CHAPTER 7

Proper Laboratory Protocol and Sample Laboratory Experiments

7.1 Preliminary Information

7.1.1 Instrument and Instrumental Settings

The topics discussed in this section are the most common instrument setting that will need to be adjusted or checked during the use of a GC, specifically a GC-MS. These will be mentioned in the experiments that follow in this section. Some topics are redundant to previous discussions but are included here for clarity purposes. While the section 7.1 concentrations 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 settings or controlled regions on a GC-MS. The first is the injector that is set at least 20 degrees higher than the final oven temperature. The column oven is set to run in either isothermal mode or in 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, temperature programmed through one or more temperature ramps, and usually 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 reason to set the oven at a relatively low temperature, as compared to the boiling point of the solvent, is 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 higher temperature of the injector and detector is to avoid recondensation of analytes on injector and detector surfaces. This recondensation would interfere with quantification due to peak tailing and can result in cross contamination between samples. The final temperature zone is the MS vacuum chamber that is set at a surprising lower temperature.
than the GC components (~150 to 250 degrees C in the quadrupole mass analyzer). This is due to the more volatile nature of analytes at the lower pressures in the vacuum chamber.

7.1.1.b Gas Flow: As noted in the GC section, all gases in GC-MS, and in most GC applications, must be 5-nine quality (99.999 percent pure). These typically include He or H₂ as a carrier gas, Ar/CH₄ for makeup gas for an electron capture detector, and H₂ and compressed air (lower grade) for flame ionization detectors in GC. For GC-MS, only He is required for the carrier gas, with CH₄ 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 gas purifiers. For the first case, imagine running the GC-MS with a high-temperature isocratic oven setting. At this temperature all contaminants in the gases will pass through the system unretained in the separation column and a high, but steady, background detector signal would result; this is not a huge problem. For the second case, imagine a temperature-programmed analysis, where initially the column oven is at a low temperature, specifically lower than the boiling point of any contaminants in the carrier gas. As carrier gas passes through the analytical column, they would be adsorbed to the stationary phase at the beginning (head) of the column. As the temperature program progresses, these contaminants would volatilize and appear as peaks in the chromatogram/spectra. The height of the contaminant peak (concentration) would be inconsistent since it would be dependent on the time, and amount of carrier gas passing through the column, between runs. The contaminant(s) would result in additional problems if it (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. This pressure is further reduced by regulators or mass flow
controllers that are internal to the instrument and are usually electronically controlled. The pressure at the head of the capillary column usually ranges from 5 to 20 psi resulting in a flow of 1 to 5 mL/minute depending on the capillary column internal diameter.

7.1.1.c. Vacuum Chamber. As the flow of He 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. After this low pressure is reached, a molecular turbo pump takes the pressure down to approximately $10^{-4}$ 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 injectors are made, the septa develops a slight perforation in it that will eventually leak carrier gas and can allow the unwanted exit of sample during an injection. Thus, 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 it barrel that interferes with the plunger. This can usually be avoided by numerous rinses between, an easy task with autosamplers. However, occasionally disassembly and rinsing of the syringe with acid, polar organic solvent, and nonpolar organic solvent is necessary. 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 ferrels, a soft hollow conical-shaped device that fits snugly around the column and fits into a receptor that is secured with a nut. As the nut is tightened, the ferrel is compressed around the column and prevents gas leaks. As the temperature program is repeatedly heated and cooled, leaks can result from the cycling expansion and contraction, so the ferrel nuts need to be tightened periodically (~weekly to monthly). Ferrels are be made of Tyflon, 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. Thus, the liners are routinely replaced with 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, or more 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, trifluorotribuylamine (PFTBA) is used. A small mass of volatilized PFTBA is introduced into the ionization chamber and the system automatically adjusts the system 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 then tuned for any mass unit.

7.1.2.g. Ion Lens. The repeller and acceleration lens may accumulate nonvolatile residues when dirty samples are analyzed. Depending on the quality of a sample and the frequency of use, lens
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 (this occurs during the MS tuning procedure).

