Reagent Name	Reqd. PPE	Critical Safety Hazards	Reactivity	Disposal
Limonene	Standard	Skin hazard, inhalation hazard, environmental hazard	Flammable	Hazardous Waste
Methanol	Standard	Toxic	Flammable	Hazardous Waste

Procedure:

1. Label a 100 mL beaker “methanol”.
2. Label eight 10 mL volumetric flasks “G”, “O”, “L”(for grapefruit, orange, and lemon), “S50”, “S25”, “S12.5”, and “S6.25” (for the standards with the concentrations in µg/mL), and leave one without a label.
3. Label a 25 mL volumetric flask “S100”.
4. Gather stoppers for the volumetric flasks.
5. Label three 7 mL vials “G”, “O”, and “L”.
6. Obtain two 5 mL beakers, label one “Limonene”.
7. Pour roughly 75 mL of methanol into the beaker.
8. Obtain a working 5 mL volumetric pipette and bulb for the methanol.

Extraction of Limonene from Fruit

9. Using a razor blade, collect a piece of the rind of a grapefruit, lemon, and orange around a fingerprint size in area into a large weigh boat. Avoid having the white flesh in the sample.
10. Bring the rind pieces and the vials to an analytical balance.
11. From each rind piece, mass and record a roughly 0.1 g sample avoiding the white flesh.
12. Place this rind piece in the appropriate vial.
13. Using the volumetric pipet, transfer 5 mL of methanol into each vial.
14. Shake each vial vigorously for 5 minutes, then let each vial rest for 5 minutes.
15. Using a P500, transfer two 500 µL aliquots from the “orange” vial and one aliquot for lemon and grapefruit to the 10 mL volumetric flask with the same label.
16. Using a transfer pipette, fill the volumetric flasks to the mark with methanol.

Preparation of Standard

$$97\% * 0.842\ \mathrm{g/mL} * \frac{200\mu\mathrm{L}}{25\ \mathrm{mL}} * \frac{153\mu\mathrm{L}}{10\ \mathrm{mL}} = 100.\mu\mathrm{g/mL}$$

17. Pour roughly 1 mL of (R)-(+)-Limonene (Sigma-Aldrich, catalog number 18316, 97% purity) into the 5 mL beaker labeled “Limonene”.
18. Using a P200, transfer 200 µL of the limonene from the beaker into an unlabeled 10 mL volumetric flask.
19. Fill the volumetric flask to the mark with methanol.
20. Pour out about 1 mL of this dilution into an unlabeled 5mL beaker.
21. Using a P200, transfer 153 µL of the dilution from the beaker into the 25 mL volumetric flask labeled “S100”.
22. Mix “S100” by 20x inversion.
23. Using the volumetric pipette, transfer 5 mL of methanol into each of the four 10 mL volumetric flasks for the standards, recovering with the stopper immediately after to avoid evaporation.
24. Use a transfer pipette to fill the “S50” flask to the mark with the contents of the “S100” flask.
25. Mix “S50” by 20x inversion.
26. Use a new transfer pipette to fill the “S25” flask to the mark with “S50”, and mix by inversion. Repeat this process to create the other two standards.

Operation of the GC-MS Machine

27. Label and fill GC vials with two replicates for each standard and one replicate for each fruit.
28. Bring them to the machine.
29. On the attached computer, open a GCMSD1 Enhanced window
30. Click the pencil icon in the "Method" section.
31. Check "Instrument/Acquisition" and leave the other two checkboxes blank.
32. For Inlet and Injection Parameters, ensure the the sample inlet is GC and the injection source is GCALS, and the "Use MS" box is checked.
33. Configure the instrument parameters according to this table.

***Table 1.* Operation Specifications for GC.**

Gas Chromatograph:	**PerkinElmer Clarus 500 GC**		
Analytical Column:	Elite-5ms (30 m x 0.25 mm x 0.25 µm)		
Injector-Port Type:	Capillary		
Injector-Port Temp:	250 °C		
Injection Type:	Split (20 mL/min)		
Syringe Volume:	5 µL		
Injection Volume:	0.5 µL		
Injection Speed:	Fast		
Rinse Solvent:	Methanol		
Carrier-Gas Program:	1 mL/min		
Oven Program:	**Temperature**	**Hold Time**	**Rate**
	80 °C	3 min	5 °C/min
	140 °C	0 min	45 °C/min
	275 °C	Hold	

***Table 2.* Operation Specifications for MS.**

Mass Spectrometer:	**PerkinElmer Clarus 560 D MS**
GC Inlet Temp:	250 °C
Ion-Source Temp:	250 °C
Function Type:	Full Scan
Full-Scan Range:	*m/z* 40-300
Full-Scan Time:	0.15 sec
Interscan Delay:	0.05 sec
Solvent Delay:	2.5 min

34. Click Apply.
35. Click Okay.

36. When it asks for an MS Tune file use “atune u”.
37. Save the method as u521-NW.
38. Go to the pencil in front of blue bottles icon under the sequence bar.
39. Delete any existing information in the sample log table and replace it with this (you can drag down cells to autopopulate with the same value.)

Type	Vial	Sample	Method/Keyword	Data file	Comments
Blank		Solvent Blank	u521-NW	25022700	
Sample		S100A	u521-NW	25022701	
Sample		S100B	u521-NW	25022702	
Sample		S50A	u521-NW	25022703	
Sample		S50B	u521-NW	25022704	
Sample		S25A	u521-NW	25022705	
Sample		S25B	u521-NW	25022706	
Sample		S12.5A	u521-NW	25022707	
Sample		S12.5B	u521-NW	25022708	
Sample		S6.25A	u521-NW	25022709	
Sample		S6.25B	u521-NW	25022710	
Sample		Orange	u521-NW	25022711	
Sample		Lemon	u521-NW	25022712	
Sample		Grapefruit	u521-NW	25022713	

40. Fill out the vial column by loading the samples into the tray and noting which place number they are placed into. Leave the comments column blank.
41. Click ok and select the running man.
42. When the samples have finished, go to file and select the folder with the datafiles.
43. Open each data file and select the area under the peak.
44. Confirm that the spectrogram of the fruits aligns with the spectrogram of the standard.
45. Go to Chromatogram > Percent Report and record the corrected area for each data file.