Poster Reprint

ASMS 2024
Poster number ThP 332

Analysis of Essential Oils Using a Comprehensive GCxGC with a Reverse Flow Modulator Combined with High Resolution GC/MS

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Introduction

Essential oils are typically analyzed to determine their chemical composition contributing to flavor and quality as well as to evaluate their authenticity and identify adulterants. Essential oils are often complex samples with high number of volatile and semi-volatile components that include many isomers with highly similar spectra and close Retention Indices (RIs) when separated on a single column. Hence, a comprehensive GCxGC approach is preferable to achieve an appropriate chromatographic separation for this sample matrix. In this study we used comprehensive GCxGC coupled to a high-resolution accurate mass GC/MS to identify the essential oil components. Here we also investigate approaches for comparison of the complex GCxGC essential oil data.

Figure 1. Agilent 7250 GC/Q-TOF

Experimental

The samples were separated on 8890 GC using a comprehensive GCxGC configuration with a reverse flow modulator (RFM). The non-polar/polar column set was a 20m x 0.1mm x 0.1um DB-1ms column (100% Dimethylpolysiloxane) coupled to a 5m x 0.25mm x 0.15um DB-17ms column (equivalent to (50%-phenyl)-methylpolysiloxane). Optimized instrumental parameters using purged splitter configuration which is preferred, are shown in Table 1.

Table 1. Instrument and method parameters

Parameter	Value
MS	Agilent 7250 GC/Q-TOF
GC	Agilent 8890 GC
Inlet/Liner	MMI, Agilent 5190-2294: 990 µL (Split, straight, wool, Ultra Inert)
Injection Mode	Split; 250:1
Injection Volume	0.5 µL
Inlet Temperature	300 °C
Column 1D & Flow	Agilent DB-1ms, 20 m x 0.1 mm x 0.1 µm; 0.2 mL/min
Column 2D & Flow	Agilent DB-17ms, 5 m x 0.25 mm x 0.15µm; 10 mL/min
Restrictor to Modulator Vent FID	Deactivated fused silica; 0.4 m x 0.05 mm
Purged Splitter Restrictor to Q-TOF & Flow	Deactivated fused silica; 0.6 m x 0.12 mm; 1.3 mL/min
Purged Splitter Restrictor to Front FID	Deactivated fused silica; 1.05 m x 0.25 mm
Modulation Delay	0.51 min
Modulation Period	5.1 sec
Injection Time	0.125 sec
Carrier Gas	Helium
Oven Temperature Program	45 °C for 1 min; 3 °C/min to 285 °C, 3 min hold
Transfer Line Temperature	305 °C
Source Temperature	300 °C
Quadrupole Temperature	150 °C
Collision Cell Gas Flows	N2, 1 mL/min + He, 4 mL/min
Electron Energy	70 eV
Emission Current	5 µA
Spectral Acquisition Rate	50 Hz
Mass Range	*m/z* 45 to 650
FID Temperature	300 °C
FID H2 Flow	30 mL/min
FID Air Flow	400 mL/min
FID Makeup Flow (N2)	15 mL/min (Front); 25 mL/min (Vent)

The data were acquired using the high-resolution 7250 GC/Q-TOF at data rate of 50 Hz. For compound identification the Unknown Analysis tool of MassHunter Quantitative Analysis software version 12.1 and the GC Image software version 2024 R1 were used. The linear retention indices (RIs) were used to increase confidence in compound identification. Statistical analysis was performed in Mass Profiler Professional (MPP) software version 15.1.

Testing GCxGC Configurations Using Diesel

As a first step, to develop a GCxGC RFM method with a goal of excellent chromatographic separation of the components while ensuring optimal carrier gas flow to the MS, a diesel sample was used. Several different configurations with variable column lengths and internal diameters were attempted, and the two of the best ones are discussed below. Note that all the configurations are using two FIDs (as vent and detector). Another point to make is that a part of the MS restrictor is heated by the oven, and another segment is heated by the transfer line and should be configured as such.

