Quantification of Limonene from Essential Oil Emissions using Thermal Desorption GC-MS Analysis

Application Note
Indoor Air Quality

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Abstract
This application note demonstrates a method for sampling and analyzing volatile organic compound (VOC) emissions of essential oils using thermal desorption (TD) GC-MS. As part of this work, VOC emissions from three different types of grapefruit essential oil were examined. Limonene, which is known to be the primary VOC emitted from grapefruit and other citrus plants, was directly quantified from emissions of in-home diffusers. For this work, limonene concentrations as high as 27 ppbv were found near the outlet of the diffuser. TD-GC-MS analysis was supplemented with headspace sampling GC-MS to further probe essential oil chemical composition. The results provide greater context into the ways in which consumer products influence indoor air quality and human health.

Introduction
Indoor air quality is both a dynamic and complex matrix filled with suspended particulate matter (PM) and volatile organic compounds (VOCs), both of which directly affect human health via inhalation to varying depths of the respiratory tract. VOCs, however, also indirectly affect human health through secondary reactions in the air that contribute to the formation of new aerosols in an indoor environment. This secondary pathway is not as readily detectable, so the affects of this pathway are often not considered by users consumer products. VOCs are ubiquitous to all types of consumer products including personal care products, cleaning products, and essential oils and fragrances with the latter being the primary interest of this application.[1]

The effects of essential oils are further debated because many essential oils are thought possess aromatherapy benefits that include stress relief, headache relief, and asthma relief. In a market that is saturated with many brands of essential oils, purists are also interested in essential oil composition to identify oil adulteration. One of the most common ways to introduce essential oils into the air is through in-home diffusers. Before essential oils can fully accepted into standard health care practices, greater understanding is needed about the concentration of VOCs emitted from essential oils and their composition.[2-5]

In this application note, VOC emissions from three different types of grapefruit essential oils were examined. The essential oils were diffused into an indoor setting using two common diffusers available on Amazon. One is a nebulizing diffuser and the other an ultrasonic diffuser. VOC emissions were sampled onto conditioned thermal desorption tubes using a CDS Air Sampling device, which comes with a built-in, adjustable vacuum pump to actively sample air through the thermal desorption tube. After the completion of sampling, thermal desorption was conducted using the CDS 7550S automated Thermal Desorber, which is combined with GC-MS. Headspace GC-MS, performed with the assistance of the CDS 6150 Pyroprobe, was used to detect the presence of any larger chemical components in the essential oils. The limonene emissions were quantified for each essential oil and the overall differences in chemical composition between oils was assessed.

Table 1:

7550S Thermal Desorber:

Valve oven:	300 °C
GC transfer line:	250 °C
Tube purge flow:	40 mL/min
Tube Rest temp.:	40 °C
Tube Dry purge temp.:	40 °C
Tube Dry purge time:	0.3 min
Tube Desorb temp.:	300 °C
Tube Desorb time:	2 min
Sample tube:	carbograph 2/carbograph 1/ carboxen 1000
Trap Rest temp.:	-10 °C with Peltier
Trap Desorb temp.:	300 °C
Trap Desorb time:	2 min
Trap Type:	Vocarb 3000
Peltier transfer line:	250 °C

GCMS QP-2010

GC conditions:

Column:	Restek Rxi 5Sil MS
Oven temp.:	35.0 °C
Injection temp.:	240 °C
Injection mode:	Split
Column Flow:	1.00 ml/min
Split Ratio:	30.0 : 1
Temp. program:	35.0 °C hold 4 min 10.0 °C/min to 150.0 °C 50.0 °C/min to 320.0 °C Hold 3.10 min

MS conditions:

Ion Source:	200.00 °C
Interface Temp.:	220.00 °C
Start m/z:	35.00
End m/z:	260.00

Experiment Setup

Three types of grapefruit essential oils, all of which are available on Amazon, were selected for this application. One oil (Oil A) was selected due to positive customer reviews while another oil (Oil B) received negative customer reviews. The third oil is labeled and marketed as a 'Fragrance Oil.'

One nebulizing diffuser and one ultrasonic diffuser, both of which a popular types of diffusers, were also used for this study. Both are also available on Amazon. Each diffuser was set for low output for each of the experiments. For the ultrasonic diffuser, 5 drops of the pure essential oil were added to 60 mL of water, which is consistent with the manufacturer recommendations. The sampling time was 20 min, which is a manufacturer suggested maximum operating time. The nebulizing diffuser instead atomizes the pure essential oil directly, so a 5 min sampling time was used. The sampling was done with the assistance of a CDS Air Sampling device containing a built-in vacuum pump. A flow rate of 700 mL/min was used for sampling, which is the