7.1.2.f. Gain on the Electron Multiplier. Another setting adjusted during the tuning process is the gain on the electron multiplier. For a given mass of tuning compound (PFTBA) a given counts per second of ions hitting the EM is expected. This is adjusted during tuning by increasing or decreasing the gain (potential) across the EM, in most cases for a continuous 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 and the gauge level should be checked monthly, and oil added if necessary. The oil should be replaced at least annually and 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 pump 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. Calibration may be even more frequently.

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 are present if mass numbers corresponding to N$_2$, O$_2$, H$_2$O, 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 will 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? Again, look for the obvious.

-Check each of the fore mentioned for problems, especially the filters and liners. And 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 and eliminate sample introduction.

-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 amu. Phenyl degradation from column degradation will be present at 281 amu. Phthalates are ubiquitous in the environment and will give an ion peak at 149 amu.

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 of a lack of detector response is a broken column. This is easily observed by cooling the oven and inspecting the column. As a precautionary rule, 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

As mentioned in section 7.1.2.f, MS instruments must be tuned frequently to ensure correct identification of ion mass to charge ratios, or essentially the value of the amu. Modern instruments have an automated sequence or menu to do this, and all that is required is to select the command from a menu and allow the instrument to proceed. As noted earlier, most instruments use trifluorotribuylamine (PFTBA) that is stored in a vial in the MS. During the tuning operation, a valve is opened allowing a small quantity, but 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 setting in the MS 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 PGTBA 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, 319, and 502 ions are used to calibration 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 (single amu values). Each of the peaks shown in Figure 7-1 are 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, IonFcus (ion focus), EntLens (entrance lens), EntOffs (entrance offset), AmuGain, and AmuOffs (amu offset). 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 mentioned above function to focus 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, one after the other, 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,O, N, O, CO and N/H,O are shown here and should be present at no more that 10 percent of the total 69 m/z ion counts. If values higher then 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. The temperature setting of the column and oven control analyte separations. 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 (petro), the subject of the next lab that illustrates calibration of an instrument.

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 mm phase coating
Injection Volume: 1.00 mL
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: 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 on 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 isothermal temperature program (150 C for 15 mintues). 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 easiest boiling analyte and program 5 C per minute to final temperature approximately10 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, isoctane, 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 facing any analytical chemist 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 analyst to analyze several compounds at one time, and 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 (petro) as our sample.

In this experiment, the analyst will (1) obtain reference standards of several components of gasoline, (2) made 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-mL, 50-mL, and 100-mL 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 mm phase

coating
Injection Volume: 1.00 mL
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 mm phase

coating
Injection Volume: 1.00 mL
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 than 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 from 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 solutions.

Procedures:

(1) Add each analyte to the flask, but filling 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.

<table>
<thead>
<tr>
<th>Analyte (&gt; 99%)</th>
<th>Boiling Point °C</th>
<th>Density of liquid</th>
<th>mL of pure analyte to be</th>
<th>Resulting ppm concentration in</th>
</tr>
</thead>
</table>
(2) All solutions injected on 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 fill 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.
<table>
<thead>
<tr>
<th>ppm</th>
<th>ppm</th>
<th>mL</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>100.0 ppm</td>
<td>2000 (2.00 mL)</td>
<td>10.00</td>
</tr>
<tr>
<td>10.0</td>
<td>100.0 ppm</td>
<td>1000 (1.00 mL)</td>
<td>10.00</td>
</tr>
<tr>
<td>4.00</td>
<td>40.0 ppm</td>
<td>1000 (1.00 mL)</td>
<td>10.00</td>
</tr>
<tr>
<td>2.00</td>
<td>20.0 ppm</td>
<td>1000 (1.00 mL)</td>
<td>10.00</td>
</tr>
<tr>
<td>1.00</td>
<td>10.0 ppm</td>
<td>1000 (1.00 mL)</td>
<td>10.00</td>
</tr>
<tr>
<td>0.400</td>
<td>4.00 ppm</td>
<td>1000 (1.00 mL)</td>
<td>10.00</td>
</tr>
<tr>
<td>0.200</td>
<td>2.00 ppm</td>
<td>1000 (1.00 mL)</td>
<td>10.00</td>
</tr>
</tbody>
</table>

Again, fill each volumetric flask in the Table 2 with the pentane solution from Step 2 that contains decane (internal standard).

