Expectations for GC-MS Lab

Since this is the first year for GC-MS to be used in Dr. Lamp's CHEM 322, the lab experiment is somewhat unstructured. As you move through the experiment, I expect that you will be able to complete the following challenges and document them in your lab notebook. I may suggest additional items in lab as the experiment progresses.

1. Collect chromatograms for the liquid fragrance sample and the sample's headspace.

2. Identify peaks in the Total Ion Chromatogram (TIC) using the mass spectral data. Justify your assignment for the peaks (saying "because the library search matched" isn't enough justification.

3. Modify the experimental protocol in some way and collect additional data to explore the impact of the modification on the experimental result. Consult with Dr. Lamp prior to executing your modification to get his approval.
   - You may consider modifying any portion of the experiment, including sampling, sample identity, separation conditions, etc.
   - In your lab report, address the following:
     - What modification did you try and why did you choose it?
     - What impact did the modification have on the experimental results? Did the impact coincide with your expectations?

4. Include in your discussion suggestions or comments that might be useful to Dr. Lamp for improving or formalizing the GC-MS experiment in the future.

The remainder of this document contains two parts

I.   **Lab Documentation for the Investigation of Imposter Perfumes using GC-MS** is a supplement to a Journal of Chemical Education article that provides some background and will serve as a starting point for our experiment.

II.   **Directions for using Agilent GC (6890) with MSD (5973)** is a series of instructions prepared by Dr. Kramer for using our GC-MS.

Again, this exercise is still in the development stage so the specifics will evolve as you move through lab.

Lab Documentation for the
Investigation of Imposter Perfumes using GC-MS

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INTRODUCTION

The goal of this experiment is to identify "active" ingredients in different fragrances. We would also like to compare name brand fragrances to their imitation counterparts and see if both fragrances really contain the same active ingredients. There are essentially 3 concepts involved in this laboratory exercise besides the underlying fundamentals of gas chromatography (GC). They are: headspace analysis, temperature programming, and mass spectral detection for the purpose of analyte identification.

In GC, a sample is typically injected into the inlet with a syringe. Usually the sample injected is a liquid, which is quickly vaporized in the hot inlet. Gaseous samples may also be injected. In the food, flavor, and cosmetics industries smells are very important. This is one example of the utility of headspace analysis. In headspace analysis, the vapor above a sample is drawn into a syringe and injected into the GC. The rationale for this type of sampling is that the molecules responsible for scent are those present in the vapor phase above the sample. If you smell a cup of coffee, the molecules that reach your nose are the volatile ones. We will use this technique to investigate the components responsible for the scent of real and imposter perfumes.

In gas chromatography (GC), components of a mixture are separated on the basis of their interaction with a stationary phase within the gas chromatography column. In this experiment, the sample is first injected into a sample inlet, volatilized, and then swept by a carrier gas through the column into a detector. The column is actually a narrow glass capillary in which the walls are coated with a nonpolar liquid film. Components of a mixture are therefore separated based on polarity as well as volatility, with more polar, volatile compounds eluting from the column more quickly than less polar, less volatile compounds.

As you might imagine, temperature dramatically influences the degree to which components of the mixture interact with the stationary phase. By changing the temperature of the oven in which the column resides, we have some control over the separation process and can manipulate it to achieve more rapid or more effective separations. Temperature programming is accomplished by ramping the temperature of the column during a separation. The advent of computer-controlled instrumentation has made temperature programming routine.

When a mass spectrometer (MS) is used as the detector, eluate from the GC column is immediately swept into an ion source in which the sample is ionized and the abundance of each ion is measured as a function of mass to charge ratio (m/z). Since the charge of most ions formed is +1, the m/z is often referred to simply as the atomic mass of the ion generated.

Electron impact ionization is a very "hard" ionization technique, meaning that it involves sufficient energy to not only ionize a molecule, but also fragment the molecule into many smaller pieces. The pattern by which a molecule fragments is characteristic of the structure and functional groups of the molecule. For example, molecules containing an ethyl group often lose the CH_2CH_3 when ionized. *Fragmentation patterns are therefore very useful for qualitatively determining the structure of the original parent molecule!*

Once the positively charged ions are formed, they are accelerated into a mass analyzer, which separates ions of different masses. There are two methods of obtaining data using GC-MS. The first method is to scan a specified range of *m/z* ratios. The data can then be plotted as the total ion abundance verses time. This plot, called a Total Ion Chromatogram (TIC) looks like a "regular" chromatogram with peaks at various retention times. Each peak is the sum of the abundance of all ions (regardless of mass) as a function of time. One can also analyze the same data to determine the masses of the fragments that are eluting from the GC column at a specific point in time. In this case, abundance is still plotted on the y-axis, but *m/z* is plotted on the x-axis to generate a mass spectrum. The fragmentation pattern contained in the mass spectrum is indicative of the compound, and ideally enables one to qualitatively identify each compound as it elutes from the GC.

