Characterization of Peppermint Essential Oils Grown in Different Areas by Gas Chromatography Ultra-High Resolution Time-of-Flight MS (GC-HRT)

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Introduction

Essential oils are hydrophobic liquids that consist of many components to create a complicated matrix which can create difficulty in isolating, identifying, and quantifying constituent analytes. Peppermint essential oils are used in many industries, including perfumes, food flavorings, alternative medicine, and environmentally-friendly insecticides. The capability to determine the identity and concentration of the various components that make up the oils are important in discovering active ingredients and maintaining quality control in products.

The peppermint oils were analyzed with two LECO TOFMS instruments. One was the Pegasus® 4D comprehensive two-dimensional gas chromatograph (GCxGC)-TOFMS with increased peak capacity and chromatographic resolution compared to conventional GC analysis. The other was the Pegasus GC-HRT ultra-high resolution TOFMS and was used to showcase the high-resolution TOFMS technique, which is well suited to analyzing complicated mixtures with semivolatile organic compounds such as essential oils due to the chromatographic separation of analytes complementing the powerful MS detector capable of providing up to 50,000 resolving power.

GCxGC-TOFMS Methodology

The *mentha piperita* peppermint essential oils were acquired from Gritman Corporation (Friendswood, TX). The two batches that were received were classified as being sourced from the Northwest (NW) and Midwest (MW) regions of the USA.

Sample Preparation	Essential oil was diluted 1:100 in methanol
Carrier Gas	Helium using corrected constant flow control
Injection Volume (µL)	1
Split Ratio	100:1
Flow Rate (mL/min)	1
Primary Column	30 m x 0.25 mm x 0.25 µm Rxi-5ms
Secondary Column	1.5 m x 0.18 mm x 0.18 µm DB-Wax
Primary Oven Ramp	40°C for 1 min then 10°C/min to 220°C with 5 min hold
Secondary Oven Ramp	+10°C offset from primary oven
Modulator Offset	25°C
Modulation Period Profile	4 s period (0.4 s hot)
Inlet Temperature	250°C
Transfer Line Temp	270°C
Ion Source Temperature	200°C
MS Detector Voltage (V)	1450
MS Acquisition Delay (s)	115
Mass Range (m/z)	40-500
Acquisition Rate (spectra/s)	200
Electron Energy for EI (V)	-70
Collection/Processing Software	ChromaTOF® 4.41

Table 1. Pegasus 4D GCxGC-TOFMS analysis conditions for the peppermint essential oils.

Figure 1. LECO Pegasus 4D GCxGC-TOFMS.

GCxGC-TOFMS Results

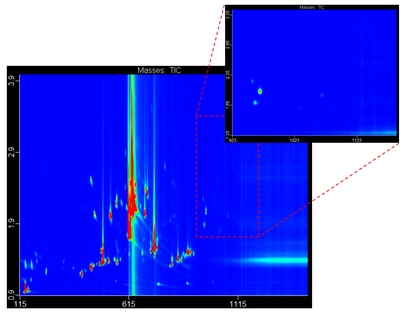

Figure 2. A contour plot representation of the GCxGC-TOFMS analysis of the NW peppermint oil. The secondary separation provides additional peak capacity to further separate compounds that coelute from the primary column. The zoomed-in region has been highlighted to contrast with the MW oil. The high concentrations of the compounds eluting at ~630 s, including menthol, led to some peak wrap-around due to exceeding the modulator's sample capacity. Diluting the sample further could prevent the wrap-around but would also lead to a loss in trace analyte detection.

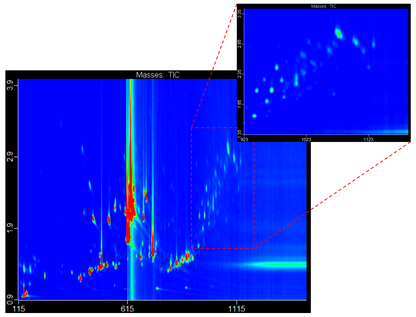

Figure 3. Contour plot of the GCxGC-TOFMS analysis of the MW oil. The zoomed-in region highlights an area that strongly contrasts with the NW oil. This oil has many components with concentrations large enough to easily distinguish even in a TIC representation in this portion of the chromatogram, whereas the NW oil has few intense peaks in this region, as shown in Figure 2.

GC-HRT Methodology

The NW and MW peppermint essential oil samples were diluted in a ratio of 1:5 with dichloromethane solvent before injection into a regular split/splitless inlet.

Carrier Gas	Helium using corrected constant flow control
Injection Volume (µL)	1
Split Ratio	100:1
Flow Rate (mL/min)	1.0
GC Column	20 m x 0.18 mm x 0.2 µm Rtx-5
Oven Ramp	40°C for 1 min then 3°C/min to 100°C then 10°C/min to 270°C
Inlet Temperature	250°C
Transfer Line Temperature	300°C
Ion Source Temperature	300°C
Interface Heater	100°C
MS Detector Voltage (V)	2481
MS Acquisition Delay (s)	None but filament off from 50-100 s
Mass Range (m/z)	40-700
Acquisition Rate (spectra/s)	10
Electron Energy for EI (V)	-74
Mass Calibration Tune Compound	PFTBA
Collection/Processing Software	ChromaTOF HRT 1.26

Table 2. Pegasus GC-HRT analysis conditions and settings for the peppermint essential oils.

