

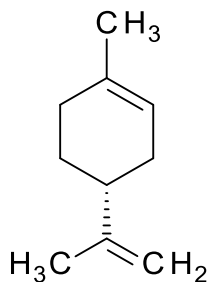
Learning Objectives:

- Extraction of organic compound(s) from natural sources
- Isolation using physical and chemical means
- Identification and quantitation of primary components – yield and purity
- Discussion of green chemistry and what a green manufacturing process is

The Problem

SC Johnson, makers of OFF®, is looking to prepare a brand-new “green” insect repellent. Their most famous OFF® product uses DEET (Diethyl-m-toluamide). DEET is a man-made product that comes from petroleum. SC Johnson began making a green product called “OFF® Botanical.” This product contains PMD, “paramenthane diol” a natural chemical that comes from *Oil of Eucalyptus*. This oil is purchased from Australia, New Zealand and Indonesia and has become very expensive due to changes in the world economy. The scientists at SC Johnson found that there is another “natural” insect repellent that is available right here in the USA. This natural compound is called limonene and is a chemical substance made by citrus fruits. It is a natural protectant against insects. That’s why oranges, unlike apples, peaches and pears, never have worm holes in them. Limonene is found in oranges, lemons, limes, grapefruits and other citrus. SC Johnson can get orange and lemon peels from the makers of *Tropicana* at a very low price. The problem is, “Which peel is better to use?” and “What is the best technique for extracting the limonene?”

(D)-Limonene (4R)-isopropenyl-1-methylcyclohexene



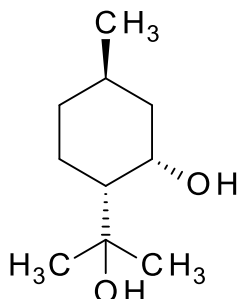
Molecular Formula = C₁₀ H₁₆

Molecular Mass = 136.23 Boiling Point = 176 °C



The actives in the OFF® Products are:

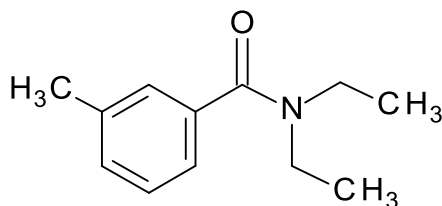
2-Hydroxy- $\alpha,\alpha,4$ -trimethylcyclohexanemethanol (PMD)
(OFF® Botanical, 5% - 10%, "Green Product")



Molecular Formula = C₁₀ H₂₀ O₂ Molecular Mass = 172.26

Melting Point = 34.5 °C

N,N-Diethyl-m-toluamide (DEET)
(OFF® Family Care Insect Repellent, 5%, "Standard Product")
(OFF® Deep Woods, 10%-30%, "Standard Product")



Molecular Formula = C₁₂ H₁₇ NO Molecular Mass = 191.27

Boiling Point = 288 °C

Experimental Background:

(+)- Limonene is found in all citrus fruits to varying degrees. The main concentration of limonene is in the zest (skin) of the fruit. (+)-Limonene is a terpene (a hydrocarbon) and is not soluble in water. It is very soluble in most organic solvents.

The limonene can be extracted from the skin in various ways. The zest can be minced in a blender and the limonene can be removed by steam distillation. The steam acts as a carrier only and does not azeotrope with the limonene. The limonene can be isolated from the distillate after steam distillation because it's not soluble in water. It will appear as a small oily layer on the top of the water layer. It can be carefully pipetted away if desired, or can be extracted with an organic solvent for further analysis (as in this experiment).



(+)-Limonene can also be extracted from the zest by Soxhlet extraction. A fiber thimble is filled with zest and isopropyl acetate is used as the extraction solvent.