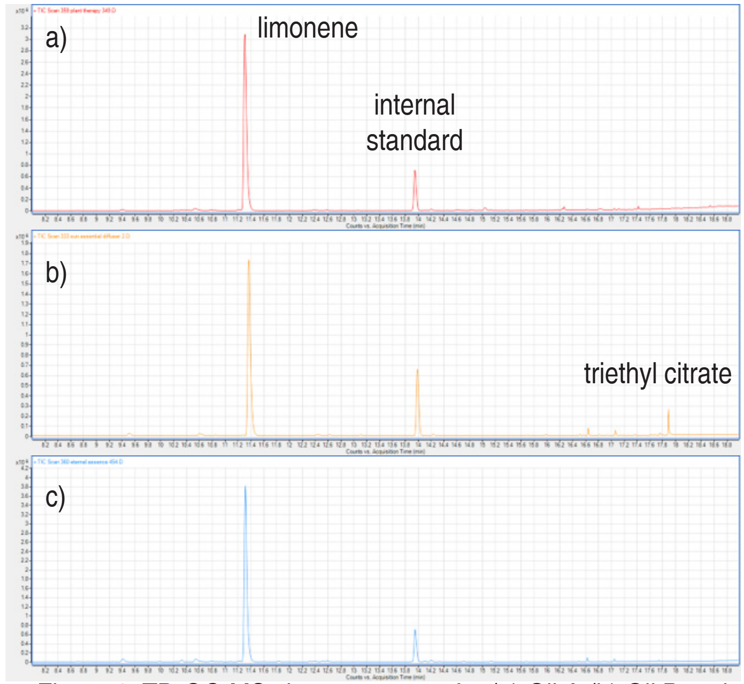

Figure 1. TD-GC-MS chromatograms for (a) Oil A (b) Oil B and (c) Fragrance Oil sampled from the nebulizing diffuser for a total time of 5 minutes.

maximum permissible flow rate with the sampling device. The thermal desoprtion tube attached to the sampling device was place 6” from the outlet of each diffuser.

A calibration curve was prepared by spiking thermal desorption tubes with 1 μL of limonene standards varying in concentration between 90 and 700 ppb. A CDS 7550S automated thermal desorber was employed with the Peltier cold trap option. Prior to loading all calibration tubes and sample tubes onto the thermal desorber, the tubes were spiked with 1 μL of 90 ppb naphthalene-d8, which served as the internal standard. Each tube was then flushed for 2 min with 100 mL/min of dry nitrogen gas to disperse the liquid standard. GC-MS was performed using a Shimadzu QP 2010. TD-GC-MS analysis was performed according to the parameters in Table 1.

To compliment the TD-GC-MS analysis of VOCs, the pure, undiluted essential oil liquid was analyzed via headspace sampling the with CDS 6150 Automated Pyroprobe coupled with GC-MS. The headspace analysis was performed by spotting approximately 1 μL of pure oil onto a piece of filter paper. A 1 mm disc was then punched from the filter paper and placed into a quartz pyrolysis tube. For this application, a pyrolysis temperature of 400°C was found to be sufficient for providing complimentary compositional information to the TD-GC-MS analysis. The CDS 6150 operating parameters are provided in Table S1.

Results and Discussions

Three types of grapefruit essential oils were diffused using the nebulizing and ultrasonic diffusers and sampled using the CDS Air Sampler and analyzed with the 7550S automated thermal desorber. Figure 1 shows an example chromatogram for each of the three types of oil. Limonene is the dominant VOC observed in each of the chromatograms, which was expected as limonene is already known be the primary VOC naturally occurring citrus plants. It is important to note that other biogenic VOCs, such as α-pinene and β-pinene, were also observed in each chromatogram, but their intensities were negligible relative to limonene in the emissions of the diffused oil.[5]

While no significant compositional differences were found between Oil A and the Fragance Oil with TD-GC-MS analysis, triethyl citrate was detected in Oil B. Triethyl citrate is frequently used as PVC plasticizer and food additive. Plasticizers, such as triethyl citrate, are sometimes added to essential oils to dilute the oil and reduce the product cost. Oil B is reviewed on Amazon as having lower quality, and customer reviews frequently cite a perceivable, negative change in the fragrance of the oil due to additives.

Figure 2 shows the calibration curve for limonene, along with the measured limonene mass for each essential oil with the nebulizing diffuser. Additionally, emitted limonene mass for Oil A is shown for the ultrasonic diffuser. Table 3 shows the calculated limonene concentration as well as the emission rate for each oil from the diffuser. For Oil A and the Fragrance Oil, the limonene concentration was found to be as high as 27 ppbv, which corresponds to an emission rate of 5.4 ppbv/min. This emission rate is nearly twice as high as it is for Oil B, which was expected due to the presence of triethyl citrate in Oil B. Comparatively, the emission rate of Oil A from the ultrasonic diffuser was 0.2 ppbv/min. A similar rate has been previously reported for an ultrasonic diffuser spiked with various essential oils.[6] Under the conditions of the experiments performed here, the nebulizing diffuser produced between 19 and 36 times higher emission rates of limonene than the ultrasonic diffuser.