GC-HRT Results

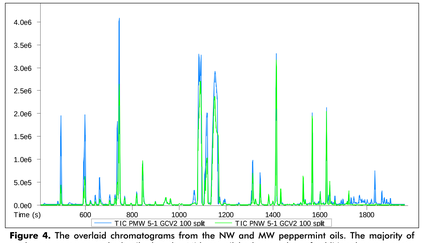

Figure 4. The overlaid chromatograms from the NW and MW peppermint oils. The majority of peaks are common to both oils, but the Midwest oil had a number of additional components, especially noticeable in the 1600-2000 s region.

Figure 5. LECO Pegasus GC-HRT.

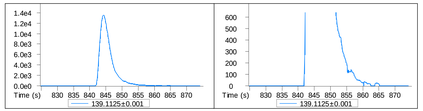

Figure 6. The 139.1125 m/z fragment of the compound cis-β-terpineol from the MW peppermint oil is displayed in the chromatogram portions above with the complete peak on the left and a zoom-in at the baseline on the right. The baseline figure demonstrates the lack of interfering noise that is achievable using the high-resolution Pegasus GC-HRT.

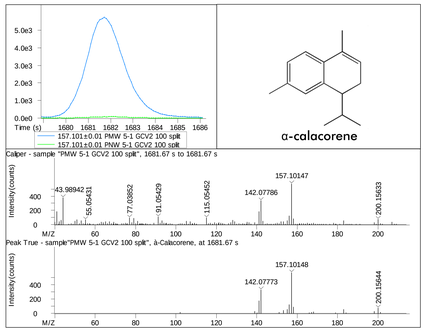

Figure 7. The overlaid chromatograms from the NW and MW peppermint oils for the compound α-calacorene are shown in the top left section, demonstrating that α-calacorene is a compound that can be used to differentiate between MW and NW oil. The structure of the molecule is shown in the top right figure. The mass spectra shown are the caliper on top and deconvoluted Peak True at the bottom. The calculated mass for M^+ ($C_{15}H_{20}^+$) is 200.15595 m/z and the measured mass is 200.15644 m/z, leading to a mass accuracy of 2.4 ppm.

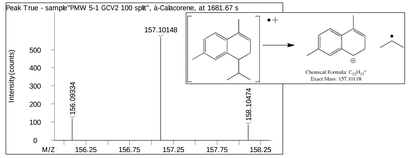

Figure 8. A zoomed-in section of the Peak True mass spectrum from Figure 7 that highlights some fragment masses including the 157 m/z fragment and it's ^{13}C isotope. The difference between masses 156.09334 and 157.10148 is 1.00814, indicating a difference of an H, while the difference between masses 157.10148 and 158.10474 of 1.00326 indicates that the heavier mass is a ^{13}C isotope. The mass accuracy of the 156, 157, and 158 ions are -0.1, 1.9, and 1.3 ppm, respectively. The structural formula at the top right corner of the figure shows a proposed fragment for the 157 ion.

GC-HRT Ultra-High Resolution Mode

The GC-HRT can also be set to run in ultra-high resolution (UHR) mode to achieve resolving power of up to 50,000 (FWHM), as opposed to the 25,000 resolution provided in high resolution (HR) mode.

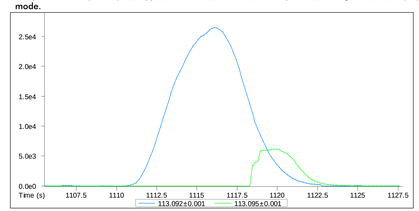

Figure 9. The utility of ultra-high resolution mode is demonstrated in this zoomed-in portion of the MW GC-HRT analysis. Ions with 113.095 m/z were isolated as a distinct peak from the 113.092 ions of the larger, overlapping isomenthone peak. The 113.09552 m/z mass is $C_8H_{13}N_2$ according to formula calculations, which is an incompatible fragment with the isomenthone peak.

