CHAPTER 7

Proper Laboratory Protocol and Sample Laboratory Experiments

7.1 Preliminary Information

7.1.1 Instrument and Instrumental Settings

This section will provide a brief introduction to the basic settings and operation of GC (with any detector other then MS) and GC-MS instrumentation. Examples of these settings will be provided in the experiments that follow in this section. Some topics are redundant to previous discussions but are included here for the purpose of clarity. While section 7.1 concentrates on GC and GC-MS applications, most of the experimental applications in sections 7.4 and 7.5 can be extended to LC.

7.1.1.a Temperature Settings: There are four main temperature controlled regions on a GC-MS. The first region is the injector, which is set at least 20 degrees higher than the final oven temperature. The column oven is set to run either an isothermal mode or in as a temperature program, with the latter being the most common. The oven temperature is usually initially set between 10 and 15 degrees below the boiling point of the solvent, held at this point during the split-less mode of the injection, followed by one or more temperature ramps, and typically held at a high final temperature to remove late eluting analytes that may or may not be of interest. Modern GC-MS systems automatically return to the starting temperature after a given time. The oven is initially held at a relatively low temperature (compared to the boiling point of the solvent) to concentrate the analytes at the head of the column. If a higher temperature is used, the solvent will rapidly volatize and spread the analytes over a broad area and decrease peak resolution. The detector is always set at a constant temperature 15 to 20 degrees above the maximum temperature of the oven. The injector and detector are held at higher temperatures to prevent recondensation of analytes onto this surfaces which would interfere with quantification due to peak tailing and potential cross contamination between samples. The final temperature region is the MS vacuum chamber. It can be set above or at a lower temperature

than the GC components (~150 to 250 degrees C in the quadrupole mass analyzer) due to the more volatile nature of analytes at low pressure.

7.1.1.b Gas Flow: As noted in the GC chapter, all gases in GC-MS, and in most GC applications, must be 5-nine quality (99.999 percent pure). These typically include a He or H_2 as a carrier gas, Ar/CH_4 for makeup gas for electron capture detectors, and H_2 and compressed air (lower grade) for flame ionization detectors in GC. For GC-MS, only He is required for the carrier gas, with CH_4 being commonly used in chemical ionization mode. Even at this purity, the gas must be purified further by passing it through a resin trap that has a high affinity for specific contaminants, including water, atmospheric oxygen in some cases, and hydrocarbons. Although the presence of these contaminants would result in a high detector background, the main reason such high purity gases are needed is due to the use of temperature programming. Two contrasting examples will demonstrate the need for high purity gas purifiers. For the first case, imagine running the GC-MS with a high-temperature isothermal oven setting. At this temperature all contaminants in the gases will pass freely through the system unretained in the separation column and a high, but steady, background detector signal would result. For the second case, imagine a temperature-programmed analysis where initially the column oven is at a temperature lower than the boiling point of any contaminants in the carrier gas. As carrier gas passes through the analytical column, contaminants would be adsorbed to the stationary phase at the beginning of the column. As the temperature program progresses, these contaminants would volatilize and appear as peaks in the chromatogram. The height of the contaminant peaks (concentration) would be inconsistent since it would depend on the time and the amount of carrier gas passing through the column between runs. The contaminants would result in additional problems if they co-eluted with an analyte of interest.

The gas pressure in the supply tank is usually between 2000 and 2500 psi (up to 17000 kPa). Instruments require that this pressure be reduced with step-down or secondary regulators that drop the pressure to 100 psi (700 kPa) or less, depending on the instrument and gas. Integrated regulators or mass-flow controllers further reduce the pressure to 5 to 20 psi at the head of the capillary column,

resulting in a flow of 1 to 5 mL/minute depending on the internal diameter of the capillary column.

7.1.1.c. Vacuum Chamber. As helium enters the MS unit, it must be evacuated to minimize secondary collisions with the ionized analytes. Two vacuum pumps are used to accomplish this. First, a rotary vacuum pump evacuates the gases to approximately 10^{-2} torr. Then a molecular turbo pump reduces the pressure to 10^{-4} to 10^{-6} torr.

7.1.2 Maintenance

7.1.2.a. Gas Filters: As noted in the previous section, ultra high purity gases are purified even further with resin filters (traps). These filters must be replaced periodically, usually after 5 to 10 tanks of gas depending on the size of the filter.

7.1.2.b. Septa. The interface where samples are introduced into the instrument is a silicone gum septum with a Teflon backing on the injector side of the septum. This allows the sharp needle of the syringe to be easily inserted into the injector chamber and the sample to be introduced. As more and more injections are made, the septa develops a slight perforation in it that will eventually leak carrier gas and allow the loss of sample during an injection. Therefore the septum must be replaced periodically, typically daily or just prior to a new run of standards and samples. Septa are relatively inexpensive so this is not a major cost issue.

7.1.2.c. Injection Syringes and Needles. Syringes can wear with time depending on the type of samples injected. Dirty samples will quickly clog the syringe by leaving residue in the barrel that interferes with the movement of the plunger. This can usually be avoided by numerous rinses between samples. However, it is sometimes necessary to disassemble the syringe and rinse with acid, polar organic solvent, and a nonpolar organic solvent. Injection needles can also plug with a piece of the septum. Most syringes come with a thin wire to remove this plug but this technique is rarely successful and the syringe is usually replaced. Syringes for manual injection are as inexpensive as $15, but autosampler syringes can easily cost $100.

7.1.2.d. Column Fitting. Columns are attached to the injector and detector ports with threaded nuts and ferrules, a soft hollow conical-shaped device that fits snugly around the column and fits into a receptor secured with a nut. As the nut is tightened, the ferrule is compressed around the column, preventing gas leaks. As the temperature is repeatedly raised and lowered, leaks can result from the cycling expansion and contraction, so the ferrule nuts need to be tightened periodically (weekly to monthly). Ferrules can be made of Teflon, ceramic, graphite, and composites of ceramic and graphite.

7.1.2.e. Glass Wool Plugs in the Injector Liner. Most injector liners have glass wool inserted into them to aid in the uniform mixing of the volatized solvent and analytes with the carrier gas. Over time (weeks to months) these liners accumulate pieces of the septum (referred to as septum worms) and nonvolatile components of the injected sample. Therefore the liners are routinely replaced when discoloration or evidence of cross contamination occurs. The frequency of replacement is directly related to the presence of nonvolatile components in the samples, and can range from weekly to yearly replacement cycles.

7.1.2.f. MS Tuning. The mass spectrometer, specifically the mass analyzer, must be calibrated with respect to mass, typically on a weekly basis. Some applications require daily tuning. Modern MS systems have an automated tuning sequence. For electron ionization systems, perfluorotribuylamine (PFTBA) is used. A small mass of volatilized PFTBA is introduced into the ionization chamber and the system automatically adjusts to correspond to its mass. Parameters such as repeller and accelerator voltages and gain on the EM are adjusted to achieve a given detector response. After this, the system can be tuned for any mass unit.

7.1.2.g. Ion Lens. The repulsion and acceleration lenses may accumulate nonvolatile residues when dirty samples are analyzed. Depending on the quality of a sample and the frequency of use, lenses will need to be taken out and rinsed with solvents, dried, and reassembled. The typical sign of a dirty lens is the need to apply higher than normal voltages to these lenses during the MS tuning procedure.

7.1.2.f. Gain on the Electron Multiplier. For a given mass of tuning compound (PFTBA), a specific counts per second of ions hitting the EM is expected. This is adjusted by increasing or decreasing the gain (potential) across the EM. As the EM ages, it may require an excess gain to be applied and the EM will need to be replaced.

7.1.2.h. Rotary Pump Oil. The rotary pump is lubricated with special grade oil. The gauge level should be checked monthly, and oil added if necessary. The oil should be replaced at least annually, sooner in high use situations and when dirty samples are analyzed. Many or most of the unionized analytes, contaminates, and solvents eventually reside in the rotary pump oil. Rotary pumps usually require semi-annual replacement due to oil leaks.

7.1.2.i. Analyte calibration. While not a normal part of maintenance, instruments are normally calibrated at least daily with analytes of interest.

7.1.3 Trouble Shooting

A variety of problems will be experienced when using a GC-MS for prolonged time. A few of the most obvious are discussed below. Instrument manuals normally come with a trouble-shooting guide.

7.1.3.a. Leak Detection. Atmospheric leaks will occur from time to time. The most likely sources of these leaks are the column fittings and the door to the MS vacuum chamber. Leaks may be present if mass numbers corresponding to N_2, O_2, H_2O, CO_2, and Ar appear in the spectra. System leaks are easily checked by setting the instrument to constant monitoring mode and then spraying canned Freon at each fitting and watching for a detector response. A readily available leak detection agent is DustOff that contains difluoroethane (CAS #75-37-6).

7.1.3.b. Contamination of the GC-MS system. Unfortunately, all systems become contaminated with time. The key to minimizing time locating the source of contamination is to systematically isolate

each system and therefore the source of contamination. A good practice is the following.

-First, look for the obvious. What was the last thing changed prior to the presence of contamination? Was a septum, liner, column, gas filter, or gas tank recently changed?

-Check each potential source for problems, especially the filters and liners. There have also been cases of contaminated 5-nine gas being delivered from suppliers and contaminated injector liners direct from the factory.

- Check the solvent for contamination by eliminating sample introduction and only running solvent"

-An easy way to isolate the injector and check for contamination, without taking it apart, is to cool the injector and conduct a temperature run without sample injection. If the contamination is not present when the injector is cooled, a contaminated injector is likely.

-Identify your contaminate with the spectra library. If your contamination is the analyte, then the contamination is likely to be on the “front-end” of the GC-MS system (syringe or injector liner). Hydrocarbon contamination from oils is possible and will be indicative when ions are present at 43, 57, 71, and 87 mass units. Siloxanes are indicative at mass units of 73 and 207 mass units. Phenyl degradation from column degradation will be present at 281 mass units. Phthalates are ubiquitous in the environment and will give an ion peak at 149 mass units.

7.1.3.c. Plugged Needle. As noted in section 7.1.2, needles frequently become plugged with pieces of septum. This is indicated when a sample is thought to be injected but no ions or peaks appear, including the solvent.

7.1.3.d. Broken Columns. Another explanation for a lack of detector response is a broken column. This is easily observed by cooling the oven and inspecting the column. Never allow the column to rub against a surface as it will wear off the protective coating of the column and promote a break in the column.

7.1.3.e. Low Sensitivity/High Gain on the EM. This is indicative of a worn out detector.

7.2 Preliminary Experiments: Getting to Know Your Instrument

7.2.1 Autotuning the MS

MS instruments must be tuned frequently to ensure correct identification of ion mass to charge ratios; modern instruments have an automated sequence or menu to do this. Most instruments use perfluorotribuylamine (PFTBA) that is stored in a vial in the MS. During the tuning procedure, a valve is opened to allow a small, consistent mass of PFTBA to enter the ionization chamber. Typical concentrations of vapor range from 1 to 10 ppm PFTBA. As PFTBA passes through the MS, the instrument optimizes several settings to obtain the maximum detector response (counts per second) for selected ion fragments of the tuning compound. Results from one of the most common brands on the market (Agilent 5975C) are shown in Figure 7.1.

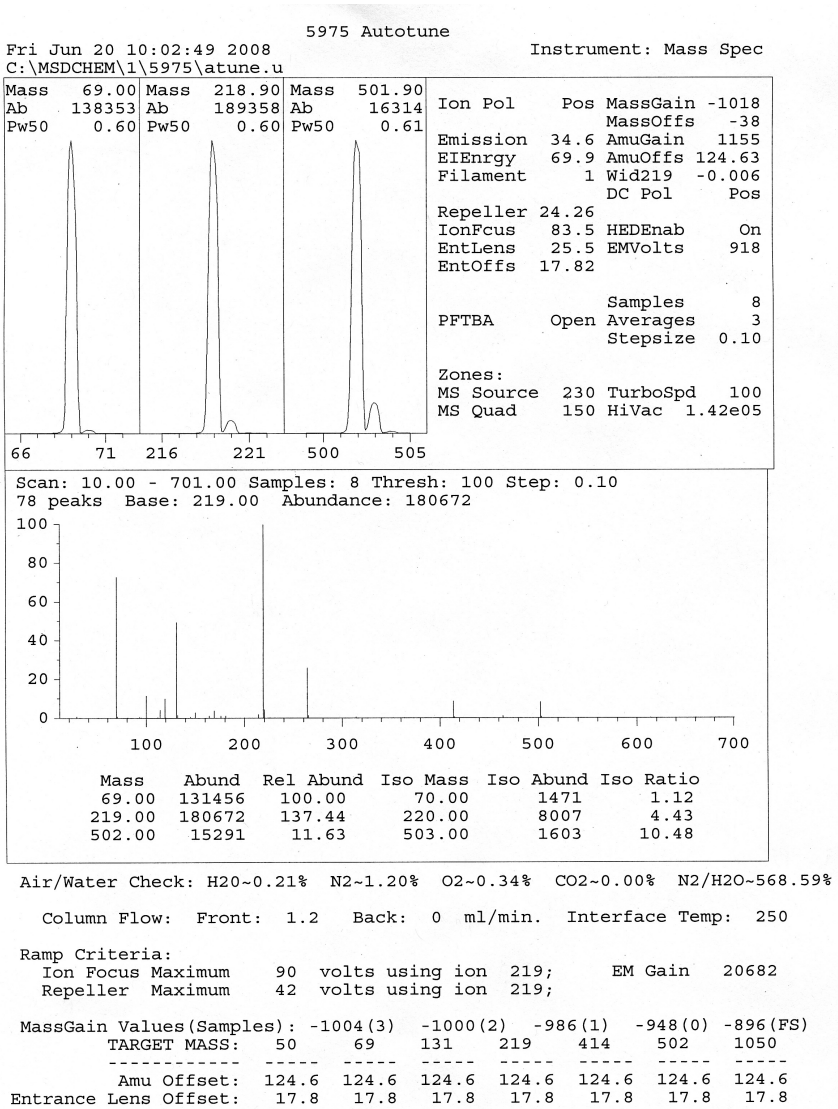


Figure 7-1. A Typical Electron Ionization Tune File from an Agilent 5875 EI-Quadrupole Mass Spectrometer.

Interpretation of the tune file. printout: The center plot in Figure 7.1 is a chromatogram of PFTBA showing the abundance of each ion as a

function of temperature. Below the plot are the observed counts per second for three m/z ratios (69 amu, 219 amu, and 502 amu) and their corresponding C-13 isotope-containing ions (the small peak to the immediate right of each tune peak) after the instrument has been successfully tuned. The counts per second for each ion are given in the "Abund" (abundance) column below the chromatogram. The 69, 219, and 502 ions are used to calibrate the m/z values over the entire range of the spectrum.

Now look at the top left-hand side of the figure. This contains expanded scale enlargements of the three m/z peaks. Recall that the quadrupole mass analyzer only yields unit mass resolution. Each of the peaks shown in Figure 7-1 is the result of 10 data point measurements evenly spread across the single amu measurement.

There are several objectives of the tune function. One objective is to calibrate the mass analyzer with respect to mass, so the instrument assigns the large peaks at 69 and 219 to these masses, while the isolated ion at 502 is calibrated to the 502 m/z value. A second objective is to obtain unit resolution as shown in the enlarged plots where the presence of C-12 and C-13 in each of the ions is resolved. A third objective of the tune is to calibrate the instrument where peak height can be used in the counts per second measurements instead of peak area since peak height calculations are faster to calculate and thus allow faster analysis. This last objective is accomplished by normalizing the width at half peak maximum for each of the three ion peaks to similar or near identical values. Each of these objectives is accomplished by sequentially adjusting the parameters listed on the top right-hand side of the figure. These include the voltages of the Repeller, Ion Focus (IonFcus), entrance lens (EntLens), entrance offset (EntOffs), AmuGain and Amu offset (AmuOffs). Recall that the repeller is located on the upstream side of the ionization source and is positively charged to "push" the ionized molecules (cations) toward the mass analyzer. Most of the inertia/velocity imposed on the ion is from charge placed on the repeller. The other lens focuses the ions into the center of the trajectory towards the mass analyzer. The mass width of the peak is primarily adjusted by the AmuGain and AmuOffs parameters. All of the other parameters shown in the top right corner of the figure are normally held constant.

Each of the parameters are adjusted sequentially until the maximum counts per second, resolution, and similar half peak width are achieved; as one parameter is changed, the instrument readjusts the previously adjusted parameters for optimum performance. Finally the EMVolts (potential applied across the electron multiplier) is adjusted so that the 69 m/z ion has a counts per second of approximately 500 000.

Leaks can be detected in the tune process by reviewing the Air/Water Check line of data located immediately below the center total ion chromatogram (TIC). The presence of H_2O, N_2, O_2, CO_2 and N_2/H_2O are shown here and should be present at no more than 10 percent of the total 69 m/z ion counts. If values higher than this are encountered, a leak is present, and is usually located at the vacuum door or column inlet fitting.

