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Application News

No. M301

Gas Chromatography Mass Spectrometry

New Approach to Analysis of Volatile Gases Formed by Intestinal Microbiota Using GCMS™ and GC-SCD

Intestinal microbiota form various volatile substances and are thought to be linked to health or disease. Analysis of these volatile substances and exploratory research to discover new biomarkers are also underway.

In this article, the gases that evolved from stool samples taken from germ-free (GF) mice and mice colonized with normal microbiota (previously germ-free mice: Ex-GF) were analyzed and a comparative study was conducted. GCMS was used for a comprehensive analysis of the evolved gases, and GC-SCD was used to analyze sulfur-based gases.

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Samples and Analysis Conditions

Fresh stool samples from GF mice and Ex-GF mice were introduced into headspace vials without pretreatment, the vials were sealed after injecting an anaerobic gas, and the samples were cultured for 24 h. The vials were then set in a headspace sampler, and the evolved gases were analyzed by GCMS and GC-SCD.

Table 1 shows the GCMS instrument composition and analysis conditions in this experiment. Table 2 shows the GC-SCD instrument composition and analysis conditions.

Table 1 GCMS Instrument Composition and Analysis Conditions

Model	:	HS-20 Trap / GCMS-QP™ 2020 NX
HS-20 Trap		
Mode	:	Trap (trap tube: Tenax® GR)
Multi injection times	:	5 times
Oven temp.	:	37 °C
Sample line temp.	:	80 °C
Transfer line temp.	:	90 °C
Trap cooling temp.	:	-10 °C
Trap heating temp.	:	280 °C
Trap waiting temp.	:	25 °C
Vial pressure	:	60 kPa
Dry purge pressure	:	60 kPa
Vial heat-retention time	:	180 min
Vial pressurization time	:	1 min
Vial pressurization Equilibrating time	:	0.1 min
Loading time	:	1 min
Loading pressurization time	:	0.1 min
Dry purge time	:	10 min
Injection time	:	1 min
Needle flush time	:	5 min
GC		
Injection mode	:	Split
Split ratio	:	1:3
Carrier gas	:	He
Carrier gas control	:	Linear velocity mode (25.5 cm/s)
Column	:	DB-WAXetr (60 m × 0.25 mm I.D., 0.25 µm)
Column temp.	:	40 °C (5 min) - 3 °C /min - 240 °C (15 min)
MS		
Ion source temp.	:	200 °C
Interface temp.	:	200 °C
Ionization mode	:	EI
Measurement mode	:	Scan (*m/z* 10 - 350)
Event time	:	0.3 s

Table 2 GC-SCD Instrument Composition and Analysis Conditions

Model	:	HS-20 / Nexis™ GC-2030 / SCD-2030
HS-20		
Mode	:	Loop
Oven temp.	:	37 °C
Sample line temp.	:	80 °C
Transfer line temp.	:	90 °C
Vial pressure	:	60 kPa
Vial heat-retention time	:	180 min
Vial pressurization time	:	1 min
Vial pressurization Equilibrating time	:	0.1 min
Loading time	:	1 min
Loading pressurization time	:	0.1 min
Injection time	:	1 min
Needle flush time	:	5 min
GC		
Injection mode	:	Split
Split ratio	:	1:3
Carrier gas	:	He
Carrier gas control	:	Linear velocity mode (25.5 cm/s)
Column	:	DB-WAXetr (60 m × 0.25 mm I.D., 0.25 µm)
Column temp.	:	40 °C (5 min) -3 °C /min - 240 °C (15 min)
Detector	:	Sulfur chemiluminescence detector (SCD)
Interface temp.	:	200 °C
Electric furnace temp.	:	850 °C
Detector gas	:	H_2 100.0 mL/min N_2 10.0 mL/min O_2 12.0 mL/min O_3 25.0 mL/min

HS-20 Trap/GCMS-QP™ 2020 NX (Comprehensive Analysis)

HS-20/Nexis™ GC-2030/SCD-2030 (Sulfur Analysis)

Analysis Results (1) (Comparison of GF and Ex-GF Mice)

Fig. 1 shows the total ion chromatogram (TIC) obtained by GCMS, and Fig. 2 shows the GC-SCD chromatogram.

