Differential Analysis of Olive Oils with Pegasus® GC-HRT and ChromaTOF-HRT® Reference Feature

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Olive Oil, GC-HRT, HS-SPME, ChromaTOF Reference, Food Fraud, High Resolution, Accurate Mass

1. Introduction

Characterization of food products, including differential analysis, is important in the food industry for quality control, brand awareness, and authentication of materials and products. GC coupled with high resolution TOFMS isolates individual analytes within complex matrices through both chromatographic and mass spectral resolution and provides confident analyte identifications with industry-leading mass accuracy. These analytical tools were employed for a non-targeted volatile analysis of aroma and flavor analytes in representative extra-virgin and light olive oil samples from a single manufacturer. Automated Peak Finding and sample comparison tools, such as "Reference," within the ChromaTOF-HRT® software provide rapid sample characterizations and differentiation to help researchers reliably determine what else is in their sample and if it should be there. These analysis tools were used to highlight and confidently identify differences between the samples which are important in differentiating oil varieties and in determining the source of unanticipated analytes in the samples. These capabilities may also facilitate detection of food fraud by readily distinguishing extra-virgin from light olive oil and other edible oils.

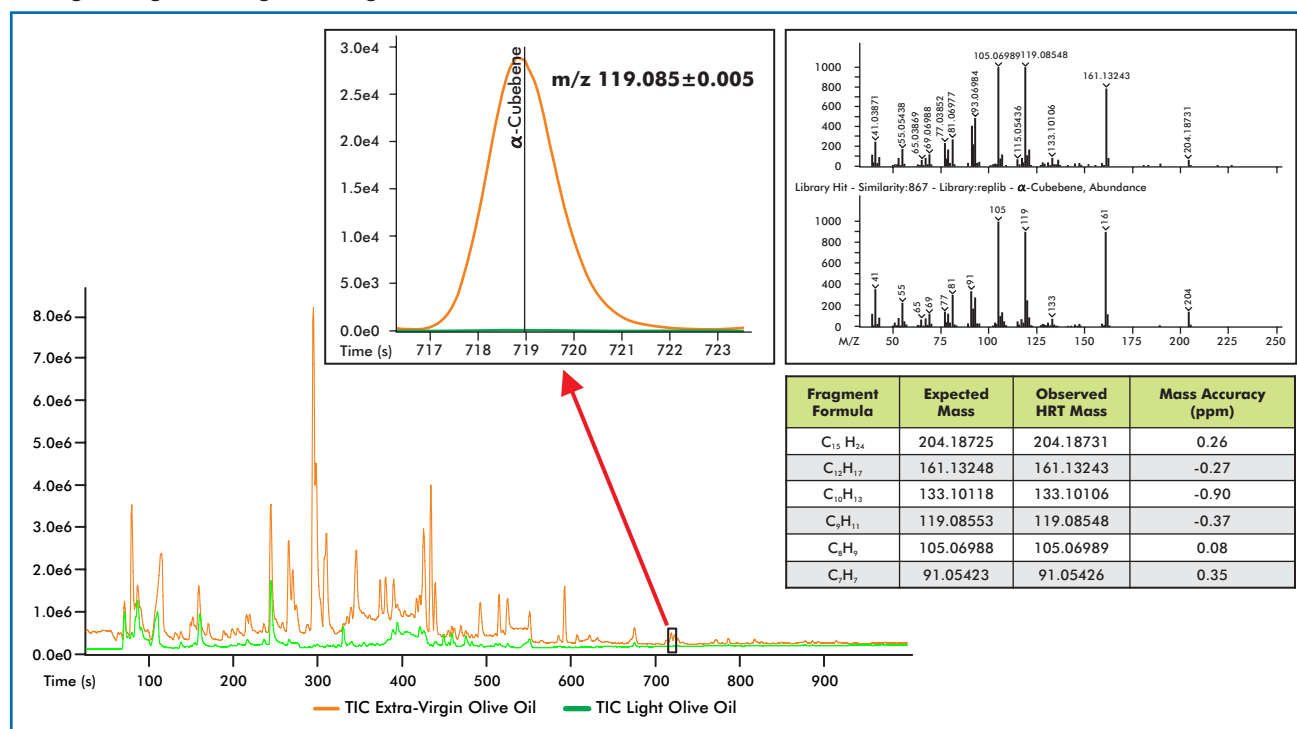| Fragment Formula	Expected Mass	Observed HRT Mass	Mass Accuracy (ppm)
$C_{15}H_{24}$	204.18725	204.18731	0.26
$C_{12}H_{17}$	161.13248	161.13243	-0.27
$C_{10}H_{13}$	133.10118	133.10106	-0.90
C_9H_{11}	119.08553	119.08548	-0.37
C_8H_9	105.06988	105.06989	0.08
C_7H_7	91.05423	91.05426	0.35

Figure 1. Representative TIC chromatograms are overlaid for extra-virgin olive oil (orange trace) and light olive oil (green trace) from the same manufacturer. Many analyte differences are apparent and ChromaTOF's Reference feature was utilized to determine analytes unique to each sample with automated processing. α-Cubebene was found in the extra-virgin olive oil and not in the light olive oil. This analyte, indicative of extra-virgin olive in these comparisons, is highlighted with an XIC chromatogram of m/z 119.085±0.005. The deconvoluted and library spectra are shown with a spectral similarity score in the 800s. The accurate mass information adds confidence to this identification with excellent agreement of the expected and observed HRT masses for the molecular ion as well as several fragment ions. The mass accuracies are all below 1 ppm.