7.2.2 Optimizing Analyte Separations with a Temperature Program

The goal of chromatography is to separate a complex mixture of compounds. Some separations are relatively simple while others require experimentation to optimize the instrumental settings. Analyte separations are controlled by the temperature settings of the column and oven. Usually the initial temperature of the oven is set at approximately 10 to 15 degrees below the boiling point of the solvent. After injection, the oven temperature may or may not be held at this value for a few minutes. Next, the oven and column temperature is increased as slow as needed to allow separation of the compounds but as fast as possible to minimize the instrument run time. Finally, after all of the analytes have reached the detector, the instrument is usually held at a high temperature to allow any high boiling compounds to exit the column. The key to an adequate separation is to determine each of these temperatures, noting the need to achieve adequate separation in a minimum amount of time, especially in an industrial setting where cost (and time) efficiency is mandatory.

In this experiment, we will show the optimization of the temperature program for a set of hydrocarbons normally found in gasoline, the subject of the next lab.

Experimental Procedures:

Chemicals and Supplies:
A Pasteur pipet for each analyte
One 10-mL volumetric flask
Neat (pure) samples of benzene, decane, ethyl benzene, n-heptane, isooctane, toluene, m-xylene, and o-xylene.

Instrumental Settings:
GC-FID Settings (Flame Ionization Detector)

Capillary Column:	DB-5 Poly(phenylmethyldimethyl) siloxane (5 % phenyl) 30 m x 0.53 mm; 1.5 μm phase coating
Injection Volume:	1.00 μL
Splitless Injection for:	1.00 min.
Split Flow Rate:	50 mL/min.
Column Flow:	1.2 mL/min.
Linear Velocity:	14 cm/s
Injector Temperature:	230^{o}C
Detector Temperature:	250^{o}C
Oven Program:	varied as described below.

Sample Preparation:

The dilution solvent will be pentane because it has a very low boiling point and most other dilution solvents would co-elute with one or more analytes. Prepare a qualitative standard, as described below, for injection into the GC.

-Add two drops of each analyte to approximately 10 mL of pentane, cap in an air-tight vial, and mix the solution.
-Inject this solution into the GC using a variety of temperature programs. Start with a relatively low temperature isothermal program (50 C) for an extended time (20-30 minutes). Next, use a relatively high temperature isothermal program (150 C for 15 minutes). You will not obtain complete separation for either of these programs. Finally, use a temperature program starting from a temperature just below the boiling point of your analyte with the lowest boiling point and program an increase of 5 C per minute to a final temperature

approximately 10 C above the boiling point of your analyte with the highest boiling point. Refer to the next experiment in section 7.2.3 for optimum temperature programming instructions. When all peaks have been separated, the elution order will be: benzene, n-heptane, isooctane, toluene, ethyl benzene, m-xylene, o-xylene, and decane.

7.2.3 Obtaining a Linear Calibration Line

After the temperature program has been optimized, the next task is to calibrate the instrument. As discussed in Chapter 1, instruments easily generate numbers but the analyst must always question the validity of numbers until they are sufficiently scrutinized. Chromatographic analysis has a special feature over most other analyses since the very nature of chromatography allows the analysis of several compounds at one time. If quantitative work is being performed, the instrument must be calibrated with respect to each analyte. This experiment will illustrate proper calibration of a GC-MS. We will use several components of gasoline as our analytes and service station samples of gasoline as our sample.

In this experiment, the analyst will (1) obtain reference standards of several components of gasoline, (2) make dilutions of the reference standards (in pentane) ranging from 1.00 ppm (parts per million) to 100 ppm, (3) inject these standards into the instrument, (4) analyze the samples (at an appropriate dilution), (5) use the software to calibrate the instrument, and (6) analyze the results (perform a linear least squares on the calibration line and calculate the concentration of each component in the gasoline sample).

Experimental Procedures

Chemicals and Supplies:
25-μL, 50-μL, and 100-μL glass microsyringes
1.00-mL and 2.00-mL Class A pipets
Eight 10-mL volumetric flasks
Two 25-mL volumetric flasks
One 250-mL volumetric flask
Neat (pure) samples of benzene, decane, ethyl benzene, n-heptane, isooctane, toluene, m-xylene, and o-xylene.

Instrumental Settings:
GC-FID Settings (Flame Ionization Detector)
Capillary Column: DB-5
Poly(phenylmethyldimethyl) siloxane (5 % phenyl)
30 m x 0.53 mm; 1.5 μm phase coating
Injection Volume: 1.00 μL
Splitless Injection for: 1.00 min.
Split Flow Rate: 50 mL/min.
Column Flow: 1.2 mL/min.
Linear Velocity: 14 cm/s
Injector Temperature: 230°C
Detector Temperature: 250°C
Oven Program: 40°C for five minutes, 4°C to 200°C, hold for 10 minutes

GC-MS Settings:
Capillary Column: DB-5
Poly(phenylmethyldimethyl) siloxane (5 % phenyl)
30 m x 0.25 mm; 0.25 μm phase coating
Injection Volume: 1.00 μL
Splitless Injection for: 0.50 min.
Split Flow Rate: 50 mL/min.
Column Flow: 1.2 mL/min.
Linear Velocity: 40 cm/s
Injector Temperature: 230°C
Detector Temperature: 250°C
Oven Program: 40°C for five minutes, 4°C to 200°C, hold for 10 minutes

Calibration and Sample Preparation:

Calibration standards containing the major components of unleaded gasoline are required. An external calibration procedure will be used, with an internal standard to correct for injector errors and detector drift. The dilution solvent will be pentane because it has a very low boiling point and most other dilution solvents would co-

elute with one or more analytes. Prepare a stock calibration standard, as described below, and use this standard to perform serial dilutions (using pentane containing decane as an internal standard) to obtain a range of calibration standards.

NOTES: (1) To minimize the volume (and expense) of GC grade solvents used, dilutions will be made with micro-syringes. This method is less accurate then when using Class A pipets, but will be sufficient for our demonstrations here. (2) All compounds used in this lab are very volatile and flammable. Work in a fume hood away form hot plates, flames, and combustion sources. To minimize volatilization of analytes during solution preparation, place approximately 10 to 15 mL of pentane in the volumetric flask. Since pentane has the lowest boiling point, it will be the first to volatilize, leaving the other analytes in solution.

Procedures:

(1) To add each analyte to the flask, fill a microsyringe to the desired volume (in Table 3.1 below), place the syringe needle on the inside neck of the flask (not in the solution), empty the syringe, withdraw it, and immediately rinse the walls of the flask with 1-3 mL of pentane. Rinse the syringe thoroughly with clean pentane and repeat the process. After all of the analytes have been added to the flask, fill it to the mark with pentane. This solution is the stock solution of each analyte.

Table 7.1 Preparation Guide for the Stock Calibration Solution.

Analyte (> 99% neat)	Boiling Point ^{o}C	Density of liquid	μL of pure analyte to be added to a 25 mL volumetric flask	Resulting ppm concentration in flask
Benzene	80	0.874	29.0	1010
Ethyl Benzene	136	0.867	29.0	1010
n-Heptane	98	0.684	37.0	1010
Isooctane	99	0.692	36.0	996

Toluene	111	0.865	29.0	1000
m-Xylene	138	0.868	29.0	1010
o-Xylene	144	0.870	29.0	1010
Decane (Internal Standard)	174	0.73	14.0	101

(2) All solutions injected into the GC must contain internal standard (decane). Make 250 mL of pentane-internal standard solution for dilutions by adding 35 mL of pure decane with a microsyringe to a 250-mL volumetric flask and then filling the flask to the mark with pentane. Cap, mix, and use to make the following solutions.

(3) Make dilutions of the ~1000 mg/L solution made in step 1, according to the table below. Fill each flask with the internal standard-pentane solution made in step 2.

Table 7.2 Preparation of GC-MS Calibration Standards.

1	2	3	4
Approx. Conc. of each Analyte (ppm)	Solution to be Used in Dilution	mL of Solution from Column 2 to be added to Volumetric Flask	Volumetric Flask Size to Use
100.	Stock 1000 ppm	1000 (1.00 mL)	10.00
80.0	Stock 1000 ppm	2000 (2.00 mL)	25.00
40.0	Stock 1000 ppm	1000 (1.00 mL)	25.00
20.0	100. ppm	2000 (2.00 mL)	10.00
10.0	100.0 ppm	1000 (1.00 mL)	10.00
4.00	40.0 ppm	1000 (1.00 mL)	10.00
2.00	20.0 ppm	1000 (1.00 mL)	10.00
1.00	10.0 ppm	1000 (1.00 mL)	10.00
0.400	4.00 ppm	1000 (1.00 mL)	10.00
0.200	2.00 ppm	1000 (1.00 mL)	10.00

(4) The compounds in pure gasoline are at too high of a concentration to be analyzed directly on the GC-MS. Most of the major constituents in gasoline are present between 5 and 20 percent on a mass basis. To dilute the gasoline to an acceptable level, add 40.0 mL to 100 mL of pentane-internal standard solution. Several samples of unleaded gasoline should be analyzed. Suggestions for selecting samples include brand, octane rating, and the presence of methanol and MTBE. Note: if methanol or MTBE are present in your sample, the calibration standards must also include these compounds.

(5) Analyze the standards and diluted samples by GC-MS using the instrumental conditions given earlier. Use the MS to identify each peak in the spectra and then calibrate your instrument. Calculate the % composition of each analyte. Finally, analyze the spectrum of each compound and review the fragmentation rules from Chapter 2.

Results:

Each compound should produce a linear calibration line over the concentration range of your external standards. Most modern instruments will do this relatively automatically. After you calculate the concentration of each analyte in your gasoline sample, convert the ppm concentrations to percent by mass. Compare this to published composition available on the Internet. NOTE: the power of chromatography is the separation of complex mixtures which we have accomplished in this experiment.

7.2.4 Electron (hard) versus Chemical (soft) Ionization.

As noted in Chapter 1, the most common form of ionization in MS is electron ionization (EI) that is considered a hard source since is creates numerous fragments and allows for a unique fragmentation pattern. Several spectral libraries and computer search/match routines are available to aid in analyte identification. In contrast, chemical ionization (CI) is a milder form of ionization. Chemical ionization is rarely used for fragmentation pattern recognition, but is used to observe or obtain the molecular mass of the molecular ion. This experiment shows the electron and chemical ionization of three compounds.

EXPERIMENTAL PROCEDURES:

Chemicals and Supplies:
A 25 ppm solution of 2,2',6'6,-tetrachlorobiphenyl in isooctane
A 50 ppm solution of cyclohexanol in methanol
A 50 ppm solution of decanoic acid methyl ester in methanol

GC-MS Settings:

Capillary Column:	DB-5 Poly(phenylmethyldimethyl) siloxane (5 % phenyl) 30 m x 0.25 mm; 0.25 μm phase coating
Injection Volume:	1.00 μL
Splitless Injection for:	0.50 min.
Split Flow Rate:	50 mL/min.
Column Flow:	1.2 mL/min.
Linear Velocity:	40 cm/s
Injector Temperature:	250°C
MS Transfer Line	
Detector Temperature:	230°C
Quadrupole Temperature:	150 °C

Oven Program: 55°C and hold for zero minutes, 5°C to 250°C, hold for ten minutes
Total Run Time: 49 min.

Procedures:
Inject the standard solutions and analyze them using the instrument conditions given above.

RESULTS:

Spectra of the three compounds for EI and CI are shown below.

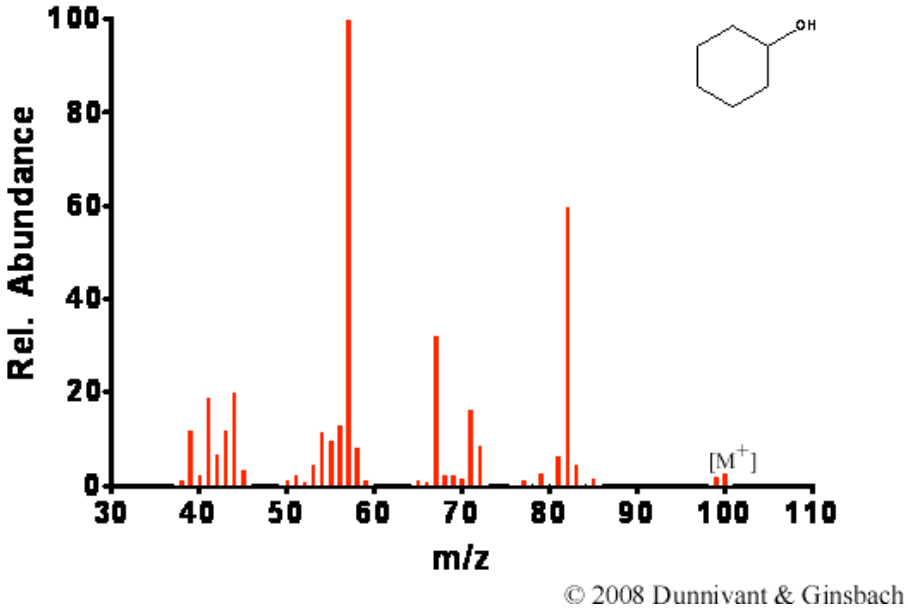


Figure 7.2. Fragmentation of Cyclohexanol by EI.

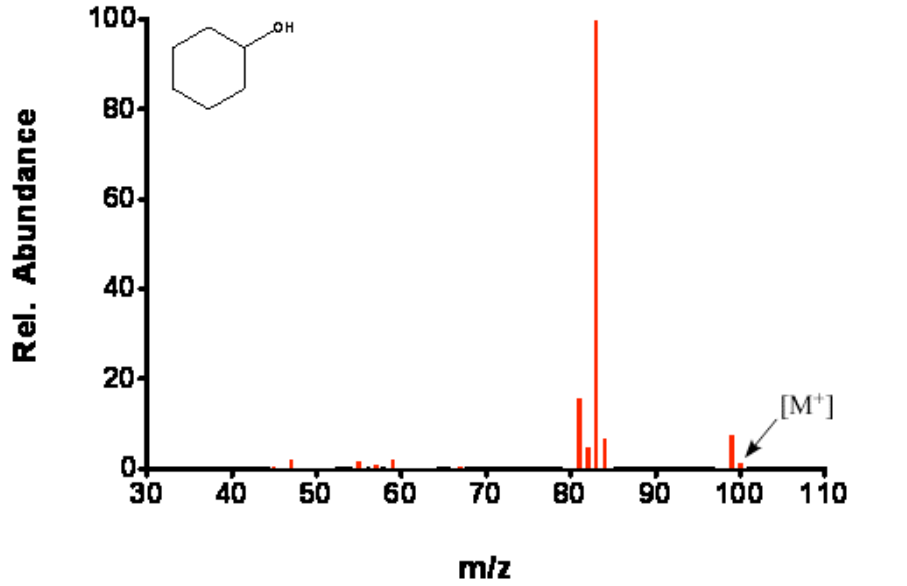


Figure 7.3. Fragmentation of Cyclohexanol by CI.

First, note the presence of the molecular ion using both ionization techniques. As expected, extensive fragmentation of cyclohexanol occurs for the EI analysis and follows the rules for fragmentation of alcohols given in Chapter 6, while minor fragmentation occurs in the CI analysis.

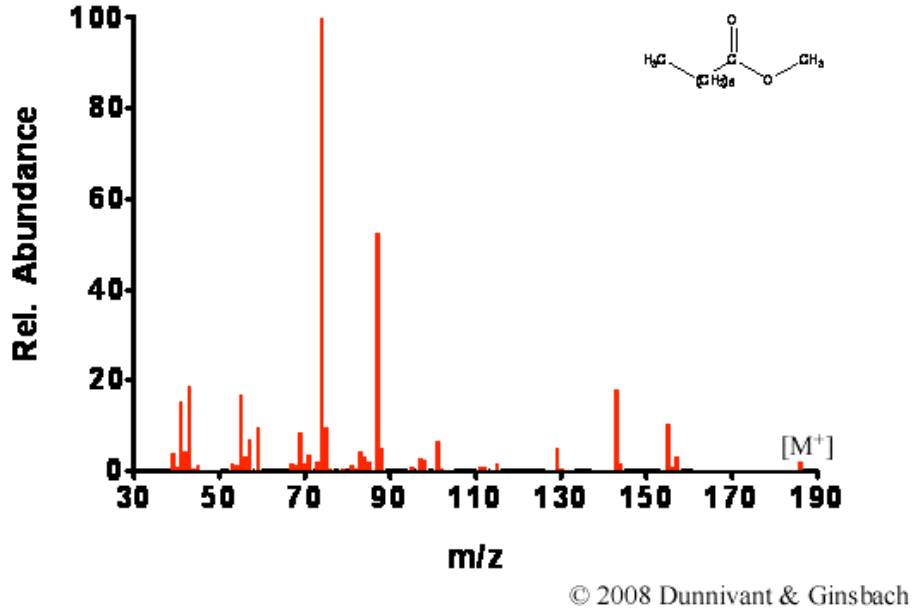


Figure 7.4. Fragmentation of Decanoic Acid Methyl Ester by EI.

