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Technical Report

GC/MS Flavor Analysis of Foods and Beverages Under Consumption Conditions

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Abstract:

To identify differences in flavor of commercially marketed alcoholic beverages, we collected fragrance components under the same conditions as during eating and drinking and measured the components by GC/MS. The measurement results were then analyzed using multivariate analysis software to determine the components characteristic of each product. In addition, we used a heat map to visually show the change in fragrance components as time elapses from immediately after opening the alcoholic beverage.

Keywords: Flavor, Fragrance, GC/MS, MonoTrap, Multivariate analysis

1. Introduction

To develop hit products in the food and beverage market, it is important to clearly differentiate them from competing products. Recently, alcoholic beverages like "chuhai" (highball of shochu, a Japanese distilled spirit similar to vodka) and cocktails that have been developed to have a more premium or authentic taste, such as with stronger fruit or other base flavors, have been especially successful in the marketplace.

Developing such products involves sensory testing based on our five senses, but due to differences in how developers sense things, their preferences, and the words used to express their impressions, sensory testing results are difficult to share with other developers. Even individually, our senses can vary depending on our health.

Given that gas chromatograph mass spectrometer (GC/MS) systems are especially capable of detecting volatile compounds, they can be used to detect the type of fragrance components contained in samples and their quantities. By using GC/MS analysis in conjunction with sensory testing, it is possible to determine how respective compounds are related to sensory testing results, which may allow smoother product development.

Because of the ease of measurements, the headspace (HS) method is used to analyze fragrance components in foods, beverages, and other liquids. Headspace analysis generally involves heating samples to increase the sensitivity of detecting fragrance components. However, since alcoholic beverages are often consumed cold, the flavor when samples are heated for headspace analysis might not be the same as the fragrances experienced at the instant they are actually consumed. Moreover, cooking food with heat can involve an oxidation reaction that can affect flavor. Consequently, it is difficult to recreate actual eating/drinking conditions using the headspace method.

MonoTrap® adsorbents (from GL Sciences) have a porous silica structure with a large surface area that results in excellent trapping capacity. That means it can trap fragrance components under a variety of conditions, regardless of the temperature or environment.

This Technical Report bulletin describes using a MonoTrap adsorbent to trap fragrance components from commercially marketed lemon-flavored alcoholic beverage products for the purpose of identifying differences in flavor at the instant they are actually consumed, and then using multivariate analysis to search through the results obtained from GC/MS analysis. It also describes the results from studying the changes in flavor as a function of time.

2. Measurement Sample Preparation

Four types of commercially marketed lemon-flavored alcoholic beverages ("chuhai") were used as measurement samples. To simulate consumer storage conditions, samples were kept stored refrigerated until immediately prior to opening. Immediately after opening the containers, 20 mL of each sample were placed in 40-mL glass vials and 200 µL of 100 µg/mL p-bromofluorobenzene was added as an internal standard. An MT holder (GL Sciences) was used to place monolithic silica adsorbent (MonoTrap DCC18 from GL Sciences) into the 40 mL glass vial caps in advance. After adding the sample, the vials were promptly sealed to trap the fragrance components (Fig. 1). Fragrance components were trapped at room temperature for 30 minutes.

Then the fragrance components were solvent-extracted from the MonoTrap material using 1 mL of diethyl ether. After sonicating the extract for five minutes, the extract was purged with nitrogen to concentrate it to a final volume of 100 µL without heating. Three specimens of each sample were prepared and analyzed by GC/MS.

More detailed GC/MS analytical conditions are shown in Table 1.

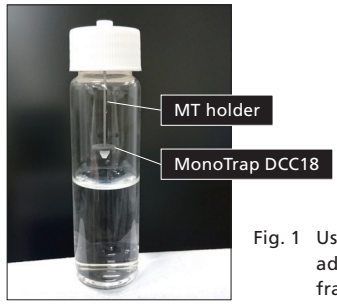

Fig. 1 Using a MonoTrap adsorbent to trap fragrance components

1 Analytical & Measuring Instruments Division

3. Results and Discussion

3-1. Analysis Results

A total ion current chromatogram (TIC) of each sample is shown in Fig. 2. The four types of products used as samples are labeled A to D.

