Application Note

Food Testing and Agriculture

Analysis of Essential Oils Using Comprehensive Two-Dimensional Gas Chromatography (GC × GC) and High-Resolution Mass Spectrometer

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Abstract

The analysis of complex samples such as essential oils can benefit substantially from comprehensive two-dimensional gas chromatography (GC × GC), which provides a high degree of chromatographic separation due to the second dimension. Combining GC × GC with high-resolution accurate mass measurement increases the number of identifiable components and enhances confidence in the results.

The focus of this study was to establish a workflow for the analysis of essential oils using GC × GC with a reverse flow modulator (RFM) combined with a high-resolution quadrupole time-of-flight (GC/Q-TOF) mass spectrometer.

Introduction

Essential oils are typically analyzed to determine their chemical composition contributing to flavor, fragrance, and quality. Additionally, these analyses help to evaluate authenticity and identify adulterants.

Essential oils are complex mixtures of volatile and semivolatile components obtained from plants either by distillation or cold expression.[1] They frequently include isomers with highly similar mass spectra and close retention indices (RIs). Therefore, separation of essential oils on a single column can be insufficient for individual component identification. The complexity of essential oil composition drives the constant search for advanced chromatographic techniques with improved selectivity. Therefore, a GC × GC approach is preferable to achieve an appropriate chromatographic separation for this sample matrix.

In GC × GC, the eluent from the primary column is frequently collected and reinjected onto a secondary column of different selectivity to achieve an orthogonal separation. This is performed by a modulating device positioned between the two columns. The modulators used for GC × GC separation are typically either thermal or flow-based. Ideally, each chromatographic peak from the first dimension should be sampled three or more times, requiring rapid second-dimension separation.[2] Therefore, TOF analyzers, offering fast data acquisition rates while maintaining high quality spectra, are the most appropriate MS when combined with a GC × GC separation technique. In contrast, when a scanning mass analyzer acquires data at its highest rate, typically near 20 Hz, spectral skewing can result in poor library matches.

Flow modulation is a cost-effective technique that enables cryogen-free GC × GC separation. The RFM is typically coupled with a flame ionization detector (FID), and is frequently used in hydrocarbon and polymer analyses, as well as in flavor and fragrance applications, among others.[3] Combining the RFM with an MS detector provides a great opportunity for more streamlined and confident compound identification. However, this technique also introduces a significant challenge due to the optimal carrier gas flow constraints of the MS, which are typically in the range of 1 to 2 mL/min.

The main goal of this study was to develop a workflow for the analysis of essential oils using a GC × GC method that combines RFM with a high-resolution accurate mass GC/Q-TOF system. Initially, the development and optimization of this GC × GC setup was performed using diesel, which is a complex but well-studied sample. Statistical analysis and approaches for the comparison of complex GC × GC essential oil data were also investigated.

Experimental

Data acquisition

The samples were separated on an Agilent 8890 GC using a GC × GC configuration with an RFM and analyzed using a high-resolution accurate mass Agilent 7250 GC/Q-TOF MS as well as an FID. A "normal" column set, combining a nonpolar primary column and a polar secondary column, was used. This set consisted of a 20 m × 0.1 mm, 0.1 µm Agilent DB-1ms column (100% dimethylpolysiloxane) and a 5 m × 0.25 mm, 0.15 µm Agilent DB-17ms column (equivalent to (50%-phenyl)-methylpolysiloxane).

To decrease flow to the MS, and to add an FID to the configuration as a second detector, the column flow must be split at the end of the secondary column by connecting it to a splitter. Two splitter types, purged and unpurged, have been explored to highlight the benefits and limitations of each configuration. In the case of a purged splitter, the column flow mixes with the makeup flow before splitting between the two detectors. In contrast, an unpurged splitter does not use makeup flow when splitting the column effluent between the two restrictors connected to the two detectors. A second FID (monitor, or vent) channel was added for method optimization and troubleshooting.

Optimized instrumental parameters using a purged splitter configuration, which is preferred due to its more straightforward setup in the acquisition software, are shown in Table 1.

Table 1. Data acquisition parameters.

Parameter	Value
MS	Agilent 7250 GC/Q-TOF system
GC	Agilent 8890 GC system
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 1 Dimensions and Flow	Agilent DB-1ms, 20 m × 0.1 mm, 0.1 µm (127-0122); 0.2 mL/min
Column 2 Dimensions and Flow	Agilent DB-17ms, 5 m × 0.25 mm, 0.15 µm (122-4711); 10 mL/min
Restrictor to Modulator (Vent) FID	Agilent deactivated fused silica; 0.4 m × 0.05 mm (160-2655-1)
Purged Splitter Restrictor to Q-TOF and Flow	Agilent deactivated fused silica; 0.6 m × 0.12 mm (CP801206); 1.3 mL/min
Purged Splitter Restrictor to Detector FID	Agilent deactivated fused silica; 1.05 m × 0.25 mm (CP802505)
Modulation Delay	0.51 min
Modulation Period	5.1 s
Injection Time	0.125 s
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	N_2, 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 H_2 Flow	30 mL/min
FID Air Flow	400 mL/min
FID Makeup Flow (N_2)	Detector FID: 15 mL/min Monitor (Vent) FID: 25 mL/min

Data processing

The data were acquired using the high-resolution 7250 GC/Q-TOF system at a data rate of 50 Hz. For compound identification, the Unknowns Analysis tool in Agilent MassHunter Quantitative Analysis software version 12.1 and GC Image software version 2024 R1 were used with the NIST23 EI library. The linear retention indices (LRIs) were used to increase confidence in compound identification. Statistical analysis was performed in Agilent Mass Profiler Professional (MPP) software version 15.1.