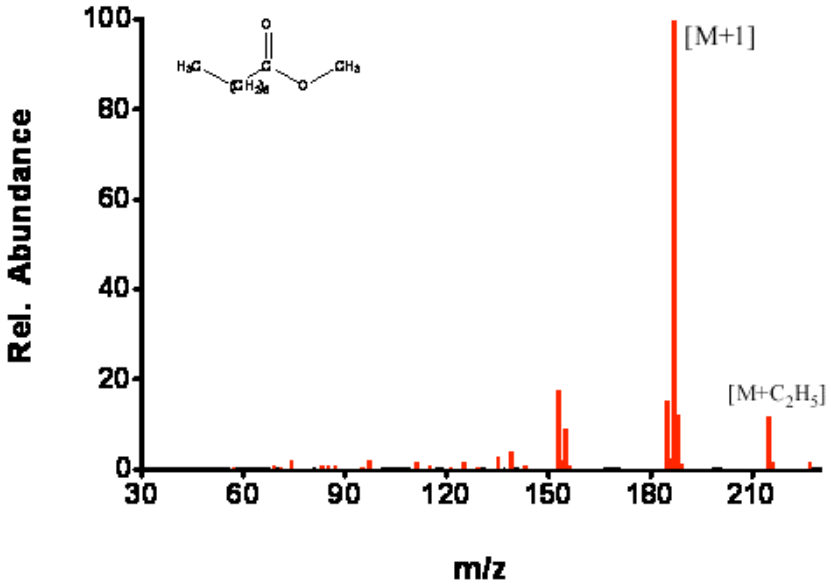


Figure 7.5. Fragmentation of Decanoic Acid Methyl Ester by CI.

Similar results are found for decanoic acid methyl ester; extensive fragmentation occurs during EI, but not during CI. Furthermore, for CI the molecular ion is more pronounced and the $M+C_2H_5$ ion is present in significant concentrations.

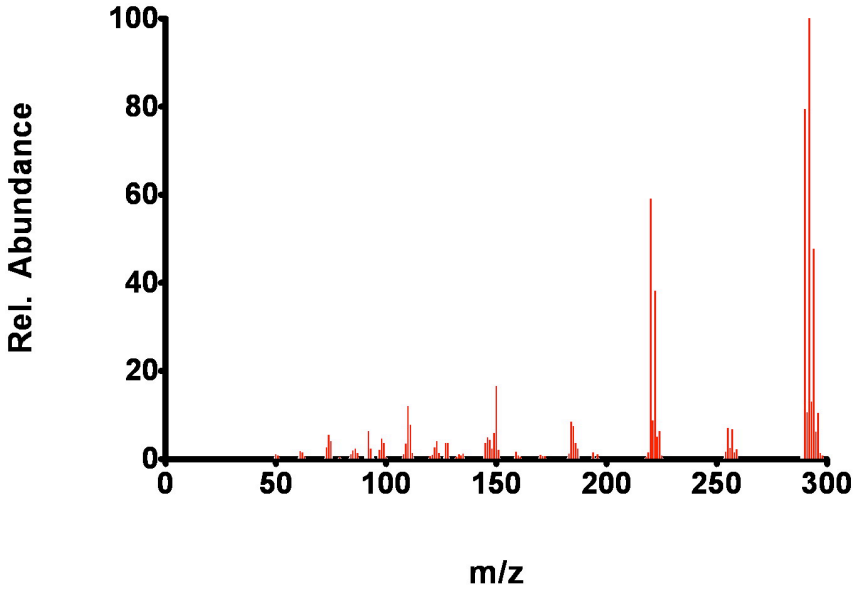


Figure 7.6. Fragmentation of 2,2',6,6'-tetrachlorobiphenyl by EI.

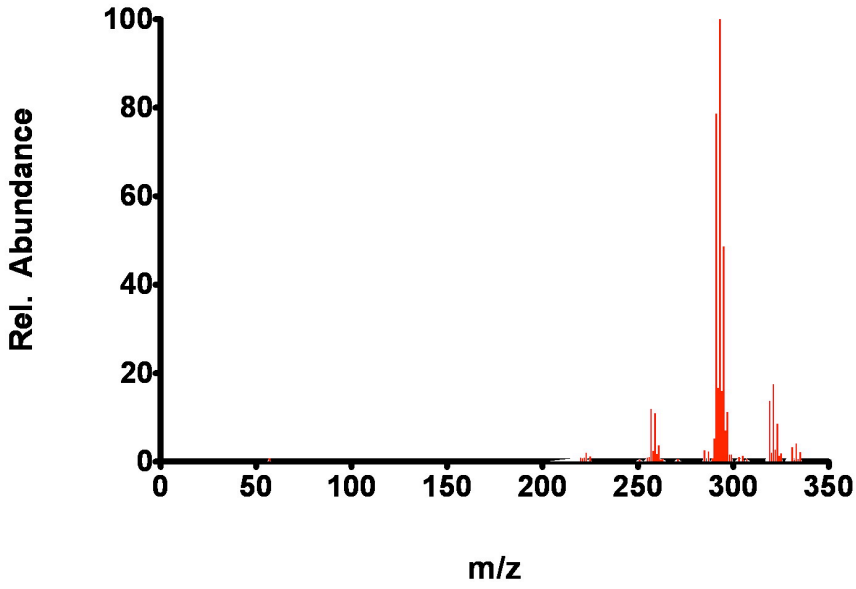


Figure 7.7. Fragmentation of 2,2',6,6'-tetrachlorobiphenyl by CI.

The analyte, 2,2',6,6'-TCB is so stable, even under the conditions in the EI chamber that the molecular ion is still a dominant peak. Again, some fragmentation occurs during EI, while additions are observed for the CI technique.

When to use EI and CI: Most MS analysis uses EI because it yields easily identified (via a fragmentation library) and unique fragmentation patterns. However, CI is used in two main cases: (1) when the point of the analysis is to obtain information about the molecular weight of the molecular ion and (2) when a better (lower) detection limit can be obtained using CI. Chemical ionization can be used in two modes, positive and negative.

As noted in section 1.5.1.2b: Chemical ionization is most commonly used to create positive ions, but some analytes, such as those containing acidic groups or electronegative elements (i.e. chlorinated hydrocarbons) will also produce negative ions that can be detected by reversing the polarity on the accelerator and detector. Some of these analytes produce superior detection limits with CI as opposed to EI, while others only give increased sensitivity (slope of the response to concentration line). Negative ions are produced by the capture of thermal electrons (relatively slower electrons with less energy than those common in the electron beam) by the analyte molecule. Thermal electrons are present from the low energy end of the distribution of electrons produced by the lower-energy CI source (~20 eV as opposed to ~70 eV in EI). These low energy electrons arise mostly from the chemical ionization process but also from analyte/electron collisions."

7.3 Concept Illustrative Experiments

7.3.1 Advantages of GC-MS over GC

Capillary columns provide superior resolution over packed columns, and while separations of complex mixtures are usually complete, some samples can be problematic. This is also why a single GC analysis for an analyte, even with a reference standard, is not conclusive, but suggestive. As discussed in Chapter 1, GC analysis (in the absence of MS detection) can be considered conclusive when a sample is analyzed twice, once on one stationary

phase and once on a different stationary phase, and when the same results from these two analyses confirm the present of an analyte based on retention time.

In contrast, gas chromatography-mass spectrometer analysis can give conclusive identification for many structures, with or without a reference standard. But MS analysis requires that a pure compound be introduced into the MS or that a GC be used to separate a complex mixture of analytes. This is the purpose of this experiment, to show the identification power of MS. Polychlorinated biphenyls (PCBs) will be used for illustration purposes here, and there are many other classes of compounds that can be used for this purpose (i.e. alkanes, aromatics, etc.). There are 209 different PCBs, ranging from monochlorobiphenyls to a single decachlorobiphenyl. PCBs are usually separated/analyzed on a non-polar column such as the polydimethyl siloxane phase (commonly referred to as HP-1, SP-1, or DB-1) or the poly(phenylmethyldimethyl) siloxane phase (commonly referred to as HP-5, SP-5, or DB-5). These columns mainly separate non-polar analytes based on boiling points and given the possibility of similar structures in PCBs (and other classes of compounds), some compounds will have similar boiling points and therefore similar retention times in the chromatogram (lack of separation). However, given the range of boiling points of the 209 PCBs a very slow oven temperature ramp (~1.0 ^{o}C per minute) is necessary that results in a long analysis time (approximately 2 hours). In this experiment the lack of separation will be illustrated for 2,2'-dichlorobiphenyl and 2,6-dichlorobiphenyl.

Experimental Procedures

Chemicals and Supplies:
A 25 ppm solution of 2,2'-DCB in isooctane
A 25 ppm solution of 2,6-DCB in isooctane
An isooctane solution containing 2,2'-DCB and 2,6-DCB (25 ppm each)

GC-MS Settings:

Capillary Column:	DB-5 Poly(phenylmethyldimethyl) siloxane (5 % phenyl)

30 m x 0.25 mm; 0.25 μm phase coating
Injection Volume: 1.00 μL
Splitless Injection for: 0.50 min.
Split Flow Rate: 50 mL/min.
Column Flow: 1.2 mL/min.
Linear Velocity: 40 cm/s
Injector Temperature: 250°C
MS Transfer Line
Detector Temperature: 230°C
Quadrupole Temperature: 150 °C
Oven Program: 70°C for two minutes, 5°C to 280°C, hold for 2 minutes
Total Run Time: 46 min.

Procedures:
Inject the standard solutions and analyze them using the instrument conditions given above.

RESULTS:

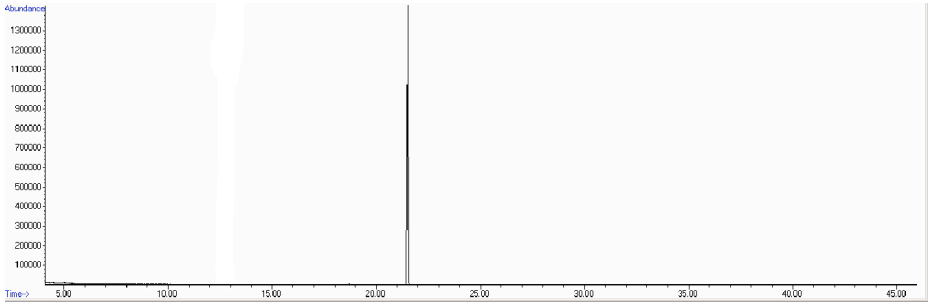


Figure 7-8. Total Ion Chromatogram of a 25ppm solution of 2,2'-dichlorobiphenyl and 2,6-dichlorobiphenyl.

Figure 7.8 shows the analysis results for a solution containing both 2,2'- and 2,6- dichlorobiphenyl. Note the lack of separation; individual injections shows that 2,6'-DCB elutes at 21.494 minutes while 2,2'-DCB elutes at 21.506 minutes. An injection of a combined solution does not resolve the two analytes. A slower temperature ramp may allow the separation of these compounds, or separation can be improved with a

longer column or with a thicker film coating. But again there are instances where gas chromatography cannot adequately separate some compounds. If only one of the compounds is present in a GC peak we may still be able identify it using MS. For example, review the two spectra below.

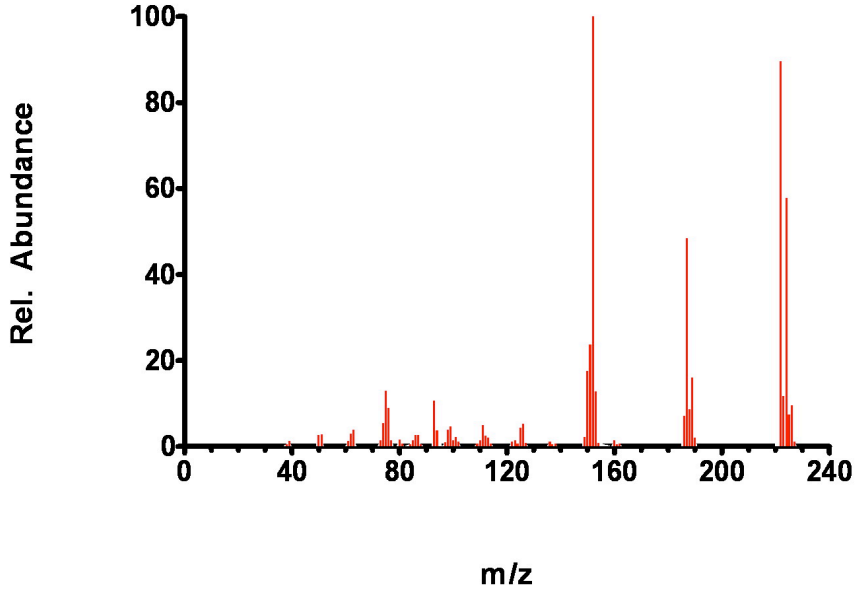


Figure 7-9. Mass Spectrum of 2,2'-dichlorobiphenyl.

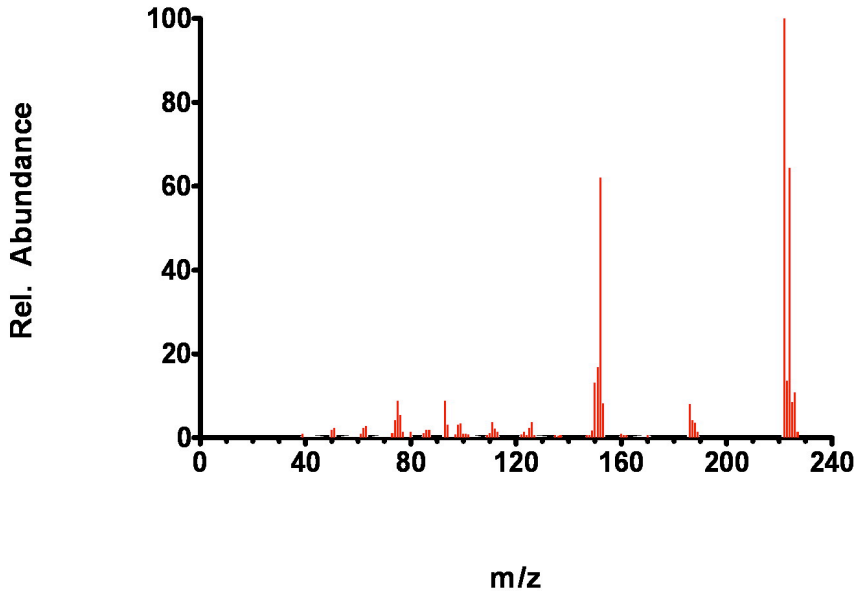


Figure 7-10. Mass Spectrum of 2,6-dichlorobiphenyl.

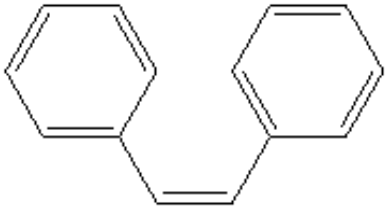
While these spectra look similar at first glance, distinct differences (relative ion abundance heights) can be noted that are used by the GC matching algorithm to identify the compound. Recall, the library search routine mainly uses two criteria to match an analysis with a known from the library spectra: presence of a m/z peak and relative heights of the m/z peaks.

Similar m/z peaks are present in each spectra but the relative proportions are distinctly different, especially in the186-190 m/z region. Thus, if only one of the compounds is present in a GC peak, it can be easily identified.

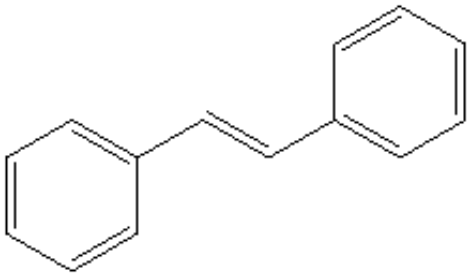
As an aside, it should be noted that if milligram quantities of the analytes could be obtained, NMR could be used to identity their presence and abundance, even in a mixed solution.

7.3.2 Advantages of GC over MS; cis- versus trans-

The experiment in Section 3.3.1 illustrated the power of MS in identifying analytes when they could not be separated by GC. This experiment will do the reverse, use GC to identify analytes that give the same spectra with MS. This is important with cis- and trans- isomers. Cis- and trans- isomers can have significantly different physical parameters due to the rotation of functional groups around a double bond. For example, cis-stilbene has a boiling point of 82-84 ^{o}C, while rotation of one benzene ring around the double bond to form trans stilbene yields a boiling point of 305-307 ^{o}C. These can easily be separated by chromatography but ~all cis- and trans- isomers yield the same fragmentation pattern in MS.



cis-stilbene



trans-stilbene

This experiment will use GC to separate and identify cis- and trans- heptene. Look up the boiling points to estimate the relative retention order.

Experimental Procedures

Chemicals and Supplies:
A 50 ppm solution of cis- and trans- in heptene

GC-MS Settings:

Capillary Column:	DB-5 Poly(phenylmethyldimethyl) siloxane (5 % phenyl) 30 m x 0.25 mm; 0.25 μm phase coating
Injection Volume:	1.00 μL

Splitless Injection for: 0.50 min.
Split Flow Rate: 50 mL/min.
Column Flow: 1.2 mL/min.
Linear Velocity: 40 cm/s
Injector Temperature: 250°C
MS Transfer Line
Detector Temperature: 230°C
Quadrupole Temperature: 250 °C
Oven Program: 80°C for two minutes, 5°C/min to 210°C, hold for 10 minutes

Procedures:

Analyze the standard solutions on a GC-MS using the instrumental conditions given above.

