Coupling Comprehensive Two-Dimensional Gas Chromatography with an Orbitrap MS for Enhanced Separation and Identification

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ABSTRACT

Two-dimensional (2D) gas chromatography (GC × GC) is a comprehensive technique for isolating and identifying compounds present in complex matrices in a single analytical run. High Resolution/Accurate Mass (HRAM) mass spectrometry has become a popular detector for GC × GC as it provides full-scan analysis with excellent sensitivity and selectivity. Trace level detection limits and chemical formula elucidation are the key performance indicators of HRAM mass spectrometry (MS) that are superior to its low resolution counterparts. With high-quality mass spectral information and accurate mass measurements, low-concentration unknown compounds can be easily detected and their elemental composition can be generated with sub ppm mass error, which dramatically increases the confidence of identification. In this study, an HRAM Orbitrap analyzer was coupled with a GC × GC using reversed flow modulation for different applications.

INTRODUCTION

HRAM gas chromatography-mass spectrometry (GC/MS) has recently become a popular tool for comprehensive sample characterization because of its high selectivity in a fullscan acquisition mode. Coeluting peaks with the same nominal mass, which interfere at nominal resolution, can be spectrally separated at high resolution, allowing for the detection and identification of more compounds in matrix. However, coeluting isomers can still be problematic as they have exactly the same masses for both their molecular and fragment ions, and also retention indices that are quite near to each other that makes for ambiguous identifications. In this case, high-resolution GC/MS alone is ineffective for separation, whereas comprehensive two-dimensional gas chromatography is an alternative tool to couple with HRAM mass spectrometry to address this issue.

GC × GC is a sequential heart-cut technique using a modulator that traps and releases portions from a primary column and reinjects them into a shorter secondary column where a different polarity phase is being used for this separation. Use of a modulation device is essential to achieve focus and reinject effluents from the primary column to the secondary column. This process can generally provide a ten-fold improvement in sensitivity with respect to unidimensional (1D) GC/MS. In this study, the INSIGHT™ reversed flow modulator from SepSolve Analytical was used as the modulation device. It contains seven ports that directly connect to a primary column, a secondary column, a bleed line, a sample loop, and a gas module. This device offers a reverse fill/flush operation comparing it with forward fill/flush flow modulators. A two-stage process is shown below in Figure 1. In the fill step, the sample loop is filled by the effluents in the forward direction from the primary column (blue arrow) while the auxiliary gas module has carrier gas passing through a normal open (NO) valve to the secondary column (red arrow). Since a bleed line is connected to the end of the sample loop, extra effluents go into the bleed line that avoid overfilling the loop, which is the key improvement in contrast to the forward flow modulator. Next, a normal closed (NC) valve is turned on. High carrier gas flow rapidly flushes the sample loop in an opposite way and passes all the effluents on to the secondary column within hundreds of milliseconds (red arrow). Due to the opposite flushing of the sample loop, it is called a reversed flow modulator. This reversed flow modulator (Figure 2) can generate relatively narrower peak shapes in the second dimension as compared to the conventional forward flow modulator and increase peak capacity, reduce baseline rise, peak tailing, and avoid breakthrough and overfilling of the sample loop.

