Supplementary Materials for

Qualitative Analysis of Citrus Fruit Extracts By GC/MS: An Undergraduate Experiment

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Typical Student Handout for this Experiment

Laboratory Handout
Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Citrus Fruit Extracts

PRELAB:
Reading

GC: Read in the lab Textbook: Technique 15 (begin pg. 711 in lab textbook), read only the Intro and sections 15.1-15.9. Also read the sections on mass spectrometry (MS.1 to MS.5) in your lab text (pp. A58-A75, in Appendix 6). Also read section 27.5-27.6 (pp. 1128-1133) in your lecture text (McMurry, 3rd ed.).,

On a separate piece of paper, answer the following, to be turned in at the beginning of the lab period.

1. What are the stationary phase and mobile phase in the gas chromatography experiment?
2. Which would you expect to elute from a GC column first, propane or hexane? Why?
3. What is a Terpene? What is the Isoprene Rule?
4. Apply the isoprene rule to the structure of Caryophyllene (Question 27.5 (c), pg. 1130 of the McMurry lecture text).

SAFETY:
Pentane is very volatile and very flammable. No flames will be permitted in the lab during this experiment.

PROCEDURE:
Extraction:
Begin by selecting a citrus fruit (Grapefruit, Lemon, Lime, Orange (Navel or Valencia), Tangerine, or Tangelo). Next, obtain a 4"x4" square of aluminum foil and record the mass of this sheet of foil. Now, transfer the citrus fruit onto the sheet of foil and then remove approximately 2.0 g of citrus peel using the cheese grater. Be absolutely certain to make sure that the rind is grated into very fine particles (use the smallest texture for the grating process and be absolutely certain to not damage the pulp of the fruit, most of the essential oils will be found in oil sacks within the peel of the fruit, not in the pulp), this is done to maximize the surface area for the extraction process. Once you have grated the citrus rind, obtain the mass of the rind and the sheet of aluminum foil together, the difference in the two masses is the net mass of the citrus rind.

Once you have obtained the mass of the citrus rind (ca. 2.0 g), add this to your separatory funnel along with 7.0 mL of pentane. Next, extract this mixture for approximately ten minutes (be certain to vent the separatory funnel frequently, as pentane is very volatile) and drain the pentane solution into a 50 mL Erlenmeyer flask. Then, repeat this procedure two more times using two additional 7.0 mL aliquots of pentane. Collect all of the pentane solutions together and then add approximately 1.0 g of sodium sulfate (Na_2SO_4) to the pentane solution. Cover the Erlenmeyer flask and allow this mixture to stand for approximately ten minutes with occasional swirling.

After the solution has dried sufficiently (it no longer appears cloudy), carefully decant the pentane solution into a pre-weighed 60 mL beaker and evaporate the pentane in a sand bath using low heat and a gentle stream of air over the mouth of the beaker. (NOTE: You must use a low heat setting for this evaporation. If you use too much heat for this evaporation, you will decompose many of the essential oils contained in your extract. In addition, you must make sure that this evaporation is performed in the fume hood, as pentane is very flammable.) Once the evaporation is complete, allow the beaker to cool to room temperature and obtain the mass of the crude citrus fruit extract. It should appear as a viscous amber oil.

Once the mass of the crude extract has been obtained, dilute the sample with approximately 1.0 mL of dichloromethane (methylene chloride) and transfer the resulting solution into a 3.0 mL conical reaction vial. Finally, inject approximately 0.25 μL of the dichloromethane solution into the GC/MS and analyze the data.

Name:_____ Lab day:_____

Lab Partner: _____

LABORATORY REPORT: GAS CHROMATOGRAPHY

GC:

Make sure your GC scan is attached. LABEL YOUR SCAN. When you have identified a peak by a retention time comparison to those compounds present in the standard sample, write the identity of this peak directly above the peak in question. Each person in each group must turn in a copy of the GC.

On this page, you will find a list of components (compounds) commonly found in citrus fruit extracts, along with their observed retention times for standard samples. As part of your analysis, you should attempt to identify these compounds in your own experimental sample using the given retention times. It is important to note that not all of these compounds will be found in every citrus fruit extract.

Instrument Model:_____ Column Type:_____

Carrier gas pressure (from regulator):_____ Column length:_____

Oven temperature program:_____

Injector temperature:_____ Detector temperature:_____

Detector type:_____

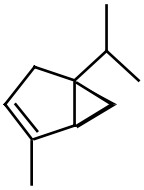
Citrus Fruit:_____

Complete the following Table. Complete the student sample column by entering the corresponding peaks from your attached GC scan in the same horizontal rows as the standard component. If you have peaks that don't correspond to any of the peaks in the standard, list them on your GC scan as "unknowns".

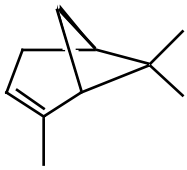
Component	Standard Sample Approx. Retention Time (min)	Student Sample: Retention time
alpha Thugene	6.050	
alpha-Pinene	6.183	
Sabinene	6.600	
beta-Pinene	6.658	
beta-Myrcene	6.700	
p-Cymene	6.842	
d-Limonene	7.133	
gamma-Terpinene	7.358	
Terpinolene	7.575	
Linalool	7.642	
Citronellal	8.008	
alpha-Terpineol	8.350	
Neral	8.592	
Geranial	8.758	
Neryl Acetate	9.208	
Geranyl Acetate	9.308	
alpha-Farnesene	9.692	
beta-Bisabolene	10.042	

The structure of each of these compounds is presented on the next page for your convenience.