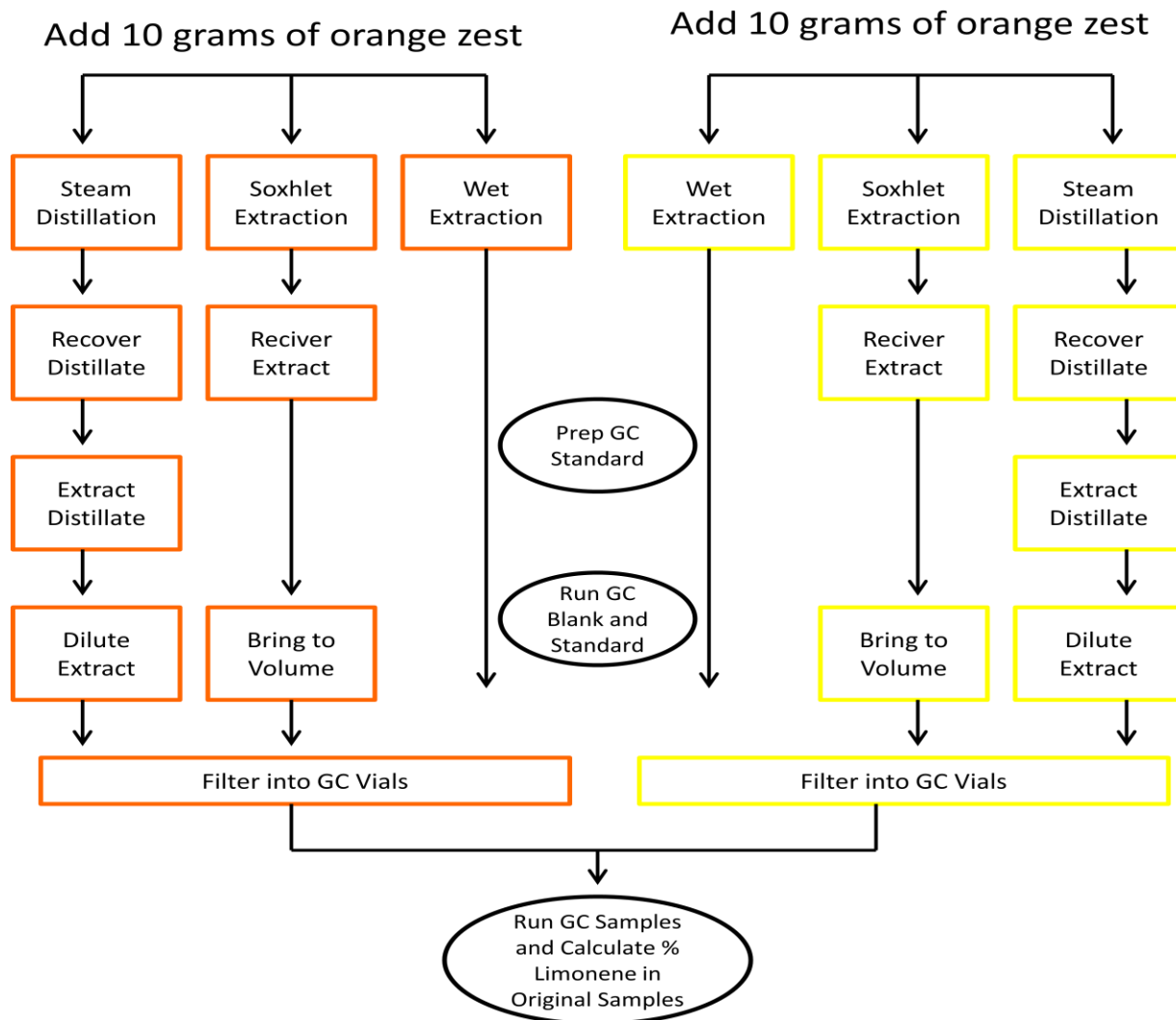
The extracts from both isolation methods can be analyzed by GC to determine the composition of the volatile fraction as well as the % limonene yield from the zest. In pure form, limonene is a colorless liquid that boils at 173°C and has a sweet, orange odor.