The first configuration (Figure 2) is using an unpurged splitter.

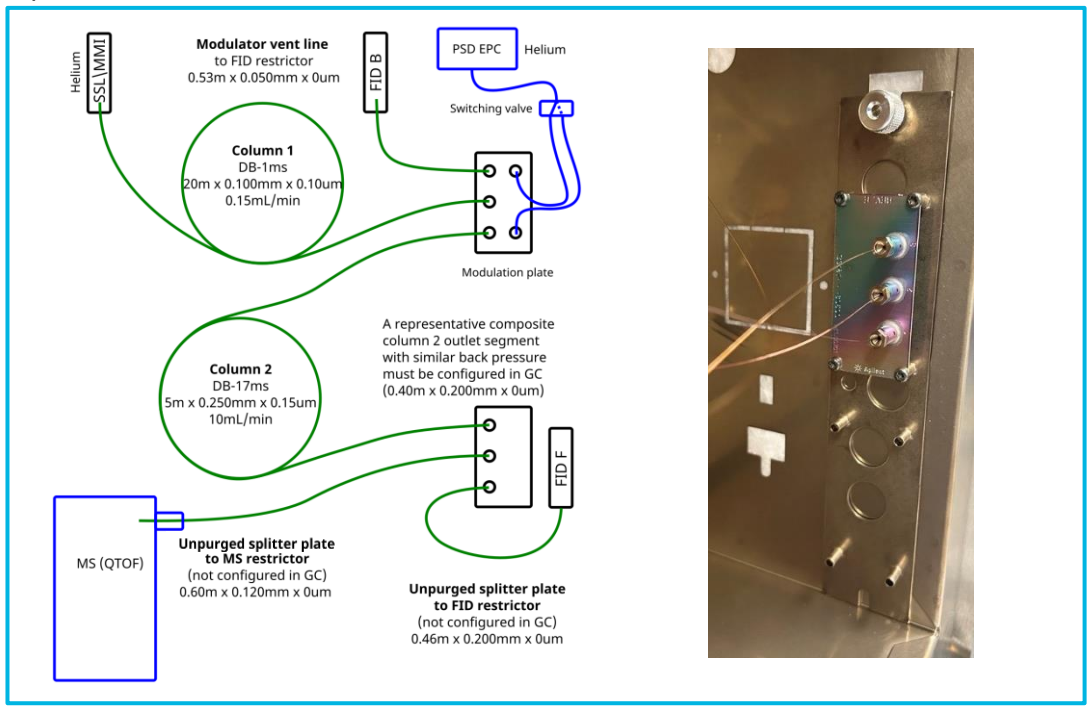

Figure 2. Setup with an unpurged splitter

Diesel separation on a 2D plot when using this configuration is shown in Figure 3. Separation of the different compound classes is clearly observed.