Monoterpenes (such as limonene, terpinene, myrcene, and cymene), sesquiterpenes (bisabolene, caryophyllene, and bergamotene), monoterpene alcohols (such as terpineol and linalool), and geranyl acetate were detected in all four samples.

In addition, aliphatic aldehydes (octanal, nonanal, and decanal) or citral were detected in some products. Detected compounds are listed in Table 2.

Table 1 Analytical Conditions

GC-MS	: GCMS-QP™2020 NX
Autoinjector	: AOC™-20i + 20s
Column	: SUPELCOWAX®10 (Length 30 m, 0.25 mm I.D., df=0.25 µm)
[GC]	
Injection unit temperature	: 280 °C
Column oven temperature	: 50 °C => (3 °C/min) => 230 °C
Injection mode	: Split (1:5)
Carrier gas	: He
Carrier gas control	: 30.0 cm/sec (constant linear velocity)
Injection volume	: 2 µL
[MS]	
Ion source temperature	: 200 °C
Interface temperature	: 250 °C
Ionization method	: EI
Data acquisition mode	: Scan
Event time	: 0.3 sec.

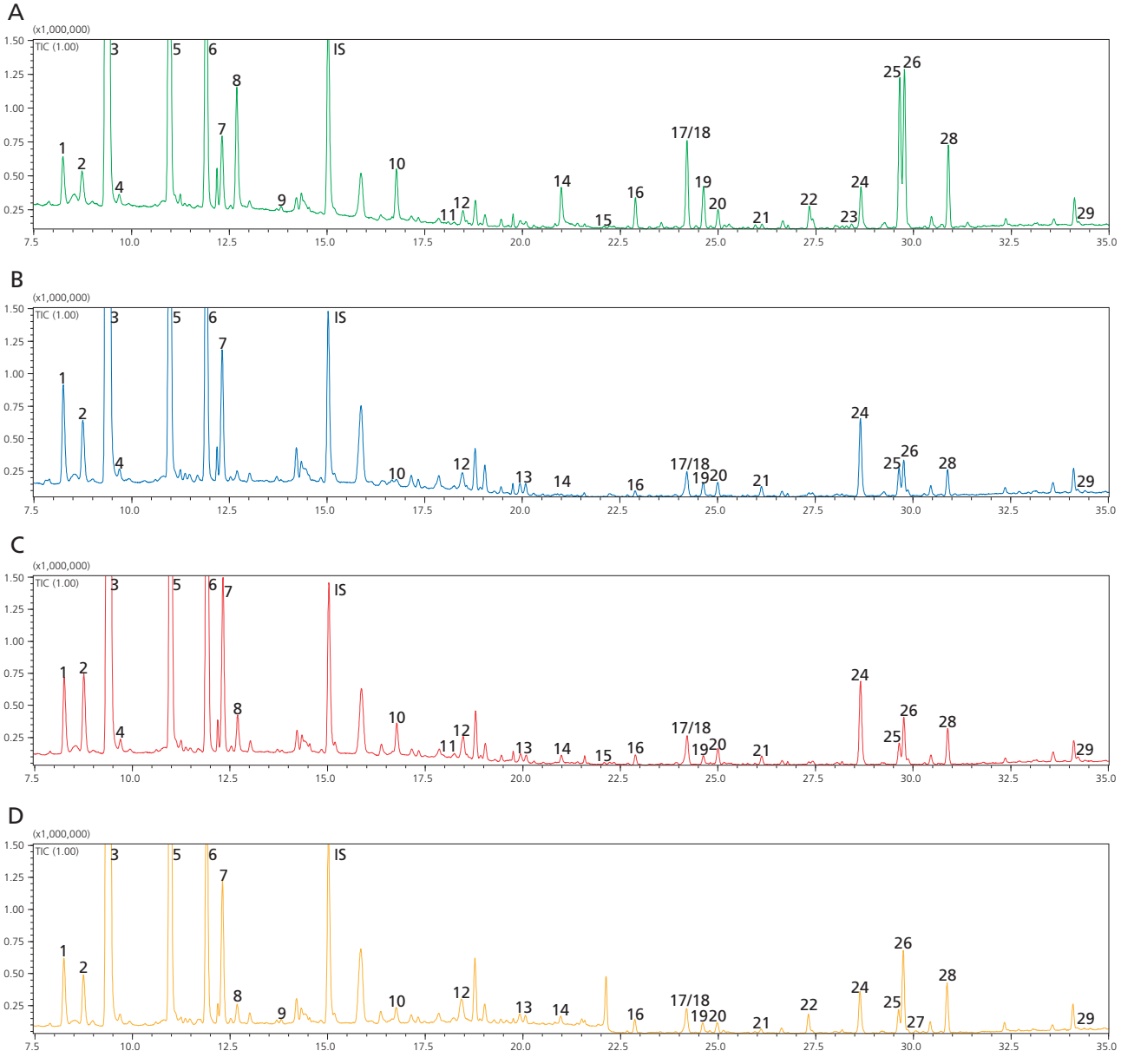

Fig. 2 Analytical results for each product
(Compound IDs correspond to Table 2. IS indicates the internal standard substance is p-bromofluorobenzene.)

Table 2 Detected Compounds

ID.	Compound Name	R.T.	*m/z*	Class
1	beta-Myrcene	8.26	93	Monoterpene
2	alpha-Terpinene	8.76	93	Monoterpene
3	D-Limonene	9.43	68	Monoterpene
4	Eucalyptol	9.70	71	Monoterpenoid
5	gamma-Terpinene	10.98	93	Monoterpene
6	para-Cymene	11.91	119	Monoterpene
7	Terpinolene	12.32	93	Monoterpene
8	Octanal	12.69	56	Aldehyde
9	Linalool ethyl ether	13.83	99	Ester
10	Nonanal	16.77	57	Aldehyde
11	1,1-Diethoxyoctane	18.10	103	Acetal
12	para-Cymenene	18.48	132	Monoterpene
13	alpha-Cyclocitral	20.07	81	Monoterpenoid
14	Decanal	20.96	57	Aldehyde
15	1,1-Diethoxynonane	22.09	103	Acetal
