Experiment 1 - Separation of Carvone and Limonene

Reading Assignment
Mohrig Chapter 19.1, 19.2, 19.5a, 19.7 (Liquid / Column Chromatography)
Review Topics: Polarimetry (Chapter 17), TLC (Chapter 18), GC (Chapter 20)

Terpenes encompass a large family of organic compounds widespread in nature and occurring in all organisms from bacteria to mammals. They are prevalent in plants, where they act as volatile chemical messengers to attract insects and also to defend the plant's territory. Mixtures of volatile and scented compounds, very rich in terpenes, can be obtained by steam distillation of plant tissues (as in the isolation of citrus oils experiment). These mixtures are called essential oils.

Carvone is a naturally occurring *ketone* found in the essential oils of caraway, dill, and spearmint in association with other **terpenoids** such as limonene. Limonene is found in spearmint, caraway, lemon, and orange oils. Carvone and limonene, both monoterpenes, have only one stereogenic center and can exist in two enantiomeric forms: *R* and *S*. Enantiomers have the same physical and chemical properties, except that they interact differently with polarized light and other chiral molecules. The two enantiomers of carvone have different smells, one fresh and minty, and the other sweet and somehow unpleasant, especially at high concentrations. The olfactory receptors that line our nasal mucus are chiral ensembles with an exquisite sensitivity to the size, shape, and chirality of the odorant. Their interaction with individual enantiomers is often specific, resulting in the production of distinctive smells. The odor difference between the two enantiomeric forms of carvone is obvious to most people.

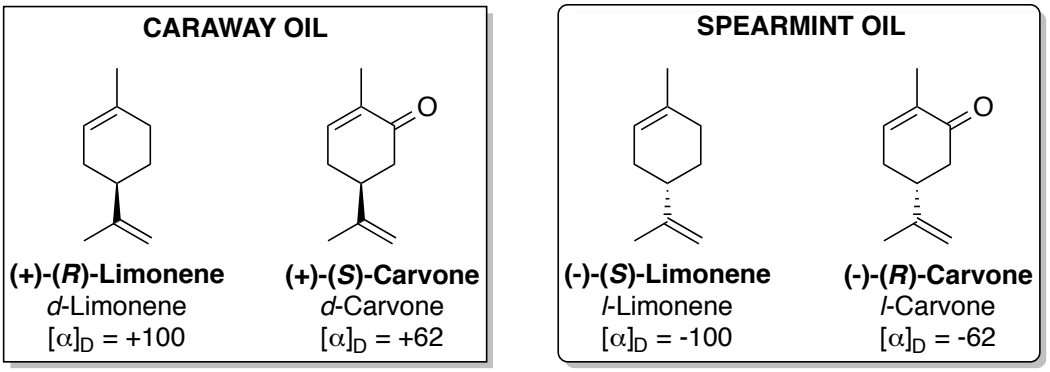


Figure 1. Structures of limonene and carvone.

(*S*)-(+)-Carvone is found in association with (*R*)-(+)-limonene in caraway oil (**Figure 1**). The composition of the oil, obtained by steam distillation from dried, ripe fruits of *Carum carvi* L., varies according to the source of the fruits but normally contains 50-55% carvone and 40-45% limonene. Thus, caraway oil has a positive specific rotation ($[\alpha]_D^{20}$ +70 to +80). (*R*)-(-)-Carvone and (*S*)-(-)-limonene are the constituents of spearmint oil. Spearmint oil is obtained by steam distillation from the partially dried leaves of the flowering plants *Mentha spicata* L. Spearmint oil normally contains 13-21% limonene and 46-70% carvone and minor components such as α- and β-pinene (4-6%) and dihydrocarvone (3-12%). In contrast to caraway oil, spearmint has a negative sign of rotation of plane-polarized light ($[\alpha]_D^{20}$ -48 to -59).

In this experiment, students will separate **carvone** and **limonene** from the essential oil of either *spearmint* or *caraway* by **column chromatography**, a type of *adsorption chromatography*. The principles of column chromatography and thin-layer chromatography (TLC) are identical. The *stationary phase* is silica (SiO_2) and the *mobile phase* is an organic solvent. In this experiment, a microcolumn will be packed with silica gel and an **eluotropic**

series of hexanes and acetone will be used to first elute limonene then carvone from the column. As in TLC analysis, this separation is based on **polarity**. The only difference is in the direction of the movement of the mobile phase. In TLC, the solvent (mobile phase) moves up the plate by capillary action, against gravity. In column chromatography, the mobile phase is added to the top of the column and moves down, with gravity. The stationary phase (SiO_2) is polar and will hold onto the more polar compounds longer. *In other words, the less polar compounds in a mixture will elute always first from a column and move farthest from the origin on a TLC plate.* The solution collected at the bottom of the column can be collected and separated over time. Students will collect limonene and carvone in separate fractions and analyze these fractions by gas chromatography (GC) and TLC.