(4) The compounds in pure gasoline (petro) 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 instrument 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.

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 that is considered a hard source since it 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 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 mm phase coating
Injection Volume: 1.00 mL
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 Cl are shown below.

Figure 7.2. Fragmentation of Cyclohexanol by EI.
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.
Similar results are found for decanoic acid methyl ester; extensive fragmentation occurs in EI, but not in CI. In addition, in CI the molecular ion is more pronounced and the M+\text{C}_2\text{H}_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 in the EI, while additions are observed for the CI technique.

When to use EI and CI: Most MS analysis use 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 systems. 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 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 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 °C per minute) is necessary that results in a long analysis time (approximately 2 hours). In this experiment the separation, or 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)
coating

Injection Volume: 1.00 mL
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.
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 the 186-190 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 mg 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 °C, while rotation of one benzene ring around the double bond to form trans stilbene yields a boiling point of 305-307 °C. These can easily be separated by chromatography but all cis- and trans- isomers yield the same fragmentation pattern in MS.

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 mm phase
Injection Volume: 1.00 mL
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.

7.4 Proper Laboratory Notebooks Protocol

Chemistry is a laboratory science and an important part of this course will to familiarize the student with proper laboratory techniques and recording of their work in laboratory notebooks. The practices we use here may seem rather detailed or “picky”, but these are minor compared to the practices used in some industrial sectors.

You must keep an elaborate and highly organized account of each lab exercise in your notebook. At the beginning of each lab, I will look for a “Things to do” list, possibly a detailed procedure, a dilutions table, etc in your lab notebook. This tells me you came prepared to lab. **Organization of your notebook:**

Sections for every lab:
- Title (in designated area)
- Purpose
Chemicals and solution used (brand, lot number, who made them)

Calculations
Cited SOP with modifications and/or detailed procedures
Data, hand written, spreadsheets, plots to be completed during each lab

(spreadsheets and plots should be neatly taped in your lab notebook)

Conclusions, what you found in this lab

A few widely-accepted details:

(1) Leave a few pages at the beginning for a table of contents (by experiment), a list of commonly used tables, and a list of commonly used figures, and appendices. Our current notebooks have a place for this. Note that at the end of the day you must initial and date each page.

(2) If someone else collects the data and you need to recopy it in your notebook, note where and when you obtained the data.

(3) Neatness is important, but you must write in procedures as you plan them (in the lab) and enter data as you collect it (not on note book paper or napkins for later transfer to your official record). EVERY LAB BOOK MUST CONTAIN ALL DATA COLLECTED IN THE LAB, NO MATTER WHO IN YOUR GROUP COLLECTED THE DATA.

(4) Record every detail in your notebook for future reference.
   Some items that may not be immediately evident, but should be included, are the type, model number, serial number of equipment (serial number only if more than one piece of the equipment is available), type of glassware used in dilutions, standard dilutions, and brand name and lot number of all chemicals used.

(5) No skipped pages are allowed in official (legally defensible) notebooks without proper procedures. Any page that contains more than 1/4 of a blank page at the bottom or top or one side of a page must have a line drawn through it and be signed and dated by you.

(6) All entries must be made with a ballpoint pen (not the alcohol-based, water soluble pens that are more commonly available). If you make a mistake, draw a single line through the mistake, and write in the correction. Do not obliterate a number that you
think is in error as you may find that you actually needed that number.
(7) Remember that the goal of this entire process is it to produce a document that you, or one of your peers, can pick up (even years in the future) and exactly reproduce your procedure and hopefully your results.
(8) Everything that you do in the lab should be recorded in your lab notebook as you do it, including lengthy procedures and calculations as you do them.
(9) Do not remove any pages from your lab notebook.