Both the TIC (Fig. 1), which is a comprehensive analysis of volatile substances, and the chromatogram (Fig. 2) analyzing sulfur-based volatile substances confirmed that the number of volatile substances detected and the amounts evolved (large peak area values) were larger in the sample originating from the Ex-GF mouse than the GF mouse, suggesting that intestinal microbiota are deeply involved in the formation of numerous volatile substances.

GCMS (Comprehensive Analysis of Volatile Substances)

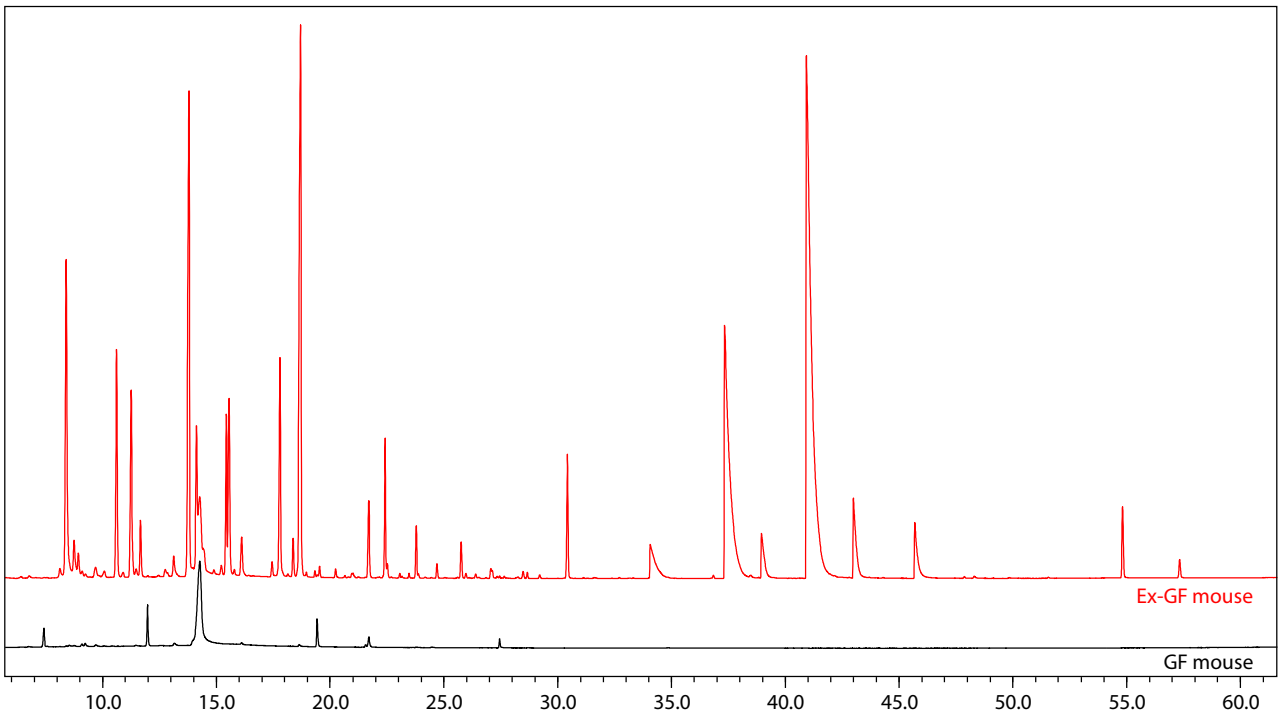

Fig. 1 Total Ion Chromatogram Obtained by GCMS

GC-SCD (Selective Analysis of Sulfur-Based Volatile Substances)

