Comprehensive Two-Dimensional Gas Chromatography with Orbitrap Mass Spectrometry Applied to Flavor and Fragrance Analysis

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ABSTRACT

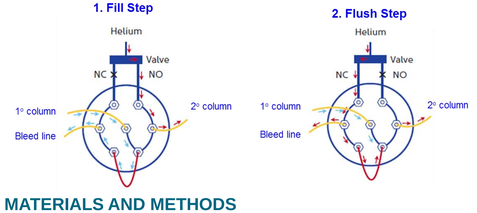
Two-dimensional (2D) gas chromatography (GC × GC) is a comprehensive technique for isolating and identifying compounds present in complex matrices in a single analytical run. High Resolution/Accurate Mass (HRAM) mass spectrometry has become a popular detector for GCxGC as it provides full-scan analysis with excellent sensitivity and selectivity. Trace level detection limits and chemical formula elucidation are the key performance indicators of HRAM mass spectrometry (MS) that is 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 confident identification. In this study, an HRAM Orbitrap analyser was coupled with a GC × GC using reversed flow modulation for essential oil analysis.

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 same masses for both their molecular and fragment ions, and also retention indices are quite near to each other makes 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 on a 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 typically 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. This device offers a reverse fill/flush operation comparing with forward fill/flush flow modulators. A two-stage process is shown below in Figure 1. Due to the opposite flushing the sample loop, it is called a reversed flow modulator. This reverse flow modulator (Figure 2) can generate relatively narrower peak shapes in the second dimension than the conventional forward flow modular and increase peak capacity, reduce baseline rise, peak tailing and avoid breakthrough and overfilling the sample loop.

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



MATERIALS AND METHODS

Sample Preparation

All the essential oils were diluted 100/1 by hexane. All the solvents were purchased from Sigma-Aldrich.

Data Analysis

Data was acquired using Thermo Scientific™ TraceFinder™ 4.1 software and processed through SepSolve ChromSpace, which allows for both quantitative and qualitative 2D GC data analysis.

RESULTS

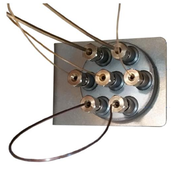
Lavender oil is the most commonly used essential oil for food, pharmaceutical, therapeutic, fragrance and beauty usages. There are several varieties and species of lavender as essential ions. The two main types are lavender and lavandin. They have similar aromas but quite different chemical composition. Lavender oil is more expensive than Lavadin oil due to the yield. Therefore, it is essential to understand and distinguish these species for safety, quality control and adulteration. In this study, four different species of lavender oil and one lavandin oil were analyzed by GC x GC-Orbitrap in Figure 3. Excellent separations were achieved for each essential oil. Monoterpenes, sesquiterpenes and terpenoids are the common types of terpenes in lavender oil. They can be easily separated on GCxGC contour plot (Figure 4 (a)). The 3D plot can be also generated and synchronized with its contour plot using ChromSpace to improve data visualization (Figure 4 (b)).

Table 1. Gas chromatograph and mass spectrometer analytical parameters. And SepSolve INSIGHT flow modulator that has seven ports on it.

Thermo Scientific™ TRACE™ 1310 GC Parameters			
Primary column	TG-5silMS 20m x 0.18mm x 0.18um	Injection Volume (µL):	1.0
Secondary column	TG-17silMS 4m x 0.25mm x 0.25um	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)
Oven Temperature Program:			
Temperature 1 (°C):	40	Hold Time (min):	0
Temperature 2 (°C):	280	Rate (°C/min)	3

Thermo Scientific™ Q Exactive ™ GC MS Parameters	
Transfer line (°C):	250
Ionization type:	EI
Ion source(°C):	250
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (*m/z*):	50-600 (EI)
Lock masses (*m/z*):	73.04680; 133.01356; 207.03235; 281.05114; 355.06990

Figure 2. SepSolve flow modulator



In Table 2, it shows the total number of compounds being identified using ChromSpace after peak detection and deconvolution. Numbers of total identified peaks with the numbers of three sub-compound classes are listed in this table for comparison. *Bulgarian* lavender contains highest amount of monoterpenes and sesquiterpenes whereas *Abralis* lavandin has the least three main classes of terpenes. *40-42* lavender oil has the highest amount of terpenoid which is mainly linalool.