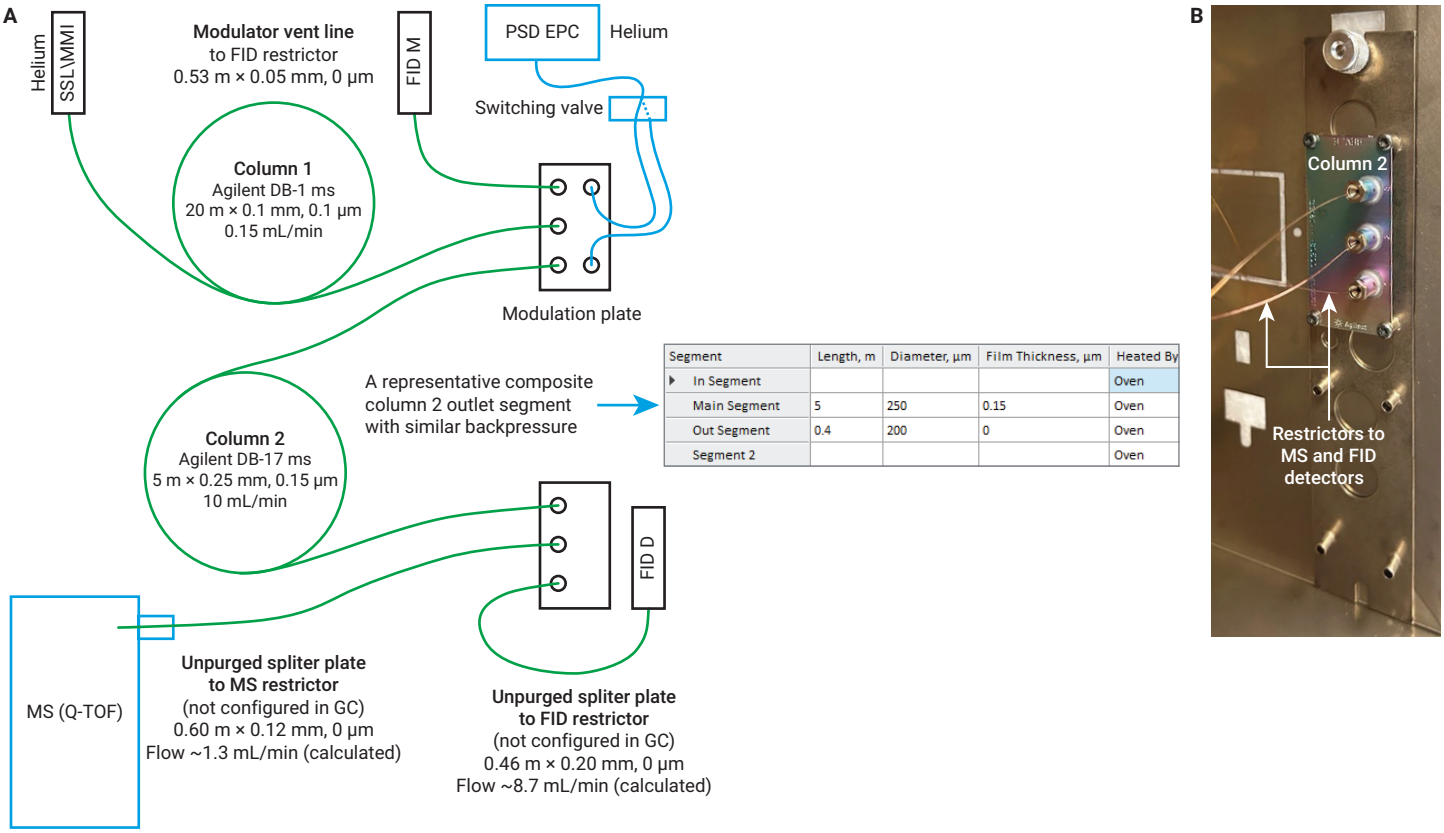
Results and discussion

Selection and optimization of GC × GC configuration

To achieve a sufficient modulation ratio when performing GC × GC, separation on the second column should be fast. This is typically achieved using a relatively short second column, which, in the context of GC × GC RFM configuration, will generate substantial flow rates incompatible with MS. Therefore, to split the flow coming off the secondary column between the MS and FID channels, either purged or unpurged splitters can be used.

Considering this, a GC × GC RFM method was initially developed and optimized using a diesel sample. Several configurations with varying column lengths and internal diameters were explored to achieve an adequate chromatographic separation of the components while ensuring optimal carrier gas flow to the MS. Figures 1 to 4 discuss the two most practical configurations. Note that both approaches use two FID channels (as a vent/monitor and a detector) in addition to the MS. Although including a monitor FID channel is not required when running GC × GC with RFM, it is critical for the initial method setup, optimization, and troubleshooting. Specifically, having the monitor FID channel configured in the setup allows for the detection of any breakthrough caused by overfilling of the modulator's sample loop, and thus further adjustment of the column/restrictor parameters, modulation and inject time accordingly. Another important consideration when configuring GC × GC with RFM is that a part of the MS restrictor is heated by the oven, while another segment is heated by the transfer line. Each segment should be configured according to its heating source. The restrictor dimensions to the MS should be chosen specifically for the transfer line temperature and flow to the MS. When splitting flow between the MS and a second detector, a constant flow to the MS should be maintained. When set up correctly, the flow splitting ratio between the MS and the second detector is constant and independent of the oven temperature profile.

The configuration using an unpurged splitter is shown in Figure 1. For proper setup, a representative composite column 2 outlet segment, with a backpressure similar to that generated by the MS and FID detector restrictors, should be configured in the acquisition software. Therefore, the MS as well as the detector FID restrictors are not configured separately.



Segment	Length, m	Diameter, µm	Film Thickness, µm	Heated By
In Segment				Oven
Main Segment	5	250	0.15	Oven
Out Segment	0.4	200	0	Oven
Segment 2				Oven

Figure 1. GC × GC RFM setup with an unpurged (two-way) splitter. (A) Schematic of the unpurged splitter configuration. (B) Photograph of the unpurged splitter plate. The RFM is installed on the other side of the GC oven.

The GC × GC chromatogram of diesel obtained using this configuration is shown in Figure 2. Excellent separation of the diesel aliphatic species was generally observed using this method. However, tri-aromatics were wrapped around (did not elute from the secondary column until the next modulation cycle) even with a modulation period of 6.3 seconds due to a slower oven ramp of 2.5 ˚C/min.

The configuration using a purged splitter is shown in Figure 3. All three restrictors employed in this setup, including for MS, vent, and detector FID, must be configured in the acquisition method.

Diesel separation using the purged splitter configuration is shown in Figure 4. This three-way splitter configuration has also provided superior separation of diesel hydrocarbons, including mono-, di- and tri-aromatics, as well as paraffins and naphthenes. No significant wraparound of tri-aromatics was observed when using a faster oven ramp rate of 4.5 ˚C/min.

