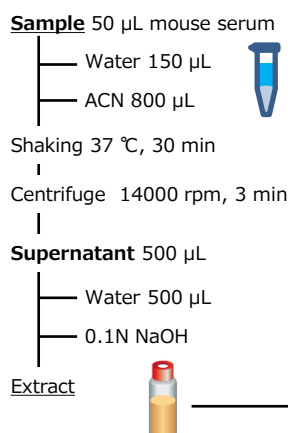


Evaluation of On-Line Solid Phase Derivatization SPE-GC/MS Analysis of Mouse Serum

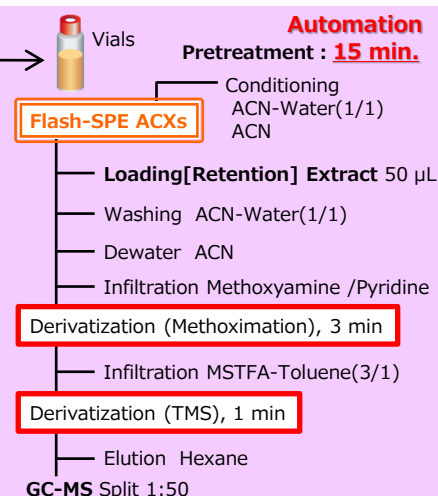
Introduction

In conventional GC/MS analysis of metabolomics, extraction, lyophilization, and derivatization were complicated and time-consuming, and the data obtained tended to be highly inconsistent. We have dramatically shortened the time required, simplified the process, and improved the accuracy by using our proprietary "solid-phase derivatization" technique. Metabolites and other substances 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 analyze metabolites in mouse serum using the SPL-M100 system, which fully automates these processes.

Pretreatment flow



Online SPE-GC : SPL-M100



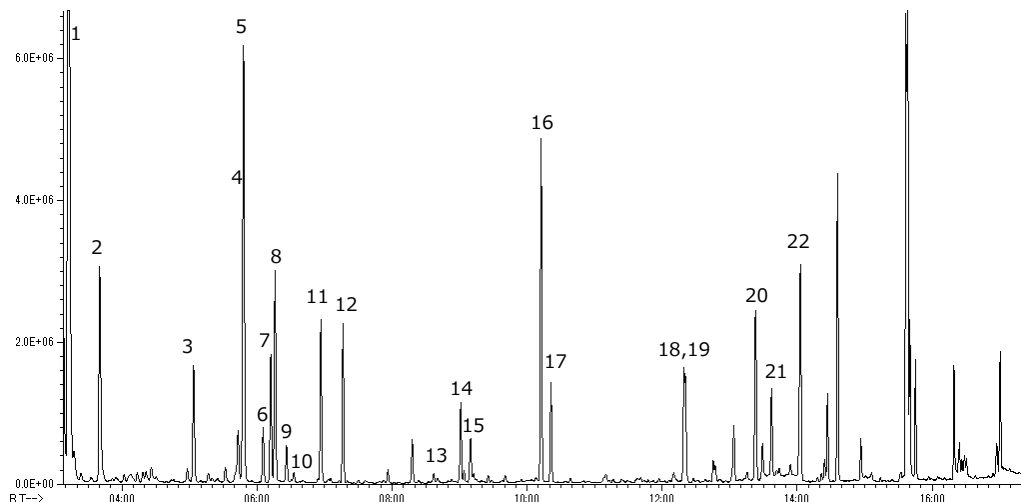
Analytical condition

PTV Injector	LVI-S250 (AiSTI Science)
Insert Type	Spiral Insert
Temp.	220 $^{\circ}$ C(0.5min)-50 $^{\circ}$ C/min-290 $^{\circ}$ C(16min)
GC-MS	
Injection 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;0.25 μ m
Oven Temp.	100 $^{\circ}$ C(2min)-10 $^{\circ}$ C/min-220 $^{\circ}$ C-30 $^{\circ}$ C/min-320 $^{\circ}$ C
Trans. Temp.	290 $^{\circ}$ C
MS	SCAN, m/z;70-470