Separation co-eluated compounds is the strength of GCxGC. Since it is couple with HRAM Orbitrap system, some co-elutions can be resolved by using deconvolution software with accurate mass measurement. One example is shown in Figure 5 (a) which the analyte 1, 2, and 3 co-eluted together on *Bulgarian* lavender contour plot. They were well separated and identified by ChromSpace where the peak list is below the contour plot. Same sample was acquired on 1D system using deconvolution software for data processing. As we can see, they were successfully deconvoluted and listed on the deconvolution peak list (Figure 5 (b)). But not all the co-elutions can be resolved by using HRAM 1D system. One example is shown in Figure 6 (a) where cuminaldehyde is submerged by a huge linalyl acetate peak. For this case, cuminaldehyde wouldn't be able to be detected on 1D system even using deconvolution software, whereas it can be clearly separated on second dimension using GCxGC. Identification was performed by library search and sub-ppm mass accuracies were achieve for each fragment ions of these two compounds (Fig 6 (b)).

GC x GC is an efficient tool for aromatic profiling especially for quality control and adulteration analysis. Among five species, *40-42* lavender is the only oil blended to produce 40% linalool and 42% linalyl acetate, which are the two primary aromas in lavender. The purpose is to create a standardized lavender aroma batch to batch and also its price is more affordable than 100% lavender oil. However, it is hard for customers to distinguish real lavender oil, blended oil or lavandin oil. Thus, it is important to using GCxGC-MS to profile essential aromas percentage for comparison. In Figure 7, it shows the intensities of linalool and linalyl acetate. For *40-42* lavender, the linalool versus linalyl acetate ratio is about 40/42, where *population* lavender has similar amount linalool but less linalyl acetate. Both *abralis* lavadin and Bulgarian lavender have both lower level of these two aromas. Terpenoids are the main aroma contributors for lavender. Three major terpenoids are charted in Figure 8. Population lavender has highest amount eucalyptol and the second highest of a-terpineol; where Bulgarian lavender has the highest of a-terpineol; English lavender has the highest of terpinen-4-ol. Lavendin oil typically has relatively lower among terpenes than lavender oils, but contains higher camphor percentage, therefore it is commonly used in soap.

Figure 3 . Contour plots of four types of lavender oils and one type of lavandin oil with their subtype names in the blue rectangular box.

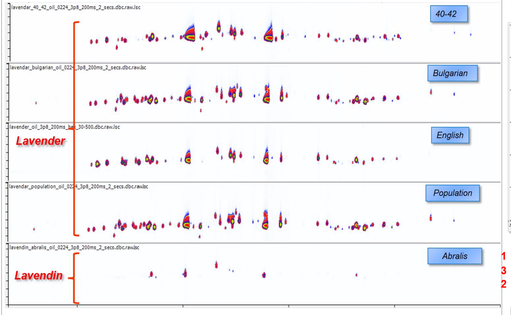


Figure 4. (a). The contour plot of *English* lavender with the three compound classes separated by stencils using ChromSpace; stencils are the black rectangular boxes that can be saved as template on ChromSpace. (b). The 3D plot of *English* lavender on ChromSpace; 3D can be synchronized with its contour plot to improve data review.

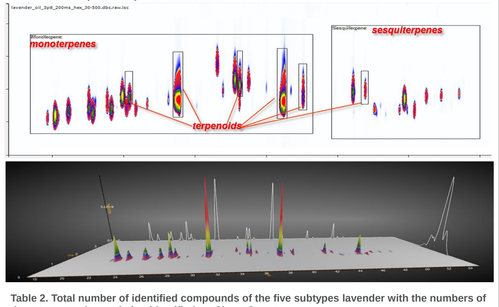
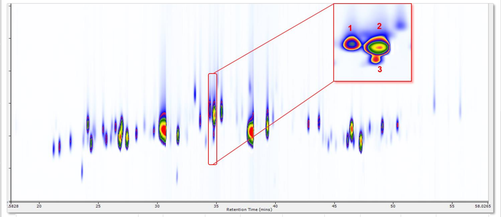


Table 2. Total number of identified compounds of the five subtypes lavender with the numbers of three terpene classes being identified on ChromSpace.