Figure 1. Fill and flush steps on the reverse flow modulator

1. Fill Step   **2. Flush Step**
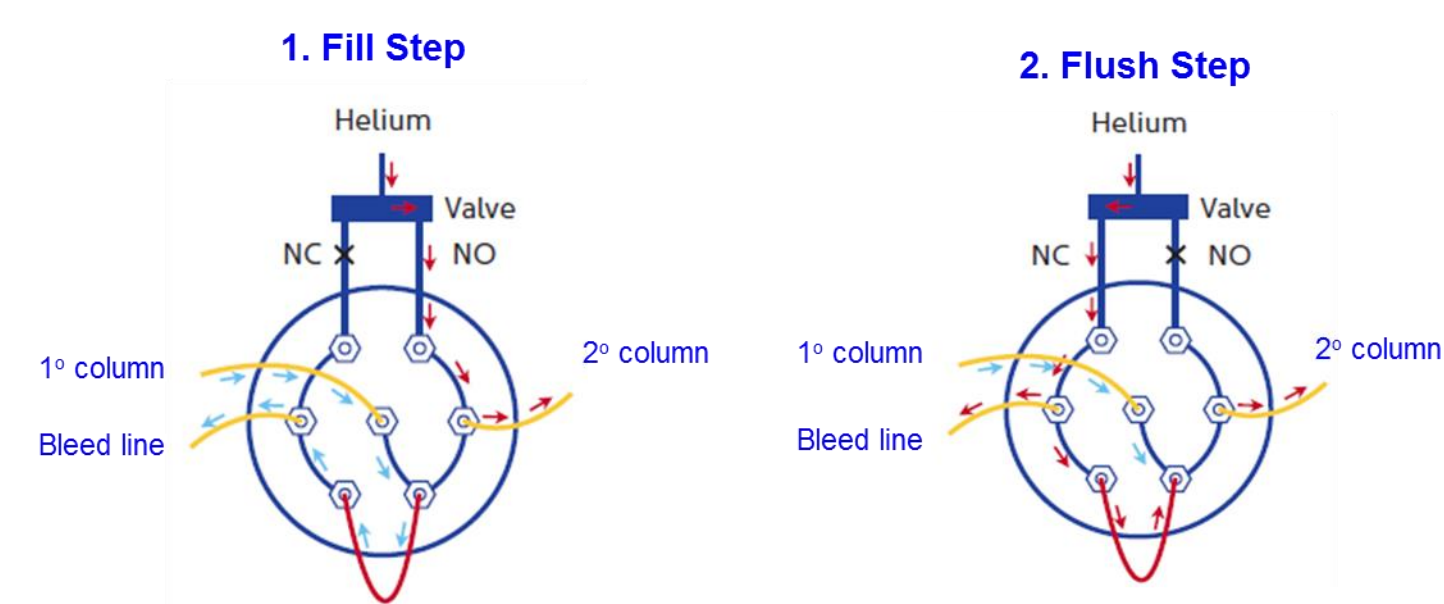
MATERIALS AND METHODS

Sample Preparation

Standards were purchased from Sigma-Aldrich including amino acids, 37 food industry fatty acid methyl esters (FAMEs). Lemon oil was diluted 100/1 in hexane.

Table 1. Gas chromatograph and mass spectrometer analytical parameters. SepSolve INSIGHT flow modulator contains seven ports.

Thermo Scientific™ TRACE™ 1310 GC Parameters

Primary column	Injection Volume (µL):	1.0
Secondary column	Liner	Single taper without glass wool
Primary column flow , (mL/min)	He, 0.5	Flow Modulator	INSIGHT™ (SepSolve Analytical)
Secondary column flow, (mL/min)	He, 20	Inlet (°C):	250
Loop (µL):	50	Inlet Module and Mode:	Split 10:1 (EI) Splitless 2min (CI)

Oven Temperature Program:

Temperature 1 (°C):	40	Hold Time (min):	1
Temperature 2 (°C):	280	Rate (°C/min)	5
Hold Time (min):	5		

Thermo Scientific™ Q Exactive™ GC MS Parameters

Transfer line (°C):	250
Ionization type:	EI / PCI
Ion source (°C):	250
Electron energy (eV):	70
Acquisition Mode:	Full scan
Reagent gas, (mL/min)	CH4, 1.5
Mass range (m/z):	50-600 (EI) 100-700 (CI)
Lock masses (m/z):	73.04680; 133.01356; 207.03235; 281.05114; 355.06990

Figure 2. SepSolve flow modulator
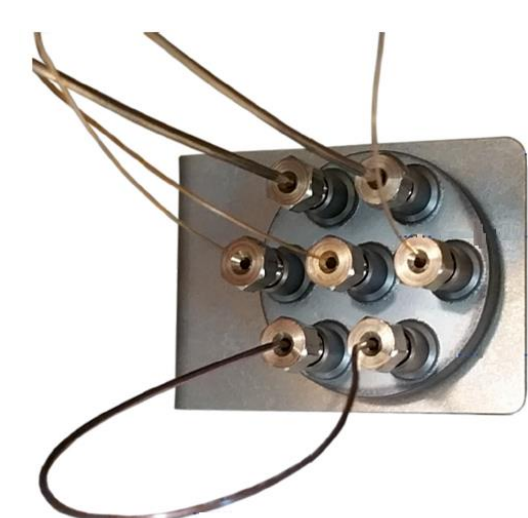
Data Analysis

Data was acquired using Thermo Scientific™ TraceFinder™ 4.1 software and processed through SepSolve ChromSpace™ software, which allows for both quantitative and qualitative 2D GC data analysis. This includes peak integration and calculation of compound concentration as well as data review and reporting. In addition, for qualitative analysis, ChromSpace can automatically perform peak detection on the whole contour plot or on a specific area defined by users. Library searching was performed by either the NIST 17 library or the HRAM GC-Orbitrap library.