RESULTS:

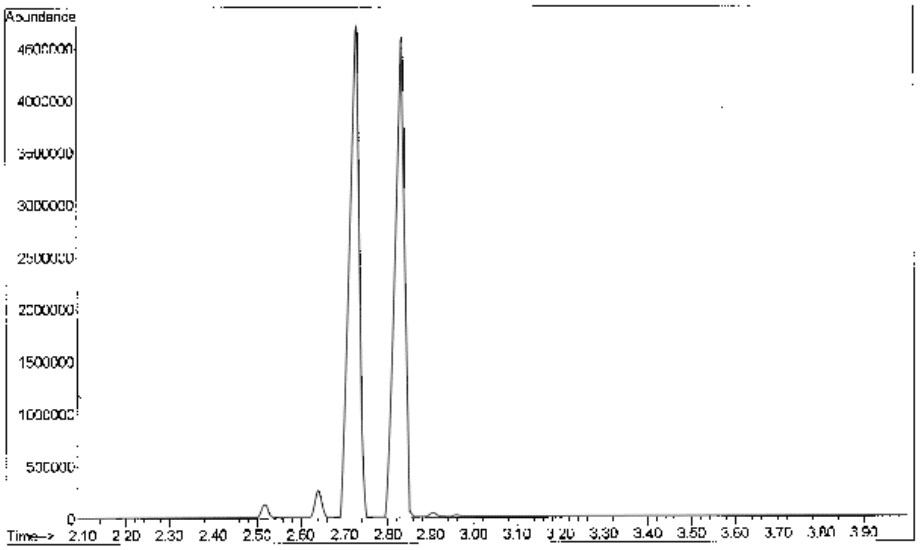


Figure 7-11. Total Ion Counts for the Analysis of cis- and trans-Heptene.

Note the dependence of the results, retention times, on boiling points. DB-1 and DB-5 columns separate exclusively based on boiling points.

7.3.3 Advantages of GC over MS; Chiral separations

One of the most difficult classes of compounds to separate is chiral compounds. In some cases these can be separated by normal capillary column GC, usually if the compound has more than one chiral center. Recall, the criteria that allows separation is if the chemical structure results in a different set of physical characteristics such as boiling point. In our design of several laboratory experiments we accidentally came across several chiral compounds that separated on a DB-5 capillary column (in the fragrance experiments in section 7.5). We know this since two ion peaks that were extremely close to each other in the chromatograph give identical and essentially exclusive identification (99% probability of a library match with limited or no additional matches). Chiral columns are available but only for a limited selection of a compound structures. In general, MS fragmentation will not distinguish between chiral compounds.

7.4 Analytical Experiments with an External Reference Standard Calibration

7.4.1 Caffeine Concentrations in Human Urine by Nathan Conroy

As the use of drugs has become more commonplace, so has the concern and apprehension toward the misuse and abuse of drugs. For example, professional athletes and Olympians are subject to random testing for performance enhancing drugs. Drug testing has even become routine as part of many job applications. Often drug analyses have to be designed to test for metabolites of the drug of interest, rather than the drug itself. Cocaine drug analyses involve not only the quantification of cocaine, by also benzoylecgonine (a metabolite of cocaine formed in the liver) and ecgonine methyl ester (both a metabolite and precursor of cocaine) [1]. Typically, urinary drug analysis procedures require the use of an internal standard that accounts for most losses during a liquid-liquid extraction to remove analyte from the protein and compound aqueous bio-layer [1-2]. The

isolated solution of analyte and internal standard is then analyzed on an instrument such as a gas chromatography-mass spectrometer (GC-MS) or high performance liquid chromatography-mass spectrometer (HPLC-MS). Laboratory analysis of many drugs requires a costly license for possession of the drug, thus most academic laboratories do not teach these extraction procedures. But similar extraction using street legal compounds can be used as surrogates. This caffeine lab procedure is designed to introduce students to the techniques and procedures used in drug analyses where caffeine is used as a surrogate for many drugs. Students will determine the concentration of caffeine in their urine after having consumed caffeinated beverages, and see how this concentration changes as a function of time; as well as across different caffeine consumption habits.

Multi-step sample preparation techniques do not quantitatively transfer an analyte from starting material to the final extraction solution; small percentage losses can occur at each step in a procedure. Therefore, when trying to determine an unknown concentration of an analyte, the analyst must account for the sum of these experimental losses using an internal standard (ISTD). A chosen internal standard should behave similarly to the analyte under reaction conditions; therefore relative losses throughout sample preparation will be equal. In other words if 20ppm ISTD (final concentration in the extraction solution) is added in a sample, but when the sample extract is analyzed and the instrument signal corresponds to only 15ppm of ISTD, we known only 75% of actual ISTD concentration was detected by the instrument. The use of an ISTD correction procedure will account for these losses in the analyte. For the case just stated, if the instrument detects a signal corresponding to 12ppm analyte, the instrument will back calculate the actual concentration of analyte in the solution to be 16ppm (a correction of +25 percent).

Experimental Procedures

The best way to ensure an equal concentration of internal standard across your samples is to deliver an equal amount of internal standard to all solutions prior to diluting. Also, make sure that the final concentration of internal standard in your sample extract is the

same concentration as internal standard used in making your calibration standards. A given volume of internal standard solution is most accurately delivered by a Hamilton-type micro-syringe where the full volume (or near full volume) of the syringe is used. Choosing an internal standard for a GC-MS analysis of caffeine is problematic because most compounds that share structural similarities with caffeine thermally degrade before volatilizing. 4-Acetylpyriding emerges on the gas chromatogram as two separate peaks, 4-Acetylpyrdine and its hydrated derivative, making a standardized integration of the peak problematic. Decyl-alcohol works as an internal standard, but is not ideal because it shares no structural similarity to caffeine. Cyclizine would likely make an appropriate internal standard for a caffeine GC-MS analysis but is more costly.

Chemicals and Supplies:

High-Resolution GC grade methanol
Caffeine
Ammonium chloride
Ammonium hydroxide
GC grade dichloromethane
Sodium chloride
High purity (99.999%) helium gas

Instrument Settings:

Front Inlet:
Mode: splitless
Inlet temperature: 250 C
Pressure: 10 psi
Purge Flow: 50 mL/min
Purge Time: 0.50 min.
Total Flow: 54.0 mL/min.

Injection Volume: 1.00 mL

Column Specifications:
HP-5MS 5% Phenyl Methyl Siloxane
Length: 30. m
Diameter: 250. mm

Film Thickness: 0.25 mm
Flow Rate: 1.2 mL/min.
Linear Velocity: 40 cm/sec.

Oven Settings:
Initial Temp.: 50 C
Initial Time: 2.00 min.
Ramp: 15.0 degrees per minute to 260 C, hold for 2.00 min.
Transfer Tube Temp.: 280 C

MS Parameters:
Solvent Delay: 4.00 min.
Ionization Source: EI
Temperatures: MS Source 230 C; MS Quad 150 C

TABLE 7.1. Approximate Peak Retention Times

Compound	Retention Time (min)
Caffeine	13.86
Decyl-alcohol	9.08
4-Acetylpyrdine	7.28

Breaking an Emulsion:

The liquid-liquid extraction used in this procedure has a tendency to form emulsions, mixtures of two immiscible liquids. They generally appear as either a cloudy combination of liquids, or as bubbly pockets at the interface between the two liquids. Emulsions interfere with the recovery of the extraction solvent and the analyte, and therefore are problematic in analytical analyses. Emulsions can be resolved or "broken" several different ways. A saturation of the aqueous phase with sodium chloride is a first defense against emulsion formation, but has not been shown to be sufficient with this procedure. Sonicating solutions is another common solution, but did not prove completely successful here. Centrifuging is another common solution. A far less costly strategy for breaking emulsions is glass wool. Glass wool can be used to break emulsions by packing the glass wool into the bottom 1cm-2cm of a transfer pipet, then filtering the emulsified layer through

the packed pipet. While glass wool does successfully break the emulsion, the likelihood of experimental loss makes it non-ideal. If all else fails time will break the emulsion; allow solutions to sit over night.

Evaporation/Concentration of the Extraction Solvent

One of the advantages of using organic solvents is that the final extraction volume can be concentrated. This results in the concentration of the analytes and improves the detection limit. The final step in the procedure below calls for concentration of the extraction solvent. Such a procedure is briefly given here.

Procedures:

Creating a calibration curve:

1. Make up solutions containing approximately 15ppm internal standard and a caffeine concentration of approximately 0.5, 1, 3, 5, 10, 15, 20, 25, and 50ppm.
3. Create a new GC method using the GC and MS conditions given above.
4. Inject samples from low to high concentration and create calibration curve using mass spectra software.

Make up an Ammonium Buffer Solution:

1. Add a few scoops of ammonium chloride to 15-20mL of High Resolution-GC grade methanol in a 25mL collection vial.
2. Place a calibrated pH electrode in the collection vial.
3. Add 28% ammonium hydroxide to the collection vial in single drop increments, until the solution reaches a pH of 9.5. If the pH exceeds 9.5, add more ammonium chloride to lower the pH.

Extraction of Caffeine from Urine:

The next step involves a liquid-liquid extraction of caffeine from a bio-aqueous layer (urine) into a methanol and dichloromethane solvent mixture. A high purity helium gas stream is used to evaporate the dichloromethane/methanol solvent (see Figure 7-12), and then the remaining material is dissolved into HR-GC grade methanol.

1. Collect a sample of urine 0.5 to 1 hour after the consumption of a drink containing caffeine.
2. Quantitatively transfer 2mL of urine to a 10mL collection vial.

3. Add ~100mg sodium chloride, 200µL ammonium buffer solution
4. Add a volume of your chosen internal standard to make the concentration equal to that used in the calibration curve, keeping in mind that the entire solution will eventually be dissolved in 200 µL.
5. Add 5mL of dichloromethane and methanol in a 9:1 volume ratio to the collection vial.
6. Mix vials vigorously for 2 minutes.
7. If necessary, use previously discussed emulsion techniques to break the emulsion.
8. Place the recovered organic layer in a KD vial and the vial in a warm water bath. (Warm water is sufficient, no hot plate is needed)
9. Use a high purity helium gas stream to evaporate the solvent. Once half the solvent has evaporated, use a transfer pipet to wash the side of the KD vial with the remaining solvent. Repeat this process again once half the remaining solvent has evaporated. Continue to evaporate to dryness.
10. Dissolve the remaining residue in 200µL High Resolution-GC grade methanol.
11. Using 100µL vial inserts in a standard automatic sampler vial, inject methanol in the GC-MS.
12. Analyze the results using MS computer software for identification and concentration using appropriate dilution factors.

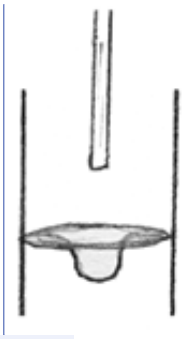


Figure 7-12. Solvent blow down.

When blowing off solvent with a gas stream, there is a delicate balance between needle height and gas pressure. The gas should

maintain a small indentation on the surface of the solvent. Submersing the needle will contaminate both the needle and your sample and too high a pressure will blow the solvent out of the KD vial. It is best to set a needle height, then start the gas pressure at 0 and increase the pressure slowly.

Mzuri Handlin 6/30/11 3:20 PM
Comment: what is this figure for? should it be referred to in the rest of the text somewhere?

Results:

Caffeine concentrations vary depending on caffeine intake, but usually are in the parts per million range. The procedure can be used in a variety of class experiments. The simplest is to extract each students' urine for a range of levels. A more interesting experiment is to have one or more students drink a cup of coffee or other relatively high dose of caffeine and follow the clearance of caffeine from their body with time.

Note that many other peaks are present in mass spectra of urine extracts.

References:

Mulé, S.J., and G.A. Casella. "Confirmation and Quantitation of Cocaine, Bezoylecgonine, Ecgonine Methyl Ester in Human Urine by GC/MS." Journal of Analytical Technology Vol.12 (1988): 153-55.

Thuyne, W.Van, and F.T. Delbeke. "Distribution of Caffeine Levels in Urine in Different Sports in Relation to Doping Control Before and After the Removal of Caffeine from the WADA Doping List." Int J Sports Med 2006 27: 745-50.

7.4.2 Analysis of Cocaine Concentration on U.S. Currency by John Nelson and David Wallace

There has been an increase in the use of cocaine in the United States since the late 1950s. Originally obtained in extremely small doses through extraction from the coca plant by oral chewing, cocaine is now harvested in large quantities and extremely high

purity. It is often found in its crystalline form. This form of cocaine is taken into the body by snorting through the nasal cavity, allowing the drug to take effect quickly. The most common method for snorting cocaine involves rolling paper money to form a straw by which the cocaine can be sucked into the nose.

The most common currency used for snorting cocaine in the United States is the one-dollar bill. Once a bill is used to snort cocaine, it can then come into contact with other bills, transferring a small portion of the residual cocaine. This proliferation of trace amounts of cocaine has led previous studies to conclude that four out of every five dollar bills have trace amounts (above 0.1 μg) of cocaine on them.

This study further examines the frequency of cocaine contamination on dollar bills and employs methodologies to increase precision in the measurement of cocaine concentration.

Experimental Procedures

This study largely employed the use of the procedure found in “Cocaine Contamination of United States Paper Currency” by Oyler J. et al. from 1996.

Ten one-dollar bills obtained from a random cash register in Walla Walla, Washington were placed in glass vials that were filled with 10.0 mL of HR-GC grade methanol. These vials were allowed to stir for a period of 24 hours to ensure that all present cocaine was dissolved from the currency. After stirring, the methanol was decanted from the vials and 10 mL of sodium acetate buffer (10 mL, 2M, pH 4.0) was added to samples and allowed to mix. The buffered samples were then filtered through solid-phase extraction (SPE) columns to extract the cocaine from any other compounds that were dissolved by the methanol. Each column was washed with deionized water (1x, 2 mL) and 0.1 M HCl (1x, 1.5 mL) and aspirated to dryness. The columns were then washed with methanol (2x, 1 mL) and aspirated to dryness again. The cocaine analytes were then eluted from the columns using a 80:20:2 ratio of methylene chloride, isopropanol, and concentrated aqueous ammonium hydroxide. The

elution solvent was added to the columns (6x, 1 mL) and allowed to drip into clean glass tubes.

The elution solvent was then evaporated to dryness using high purity helium. This concentration of the elution solvent will provide a higher signal to noise ratio when analyzed by gas chromatography (GC). 100 µL of methanol was added to the glass tubes to allow the cocaine analyte to redissolve. The methanol was then transferred to glass gas chromatography (GC) vials for analysis.

An external calibration curve was made with concentrations of 10, 25, 40, 50, and 100 ppm cocaine. Five samples were analyzed by GC-MS using a temperature program starting at 180 ^{o}C and ending at 250 ^{o}C with a ramp of 5 ^{o}C per minute. The analyte concentration was measured by comparing the analyte response to the calibration curve responses to achieve part-per million concentration levels.

This procedure was repeated with the use of a derivatizing agent (BSTFA with 1% TMCS) to increase the signal strength of the analyte. The derivatizing agent was added to the final 100 µL of methanol in equal volume and allowed to heat in a 50 ^{o}C oven for 1 hour. The derivatizing agent was also added to the external calibration standards.

Results

Two separate calibration plots were constructed from the calibration standards of each run. A linear regression line was fit to each of these plots from which the concentration of cocaine in each of the corresponding samples was determined. Five samples were run along with the calibration standards without derivatizing agent, and four derivatized samples were run with the derivatized standards. Table 7-2 shows the cocaine concentrations obtained for each sample. The derivatized samples and calibration curve yielded substantially better results and detection limits than samples run without derivatizing agent—for this reason, the use of a derivatizing agent is crucial to obtaining precise and accurate results. Data for both calibration lines are reproduced below.

Table 7.2 Instrument Calibration and Extraction Results

Cocaine Calibration - Underivatized

Concentration (ppm)	Response	Slope	Y Intercept	R2
25	96	316	-9182	0.99359
40	2136			
50	6082			
100	22810			

Sample Number	Response	Concentration (ppm)	Amount On Bill (mg)
1	2718	37.707	0.37707
2	1161	32.7734	0.327734
3	4633	43.775	0.43775
4	1740	34.6081	0.346081
5	8352	55.5593	0.555593

Cocaine Calibration - Derivatized

Concentration (ppm)	Response	Slope	Y Intercept	R2
10	1580	261	-604	0.989426
25	5786			
40	11947			
50	10947			
100	25540			

Sample Number	Response	Concentration (ppm)	Amount On Bill (mg)
1	13589	52.0651	0.520651
2	14100	54.0229	0.540229
3	12512	47.9387	0.479387
4	3795	14.5402	0.145402

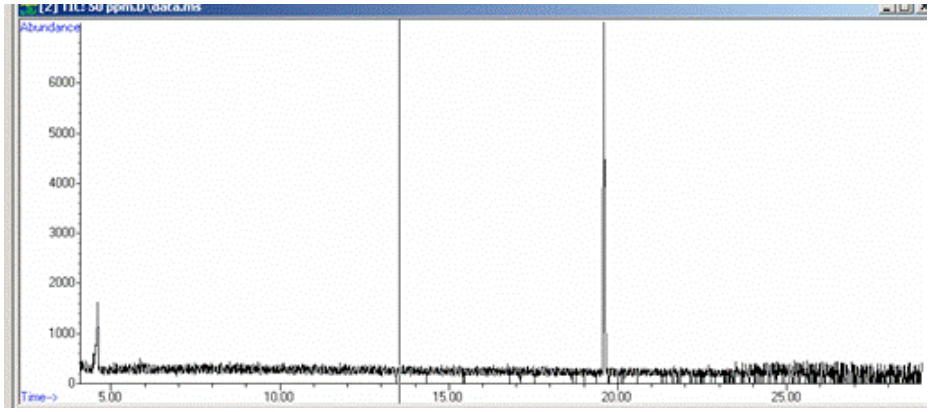


Figure 7-13. Total Ion Chromatogram of a Cocaine External Calibration Standard. The retention time of cocaine is just under 20 mintutes.

