

Experiment 2: ISOLATION AND ANALYSIS OF ESSENTIAL OILS A (mostly) GREEN-CHEMISTRY APPROACH

* Watch video on steam distillation on CHEM 146A website

In this experiment students will isolate the essential oil of either lavender, rosemary, peppermint, or spearmint. Steam distillation will be used to obtain the oil from lavender flower or leaves of rosemary or mint. **Essential oils** are mixtures of volatile compounds made by plants to communicate with their environments. They are used to attract insects and other animals to help in the fertilization and propagation processes. Some are also herbicides used by plants to defend their territory from aggressive vegetation. These mixtures are called oils because they are immiscible with water and, being lighter than water, separate as the upper layer.

Distillation of freshly obtained plant material with water will produce a mixture that consists largely of water and small amounts of essential oil. The distillate may look cloudy because of the emulsification of the oil in water, but if left to settle, it will (hopefully) separate into two layers: a large aqueous bottom layer and a small oily upper layer. The separation of the oily upper layer from the bottom aqueous layer can be difficult, especially if the volume of oil is small. To circumvent this problem, the oil is usually separated from the water by liquid-liquid extraction with an organic solvent. This process is very effective in removing the oil from the water but requires the use of organic solvents, which are potentially toxic and expensive and must be handled with special precautions.

The quest for finding safer and environmentally friendlier chemicals to carry out common laboratory operations is known as **green chemistry**. One of the principles of green chemistry is the use of safer solvents or elimination of solvents altogether if possible. In this experiment, students will attempt to isolate an essential oil from fresh lavender, rosemary, or mint. Ideally, the steam distillation will produce enough oil to allow separation in a buret, however, solvent-based extraction may be necessary.

Citrus oils were isolated by students *via* steam distillation in CHEM 8L and consist largely of volatile hydrocarbons (**Figure 1**). Any *essential oil* has a particular combination of chemicals that makes its smell unique and often times easily recognizable. It is common for natural plants extracts to contain compounds having carbon atoms in multiples of five. This broad class of compounds is called **terpenes**. They are biosynthetically derived from two or more *isoprene* units. There are several different ways for plant metabolism to combine two five-carbon isoprene units into a ten-carbon *monoterpene*, hence the occurrence of many different isomers. The most prominent components in citrus oils are α -pinene, β -pinene, myrcene, limonene, γ -terpinene (**Figure 1**). Lavender, rosemary, and mint contain very different mixtures of terpenes!

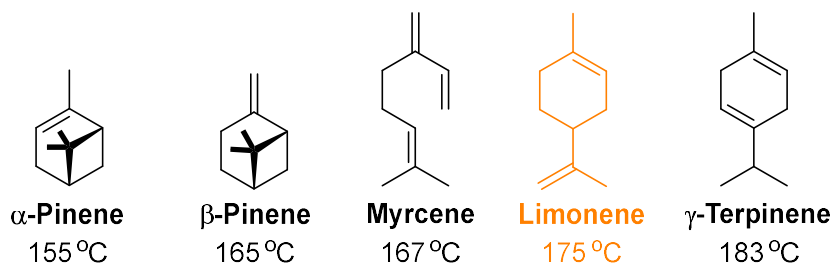
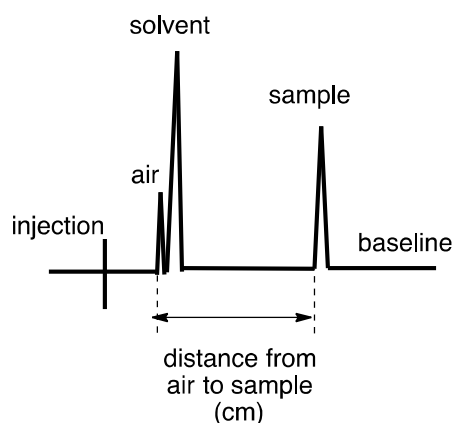


Figure 1. Monoterpenes found in citrus oils and their normal boiling points

The isolated oil will be analyzed by gas chromatography (GC) to determine its composition. The components of the oils usually come out of the GC column in the order of their boiling points. More volatile compounds come out first and less volatile compounds last. There are exceptions to this rule. To identify the components of the oil, authentic samples of the compounds will be injected individually (these authentic samples are called **standards**) and compare their retention times with the retention times of the peaks in the essential oil. The areas under the peaks will be calculated to determine the percent composition of the oil.