Basic Analytical Scheme

Limonene Extraction/Analysis - Analytical Scheme

Fresh Oranges and Lemons

Zest fruits and combine like zestings (~35g/ for each fruit)





Equipment and Materials:

Soxhlet Extraction Set-up

1 ring stand
1 clamp 24/40
2 large 3-prong clamps
1 ring clamp
250mL heating mantle
250mL round bottomed flask 24/40
soxhlet extractor body 24/40 to 35/50
extraction thimble – 33mmx80mm
condenser - 35/50
mini lab jack
small jar of boiling chips (Teflon)
cork ring
2 large almond-shaped stir bars
small squirt bottle of isopropyl acetate
small glass funnel
100mL graduated cylinder – glass
glass wool
200mL volumetric flask with plastic stopper
2mL glass autosampler vial (8mm x 40mm)
3mL plastic transfer pipette
5mL “syringeless” filter device
120V rheostat
tygon tubing for coolant
50mL glass beaker
glass crystallization dish 125cmx65cm

Steam Distillation Set-Up

1 ring stand
2 3-prong clamps
250mL heating mantle
250mL round bottomed flask 24/40
small jar of boiling chips (Teflon)
10/30 thermometer adapter
8” x 1/8” O.D. J-type thermocouple
2-way J-type temperature controller
2 - 50mL pyrex culture tubes with screw caps
100 mL graduated cylinder
200mL glass beaker
one-piece 300 mm distillation head 24/40
cork ring green
green Keck clamp for elbow
29/42 collection “elbow”
powder addition funnel (plastic)
DI water bottle – 500mL
mini lab jack
antifoam B
10mL. graduated pipette
5mL. graduated pipette
50mL volumetric flask with plastic stopper
5mL “syringeless” filter device
2mL glass vial (8mm x 40mm)
tygon tubing for coolant

Simple “Manual” Extraction

250mL. Erlenmeyer flask with plastic stopper
100mL. graduated cylinder - glass

**For All Set-Ups:**

4 Fume hoods (needed for soxhlet extractions and all other solvent work)
4 x Plastic Waste Collection Beakers (one for each hood)
2 x Electronic Balances (2 decimal places)
Kimwipes/Tissues
8 Scoopulas
4 spoon/spatulas
8 small fine graters (zesters)
plastic weighing boats
fresh oranges (4) and lemons (7)
8 pairs of heavy duty rubber dishwashing gloves
4 aluminum baking pans
1 circulating bath for cooling condensers (30% ethylene glycol in water)
1 extra glass bottle (500mL) containing isopropyl acetate
aluminum foil for insulating soxhlet arm and distilling head
4 calculators
Sharpie permanent markers in all hoods and at all stations
one plastic test tube rack for each of the corner hoods.

For GC Analysis:

10mL volumetric flask with plastic stopper	3mL plastic transfer pipette
limonene reference material (97%)	10 μ L - 100 μ L autopipettor with tips
autosampler vials	

Individual Hood and Bench Set-Ups:

See the accompanying photos. The set up descriptions will follow. There will be 2 portable hoods on one side of the bench. These will contain the soxhlet extraction setups. The 2 small hoods off either corner of the bench will be for manual extractions, solvent dilutions and reference standard preparation.

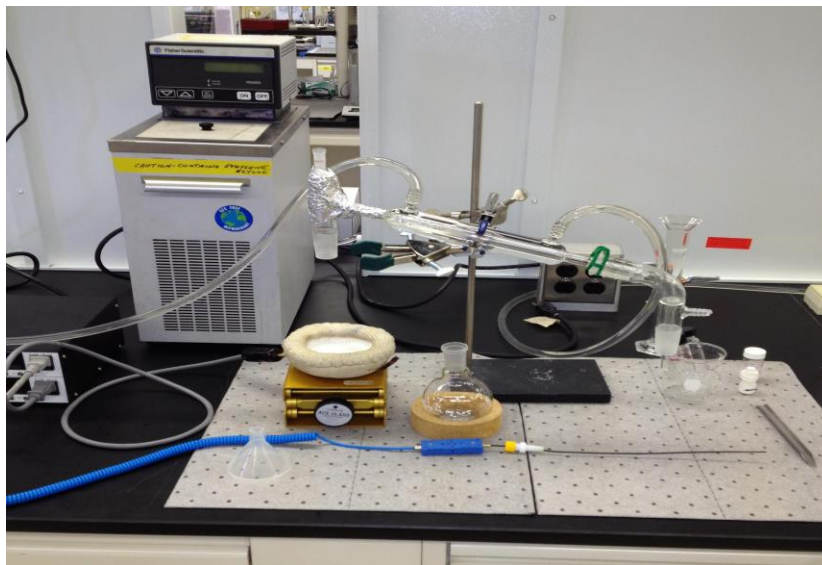
Vial and container labeling conventions – it is recommended the letters “O” and “L” be used to designate solutions related to oranges and lemons, respectively. The extraction procedures can be designated as “M” for manual, “S” for soxhlet and “D” for steam distillation.