RESULTS

Flavor and Fragrances : terpene analysis

Terpene hydrocarbons such as monoterpenes and sesquiterpenes are widely seen in citrus oils. The determination of the essential oil volatile profile is essentially important for evaluating quality and authenticity. The separation of very complex citrus oil matrices through 1D GC/MS is difficult as co-elutions are inevitable even when sophisticated deconvolution software has been employed. For this reason, 2D GC/MS provides second dimension separation, which increases resolution, peak capacity, and selectivity especially for terpene isomers that have exactly the same molecular and fragment ions so that even HRAM GC/MS would not be readily able to differentiate them. In this case, a lemon oil extraction was used as an example. Limonene, a lemon-like odour monoterpene, is the most abundant terpene in lemon oil and has been widely used as an additive in industrial cleaning solvents and in cosmetics. To correctly detect trace amounts of monoterpene components in lemon oil is also critical for quality control. Ocimene, a monoterpene with a pleasant odour appreciated in perfumery, is most frequently found in essential oils. The comparison of their high resolution spectra (Figure 2) acquired on a Q Exactive GC-MS shows they share almost the same fragment ions.

Furthermore, their retention indices (RIs) on a semi-non-polar column are nearly identical (1030 vs 1037) (Table 2), which makes it extremely difficult to separate and firmly identify on a 1D GC/MS when lacking available standards. However, their RIs on a polar phase column are relatively large (1200 vs 1250), and that could help for separation on a 2D GC/MS.

Figure 2 . Comparison of the spectra of limonene and ocimene acquired on Q Exactive GC/MS.
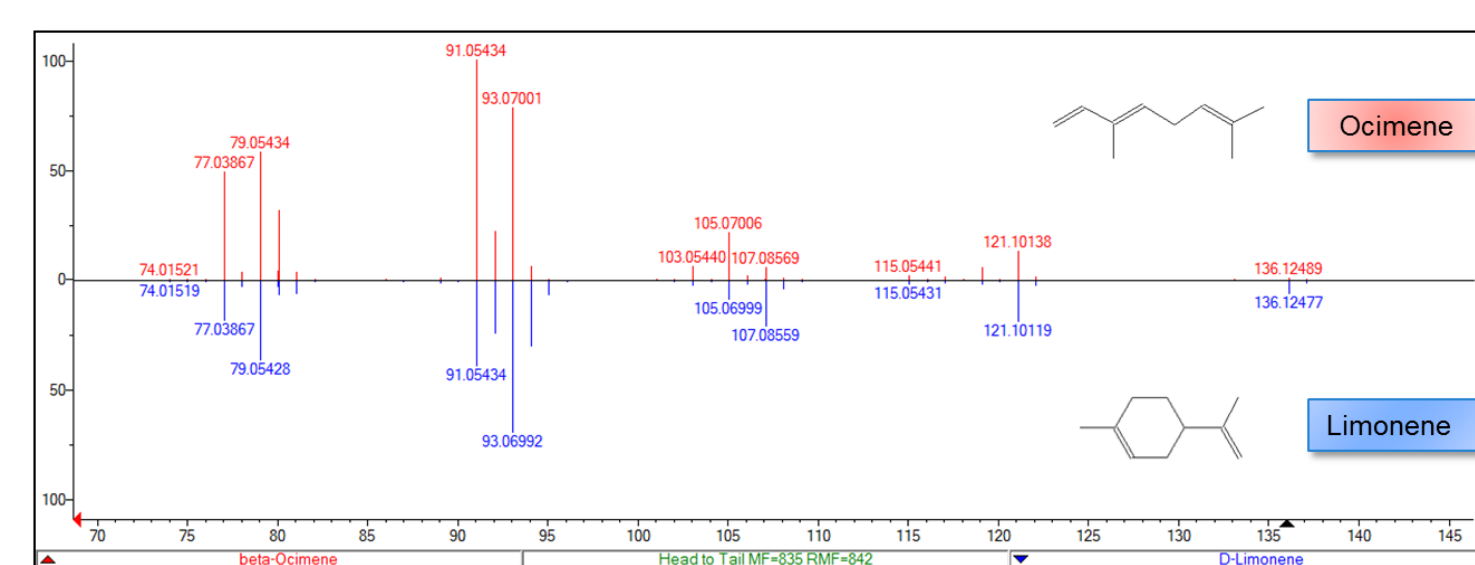

Table 2. Retention indices of limonene and ocimene on semi-non-polar and polar columns from the National Institute of Standards and Technology (NIST) library.