The monitor FID channel showed no peaks with both purged and unpurged splitter approaches, indicating proper modulator configuration. An example demonstrating a clean FID channel is shown for the three-way splitter configuration (Figure 5).

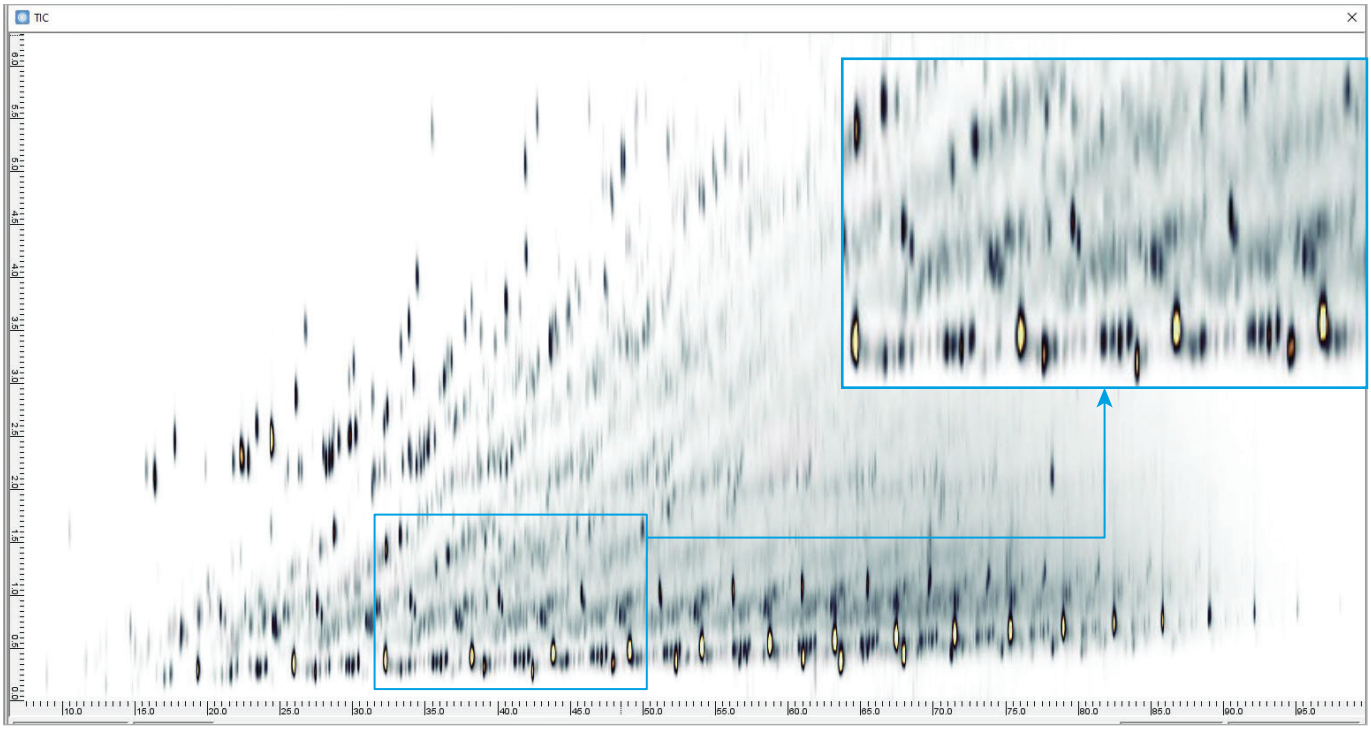


Figure 2. Diesel separation using an unpurged splitter setup. The modulation period was 6.3 seconds with an oven temperature of 40 ˚C for 6 minutes and a ramp rate of 2.5 ˚C/min.

