Development of online SPE-GC-MS system with automated SPE-based derivatization method for metabolome analysis.

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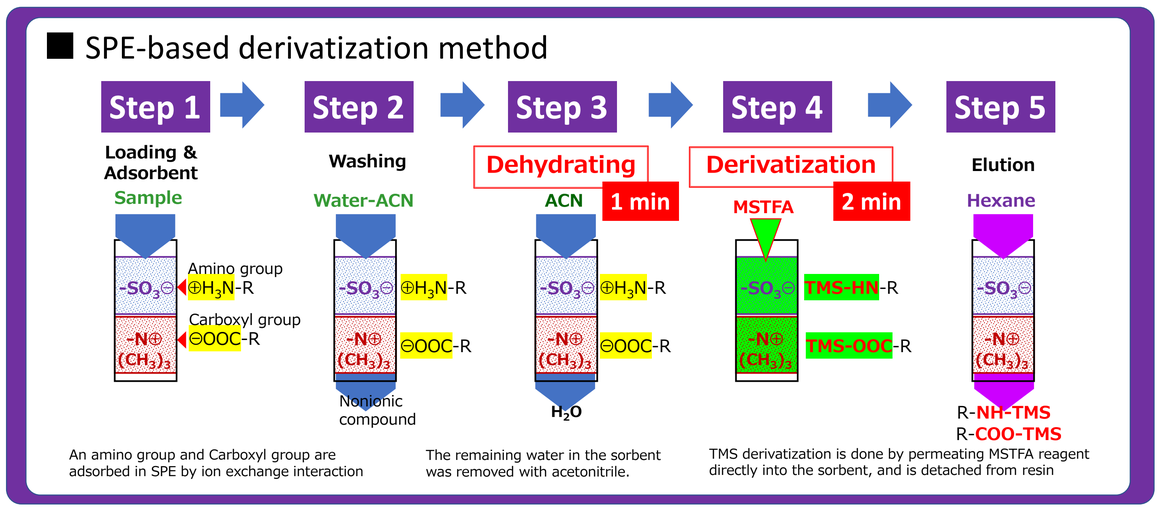
【Abstract】

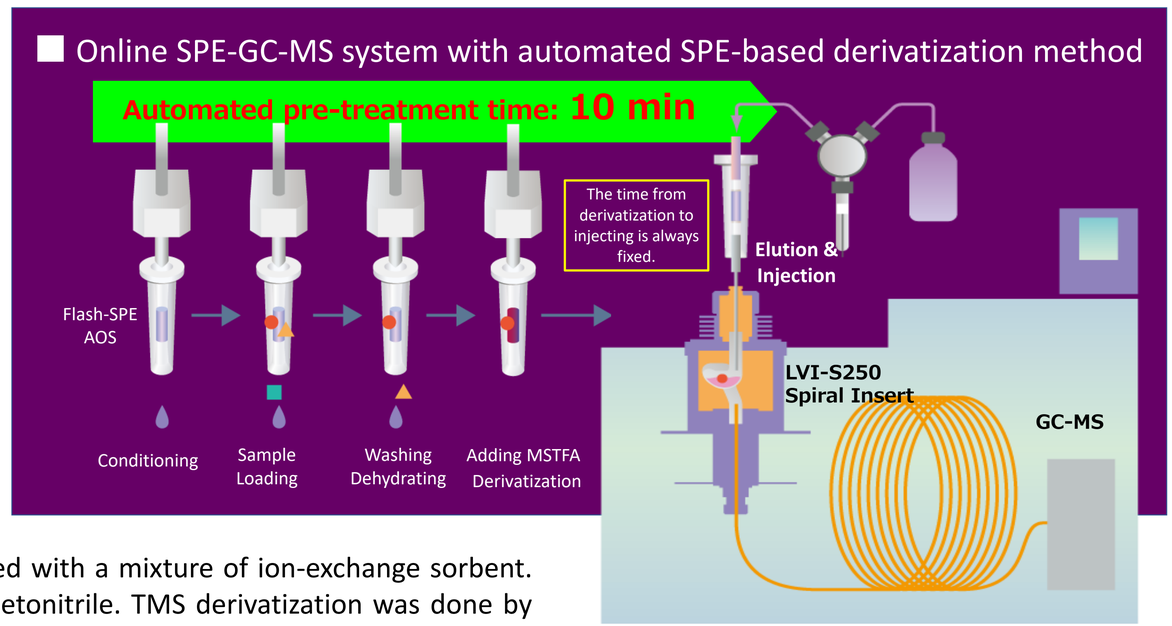
Due to complicated sample preparation procedures that include centrifugal concentration, freeze-drying and derivatization, the current metabolomics protocol using GC-MS, requires not only longer processing time but also advanced technical skill. In addition, some silylated metabolites are unstable and the time required for complete silylation varies depending on the metabolite. Therefore, the time gap between derivatization and actual GC-MS analysis can potentially be a source of error. In this study, **automated SPE-based derivatization** method coupled with GC-MS was developed to accomplish an extremely rapid sample preparation. The derivatization method using SPE-gel has a high reaction efficiency and can provide sequential sample introduction to keep the interval from silylation to GC-MS injection constant.