Name	R.T. (s)	Base Mass	Quant S/N	Area	Full Width at Half Height	Formula	Mass Accuracy (ppm)	Found in NW oil?
Butanal, 3-methyl- (chocolate, peach, fatty)	122.330	44.02598	1212	213656	1.72	C5H10O	2.9	Yes
Butanal, 2-methyl- (musty, chocolate, nutty)	127.448	41.03894	937	183559	1.84	C5H10O	3.3	Yes
Furan, 2-ethyl- (sweet, burnt, earthy, malty)	148.472	81.03268	1058	177141	1.53	C6H8O	4.1	Yes
Butanoic acid, 2-methyl-, ethyl ester	338.159	57.07010	290	77149	2.52	C7H14O2	3.9*	No
Pentanoic acid, ethyl ester (sweet, fruity, acidic, pineapple, apple, green, berry, tropical)	344.878	88.05233	62	15271	2.22	C7H14O2	5.1*	Yes
α-Pinene (woody, piney)	598.243	93.07020	47643	12208710	2.55	C10H16	3.5*	Yes
1-Octen-3-ol (earthy, green, oily, vegetative, fungal)	621.665	57.03394	4125	2113661	4.15	C8H16O	7.9*	Yes
Ethanol, pentamethyl-	660.513	59.04946	18738	7022771	3.15	C7H16O	5.4*	Yes
α-Terpinene (citrusy, woody, piney)	703.996	121.10169	3197	991108	3.05	C10H16	4.6	Yes
β-Limonene (terpene, pine, herbal, peppery)	736.646	68.06244	25294	8321724	3.28	C10H16	5.3	Yes
Eucalyptol (eucalyptus, herbal, camphor)	743.984	43.01820	57241	17808352	3.1	C10H18O	5.6	Yes
cis-β-Terpineol (pungent, earthy, woody)	844.185	71.04954	9209	3326024	3.25	C10H18O	5.9	Yes
α-Terpinolene (sweet, fresh, piney, citrus)	898.502	93.07053	949	277862	2.92	C10H16	7.9	Yes
3-Octanol, acetate (fresh, woody, green, grapefruit, rose, apple, mint)	1011.736	43.01816	818	258252	3.13	C10H20O2	7.4*	No
Menthofuran (pungent, musty, nutty, earthy, coffee)	1109.510	108.05662	31250	11836271	3.76	C10H14O	-2.7	Yes
cis-3-Hexenyl isovalerate (fresh, green, apple, fruity, tropical, pineapple)	1308.378	67.05436	3775	754892	1.99	C11H20O2	2.0*	No
Pulegone (peppermint, camphor, fresh, herbal)	1313.189	81.06997	13115	4028250	2.99	C10H16O	2.3	Yes
Carvone (spice, bread, caraway)	1325.531	82.04136	2505	879561	3.19	C10H14O	0.5*	Yes
Piperitone (peppermint, mint)	1344.693	82.04141	18776	5207817	2.67	C10H16O	2.5	Yes
Menthol (peppermint, cool, woody)	1381.375	81.06999	1430	334456	2.31	C10H20O	1.4*	Yes
Dihydroedulan I	1399.532	179.14365	941	234882	2.47	C13H22O	2.2	Yes
Menthyl acetate (herbal, mint, rose)	1413.819	43.01810	3622	9487453	2.47	C12H22O2	6.0*	Yes
Thymol (herbal, thyme, phenolic, medicinal, camphor)	1414.663	135.08084	503	128059	2.33	C10H14O	3.7	Yes
Eugenol (sweet, spicy, clove, woody)	1500.202	164.08336	163	44399	2.37	C10H12O2	1.1	Yes
α-Copaene (woody)	1517.987	105.06999	737	144888	1.96	C17H19NO	1.1*	Yes
β-Bourbonene (herbal, woody)	1528.523	81.06996	11547	2533470	2.06	C15H24	1.0*	Yes
Acetic acid, decyl ester	1556.074	43.01805	578	111871	1.9	C12H24O2	4.9*	No
Caryophyllene (spicy, woody, terpenic)	1567.072	41.03882	16279	3463713	2.12	C15H24	1.2	Yes
β-Myrcene (herbaceous, woody)	1601.121	93.06989	3470	745478	1.97	C10H16	0.1*	Yes
Germacrene D	1627.839	161.13258	23752	4816599	1.98	C15H24	-1.0	Yes
Elixene	1641.894	121.10125	4742	937340	1.96	C15H24	0.6*	Yes
α-Calacorene (woody)	1681.672	157.10148	596	109113	1.81	C15H20	2.4	No
Ledol	1723.284	41.03873	1173	211526	1.8	C15H26O	3.7	Yes
beta-Patchoulene (perfuming agent)	1732.443	119.08533	758	150159	1.97	C15H24	-0.3	No
Cadalene (from sesquiterpenes)	1784.193	183.11653	116	22379	1.76	C15H18	-0.9	No

Table 3. 35-selected odor and fragrance compounds located in the MW oil sample based on the high-resolution GC-HRT analysis. The mass accuracies marked with a (*) indicate the mass accuracy is based off of the base mass, as opposed to the M^+ mass. While most compounds were also found in NW oil, the final column shows some examples of compounds that were not. The odor characteristics were primarily from *www.thegoodscentcompany.com's* online database of flavor and fragrance compounds.

Conclusions

The peppermint essential oil extracts were successfully analyzed using both types of LECO GC-TOFMS instrumentation, including the new Pegasus GC-HRT. The Pegasus 4D GCxGC-TOFMS found ~600 peaks at S/N=50 associated with the peppermint oil and the Pegasus GC-HRT found 643 peaks in high-resolution mode with S/N=5. The GC-HRT platform can produce spectra with an extremely low noise floor to improve detection limits compared to unit-mass instrumentation, as well as provide additional mass information to confirm or discredit mass formula assignments. As demonstrated above, locations in the chromatogram that require closer scrutiny can be analyzed in ultra high-resolution mode for additional separation power to target similar but distinct masses.