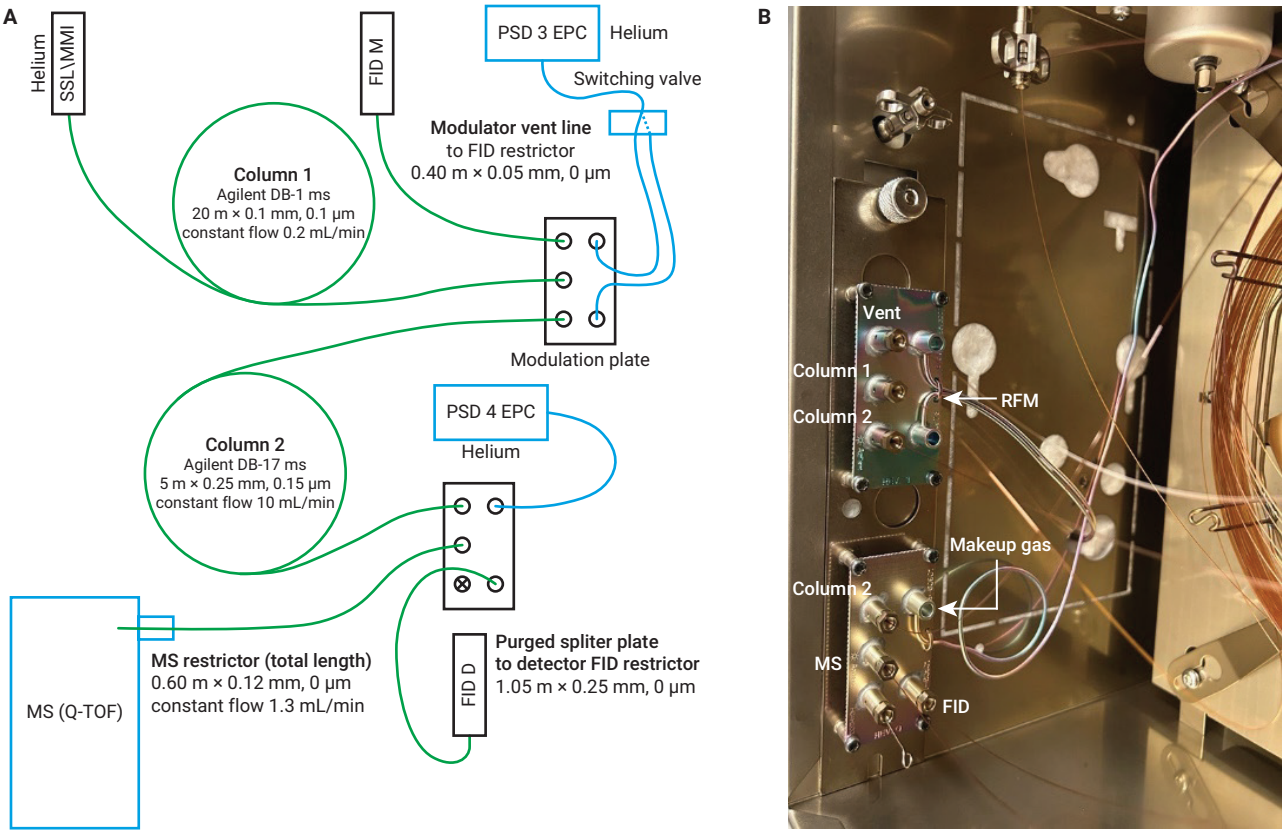


Figure 3. GC × GC RFM setup with a purged splitter. (A) Schematic of GC × GC when using the purged (three-way) splitter configuration. (B) Photograph of the GC oven with purged splitter and reverse flow modulator plates.

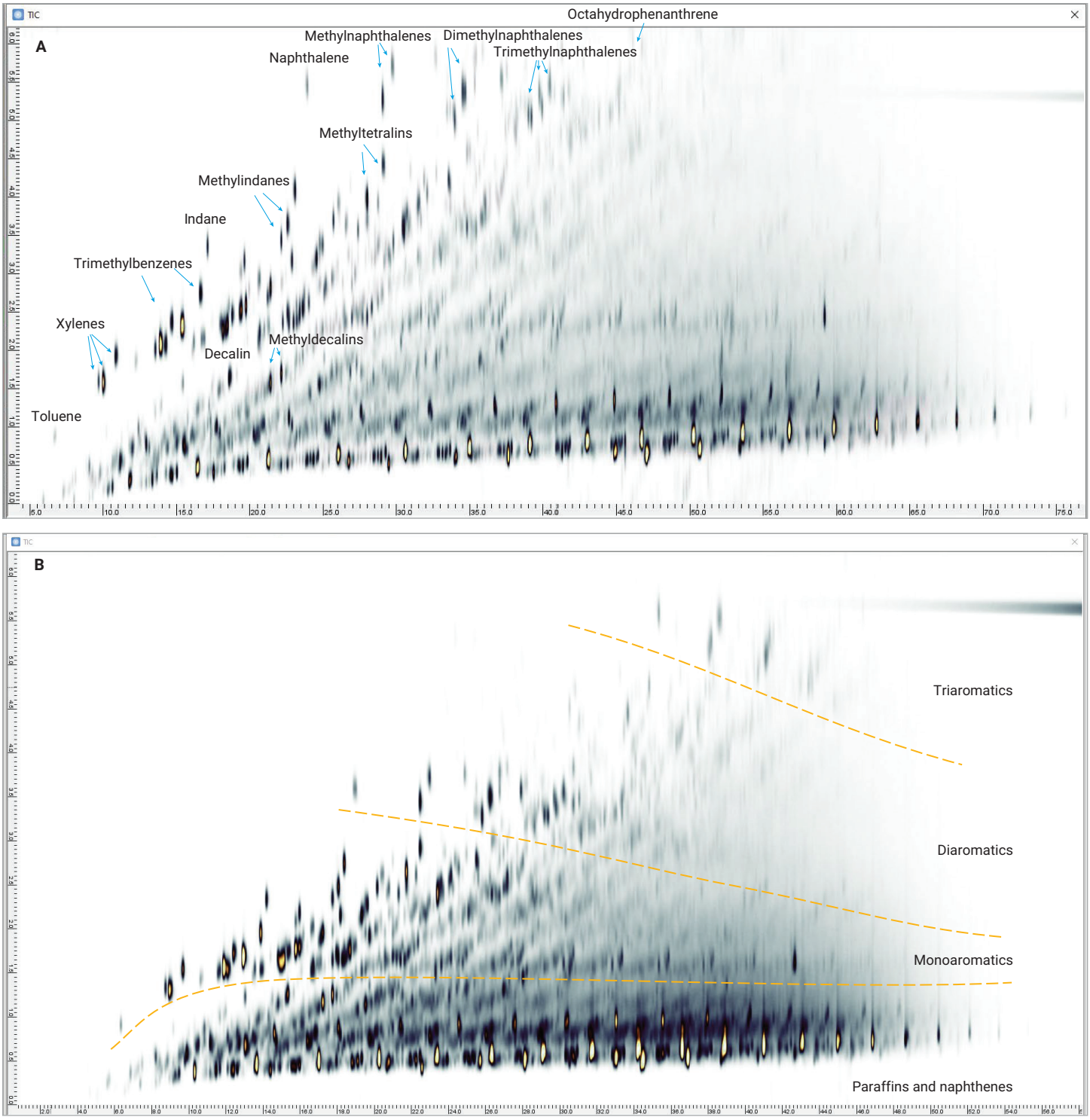


Figure 4. Diesel separation using a purged splitter setup. The modulation period was 6.3 seconds with oven ramp rates of (A) 3 ˚C/min and (B) 4.5 ˚C/min.