RI	Semi non polar	Polar
Limonene	1030±2	1200±7
Ocimene	1037±7	1250±4

The result, as shown in Figure 3, illustrates the excellent separation of the trace of ocimene from a concentrated limonene peak. These two analytes were fully separated on the secondary column. Also, cymene was separated from limonene peak as well. Library search was performed against the HRAM GC-Orbitrap library (Figure 4) and a standard was also analyzed for confirmation. Sub-ppm mass accuracies were achieved across all compounds not only on molecular ions but also on fragment ions (Figure 5).

Figure 3. The separation of limonene and ocimene acquired on GC × GC-Orbitrap MS with less than 1 ppm mass accuracies on their molecular ions. Data was processed on SepSolve ChromSpace.
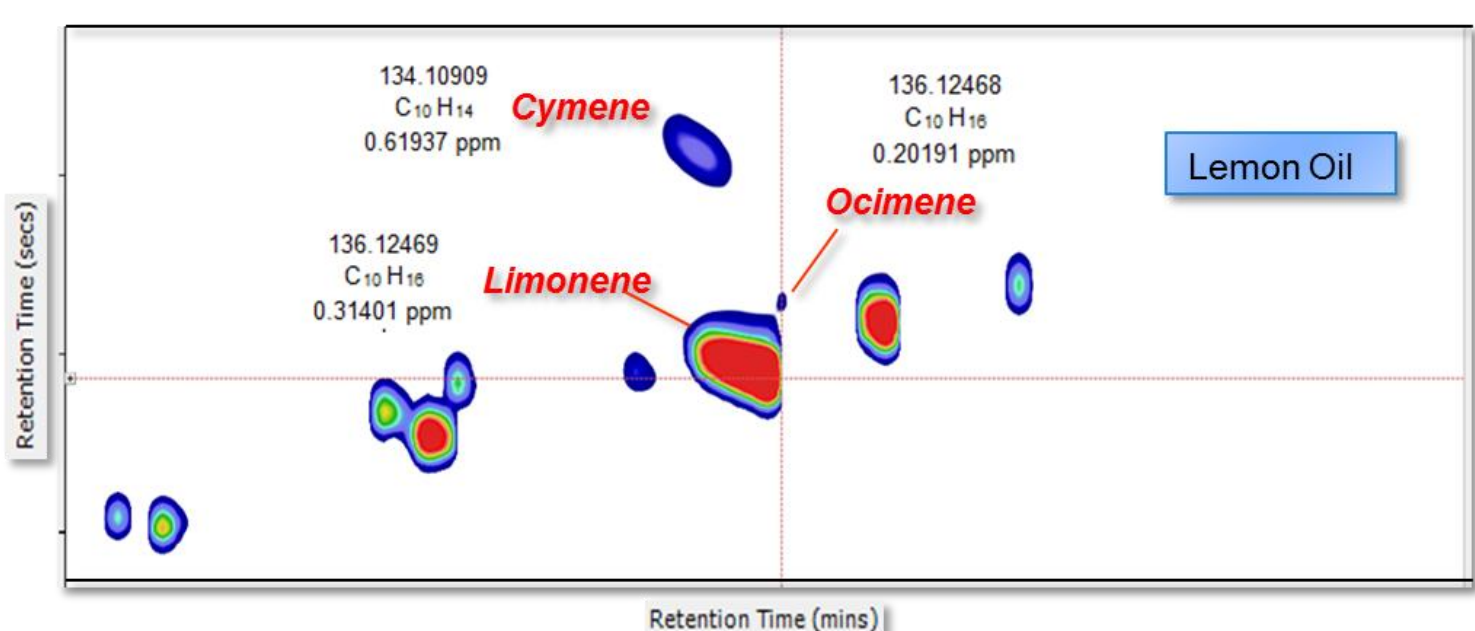

