Compositional Comparison of Beers by On-Line Solid Phase Derivatization SPE-GC/MS

Introduction

In conventional GC/MS analysis of metabolomics, extraction, lyophilization, and derivatization were complicated and time-consuming, and the data varied from person to person. To solve this problem, we have dramatically shortened the time required, simplified the process, and achieved high accuracy with our proprietary “solid-phase derivatization method”. Amino acids and organic acids are held in the solid phase by ion-exchange interactions, and then passed through acetonitrile to dehydrate and wash the solid phase. In this study, we attempted to compare the composition of beers using the SPL-M100 system, which fully automates these processes.

Sample

A. Beer

Malt content of more than 50% and less than 5% of secondary ingredients

B. Third-category beer

Malt less than 50% or more than 5% of the secondary ingredients, plus spirits derived from wheat, or made from ingredients other than wheat or malt

C. Non-alcoholic

Alcohol content less than 1%.

Pretreatment flow

Sample 50 µL
- Add Water 150 µL
- Add ACN 800 µL

Shaking 37 ℃, 5 min

Centrifuge 14000 rpm, 3 min
- Add 0.1N NaOH

Extract

On-line solid-phase derivatization

SPE-GC : SPL-M100

Automation
pretreatment : 15 min

Vials
- Conditioning ACN-Water(1/1) ACN
- **Flash-SPE ACXs**
- **Load[Retention]** **Extract** 50 µL
- Washing ACN-Water(1/1)
- Dewater ACN
- Infiltration Methoxyamine / Pyridine
- SPD Methoximation, 3 min
- Infiltration MSTFA-Toluene(3/1)
- SPD TMS, 1 min
- Elution Hexane

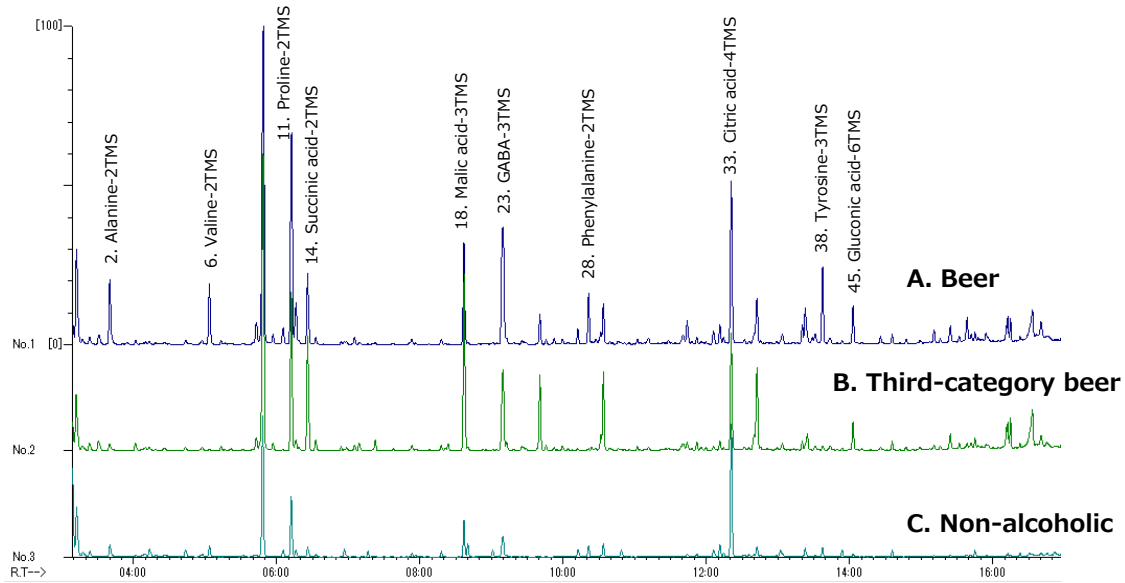
GC-MS Sprit 1:50

SPE-GC-MS system for metabolome analysis
SPL-M100 / GCMS-TQ8040NX

Analytical condition

PTV Injector	**LVI-S250 (AiSTI Science)**
Insert Type	Spiral Insert
Injector Temp.	220℃(0.5min)-50℃/min-290℃(16min)
GC-MS	
Inlet Mode	Split 1:50
Flow Mode	Constant Flow, 1 ml/min
Pre-Column	0.25mm i.d. x 1m
Column	Vf-5ms, 0.25mm i.d. x 30m, df;0.25µm
Oven Temp.	100℃(2min)-10℃/min-220℃-30℃/min-320℃
Trans. Temp.	290℃
MS Method	SCAN, m/z;70-470

Comparison of SCAN total ion chromatograms for each beer by this method



SPL-M100
for SPE-GC system

Sample

Information

【Sample】
- ・Beer
- ・Third-category beer
- ・Non-alcoholic

【Target】
- ・Amino acids
- ・Amines
- ・Nucleobases,
- ・organic acids

AiSTI SCIENCE

Product

SPE-GC interface
SPL-M100

Solid-phase cartridge
Flash-SPE

GC Lage volume injection
LVI-S250

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■Comparison of the amount of ingredients in each beer category (Beer, Third-category beer, Non-alcoholic)

■Reproducibility of peak area values for beer using this method (n=5)