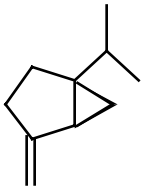
The Structures of Common Essential Oils Observed Performing GC/MS Analysis of Citrus Fruits



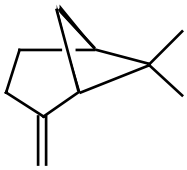
alpha -Thujene



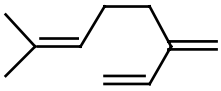
alpha-Pinene



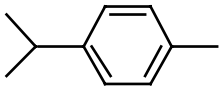
Sabinene



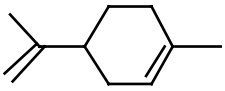
beta-Pinene



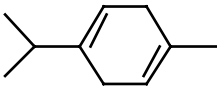
beta-Myrcene



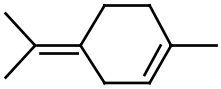
p-Cymene



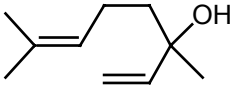
d-Limonene



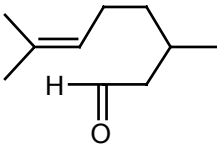
gamma-Terpinene



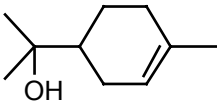
Terpinolene



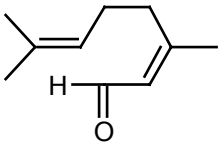
Linalool



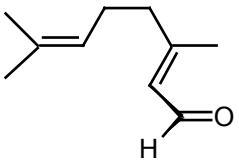
Citronellal



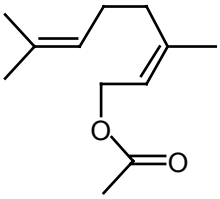
alpha-Terpineol



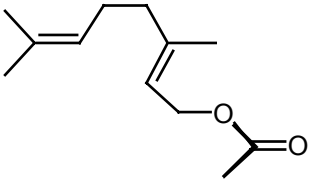
Neral*



Geranial*

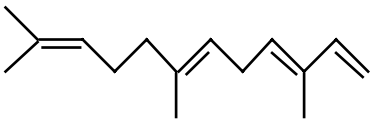


Neryl Acetate

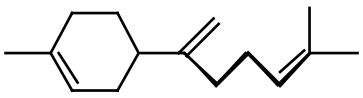


Geranyl Acetate

(*Z* and *E* isomers of citral)



alpha-Farnesene



beta-Bisabulene

Name:_____ Lab day:_____

LABORATORY REPORT: GAS CHROMATOGRAPHY, CONTINUED

Citrus Fruit Used: _____

Mass of Citrus Peel Used: _____

Mass of Citrus Oil Recovered: _____

Percent Recovery: _____

Questions:

1. Examine the structures of the eighteen compounds pictured on the previous page. These compounds have different classifications of terpenes. Indicate which of these terpenes and terpenoids are monoterpenes and which are sesquiterpenes. Explain. Which of the compounds listed on the previous page are erpenes? Which of the compounds listed on the previous page are terpenoids

2. Alpha Pinene has a boiling point of 155-156°C, while beta Pinene has a boiling point of 165-166°C. Compare these boiling points to the standard retention times presented in the table. Does the order of elution (value of the retention times) make sense relative to the boiling points? Explain.

3. Apply the isoprene rule to find the isoprene units in the following compounds:
   (a) alpha-Farnesene
   (b) gamma-Terpinene
   (c) alpha Pinene

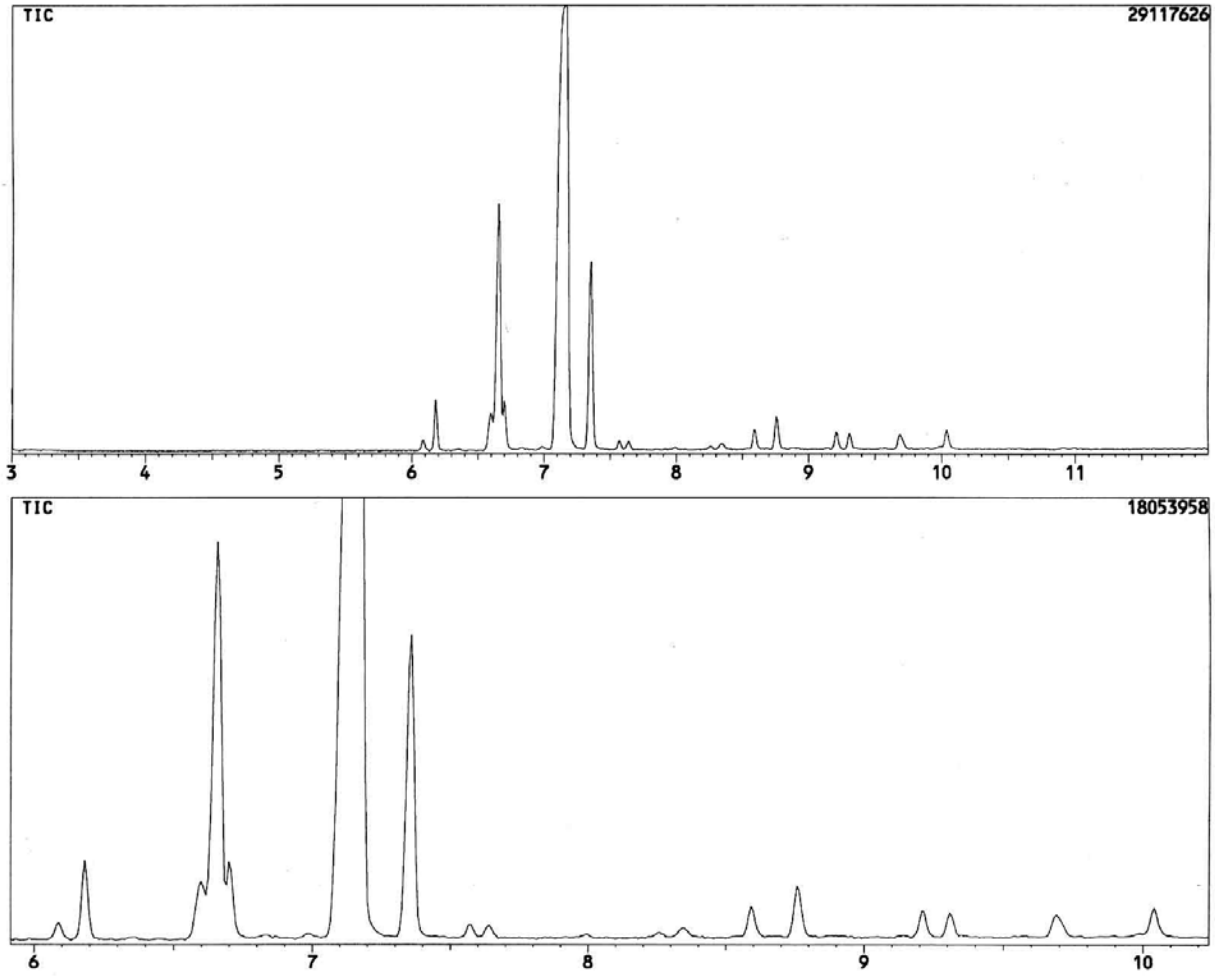
4. Examine the labeled gas chromatograms for a different citrus fruit. What peaks (compounds) do these two different citrus fruits share in common? What are the differences? Does it appear that the compounds are present in the same relative (approximate) amounts?