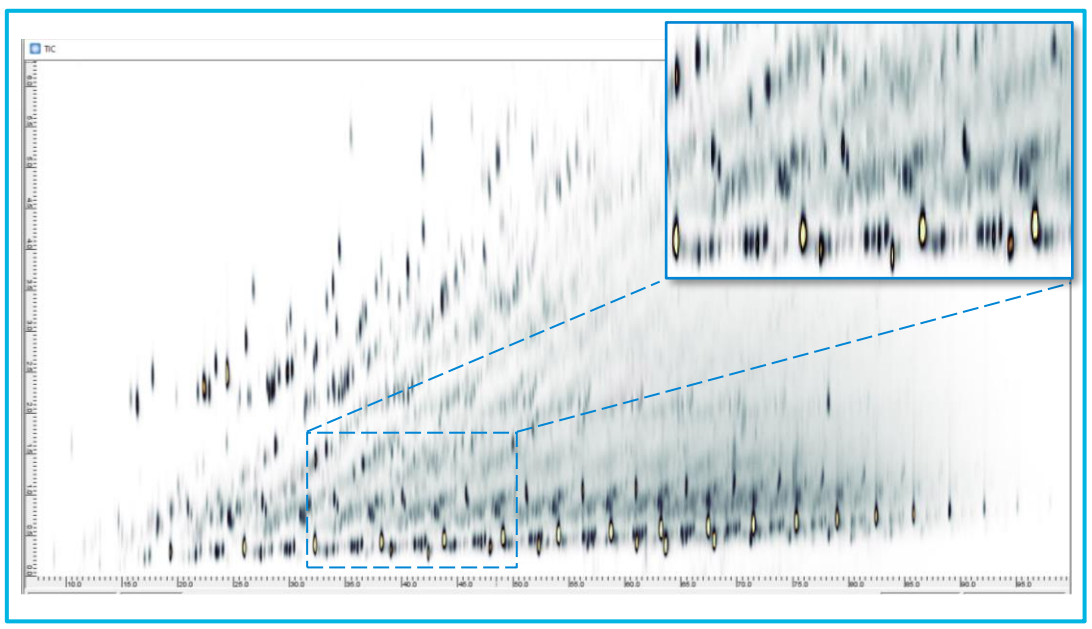

Figure 3. Diesel separation when using an unpurged splitter setup. Modulation Period 6.3 sec, oven: 40 ˚C for 6 min, ramp 2.5 ˚C/min.

The second configuration (Figure 4) is using a purged splitter. The diesel separation using this configuration is displayed in Figure 5.

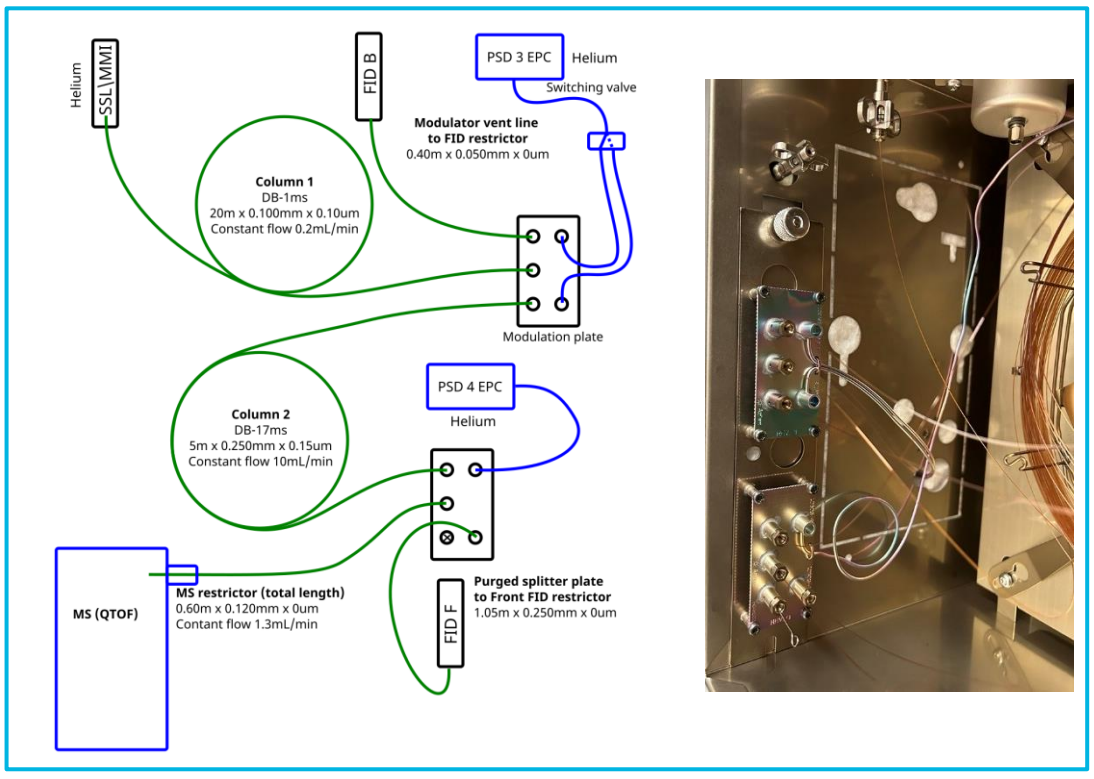

Figure 4. Setup with a purged (3-way) splitter.