【Experimental】

■ SPE-based derivatization method



■ Online SPE-GC-MS system with automated SPE-based derivatization method



Amino acids and organic acids from the extracted samples were loaded into the SPE column filled with a mixture of ion-exchange sorbent. After washing with acetonitrile/water, the remaining water in the sorbent was removed with acetonitrile. TMS derivatization was done by permeating MSTFA reagent directly into the sorbent. A needle was automatically connected to the SPE cartridge and inserted into the GC-MS. Then, the TMS-derivatized metabolites were eluted with n-hexane, and injected directly into GC-MS with LVI spiral insert.

Automation

Sample : 50µL（50mg）
- Added Water 150µL
- Added ACN 800µL

Shaking （37℃, 30min）

Centrifuging（14000rpm, 5min）

Aliquot of upper extract : 250µL
- Added Water 250µL

Vial

Online SPE-GC-MS System

Vial

Aliquot of extract 40µL
- Conditioning with Water-ACN 100µL

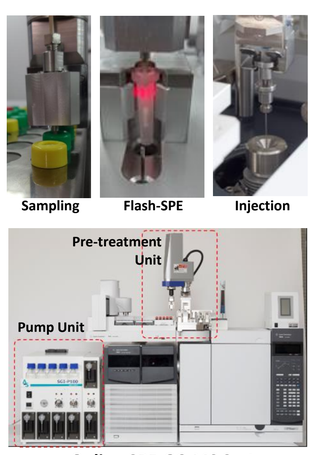
Flash-SPE **AOS**-3mg
- Wash with Water-ACN 100µL : **Cleaning**
- Wash with ACN 100µL : **Dehydration**
- **Added Methoxyamine-Pyridine-MSTFA 8µL**