2. Experimental

Samples: Extra virgin and light olive oils from a single manufacturer were analyzed. Two grams of each sample were pipetted into 10 mL SPME vials and sealed with septum caps.

HS-SPME conditions: HS-SPME sampling was performed with a divinylbenzene/carboxen/polydimethylsiloxane (50/30 μm DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) fiber. Each sample was incubated at 40°C for 5 min prior to 25 min of extraction at the same temperature.

| GC-TOFMS (Pegasus® GC-HRT) Conditions	
Injection	SPME desorption for 2 min @ 250°C
Carrier Gas	He @ 1.5 ml/min
Column	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 μm coating (Restek, Bellefonte, PA, USA)
Temperature Program	2 min at 40°C, ramped 12°C/min to 250°C, held 3 min
Transfer Line	Temperature set to 250°C
Acquisition Mode	High Resolution, R = 25,000 (FWHM)
Mass Range	33-400 m/z
Acquisition Rate	6 spectra/s
Source Temp	250°C

Data analysis: Data were analyzed with LECO'S ChromaTOF-HRT software. ChromaTOF's Reference feature was employed as a feature selection tool to determine analyte differences between the extra-virgin and light olive oils. This analysis tool computes the relative concentration of analytes in a sample with respect to a user-specified reference and classifies each analyte peak to indicate the similarity to the reference. Analytes that are present in both the reference and the sample are tagged "Match" or "Out of Tolerance" depending on their relative concentrations compared to a user-specified threshold. "Not Found" and "Unknown" indicate analytes that are only present in the reference or the sample, respectively.

3. Results and Discussion

Characterization and differentiation of extra-virgin and light olive oil samples from the same manufacturer were explored with LECO's Pegasus GC-HRT. Analytes in these samples were determined with ChromaTOF's automated processing tools and many compound types were detected, including esters, alcohols, aldehydes, ketones, and terpenes, etc. These types of analytes have important flavor and aroma properties and may be useful to understanding the sensory properties of the samples.

Additionally, representative TIC chromatograms for each sample were overlaid in Figure 1 and many differences are apparent. The Reference feature in the ChromaTOF-HRT software was utilized to further characterize the differences in an automated way. This tool aids with feature selection to identify sample-distinguishing analytes that may become candidates for detecting food fraud. In this case, the Reference feature was applied with the extra-virgin olive oil specified as the reference and the light olive oil as the sample. Peaks in the sample classified as "Not Found" or "Out of Tolerance" (with small concentration values) are indicative of extra-virgin olive oil. Conversely, "Unknown" and "Out of Tolerance" (with large concentration values) are indicative of light olive oil.

The analyte highlighted in Figure 1, α-cubebene, is a sesquiterpene with herbal odor properties that was an example of a "Not Found" analyte (present in extra-virgin, but not in light olive oil). The XIC chromatogram shows the analyte was only measured in extra-virgin olive oil and not the light olive oil. Two "Out of Tolerance" examples with low and high concentrations are shown in Figures 2 and 3, respectively. Phenylethyl alcohol is a compound that was "Out of Tolerance" with a low concentration value (14.74%) and heptane is an "Out of Tolerance" compound with a high concentration value (535.4%) relative to the reference. Phenylethyl alcohol has floral odor properties and was indicative of extra-virgin olive oil while heptane has sweet and ethereal odor properties and was indicative of the light olive oil. In each case, identification was determined by matching to NIST library databases and the accurate mass information for the molecular and fragment ions provided confidence, with mass accuracies less than 1 ppm.
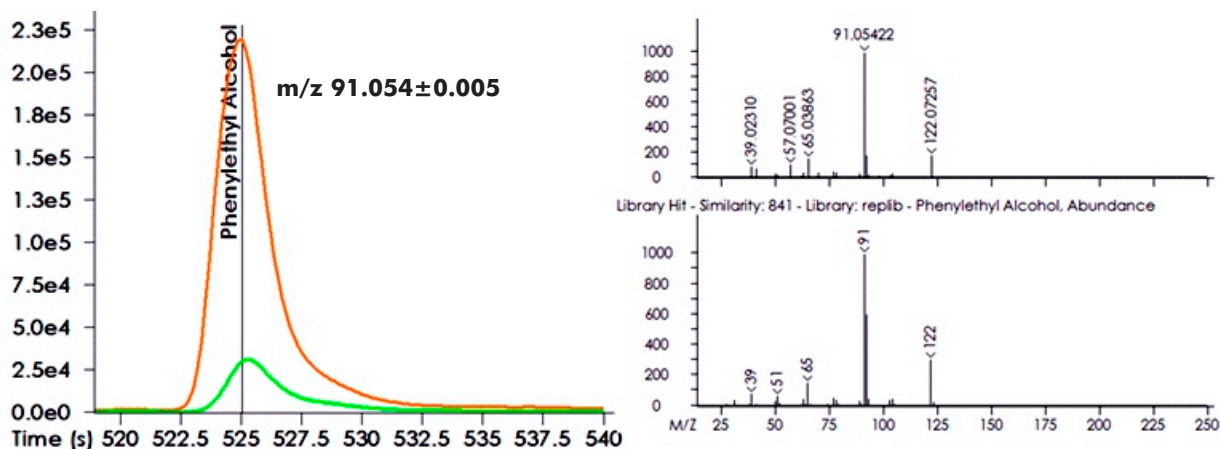
Fragment Formula	Expected Mass	Observed HRT Mass	Mass Accuracy (ppm)
$C_8H_{10}O$	122.07262	122.07257	-0.40
C_7H_8	92.06205	92.06203	-0.26
C_7H_7	91.05423	91.05422	-0.04
C_5H_5	65.03858	65.03863	0.77

