Using GC/MS for Qualitative Determination of Volatile Compounds

Required Reading
In addition to reading this project description and answering questions in the prelab section, you should read Chapter 30E and Chapter 31 in order to understand the instrument operation and sample preparation. Pay close attention to Table 31-1. Figures 31-4, 31-11 and 31-12 will help you to understand the instrument used in this work; study them carefully.

I. Background
After years of preparation, Dr. Evil released his own line of fragrances. Commercials flooded the TV screens with Frau Farbissina advertising perfumes “No. 5 Evinel”, “Coco Evinel”, and of course “Evillure”. Despite the questionable scent bouquet (a convolution of candy and carpet cleaner scents), the fragrances became an overnight success with millions of bottles already sold. The world is in peril once again: in a video call from Dr. Evil’s secret lair to various world leaders he explains that anyone who has ever used his perfumes would soon turn into a robot, who only listens to Dr. Evil. He demands a billion gagillion fafillion dollars for the antidote. The world leaders are not sure whether should get the cash ready, however. They suspect that he simply mixed common and harmless chemicals in his perfume. They request immediate help from the UCI analytical chemistry students in testing this theory. With an ancient but still functioning GC/MS instrument at their disposal, the students get to work. The world leaders anxiously await the results…

II. Introduction to GC/MS Methods
Gas chromatography (GC) is routinely used to separate mixtures of volatile compounds before their analysis. A gas sample or vaporized liquid sample is injected into a chromatographic column where the separation of analyte components takes place. The time it takes for the individual compounds to be eluted from the column (retention time) depends on the relative polarities of the analyte and the stationary-phase molecules. The degree of separation can be controlled by choosing an appropriate stationary phase, column temperature, carrier gas flow rate, and other parameters. Altering the temperature has the greatest effect on column resolution and efficiency, as you will see in this lab.

The stationary phase in GC is commonly a packing of inert, small-diameter particles coated with a very thin layer (0.1-5 µm) of nonpolar liquid. Polydimethyl siloxane (Figure 1), with some of the –CH₃ groups altered to match the polarity of the analytes is a common stationary liquid phase. The mobile phase is an inert gas such as argon, helium or nitrogen that only carries the analyte molecules through the column. The carrier gas does not interact with the analyte and column packing material. In this lab, ultrahigh purity helium is used as the carrier gas.

The retention time (the time it takes to pass through the column) for an analyte is based on the time spent in the stationary phase vs. the mobile phase, with longer retention times for analytes with polarities closer to that of the stationary phase. In the sample chromatogram shown in Figure 1, two different molecules have distinct retention times, $t_1$ and $t_2$. Dead time, $t_0$, is the time it takes for the carrier gas to go through the column.
The analyte peaks tend to broaden as they pass along the column, resembling Gaussian peaks. This is due to the random motions of molecules as they migrate down a column, passing in and out of the stationary phase. This peak broadening affects the efficiency of the column as well as its ability to distinctly separate the peaks of two different analytes (the resolution). It can be a problem for complicated mixtures. In Figure 1, molecules are well separated in spite of the substantial peak broadening because there are just two components in the mixture. We would not be able to separate a more complicated mixture with such broad peaks, however.

The molecules leaving the column are measured in real time by a sensitive detector. An ideal detector for GC applications should have the following characteristics:

1. Suitable sensitivity, typically in the range $10$ to $10^{-6}$ ng s$^{-1}$.
2. A linear response to analytes that extends over several orders of magnitude.
3. Good stability and reproducibility.
4. A temperature range from room temperature to at least 400 °C.
5. A short response time that is independent of the flow rate.
6. Similarity of response toward all analytes, or alternatively, a highly predictable and selective response toward one or more classes of analytes.

Unfortunately, a detector exhibiting ALL of these characteristics has not been developed yet, but a number of detectors exist that work well for a particular application. Among these, mass spectrometry (MS), electron capture (ECD), and flame ionization (FID) detectors are most often used. In this lab, you will use an MS detector to identify volatile compounds in a mixture of fragrant chemicals based on their retention time and mass-spectrum.
Electron impact (EI) ionization is used to convert neutral analyte molecules into ions before analyzing them with a mass spectrometer. Commonly, the molecular ion produced by the collision of the parent molecule with an electron has excess energy and fragments into smaller molecular ions. The fragmentation pattern is used as a qualitative identification method, and many instruments have a library of references for automatic comparison. For example, Figure 2 shows a mass spectrum of ethyl butyrate, one of your primary standards. Note that normal isotopic configuration of ethyl butyrate (\(^{12}\text{C}_6^{16}\text{O}_2^{1}\text{H}_{12}\)) has a molecular weight of 116.08 atomic mass units. However, there is only a very small peak corresponding to m/z = 116 in its electron impact mass spectrum. This happens because molecules commonly fragment upon electron impact ionization:

\[
\text{Molecule} + e^- \rightarrow \text{Molecular ion}^+ + \text{neutral fragment(s)} + 2 e^-
\]

