

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

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Abstract: Rinds from a variety of citrus fruits (grapefruit, lemon, lime, navel and Valencia orange, tangerine, and tangelo) were extracted and analyzed using GC/MS. In this experiment, sophomore organic chemistry students explore extraction techniques and gas chromatography with mass spectrometry detection as well as the theory associated with these methods. Furthermore, the experiment provides students with an opportunity to learn about essential oils and terpenes. Background information on terpenes, the extraction protocol, and GC/MS conditions used for analysis is provided. A summary is given of the most frequently observed terpenes and terpenoids identified for each citrus fruit mentioned above.

Introduction

From an industrial standpoint, the use of GC/MS in the analysis of complex mixtures, both from a qualitative and quantitative standpoint, has become commonplace. Accordingly, there is a greater need to introduce this valuable analytical tool into the undergraduate curriculum. Furthermore, it is essential for students to become familiar with those laboratory techniques that are commonly encountered in nonacademic settings. Gas chromatography, coupled with mass spectrometry detection, has found a variety of analytical uses, which include qualitative analyses of illicit drug samples and forensics evidence [1], trace analyses of pesticides and other toxic residues present in soil and ground water samples [2], and performing quality control analyses in both the pharmaceutical and food product industries [3, 4]. Thus, it has become essential to introduce these basic analytical techniques to students to better prepare them for future careers in industrial settings.

Analysis of essential oils offers a fragrant, interesting, and instructive opportunity for students to explore a variety of experimental and instrumental techniques. These essential oils, derived from natural sources, are found in many common household products, including fruit juices, spices, flavor components (natural flavorings) in beverages and bakery products, and fragrances in incense and many household cleaning products.

Many of these essential oils belong to a family of compounds known as terpenes and terpenoids. Terpenes are small organic hydrocarbon molecules; they may be cyclic or acyclic, saturated or unsaturated. Terpenoids are oxygenated derivatives of terpenes, which may contain hydroxyl groups or carbonyl groups. Regardless of their structural diversity, terpenes and terpenoids share certain structural similarities.

They contain multiples of five carbon atoms (i.e., hemiterpene contains 5 carbon atoms; monoterpene, 10; sesquiterpene, 15; diterpenes, 20; etc.). Rather than having a haphazard arrangement of these carbon atoms, terpenes and terpenoids may be thought to be assembled from isoprene units linked together in a head-to-tail fashion, as demonstrated for the molecule limonene shown in Figure 1 [5].

Terpenes, such as limonene, and terpenoids, such as neral or geranial (the *E* and *Z* isomer of citral, respectively, Figure 2) may be found in abundance in oil sacs located in the outer, colored or flavedo portion of the rinds of many common citrus fruits [6]. These essential oils may be readily isolated through a variety of methods, such as cold pressing [7], steam distillation [8], or extraction [9]. Thus, the isolation and analysis of these essential oils presents the opportunity to introduce students to a variety of laboratory techniques. In addition, it helps to familiarize them with essential theory concerning both gas chromatography and mass spectrometry.

Experimental Section

Preliminary remarks. This experimental protocol has been developed with the understanding that the students will have had one year of general chemistry laboratory. In the interest of time, it is also advisable for students to have already performed and previously been introduced to the concept of extraction (not necessarily solid/liquid extraction). Thus, it will only be necessary to present a prelaboratory lecture regarding terpenes, gas chromatography, and mass spectrometry detection.

Safety. During the experiment, it is advised that students wear their safety goggles and make use of either latex, rubber, or vinyl gloves. Moreover, it is essential that no flames be permitted in the laboratory during the extraction and evaporation portions of this experiment as pentane is very flammable.