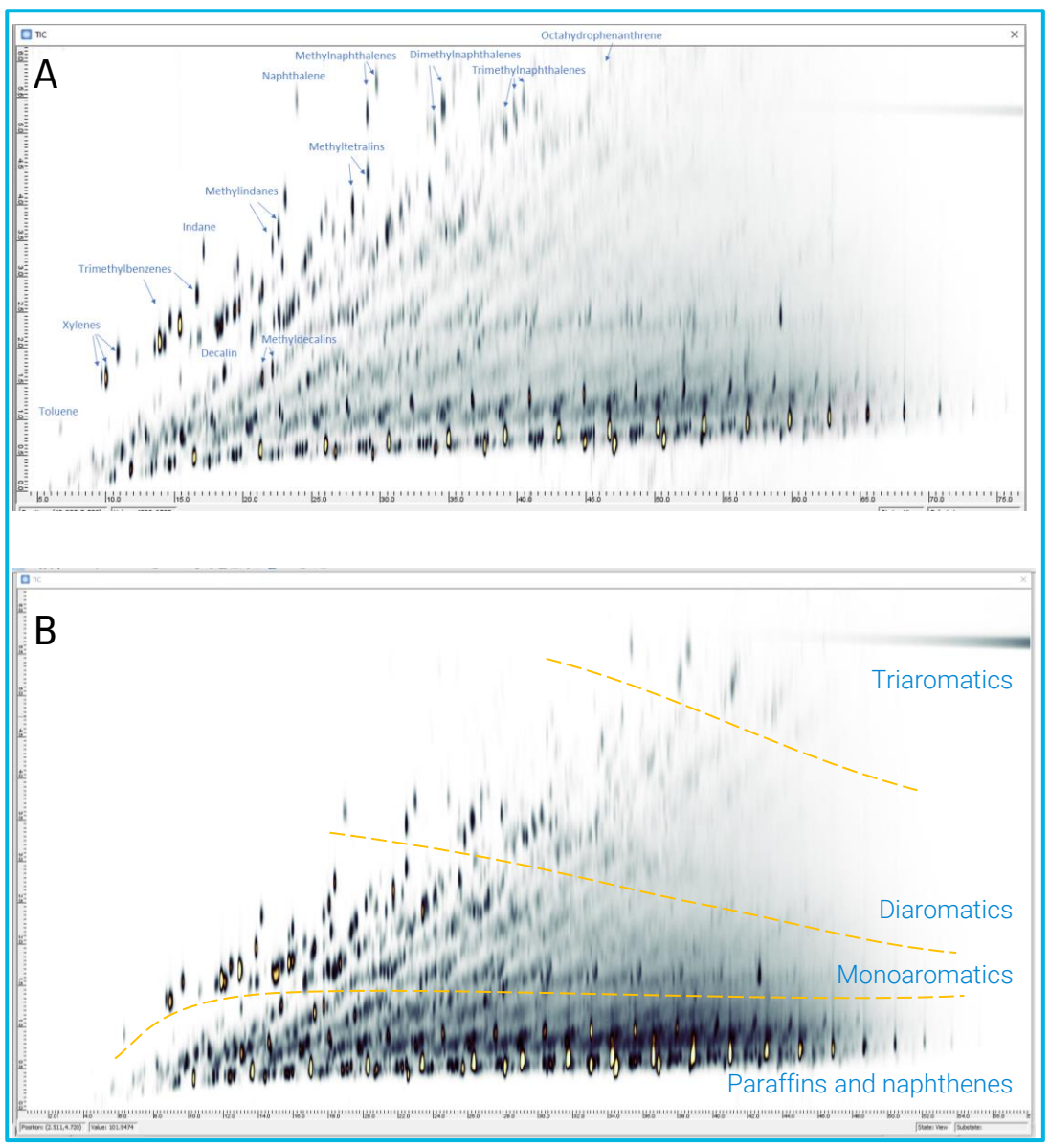

Figure 5. Diesel separation when using the purged splitter setup. Modulation Period 6.3 sec A) Oven ramp 3 ˚C/min, B) Oven ramp 4.5 ˚C/min.

Both configurations have provided the superior 2D separation of diesel hydrocarbons, including mono-, di- and triaromatics as well as paraffins and naphthenes, and clean FID monitor channel was observed.

Results and Discussion

Workflow for Determination of Composition and Comparison of Essential Oils Using MPP

Ginger and juniper berry essential oils have been analyzed using both configurations described above, and the resulting 2D chromatograms looked comparable. First, to determine their chemical