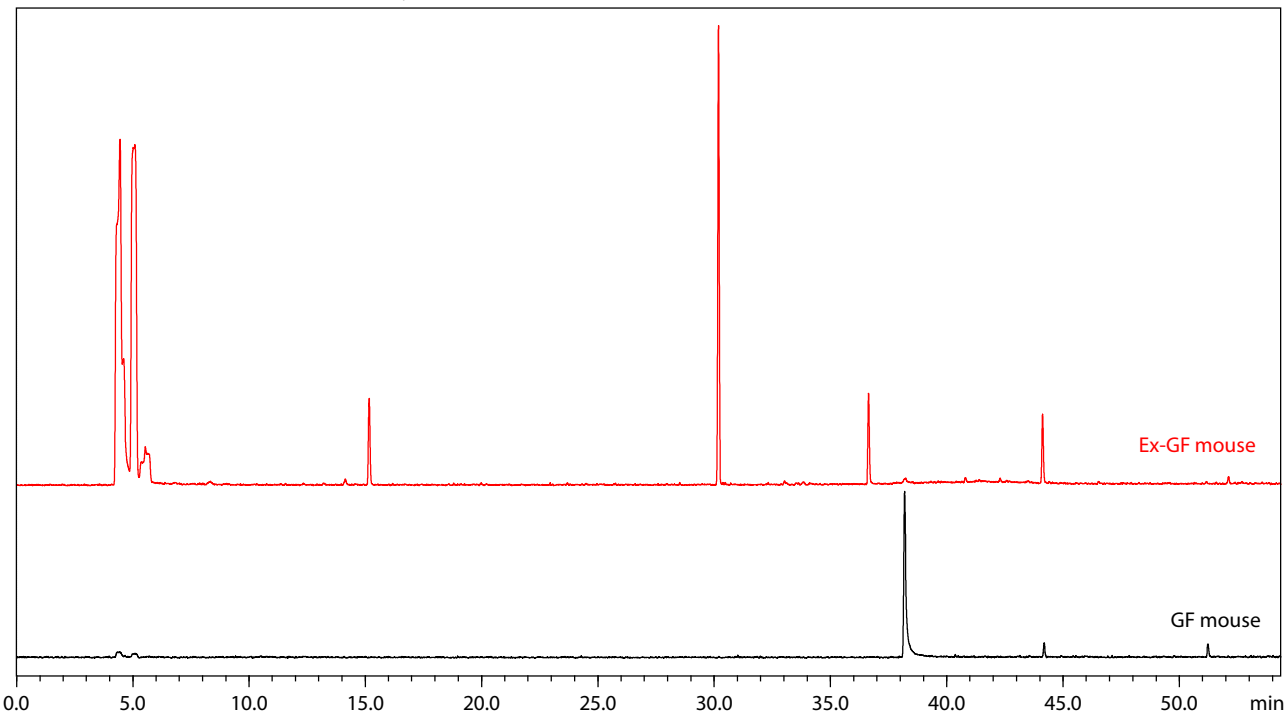

Fig. 2 Chromatogram Obtained by GC-SCD

Fig. 3 shows a comparison of the SCD and GCMS chromatograms. In the data analysis, H_2S could be detected/identified from the GC-SCD chromatogram, but was missed in the GCMS analysis, as it was overlapped with another compound in the TIC. GC-SCD could also detect/identify the sulfer compounds ethanol, 2-(methylthio)- and 1-propanol, 3-(methylthio)-, which have weak peak intensities and thus have a high possibility of being overlooked in GCMS analysis.

As shown by this experiment, sulfur compounds could be identified easily by using a combination of GC-SCD and GCMS.