For example, in the case of ethyl butyrate, the largest peak appears at m/z = 71, which corresponds to a loss of C\(_2\)H\(_4\)O fragment from the molecule upon ionization (Figure 3). You will be required in this lab to think about the possible mechanism by which your analytes (5 flavor compounds) are ionized and fragmented in the ionizer. Electron impact mass spectra for a large number of organic molecules are available from the NIST Chemistry WebBook.
Figure 3: Selected ionization pathways for methyl butyrate leading to the appearance of m/z 71, 88, 116.

The mass spectrometer used in this work is a Quadrupole Mass Spectrometer (QMS). Discussion of how this instrument works goes beyond the scope of Chem 151L; those of you who take Chem 152 later on will get better acquainted with the principles of QMS operation. The QMS used in this project is capable of 1 m/z resolution over the range of 10-500 m/z. It is capable of taking full mass spectra for analytes coming out from the column on a millisecond timescale, making it possible to observe the mass spectra in real time.

III. Instrument

The Hewlett Packard 5800 GC / 1800A MS system is used in this experiment. It contains an HP-5 capillary column that is composed of (5%)-Diphenyl-(95%)-dimethylpolysiloxane copolymer. This means that 5% of methyl groups in the compounds shown in Figure 1 were replaced by phenyl groups. This is an example of a nonpolar stationary phase. Read the manual for operating the GC/MS instrument before you start while you are waiting for your TA to help you get going.

Follow the directions to load method CH15109.m, which contains parameters for the GC and MS. Click on /Method/Edit to examine the parameters. It is important to verify that the method parameters are as follows (if they are not set them to the appropriate values):

- Injector temperature: 200°C
- Detector temperature: 250°C
- Splitless injection selected
• Oven max: 300°C
• Manual injection
• Column: 30.0 m, 0.25mm ID
• Helium carrier gas; 1.0 mL/min flow rate
• Solvent Delay: 0.00 min
• Mass Range: 45 to 200 m/z

The following parameters are set for the temperature program:
• Initial Temperature: 50°C
• Initial Time: 2.0 min
• Level 1: rate = 25.0; Final = 170 °C; time = 2 min
• Other levels set to zero

This means that the column will initially remain at 50°C for 2.00 minutes, and then the temperature will increase at rate of 25.0 degrees per minute until it reaches 160°C, where it will remain for 1.60 minutes. Programmed this way, one chromatogram should take about 8 minutes to complete. This is sufficient to fully separate all flavor compounds you are going to deal with in this lab.

IV. Primary Flavor Compound Standards and Unknown Sample

In this lab you will be analyzing five primary flavor compounds, and you will use them to aid in the identification of the volatile compounds in the unknown sample. You will be given the following primary flavor compounds in small septum-sealed vials:

1. Ethyl Butyrate
2. Isoamyl Acetate
3. Benzaldehyde
4. Limonene
5. Cinnamaldehyde

The vials will be numbered 1-5. Do NOT dispose of these vials at the end of the lab because multiple groups can use the same standards.

The unknown will be in an unlabeled vial. It will contain several of your primary standards, and you will have to figure out which ones based on your GC/MS work. You can open all vials and smell all of the flavors. If you have a good nose you will be able to accurately predict the composition of your unknown mixture based on its smell. You will then confirm your prediction with GC/MS analysis.

V. Syringe Preparation (this part can be skipped with new syringes)

You will be given a 10-50 µL gastight syringe (and possibly a few other syringes) for injection of both the primary flavor components and your unknown mixture. You should examine each syringe before proceeding further:

1. The syringe should be cleaned a few times with methylene chloride. Draw some methylene chloride into the syringe. Once you have drawn up methylene chloride into the syringe, release it into a labeled “syringe rinse” container. You may repeat this cleaning procedure later on if your chromatograms contain a lot of impurity peaks (e.g., you see two significant peaks instead of one when injecting one of your standards).
2. Now, draw up some lab air into the syringe, dip the needle into methylene chloride, and inject the lab air into the liquid. You should see tiny bubbles. If unsuccessful, there is a poor fit between the plunger and syringe. Consult your TA about replacing the syringe.