compositions, the compounds were identified in the Unknowns Analysis software using NIST23 library with RI matching. Accurate mass information in conjunction with the library hit formulas helped to confirm compound ID and eliminate false positives (Figure 6).

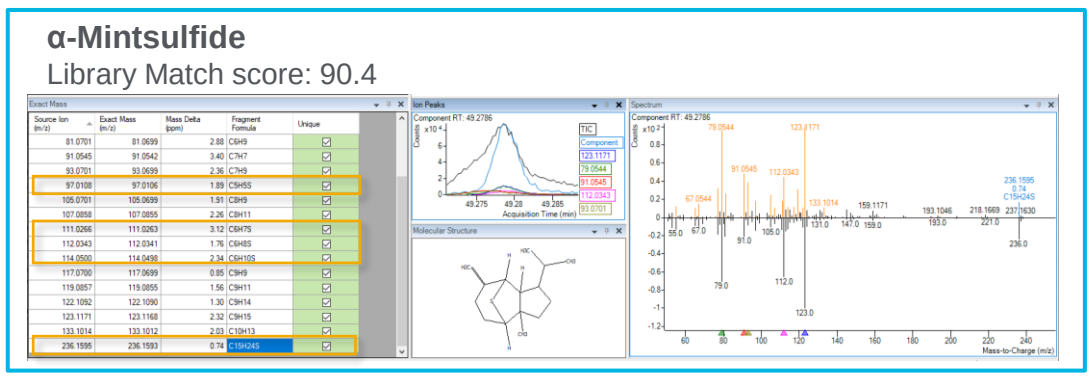

Figure 6. Confirmation of the compound ID in the Unknowns Analysis with accurate mass. ExactMass tool confirming the library hit by annotating ions in the spectrum with fragment formulas corresponding the molecular formula of the hit based accurate mass match.

The GCxGC data were also visualized in GC Image software, and the compound ID and chemical classes were mapped on the 2D plot (Figure 7).