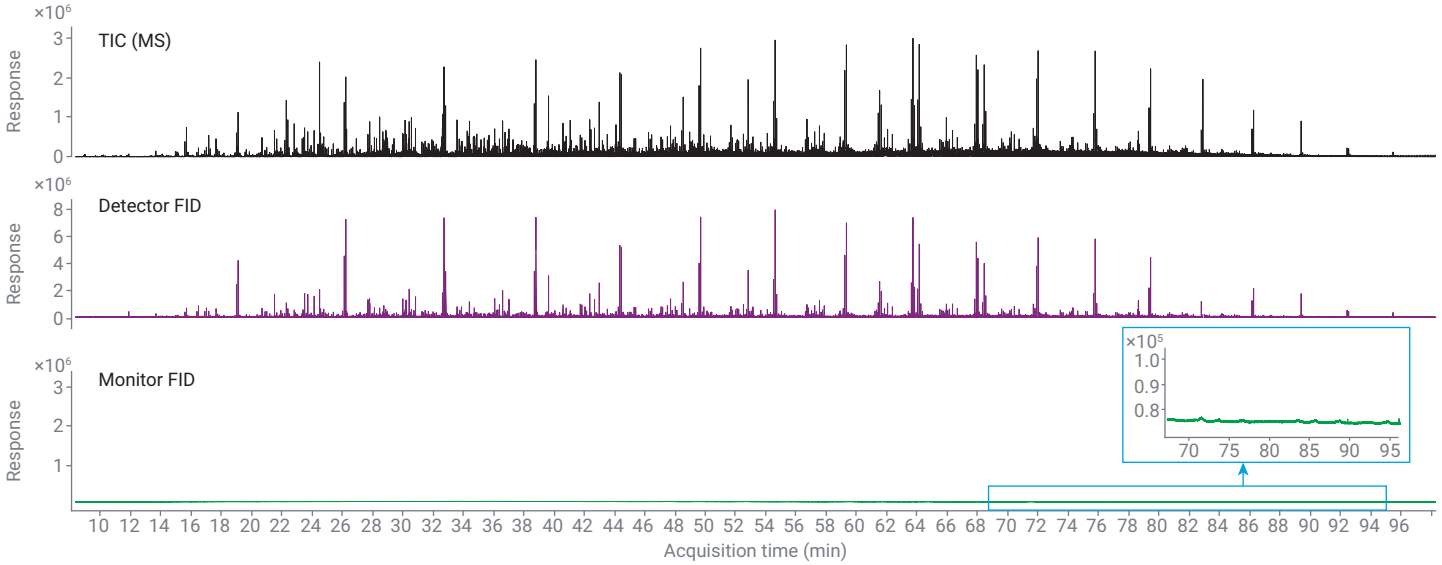


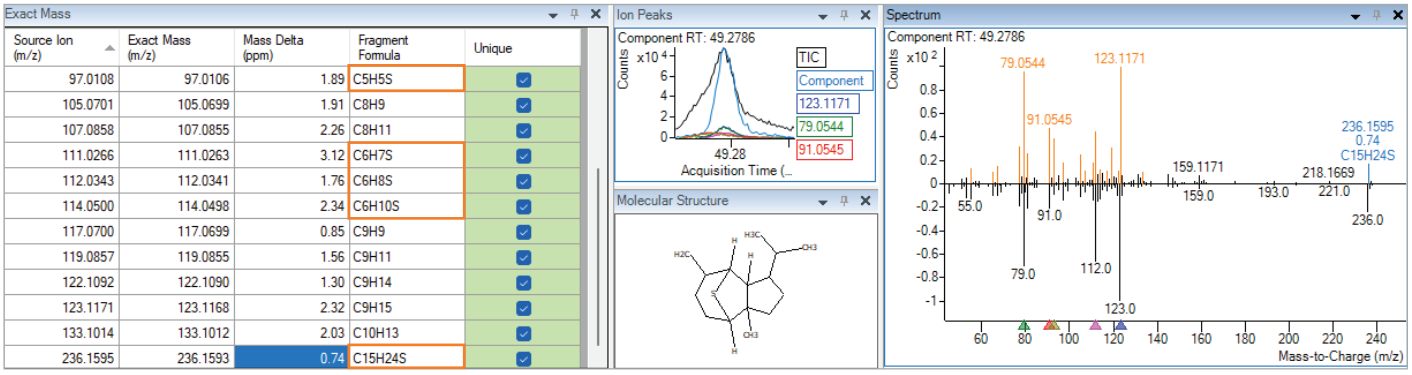
Figure 5. An example of a diesel total ion chromatogram (TIC) from the GC/Q-TOF MS channel as well as FID signals from the end detector and the monitor FID channels.

Workflow for determining and comparing the compositions of essential oils

Ginger and juniper berry essential oils (obtained from Sigma-Aldrich) were analyzed on the GC/Q-TOF system using the GC × GC RFM configurations with the unpurged and purged splitters. Both approaches resulted in comparable GC × GC separations. To determine the chemical compositions of the essential oils, the compounds were identified with the Unknowns Analysis software using the NIST23 library with LRI matching.

To further increase confidence in compound identification, the ExactMass tool in Unknowns Analysis was used. The tool assigned accurate mass fragments from deconvoluted spectra with formulas, based on the library hit where possible, within a small mass error window. This approach helped to either confirm the hit or reject the compound ID in case of a false positive (Figure 6).

A α-Mintsulfide
Library match score: 90.4, RI Δ 13, *m/z* match ✓



B n-Propylcyclohexane
Library match score: 78.2, RI Δ 5, *m/z* mismatch ✗

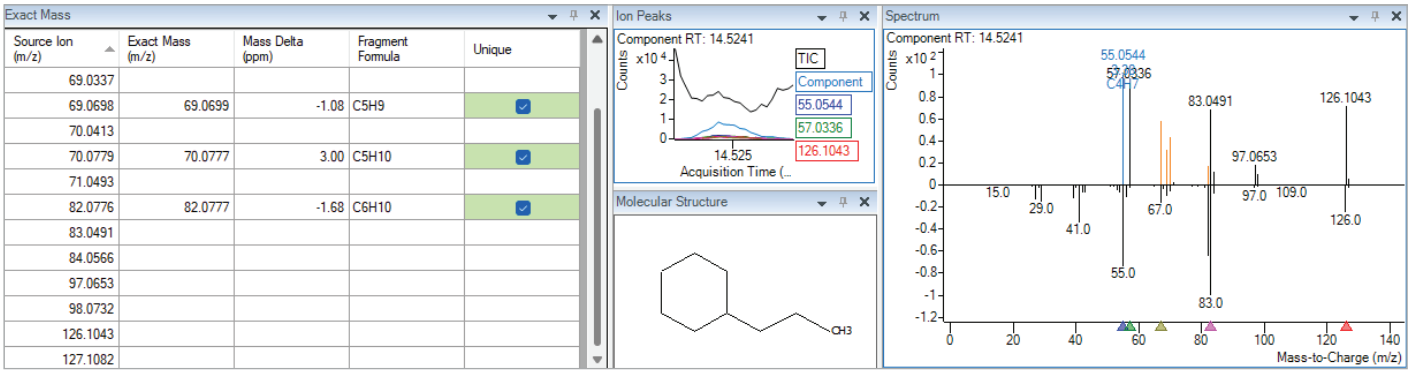


Figure 6. Confirmation of compound ID in the Unknowns Analysis tool using accurate mass information. The ExactMass tool available in Unknowns Analysis (A) confirms or (B) rejects library hits. This decision is based on whether formulas can be assigned to the most specific accurate mass fragment ions in the spectrum, considering the elemental composition of the library hit. The orange rectangles in panel A highlight fragment formula annotations containing the heteroatom sulfur.