5. How does the mass spectrum tell you what compound a given GC peak corresponds to?

Typical Student Chromatograms

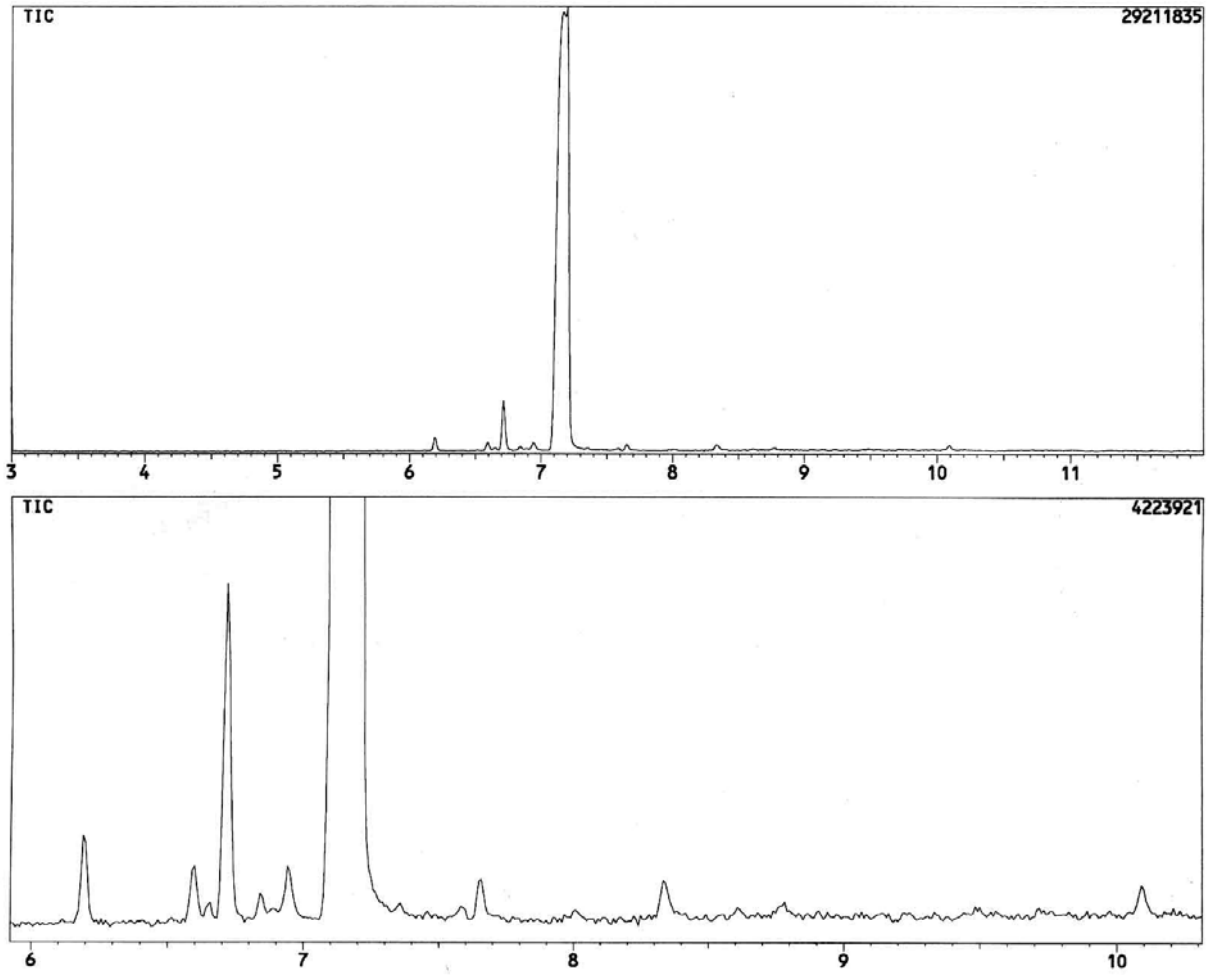
*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D39 00/05/12 13:32:08

Sample : lemon oil
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :

