Comparison of Two Lemon Oils by GC-TOFMS

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Key Words: TOFMS, Quantification, Extended Range Calibration

1. Introduction

Lemon oil is an essential component in many food, flavor, and fragrance consumer products. The purpose of this study was to compare cold-pressed lemon oils from two different geographical sources, Argentina and California. The components β-pinene, limonene and γ-terpinene were identified by Chamblee et al (1991) as the major components of Lemon Oil and are the focus of this study.

2. Instruments and Methods

In this study, measurements were made with a LECO Pegasus® HT GC-TOFMS system. This system consists of an Agilent 6890 gas chromatograph with a LECO Pegasus HT Time-of-Flight Mass Spectrometer (TOFMS) as a detector. For all analyses in this study, the analytical column was a 10.2 m x 0.18 mm ID x 0.18 μm df DB-5. Helium was used as the carrier gas at a constant flow of 1.0 mL/minute. The transfer line to the TOFMS consisted of the last 20 cm of the analytical column. The detector was optimized to have a S/N ratio of ≥10 for a 2 pg/μL injection of hexachlorobenzene with a detector voltage of 1850 volts.

For non-quantitative analyses, 1 μL samples of each lemon oil were diluted 1:5 in dichloromethane (DCM) for minor peaks, and 1:1000 in DCM for the major peaks. Samples were then injected using the split mode of the split/splitless inlet with a split ratio of 100:1 for minor peaks, and 200:1 for the major peaks. For quantitative analyses, the individual lemon oils were diluted in DCM to generate calibration curve standards ranging from ~8.5 pg/μL to ~85 ng/μL for each of the major components. Standards for the calibration curve consisted of three 1 μL injections made in splitless mode.

3. Results

The variation in concentration between the three major peaks and the remaining components necessitated two analyses for each lemon oil. In the first, the lemon oil samples were diluted 1:5 in DCM and the minor peaks were observed (see Figure 1).
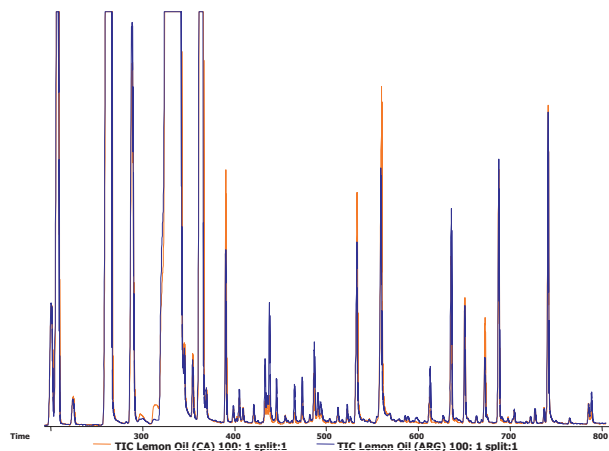

Figure 1: Overlay of the TIC's showing minor peaks of two different lemon oils demonstrating excellent reproducability of retention times.

For the major peaks, the lemon oil was diluted 1:1000 in DCM so that the three major peaks were in the linear range of the calibration curve (see Figure 2). Due to detector saturation at higher concentrations, extended-range calibration curves were used. The extended-range calibration curve allows for compensation for the saturation of the detector at higher concentrations through the use of ions that are less abundant. This prevents detector saturation, causing the curve to be non-linear at higher concentrations. For β-pinene, from 8.49 pg/μL to 849 pg/μL, m/z 93 was used for quantification, and the less intense m/z 77 was used from 849 pg/μL to 84.9 ng/μL. The R^2 for the lower portion of the curve was 0.9999 and the R^2 for the upper portion of the curve was 0.9939.
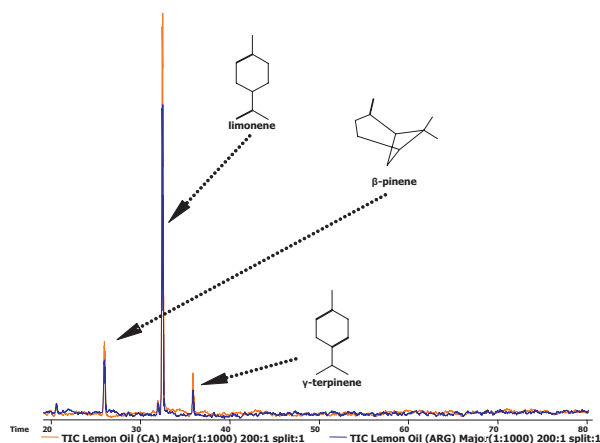

Figure 2: Overlay of the TIC's showing the major peaks of two different lemon oils.

Figure 3 shows TOFMS spectral information for the library identification match compared to that of the limonene standard used in the calibration curve.

Table 1 shows the reported concentrations of each of the three major components for each type of lemon oil. The concentrations obtained from the calibration curve (in pg/μL) are then corrected to account for sample dilution and the actual concentration is reported in mg/μL. Quantitation is automatic once the appropriate associated calibration curve is completed. The percentage contribution of each peak to the total area of the three components is also listed.