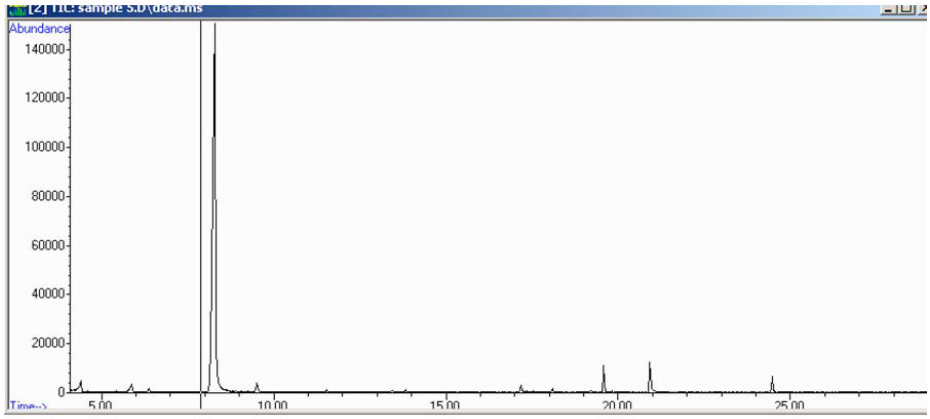


Figure 7-14. Total Ion Chromatogram of a Sample Extract. Again, the retention time of Cocaine is near 20 minutes.

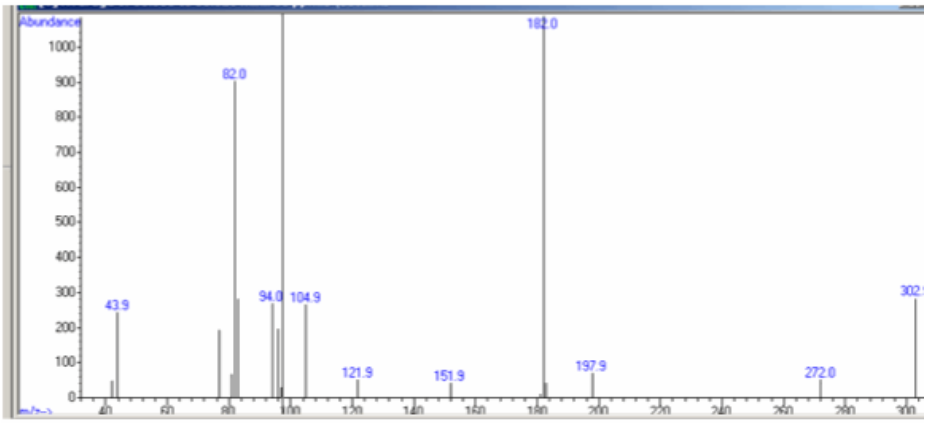


Figure 7-15. The Mass Spectrum and Fragmentation of Cocaine.

References

Jenkins, A.J. 2001. Drug Contamination of US Paper Currency, Forensic Science International, Vol. 21, pp. 189-193.

Oyler, J., W.D. Darwin, and E.J. Cone. 1996. Cocaine Contamination of United States Paper Currency, J. of Analytical Toxicology, Vol 20, July/August, pp. 213-216

7.5 Analytical Experiments without an External Reference Standard; Conformational Identification without Quantification.

One of the most powerful applications of an MS system is its ability to identify an analyte without a reference compound. In GC (with other non-conclusive detectors), reference compounds are needed to determine the retention time, the criteria for identification in GC. In MS, the spectrum is the identifier since it can be compared to thousands of reference spectra and a unique match is normally achieved. The laboratory exercises below illustrate the power of MS in identifying unknown analytes in a variety of samples.

7.5.1 Identification of Components in Liquors and Distilled Spirits

Distilled Spirits contain a range of flavors that can be identified by GC-MS. Not only can an analyst tell what type of liquor is present

(i.e. gin versus whiskey) but they can also compare the presence and abundance of select flavor compounds between different brands of a given type of liquor.

NOTE/WARNING: Some liquors contain nonvolatile components that will coat out on the glass liner in the injector port. The injector liner may need to be cleaned or replaced after completing this laboratory exercise to avoid damage to the GC column.

Experimental Procedures

Chemicals and Supplies:

Pure samples of a variety of liquors. One approach is to contrast types of liquors (rum versus gin versus whiskey). Another approach is to contrast brands (spiced liquor versus pure liquor).

GC-MS Settings:

Capillary Column:	DB-5 Poly(phenylmethyldimethyl) siloxane (5 % phenyl) 30 m x 0.25 mm; 0.25 μm phase coating
Injection Volume:	1.00 μL
Splitless Injection for:	0.50 min.
Split Flow Rate:	50 mL/min.
Column Flow:	1.3 mL/min.
Linear Velocity:	42 cm/s
Injector Temperature:	250^{o}C
MS Transfer Line	
Detector Temperature:	230^{o}C
Quadrupole Temperature:	150 ^{o}C

Oven Program: Initial Temp. at 60.0^{o}C for zero minutes, 2.0^{o}C to 150^{o}C, hold for zero minutes. Post Temp. at 280^{o}C for 10 minutes.

Total Run Time: 45 min.

Procedures:

Analyze a variety of liquor samples on a GC-MS using the instrumental conditions given above.

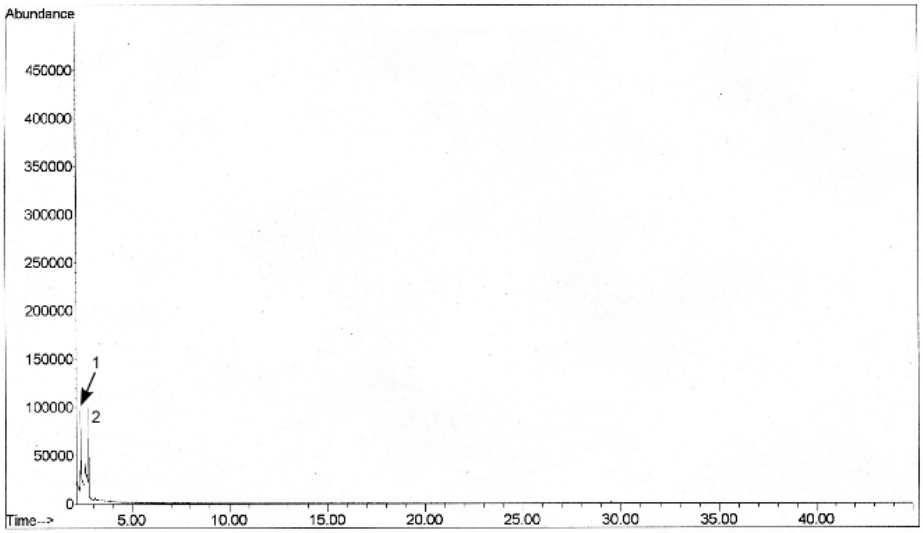
RESULTS:

It is relatively easy to distinguish between most types of liquors. However, pure liquors (unspiced) such as rum and vodka produce similar chromatograms and only contain ethanol, water, and a few trace longer chain alcohols. Other liquors, as shown below, are easily distinguished.

All of the compounds identified in the chromatogram below were conclusively identified by the spectral library (typically 99 percent confidence/probability) and are known to be present in the liquors based on a scientific literature search or from common information found on company web sites or web searches. An interesting project is to Goggle some of the compounds identified in the spectra and research their origin and why they are added to a specific liquor.

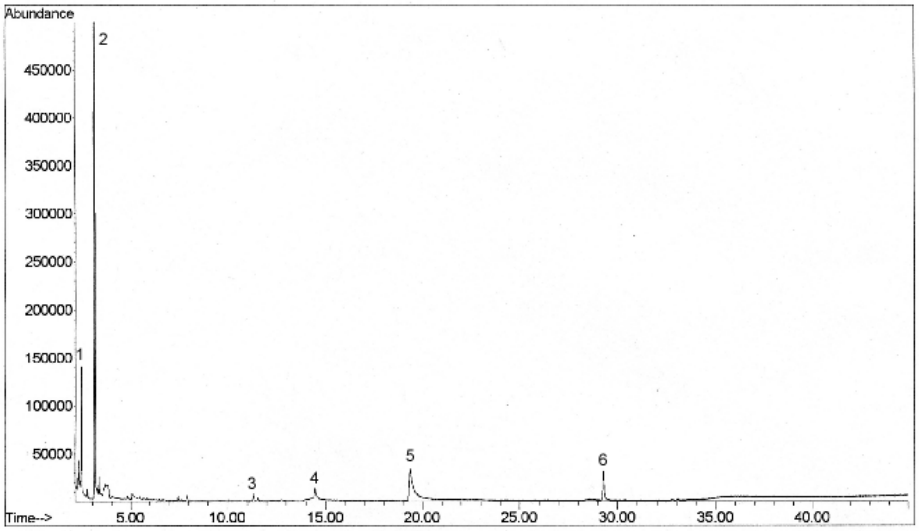
Rum: Rum is a fermented beverage made from sugarcane byproducts such as molasses and juice. After fermentation, it is distilled as a clear liquid. Double distillation yields the light rums, while single distillation will yield darker rums that were originally thought of as being of lower quality. From here the process becomes brand specific and the initial rum can be aged in a variety of barrels, including oak to impart strong flavors, or filtered through charcoal to remove colors. Spices or color agents are then added.

Figures 7-16, 7-17, and 7-18 are chromatograms for Bacardi Gold, Captain Morgan, and Citrus Rum, respectively. Note the lack of compounds in the relatively pure Barcardi Gold rum, only 3-methyl-1-butanol and acetic acid are present in measurable quantities. Barcardi Gold has little presence of the oak flavor compounds such as those found in the other two rum beverages. Captain Morgan's flavor is characterized by additional compounds, most notably oak flavors and vanilla. Citrus Rum contains almond, orange, cocoa, fruit, and lemon flavors, as well as extracts from the oak barrel aging process.



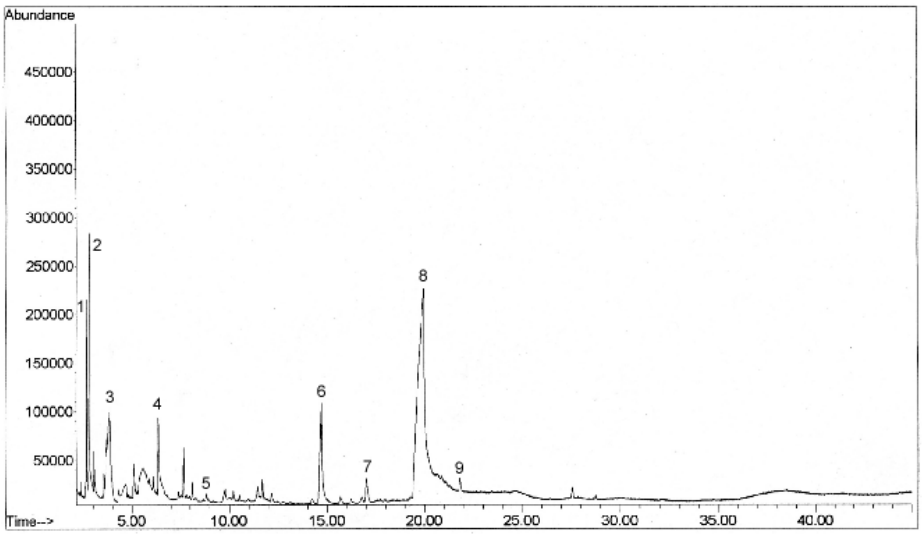
1) 3-methyl-1-butanol
2) acetic acid

Figure 7-16. Chromatogram of Barcardi Gold.



1) acetic acid
2) 1,2-dihydroxypropane
3) 5-methylbenzene-1,3-diol
4) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (toasty caramel aroma of heated oak)
5) 5-hydroxymethylfurfural (wood flavor)
6) 4-hydroxy-3-methoxy-benzaldehyde (vanilla flavor)

Figure 7-17. Chromatogram of Captain Morgan.



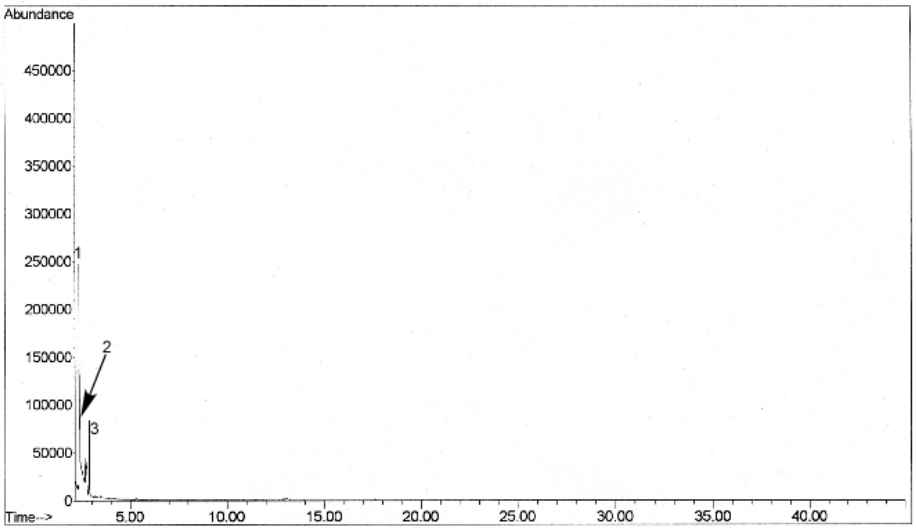
1) formic acid
2) acetic acid
3) 2-furaldehyde (wood flavor)
4) benzaldehyde (almond flavor)
5) limonene (orange flavor)
6) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (cocoa flavor)
7) α-terpineol (fruit juice flavor)
8) 5-hydroxymethylfurfural (wood flavor)
9) citral (lemon flavor)

Figure 7-18. Chromatogram of Citrus Rum.

As a side note, most of the components shown in these figures are in the parts per million range of concentrations.

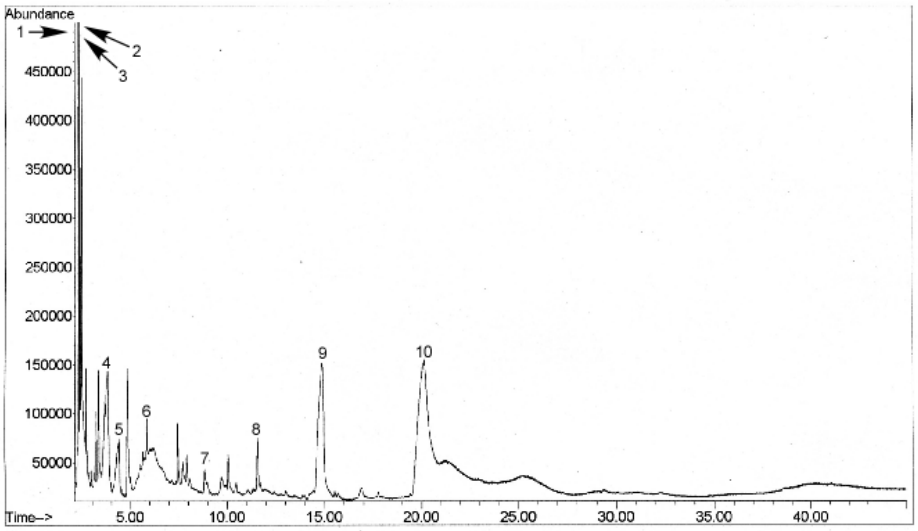
Whiskey: Whiskey (originally Whisky from its origin with Irish monks) refers to a broad range of alcoholic beverages that are distilled from fermented grain mash and aged (matured) in oak barrels (casks). The age of a whiskey refers to its time in the cask (between fermentation and bottling) and the length of aging greatly affects its chemical makeup and taste from the extraction of wood components from the cask. These components include lacone (3-methyl-4-octanolide) that has a coconut aroma, and numerous phenolic compounds. Grains of choice include barley, malted barley, rye, wheat, and corn, and it may be fermented from single or blends of grains. Published flavoring chemicals include carbonyl

compounds, alcohols, carboxylic acids and their esters, nitrogen- and sulphur-containing compounds, tannins and other polyphenolic compounds, terpenes, and oxygen-containing heterocyclic compounds and esters of fatty acids. The nitrogen compounds include pyridines, picolines and pyrazines. After distillation, the flavoring compounds that are common among different brands of whiskey include fusel oils that are higher alcohols that are actually mildly toxic and have a strong disagreeable smell and taste in high concentrations. Hence, these are commonly removed by charcoal and linen filtration. Other common flavor agents in whiskey are acetals, such as acetaldehyde diethyl acetal (1,1-diethoxyethane), the principal flavor agent in sherry. The presence of a buttery aroma is due to diketone diacetyl (2,3-butanedione). Some whiskey blends contain specific flavor agents. Use the chromatograms given below to confirm the presence of these known whiskey components.