Zesting stations (set up on bench)



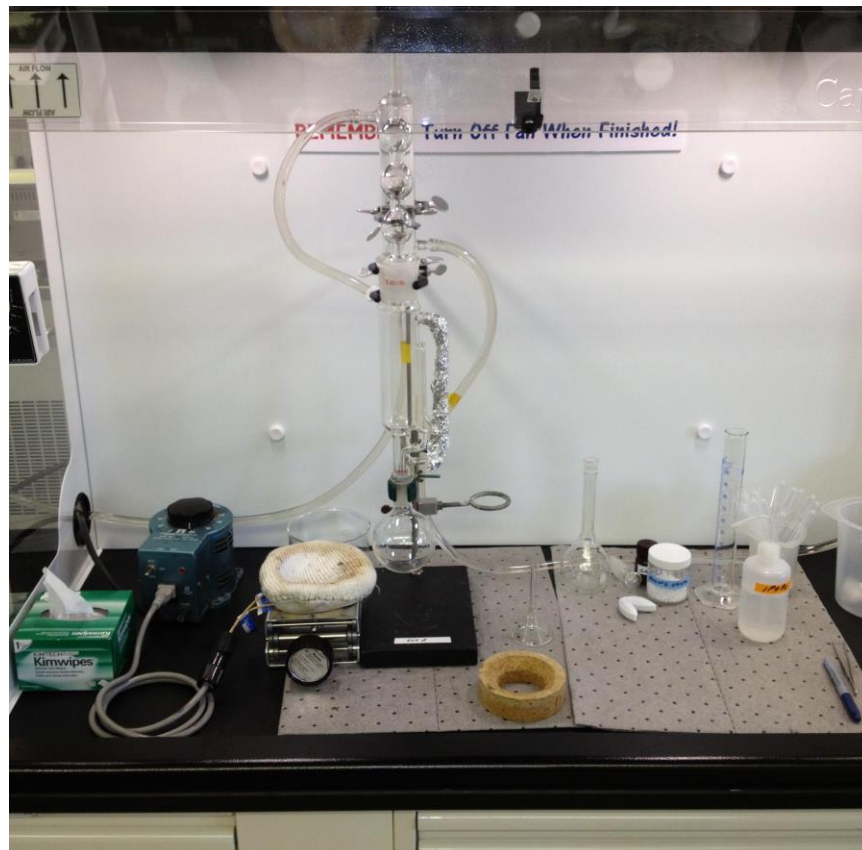
- Place a digital balance on each end of the bench along with some plastic weighing dishes.
- Place one aluminum pan on each corner of the bench (4 in all).
- On the side of each pan (8 in all) place a pair of heavy rubber gloves and a scoopula.
- Place 2 graters in each pan.
- There should be some kimwipes or tissues near by to clean up any messes.
- One end of the bench will receive 4 oranges and the other 7 or 8 lemons.
- Place a calculator near each pan for later use.