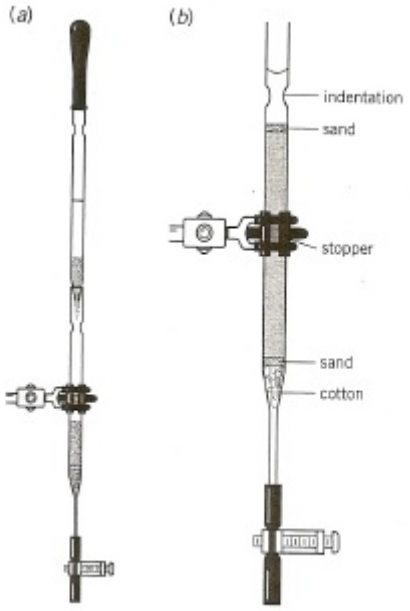
Notebook Preparation – see sample notebook page online

- *Purpose:* Write the purpose of the experiment in one complete sentence. Draw the process scheme and structures of limonene and carvone at the top of the page.
- *Reagent Table:* Make a table with the physical properties of silica (SiO_2), carvone, limonene, hexanes (*n*-hexane), and acetone. Include amount to be used (mL), MM, b.p., density, and a one-word hazard description (flammable, corrosive, etc.) that can be found in the safety table at the end of the procedure.
- *Procedure:* Hand-written, step-by-step procedure with micro-column diagram (**Figure 2**), GC table (in-lab #2), and list of materials (chemicals, glassware, equipment, etc.).
- *Clean-up & Safety:* Copy the table at the end of the procedure into your notebook.

PROCEDURE – work in pairs

Separation of Carvone and Limonene

There will be a demo for the setup in the beginning of lab. A short-tipped disposable Pasteur pipet makes an appropriate column for microscale column chromatography. Put a small piece of tygon tubing of the tip of the pipet (**Figure 2**). Clamp the tubing with a screw clamp. Put a notched stopper around the center of the pipet (column) and clamp the stopper in a vertical position to a ring stand. Place a small cotton plug at the bottom of the column and cover the cotton with a thin layer of sand (2 mm). Add hexanes to the column and let about 1 mL of the solvent run through. This will get rid of air bubbles trapped in the cotton. Recycle this solvent for the next step. Be sure to leave a little solvent behind in the microcolumn.



Microcolumns run very quickly, so it is important that you have everything prepared (solutions, pipets, and receiving containers) before starting the column. In six labeled test tubes obtain the following (these solvents will be used in succession to run the column):

(1), (2), & **(3)** - 2 mL hexanes each

(4), **(5),** & **(6)** - 2 mL 10% acetone in hexanes each

Cover the test tubes with a small piece of parafilm to prevent evaporation. Label six scintillation vials 1-6. These vials will be used to collect the column fractions. A fraction is complete when a test tube of solvent has been used up. For example, you will elute from the column into "Fraction 1" until the first 2 mL hexane portion has been delivered. You will not run the column dry so not all of that 2 mL hexane will end up in "Fraction 1."

Figure 2. Microcolumn diagram – include in notebook.

Packing a microcolumn: Obtain about 1.5 g of silica gel by comparison to the 1.5 g sample provided. Mix the measured amount of silica gel with just enough hexanes to make a pourable slurry, but not so much that there is a large excess of solvent. Fill the microcolumn to the very top with hexanes. Use a plastic pipet with the tip cut off to transfer the slurry into the microcolumn from above by simply resting the plastic pipet on top without creating air bubbles. If done correctly, the silica gel will be free-flowing and settle to the bottom of the microcolumn, like snow falling. Bubbles can be tapped out using a microspatula. Repeat this process of transferring slurry into the microcolumn as many times as necessary until the adsorbent is just below indentation level of the Pasteur pipet (see **Figure 2b**). A small portion of sand is added on top of the column (about 3mm high) and the column is ready to be loaded with sample. DO NOT LET THE COLUMN RUN DRY.