Note that several fragment formula annotations (highlighted in the orange rectangles for the library hit shown in Figure 6A) contain a heteroatom, in this case sulfur. The presence of heteroatoms in assigned fragment formulas is another important indication that the identified elemental composition of the compound is likely accurate.

Compound identification in essential oils was further facilitated by mapping chemical classes of compounds on a 2D plot. This visualization of the GC × GC data was performed using GC Image software (Figure 7). As shown in Figure 7A, compound classes are clearly clustered on the 2D plot, and the specific coordinates (retention times) in the first and second dimensions help to provide additional clues to compound structure.

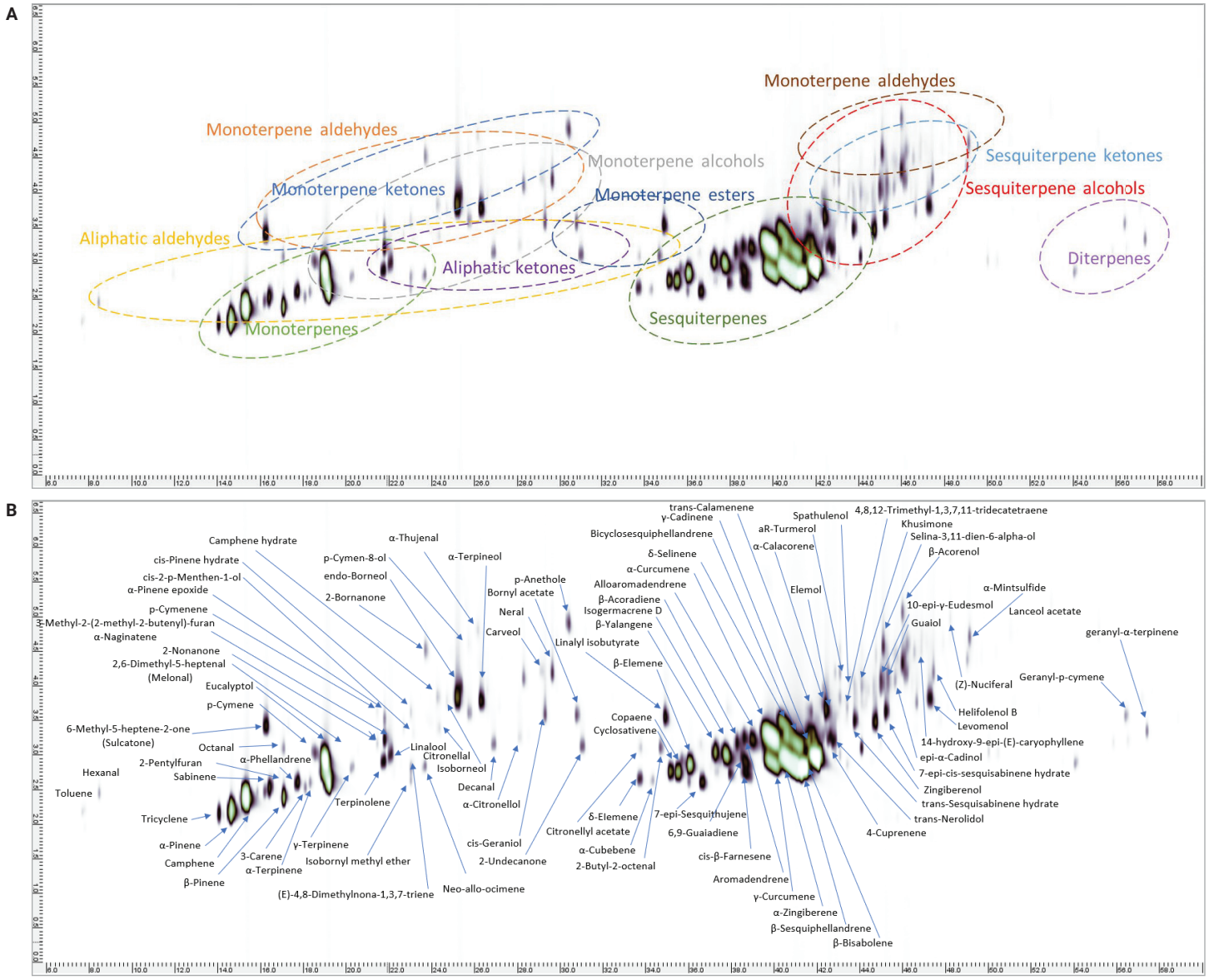


Figure 7. (A) Compound classes and (B) individual components mapped on a 2D chromatogram of a ginger oil sample. The modulation period was 6.7 seconds with an oven ramp rate of 2.5 ˚C/min.

On the 2D chromatogram, compounds aligned on the same vertical line are coeluting in the first dimension. Therefore, without additional chromatographic separation in the second dimension or using a different polarity column, these compounds would be challenging to accurately identify.

An example of such a case is illustrated in Figure 8, which shows the second-dimension separation of three terpenoids (from the same modulation period) along with their deconvoluted spectra, resulting in reliable library matches.