Figure 4. Comparison of acquired and library (HRAM GC-Orbitrap library) spectra to confirm target analyte ocimene.
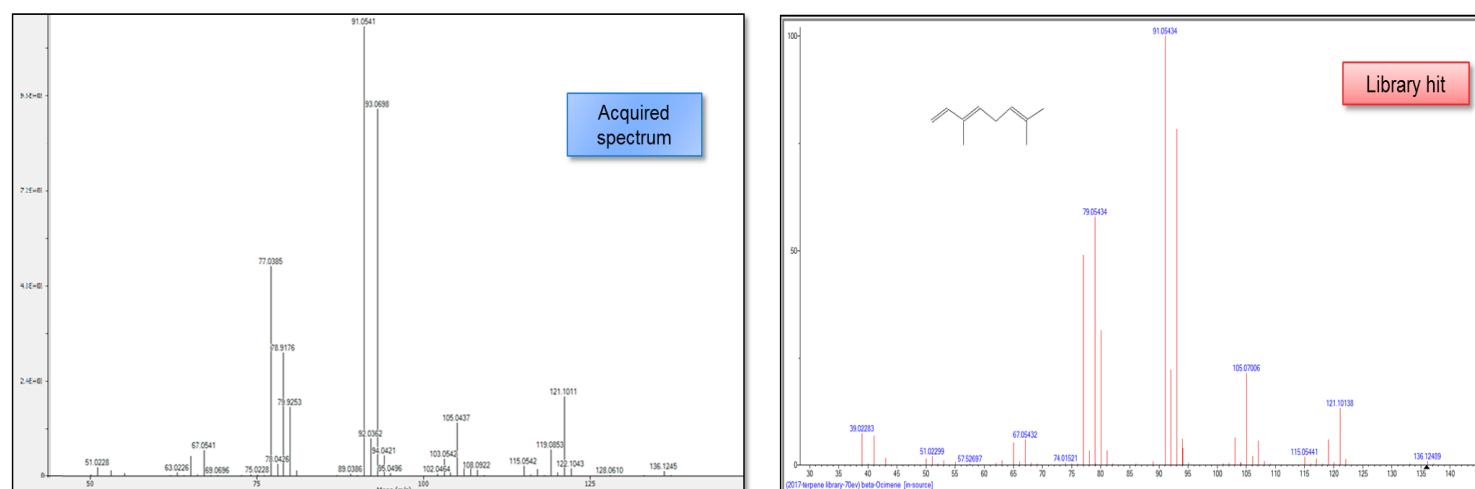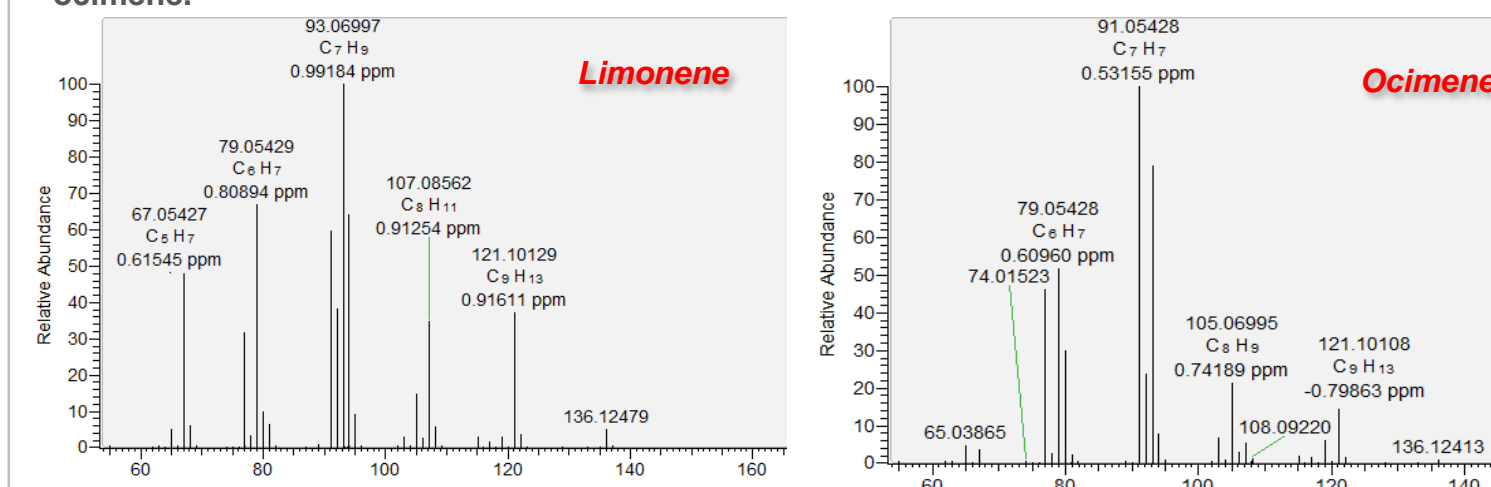

Figure 5. Sub-ppm mass accuracies were maintained on all fragment ions of limonene and ocimene.

Foodomics: food industry FAMEs Analysis

Fatty acids are fundamental to human nutrition. They are key intermediates in the formation of energy in the human body and significantly reduce the risk of diabetes and heart disease. In the food industry it is challenging to determine the absolute amount of both cis and trans double bond isomers of unsaturated fatty acids, and the trans isomers have significant negative health effects. Fatty acid methyl esters (FAMEs) are the typical formation of fatty acids to be analyzed on GC/MS. In this case, GC×GC-Orbitrap MS was used to differ FAMEs by their carbon numbers, and particularly to separate unsaturated isomers based on the position of their double bonds. The contour plot shown in Figure 6 illustrates all the saturated and unsaturated FAMEs that were separated and identified. The retention pattern of different compound classes provides additional information to assist with the identification of structurally related compounds (in red rectangular boxes). Surface plot (3D plot) shown in Figure 7 is also generated and synchronized with the contour plot which provides a three-dimensional perspective of this GC × GC data.