Extraction protocol. The extraction method employed is a modification of a method previously reported by Garner [9]. After selecting an appropriate citrus fruit, the rind was freshly grated using the finest texture of a common cheese grater. Care must be taken during the grating of the citrus fruit. It is essential to grate *only* the flavedo, the colored portion of the peel, and avoid abrading the

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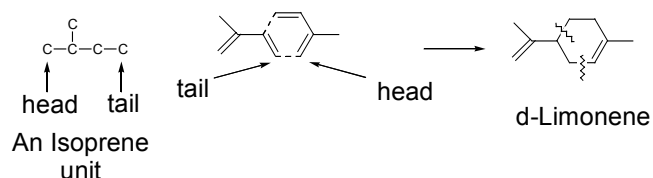


Figure 1. Isoprene units in the formulation of terpenes and terpenoids.

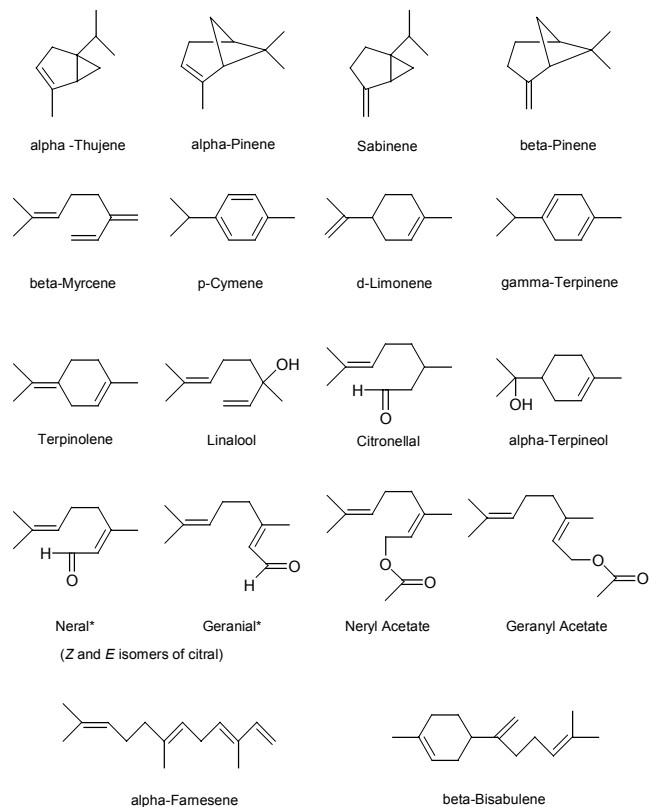


Figure 2. Names and structures of terpenes and terpenoids observed in citrus-fruit extracts. Neral and Geranial represent industrially important quality assurance indicators for cold pressed lemon oils [7].

albedo, or white portion of the inner peel. It is also essential to avoid abrading the pulp to avoid excessive water contamination. Approximately 2.5 g of the finely grated peel was placed into a 125-mL separatory funnel. The rind was then extracted three times with 7-mL portions of pentane for 10-min intervals, being certain to frequently vent the separatory funnel. (Pentane was used for the extraction as opposed to the higher boiling hexane because pentane could be more readily removed by evaporation without risking oxidation of the terpene extracts.) The combined extracts were then dried over anhydrous sodium sulfate (approximately 1 g) for 15 min. The resulting solution was filtered through a powder funnel containing a plug of cotton to ensure complete removal of the sodium sulfate. The extract was then transferred to a tared 50-mL beaker, and solvent removal was accomplished over low heat, ca. 35 °C, (sand bath, in a fume hood) using a *very* gentle stream of air to avoid evaporation of the more volatile components of the citrus essential oils. After evaporation of the pentane, the essential oils were weighed and a percent recovery was calculated. Typical yields for this extraction protocol were between 30 and 130 mg of oil (1.2–5.0% recovery, based upon initial mass of rind used during extraction).

Gas Chromatographic Analysis of Citrus-Fruit Extracts. The crude citrus oil was diluted with 1.0 mL of dichloromethane, and 0.25 μ L of the resulting solution was injected (split injection, 92:1) into the GC/MS. The time interval between evaporation and injection was minimized in order to prevent oxidation of the essential oils.