A second method of acquiring data on the GC-MS is called Selective Ion Monitoring, or SIM. In SIM mode, one would select a few *m/z* ratios that are unique to the compound of interest. The mass filter is then set to detect those select fragments with specific *m/z* ratios, ignoring all others. This mode minimizes the chance that co elution of an interferent will compromise the detection of a primary species of interest. It also provides maximum sensitivity because the detector spends more time looking for those particular *m/z* fragments. SIM mode is both more sensitive and selective, but the ability to identify analytes based on mass spectral libraries is sacrificed because an entire mass spectrum is not collected.

EXPERIMENTAL METHODS

Hazards note: Some perfumes may be irritating and even toxic to people with severe allergies. It is recommended that liquid perfumes be dispensed into septum vials in a fume hood using gloves. Keep perfumes away from extreme heat and open flames.

If your instructor has not already done so, tune the mass spectral detector.

Obtain 2 small septum vials. In one vial place approximately 0.5 mL of a perfume sample. In the second vial, place approximately 0.5 mL of the corresponding imposter perfume. As you dispense the real and imposter perfumes, smell them, describe the scents and identify any olfactory differences.

Select the appropriate stored method file to control the GC-MS instrument. Check each parameter to become familiar with the operating software and be sure your fellow comrades didn't change it!

Injector: we will be making manual injections.

Inlet: temperature 250 °C; split mode; split ratio 50:1
Mobile Phase Flow Rate: 1.0 mL/min
Oven (controls column temp):   140 °C hold for 20 min
Auxiliary (transfer line from GC to MS):   temperature 280 °C
MS detector: 3.00 minute solvent delay
                                                Scan mode from 20 to 500 *m/z*

Step by Step Instructions for Data Collection and Evaluation

1. Once you've become familiar with the software and confirmed your operating conditions, prepare to inject 1 μL of a liquid perfume sample. Let the instructor show you how to inject the first time. Collect a Total Ion Chromatogram (TIC) of the sample. Make note of the directory in which you save this and all subsequent chromatograms.
2. After you have collected the first TIC, modify the operating parameters to include a temperature ramp during the run according to the following:
   a. Initial Temp. 60 °C
   b. Hold it at 60 °C for 2 minutes
   c. Ramp at 20 °C/min up to 250 °C
   d. Hold at 250 °C for 3 minutes.
3. Inject the identical sample as in step 1 - 1 μL of liquid perfume sample.
4. Clean the syringe with methanol or ethanol.
5. Inject 1 μL of the imposter perfume under identical conditions.
6. While chromatograms are being acquired, you may begin analyzing chromatograms already collected. Be sure to make the following part of your analysis:
   a. Compare the isocratic (fixed temp) and temperature programmed chromatograms.
   b. Zoom in on the chromatograms to investigate small time windows with multiple peaks.
   c. Display mass spectra for several peaks.
   d. Remember each molecule results in a unique fragmentation pattern. Therefore, the pattern from the mass spectrum can be used to identify the molecule. You can compare your mass spectra with those in a mass spectral library. The probability that your mass spectrum matches one in the library should be noted. A probability greater than 90 indicates that there is a high probability that the two compounds are the same, while probabilities less than 50 indicate that substantial differences exist between the unknown and reference mass spectra.
   e. While looking at the mass spectrum for one of the peaks in a congested area of the total ion chromatogram, make note of the *m/z* of two or three of the most abundant fragments. You will use these to perform selected ion monitoring (SIM) later in this experiment.
   f. Using the mass spectral library search feature identify 5 of the components of the perfume sample. Only trust matches that have a probability of 90 or better. Report what scent the volatile "active ingredient" of your fragrance provides. Does it provide a floral, woody or spicy "note?" This will require a little literature searching. Several great articles about this have appeared in *Journal of Chemical Education*, *Analytical Chemistry* and *Chemical and Engineering News* recently. The *Sigma Aldrich Catalog of Fragrances and Flavors* is an excellent resource

for this type of information; as is the internet, particularly a site by the Good Scents Company!

    g. View the perfume and imposter on the same set of axes in order to better determine compositional differences between the two samples.