No.	Compound name	1	2	3	4	5	*Ave.*	RSD,%
2	Alanine-2TMS	9,045,768	8,560,499	8,976,348	8,793,088	8,986,315	*8,872,404*	**2.2**
3	Oxalic acid-2TMS	435,910	386,678	455,303	423,232	421,322	*424,489*	**5.9**
5	Malonic acid-2TMS	246,825	250,550	283,014	263,870	278,290	*264,510*	**6.1**
6	Valine-2TMS	7,482,140	7,144,291	7,462,783	7,319,498	7,527,203	*7,387,183*	**2.1**
9	Leucine-2TMS	2,281,092	2,177,498	2,331,331	2,251,281	2,341,946	*2,276,630*	**2.9**
10	Isoleucine-2TMS	1,769,980	1,684,710	1,753,759	1,723,011	1,773,770	*1,741,046*	**2.1**
11	Proline-2TMS	37,381,457	36,443,437	36,453,744	36,790,934	37,089,023	*36,831,719*	**1.1**
12	Glycine-3TMS	3,744,069	3,605,424	3,724,203	3,729,635	3,745,664	*3,709,799*	**1.6**
14	Succinic acid-2TMS	9,348,320	9,158,144	9,295,211	9,397,023	9,504,533	*9,340,646*	**1.4**
15	Fumaric acid-2TMS	191,564	188,831	184,723	190,778	186,857	*188,551*	**1.5**
16	Serine-3TMS	165,095	151,296	166,918	161,272	177,821	*164,480*	**5.8**
17	Threonine-3TMS	11,112	11,557	12,044	11,903	13,715	*12,066*	**8.2**
18	Malic acid-3TMS	1,693,758	1,661,588	1,710,883	1,697,603	1,746,216	*1,702,010*	**1.8**
20	Aspartic acid-3TMS	139,759	133,693	116,042	138,842	141,339	*133,935*	**7.8**
21	Methionine-2TMS	7,455	7,976	7,116	6,761	7,765	*7,415*	**6.6**
22	4-Hydroxyproline-3TMS	43,411	35,023	40,200	36,148	40,477	*39,052*	**8.8**
23	GABA-3TMS	6,001,936	6,116,484	5,890,392	6,319,461	6,089,764	*6,083,607*	**2.6**
24	Cytosine-2TMS	93,316	90,716	91,565	91,061	93,332	*91,998*	**1.4**
25	Threonic acid-4TMS	95,914	85,688	102,953	96,623	94,752	*95,186*	**6.5**
26	Ketoglutaric acid-MO-2	101,455	100,364	95,273	97,427	107,090	*100,322*	**4.5**
27	Glutamic acid-3TMS	953,604	932,080	791,415	915,642	930,802	*904,709*	**7.2**
28	Phenylalanine-2TMS	2,418,894	2,301,757	2,380,551	2,353,220	2,426,315	*2,376,147*	**2.1**
30	Asparagine-3TMS	19,423	19,581	17,856	18,634	19,954	*19,090*	**4.4**
31	Putrescine-4TMS	530,322	556,091	481,363	574,139	523,674	*533,118*	**6.6**
32	Shikimic acid-4TMS	279,637	272,929	319,044	316,435	294,930	*296,595*	**7.0**
33	Citric acid-4TMS	2,683,364	2,576,903	2,619,719	2,657,033	2,666,969	*2,640,798*	**1.6**
34	Quinic acid-5TMS	162,309	142,400	156,146	154,321	147,561	*152,547*	**5.1**
35	Adenine-2TMS	760,836	755,687	770,628	793,754	794,590	*775,099*	**2.4**
36	Lysine-4TMS	1,347,973	1,356,989	1,303,882	1,428,463	1,315,811	*1,350,624*	**3.6**
37	Histidine-3TMS	210,251	218,994	218,757	246,916	228,676	*224,719*	**6.2**
38	Tyrosine-3TMS	7,780,479	7,286,124	7,428,618	7,444,638	7,661,244	*7,520,221*	**2.6**
39	Guanine-3TMS	692,918	625,086	694,491	676,205	697,726	*677,285*	**4.5**
41	Tryptophan-3TMS	316,641	333,292	274,695	334,574	311,702	*314,181*	**7.7**
44	Adenosine-4TMS	86,733	84,366	85,527	83,393	83,617	*84,727*	**1.6**
45	Guanosine-5TMS	679,723	657,820	648,207	629,491	645,907	*652,230*	**2.8**

※To evaluate the reproducibility of the device, no correction is made with internal markers, etc.

【Result and Discussion】

The RSD. (reproducibility) of each component was all less than 10%, confirming high accuracy. The TIC (total ion chromatogram) shows good peak shape and separation, indicating that the washing and dehydration by solid-phase extraction functionally work and that derivatization is efficiently performed in the solid phase. This method is considered to be a highly accurate analytical method quantitatively after solid-phase extraction.

The solid-phase derivatization of this analytical method simplifies the extraction process, improves accuracy, and shortens the time required. In addition, the method overcomes the weak points of complicated and time-consuming pretreatment, and is expected to maximize the advantages of GC/MS, such as high separation, high qualitative ability, and a full database.

As a result of quantitative comparison (area value ratio) of each sample, differences by sample could be clearly determined. The data obtained from the highly accurate analytical method can contribute to highly reliable analytical results, which can be utilized in the future development of beer beverages and other products.