Loading and running the microcolumn: After the column has been packed, open the column outlet (screw clamp) until the solvent level comes down the top of the sand (not lower) and load the column with 150 μL (0.15 mL) of the oil. Open the screw clamp briefly to allow the oil to load into the column, then close. Add a few drops of hexanes, open the clamp to allow residual oil to load onto the column, and close the clamp before the solvent level gets too low.

Running the microcolumn: Careful not to disturb the stationary phase, add the remainder of the first fraction to the column portion-wise (don't over-flow!). Start collecting the elutent in the vial labeled "Fraction 1". *Without letting the column to run dry*, and after the sample has penetrated the column (liquid level right above the sand), add the first solvent from the test tubes (2 mL hexanes) in portions using Pasteur pipet. Keep collecting the elutent in the vial labeled "fraction 1" until 2 mL has been added to the column. Do not let the column run dry in between fractions. Add the next solvent, and begin collection into "fraction 2" and so on until all six solvents portions have been eluted. Allow the column to run dry when collecting "fraction 6".

Polarimetry: Report the optical rotation and concentration of the unknown oil (values provided in lab). Calculate the specific rotation (path length is 2 dm).

TLC Analysis: Spot two separate TLC plates with two lanes each: fractions 1, 5, 6, and a diluted sample of crude oil (1 drop in 2 mL hexanes in a test tube). The plate can be spotted before, during, and after running the column to save time. Keep the spots tight and small by briefly touching the capillary tube *once* to the plate to release sample. Run the plate in a TLC chamber containing hexanes as the mobile phase in the fume hood. After evaporation of solvent from the plate, visualize by placing the plate in a capped iodine chamber for 2 minutes. Crystalline iodine is volatile and will stain the areas of the plate containing limonene and carvone. Immediately sketch this plate into your notebook and calculate R_f values as these spots will fade quickly. If fractions 1, 5, and/or 6 did not contain sample, spot and run another TLC plate with fractions 2-4. This must be done before GC analysis to determine which fractions to inject.

GC analysis: Write a sample calculation for retention time, peak area, and percent composition in your notebook (use '# cm' in place of actual data). Reproduce the GC Table from the in-lab questions into your notebook. Analyze the crude oil by GC using a column of medium polarity at 150° C. Determine the percent composition of limonene and carvone in the unknown oil. Place a boiling chip in the fractions containing limonene and carvone (determined from TLC, likely 1 or 2 & 5 or 6). Evaporate the solvent by placing the vials in a sand bath on a hot plate in the fume hood, until about a drop liquid remains. It is ok to evaporate to dryness, but not to burn the sample! Simply at one drop of hexane to the sample and swirl to make a concentrated solution. Inject 0.2 uL of the appropriate fractions by GC. Determine the percent composition of carvone and limonene, if not 100%. Report your data in table format (see in-lab #3) and attach the chromatograms to the back of the lab report.

Table 1. Clean-up and safety notes

Clean-up	**Safety**
Liquid waste: acetone, hexanes, and fractions	Acetone & hexanes are *flammable*
Solid waste: pipets, columns, dry silica KEEP THE SCREW CLAMPS	Caraway and spearmint oils are *irritants*
Wash glassware, put away equipment, and wipe bench tops	Silica is a fine powder that is a *respiratory irritant.* Be careful not to inhale this powder!
ALL STUDENTS IN ANY SECTION WITH GLASSWARE (INCLUDING PIPETS) IN ANY OF THE TRASHCANS WILL **LOSE 5 POINTS** FROM THEIR LAB REPORT	
** **Check with your TA before leaving** (show them your results and drawer) or you will receive zero points on the neatness, organization, & technique section of the report **	

Introduction: Pre-lab Questions – *Turn in typed responses in the beginning of lab. Your TA will keep this until the report due date. Use complete sentences and keep your responses as brief as possible.*

1. State the approximate percent composition of limonene and carvone in both spearmint and caraway oils. Report the specific rotation of both oils.

2. THE STATIONARY PHASE: What stationary phase is used in the column and in TLC analysis? Is this substance considered polar or non-polar?

3. THE MOBILE PHASE: What solvents (mobile phases) are used in the column? What is the mobile phase for TLC? Are each of these solvents considered relatively polar or non-polar?

4. THE SAMPLE: Column chromatography and TLC are techniques used for separating compounds from a mixture based on polarity. What functional groups are found in limonene and carvone? Which is more polar: limonene or carvone?