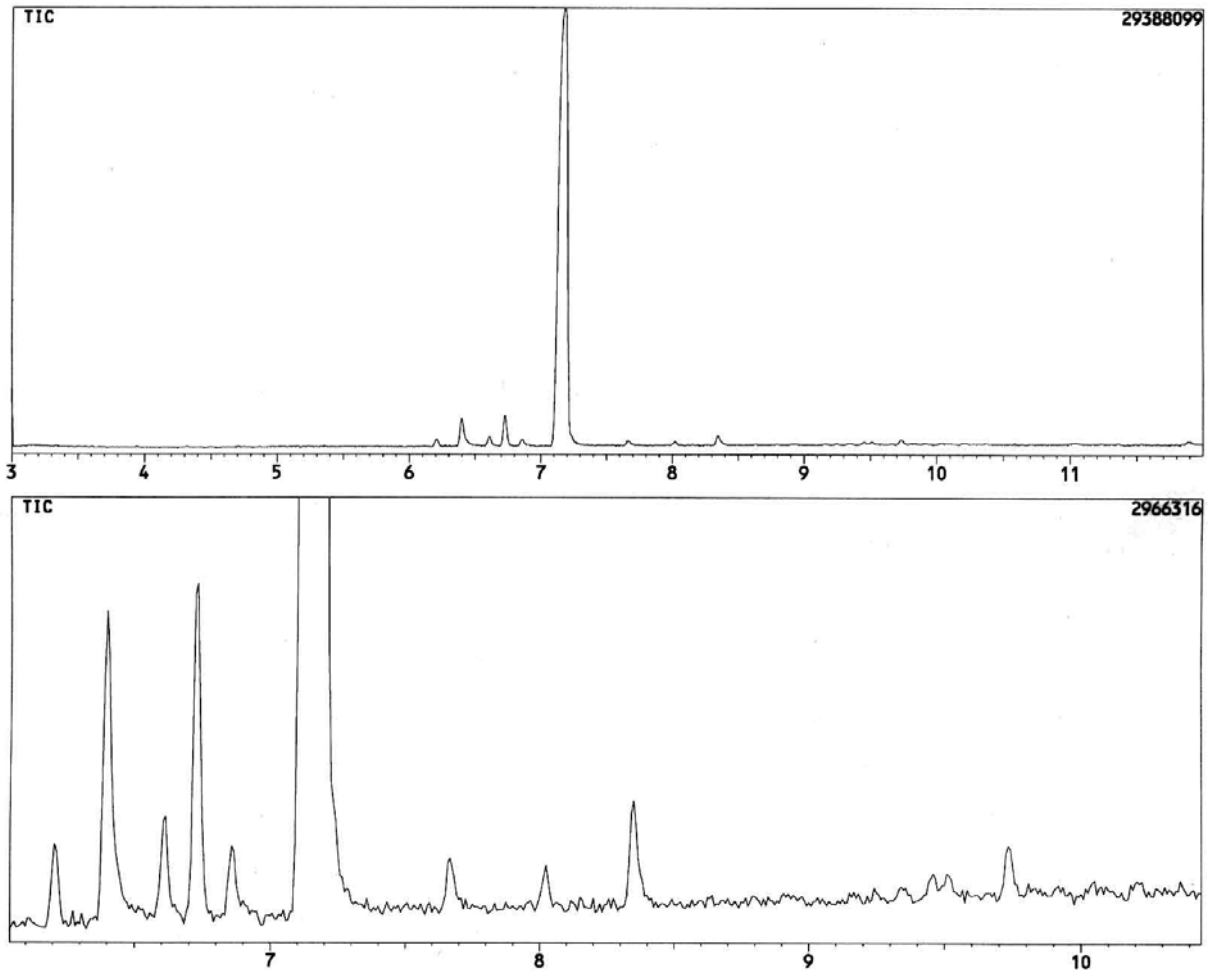


*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D41 00/05/12 14:19:03

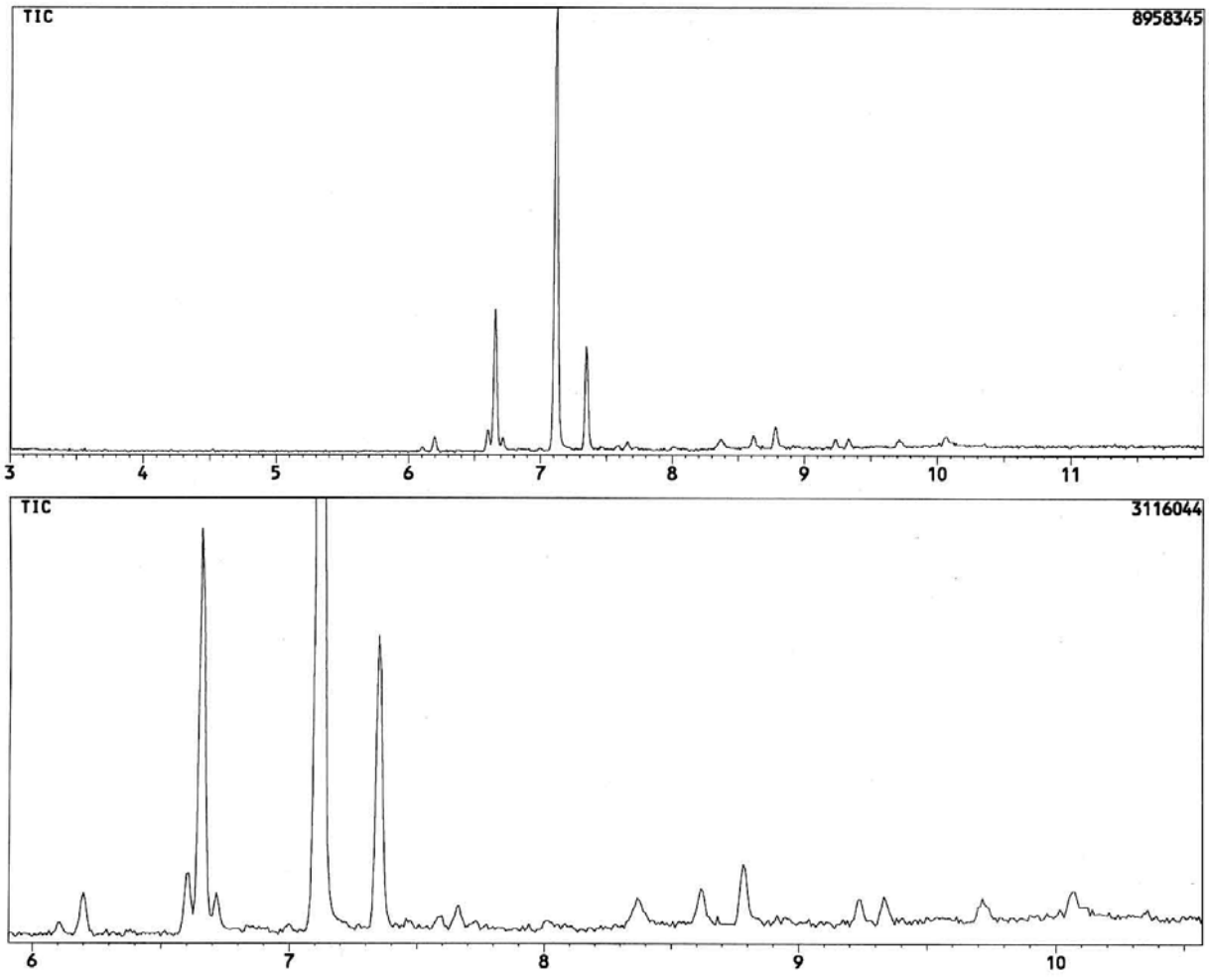
Sample : orange oil
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :