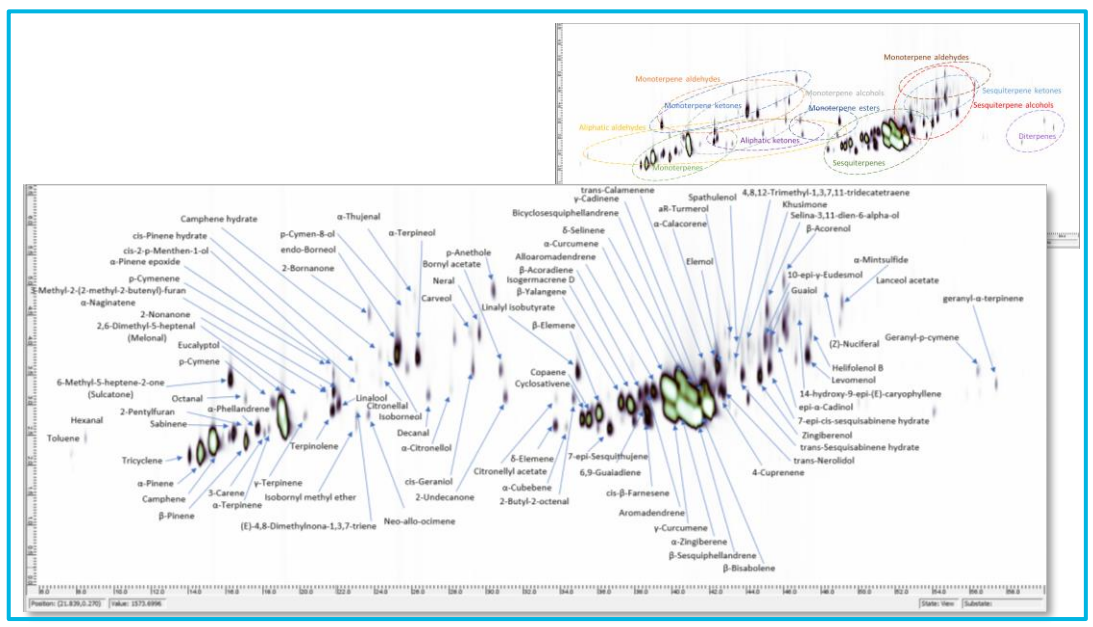

Figure 7. Individual compounds and compound classes mapped on 2D chromatogram of the ginger oil sample. Modulation Period 6.7 sec, oven ramp 2.5 ˚C/min.

Among compound classes identified in the essential oils samples there were aliphatic aldehydes and ketones, monoterpenes and monoterpene aldehydes, ketones, alcohols and esters, sesquiterpenes and sesquiterpene alcohols and ketones, as well as diterpenes. The current approach has also allowed for accurate identification of the major essential oil components (e.g. alpha-zingiberene) as well as minor trace components (e.g. α-mintsulfide) in a single run.

To perform statistical analysis, compound annotations were exported from the Unknowns Analysis as CEF files and imported into the MPP. Duplicate component IDs, produced from several modulations across the same component peak have been automatically merged during alignment. Principal Component Analysis (PCA) plot for juniper berry and ginger oil samples is shown in Figure 8.

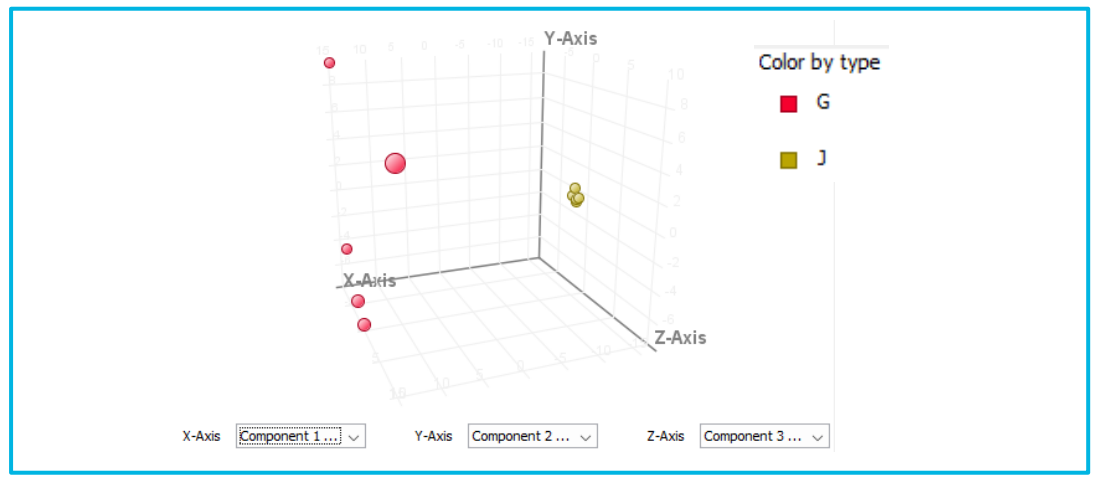

Figure 8. PCA plot demonstrating clear clustering of juniper berry (J) and ginger (G) oil samples.