Steam Distillation station (pre-assembled on bench)



- Mount clamp to ring stand. Clamp the one-piece distilling head in place with a 3-prong clamp. Make sure the distilling head is at least 12" above the base.
- Connect water lines and add a second 3-prong clamp under the distilling head to be used to hold the 250mL round bottomed flask.
- Attach the 250mL round bottomed flask to the distillation head joint and secure with a 3-prong clamp.
- Attach the 29/42 collection elbow and secure with a green Keck clamp.
- Make sure the joints fit snugly together.
- Place the mini- lab jack under the flask and the heating mantle on top of the jack.
- Crank the jack upward until the mantle meets the bottom of the flask. (Note: There must be enough clearance so that if overheating occurs, the jack can be cranked down far enough for the bottom of the flask to be clear of the heating mantle.)
- Pre-grease and insert the 10/30 thermometer adapter with the 8" x 1/8" O.D. J-type thermocouple attached (held in place with a green GC septum) into the adapter opening of the distilling head.
- The bottom of the thermocouple should be an inch above the bottom of the flask.
- This thermocouple is used to regulate the still pot temperature.
- Fill one of the 50mL culture tubes with 35mL of water and using a marker, trace a line around the tube at the water mark. This will serve as the desired stopping point for the distillation.
- Place the pre-marked 50 mL tube below the receiving tube on the end of the distillation apparatus to collect the distillate.
- Set controller temperature to 110°C. The "Output Power Level" knob should be initially set to "2L", then turn down to "330-2L" when boiling initiates.
- Keep controller off before and while the flask is having zest and water added to it.
- Place a small container of boiling chips and a small bottle of defoamer by each setup.
- Place a 500mL squirt bottle of water, a plastic funnel and a scoopula by each setup.
- Wrap some aluminum foil around the section of the distillation head just before the condenser to insulate it.

Soxhlet Extraction Station (pre-assembled in hood)



- Place a squirt bottle of isopropyl acetate in each hood.
- Place a small jar of boiling chips and a small container of glass wool in each hood.
- Place a 200mL. volumetric flask with plastic stopper in each hood.
- Place a 100mL graduated cylinder (glass) in each hood.
- Place a plastic beaker containing some syringe/filters in each hood.
- Place a small glass funnel in each hood.
- Place a new extraction thimble in each hood.
- **Carefully** place the two stirring bars in the bottom of the extractor
- Mount 3-pronged clamp to the ring stand about midway up the bar and clamp the extractor body so that the bottom of the extractor is about 12" above the counter top.
- Place the condenser above the extractor body and connect the water cooling hoses.
- Connect a second 3-pronged stand clamp to the ring stand and place it at the top of the extractor body around the 34/40 joint.
- Connect the round bottomed flask to the extractor body.
- Tighten the 24/40 clamp around the flask neck making sure the joint connection remains tight.
- Put the heating mantle on the mini-jack and plug the mantle into the rheostat.
- Attach a ring clamp (for holding a funnel) to the ring stand.
- Wrap some aluminum foil around the outer "arm" of the extractor to insulate it.



Experimental Procedures:

Zest Preparation : (instructor should briefly demonstrate proper technique)

- Carefully push the fruit against the grater and slide it along its length.
- You should grate until a "scrape" is observed; the bright shiny color has been removed and there is a lighter, dull area remaining.
- Keep rotating the fruit and adjusting the position so that only fresh skin is zested. Do not go back over previously zested areas or too much skin will be removed.
- Tare the plastic weighing boat on the balance.
- Periodically collect the zests from the aluminum pans using the scoopulas to scrape it up.
- Add the zest to the boat until there is **35 ±0.1 grams of zest**. They should **record the weight** on their data sheet.

Turn on the circulating bath on. Wait 3 seconds for the word 'OFF'. Set temperature to 5°C, hit return to save. Attach cooler all condensers, which are connected in series.

Soxhlet Extraction Teams:

- Place the paper thimble on the balance and press the tare button.
- Using a metal scoop, transfer 10 grams +/-0.1g of the zest into the paper thimble, gently packing it down lightly. Record the weight on your data sheet.
- Put some glass wool on top of the zest to keep the small particles in place.
- Remove the extractor body from the condenser and place the thimble inside.
- Add 2 Teflon boiling chips to the round bottom flask. and reconnect to extractor.
- Using a 100mL graduated cylinder, measure and add 175mL of isopropyl acetate to the round bottom flask.
- Carefully reconnect the flask, extractor body and condenser.
- Turn on the rheostat and turn the dial to 80 to 90.(adjust to maintain even boil)
- Watch the extraction process. Take mental notes on the clarity and color of the solution as the extraction progresses.
- After approximately 30 - 40 minutes (*the instructor will let you know*), turn off the power.
- Disconnect the round bottom flask from the extractor and lower it down into a crystallization dish containing some cold water.
- After it has cooled, transfer the contents of the flask and the extractor into a 200mL volumetric flask with the aid of a glass funnel.
- Bring up to the mark by slowly adding isopropyl acetate using the squirt bottle. Mix.
- Transfer about 1mL to a GC autosampler vial, filtering the sample with the "syringeless" filter. (*sometimes this takes two sets of hands*). Label the vial.
- With the help of the instructor, put your sample into the GC autosampler.