16	Linalool	22.88	71	Monoterpene alcohol
17	cis-alpha-Bergamotene	24.20	119	Sesquiterpene
18	Fenchyl alcohol	24.22	81	Monoterpene alcohol
19	Caryophyllene	24.62	69	Sesquiterpene
20	Terpinen-4-ol	24.99	71	Monoterpene alcohol
21	beta-Terpineol	26.12	71	Monoterpene alcohol
22	Citronellyl isobutyrate	27.33	81	Ester
23	Viridiflorene	28.42	105	Sesquiterpene
24	alpha-Terpineol	28.65	121	Monoterpene alcohol
25	beta-Bisabolene	29.64	161	Sesquiterpene
26	trans-Geranyl acetate	29.76	80	Ester
27	Citral	30.08	69	Monoterpenoid
28	cis-Geranyl acetate	30.88	69	Ester
29	para-Cymen-8-ol	34.22	135	Monoterpene alcohol

IDs: Indicated in Fig. 2; R.T.: Retention time; *m/z*: Value used for data analysis by Signpost MS

3-2. Analysis by Signpost MS Multivariate Data Analysis Software

Multivariate analysis is a statistical technique for comparing multiple samples to determine variables that most appropriately describe the differences between those samples, so that differences can be evaluated objectively.

The results obtained from GC/MS analysis were analyzed by multivariate analysis using Signpost MS™ (Reifycs Inc.) software (Fig. 3).

Signpost MS is software that can extract ion information detected from acquired data as data spots, plot the data spots based on retention time and *m/z* values, and then compare the differences between samples. It enables a variety of data analysis techniques for visually evaluating differences between different sets of data, such as pairwise plot (comparison of two groups), hierarchical clustering, principal component analysis (PCA), and transition chart. Scan data files acquired with GCMSsolution (qgd files) can be loaded directly into Signpost MS.