SPE-GC-MS system for metabolome analysis

SPL-M100 / GCMS-TQ8040NX



- | | | | | |
|------------------------|----------------------|--------------------------|----------------------|----------------------|
| ① Lactic acid-2TMS | ⑥ Isoleucine-2TMS | ⑪ Serine-3TMS | ⑱ Glutamic acid-3TMS | ⑳ Tyrosine-3TMS |
| ② Alanine-2TMS | ⑦ Proline-2TMS | ⑫ Threonine-3TMS | ⑲ Phenylalanine-2TMS | ㉑ Gluconic acid-6TMS |
| ③ Valine-2TMS | ⑧ Glycine-3TMS | ⑬ Malic acid-3TMS | ⑳ Ornithine-4TMS | |
| ④ Leucine-2TMS | ⑨ Succinic acid-2TMS | ⑭ Aspartic acid-3TMS | ㉑ Citric acid-4TMS | |
| ⑤ Phosphoric acid-3TMS | ⑩ Glyceric acid-3TMS | ⑮ Pyroglutamic acid-2TMS | ㉒ Lysine-4TMS | |

Figure1 SCAN total ion chromatogram of mouse serum using this method



SPL-M100
for SPE-GC system

Sample



Information

- [Sample]**
- Mouse serum
- [Target Component]**
- Amino acid
 - Amine
 - Organic acid

AiSTI SCIENCE

Product

Online SPE-GC
SPL-M100

Solid Phase cartridge
Flash-SPE

GC Large Volume Injection
LVI-S250



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AiSTI Application Note

Table 1 Recovery Test of Mouse Serum with this System

No.	Compound names	Standard	Operation Blant	Mouse serum	Mouse serum+ST	Recovery% (A-M)/(ST-B)
		ST	B	M	A	
1	Lactic acid-2TMS	2,641,331	2,044,267	14,805,790	11,895,241	-
2	Alanine-2TMS	2,526,424	84,451	4,971,432	6,847,093	77
6	Valine-2TMS	3,223,917	8,177	2,224,994	5,182,605	92
9	Leucine-2TMS	3,806,169	9,760	3,940,497	7,217,079	86
10	Isoleucine-2TMS	3,483,014	5,009	958,681	4,444,077	100
11	Proline-2TMS	3,349,273	6,613	3,008,108	5,928,827	87
12	Glycine-3TMS	2,822,020	38,903	3,192,716	5,175,424	71
14	Succinic acid-2TMS	5,442,569	51,260	607,407	6,159,930	103
15	Fumaric acid-2TMS	1,886,256	3,365	11,533	1,967,128	104
16	Serine-3TMS	1,763,290	19,550	1,451,277	3,032,036	91
17	Threonine-3TMS	584,437	2,454	484,176	957,064	81
18	Malic acid-3TMS	610,728	1,773	18,302	629,151	100
20	Aspartic acid-3TMS	2,929,674	5,116	784,543	3,822,406	104
21	Methionine-2TMS	321,403	1,578	77,627	321,294	76
22	4-Hydroxyproline-3TMS	2,400,683	122	54,733	1,997,473	81
23	GABA-3TMS	808,381	5,804	9,875	776,441	96
25	Threonic acid-4TMS	331,448	417	27,125	202,330	53
27	Glutamic acid-3TMS	2,350,172	2,555	3,437,586	6,150,075	116
28	Phenylalanine-2TMS	1,457,813	1,404	690,541	2,098,341	97
31	Putrescine-4TMS	4,356,542	1,610	9,512	4,597,459	105
33	Citric acid-4TMS	911,581	660	216,437	1,160,422	104
36	Lysine-4TMS	1,587,702	1,259	940,190	2,675,748	109
37	Histidine-3TMS	2,194,472		10,446	3,240,581	147
38	Tyrosine-3TMS	4,650,501	1,959	1,175,034	5,939,201	102
41	Tryptophan-3TMS	230,794	0	29,096	398,017	160
43	Cystine-4TMS	2,142,088	0	14,728	2,196,172	102