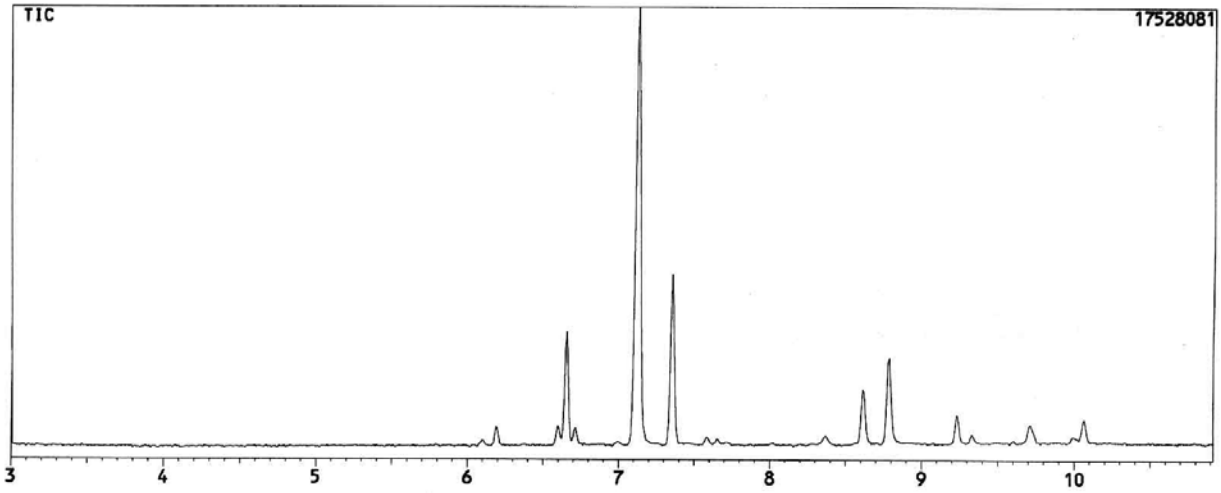
*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D15 00/05/10 16:50:17
Sample : grapefruit
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :

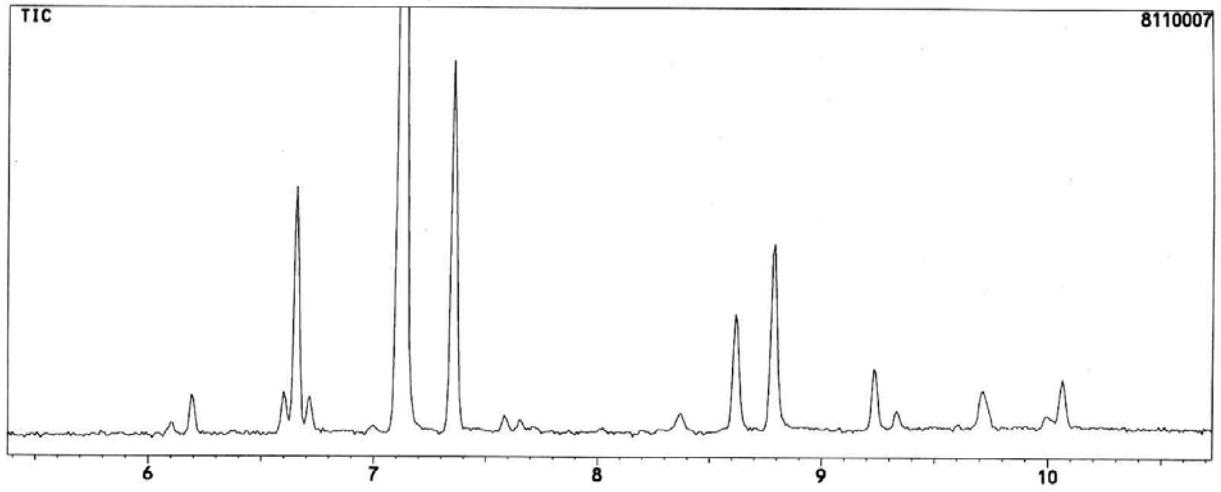


*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D36 00/05/12 12:42:35
Sample : lemon
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :



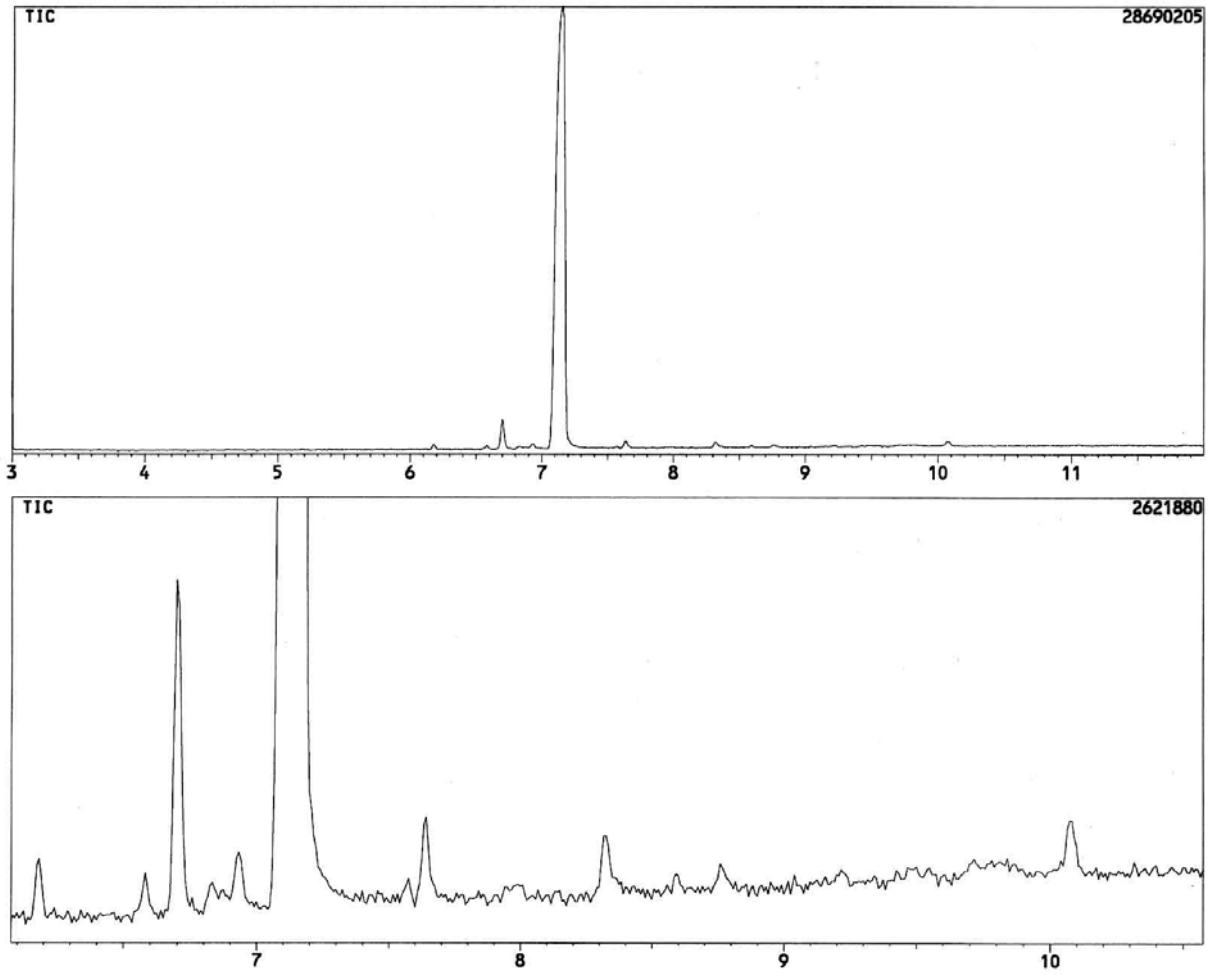
*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D03 00/05/09 16:18:35
Sample : lime
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :





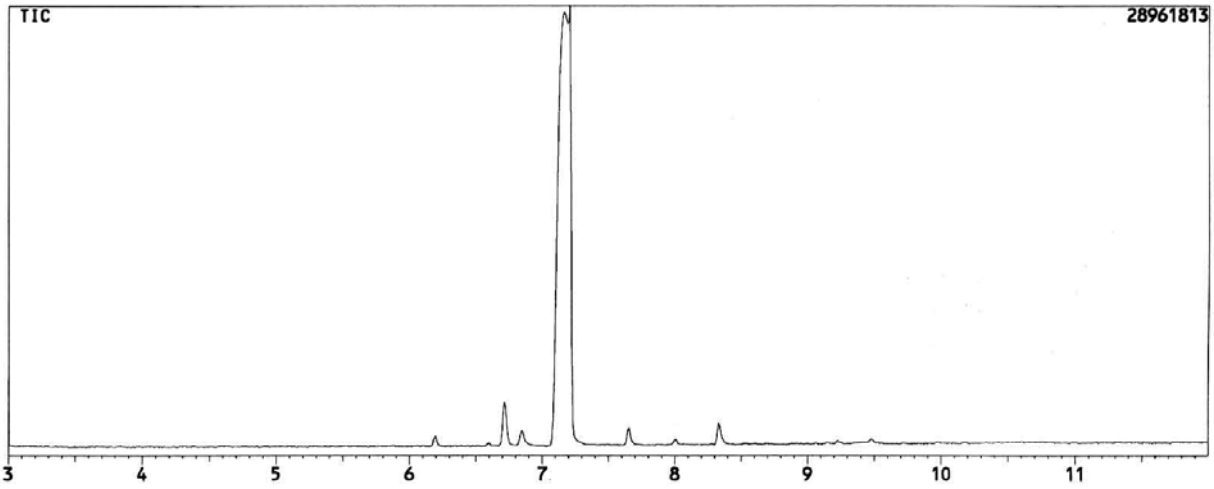
*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D30 00/05/12 10:41:31