Table 1: Quantification Results for the Major Components in Lemon Oil

Cold-pressed Californian Lemon Oil: Major Components			
Compound	Reported Conc. (pg/µL)	Conc. Corrected for Dilution (mg/ µL)	% Contribution
β-pinene	663.53	132.71	14.88%
limonene	3571.57	714.31	80.09%
γ-terpinene	224.35	44.87	5.03%

Cold-pressed Argentinean Lemon Oil: Major Components			
Compound	Reported Conc. (pg/µL)	Conc. Corrected for Dilution (mg/ µL)	% Contribution
β-pinene	416.74	83.35	13.53%
Limonene	2576.36	515.72	83.72%
γ-terpinene	84.78	16.96	2.75%

Peak True - sample "Lemon Oil (ARG) Major(1:1000) 200:1 split:1" peak 4, at 324.676 s

(A)

Reference Spectrum - Calibration "curve 3 points extended" Analyte

(B)
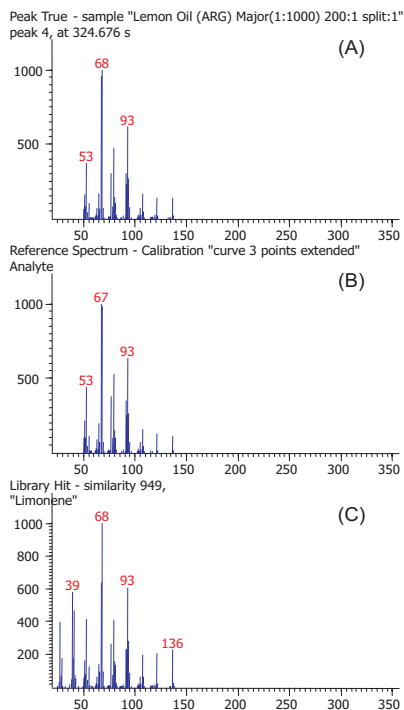
Library Hit - similarity 949, "Limonene"

(C)

Figure 3: Mass Spectral Identification. The deconvoluted spectrum is shown as the "Peak True" in (A). The reference spectrum from the associated calibration curve is shown in (B). The spectrum of the library hit from the NIST-MS library is shown in (C).

Figure 4 shows an example of how True Signal Deconvolution™ can be used to identify coeluting peaks where a single channel detector shows only one peak, as in the Total Ion Count (TIC) trace.
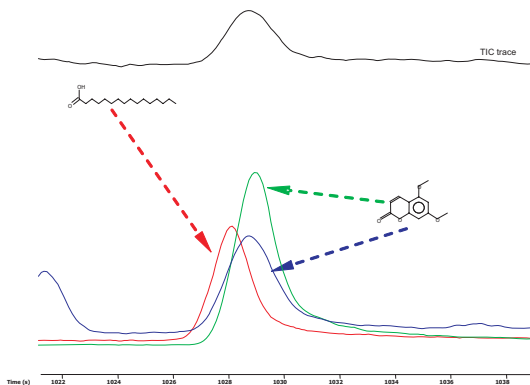
TIC trace

Figure 4: Example of True Signal Deconvolution of coeluting peaks utilizing ions unique to each peak. The ability to deconvolute these peaks using unique ions is made available by using the full-range mass spectra, and the necessary data acquisition rate (20 spectra/second) available from TOFMS.

Figure 5 shows a chromatogram of the minor peaks of Argentinean lemon oil. Sample TOFMS structure identifications of some peaks are shown. These identifications were made using the NIST-MS database with a spectral similarity score of at least 850 out of a possible 1000.
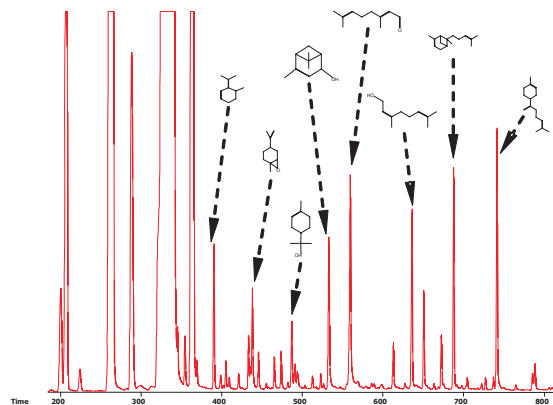
Figure 5: TOFMS structure identification of selected minor peaks.

4. Conclusions

Essential oils are complex samples that present challenges to the chromatographer. In order to attempt to minimize the amount of coelutions, longer analysis times would be necessary. The ability to deconvolute coeluting peaks rather than having to chromatographically separate all peaks greatly decreases analysis time and increases throughput. GC-TOFMS is advantageous in that its high full-range spectral acquisition rate allows for the True Signal Deconvolution of coeluting peaks. The ChromaTOF® software package used in conjunction with the LECO Pegasus GC-TOFMS automatically deconvolutes the obtained spectra during data processing. The TOFMS also shows an excellent dynamic range. In this study, components displayed S/N ratio of >250 for concentrations in the low pg/µL range. The use of the NIST-MS library also allows for the possibility of identifying components without having to run the associated standard for identification.