Main types	Subtypes	Total identified compounds	Monoterpene	Sesiquterpene	Terpenoid
Lavender	*40-42*	378	58	62	37
	Population	358	55	58	20
	English	328	53	40	12
	Bulgarian	375	63	65	22
Lavandin	*Abralis*	143	33	9	6

Figure 5. (a) The example of three co-eluted compounds on *Bulgarian* lavender contour plot with their identifications listed in the table (b). Same sample acquired on 1D GC-Orbitrap and the data was processed on Deconvolution software where these three co-eluted compounds can be deconvoluted as well.



	Compound	MF	Probability	CAS #	Formula	Exact mass	RT	^1t_R	^2t_R	Area
1	Borneol	832	78.48	507-70-0	C10H18O	154.136	34.6731	34.5433	1.7887	1.79029E+09
3	Propanoic acid, 2-methyl-, hexyl ester	672	48.3	2349-07-7	C10H20O2	172.146	34.794	34.77	1.4382	2.70287E+08
2	Terpinen-4-ol	672	7.92	562-74-3	C10H18O	154.136	34.8342	34.8058	1.7087	1.305E+10

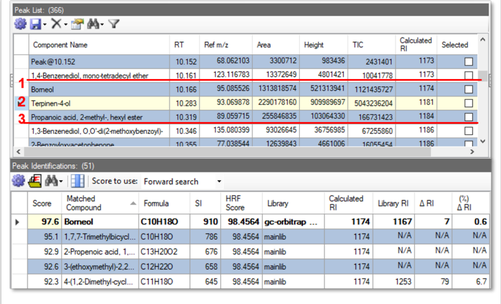
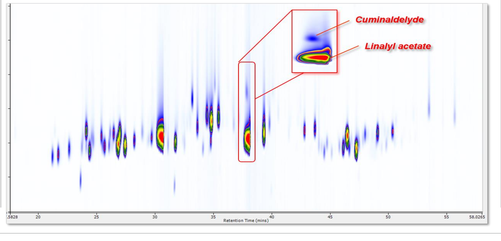
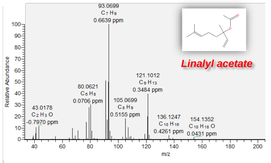


Figure 6. (a). The example of two co-eluted compounds are separated on *Bulgarian* lavender contour plot (b). The spectra of these two co-eluted compounds with sub-ppm mass accuracies for each fragment ions which increased the confident identification.





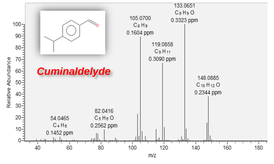


Figure 7. Comparison of the relative intensity of linalool and linalyl acetate among five species lavender

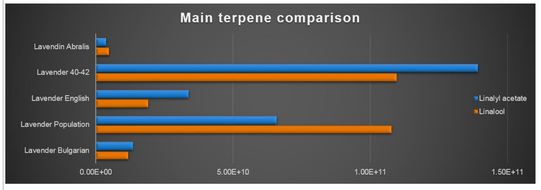
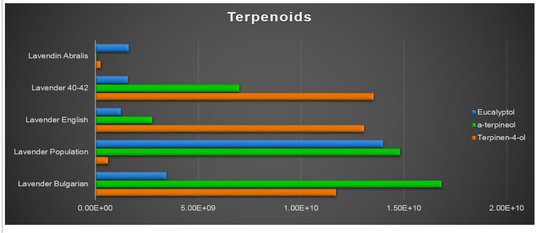


Figure 8 . Comparison of the relative intensity of three main terpenoids among five species lavender



CONCLUSIONS

HRAM GC Orbitrap mass spectrometry coupled with flow modulated GC × GC modulator was employed in the full characterization of essential oil analysis. The benefits utilizing GC × GC-Orbitrap are listed below:

- Sub-ppm mass accuracy for chemical formula elucidation and further increase the confident identification
- Highest sensitivity for trace-level compounds identification especially for unknown screening analysis
- Efficient separation of co-eluted isomers or chemical structure similar compounds
- Efficient tool for aromatic profiling

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