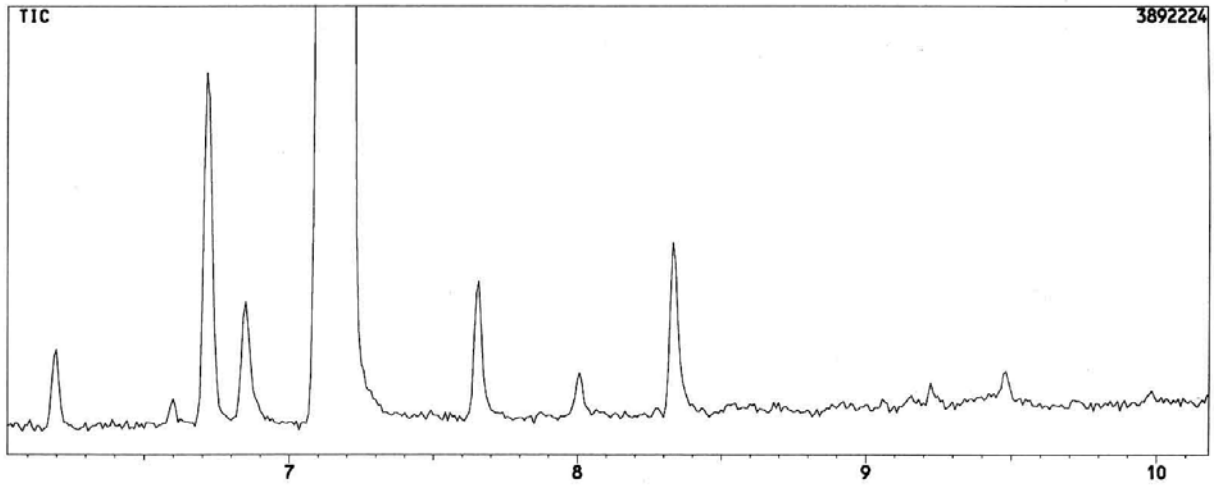
Sample : navel orange
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :



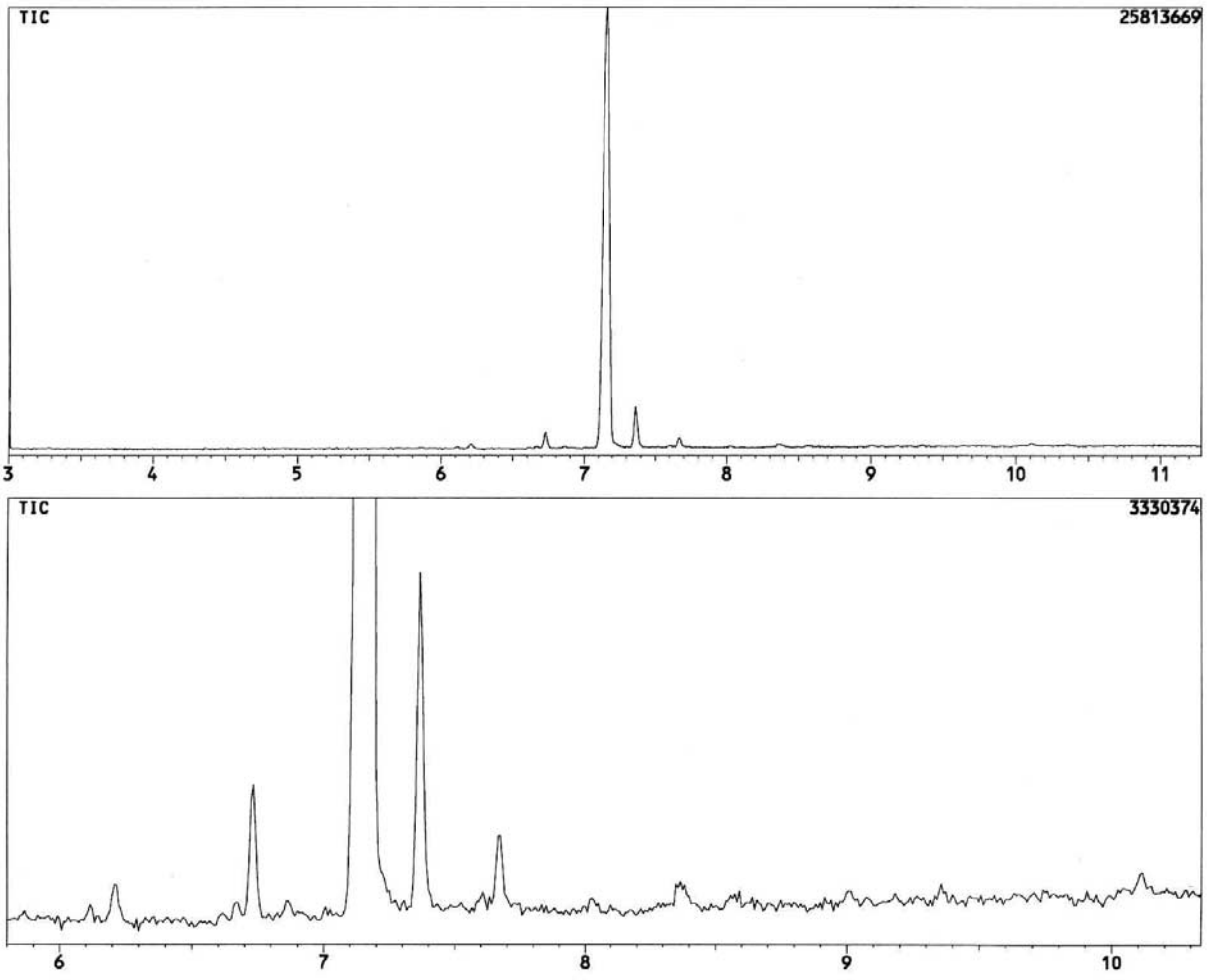
*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D34 00/05/12 12:04:43