CLEAN-UP (*may be performed by instructors*)



- Remove the round bottomed flask from the extractor body.
- Rinse the flask by adding about 20mL of acetone and swirling the flask.
- Dump acetone into an approved waste collection container.
- When the flask looks clean, go to the sink, rinse it with water and put the flask on the drying rack.

Steam Distillation Teams:

-
- Tare a plastic weighing boat on the balance.
- Weigh the 10 grams +/-0.1g of zest into the weighing boat and record the weight on your data sheet.
- Add the zest to the 250mL. round bottom flask.
- Using a 100mL. graduated cylinder, add 100mL. of DI water to the zest.
- **Add 2 boiling chips and 3 drops of Antifoam B to the liquid in the flask.**
- Mix the contents by gently swirling the flask.
- Reconnect the flask and raise the mini-jack and heating mantle.
- Place a 50mL screw cap culture tube (the one with the water level mark) under the receiver end of the distillation apparatus.
- Turn on the heating controller. (temperature settings are above)
- It is usually easiest to keep the tube in the 200mL beaker to help hold it steady.
- Collect approximately 35mL. of distillate (approximately up the mark). Cap and label the vial. Carefully put the other vial under the receiver and collect another 10mL. or so. (*observe whether oil is still distilling over. Does the second distillate sample smell of citrus? – Note – this sample will NOT be analyzed*)
- In a fume hood, use a 10 mL graduated pipette to add 10 mL of isopropyl acetate to the distillate/oil mixture.
- Cap the vial and grasping it firmly, mix by inverting and gently swirling the container. You should see tiny oil droplets mixing with the water. After 2 minutes, set the container down in a test tube holder and allow the layers to separate.
- Fill a 10mL. volumetric flask about half full with isopropyl acetate.
- Using a 1mL. automatic pipette, **carefully** transfer mL. of the upper (solvent) phase from the vial into the volumetric flask. **Note – it helps if the instructor holds the lower end of the pipette to assure it is submerged in the solvent layer but not allowed to penetrate or suck up the water layer.** Dilute to the mark with isopropyl acetate and mix by inverting several times.
- Transfer about 1mL to a GC autosampler vial. Label the vial.
- With the help of the instructor, put your sample into the GC autosampler.

CLEAN-UP (*may be done by the instructors*)



- When the round bottomed flask is room temperature, loosen the clamp and remove it from the distilling head.
- The contents can go down the drain and the flask can be washed in the sink with normal cleanser and water.

“Manual” Solvent Extraction:

This simple sample preparation can be prepared by whichever extraction team has gotten their setup extracting first and needs something to fill their time.

- Tare a plastic weighing boat on the balance.
- Weigh the zest into the weighing boat and record the weight on your data sheet.
- Add the zest to the 250mL. Erlenmeyer flask round and record the weight on your data sheet.
- Using a 100mL. graduated cylinder, add 100mL. of isopropyl acetate to the zest.
- Stopper the flask and periodically swirl the contents to extract.
- Transfer about 1mL to a GC autosampler vial, filtering the sample with the “syringeless” filter. (*sometimes this takes two sets of hands*). Label the vial.
- With the help of the instructor, put your sample into the GC autosampler.