Derivatization in SPE 2min
- Elute Acetone-Hexane 40µL

GC-MS : LVI-Split（1:50）

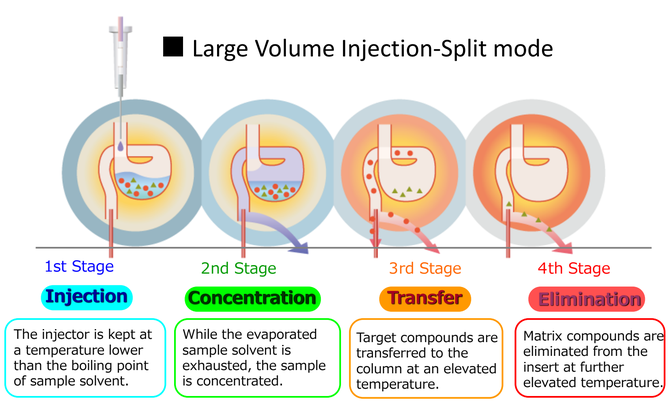
■ Condition of SPE-GC-MS System

SPE-GC Interface	**SGI-M100 ;AiSTI Science**
SPE Cartridge	Flash-SPE AOS ;AiSTI Science
Sample Volume	40µL
PTV Injector	**LVI-S250 ;AiSTI Science**
Insert	Spiral Insert ;AiSTI Science
Injector Temp.	80℃(0.2min)-50℃/min-290℃(38min)
GC	**Agilent 7890B**
Pre-column	Deactivated silica capillary tube, 0.25mm×0.3m
Column	DB-5MS, 0.25mm i.d.×30m, df; 0.25µm
Column Oven Temp.	60℃(2min)-15℃/min-240℃-20℃/min-310℃(1min)
Inlet Mode	Split
Split ratio	50
Gas Saver Flow	15 mL/min
Gas Saver Time	6 min
MS	**Agilent 5977B**
Detector Temp.	290℃
MS Method	SCAN; 70 - 500 m/z



Online SPE-GC-MS System

■ Large Volume Injection-Split mode



The spiral shaped insert makes it possible to accept all of the injected eluate(40µL) from the SPE cartridge.

【Results】

Evaluation with the standard solution

◆ Derivatization Time

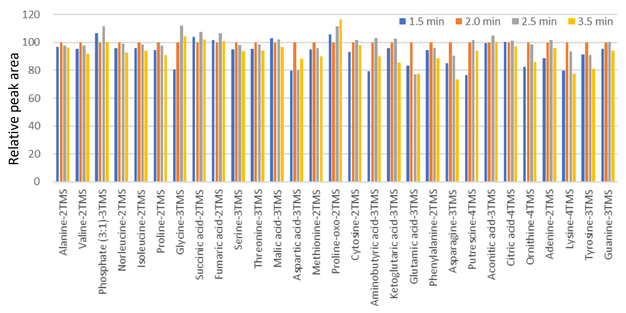


Fig. 1. Relations of derivatization time in sorbent and the relative peak area.

◆ Derivatization Reagent

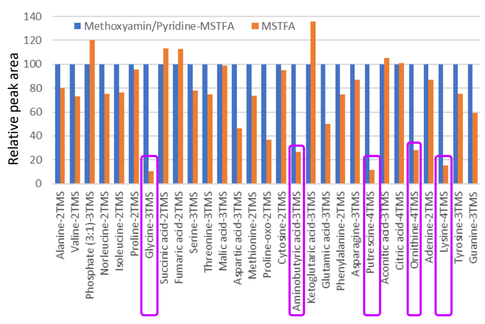
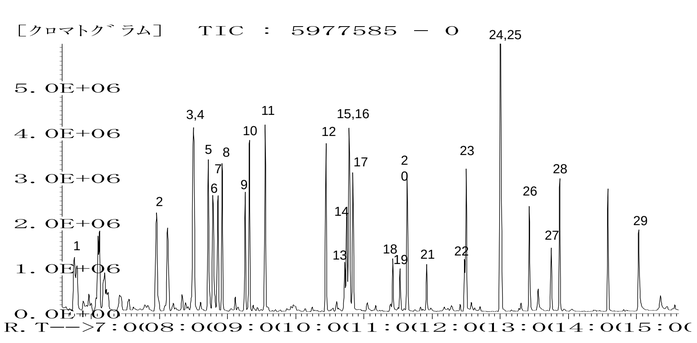
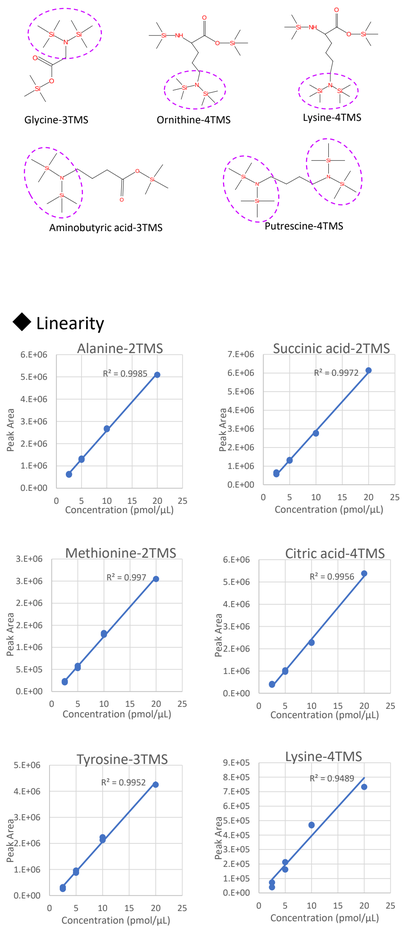