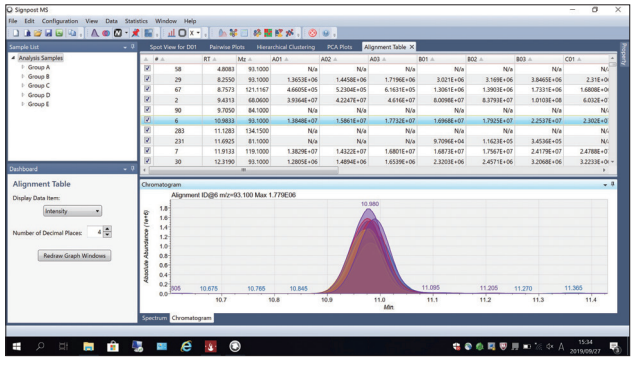

Fig. 3 Signpost MS data analysis window

3-3. Principal Component Analysis

Fig. 4 shows the results of using a score plot from principal component analysis (PCA) to investigate the differences between the four types of lemon-flavored alcoholic beverages. The compound intensity values detected from each sample were normalized based on the internal standard substance p-bromofluorobenzene.

The contribution rate was 60.8 % from the first principal component and 24.3 % from the second principal component. There was a positive distribution of the first principal component in A and a negative distribution in B and C. Fig. 5 is a principal component analysis loading plot. The components with a mainly positive distribution for the first principal component were sesquiterpene, ester, and aliphatic aldehyde, which were detected significantly in A. In contrast, the components with a negative distribution were mainly monoterpene and monoterpene alcohol, which were detected significantly in B and C. The redder the color, the lower the p-value and the larger the significant difference, whereas the bluer the color the higher the p-value. The gray color indicates components included only in specific samples.

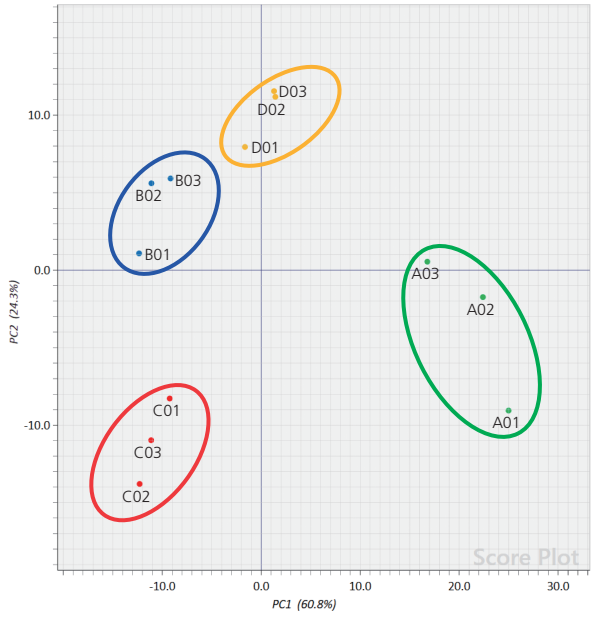

Fig. 4 Score plot distribution of each product

Fig. 5 Loading plot distribution of each compound

To analyze the similarity between samples and relative intensity of detected compounds in more detail, the Hierarchical Clustering statistical analysis tool was used to display the quantities detected in each product as a heat map and create a dendrogram (Fig. 6). A comparison of the four types of products shows that B and C are the most similar and A the most dissimilar.

Products B and C were characterized by detection of more monoterpenes such as D-limonene, para-cymene and beta-myrcene, fenchyl alcohol, and terpineol, but lower quantities of other components, such as beta-bisabolene or other sesquiterpenes, geranyl acetate, or linalool. Though B and C were similar, there were differences in the amount of nonanal, octanal, and other aliphatic aldehydes detected.

Product A was characterized by detection of lower quantities of monoterpenes, fenchyl alcohol, and terpineol, but higher quantities of components such as beta-bisabolene, caryophyllene, cis-alpha-bergamotene and other sesquiterpenes, geranyl acetate and other esters, nonanal and other aliphatic aldehydes, and linalool. With a few exceptions, most of the components detected in higher quantities in products B and C were detected in lower quantities in A. Conversely, components with lower quantities in B and C were detected in higher quantities in A.