At a given set of GC conditions (temperature and type of column) *on the same instrument*, one compound will consistently exit the column in the same amount of time after injection. This is known as the **retention time, t_R** (the amount of time the compound is retained on the column). For example, you will inject pure limonene and observe one major peak. There is no timer on the recorder, but it is set to a certain speed (2.5 cm/min). The distance (cm) from the start of the injection to the beginning of the peak can be converted to its retention time in minutes (**eq 1**) then converted into seconds. For better precision, students should calculate **corrected retention times (t_R')**, where the distance is measured using the air peak as the beginning of the run. You can expect there to be several peaks in the chromatogram of citrus oil, for example, which correspond to the various components isolated in the distillation step. If the citrus oil contains any limonene, then you will expect to see a peak at the exact time as the standard. Once the peaks are identified, calculate the percent composition of each component in the citrus oil using the area under the curves (integration) of each of the peaks (**eqs 2 & 3**).



$$t_R' \text{ (sec)} = \frac{\text{distance from air to sample (cm)}}{2.5 \text{ cm}} \times \frac{1 \text{ min}}{2.5 \text{ cm}} \times \frac{60 \text{ sec}}{1 \text{ min}} \quad (1)$$

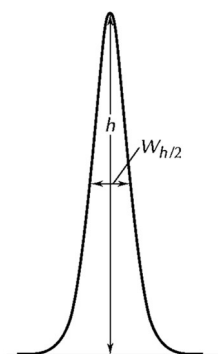


FIGURE 20.13
Determining peak area: h = height;
 $W_{h/2}$ = width at half-height.

$$\text{Integration: Peak Area} = h \times W_{h/2} \quad (2)$$

$$\% \text{ Composition of A} = \frac{\text{Area of component A}}{\text{Total area of all components}} \quad (3)$$

Notebook Preparation

Distillation & GC

- *Purpose*: One-sentence description of the purpose of Day 1. The reaction scheme should begin with a picture of the plant-of-choice, follow with boiling water over the reaction arrow and the structures of 2-5 potential components.
- *Reagent Table*: plant material (starting material) and essential oil (product) in addition to the properties of the top 2-5 terpenes that should be present in the oil (MW, bp, density, and one-word hazard from safety table).
 - *Look up the expected percent composition of your plant and do your own reading on specific details for steam distillation of that plant*
- *Procedure*: Hand-written, step-wise, and in your own words; include **Figure 2**.
- *GC Table*: re-create the table from the post-lab questions
- *Safety & Clean-up* – Copy pertinent notes from the table after the procedure.

PROCEDURE - ISOLATION & ANALYSIS OF CITRUS OILS

**Coordinate with your lab partner to hunt down locally grown lavender flower, rosemary, spearmint, or peppermint.

Amounts of material: a lot! You'll fill a 1-L round bottom flask about $\frac{1}{2}$ to $\frac{2}{3}$ of the way.

Lavender will ideally have some brightly colored flowers on the plant, though that's not required – take the buds off the stem in lab and cut the stems into ~1 inch pieces.

Rosemary essential oil is produced on the outside of the leaves. Keep the leaves intact and on the stems and cut into ~1 inch pieces in lab.

Mint leaves should be pulled off the stems and cut into small pieces (put leaves into a cup and cut with scissors – please do this at home and store in the fridge in a closed container). Keep several ~1 inch pieces of stem and bring to lab.

Record the amount of plant material added to 1-L round-bottom flask (RBF), including stems - set on a cork ring using a wide-mouth plastic funnel and stir rod to poke the flowers or leaves through. **Add 250-500 mL of distilled water** (depending on how much flower or leaves you have - do not exceed $\frac{2}{3}$ of the flask volume) to the flask and add a few boiling chips. Assemble a simple distillation apparatus, beginning with the 1-L RBF (**Figure 2**). If you forget to add the distilled water before heating, it will scorch the flask. *You will not be allowed to start over, and your report will be penalized.* Copy **Figure 2** into your notebook. *There will be no in-lab demo. Please watch the steam distillation video on the 146A website. This must be set up starting with the flask already containing plant material and water. If you would like more detailed procedure on assembling the distillation apparatus, please read Experiment 2 on the CHEM 8L website.*

Your TA must OK your apparatus before you turn on the heat. Heat the distillation flask using a heating mantle, starting at a medium setting. Adjust the heat supply so that the liquid distills at an approximate rate of one drop per second. Distilling too quickly with too high heat will severely decrease recovery of oil. Be patient - it may take 20-30 minutes for the first drop. Collect at least 200 mL of distillate, depending on how much water was added in the beginning. Read and record the temperature throughout the distillation (first drop and the final temperature). Students may inject and analyze appropriate GC standards (depending on the plant) in this down time. Someone must be watching the distillation apparatus.