Instrumental Analysis Section:

Prior to analyzing the sample extracts, the GC (gas chromatograph) must be brought on line, a suitable blank run to establish system integrity, then a calibration standard prepared and run. The data system will update the calibration factor (limonene response factor) automatically. **The students can fill the autosampler vials with “blank” isopropyl acetate as well as prepare the reference standard (one solution is split and used on both GC’s).**

Bring the GC online by:

- turn on the main GC power switch
- open the main valve on the helium cylinder and valve on manifold
- open the valves to bring hydrogen to the instrument (outlet from generator and manifold)
- open the valves to bring air to the instrument (outlet from house air and manifold)
- turn on the data system PC
- open the TCNav icon
- logon in the “manager” mode on the drop-down menu (password is on PC mouse pad)
- “bind” the instrument and setup the method called “limonene”
- sample information can be loaded into the sequence called “limonene”
- after the GC has stabilized at its initial temperatures, ignite the flame (if manual) by
 - o turning the air knob on the GC fully clockwise (to close)
 - o push the ignitor plug (found under the cover to the right) onto the detector
 - o while pushing down fully, open the air knob – you will hear a “pop” when ignition occurs

**GC Parameters:**

column – 30mx0.53mmID DB-624 (3.0µm film)
oven temperature profile - 120°C for 3.00 min.; 30°C/min. to 180°C (5 minute run)
injection port - 225°C
detector (FID) - 250°C
helium carrier gas – 14.0 psi head pressure
hydrogen flow – 30mL/min.
air flow – 300 mL/min.
split ratio – 2:1

detector range – 20

1µL direct auto injections – split mode
data system attenuation – (-1)
all data system methods, sequences and report formats are designated as “limonene”

GC “blank” – isopropyl acetate (same as used in extractions)

Quantitative Standard: Fill a 10mL volumetric flask approximately half full with isopropyl acetate. Using a 10µL - 100µL autopipettor, add 100µL of limonene to the flask and dilute to volume with isopropyl acetate. This will make a standard that is 8.40mg/mL

$$\frac{(100\mu\text{L})(0.840 \text{ mg}/\mu\text{L})}{10\text{mL}} = 8.40 \text{ mg/mL}$$

Instructor Notes:

Assignments:

- Break the group up into 4 sets of 2 and assign an instructor to each set.
- Primary instructor goes over procedures, safety, and assignments.
- Primary instructor assists secondary as needed.
- All secondary instructors are responsible for watching their group prepare fruit.
- All should assist in glassware setup/connecting as needed.

Discussion points:

Remember- We need to know, which is the better fruit to use and what is the most effective extraction technique to use. Make judgments based on yield, purity, time, energy needs, and waste generated.

Insider Points



Soxhlet Extraction:

This extraction will provide “dirtier” material than by steam distillation. All polar components in the zest will be extracted. There might not be evidence by GC, but if the extract is isolated, it might be more evident. Make sure students note the darkening of the extraction liquid over time.

Steam Distillation:

When heating the set point temperature should be 103°C and the **Output Power Level** set to “> 2 L”, otherwise the distillation will not go fast enough. Expect about 0.5-1mL of oil.

Validation Discussion

Two goals of these experiments are to compare the amount of limonene collected by steam distillation versus the Soxhlet extraction and manual extraction and also, to find if the orange or lemon zest yields a higher purity oil. The Gas Chromatograph (GC) will separate the individual components in each sample and quantify the amount of limonene present as well as provide information as to the overall purity of the volatile fraction.

Three standard solutions of 0.75 mg / mL, 0.30 mg / mL and 0.075 mg / mL were prepared and 1 μ L of each solution was analyzed on the GC. These different concentrations are used to provide evidence that the amount of area reported by the GC increases by the same amount for each increase in concentration of the limonene. Mathematically, this means the ratio of the area divided by the concentration is a constant. If a 1 μ L injection of a sample is compared to the standard, then