Product D was characterized by detection of higher quantities of D-limonene, terpinene, and other components, and lower quantities of beta-bisabolene and other sesquiterpenes, which was similar to products B and C. However, detection of lower quantities of para-cymene and para-cymene-8-ol and higher quantities of citronellyl isobutyrate and geranyl acetate was similar to product A. In addition, only product D contained high quantities of citral.

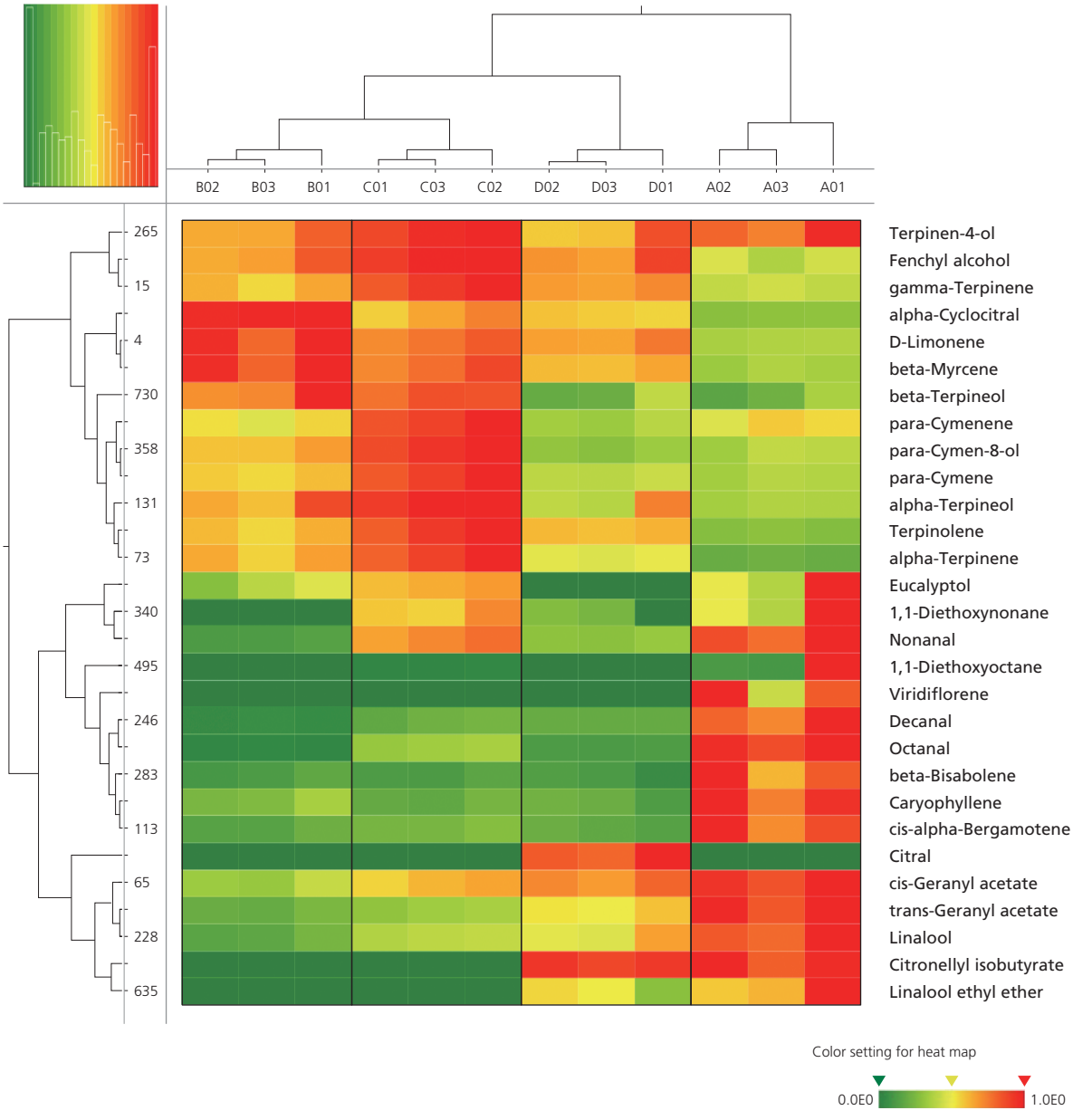

Fig. 6 Comparison of quantities detected in products based on a heat map created using statistical analysis tools in Signpost MS (Component concentration is indicated by color gradation. Using the highest detection level (red) as a reference, components with half that detection level are indicated with yellow and components with one-tenth or less of the highest level are indicated with green.)

4. Change In Fragrance Components Over Time After Opening

4-1. Sample Preparation

The fragrance components after opening were measured by GC/MS as a function of time for the purpose of determining how the flavor changes over time.

Samples of one of the alcoholic beverage products compared above were prepared. The samples were kept stored refrigerated until just before opening. After opening, the entire quantity was poured into a cup. Immediately after opening the beverage container and at 30-minute intervals thereafter, 20 mL of sample was placed in a 40 mL glass vial and the fragrance components were trapped. The components were trapped immediately after pouring the chuhai sample (zero minutes after opening), and then after 30, 60, 90, and 120 minutes had elapsed.

The fragrance components were trapped and extracted using the same methods as described in 2-1. They were trapped with a Mono-Trap adsorbent for 30 minutes at room temperature, so that the change in fragrance components in an actual drinking/eating environment could be evaluated directly. GC/MS analytical conditions are indicated in Table 1.

4-2. Results

To visually identify how much fragrance components in respective samples decreased as a function of time, Signpost MS software was used to display the data as a heat map (Fig. 7). Overlays of time-series mass chromatograms for representative compounds detected are shown in Fig. 8.

There was almost no difference between the quantities of fragrance components trapped during the 30 minutes after opening (at zero minutes) and components trapped for 30 minutes after 30 minutes had already elapsed. This result suggests that there is almost no loss in fragrance about one hour after opening the beverage container. The heat map confirms that after about one hour of elapsed time, the fragrance decreases with time. In terms of specific components, the results suggest that terpene alcohols and esters decrease more gradually than monoterpenes and sesquiterpenes.