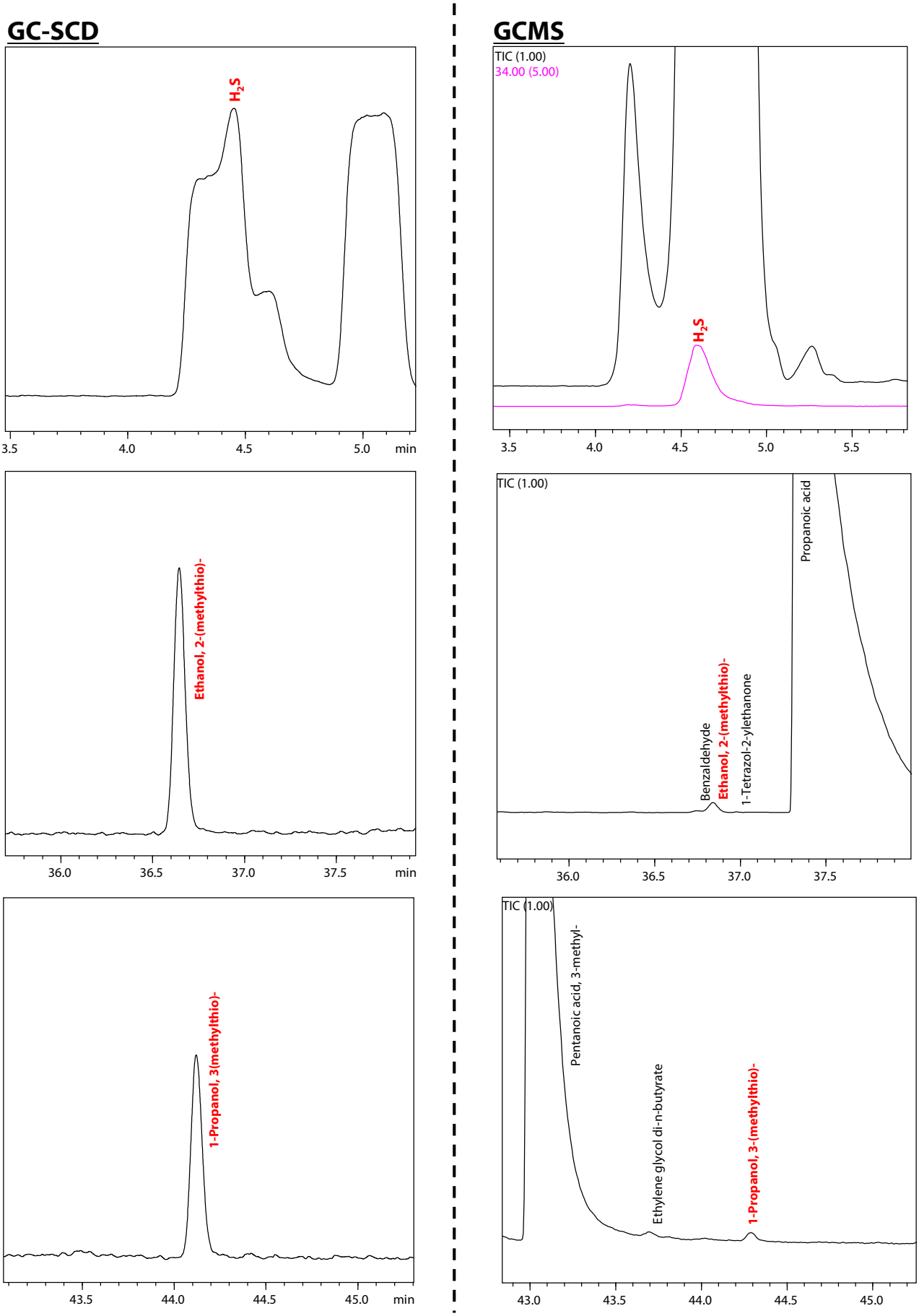

Fig. 3 Chromatograms Obtained by SCD and GCMS (Red: Sulfur Compounds)

Analysis Results (2) (Comparison of Individual Differences in Ex-GF Mice)

Fig. 4 shows the TICs obtained by GCMS when two samples each were recovered from six Ex-GF mice.