Approximately 150 identified compounds (and over 300 components) were found at significantly different levels between ginger and juniper berry oils, as shown on volcano plot in Figure 9.

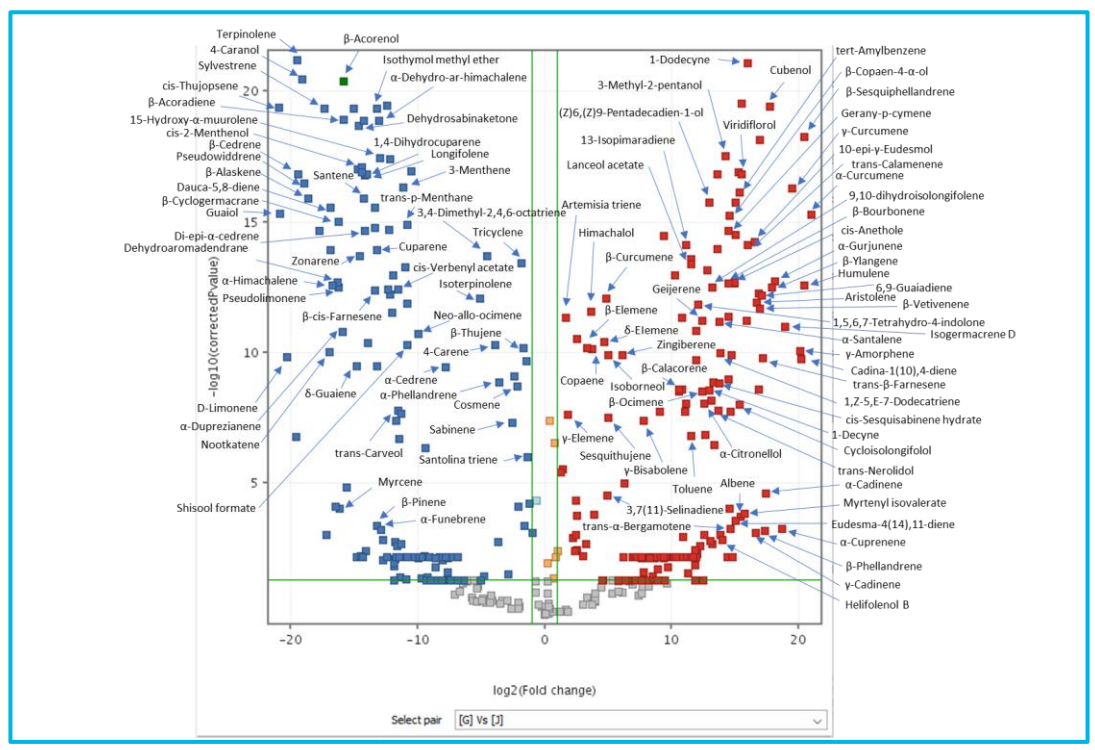

Figure 9. Volcano plot comparing the compositions of ginger oil vs juniper berry oil when using Fold Change cutoff of 2, and p-Value cutoff of 0.05.

Conclusions

- Several comprehensive GCxGC configurations and method using the high-resolution GC/Q-TOF and RFM were developed and optimized using a diesel sample.
- Essential oils were analyzed using the optimized GCxGC approach, and the workflows for compound identification and statistical analysis were evaluated.

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DE87353504

Published in USA, May 31,2024

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