APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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The Extraction and Quantification of Limonene from Citrus Rinds Using GC/MS

Introduction

D-Limonene, shown in Figure 1, is a common naturally occurring compound with a citrus scent. It is often used as an additive in food products and fragrances, and is classified by the U.S. Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS)[1]. It has also been approved by the U.S. Environmental Protection Agency (EPA) for usage as a natural pesticide and insect repellent[1]. Limonene has also been studied for its anti-carcinogenic properties[2]. Orange oil, which contains a considerable amount of limonene, has numerous applications including a combustant in engines[3], a powerful degreaser in cleaning applications, and a natural pesticide[4]. These uses may require a known concentration of limonene with a limited amount of impurities. This exemplifies the need for a reliable method of extraction of limonene from its natural source, citrus rinds, followed by a quantitative analysis of the extract for limonene and possible impurities.

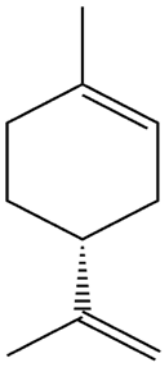

Figure 1. Molecular structure of limonene.

A method for the extraction and quantification of limonene from citrus fruit peels is discussed in this applications note. Beyond demonstrating the use of GC/MS in the analysis of citrus fruit for limonene content, this application demonstrates a simple, inexpensive technique to introduce students to method development, calibration and quantification using a chromatographic technique. The analysis of citrus fruit for limonene may be an ideal laboratory assignment at the undergraduate level. The techniques used are safe, simple and easy.

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Experimental

External Calibration Curve

A limonene standard (SPEX CertiPrep®, Metuchen, NJ) with a concentration of 1000 µg/mL was diluted to 100 µg/mL by a 10:1 dilution with methanol. The remaining solutions were prepared by serial 2:1 dilutions resulting in final limonene concentrations of 50, 25, 12.5, and 6.25 µg/mL.

Extraction of limonene

Samples of lemon, orange, and grapefruit rinds were carefully collected using a razor blade. The samples were checked to ensure that none of the white flesh under the rind was included in the sample, as shown in Figures 2 and 3. The white flesh contributes to the mass of the sample but contains little limonene; this makes the rinds appear to have a lower limonene concentration. Then each sample was cut down to a mass of approximately 0.1 g. The rind samples were each placed in 7 mL vials with 5 mL of methanol. The vials were shaken vigorously for 5 minutes and then allowed to stand for an additional 5 minutes. After the 10-minute extraction was complete, 0.5-mL aliquots of methanol from each vial were diluted volumetrically (20:1 for lemon and grapefruit rinds, and 10:1 for orange rind). These dilutions were necessary in order to prepare solutions with concentrations of analyte within the range of the previously prepared calibration curve.

Analysis and quantification of limonene

The analysis of the standards and samples was performed with a PerkinElmer® Clarus® 560 D GC/MS, using the parameters shown in Table 1. The GC was fitted with a capillary injector port using a 4-mm standard glass liner packed with quartz wool configured for split operation (PerkinElmer Part No. N6121010). A PerkinElmer Elite™-5ms (30 m x 0.25 mm x 0.25 µm) column (PerkinElmer Part No. N9316282) was used throughout; the details of the method are shown in Tables 1 and 2.

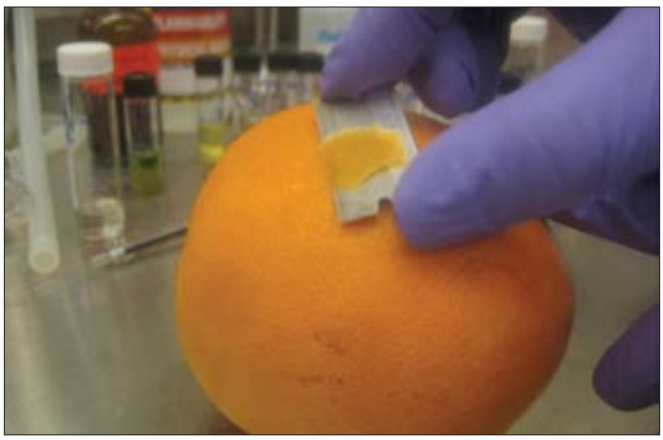

Figure 2. Example of an orange rind being cut with a razor blade.

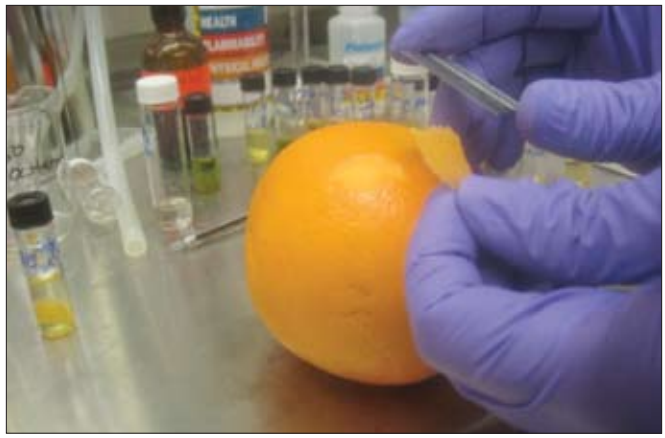

Figure 3. Example of a good sample of orange rind. (None of the white flesh is on the sample).