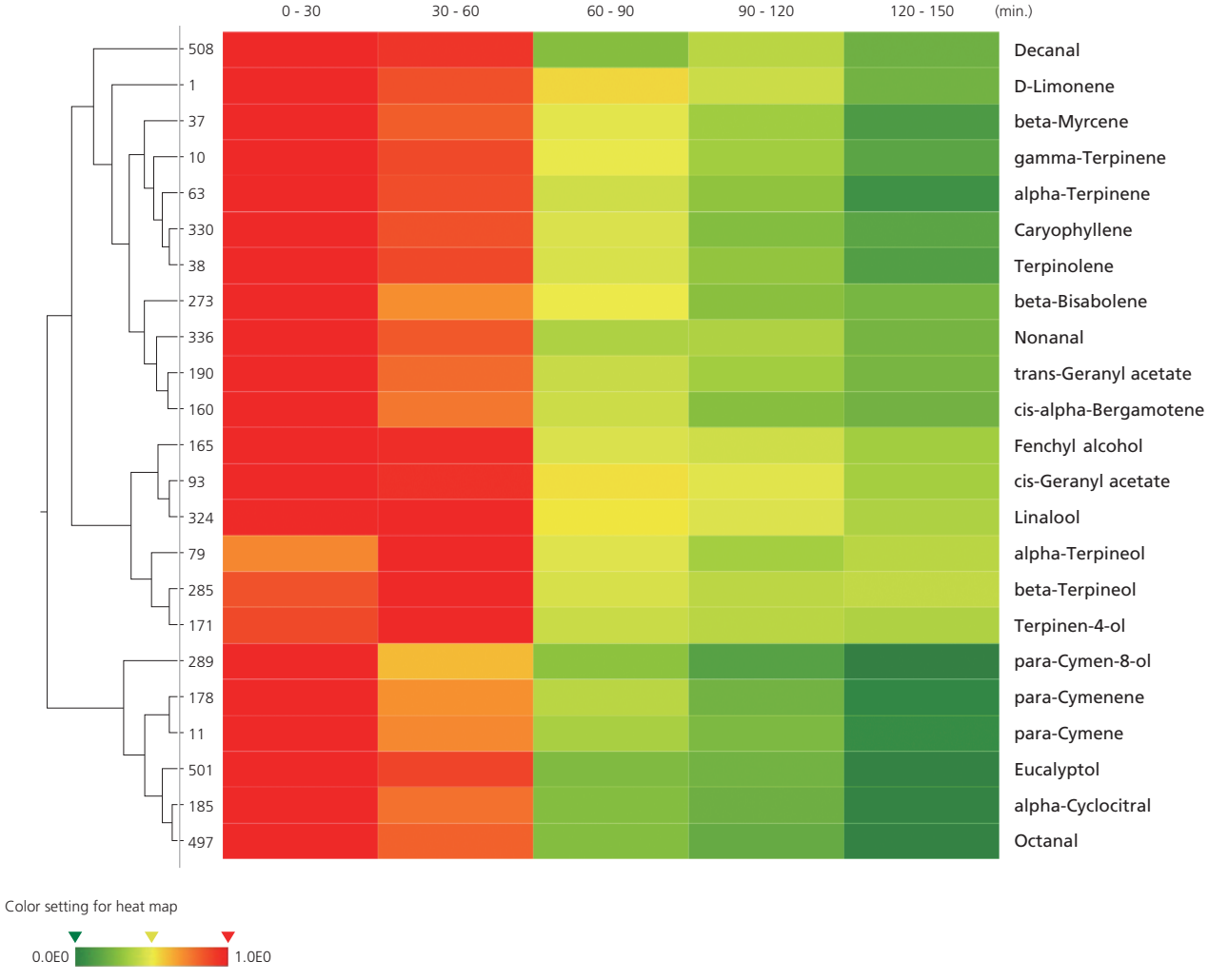

Fig. 7 Heat map visualization of changes in fragrance components over time
(Component concentration is indicated by color gradation. Using the highest detection level (red) as a reference, components with half that detection level are indicated with yellow and components with one-tenth or less of the highest level are indicated with green.)