3. Before the first injection, be sure to wipe the needle off using a Kimwipe. It is necessary to remove as much residual methylene chloride from the syringe as possible. This is best done by drawing up some air and releasing it a number of times. Make sure you do not see any residual liquid methylene chloride in the syringe.

VI. File Locations and file names

This computer runs on Windows 3.1. Unlike Windows Vista or Windows XP, this operational system has very tight restrictions on naming the files. File names cannot contain more than 8 characters before the period in front of the file extension. Therefore, please pay very close attention to the location and names of your files.

- The correct path to the method file: C:\DATA\151L\CH15109.m
- Data files must be saved in the following folder: C:\DATA\151L\2011\,
- Please give the following names to each of your data files: MMDDtdXX.d where MM = today’s month; DD = today’s date; td = am or pm depending on whether you are in the morning or afternoon section, and XX is the index of your file 01, 02, 03, 04, ...
- For example, if you do this experiment on Monday afternoon of November 7 please call your first file: C:\DATA\151L\2011\1107am01.d
- Your second file will be C:\DATA\151L\2011\1107am02.d etc.
- Make detailed notes in your lab notebook as to what each file corresponds to.

VII. Chromatogram of air (with residual CH$_2$Cl$_2$ vapor)

Because the GC/MS instrument is very sensitive, it can detect pretty much anything you can smell and then some. You can check the cleanliness of the syringe and GC/MS system by injecting some lab air into the instrument. This is a useful check whenever you suspect contamination peaks.

Draw up 5-10 µL of lab air into your syringe. Use Acquire Sample/One Sample to type your file name and sample description (be sure to follow the file naming convention described above). Select Run Method to prepare the computer/GC for sample acquisition. Wait for the GC/MS instrument to become ready (green light will come on when ready). Inject the air and press GC’s START button immediately to start the run.

When the chromatogram is finished, use GCD TOP’s Review Data to bring up the chromatograms. If the syringe is clean, you will get very low signal at all retention times—signal that sort of looks like noise. One peak you might see is that from residual methylene chloride (CH$_2$Cl$_2$) vapor that did not fully evaporate from your syringe. Another peak that often appears with this column is a peak at around 7.5 min arising from column “bleeding”. If you see a lot of additional peaks, your syringe is most likely still dirty, and needs another round of cleaning.

Methylene chloride is easy to recognize from its mass spectrum, which contains a characteristic triplet of peaks at m/z = 84, 86, 88 and a doublet of peaks at m/z = 49 and 51 (you will be asked to assign these peaks to specific isotopes in your report). Write down the retention time for methylene chloride; you do not want to have it confused with one of your flavor compounds later. If you do not observe methylene chloride in your chromatogram, withdraw 5-
10 µL of headspace air (NOT liquid!) above the CH₂Cl₂ solution and record another chromatogram with this sample.

When withdrawing headspace above the liquid into your syringe, be sure NOT to touch the vial wall, liquid sample, or puncture through the contaminated vial cap septum. Injecting the liquid instead of air will overload the instrument, and may damage the mass spectrometer. This comment applies to the CH₂Cl₂ injection as well as all injections described below.

Note that we intentionally block the air peaks out by selecting the lower limit for the mass spectrometer to be m/z 45. Therefore, we are not going to see any standard air components: oxygen (m/z = 32), nitrogen (m/z = 28), water (m/z = 18), argon (m/z = 40), and CO₂ (m/z = 44). Starting at m/z = 45 presents no problem for detection of flavor compounds because their mass spectra contain plenty of peaks above m/z 45.

Print out one copy of your chromatogram with the mass spectrum corresponding to your methylene chloride peaks.

VIII. Unknown Sample Injection

It is important to clean the syringe between all injections (in this part of the lab and in the next). Some of the compounds you are using are “sticky” and if not cleaned away will show up in the next chromatogram. For example, if you inject benzaldehyde, and then inject limonene, the peak from benzaldehyde may still be visible in the chromatogram. Therefore, if your syringe is not clean you may make incorrect deductions about the composition of your trial mixture. To avoid these problems, clean the syringe with CH₂Cl₂ and dry it by filling it with air and expelling it multiple times. In order to save time, clean the syringe while you are waiting for the previous chromatogram to complete.