Table 1. GC/MS Parameters

Oven	
Injection temperature	200 °C
Detector interface temperature	260 °C
Initial temperature	50 °C for 3 min.
Ramp	30 °C per min.
Final temperature	250 °C for 1 min.
Column	
Column length	30 m
Column diameter	0.25 mm
Carrier gas	Helium, 99.9999% purity
Carrier gas pressure	28.7 Kpa
Column flow	0.7 mL per min
Linear velocity	30.6 cm per s
Split ratio	92
Total flow	66.1 mL per min
Mass Spectrometer	
M/z range	20 to 350 amu
Scan interval	0.5 s
Threshold	1000
Scan speed	1000 amu per s
Solvent cut	3 min
Detector	1.0 kV

using an interfaced Pentium II computer running Shimadzu's Class 5000 software, which referenced NIST libraries of mass spectra. (NIST12.LIB and NIST62.LIB, V1.0, P/N 225-01860-93) The capillary column used was a RESTEX XT-5 capillary column (95% dimethyl and 5% diphenyl polysiloxane), 30 m in length, 0.25- μ m i.d., and 0.25- μ m film thickness. For exact instrumental conditions, refer to Table 1.

Results and Discussion

All citrus oil terpenes and terpenoids were identified using an averaged total-ion mass spectrum, corrected for background and compared directly to an NIST database of mass spectra. Moreover, samples obtained from cold-pressed citrus-fruit peels (orange and lemon, courtesy of Sunkist Growers) were analyzed using this method, and the chromatograms were correlated against chromatograms obtained from the Sunkist Growers Analytical Laboratories. Component identities were assigned based both upon comparisons using the NIST database, as well as retention times, in order to accurately identify terpenoid components. A summary of the terpenes and terpenoids observed, their retention times, and their occurrence in a particular citrus fruit are summarized below in Table 2. Please note that seasonal variations, stage of ripeness, and geographical origins of the citrus fruits may result in variations in the total percent recovery of the terpene extract, the relative amounts of terpenes observed, and the likelihood of occurrence of a given terpene [6].

Students were provided with a composite list of all terpene and terpenoid components identified using these methods as well as a corresponding list of standard retention times. Students were then asked to compare their observed retention time values to the standard values and make peak assignments based upon these comparisons (In the absence of a MS detector, this method may be extended to chromatographs which possess either FID or TCD detectors). Alternatively, students could be asked to identify these components using the

Table 2. Common Terpenes and Terpenoids Found Using GC/MS Analysis of Citrus-Fruit Extracts

Component	Retention Time (min)	Grapefruit	Lemon	Lime	Navel Orange	Valencia Orange	Tangerine	Tangelo
α -Thujene	6.050		X	X				X
α -Pinene	6.183	X	X	X	X	X	X	X
Sabinene	6.600	X	X	X	X	X	X	
β -Pinene	6.658		X	X				X
β -Myrcene	6.700	X	X	X	X	X	X	X
<i>p</i> -Cymene	6.842	X			X	X	X	X
<i>d</i> -Limonene	7.133	X	X	X	X	X	X	X
γ -Terpinene	7.358		X	X				X
Terpinolene	7.575		X	X	X			
Linalool	7.642	X	X	X	X	X	X	X
Citronellal	8.008	X	X		X		X	X
α -Terpineol	8.350	X	X	X	X	X	X	X
Neral	8.592		X	X	X	X		
Geranial	8.758		X	X	X	X		
Neryl acetate	9.208		X	X			X	
Geranyl acetate	9.308		X	X				X
α -Farnesene	9.692	X	X	X				
β -Bisabolene	10.042		X		X	X		X

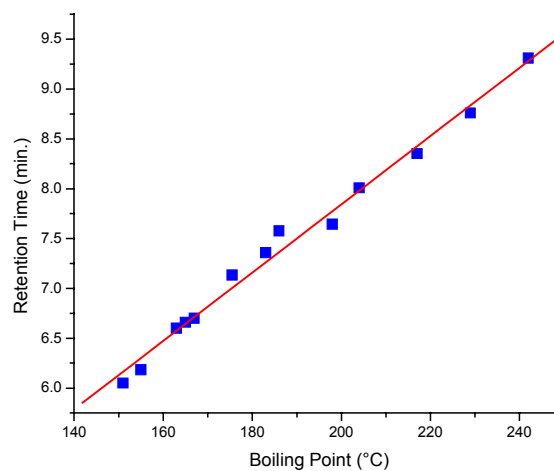
(An X denotes the presence of a particular terpene or terpenoid in the indicated citrus-fruit extract)