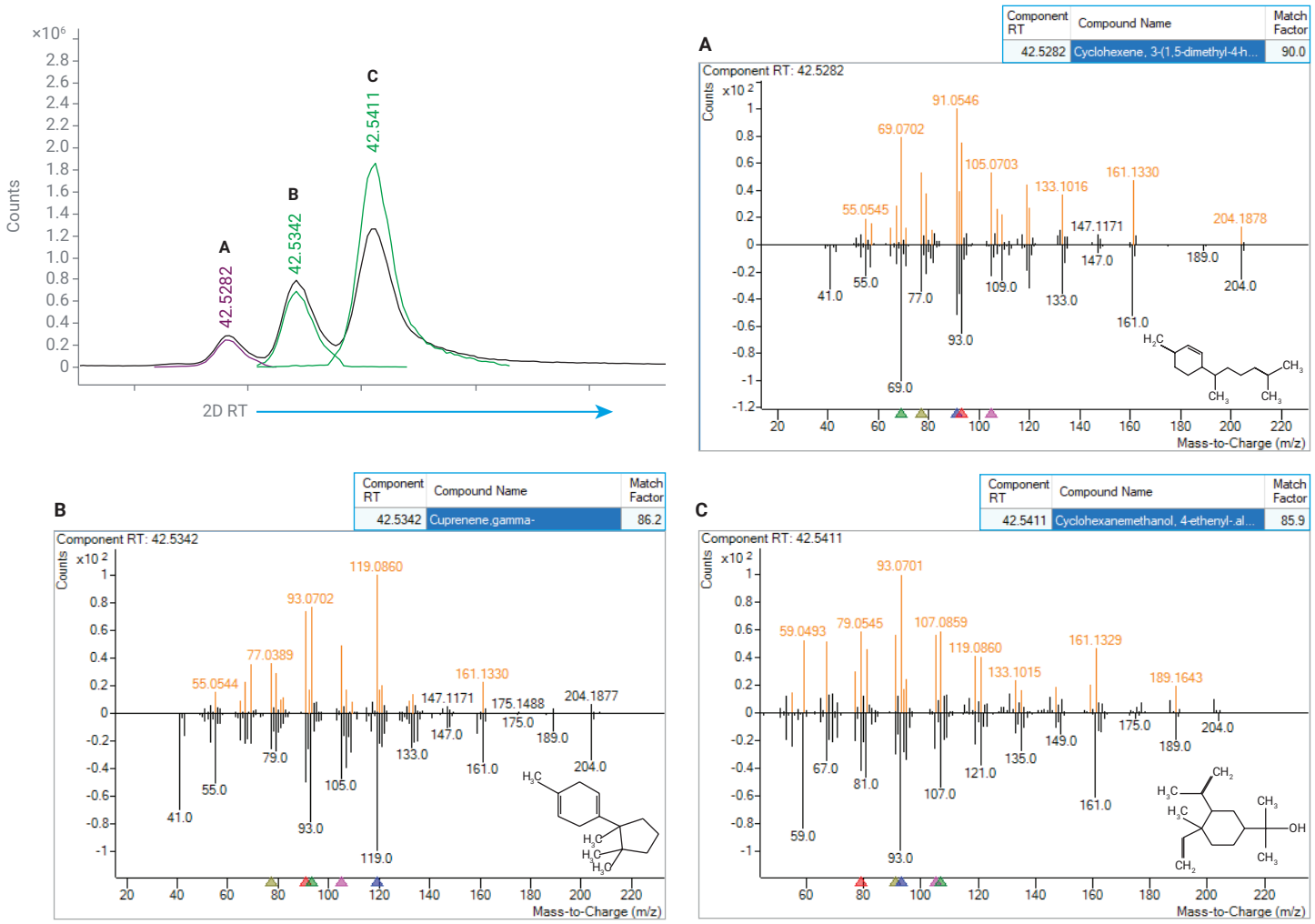


Figure 8. Separation of ginger oil terpenoids (A, B, and C) from the same modulation on the second dimension.

Among the compound classes identified in the essential oil samples 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 also allowed for the accurate identification of major essential oil components (for example, alpha-zingiberene) as well as minor trace components (for example, alpha-mintsulfide) in a single run.

The separation power of GC × GC technology could also help to improve statistical analysis, although the presence of the second dimension brings an additional degree of complexity to the data processing. To perform statistical analysis, compound annotations were exported from Unknowns Analysis as compound exchange format (CEF) files and imported into the MPP software. Duplicate component IDs, produced from several modulations across the same component peak, are automatically merged during the alignment process in MPP.

To compare the compositions of ginger and juniper berry oils, a principal component analysis (PCA) plot was used to evaluate the sample grouping (Figure 9). Ginger and juniper berry oil samples show distinct clustering.

The volcano plot in Figure 10 shows that approximately 150 identified compounds were found at significantly different levels between ginger and juniper berry oils.