Table 2 Reproducibility of mouse serum by this system

No.	Compound names ^v	U1	U2	U3	U4	U5	Ave.	RSD,%
1	Lactic acid-2TMS	14,649,682	15,089,600	13,841,394	15,639,698	14,808,578	14,805,790	4.4
2	Alanine-2TMS	5,118,252	4,719,988	4,790,287	5,126,762	5,101,869	4,971,432	4.0
6	Valine-2TMS	2,261,504	2,125,119	2,152,889	2,285,552	2,299,904	2,224,994	3.6
9	Leucine-2TMS	4,005,604	3,817,067	3,780,734	4,039,212	4,059,866	3,940,497	3.3
10	Isoleucine-2TMS	971,384	928,534	931,475	980,243	981,767	958,681	2.8
11	Proline-2TMS	3,078,401	2,818,958	2,910,867	3,100,657	3,131,659	3,008,108	4.5
12	Glycine-3TMS	3,243,322	2,973,200	3,083,005	3,312,366	3,351,687	3,192,716	5.0
14	Succinic acid-2TMS	609,722	610,940	585,493	627,168	603,713	607,407	2.5
15	Fumaric acid-2TMS	11,626	11,149	10,691	11,947	12,254	11,533	5.4
16	Serine-3TMS	1,466,003	1,327,848	1,366,049	1,533,536	1,562,951	1,451,277	7.1
17	Threonine-3TMS	497,778	441,900	461,299	506,740	513,163	484,176	6.4
18	Malic acid-3TMS	19,076	18,062	17,173	18,728	18,470	18,302	4.0
20	Aspartic acid-3TMS	745,837	821,214	711,854	825,260	818,549	784,543	6.7
21	Methionine-2TMS	71,255	66,983	76,059	92,889	80,950	77,627	12.9
22	4-Hydroxyproline-3TMS	54,583	47,943	51,238	58,569	61,333	54,733	9.9
23	GABA-3TMS	10,265	8,742	9,675	10,265	10,430	9,875	7.0
25	Threonic acid-4TMS	28,177	28,501	22,545	29,638	26,765	27,125	10.2
27	Glutamic acid-3TMS	3,365,311	3,486,693	3,156,198	3,603,886	3,575,843	3,437,586	5.3
28	Phenylalanine-2TMS	682,545	669,938	666,324	718,207	715,692	690,541	3.6
31	Putrescine-4TMS	9,970	9,716	9,256	8,922	9,694	9,512	4.4
33	Citric acid-4TMS	212,800	218,960	209,116	224,337	216,971	216,437	2.7
36	Lysine-4TMS	926,298	906,996	922,350	974,013	971,295	940,190	3.2
37	Histidine-3TMS	10,802	9,595	9,636	10,237	11,959	10,446	9.4
38	Tyrosine-3TMS	1,192,371	1,093,010	1,123,091	1,241,557	1,225,141	1,175,034	5.5
41	Tryptophan-3TMS	29,187	29,591	28,393	29,027	29,282	29,096	1.5
43	Cystine-4TMS	15,113	14,209	13,349	15,634	15,333	14,728	6.4

【Results and Considerations】

The RSD. (reproducibility) of each component is less than 13%, indicating high accuracy. Peak shape and separation are also excellent in TIC. From these results, it can be inferred that washing and dehydration by solid-phase extraction functionally work, and derivatization is efficiently performed in the solid phase. The good results obtain even with samples containing a large amount of lipids, etc., will reduce GC/MS maintenance labor. The solid-phase derivatization of this analytical method improves the accuracy and shortens the time required for analysis, and automation of the method is expected to increase efficiency. The method is expected to be applied to the analysis of metabolites in biological samples in the future, as it can maximize the advantages of GC/MS, such as high resolution, high qualitative ability, and a full database, by overcoming the complexity and long time required for pretreatment, which is a weak point of the method.