Table 3. A Comparison of Retention Times to Available Normal Boiling Point Data

Terpene/ Terpenoid	Retention Time	Normal Boiling Point	Reference
α -Thujene	6.050 min.	151-153 °C	10, p 147
α -Pinene	6.183 min.	155-156 °C	11, p 1940
Sabinene	6.600 min.	163-165 °C	11, p 2085
β -Pinene	6.658 min.	165-166 °C	11, p 1941
β -Myrcene	6.700 min.	167 °C	11, p 1734
<i>d</i> -Limonene	7.133 min.	175.5-176 °C	10, p 94
γ -Terpinene	7.358 min.	183 °C	10, p 95
Terpinolene	7.575 min.	186 °C	11, p 2333
Linalool	7.642 min.	198-200 °C	10, p. 12
Citronellal	8.008 min.	204-205 °C	10, p 15
α -Terpineol	8.350 min.	217-218 °C	11, p 2332
Geranial	8.758 min.	229 °C	11, p 1060
Geranyl acetate	9.308 min.	242-245 °C	11, p 1061

Instrumentation and Conditions. All samples were analyzed using a Shimadzu GC-17A gas chromatograph coupled to a QP5000 mass-selective detector. Parameters were entered and data analyzed library search feature of the supporting software to perform comparisons to those mass spectra present in a database. This perform a more "real-world" analysis in determining the identities of each component; however, this would depend greatly upon instrument availability and student enrollment in the particular laboratory section.

Because organic chemistry laboratory texts explain the separation of a volatile mixture using GC as a function of either the vapor pressure or the boiling points of the individual compounds, a comparison of both the boiling points and the retention times of the terpene components is presented in Table 3. Based upon available data, it can readily be seen that the order of retention times (shortest to longest) gives excellent correlation with the available normal boiling points (lowest to highest) provided for these terpenes. Accordingly, an alternate method of student analysis might be to provide the students with a list of the these boiling points to be utilized in assigning identities to each peak in the chromatogram. A plot of

**Figure 3.** Citrus Oil Terpene and Terpenoid Boiling Point vs. Retention Time

available boiling point data versus retention times is provided in Figure 3.

Conclusion

To date, a total of 62 students, working in pairs in a sophomore organic chemistry laboratory, have performed this experiment. The extraction protocol typically took a total of 1.5 h for completion, and each GC run generally required a total of 11 min for all detected terpenes and terpenoids to elute off of the column. Thus, it is desirable to run this experiment in conjunction with another short experiment or to use the additional time to complete a previous weeks experiment.

Results varied with the care taken in rind grating, fruit ripeness, and extraction techniques [6]. It is essential for all chromatograms to be obtained the day the extraction is performed in order to avoid excessive oxidation of the terpene extracts (*p*-cymene is observed as a result of oxidation of terpene residues). All of the factors listed above may result in decreased yield of essential oils, a reduction in the number of

observed peaks, or a reduction in the intensity of observed peaks.

This extraction and analysis protocol lends itself to a variety of analysis techniques, depending upon available detectors (TCD, FID, or MS). Student analysis of GC data for citrus-fruit extracts may be made based on boiling point, retention time comparisons, or by comparison of mass chromatograms to those present in an NIST or equivalent database program. This would provide students with a more real-world analysis of a industrially relevant sample.

In addition, students could be asked to compare chromatograms from different citrus fruits in order to determine which fruits have the most natural-flavoring components. Moreover, this method might also be used to compare differences in both California and Florida citrus fruits in terms of terpene content or relative percentages of terpene compositions.

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Supporting Material. The student laboratory handout is available as 610028dss1.pdf (<http://dx.doi.org/10.1007/s00897000450b>).

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