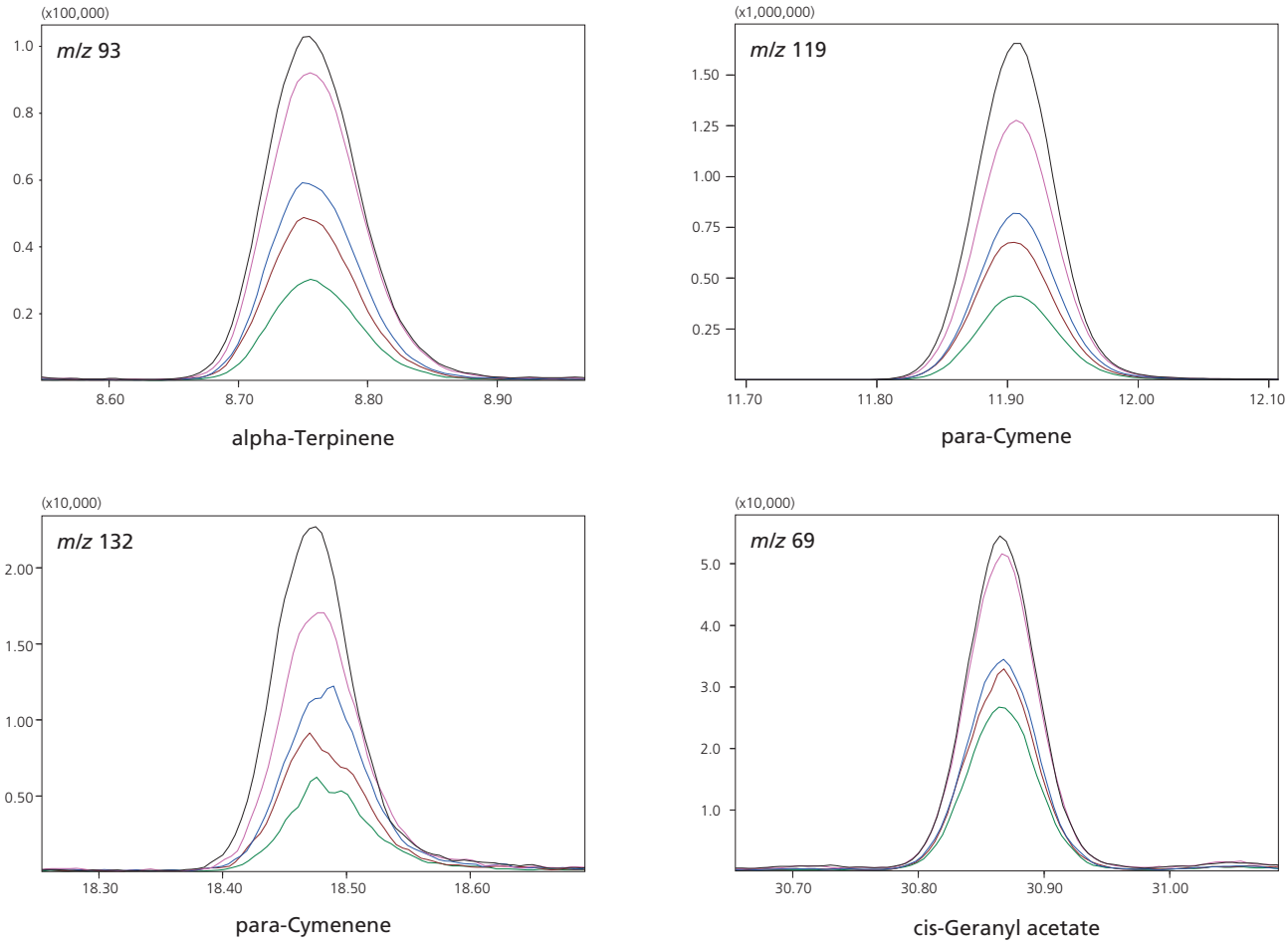

Fig. 8 Mass chromatograms of representative fragrance components at various times
Black: 0 to 30 minutes elapsed; Pink: 30 to 60 minutes elapsed; Blue: 60 to 90 minutes elapsed;
Brown: 90 to 120 minutes elapsed; Green: 120 to 150 minutes elapsed

5. Conclusion

Using a MonoTrap monolithic silica adsorbent, fragrance components could be trapped under actual eating/drinking conditions, without heating or otherwise pretreating samples. Furthermore, by using Signpost MS multivariate analysis software, similarities and differences between products and characteristic components of products could be determined visually, even if the target components were not specified in advance. In conjunction with sensory testing performed during product development, the GC/MS results can be used to corroborate sensory testing results.

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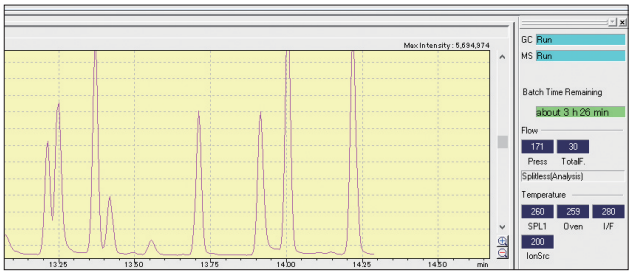

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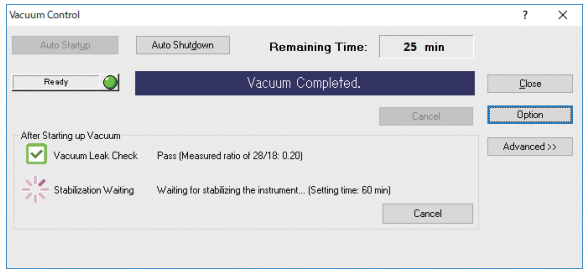

Time management for auto startup and shutdown

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