7. Set the MS detector to operate in SIM mode at the *m/z* chosen in step 6e. All other conditions should remain identical.
   a. As before, inject 1 uL of the genuine perfume.
   b. When this SIM chromatogram is complete, return the MS to operate according to the original SCAN settings.
   c. Compare the SIM and TIC chromatograms.
8. Before doing headspace analysis of the perfume, obtain a 50 or 100-μL gas-tight syringe. Change the inlet mode to:
   a. Pulsed Splitless
   b. Inj. Pulse Pressure: 30 psi
   c. Pulse time: 1.0 min.
   d. Purge Flow: 50 mL/min.
   e. Purge Time: 0.9 min.
      i. Note: If your instrument does not have automated pulsed splitless capability, you may want to warm both the samples and the gas-tight syringe in a 40 $^{\circ}$C oven to increase headspace concentrations of perfume components.
9. Perform headspace sampling by inserting the syringe into the septum, but not under the surface of the liquid. Draw 50 μL of the VAPOR above the liquid surface into the syringe. Inject the gas sample into the GC. Collect a TIC.
   a. View the headspace chromatogram and the liquid chromatogram on the same plot. Based on a comparison of these chromatograms, what can be said about the volatility of most of the components of this mixture?
10. Collect a headspace TIC of the imposter to the perfume you have selected.
    a. Overlay the real and imposter headspace chromatograms. Identify (using the mass spec library) as many components as you can that are present in the real perfume but missing from the imposter, or vice versa. Why do you think these are missing? OR Why do you think these are present?
11. Clean both syringes with methanol or ethanol.

Guide for the Lab Report
Submit a Title and Abstract.
Focus on the benefits of using a mass spectral detector in the theory section.
You should include separate model numbers for the GC and the MSD (mass spectral detector).
The procedure should focus primarily on how you obtained the headspace sample.
Data to present:
        TIC of perfume – isocratic
        TIC of perfume – T programmed
        TIC of imposter – T programmed (these could be overlaid)
        SIM chromatogram (identify the major peak)
        Table of Retention Times, Peak Identities, Characteristic Odors (if found)
        TICs of real and imposter headspace samples (can be overlaid).
        Identity of extra or missing components in imposter perfume.
Results and Discussion

How might extra components contribute to the scent? How would the missing components affect the scent? Does your analytical evaluation support your olfactory observations?

Compare the SIM and TIC chromatograms. Consider the y-axes on these chromatograms. Which provides a larger signal for the analyte on which you focused for the SIM? Why does SIM mode offer greater sensitivity?

Directions for using Agilent GC (6890) with MSD (5973)

If the computer isn't on, log-in with username "Administrator" and password "administrator". Check to make sure the gas line is open and set to at least 20 psi. You may need to adjust the pressure later to satisfy the pressure needed by your method.

From the desktop, double-click "1 GCMS" icon. Three windows will open – leave all three open. We will assume that the MSD is already pumped down and ready. If it is not, please see your instructor or Dr. Kramer for help.

Tuning the instrument
If it has been more than 24 hours since the instrument has been tuned, you need to perform a quick tune to confirm the settings.
1. In the "Instrument Control" window, choose "View – Manual Tune."
2. In the "Manual Tune" window, choose "Tune – Quick Tune."
3. The MSD will click on and perform a scan of three ions from a standard stored in the instrument. It will adjust to make the peaks as narrow as possible and as accurate as possible.
4. If the scan completes, you are ready to move on. If it does not, please see your instructor.
5. Go to "File – Save Tune Values" to save the tune. Choose "atune.u" for the file name.

Setting up a method
In order to run the instrument, you will set up a method in Chemstation.
1. If you are not in the "Instrument Control" window, open it by choosing "View – Instrument Control."
2. If you plan to use a stored method, choose "Method – Load" and choose your method. If you plan to start a new method, choose "Method – Edit Entire Method."
3. In the "Edit Method" window, make sure that only "Method Information" and "Instrument/Acquisition" are selected. Select "OK."
4. In the "Method Information" window, enter any comments about the method and make sure that only "Data Acquisition" is selected. Select "OK."
5. In the "Inlet and Injection Parameters" window, make sure that "GC" is chosen as the inlet, "Manual" is chosen as the injection source (unless an auto-injector is to be used), "Front" is chosen as the injection location and an "x" is in the box next to "Use MS." Select "OK."
6. Now you will edit each step of the analysis. These parameters can also be edited individually later by choosing the icons on the "Instrument Control" screen. The parameters you will most likely want to edit are:
   a. Inlets: Two options are available, split or splitless. For a "Split" run, choose "Split" and set the ratio below. For a "Splitless" run, choose "Splitless." On this screen you should set the heater temperature, type of carrier gas, pressure and total flow for the inlet.