Fig. 2. Relations of the derivatization reagent and the relative peak area.



1.Alanine-2TMS
2. Valine-2TMS
3. Phosphate-3TMS
4. Leucine-2TMS
5. Isoleucine-2TMS
6. Proline-2TMS
7. Glycine-3TMS
8. Succinic acid-2TMS
9. Fumaric acid-2TMS
10. Serine-3TMS
11. Threonine-3TMS
12. Malic acid-3TMS
13. Aspartic acid-3TMS
14. Methionine-2TMS
15. Proline-oxo-2TMS
16. Cytosine-2TMS
17. Aminobutyric acid-3TMS
18. Ketoglutaric acid-3TMS
19. Glutamic acid-3TMS
20. Phenylalanine-2TMS
21. Asparagine-3TMS
22. Putrescine-4TMS
23. Aconitic acid-3TMS
24. Citric acid-4TMS
25. Ornithine-4TMS
26. Adenine-2TMS
27. Lysine-4TMS
28. Tyrosine-3TMS
29. Guanine-3TMS

Fig. 3. The SCAN total ion chromatogram of standard solution using SPE-GC-MS system with automated SPE-based derivatization method.
＊Concentration of standard solution is 0.01nmol/µL



◆ Linearity

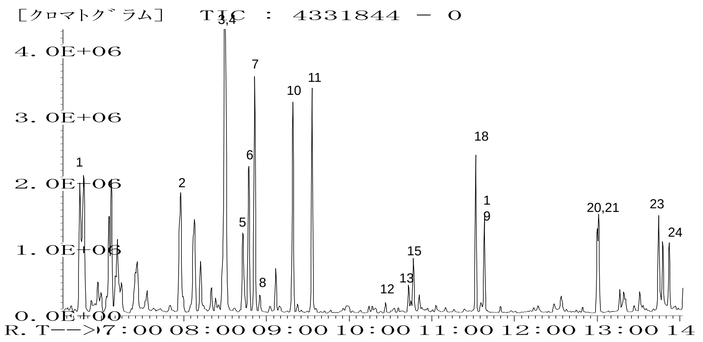
Fig. 4. Relations of concentration in vial and the peak area.

◆ Reproducibility

Table 1. Reproducibility of peak area with standard solution using SPE-GC-MS system.