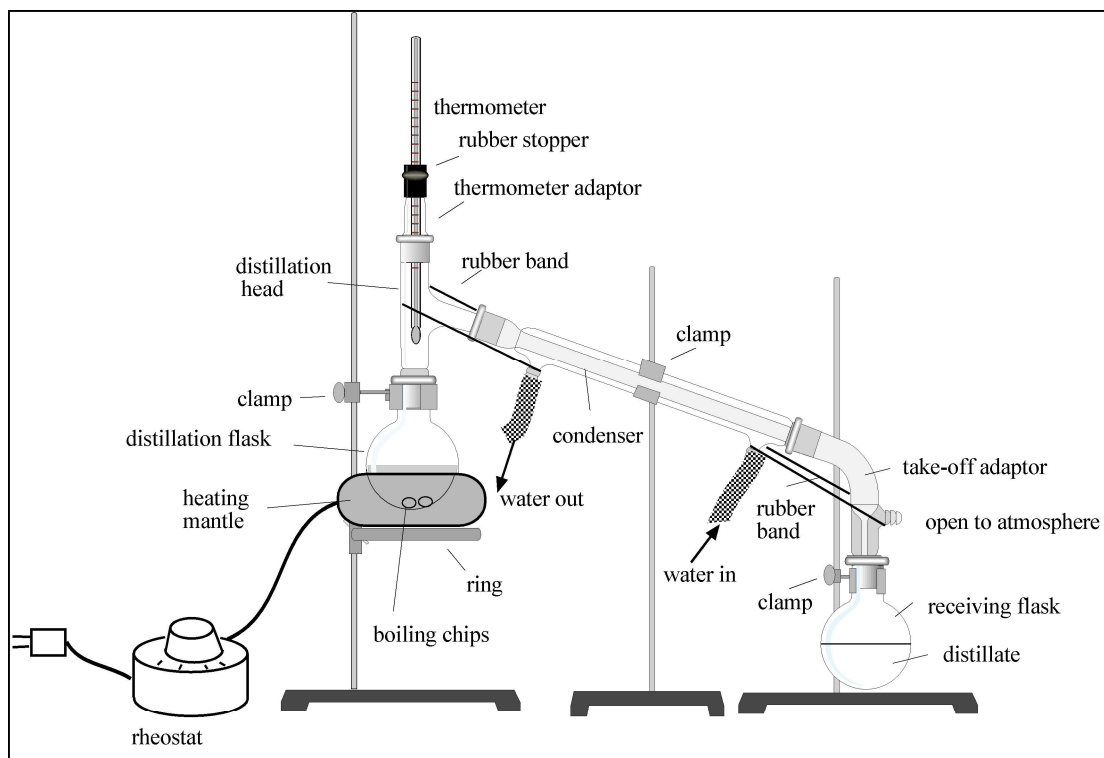


Figure 2. A simple distillation apparatus used for steam distillation. *Sketch this labeled diagram into the procedure portion of your notebook (omit boiling chips).*

Stop the distillation by turning off and unplugging the heating mantle then carefully lowering the ring support for the heating mantle. You will need a spotter to help with this. You may take off the receiving flask, but wait for the entire system to cool before disassembling. Place a beaker under the take-off adaptor to collect any further distillate.

Consult an instructor to decide whether there is enough apparent oil for separation in a buret or if solvent extraction is necessary.

Buret separation: Fasten a 50-mL buret to a ring stand using a buret clamp. Use a funnel to add distilled water to leave a few milliliters of space above the liquid in the buret. *Slowly* add a few milliliters of the orange distillate to the buret, being careful not to overflow. Allow the liquid to settle, then drain to the 45 mL mark, catching the liquid in an Erlenmeyer flask. Repeat the add – settle – drain process until all of the distillate has been transferred. Add 10 mL of water to the flask, swirl to dislodge any drop adhered to the wall and transfer the liquid portion-wise to the buret. Repeat this operation with another 10-mL portion of water. This process should limit the formation of emulsions, allowing the orange oil to neatly rest on top of the water. This is time consuming, but worth the result!

Let the system settle for at least 5 minutes, or until phase separation of oil on top of water is apparent. There may be some air bubbles or oil droplets stuck along the buret walls, however, we have found that attempting to collect this oil has an overall negative effect on recovery. The level of the liquid should be near the 50-mL marking so that you can collect the oil from the top layer by pipet. Label a small screw-cap vial with your name and the name of the oil. Weigh it and keep it handy.