Figure 2. Phenylethyl alcohol, an "Out of Tolerance" analyte indicative of extra-virgin olive oil, is shown. XIC 91.054±0.005 chromatograms show the analyte present at higher levesls in extra-virgin olive oil (orange) than in the light olive oil (green). The deconvoluted and library spectra are shown, as well as accurate mass information for the molecular and fragment ions.
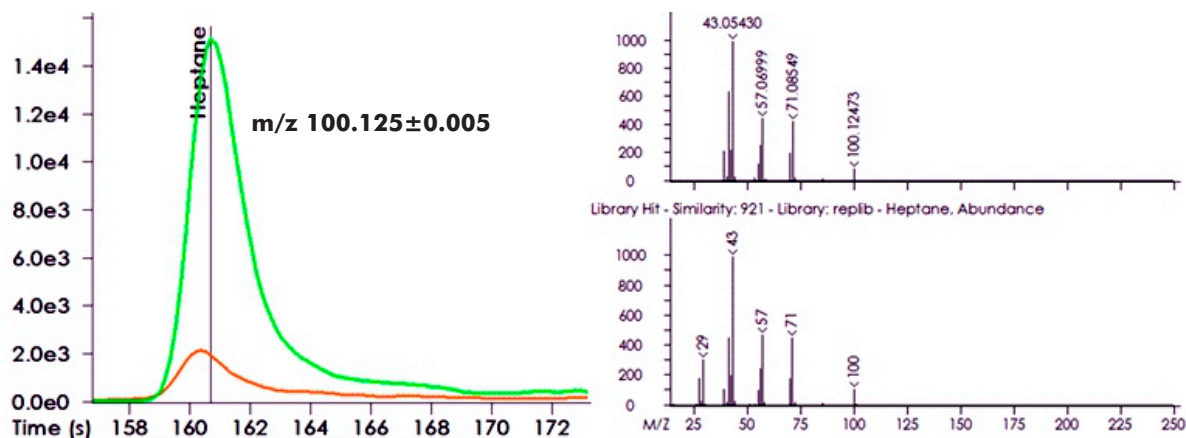
Fragment Formula	Expected Mass	Observed HRT Mass	Mass Accuracy (ppm)
C_7H_{16}	100.12465	100.12473	0.82
C_6H_{13}	85.10118	85.10117	-0.08
C_5H_{11}	71.08553	71.08549	-0.57
C_5H_{10}	70.07769	70.07770	-0.11

Figure 3. Heptane, an "Out of Tolerance" analyte indicative of light olive oil, is shown. XIC 100.125±0.005 chromatograms show the analyte present at higher levels in the light olive oil (green) than in the extra-virgin olive oil (orange). The deconvoluted and library spectra are shown, as well as accurate mass information for the molecular and fragment ions.

Life Science and Chemical Analysis Solutions

4. Conclusion

This study demonstrates how LECO's analytical tools deliver the capabilities to characterize complex food samples and facilitate detection of food fraud. The Pegasus GC-HRT provided the industry-leading mass accuracy and resolution necessary to isolate individual analytes within the complex sample matrix and confidently identify analytes. Individual analytes were identified using the automated data processing tools in LECO's ChromaTOF-HRT software, with mass accuracies of less than 1 ppm routinely achieved to complement library search results. A comparison between an extra-virgin and light olive oil sample was explored to demonstrate a differential analysis approach utilizing ChromaTOF's "Reference." This tool rapidly determined analyte similarities and differences between the olive oil samples. A collection of representative differences were highlighted here, with many more observed in these data. This data analysis approach offers feature selection capabilities to determine analytes that are characteristic of each sample type. These sample-distinguishing analytes may be candidates for detecting food fraud in mislabeling of edible oils.