A principal component analysis of the data for a total of 121 compounds obtained from the GCMS analysis was also carried out using the multivariate analysis software SIMCA®15 (Infocom Corporation). Fig. 5 shows the score plots of the multivariate analysis results. In the score plots, a tendency of samples from the same individual to form minimum clusters and separation between the individuals could be observed. Since these test results clarify the fact that it is possible to detect individual differences in the volatile substances originating from intestinal microbiota, application to the search for new biomarkers using disease-model mice is expected.

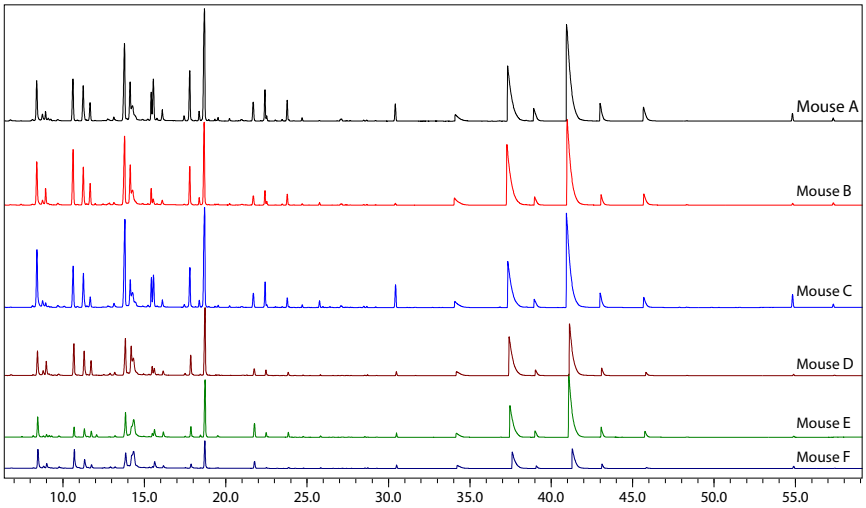

Fig. 4 Total Ion Chromatograms of Samples from Ex-GF Mice

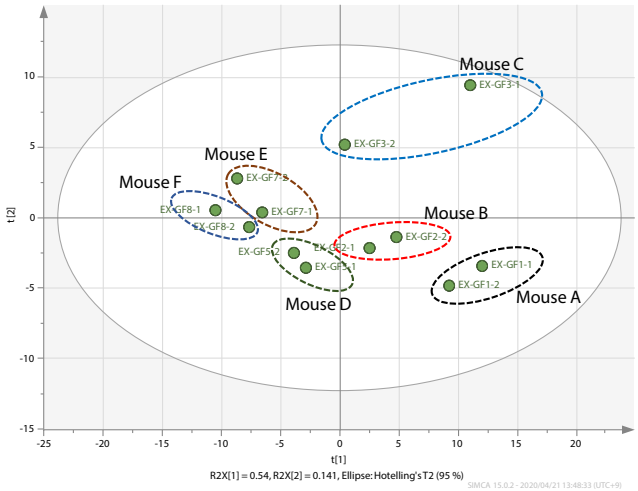

Fig. 5 Results of Multivariate Analysis of Analysis Results for Ex-GF Mice (Score Plots)

Conclusion

By using GCMS and GC-SCD, it was possible to analyze the volatile gases formed by intestinal microbiota without pretreatment of the samples. GCMS was used in the comprehensive analysis, and GC-SCD enabled analysis of sulfur-based components, which are difficult to detect by GCMS. It was also possible to confirm the existence of individual differences in the volatile components that evolve from samples taken from mice colonized with normal intestinal microbiota (Ex-GF), demonstrating the possibility of biomarker searches by this technique.

<Acknowledgement>
We wish to thank Dr. Mitsuharu Matsumoto, Principal Investigator of the Kyodo Milk Industry Co., Ltd. for his cooperation in conducting these measurements, including providing the samples.

First Edition: Jul. 2020

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