

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

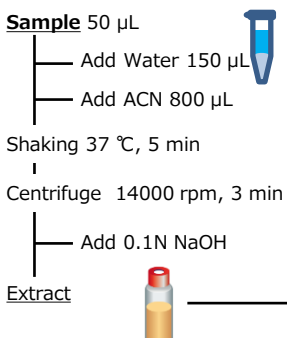
B. Third-category beer

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

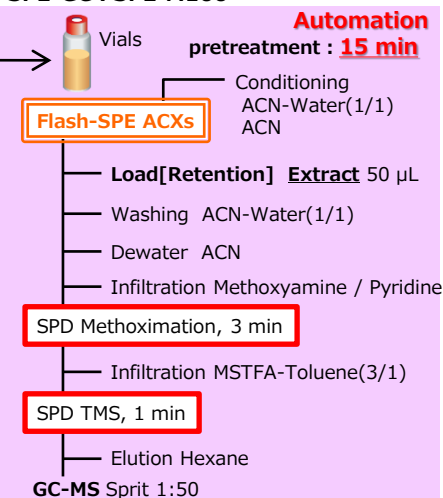
Alcohol content less than 1%.

Pretreatment flow



On-line solid-phase derivatization

SPE-GC : SPL-M100



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°C(0.5min)-50°C/min-290°C(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°C(2min)-10°C/min-220°C-30°C/min-320°C
Trans. Temp.	290°C
MS Method	SCAN, m/z:70-470



SPL-M100
for SPE-GC system

Sample



Beer

Information

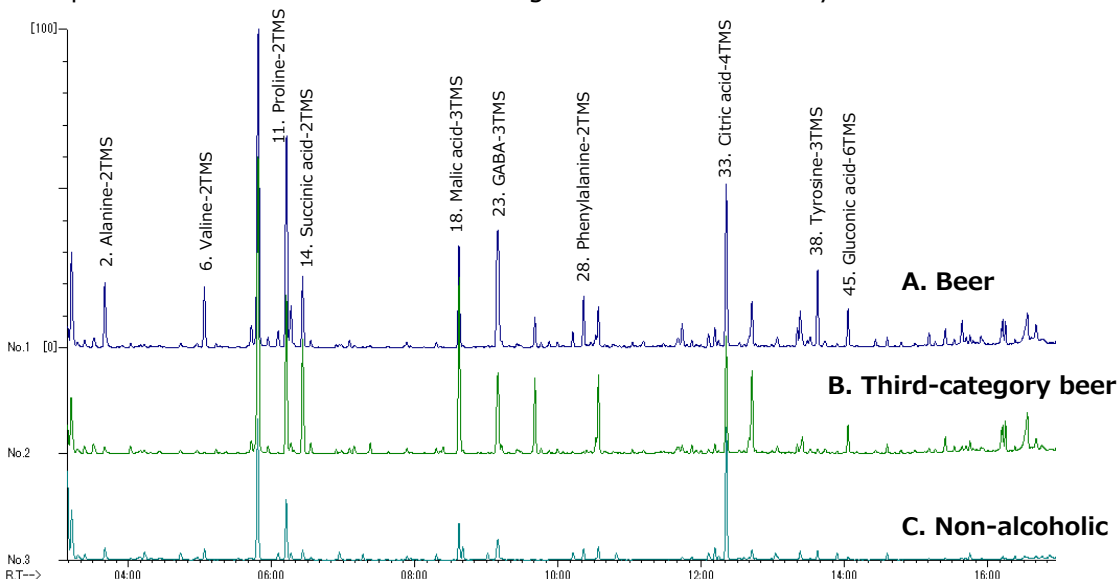
[Sample]

- Beer
- Third-category beer
- Non-alcoholic

[Target]

- Amino acids
- Amines
- Nucleobases,
- organic acids

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



AiSTI SCIENCE

Product

SPE-GC interface
SPL-M100

Solid-phase cartridge
Flash-SPE

GC Luge volume injection
LVI-S250



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AiSTI Application Note

■ 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.