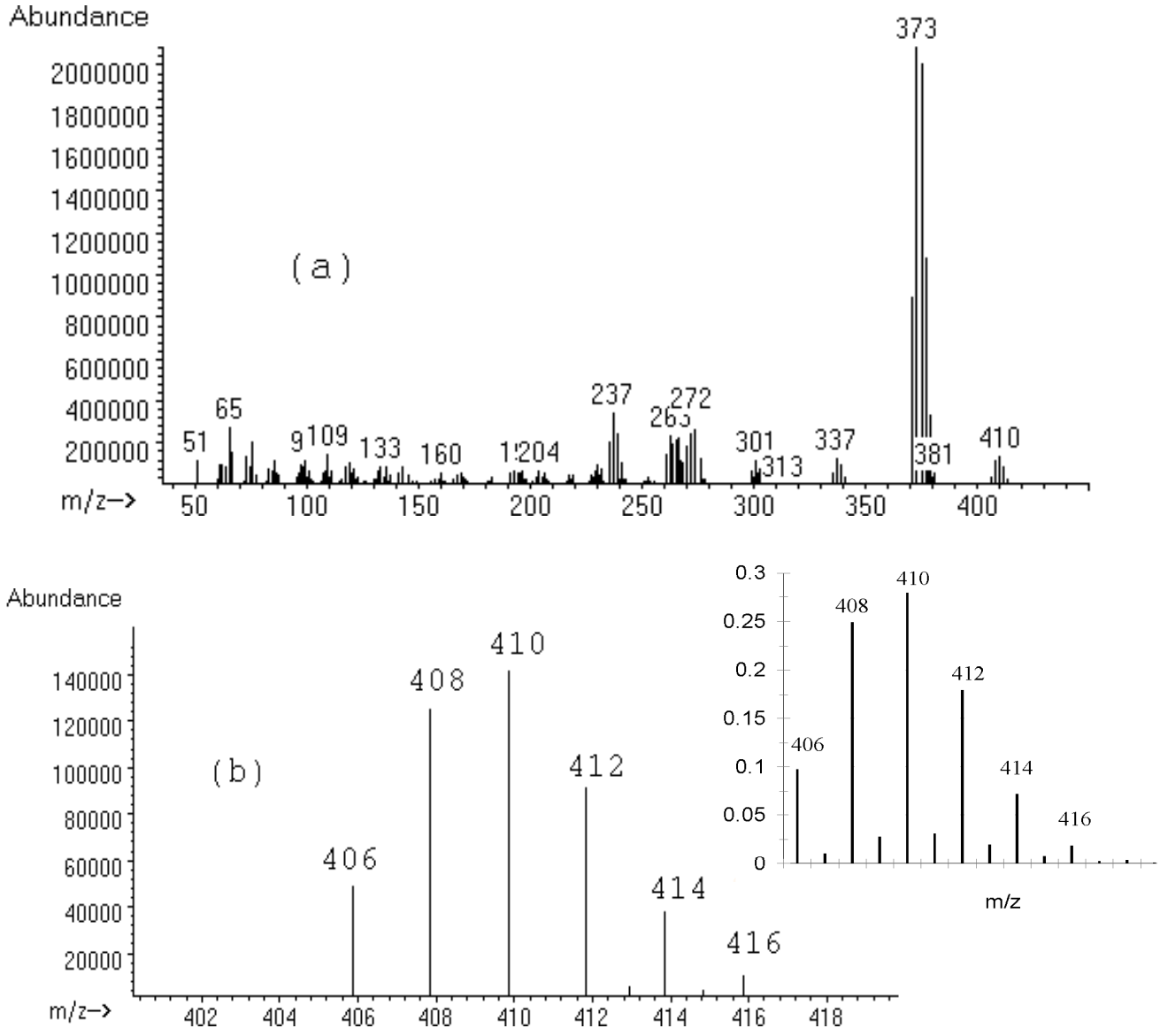

Figure 2. (a) Mass spectrum of α-chlordane. (b) Expanded region of the molecular ion of (a). Inset shows calculated isotopic ratios (isopro 3.0) of the chlordane molecular ion for comparison.

The formula now stands at $C_{10}Cl_8$. Since the mass of this fragment equals 404 (to the appropriate number of significant figures), it is observed that the missing mass equals 6, corresponding to six hydrogen atoms, and thus the molecular formula is $C_{10}H_6Cl_8$.

(d) At this point, it is time to confirm the analysis and actually identify the compound. By logging onto to the Chemistry WebBook of the NIST (http://webbook.nist.gov/chemistry/), students can enter their formula under "Search Options." Chlordane is matched within a few seconds. By scrolling down to "Other Data Available" and clicking on "Mass Spectrum," students can observe a spectrum virtually identical to their own (Figure 2), thus unambiguously identifying their unknown pesticide as chlordane. The aforementioned mass spectrum can be printed out and submitted with the laboratory report as evidence corroborating the identification.

Discussion

The practical value of the information available on the Internet to the chemical educator is amply demonstrated by this experiment. The use of freeware programs allowed data manipulation without tying up instrument time; this offline processing increased student access to the instrument and allowed each student to analyze his or her data independently. The NIST Chemistry WebBook is a valuable resource—reference data for many compounds are readily available in a single location. Much library leg work is obviated, thus greatly reducing student frustration. The presence of such data allows a structure to be determined without having to solve, ion-for-ion, the total mass spectrum, which most would agree to be beyond the level of senior instrumental analysis students. However, extra credit can be assigned to those who will search the literature (also online) for the chlordane mass spectrum and use that information to report, with proper referencing, the

identities of major ions and, depending upon the depth the instructor desires, mechanisms by which the ions are generated. Note how the question posed by the laboratory exercise was answered without the instructor having to provide the identities of the compounds in the EPA 508/508.1 standard. In pre-Internet days, this task would have been either much more difficult (using the mass spectrum to piece the molecule together) or else trivial (revealing the components of the standard to the students).

Our students find the environmental aspects of exercise quite engaging, and, though the actual sample preparation is somewhat contrived due to the high levels of contaminant found in the doped grapes, it can be made more realistic at the discretion of the instructor through the use of much smaller amounts in conjunction with microextraction columns and other preconcentration methods. Such an approach would require the use of two laboratory periods; it is our intent to implement these options in our next iteration of this experiment.

The exercise described above does not actually require the GC/ECD instrument for analysis; however, we use it to demonstrate the capability of the instrument and to expose the students to the technique. In addition, we found that breaking students up into teams allowed them to participate integrally with very little idle time.

The experiment can easily be made quantitative if desired, since the concentration of each of the standards in EPA 508/508.1 is known to be 1000 μg/mL in methyl *t*-butyl ether.

Chlordane need not be the compound used for this experiment. For example, departments possessing nitrogen–phosphorus detectors might wish to use similar methods with organophosphate pesticides. Note, however, that many pesticides are highly toxic to humans as well as pests; all samples and wash solvents from syringe needles and glassware that have come in contact with the samples must be collected and disposed of by a reputable waste-disposal firm properly licensed to do so. If chlordane is used, a volume of 500 μL of commercial preparation is sufficient to repeat this experiment for at least 10 years; this amount can usually be found at and borrowed from a local pest-control concern.

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