Your syringe should be dry and clean before injecting your unknown (otherwise you may have excessively large signal from CH₂Cl₂). Draw up 5-10 µL of headspace air above your unknown, and inject it into the GC injector. Press GC’s START button immediately to start the run. When the chromatogram is finished, use GCD TOP’s Review Data to bring up the chromatograms. There may be up to 5 different peaks in the chromatogram of your unknown sample (in addition to possible methylene chloride peaks). Analyze the unknown twice for reproducibility. For each run, print out one copy of your chromatogram with the mass spectrum corresponding to the strongest observed peak.

IX. Primary Flavor Component Injection

You will inject all 5 primary flavor components individually to measure their retention times. Remember that you will be injecting head space air above the liquid samples, NOT the liquid. For each primary standard, inject 5-10 µL of headspace air using your syringe. When the chromatogram is finished, use GCD TOP’s Review Data to bring up the chromatograms. In each case, you should observe a single peak with retention time somewhere in the range of 2-8 min. There can be more peaks if impurities are present. Make a note of the retention times and the widths of all significant peaks in your lab notebook. Right click on each peak to view the corresponding mass spectrum. Make sure that the peak positions in the mass spectrum match the expected peak positions for this standard from the NIST Chemistry WebBook. For each primary
standard, print out **one copy** of your chromatogram with the mass spectrum corresponding to the peak.

**Note:** the normal peak width should be about 0.2-0.5 min. If you inject too much sample, the width of the peaks will be very wide, and the top of peaks will appear to be flattened. Reduce the injected amount if this is the case.

**Note:** if your peak is very small and noisy, try heating up the sample vial in warm water before collecting headspace air from it, and re-recording the chromatogram. This will be necessary for cinnamaldehyde and possibly for some other primary standards.

### X. Variation of Temperature Program

In this part of the lab you will investigate the effect of the column temperature on the retention times. You will be experimenting with a mixture of **all 5 flavor compounds** to examine the effect of temperature on all of them simultaneously. Your TA will provide you with this mixture in a septum-sealed vial.

Before every injection you will need to edit the method. In order to avoid overwriting the CH15109.m method used in the previous steps, load the method called CH151XX.m and modify its parameters before every new injection as required. **Do not create additional methods using “save as”!!** Run the chromatogram of 5-10 µL of headspace air collected above the mixture under the following column temperature conditions:

<table>
<thead>
<tr>
<th>Trial number</th>
<th>Initial temperature</th>
<th>Initial time</th>
<th>Level 1 rate</th>
<th>Final temperature</th>
<th>Hold time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 °C</td>
<td>2 min</td>
<td>25 °C/min</td>
<td>170 °C</td>
<td>2 min</td>
</tr>
<tr>
<td>2</td>
<td>50 °C</td>
<td>2 min</td>
<td>18 °C/min</td>
<td>170 °C</td>
<td>2 min*</td>
</tr>
<tr>
<td>3</td>
<td>50 °C</td>
<td>2 min</td>
<td>32 °C/min</td>
<td>170 °C</td>
<td>2 min</td>
</tr>
</tbody>
</table>

* Be prepared to extend the chromatogram if you do not see the cinnamaldehyde peak come out.

Note that Trial 1 uses the same conditions as you used in the previous part. Run 2 and 3, decrease and increase the rate of the temperature change. In all cases, you should see all 5 flavor compounds come out during this run, at the same retention times as you measured in section IX. In each case, you will need to wait before all 5 compounds come out of the column before stopping the run.

For each run, print out **one copy** of your chromatogram with the retention times shown in your printout.

### XI. Clean up

Make sure the syringes are clean and working. If they do not function well, let your TA know so that they can be replaced. Sample vial cap and septum can be reused. Wipe them clean with hot water and methylene chloride. It is difficult to get the sample vials cleaned. Dispose of them into the broken glass box.

**Important:** At the end of the lab, you will be required to show your TA printed copies of all chromatograms/mass spectra showing significant peaks (one CH$_2$Cl$_2$ run, five standard runs, two unknown runs, and all the chromatograms recorded in part X). After your TA approves your results, you should take the printouts with you because you will need them for your report.
preparation. Include them with your report. You only need one set of print outs for the whole group; you do not need one for every student in the group.

XII. What to Submit in Your Report

Specific sections, tables, and graphs that need to be included in your report are described below. Unlike the other lab reports, this report will require a lot of writing, and not too many calculations. Be sure to spell-check your writing; points may be deducted for repeated mistakes as well as for poor penmanship.

As usual, turn in your prelab (5 pts) at the beginning, and your duplicate copies of all the notebook pages (5 pts) at the end of the laboratory session to your section TA.