Figure 6. Food industry FAMEs were separated on the GC×GC-Orbitrap MS. Red rectangles represent compounds by different carbon numbers. The green rectangle shows solvent and column bleed peaks. Data was processed on SepSolve ChromSpace.
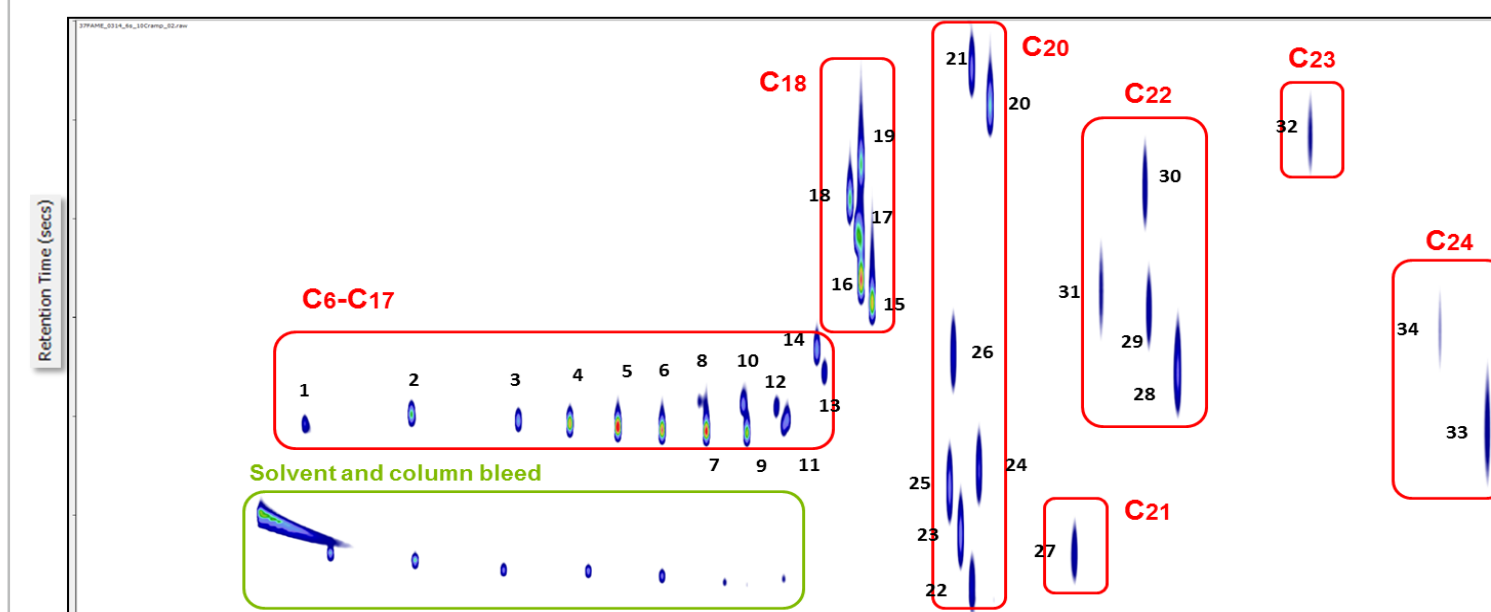