b. <u>Column</u>: For Mode, choose "Constant Flow." For Inlet, choose "Front." For Detector, choose "MSD."

c. <u>Oven</u>: On this screen, you can set the starting temperature and any temperature gradient using the table of times and temperatures. Any changes you make will be expressed in the graph of temperatures at the top of the screen. Make sure that your temperatures will not go over the "Oven Max" chosen on this screen.

d. <u>Aux</u>: This refers to the MSD. On this screen you should set the temperature for the transfer line to the MSD.

7. After editing all of the instrument parameters, select "OK."

8. In the "GC Real time plot" window, make sure that no signals are selected. These would only apply if you were using a different GC detector. Select "OK."

9. In the next window, make sure to select "atune.u" or the name of the tune file you have saved previously.

10. In the "MS SIM/Scan Parameters" window, you can set up the parameters for the mass spectrometer. The instrument can be run in "Scan" mode for a total ion chromatogram (TIC) or in "SIM" mode for selected ion monitoring.

a. Set the solvent delay. Three minutes is usually sufficient. This delay keeps the MSD from turning on until after the solvent in a liquid sample has passed. If you are injecting a gaseous sample, it is unnecessary.

b. <u>Scan</u>: If you would like to adjust the range of m/z ratios that the instrument will monitor or the amount of time it will spend on each m/z, do so in the table at the bottom of the screen. Most likely, you will not need to adjust this table.

c. <u>SIM</u>: If you know the compounds you will be detecting and have their mass spectra, you may use this screen to choose specific ions to be detected at different times during the run.

11. After selecting "OK," you will be given a place to save your method. Type in a method name. All names used in this program must be 8 characters or less.

Running a Method
Make sure your method is loaded.

1. Choose "Method – Run" in the "Instrument Control" window.

2. Choose a name for your file. To find a list of files and folders, type "?" in the "File name" box.

3. Fill in the rest of the boxes with information about your run.

4. Choose "Run Method" (not "OK") to start the method.

5. When "GC Ready" window appears, prepare your sample to be injected. See your instructor for information about appropriate volumes used. Make sure to get rid of any bubbles in the syringe.

6. On the GC itself, press the "Prep run" button. When the "pre-run" light turns on, quickly inject your sample by piercing the septum in the front inlet and pressing the plunger of the syringe. **Immediately** press the "Start" button on the GC.

7. **Do not choose** "override solvent delay" unless you are injecting a gas into the GC. Overriding the solvent delay when injecting a liquid sample will greatly reduce the life expectancy of the detector. Ignore the window.

8. Your chromatogram will appear in the "Total Ion" window. Double click on it to expand the window.

Data Analysis

During a run, from the "MS Top/Enhanced" window, choose "Open New Data Analysis." Otherwise, open a data analysis window by choosing "View – Data Analysis (offline)."

1. If you would like to analyze your chromatogram as it is being collected, choose "Take Snapshot" under the "File" menu. Otherwise, choose "Load Data File" under the "File" menu.

2. To display the mass spectrum at any point in a chromatogram, double **right** click on a point in the chromatogram. The spectrum will be displayed below. You may also average several points together by drawing a rectangle with the **right** mouse button clicked.

3. To search the library for a given spectrum:
   a. Choose "Spectrum – Select Library."
   b. Type "?" in one of the boxes. Scroll down to choose "Wiley275.L" as the library to search.
   c. Double **right** click on a spectrum and a list of potential library matches will be displayed along with the probability of a match ("Qual" column – nearer to 100 is better). To find out more information about a certain choice, highlight the compound and then click the "Text" button.

4. To determine the area of the peaks in the chromatogram:
   a. Select "Chromatogram – Integrate." The retention time of the peaks will be listed next to each peak.
   b. If too many or too few peaks are chosen, go to "Chromatogram – Select Integrator." Choose "RTE Integrator." Choose "Chromatogram – MS Signal Integrator Parameters" and change the minimum peak area detected (increase if too many peaks are chosen, decrease if too few were chosen). Integrate again by selecting "Chromatogram – Integrate."
   c. To display the areas of each peak, choose "Chromatogram – Integration Results."