Although limonene is the primary VOC present in citrus, limonene also is naturally emitted, in lower concentrations, from other species of broad-leaved and coniferous tress. Peak outdoor concentrations of limonene in the mid-Atlantic region of eastern United States, where CDS Analytical headquarters is located, is 0.2 ppbv. This is up to 135 times lower than the limonene emitted from the nebulizing diffuser in this application. Despite low outdoor concentrations, limonene is understood to be one of the most reactive biogenic VOCs due to both endo- and exocyclic double bonds and accounts for 80% of daytime reactivity with atmospheric ozone. Through the use of consumer products such as essential oil diffusers, the reaction rates of highly reactive VOCs, such as limonene, would be expected to increase significantly in an indoor environment.[7]

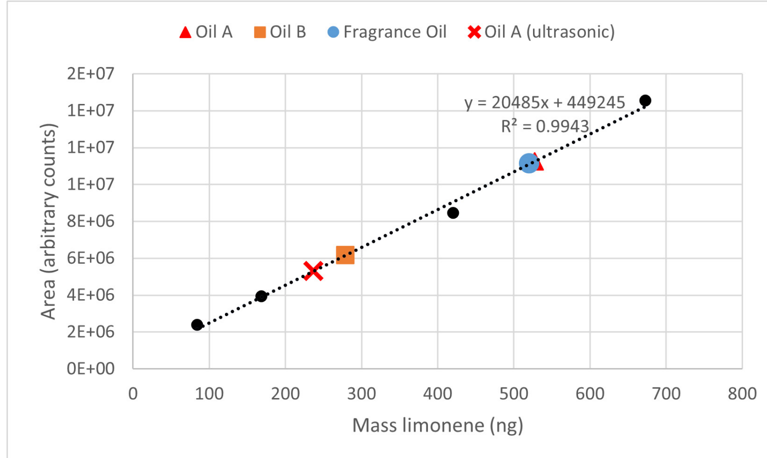

Figure 2. Calibration curve for limonene and the sampled mass for each of the three essential oils with the nebulizing diffusers and Oil A with the ultrasonic diffuser (n=3).

Table 3. Concentration (ppbv) and emission rate (ppbv/min) for each of the three essential oils with the nebulizing diffuser and Oil A with the ultrasonic diffuser.

	Concentration (ppbv)	Emission Rate (ppbv/min)
Oil A	27± 5	5.4 ± 0.9
Oil B	14 ± 3	2.8 ± 0.6
Fragrance Oil	27 ± 0	5.4 ± 0.0
Oil A (ultrasonic)	3 ± 1	0.15 ± 0.04

Lastly, the CDS 6150 Pyroprobe was used to conduct headspace sampling to scan for the presence of any larger organic components that may have been incorporated into any of the three grapefruit essential oils. While Oil A and Oil B showed no extra additives to the oil, the Fragrance Oil (Figure 3) showed the presence of bis-(2-ethylhexyl) ester hexanedioic acid, or DEHA ($C_{22}H_{42}O_4$). The corresponding chromatogram for headspace sampling conducted at 400°C is shown in Figure 3. DEHA is known to be used as a PVC plasticizer and is also used in food packaging and cosmetic products.

Conclusions

This application note demonstrates how methods for sampling and TD-GC-MS analysis can be used for quantifying VOCs emitted from consumer products, such as essential oil diffusers. Limonene emissions were quantified for three types of grapefruit essential oil with both a nebulizing and ultrasonic diffusers. Even when set to low output, the nebulizing diffuser was found to emit 36 times more limonene than the ultrasonic diffuser. This is also 135 times more than peak outdoor concentrations of limonene in the eastern United States. The use TD-GC-MS analysis here provides additional insight to how the use of consumer products may effect overall indoor air quality.

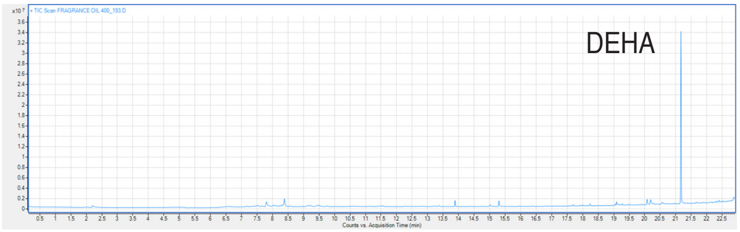

Figure 3. Headspace GC-MS collected with the CDS 6150 Pyroprobe for grapefruit Fragrance Oil at 400°C.

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Table S1:

6150 Pyroprobe headspace analysis

Valve oven:	300 °C
GC transfer line:	325 °C
Initial Temp.	Ambient
Final Temp.	400 °C
Temp. Ramp Rate	Maximum

Thermo 1310 ISQ GC-MS

GC conditions:

Column:	Restek Rxi 5Sil MS
Oven temp.:	40.0 °C
Injection temp.:	360 °C
Injection mode:	Split
Column Flow:	1.25 ml/min
Split Ratio:	75 : 1
Temp. program:	15.0 °C/min to 320.0 °C Hold 2min

MS conditions:

Ion Source:	230.00 °C
Interface Temp.:	300.00 °C
Start m/z:	35.00
End m/z:	600.00