***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

Discussion

Mass spectra for the limonene standard and limonene in the extract are shown in Figure 4. The limonene spectrum and retention time in the standard matched those of the fruit extract, and a NIST library search also supported the identification as limonene. The chromatogram for *m/z* 136 was chosen for quantification because it is a unique, high *m/z* peak that is relatively abundant; higher *m/z* peaks generally experience a better signal-to-noise ratio.

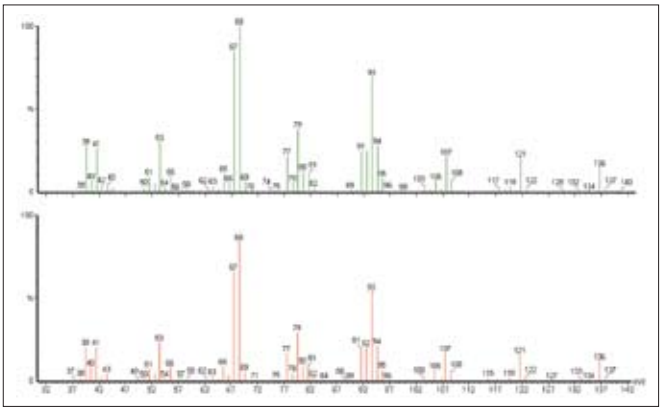

Figure 4. Mass spectrum for limonene in the rind extract (top) and in the standard (bottom).

The extracted *m/z* 136 ion chromatogram for the orange sample is shown in Figure 5. The amount of limonene in each sample was quantified by plotting a calibration curve using the instrument response at *m/z* 136, shown in Figure 6. The linear regression analysis of the calibration curve in Figure 6 yielded Equation 1, which was used to calculate the concentration of limonene in the sample. These concentrations were then used to calculate the concentrations of the undiluted solutions, which were then used to determine the wt/wt % of limonene in each fruit's rind; these results are shown in Table 3.

Equation 1: $y = 941.4172x + 2317.1604$

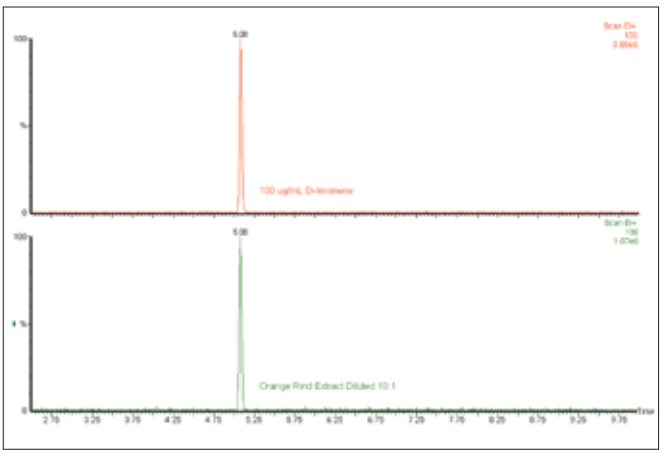

Figure 5. The extracted ion chromatogram for the 100 µg/mL limonene standard (top) and the diluted orange extract (bottom) at *m/z* 136.

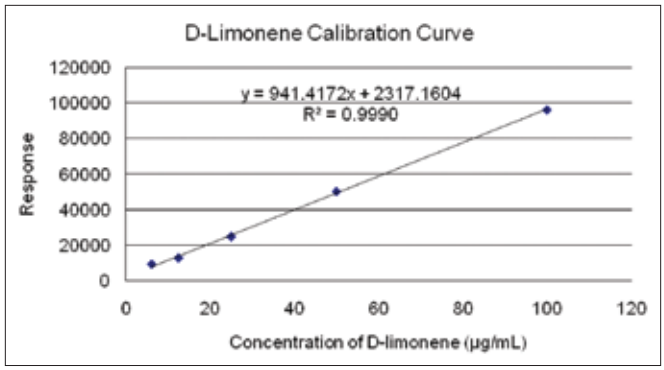

Figure 6. Calibration curve of limonene used to quantify the samples.

***Table 3.* Intermediate and Final Results for the Analysis of Lemon, Grapefruit and Orange Rinds.**

	Diluted Conc. (µg/mL)	Undiluted Conc. (µg/mL)	Mass Extracted (µg)	Mass of Sample (g)	% wt/wt
Lemon	35.66	713.2	3566	0.1199	2.97
Grapefruit	44.55	891.0	4455	0.1557	2.86
Orange	35.62	356.2	1781	0.1096	1.63

Conclusion

This application note demonstrates a simple extraction and quantification method for limonene using GC/MS. The limonene extraction and calibration curve preparation were discussed, as well as the method for analysis. The results obtained by following this method were presented along with the final %wt/wt of oil in the rinds. It was discovered that while all of these fruits had limonene in their rinds, lemon contained the highest concentration. Students conducting this analysis will gain valuable experience in sample preparation, solid-liquid extractions, and one of the most sensitive analytical techniques for the analysis of volatile compounds.

References

1. Limonene. R.E.D. Facts. 1994. USA EPA: http://www.epa.gov/oppsrrd1/REDs/factsheets/3083fact.pdf

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3. Cyclone Power Technologies Inc. *Why It's Better* 2009. http://www.cyclonepower.com/better.html

4. Orange Oil: http://en.wikipedia.org/wiki/Orange_oil

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