1) 3-methyl-1-butanol
2) 2-methyl-1-butanol (shoulder peak)
3) acetic acid

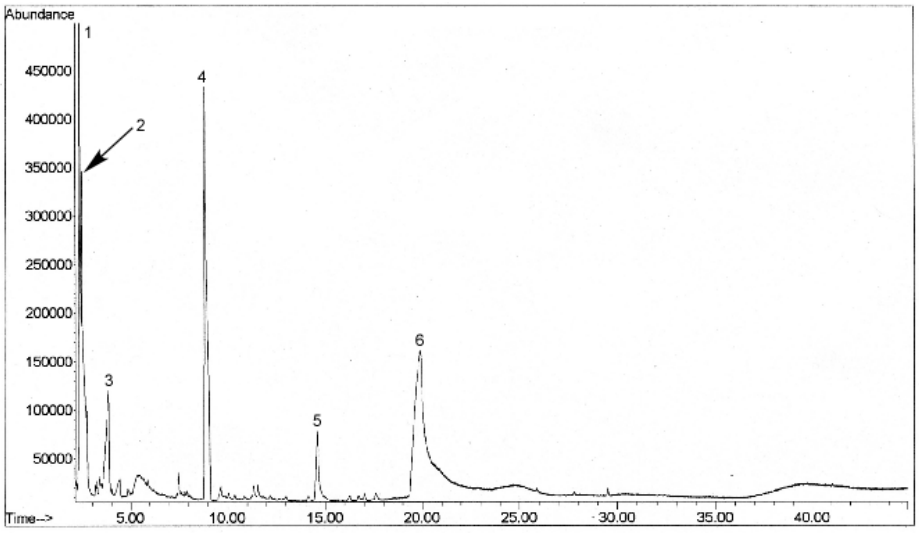
Figure 7-19. Chromatogram of Crown Royal.



1) formic acid
2) 3-methyl-1-butanol
3) acetic acid
4) 2-furaldehyde (wood flavor)
5) 2-hydroxymethylfuran (burnt flavor)
6) 1,2-cyclopentanedione (wood flavor)
7) limonene (orange flavor)
8) Methyl 2-furoate (fruity flavor)
9) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (cocoa flavor)
10) 5-hydroxymethylfurfural (wood flavor)

Figure 7-20. Chromatogram of Southern Comfort.

Cognac: The cognac used here is Grand Marnier, a blend of cognac. Cognacs are brandies produced from specific white grape varieties. Most cognacs are distilled twice and aged in oak barrels. After the review of the liquors above, the student should be able to predict some of the compounds present in cognac.

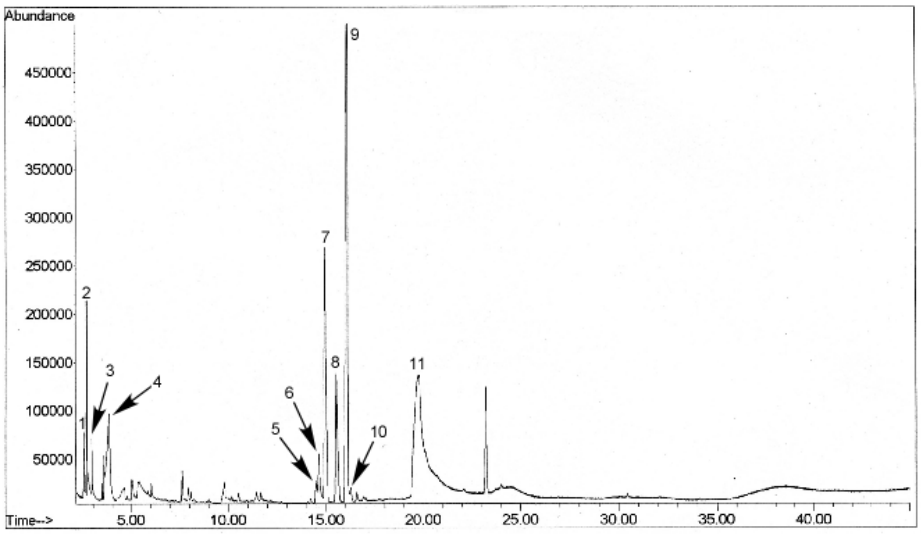


1) 1-pentanol
2) acetic acid (shoulder peak)
3) 2-furaldehyde (wood flavor)
4) limonene (orange flavor)
5) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (cocoa flavor)
6) 5-hydroxymethylfurfural (wood flavor)

Figure 7-21. Chromatogram of Grand Marnier.

Note that most of the flavor compounds come from the oak barrel or are specifically added.

Peppermint Schnapps: Schnapps is usually a clear, colorless beverage with a light fruit flavor since it is fermented from fruit. The schnapps used in this experiment is infused with peppermint leaf extract or specific flavors (chemicals) found in peppermint. Note the dominant mint flavor compound in the chromatogram.



1) formic acid
2) acetic acid
3) 1-hydroxy-2-propanone
4) 2-furaldehyde (wood flavor)
5) isopulegol (mint flavor)
6) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (wood flavor)
7) D-menthone (mint flavor)
8) L-menthone (mint flavor)
9) 5-methyl-2-(1-methylethyl)-cyclohexanol (mint flavor)
10) 4-carvomenthenol (mint flavor)
11) 5-hydroxymethylfurfural (wood flavor)

Figure 7-22. Chromatogram of Peppermint Schnapps.

7.5.2 Identification of Fragrances

Fragrances/perfumes provide a “Holy Grail” for GC-MS analysis. As noted in many movies or from a trip to a European fragrance shop (perfumery), a near infinite variety of combinations of fragrances can be made. In this laboratory exercise, “name brand” fragrances will be compared to their more inexpensive counterparts in an effort to determine if a difference exists in their “fingerprint” based on GC-MS. A fingerprint, in this context, is a characteristic chromatogram of a complex mixture of compounds.

Perfumes consists of (1) primary scents at the parts per million concentration, (2) modifiers that alter the primary scent to give the perfume a certain desired character, (3) blenders (ingredients that smooth out the transitions of a perfume between different bases; top, middle, and base notes of a fragrance may have separate scents), and (4) fixatives (natural or synthetic substance used to reduce the evaporation rate).

Sources of primary scents include: (1) Plant sources (bark, flowers and blossoms, fruits, leaves and twigs, resins, roots, rhizomes and bulbs, seeds, woods, (2) Animal sources (Ambergris which are lumps of oxidized fatty compounds, Castoreum from the odorous sacs of the North American beaver, Civet Musk obtained from the odorous sacs of the animals related to the Mongoose, Honeycombs, Musk originally derived from the musk sacs from the Asian musk deer), (3) and 0ther natural sources (extracts of lichens and seaweed). Synthetic sources of the natural compounds mentioned above are used today, as well as calone, linalool and coumarin from terpenes, and salicylates (orchid scents) are also used today.

Experimental Procedures

Chemicals and Supplies:

A variety of perfume samples can be analyzed. In this experiment, Light Blue by Dolce and Gabbana, Shades of Blue by Belcam, Drakkar Noir by Guy Karoche, Classic Match by Belcam, Unforgivable by Sean John, Unjustified by Belcam, and Bring It by Parfums were used.

GC-MS Settings:

Capillary Column:	DB-5 Poly(phenylmethyldimethyl) siloxane (5 % phenyl) 30 m x 0.25 mm; 0.25 μm phase coating
Injection Volume:	1.00 μL
Split Mode of Injection	
Split Flow Rate:	131 mL/min.
Column Flow:	1.3 mL/min.

Linear Velocity: 42 cm/s
Injector Temperature: 250°C
MS Transfer Line
Detector Temperature: 230°C
Quadrupole Temperature: 150 °C
Oven Program: Initial Temp. at 40.0°C for zero minutes, 2.0°C to 280°C, hold for five minutes.
Total Run Time: 125 min.

Procedures:
Analyze a variety of perfume samples on a GC-MS using the instrumental conditions given above.

Results

(1) The relatively expensive “Light Blue” and a generic blend “Shades of Blue”:

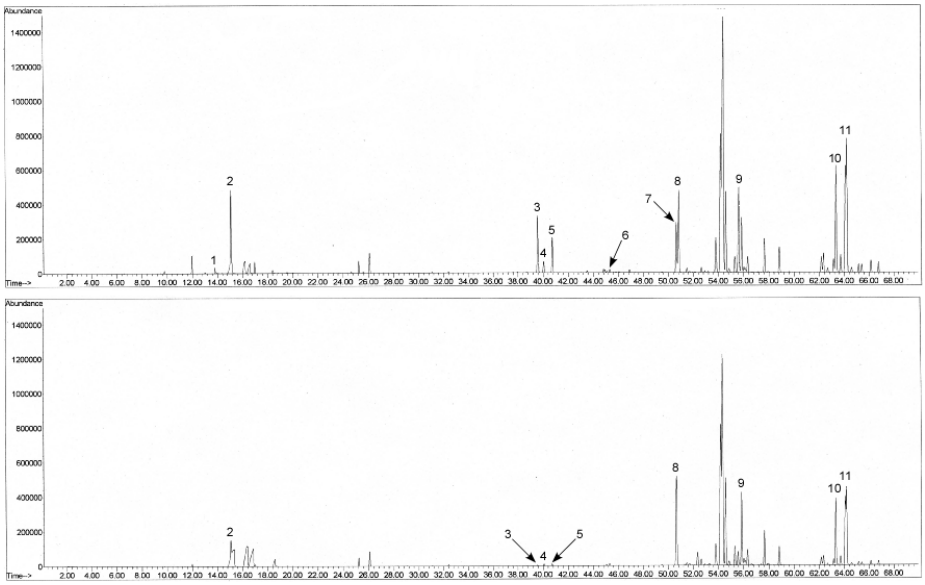
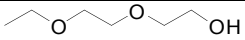
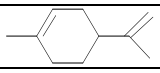
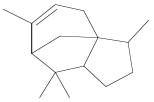
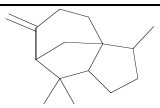
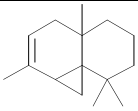
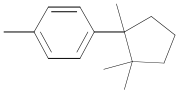
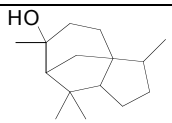
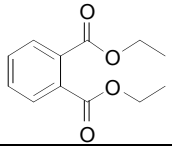
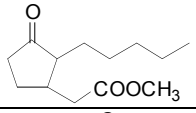
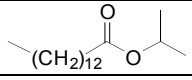
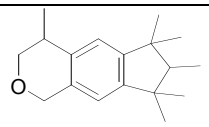


Figure 7-23. Chromatogram of Light Blue (top figure) and Shades of Blue (bottom figure).

Note the presence, absence, or reduced concentrations of the components between the two perfumes. Names and chemical structures for the numbered components are given in the following table.

Table 7.5. Labeled Components in Chromatograms of Light Blue by Dolce & Gabbana vs Shades of Blue by Belcam.

	Name	Structure
1)	diethylene glycol monoethyl ether (preservative)	
2)	limonene (lemon scent)	
3)	a-cedrene (wood scent)	
4)	b-cedrene (wood scent)	
5)	thujopsene (wood scent)	
6)	cuparene (wood scent)	
7)	cedrol (wood scent)	
8)	diethyl phthalate (preservative)	
9)	methyl dihydrojasmonate (jasmine)	
10)	isopropyl myristate (skin binder)	

11)	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-g-2-benzopyran (musk scent)	

(2) Drakkar Noir and the generic “Classic Match”:

Drakkar Noir is a blend of citrus, lavender, spices and woods. Top notes are citrus, middle notes are woody and herbaceous and base notes are woody warmed and spiced with aromatic coriander and juniper berries, strengthened by sandalwood, patchouli and fir balsam.

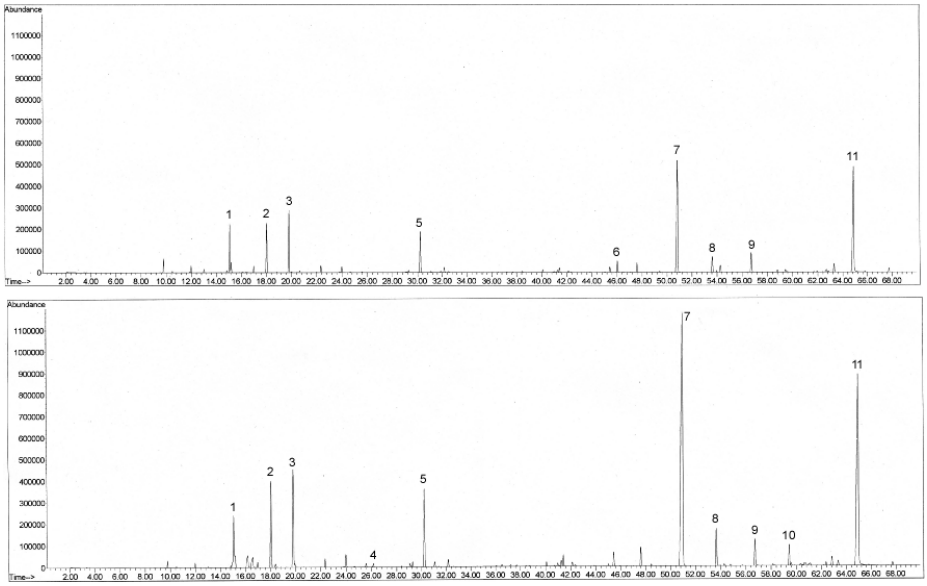
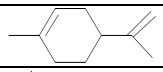
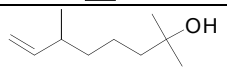
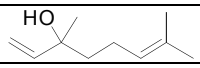
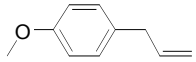
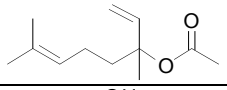
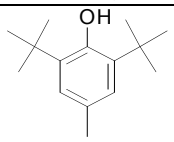
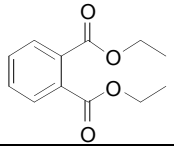
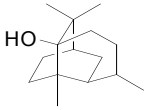
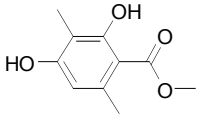
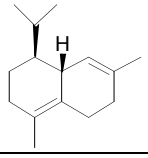
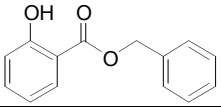


Figure 7-24. Chromatogram of Drakkar Noir (top figure) and Classic Match (bottom figure).

Table 7.6. Labeled Components in Chromatograms of Drakkar Noir and Classic Match.

	Name	Structure

1)	limonene (lemon scent)	
2)	dihydromyrcenol (lime scent)	
3)	linalool (spicy floral scent)	
4)	4-Allylanisole (minty sweet scent)	
5)	linalyl acetate (sweet scent)	
6)	2,6-ditertbutyl-4-methylphenol (antioxidant)	
7)	diethyl phthalate (preservative)	
8)	patchouli alcohol (woody scent)	
9)	verymoss (woody scent)	
10)	d-cadinene (woody scent)	
11)	benzyl salicylate (floral scent)	

(3) Unforgivable by Sean John vs Unjustified by Belcam Inc. vs Bring It by Parfums de Coeur

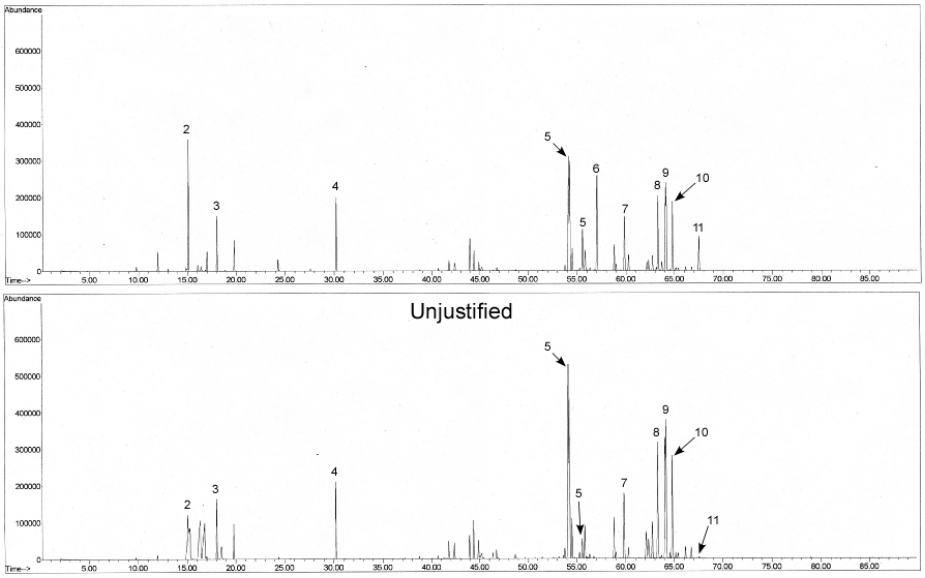


Figure 7-25. Chromatogram of Unforgivable (top figure) and Unjustified (bottom figure).