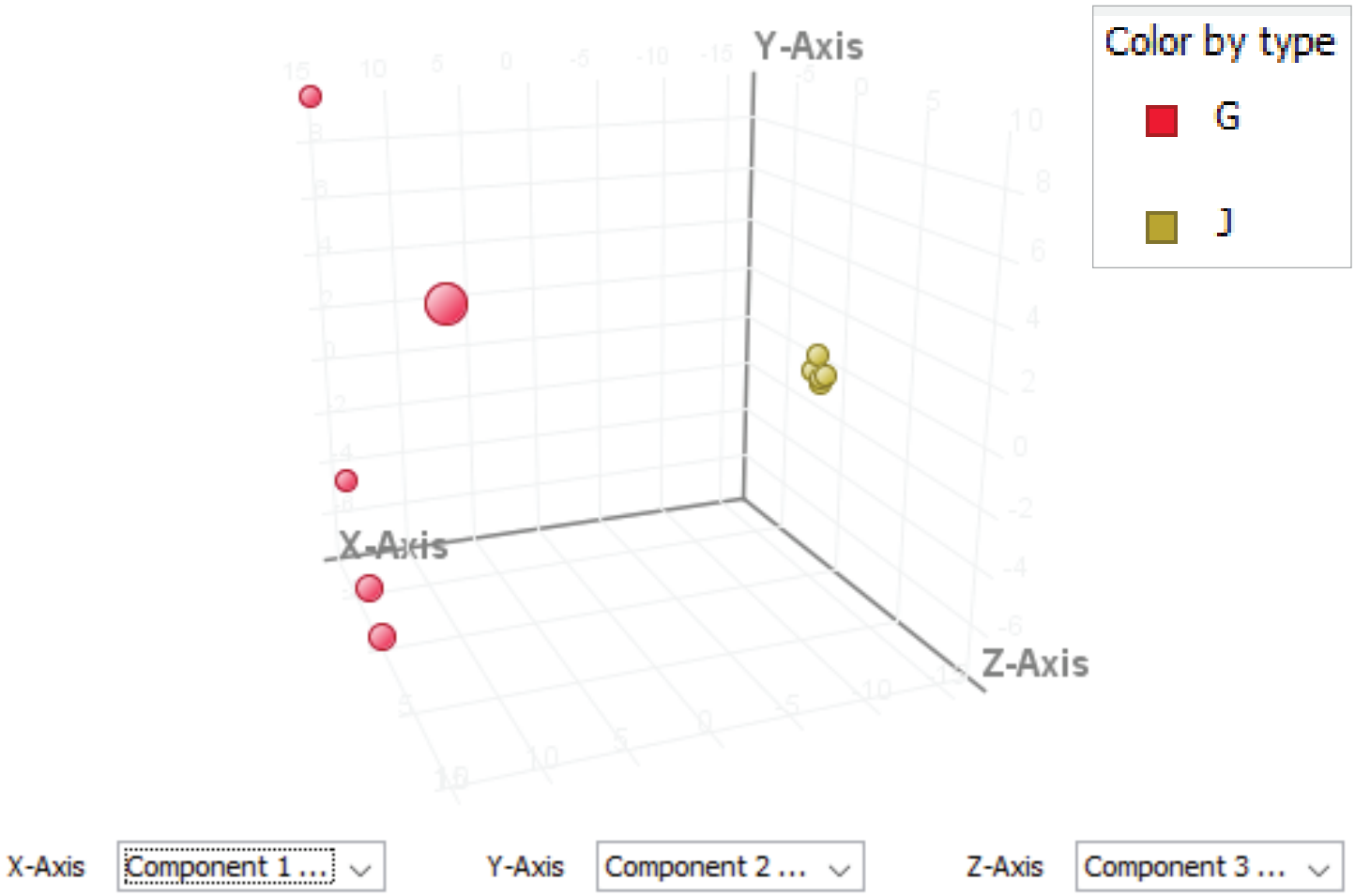


Figure 9. PCA plot demonstrating clear clustering of ginger (G) and juniper berry (J) oil samples.

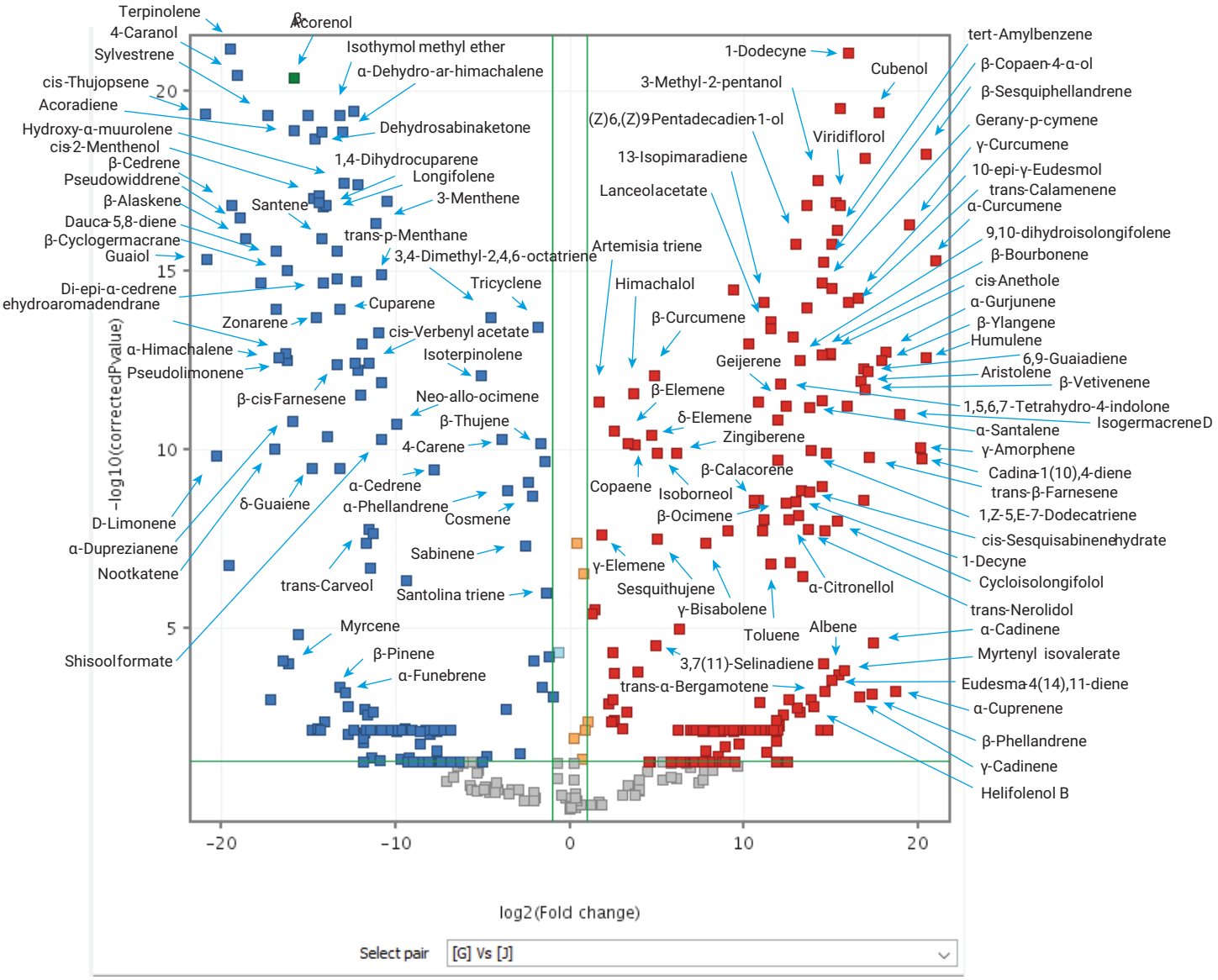


Figure 10. Volcano plot comparing the compositions of ginger and juniper berry oils, using a fold change cutoff of 2 and a p-value cutoff of 0.05.

Conclusion

In this application note, a method for the analysis of essential oils using several comprehensive GC × GC configurations with a reverse flow modulator (RFM) and a high-resolution Agilent 7250 GC/Q-TOF system was developed. Method optimization was performed using a diesel sample. Essential oil components, separated by GC × GC, were reliably identified using the Unknowns Analysis tool. Agilent Mass Profiler Professional (MPP) software successfully determined the differences in chemical composition between the essential oils.

References

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