Use a pipet to carefully collect the layer of oil from the top of the buret, avoiding the water as much as possible. You will likely not be able to collect all of the oil so just do your best! Transfer the oil to the pre-weighed vial. Carefully remove any visible droplets of water in the bottom of the vial using a pipet. Weigh the vial with the oil and determine the mass by difference.

Solvent extraction: transfer most, if not all, of the emulsion to the largest separatory funnel you can find. Extract this with 3 portions of diethyl ether (no more than ~100 mL total), allowing several minutes before each separation. Wash the combined organic extracts with brine, dry (MgSO_4), filter, and concentrate *in vacuo* (rota-vap).

Day 1 and/or 2 – GC ANALYSIS OF CITRUS OIL

Standards will be injected first then your oil. Record the chart speed and all other conditions posted on the bulletin board above the instrument (oven temperature, etc.). Use care when handling the sharp, delicate needles. Rinse the syringe three times with the sample before each run to treat the syringe and remove air bubbles. Load 0.2 μL of sample into the syringe and pull back to the 10 μL mark with air per TA instructions. Write the sample name on the chart paper then turn on the chart recorder. Turn the knob on the recorder to mark the beginning of the run and quickly inject the sample with air into the HOT injection port. The air or acetone peak will be used as the starting point for the **corrected retention time**. Rinse the syringe three times with acetone and clean the outside of the needle with a Kim wipe to remove traces of liquid after each injection to avoid cross-contamination. **Take note of the least count and ILE of the needle.**

Inject pure samples of **limonene, α -pinene, β -pinene, and γ -terpinene**, rotating with other groups for fair sharing of the instruments as instructed by the TA. Allow all the components to come out before performing the next injection. Myrcene is not injected due to unwanted interaction with the column. **Measure the corrected retention times of each standard (no integration) BEFORE PERFORMING THE INJECTION OF YOUR OIL.** Check with your TA then inject your oil and identify the peaks by comparing corrected retention times to the standards. Determine which compounds are the main components of your oil. If any peaks are in question, spike your sample with a standard (procedure below). Calculate the percent composition of your oil. *This analysis must be done during this lab period in case further injections are necessary.* One student in each pair should turn in the clearly labeled GC charts. The other student adds a clear note directing the TA to their partner's report for the charts.

Heads up: lavender contains many components and it may not be possible to analyze the chromatogram for percent composition and peak identification may be difficult or impossible. Do your best! For learning objective's sake, you may inject another student's oil or a provided sample of spearmint or caraway oil. This goes for any other essential oil with a messy GC chart.

Although not ideal, standard peaks can still be interpreted if the peaks level off at the top because only the retention time is needed. However, if the peaks from the citrus oil are too small to interpret or if sample peaks (not air/solvent peaks) level out at the top, repeat the injection. The intrinsic error is very high because the sample volume is so small. Often, **simply repeating an injection at the same volume will provide data that is easier to interpret. Do not increase sample volume beyond 0.2 μL .** Measure the retention times of the peaks and calculate the area under the curve (integration).

Table 1. Clean-up and Safety

Clean-up	Safety
<p>Allow the distillation apparatus to cool completely before disassembling. All ring stands, clamps, etc. should be put back in an organized manner.</p> <p>Taking the hoses off of the condenser: disconnect the condenser on both ends, clamp the hoses to stop water flow, then carefully remove the hoses OVER THE WATER TROUGH with a back-and-forth motion.</p>	
Strain the distilled orange peels through a strainer into the sink, using water to aid the transfer with shaking. When the strainer becomes full, transfer to the specified compost container.	Use caution with the heating mantle and distillation apparatus. Slowly increase heat, which should never go past a medium setting.
Dispose of the aqueous layer from the buret down the drain.	Terpenes are irritants.
Keep the oil in a capped, labeled vial in your drawer. Dispose of the oil in the same labeled vial in the container provided after analysis.	Limonene is a possible carcinogen.
Dispose of pipets in the glass waste box.	Acetone is flammable.
Rinse syringes with acetone.	GC Needles are sharp, delicate, and expensive – handle with care.
<p>Every Day: Thoroughly wipe down bench tops with a sponge and dry with a paper towel. Wash and rinse all glassware. Return shared glassware and equipment to where you found it in an organized fashion. All students in the section will lose points if even one student leaves a mess.</p>	
<p>Keep the instrument room clean and free of personal belongings. No more than 6 students should be in the instrument room at any given time. GC kits should be kept clean and organized. Cap the markers after completing all GC runs.</p>	