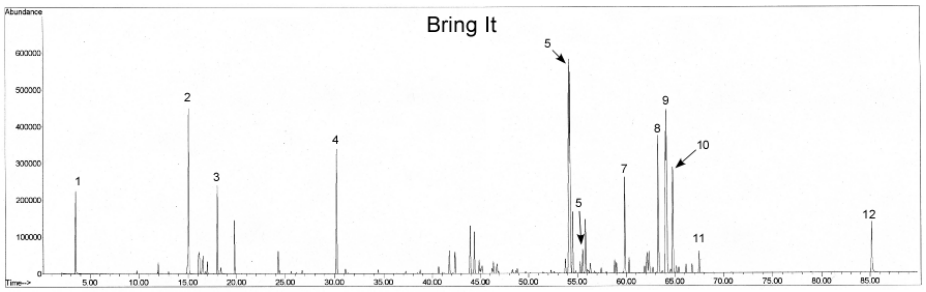
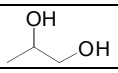
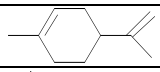
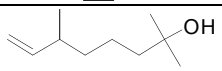
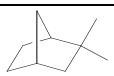
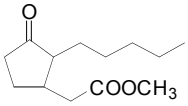
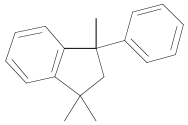
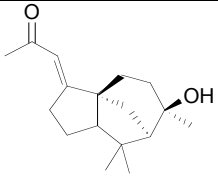
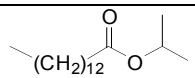
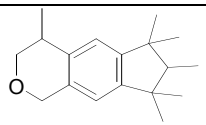
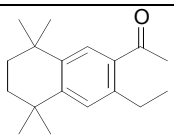
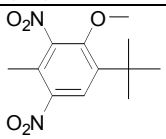
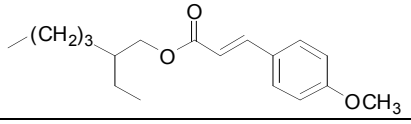


Figure 7-26. Chromatogram of Bring It.

Table 7.7. Labeled Components in Chromatograms of Drakkar Noir and Classic Match.

	Name	Structure
1)	propylene glycol	

2)	limonene (lemon scent)	
3)	dihydromyrcenol (citrus scent)	
4)	tricyclene (citrus scent)	
5)	methyl dihydrojasmonate (jasmine)	
6)	1,1,3-trimethyl-3-phenylindan	
7)	acetyl cedrene (musty scent)	
8)	isopropyl myristate (skin binder)	
9)	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-g-2-benzopyran (musk scent)	
10)	Versalide (musky scent)	
11)	6-tert-butyl-3-methyl-2,4-dinitroanisole (musky scent)	
12)	Octyl 4-methoxycinnamate (keratin binder)	

Comparisons of the “real” versus “fake” perfumes show distinct similarities with respect to presence of peaks and their “fingerprint”. However, closer inspection of each peak shows differences. These subtle differences change our olfactory perception of their “smell”. Note the identification of the components in each table and the type of the compounds present and their purpose.

7.5.3 SPME-GC-MS Analysis of Wine Headspace by Bailey Arend

For many consumers, the aroma of a wine is nearly as important as the flavor. The wine industry is obviously interested in producing wine with pleasing and abundant aroma. More than 1000 compounds have been identified in the headspace of wine, including alcohols, esters, carbonyls, acids, phenols, lactones, acetals, thiols, terpenols and many more (Weldegergis, et al, 2007; Polaskova, et al, 2008)] Although human senses can detect surprisingly small concentrations of certain volatile organic compounds in wine headspace, analytical instrumentation provides a more specific and precise way to measure the headspace character of wine.

The complex matrix of wine, as well as the low concentration of some of the volatile compounds presents further obstacles in the characterization of wine aroma. To analyze many of the compounds, sample enrichment techniques must be employed (Weldegergis, et al., 2007) liquid-liquid extractions using organic solvents (Andujar-Ortiz, et al., 2009; Ortega-Heras, et al., 2002) and solid phase extraction (SPE) (Andujar-Ortiz, et al., 2009; Dominguez, et al., 2002) are both effective for wine analyses, however solid-phase micro extraction (SPME) presents a major advancement in volatile compound analyses.

SPME was first introduced in 1989 by Belardi and Pawliszyn for analysis of organic pollutants in water. The original method involved immersing fiber coated with fused-silica stationary phase directly in the liquid analyte[6]. Analytes are sorbed/adsorbed onto the solid phase, which can then be inserted directly into a gas chromatograph (GC) injector, where the analytes are thermally desorbed and loaded onto the GC column. The newer SPME has drawn much attention for being versatile, yet simple. The technique does not require expensive, high-purity, toxic organic solvents generally associated

with instrumental analysis and eliminates many possible sources of error.

SPME was first modified for headspace analysis in 1993. The new method exposed the coated fiber to the sample headspace only, which was found to shorten the extraction time while maintaining detection limits in the ppt range (Zhang and Pawliszyn, 1993). The driving theory behind any SPME is the partition coefficient of the analyte between the coating and the solvent or vapor. The partition coefficient along with the large difference in volume between the coating and headspace volume result in impressive concentration factors. The mass of analyte adsorbed to the coating (n) is given by

$$n= C_oV_1V_2K_1K_2/(K_1K_2V_1+K_2V_3+V_2),$$

where C_o is the original concentration in the liquid phase, and the volumes of the three phases in equilibrium are as follows: V_1 for the coating, V_2 for the liquid phase, and V_3 for the headspace (Zhang and Pawliszyn, 1993). Minimizing the ratio of headspace to sample volume ($V_3<<V_2$) and maximizing the affinity of the coating for analyte (large K_1) can boost the amount of adsorbed analyte. This allows direct injection of analyte to the GC without risking instrument damage by injecting concentrated and sugary wine matrix. HS-SPME also avoids many complications of matrix effects, even allowing analysis of solid samples and human blood (Cardinali et al., 2000), as long as the analyte is volatile (Zhang and Pawliszyn, 1993). Tat et al. found that 50/30µm Divinylbenzene/Carboxen/Polydimethylsiloxane coated fiber gave the most sensitive and repeatable results for the analysis of wine headspace (Tat, 2005).

Aside from fiber coatings and volume ratios, other parameters that can affect the sensitivity of HS-SPME are exposure time, temperature, and pH of the sample solution (wine). Exposure time is logically related to the concentration of analyte sorbed to the fiber. Sufficient time must be given for the system to reach equilibrium before the equation above is valid. Temperature governs the fraction of analyte present in the headspace and available for adsorption. Many methods immerse the extraction vial in a heated water bath (Tat, 2005), however, care must be taken that the fiber, headspace and condensed phase are all in thermal equilibrium. The pH values

have been adjusted in some studies (Boutou and Chatonnet, 2007) to allow multiple classes of molecules to be in their most analyzable form. For example, to simultaneously adjust for pyrazines (which are best analyzed at neutral to basic pH values, pKa ~ 0.50) and phenols (pKa ~25, which are best analyzed at low pH values) Boutou and Chatonnet adjusted all samples to a pH value of 7.

The method of Boutou and Chatonnet was also sufficient for analysis of contaminants that cause off flavors in wine. Compounds such as 2,4,6-trichloroanisole, 2,3,4,6-tetrachloroanisole, 2,4,6-tribromoanisole have olfactory perception thresholds near 10 ng L^{-1} and give wine a “barnyard” character (Boutou and Chatonnet, 2007). Wines with these contaminants are referred to as “corked” and are quite undesirable. Analysis of such contaminants can determine the origin of contamination and improve wine production techniques (Boutou and Chatonnet, 2007).

Materials and Methods:

Sampling conditions (adopted from Tat, 2005)

Sample wines and all equipment were stored at room temperature to ensure thermal equilibrium and minimize thermal differences between samples. Thirty-two (32.0) mL of sample wine was pipetted into a 40-mL glass vial equipped with a septum. The septum was pre-punctured with a sharp, hollow needle to avoid contaminating or breaking the fiber by contact with the septum. The extraction fiber was a Supelco 50/30µm Divinylbenzene/Carboxen/Polydimethylsiloxane Stableflex fiber conditioned at 270°C in the GC inlet for 1 hour. The fiber was inserted into the vial via the septum before being exposed. The fiber was exposed to the headspace while the wine was stirred. The extraction was performed at 25°C for 15 minutes. When finished, the fiber was immediately inserted into the gas chromatograph injector, where it remained for the entire duration of the temperature program.

Instrumental Parameters (adopted from Boutou and Chatonnel, 2007)

GC/MS analysis was performed by:
Instrument: Agilent 19091S-433

Column: HP-5MS 5% Phenyl Methyl Siloxoane 30.0m x 250 μm x 0.25 μm nominal capillary column.
Carrier gas: helium (ultra high purity, 99.999% passed through hydrocarbon traps) programmed to flow at a constant linear rate of 54.1 mL/min for the entire run. The injector lining was ensured to be long enough to allow full insertion of the fiber and operated by manual injection. The injector was operated in splitless mode at 270°C for the entirety of the each run.

The oven program started at 50°C for 2.0 min, and then increased at 3.0°C min^{-1} to 190°C. The temperature was than increased at 50°C min^{-1} to 320°C where it was held for 5min. Detection was performed by an Agilent 5975C inert EI/CI MSD quadrupolar mass detector with EI ionization (source temperature 230°C, quadrupole temp. 150°C, energy of constant ionization 70). The entire method required a total of 56 min.

Results:

This study was conducted to demonstrate the ease and versatility of HS-SPME for student chemists. For this reason, no adjustments were made to the wine samples and the recommended heating of the samples was not performed. Although the range of detectable analytes was much smaller than other methods have reported (Tat, 2005; Boutou and Chatonnel, 2007), the quick and simple method returned multiple analyte peaks for each wine tested.

It was found to be important to thermally clean the extraction fibers before each exposure. Samples of Black Box Merlot were run without cleaning the fiber and unexpected compounds were observed (shown in Figure 1). Many silica compounds were found in the blank runs, which could possibly be attributed to degradation of the fiber coating (shown below in Figure 2). If the fiber was “baked out” directly before exposure, the presence of these compounds was minimized (shown below in Figure 3).

The method was repeated 5 times on Black Box 2007 California Merlot and the results were found to be reproducible. One advantage of using boxed wine is that the affects of oxidation are eliminated. Samples may be drawn weeks apart, whereas opening a bottled wine

introduces oxygen to the entire bottle and could affect the composition of the wine (Simpson, 1978).

While the method was found to be repeatable, only a limited number of identifiable compounds were recovered for each sample. These compounds are shown in Figure 7-26 below and include 3-methylbutyl acetate, ethyl hexanoate, 2-phenylethanol, diethyl succinate, ethyl octanoate and ethyl decanoate. The chromatogram for Chardonnay (Buckley's Cove, South Eastern Australia, 2009, Figure 6) does not contain 2-phenylethanol and diethyl succinate which were found in all red wine samples. It is possible that the lack of these two compounds could be a signifier of white wine.

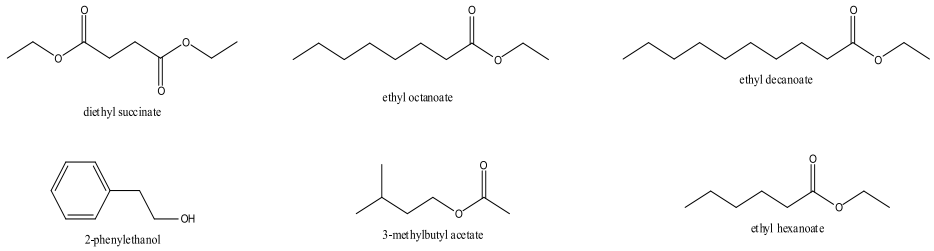


Figure 7-26**.** The compounds repeatedly found in all red wine samples by the proposed method.

It was thought that a higher-quality wine would have a stronger bouquet and yield more volatile compounds, however the chromatogram of Red Table Blend from Walla Walla Village Winery (82%Cabernet Sauvignon, 9% Merlot and 9% Cabernet Franc, Figure 7) did not show additional peaks. This method was also insufficient to characterize contaminants in a sample of corked wine from Foundry Vineyards in Walla Walla (2005 Red Wine), (shown in Figure 8). It is likely that heating of the samples or adjustment of the other parameters, such as pH, would increase the range of compounds detectable by this method.

Although this simplified method does not engage the full potential of HS-SPME techniques, it is sufficient to demonstrate the theory and application of such techniques in a college/university laboratory.

Additional streamlined methods may be developed for other consumable complex matrices such as whiskey or vinegar.

Chromatograms

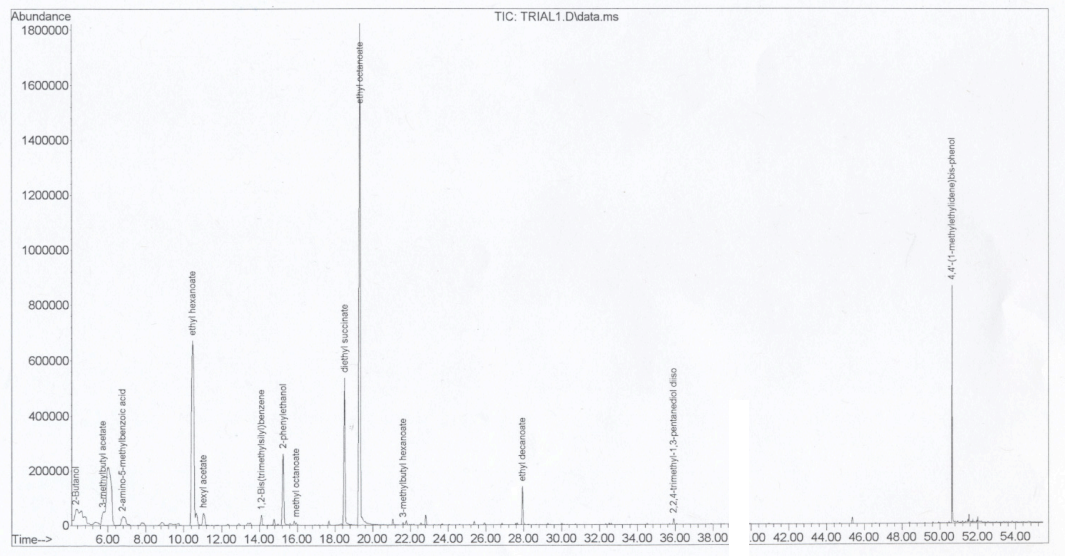


Figure 7-27**.** The chromatogram for Black Box California Merlot, 2007. No blank was run and unusual peaks were observed.

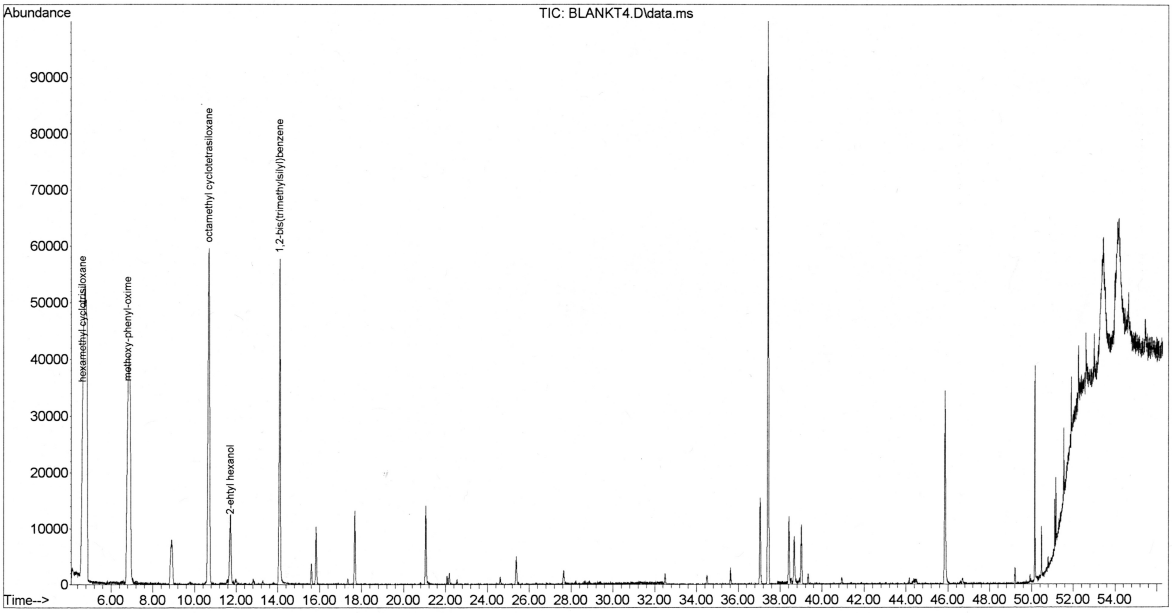


Figure 7-28. The fiber was subjected to an entire run without exposure to any sample. Multiple contaminants were observed, demonstrating the need to run blanks and thermally clean the fiber between samples.