Figure 7. 3D plot of the whole chromatogram view of food industry FAMEs.
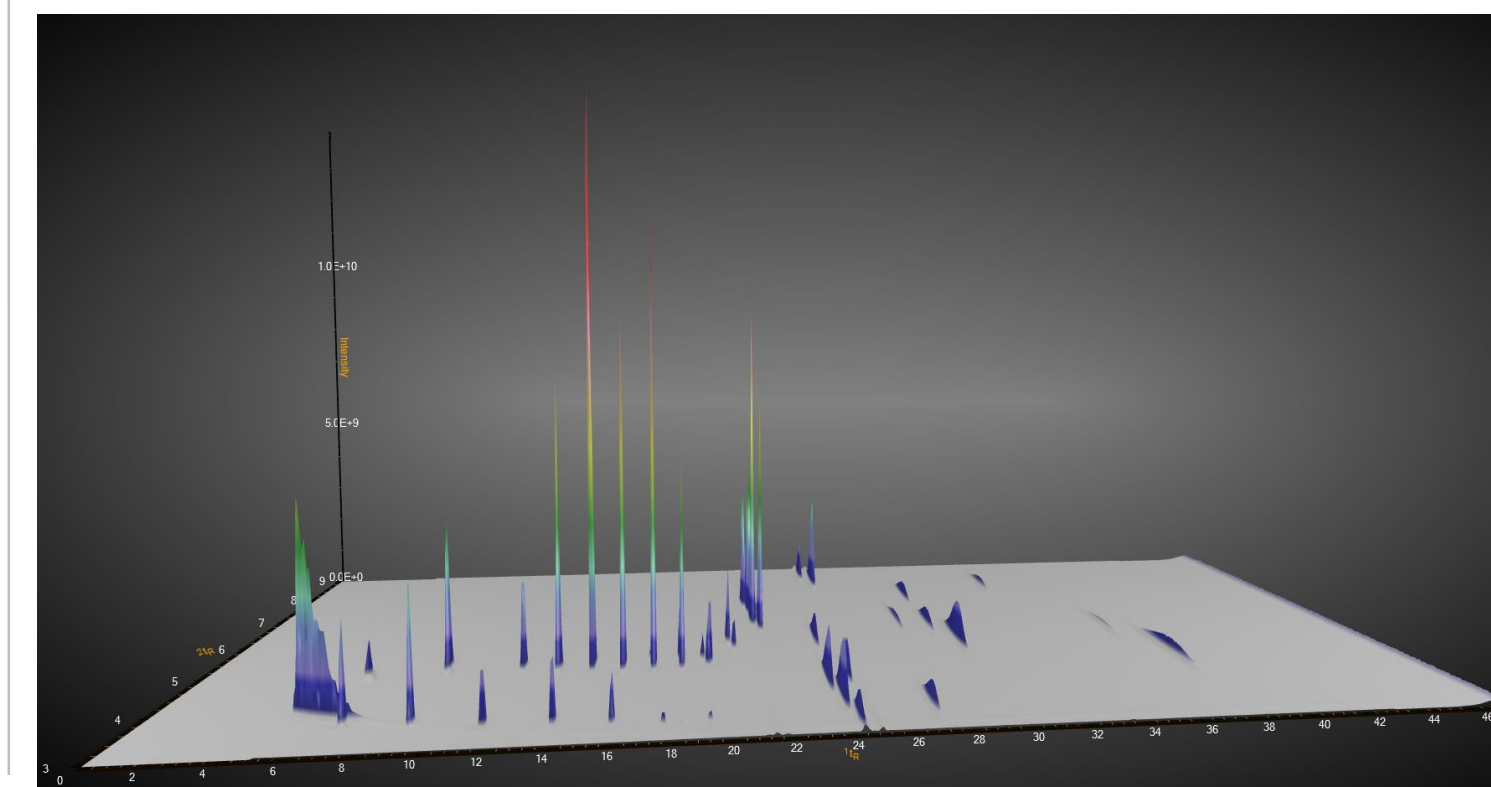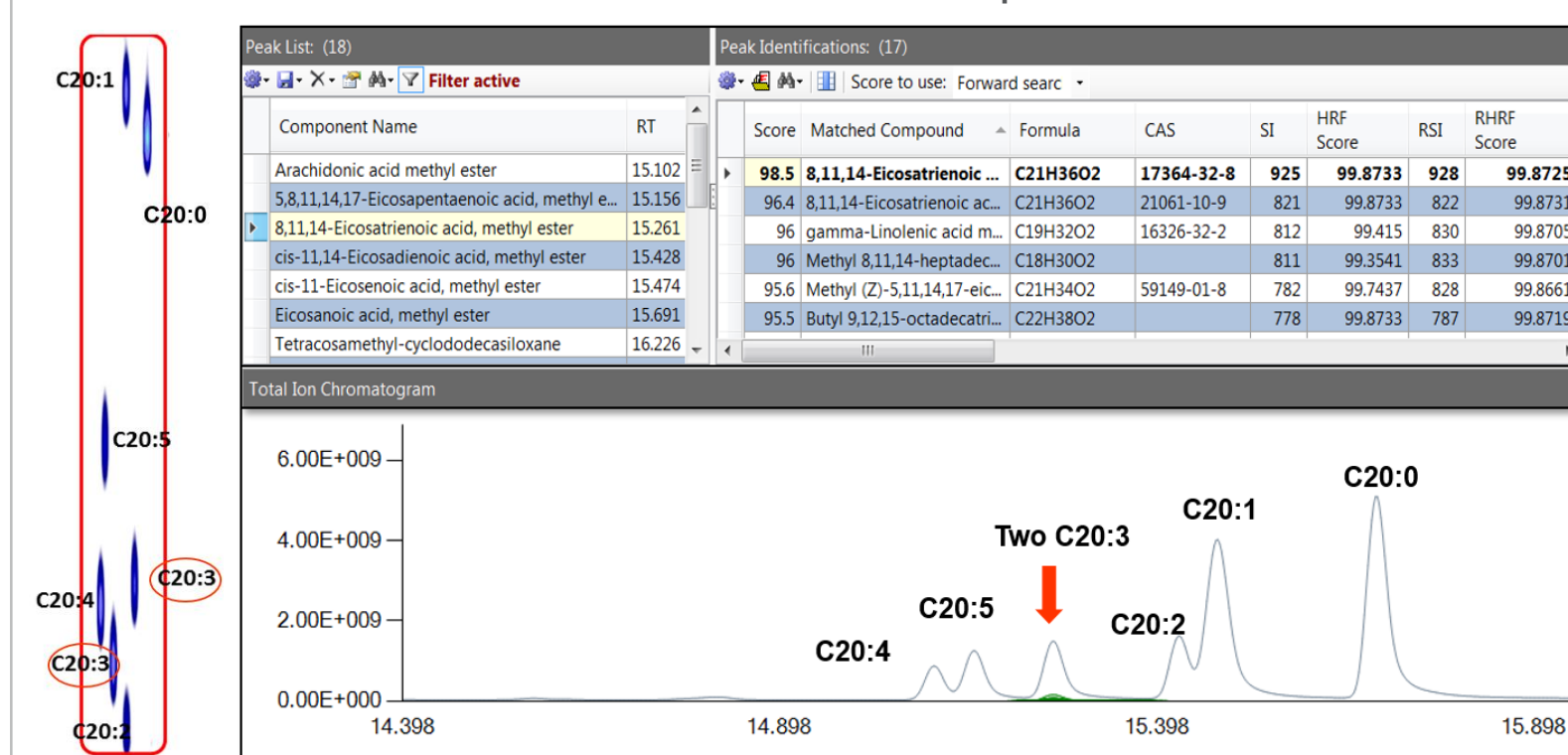