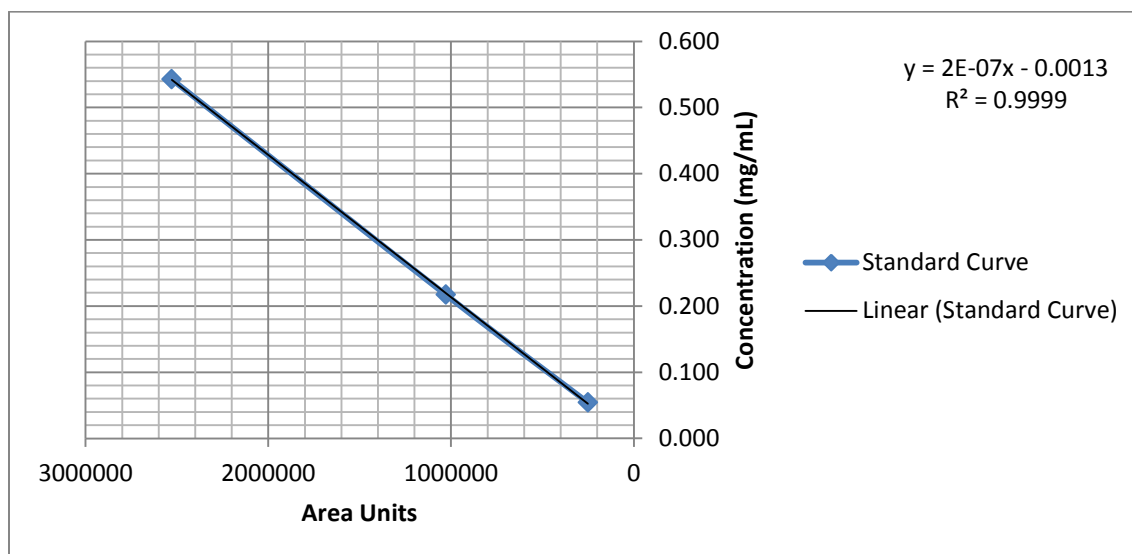
$$\frac{Area_{Std}}{Conc_{Std}} = \frac{Area_{Smp}}{Conc_{Smp}}$$

By rearranging this equality, the concentration of the sample can be determined from the area of Limonene recorded by the GC.

$$Conc_{Smp} = \frac{Conc_{Std}}{Area_{Std}} \times (Area_{Smp})$$

While the standards cover a 10 x change in concentration, the sample solutions will have to be diluted so that the amount of Limonene is within the range validated by the standards. The idea of validation is important in analytical methods. A solution that is known to have the chemicals of interest is prepared, and then the ability of the scientific instrument to report these results is checked during the experiment.

Typical Calibration Curve



Points to Cover with Students

Everything you do in the factory costs money. The longer the process takes and the more processing that is needed, the greater the costs. It's best to pick a fruit that has the most extractable limonene. It is also best to pick a method that gives you the purest limonene (fewer extra peaks by GC).

When answering the below questions, assign a question or two to each student so they all have to pay attention to the discussion.

- Which fruit yields more limonene by each extraction method?
- Which fruit yields higher purity limonene by each extraction method?
- Which extraction method generally gives you more limonene?
- Which method is more efficient (quality and quantity)? Why?
- Which method uses less energy to get maximum limonene?
- Which method uses a safer solvent?
- Which method has "cleaner waste?"
- Which method is greener? Why?

Future Activities:

Supercritical Fluid CO₂ Extraction
Isolation and sensory evaluation of extracted material
Use of rotary evaporative technique

Limonene Extraction from Citrus Peels



-Data Sheet-

Analysts Names _____

Date _____

Fruit Analyzed _____

Mass of Zest Used (grams) _____

Extraction Technique _____

Amount of Limonene in Extract (mg/mL) _____ (by GC)

Total Limonene from Sample (grams)

$$\frac{(\text{Amount in Extract mg/mL}) \times (\text{total volume of solvent})}{(1000\text{mg/gram})} = \text{_____ grams}$$

Volume = 100mL for steam distillation and manual extraction techniques

Volume = 200mL for soxhlet extraction technique

$$\text{Yield from Zest (weight \%)} = \frac{(\text{total grams})}{(\text{mass of zest})} \times 100 = \text{_____ \%}$$

Purity of Limonene (% from GC chromatogram area %) _____ %

Bonus Question: Do you prefer orange juice or lemonade? _____

Knock Knock

Who's there?

Orange

Orange who?

Orange you glad you did this experiment?!!!!!!