- (1 pts) The **Title page** of your report should include the title of the project, your name, date, your UCI ID number, your section TA's name, your lab section, and names of your group members.
- (4 pts) Start your report with a short (3-4 sentences) **Abstract** that explains the procedure and summarizes the main result of your measurement (in this case, it will be the content of the “perfume” you were analyzing).
- (20 pts) In the **Introduction** section, discuss the principle of operation of a GC/MS instrument. What major components does it have? What kind of mobile and stationary phases does it uses for the separation? What is a typical length and diameter of a GC column? What is the role of the column temperature? What happens with the separated gases as they come out from the column and enter the MS sector? Briefly describe the operation of the ionizer and MS detector.
- (3 pts) In the **Experimental** section, describe only the deviations from the procedure, if there were any.
- (15 pts) Your **Results** section should contain a detailed table which contains information about all injections of the individual standards. Here is how this table may look like:

<table>
<thead>
<tr>
<th>What is injected?</th>
<th>Injection volume</th>
<th>Retention times observed</th>
<th>Strong m/z peaks in the major GC peak</th>
<th>Observations and notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab air</td>
<td>5 µL</td>
<td>XX min (whatever you measure)</td>
<td>49, 51, 84, 86, 88, …</td>
<td>One significant peak due to CH₂Cl₂; no other impurities detected</td>
</tr>
<tr>
<td>Headspace gas above</td>
<td>5 µL</td>
<td>XX min (whatever you measure)</td>
<td>XX, YY, ZZ, …whatever peaks you observe</td>
<td>Large peak due to benzaldehyde; sizable peak due to CH₂Cl₂</td>
</tr>
<tr>
<td>benzaldehyde</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headspace gas above</td>
<td>5 µL</td>
<td>XX min (whatever you measure)</td>
<td>XX, YY, ZZ, …whatever peaks you observe</td>
<td>Large peak due to limonene; small peak due to residual benzaldehyde from the previous injection</td>
</tr>
<tr>
<td>Limonene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Your **Discussion** section (45 points total, distributed as follows):
  - (10 pts) Based on the natural abundances of the $^{35}$Cl and $^{37}$Cl isotopes (look them up), predict the expected relative intensities of the peaks corresponding to
different isotopic modifications of CClH⁺, CClH₂⁺, CCl₂⁺, CHCl₂⁺, CH₂Cl₂⁺ that may be produced from ionization of CH₂Cl₂. Ignore different natural abundances of isotopes of C and H atoms in your estimations. Examine the relative intensities of the peaks observed in your MS spectrum of CH₂Cl₂. What are the major ions formed during the electron impact ionization of this molecule?

b. (10 pts) Go to the NIST webbook and find the mass spectra for each of your fragrance standards so that they can be positively identified. Include the NIST images of the mass spectra in your report to facilitate the discussion of your results (see example in Figure 2). Compare the major peaks in the mass spectra you observed with those in the reference mass spectra in the NIST database. Do you get a good match with your observations? **Note that you do not need to discuss details of the mass spectra, and why specific mass peaks occur, you will be asked to do this just for one of the compounds later in part “f”**.

c. (5 pts) Identify the compounds in your unknown mixture by comparing both retention times and mass spectra for each unknown peak with those of the standards. How certain you are in your identification? Remember, you have more than the GC/MS at your disposal. You have your sense of smell as well. You can probably determine the identity of the unknown flavor before using GC/MS. Comment on how well your nose compares to the $60,000 instrument.

d. (5 pts) Explain why your standards come out in this particular order of retention times. Is there a correlation to molecular size? Functional groups? Polarity? Boiling point? Vapor pressure? Something else? What appears to be the most important factor in determining the retention times?

e. (5 pts) Describe the effect of the different temperature ramp rates on the retention times. Include a table (or graph) of the retention times for each of the compounds as a function the Temperature-ramp rate. Explain the observations.

f. (10 pts) Discuss the ion fragmentation pathways for one of the flavor compounds (except methyl butyrate because it was already discussed above) after the molecule is hit with 70 eV electrons. Draw the chemical equations leading to the appearance of the major fragments. Your drawing should be done in ChemDraw, ChemSketch or similar program, and be similar to Figure 3.

- In the **References** section (2 pts), list all the information sources you used in preparation of this report. For journal articles, list the authors, title, journal name, volume, year, and pages. For books, list the title, authors, year, and publisher. For on-line sources, cite the web-link. Index all references in the order of appearance.