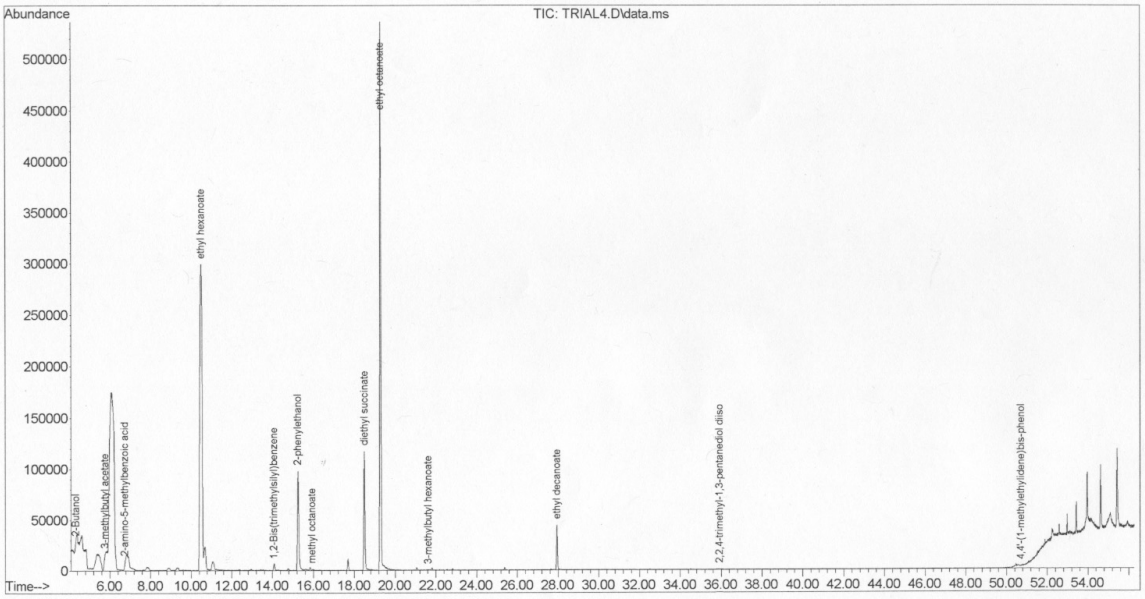


Figure 7-29**.** The chromatogram for Black Box California Merlot, 2007. The fiber was exposed to the sample immediately after subjecting it to a blank run. Fewer contaminants were observed.

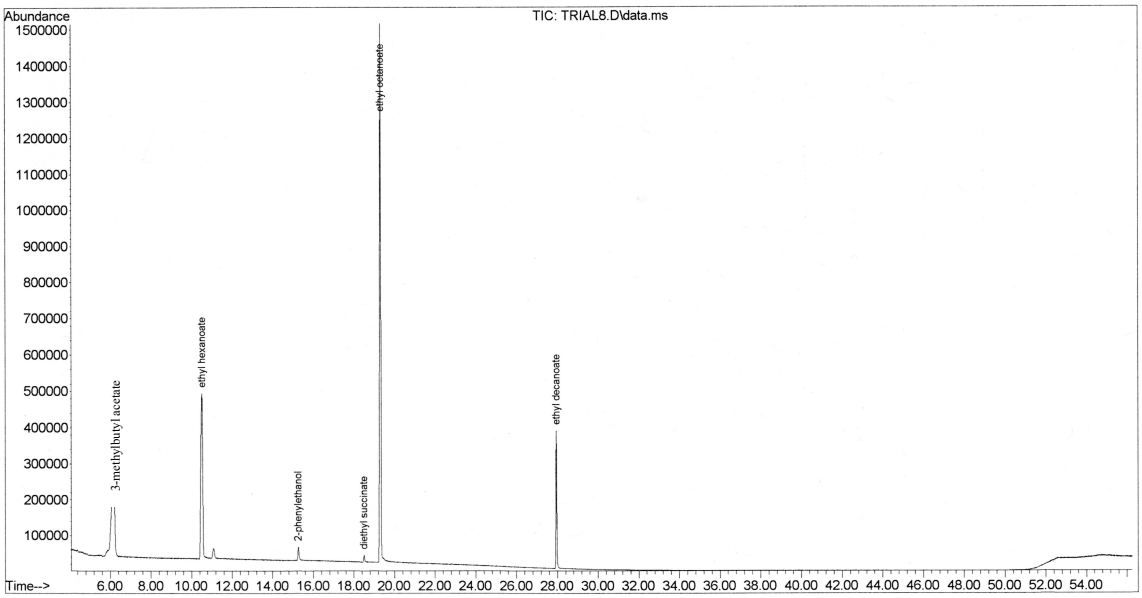


Figure 7-30**.** The chromatogram for Buckley's Cove 2009 Shiraz from South Eastern Australia. Relatively few volatile compounds were detected.

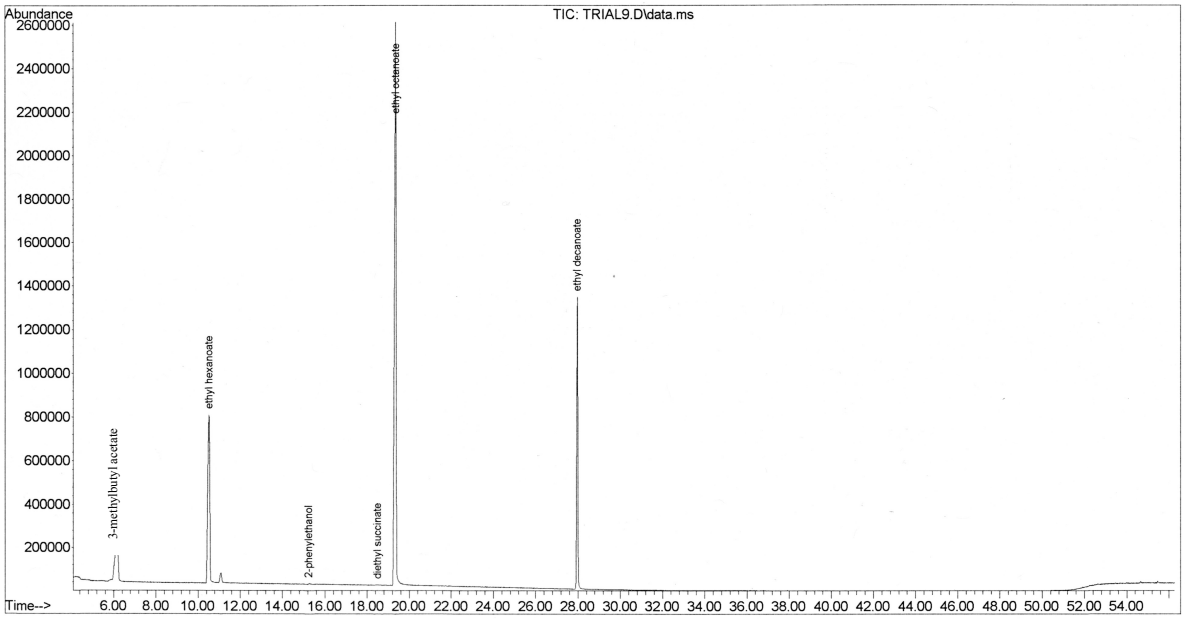


Figure 7-31**.** The chromatogram for Buckley's Cove 2009 Chardonnay from South Eastern Australia differs slightly from Buckley's Cove Shiraz (Figure 4). 2-Phenylethanol and diethyl succinate that were found in the Shiraz were not observed in this sample.

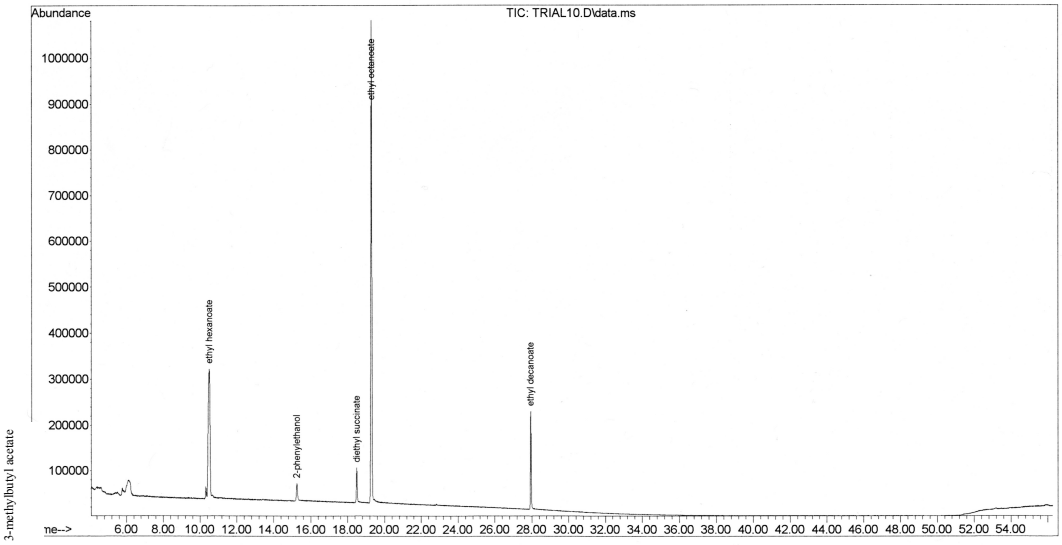


Figure 7-32**.** The chromatogram for Walla Walla Village Winery's Red Table Blend (82%Cabernet Sauvignon, 9% Merlot and 9% Cabernet Franc). Contrary to our prediction, no additional compounds were detected in the higher-quality wine. Changing the sample parameters could aid the detection of additional volatiles.

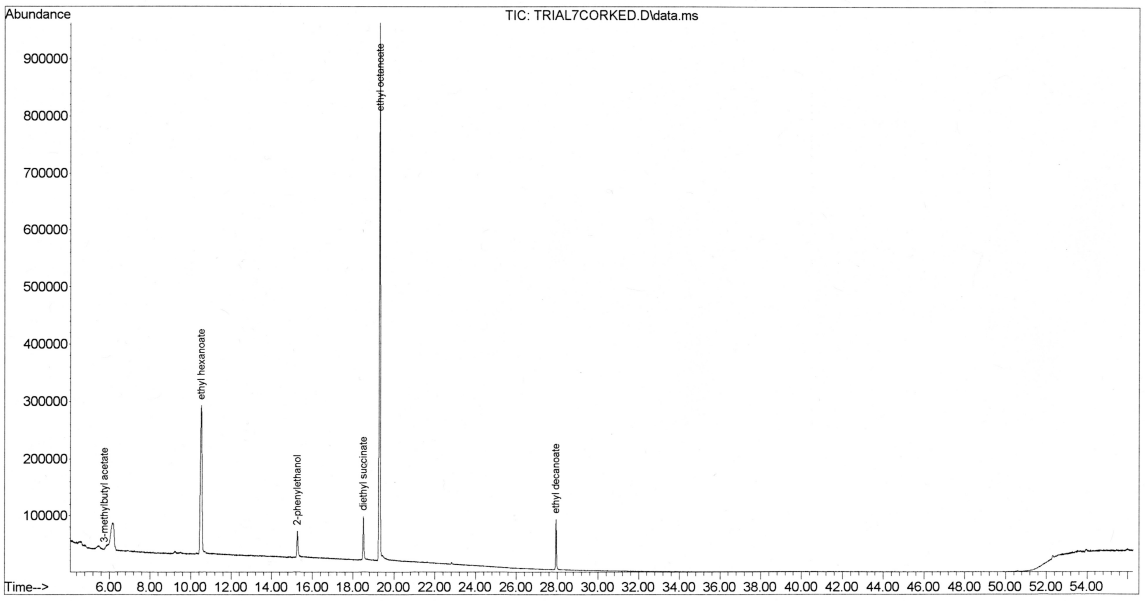


Figure 7-33**.** The chromatogram for Foundry Vineyards 2005 Red Wine. “Corked” wines have a displeasing aroma caused by trichloroanisole or tribromoanisole (Boutou and Chatonnet, 2007). The streamlined method could not detect the presence of these compounds, however changing the sample parameters such as temperature or pH could improve detection of these contaminants.

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7.5.4 An Extraction Procedure for the Investigation of Pesticide Residues on Strawberries by Kyle Byrd-Fisher

Agriculture is as vital to the health of the United States and the world as ever. Over the course of the 20th century, the scale of food production in the United States increased dramatically to cope with the increased demand of the larger population. An increase in the scale of food production is more cost effective for farmers, but it does have some very significant draw-backs. Most notably, large-scale production carries with it the cost of crop vulnerability. If a pest is mobile or transferable, such as an insect or fungi, it has the potential to damage a much larger quantity of food. For this reason, the ability to contain pests or eliminate them is of vital importance to the modern farmer. This is how pesticides have found a home in modern agriculture, especially within the United States.

To better give an indication of the pervasive use of pesticides, in 2006, of the states surveyed by the United States Department of Agriculture Pesticide Data Program (USDA PDP), 98 percent of all head lettuce producing acreage received applications of insecticide, 63 percent of head lettuce acreage received applications of herbicide, and 87 percent of head lettuce acreage received applications of fungicide (United States, 2010). One of the issues with the broad use of pesticides is that the health effects of human consumption have often not been sufficiently studied by the time a pesticide is approved by the Environmental Protection Agency (EPA). In the case of azinphos methyl, which was approved as a coddling moth insecticide in 1959, it was not until 2006 that the EPA decided to phase it out due to its consumption and application toxicity (United States EPA). Pesticides are currently regulated by three separate branches of the Federal Government: the EPA is responsible for approving pesticides for use and setting tolerance levels on their presence in or on food, the USDA is responsible for monitoring the pesticide residue levels in food with the PDP, and the Food and Drug Administration (FDA) is responsible for enforcing standards with the Total Diet Study. This paper is concerned with the monitoring aspects of pesticide regulation, specifically extraction and identification methods for determining the presence of pesticide residues on the surfaces of fruit. The focus is on strawberries, which, like head lettuce, have significant quantities of pesticides applied on them every year.

Pesticides on Strawberries

In 2008, 2.5 billion pounds of strawberries were produced domestically, the vast majority coming from California (United States, DoA, 2008). Strawberry monitoring by the PDP in 2008 came in the form of 741 samples analyzed with a total of 113,071 analyses performed. Of these analyses, 3.3 percent detected pesticide residues, with 46 different pesticide detections reported[1]. Of these detections, 16 pesticides were detected on more than 5 percent of the samples, with the top three pesticide detections being boscalid, captan, and myclobutanil in that order (Anastassiades, 2003).

Extraction Standard Operating Procedure:

Strawberry samples were obtained from Safeway, which purchased them from Boskovich Farms of Oxnard, CA. A sample size of seven strawberries was obtained by washing the surface of each strawberry twice with methylene chloride. No prior wash with water was conducted. The methylene chloride wash was evaporated overnight and reconstituted in 10 mL of methylene chloride. The reconstituted methylene chloride organic wash was then put into a separatory funnel and washed twice with a concentrated aqueous sodium chloride solution. The remaining organic layer was then filtered through a syringe filter into a GC vial, totaling 0.5 – 1.0 mL. A methylene chloride blank was created and run on an Agilent 6890N GC-MS (quadrupole) alongside the sample. The temperature program was set to run at 90°C for 2 minutes before ramping by 2°C per minute to 270°C.

Results:

This extraction procedure produced a chromatogram showing evidence of three sharp peaks. The first peak had a retention time of 52.716 minutes, a percent area of 19.58%, and an approximate number of counts of 300,000. The library search report conclusively identified this compound (quality of 96) as *cis*-1,2,3,6-tetrahydropthalimide also known as *cis*-4-Cyclohexene-1,2-dicarboximide. This is a breakdown product of captan, which was identified as the third peak. The second peak had a retention time of 74.38 minutes, a percent area of 4.81%, and an approximate number of counts of 80,000. The library search report conclusively identified

this compound (quality of 98) as cyprodinil or 4-cycloproplyl-6-methyl-N-phenyl-pyrimidinamine. This compound is an insecticide commonly applied to the foliage of grapes, almond trees, and stone fruit targeting scab and brown rot blossom. The third peak had a retention time of 81.401 minutes, a percent area of 75.61%, and an approximate number of counts of 1,200,000. The library search report conclusively identified this compound (quality of 99) as captan. Captan was listed above as the second most common pesticide found on strawberries and is also listed as a Pesticide Action Network (PAN) bad actor and a probable carcinogen (*EXTOXNET, 2011*). Captan is a broad-spectrum fungicide applied on many fruits and vegetables, and is also applied to the surfaces of fruit after harvesting to improve the fruit's appearance (*PAN Pesticide Database, 2011*).

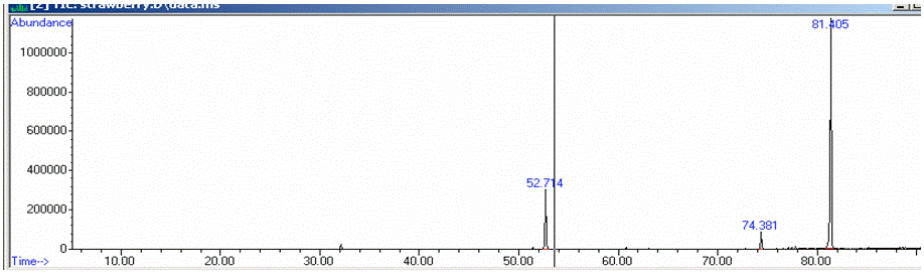

Figure 7-34. Total Ion Chromatogram of Strawberry Extract.

Conclusion

This investigation was not meant as a quantitative study as in the PDP analyses, but rather as a proof of extraction/concept and analyte identification without an external standard. Finding that pesticides residues can be readily identified with an easy extraction procedure is a strong starting point for a more extensive quantitative project.

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