5. Which compound, limonene or carvone, should elute first from the silica gel column (fraction 1)? Which will travel farther on the TLC plate? *Briefly* justify your answers, taking into account your responses from pre-lab #2-4.

6. In this experiment, the column will be run first with hexanes then with 10% acetone in hexanes to separate the oil. The oil *cannot* be successfully separated if the solvents are added in the reverse order (10% acetone in hexanes then pure hexanes). Explain why reversing the order of solvents wouldn't work and what would be in fractions 1 and 5 if that were the case (*hint: read section 19.7 in the Mohrig text*).

7. Report the boiling points of limonene and carvone. Which will exit the GC column first?

Results: In-lab questions – *Copy an abbreviated version of these questions into your notebook (the underlined portions highlight the results that should be recorded in the lab). Complete all calculations in the lab & type responses in complete sentences for the report.*

1. Report the provided concentration and observed rotation of your oil and calculate the specific rotation (see Chapter 17.3). List the unknown code and the identity of your oil based on polarimetry.

2. Report the GC results of the crude oil and fractions from your separation. Calculate the percent composition of carvone and limonene in the crude oil and fractions 1 and 5. Show one sample calculation each for retention time, integration, and percent composition. Report the final results in table format.

Table x. GC Results – *reproduce in the notebook before lab*

Sample	R_t (sec)	ID (Limonene or Carvone)	Integration (cm^2)	% Composition
Oil				

(*Continue the same table with additional rows for other samples – fractions*)

3. Report the retention factor (R_f) values for limonene and carvone. Include a sketch of the TLC plate(s) in this section of the report (copy from the lab notebook). How is the elution order of limonene and carvone from the column related to R_f values on TLC?

4. Comment on the purity of fractions 1, 5, & 6 based on your GC and TLC results. Was the column separation effective? Did the compounds elute or move in the expected order? Was it necessary to analyze fractions 2-4? Use your data to support your conclusions.

5. Go to pubs.acs.org and perform a search on "Limonene." *Choose any journal article* (not a book, website, or other type of source) and provide just the citation in the proper ACS format (see Technical Writing Guidelines).

Abstract – see *Technical Writing Guidelines* for writing the abstract. You should have put a draft together the first day of lab. Incorporate your results in place of the sample data. There is no need to include TLC data in the abstract either, as this is secondary to GC data.

LAB TECHNIQUE - *agreement signed on the first day of lab, including the main points below*

Safety & Clean up
- Wear lab coat, goggles, & gloves during the entire experiment.
- Take off gloves while washing glassware.
- Bench tops and isles should be free of clutter (non-lab-related belongings, ex. Cell phones)
- All glassware thoroughly washed and put away in drawer in an organized manner
- Student work space is clean – wipe down counters, leave drawer closed but *unlocked*
- Community work spaces clean – fume hoods, side counter-tops – all students responsible!

Technique
- Proper use of equipment, per TA demo and instructions
- Fume hood usage – work 6 inches into hood, no heads in the hood, no kneeling on the ground
- Proper waste procedures followed
- Careful not to spill chemicals or break glassware

General
- Proficiency - apparent preparation and understanding of the procedure
- All glassware labeled with contents and student name

GOLDEN RULE: Picture perfect drawers – see bulletin board – 1 point off for extra, missing, or dirty items

Exp 1 - Separation of Limonene and Carvone, *Due date in syllabus*

Name _____

Section Day _____ Time _____

TA Name _____

CHEM 8M GRADING RUBRIC - Use as cover page for report

SECTION	INSTRUCTOR COMMENTS	POINTS ASSIGNED
IN-LAB QUIZ		**/ 5**
LAB REPORT		
ABSTRACT One paragraph, typically four-six sentences: Purpose, procedure, main result(s), and conclusion(s).		**/ 10**
INTRODUCTION Original responses to pre-lab questions with TA initials		/ **30**
RESULTS The main results are stated, as outlined in the in-lab questions, using complete sentences.		/ **30**
EXPERIMENTAL SECTION The experimental details (including final amount used and obtained) are *briefly* described in a few sentences.	NONE	0 / 0
NOTEBOOK PAGES Proper format: purpose, scheme, chemical info table, procedure, safety and clean-up notes.		/ **15**
NEATNESS, ORGANIZATION, & LAB TECHNIQUE Proper order and format (see syllabus for full descriptions of each section), spelling & grammar. Safety rules followed, equipment handled properly.		**/ 10**
	LAB REPORT TOTAL	**/ 100**