Figure 8. Example shown GC × GC separated two FAMEs C20:3 isomers on ChromSpace, whereas in Deconvolution software these two isomers co-elute in one peak and can't be deconvoluted.

Isomers with double bonds in the same position but in a different formation were also well separated. Figure 12 shows the separation of the class of C20 FAMEs. The separation of two isomers of C20:3 (Figure 8) was firmly established on a 2D contour plot, whereas co-elution of these two isomers was observed on a 1D chromatogram, and even deconvolution software would not be able to separate them. In order to further confirm all the FAME compounds, positive chemical ionization (PCI) analysis using methane as the reagent gas was performed. Each compound was confirmed by their pseudo-molecular ions [M+H]+. Excellent mass accuracy was maintained in PCI mode (Table 3).

FAME	RT₁(min)	RT₂(s)	Formula	Exact Mass [M+H]+	Mass Accuracy (ppm)
C₂₀:0	24.953	3.172	$C_{21}H_{43}O_2$	327.3258	-1.01
C₂₀:1	24.502	3.566	$C_{21}H_{41}O_2$	325.3101	0.69
C₂₀:2	24.419	4.330	$C_{21}H_{39}O_2$	323.2945	-0.13
C₂₀:3*	24.083	4.852	$C_{21}H_{37}O_2$	321.2788	-0.97
C₂₀:3**	24.583	5.525	$C_{21}H_{37}O_2$	321.2788	-0.92
C₂₀:4	23.813	5.358	$C_{21}H_{35}O_2$	319.2632	-0.39
C₂₀:5	23.915	6.690	$C_{21}H_{33}O_2$	317.2475	0.64

*: (all-cis-8,11,14)-methyl eicosatrienoate;
**: (all-cis-11,14,17)-methyl eicosatrienoate

CONCLUSIONS

HRAM GC Orbitrap mass spectrometry coupled with flow modulated GC × GC modulation was employed in both EI and CI acquisition modes for the full characterization of flavor and fragrances analysis and foodomics. The benefits utilizing GC × GC-Orbitrap are listed below:

- Excellent mass accuracy in both EI and CI modes for chemical formula elucidation and further increase in confident identification
- Highest sensitivity for trace-level compound identification especially for unknown screening analysis
- Highest repeatability using flow modulator for GC × GC
- Efficient separation of co-eluted isomers or structurally similar compounds

TRADEMARKS/LICENSING

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