Sample : tangerine
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :

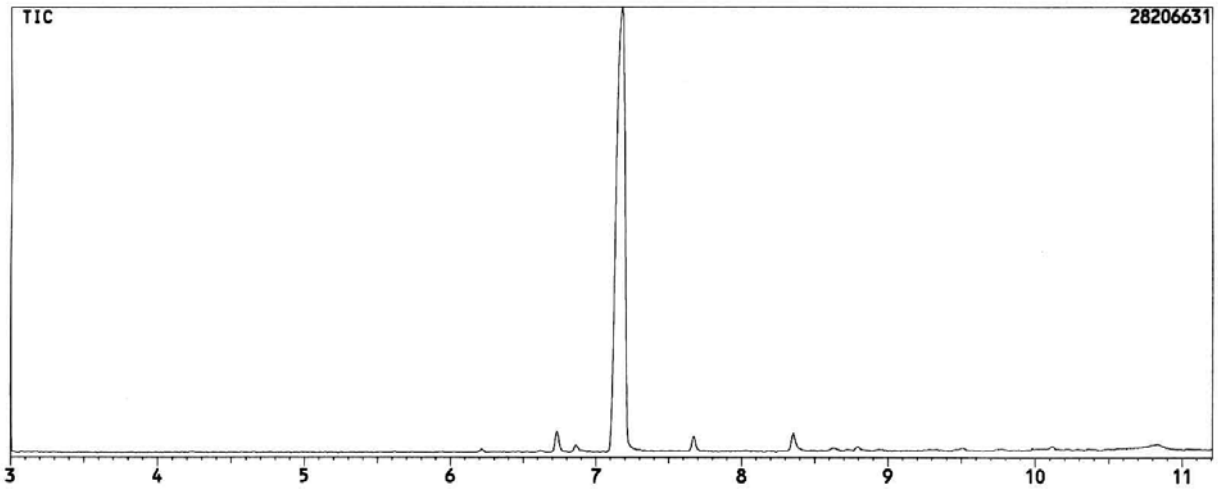


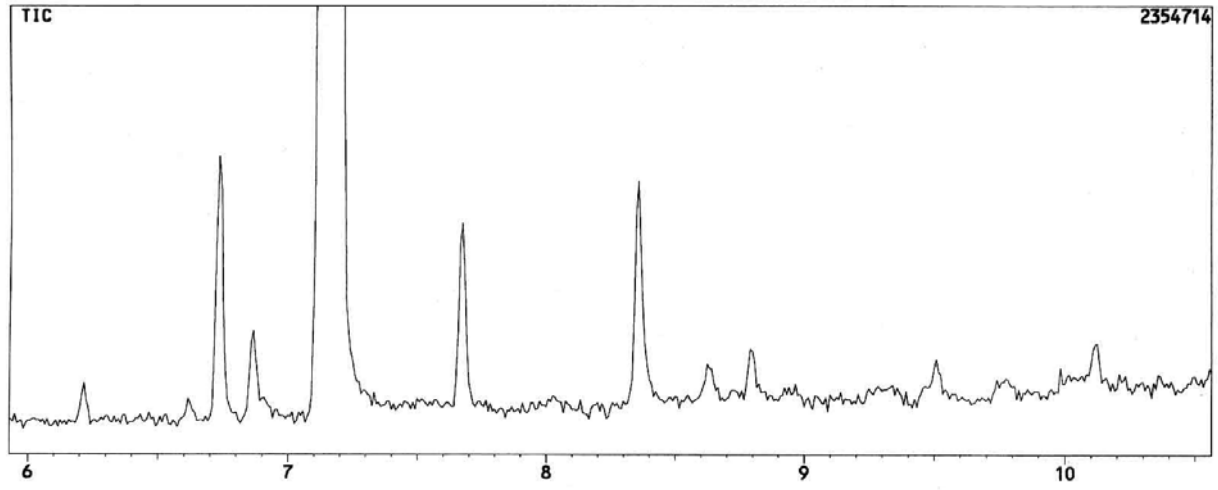


*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D18 00/05/10 17:47:03
Sample : tangelo
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :



*** CLASS-5000 *** Report No. = 1 Data : 223CITR.D16 00/05/10 17:09:45
Sample : valencia orange
ID :
Sample Amount : 1
Dilution Factor : 90
Type : Unknown
Operator : smith
Method File Name : CITRUS.MET
Vial No. : 1
Barcode :





Experiment Preparations:

For a class of twelve students, or twenty four students working in pairs, the following will be required:

A variety of fresh, ripe citrus fruits (one per student or lab group) including: grapefruit, lemon, lime, navel orange and Valencia orange, tangerine, and tangelo.

Cheese grater: four is ideal for this class size.

Aluminum foil or wax paper

125 mL separatory funnel with stopper, one per lab group.

275 mL of pentane

15 g of anhydrous sodium sulfate

2 sand baths placed in a well ventilated area, preferably a fume hood

20 mL of dichloromethane

10 μL GC syringe

Safety:

No flames should be permitted in the laboratory during this experiment.

Each student should wear appropriate eye protection and provided with either latex or vinyl gloves.

Waste:

A non-halogenated waste container, for excess pentane.

A halogentated waste container, for dichloromethane and dichloromethane/citrus oil waste.

A solids waste container for citrus peels (which have been extracted with pentane)

A solids waste container for anhydrous sodium sulfate.

Calculations:

Percent recovery: (mass of citrus oil obtained/mass of citrus peel)x100