No.	Compound	1	2	3	4	5	6	7	8	9	Ave.	RSD, %
1	Alanine-2TMS	2,780,202	2,814,678	2,805,838	2,570,446	2,663,543	2,676,876	2,632,581	2,692,127	2,718,118	*2,706,045*	**3.0**
2	Valine-2TMS	3,231,804	3,290,271	3,270,689	2,966,049	3,107,653	3,132,760	3,054,963	3,085,131	3,160,769	*3,144,454*	**3.4**
3	Phosphate (3:1)-3TMS	1,980,261	1,945,488	1,842,146	1,762,489	1,746,163	1,658,840	1,585,771	1,679,471	1,991,253	*1,799,098*	**8.3**
4	Norleucine-2TMS	3,860,754	3,995,800	3,961,145	3,572,341	3,750,253	3,798,730	3,712,869	3,729,371	3,834,508	*3,801,752*	**3.4**
5	Isoleucine-2TMS	3,166,213	3,281,486	3,263,756	2,942,720	3,112,611	3,105,804	3,057,182	3,062,330	3,129,208	*3,124,590*	**3.4**
6	Proline-2TMS	3,326,569	3,445,278	3,452,215	3,055,493	3,264,503	3,272,235	3,230,054	3,247,932	3,297,886	*3,288,018*	**3.6**
7	Glycine-3TMS	2,170,649	2,352,541	2,219,378	2,118,024	2,229,077	2,288,729	2,328,562	2,291,359	2,432,007	*2,270,036*	**4.3**
8	Succinic acid-2TMS	3,020,526	3,101,538	3,047,906	2,891,328	2,874,284	2,677,718	2,840,826	2,917,558	3,180,044	*2,950,192*	**5.2**
9	Fumaric acid-2TMS	1,768,634	1,813,725	1,790,816	1,626,601	1,697,384	1,608,388	1,635,905	1,710,549	1,825,272	*1,719,697*	**4.9**
10	Serine-3TMS	2,012,774	2,110,285	2,078,505	1,857,176	1,969,420	1,978,512	1,918,379	1,950,455	1,968,792	*1,982,700*	**3.9**
11	Threonine-3TMS	1,040,407	1,085,291	1,075,400	963,085	1,019,290	1,028,004	988,509	997,181	1,024,209	*1,024,597*	**3.8**
12	Malic acid-3TMS	485,209	496,725	505,695	464,603	471,484	446,942	451,563	459,884	497,251	*475,484*	**4.5**
13	Aspartic acid-3TMS	527,945	521,172	605,941	439,870	548,152	689,805	622,430	590,812	358,848	*544,997*	**18.3**
14	Methionine-2TMS	1,317,135	1,376,552	1,320,877	1,165,099	1,233,662	1,299,449	1,267,142	1,274,606	1,279,034	*1,281,506*	**4.6**
15	Proline-oxo-2TMS	1,972,283	2,178,513	2,188,232	2,213,001	2,171,464	2,218,260	2,414,150	2,348,929	2,386,591	*2,232,380*	**6.1**
16	Cytosine-2TMS	1,164,055	1,199,619	1,211,399	1,081,564	1,154,429	1,179,900	1,130,354	1,140,346	1,191,227	*1,161,433*	**3.5**
17	Aminobutyric acid-3TMS	1,903,218	2,080,359	1,832,270	1,718,333	1,911,954	1,983,878	1,952,195	1,884,295	2,036,145	*1,922,516*	**5.6**
18	Ketoglutaric acid-3TMS	179,954	187,293	167,292	138,034	169,160	156,600	151,045	147,256	178,112	*163,861*	**10.1**
19	Glutamic acid-3TMS	486,088	482,193	528,880	375,004	494,429	585,775	510,207	483,426	320,272	*474,030*	**16.8**
20	Phenylalanine-2TMS	1,553,897	1,642,952	1,616,874	1,422,103	1,528,499	1,564,042	1,507,985	1,508,373	1,520,941	*1,540,630*	**4.2**
21	Asparagine-3TMS	264,587	293,568	269,785	215,342	260,091	264,791	258,735	249,746	263,337	*259,998*	**7.9**
22	Putrescine-4TMS	1,097,163	1,143,662	1,022,892	1,069,833	1,140,680	1,218,672	1,113,605	1,075,243	1,130,409	*1,112,462*	**5.0**
23	Aconitic acid-3TMS	1,068,411	1,095,208	1,085,018	973,865	1,026,875	1,009,638	1,007,640	1,025,144	1,085,668	*1,041,941*	**4.1**
24	Citric acid-4TMS	2,509,279	2,585,329	2,551,370	2,326,885	2,437,909	2,410,546	2,372,287	2,395,838	2,470,814	*2,451,140*	**3.5**
25	Ornithine-4TMS	948,801	1,074,181	928,962	889,675	963,445	1,025,487	1,049,537	1,005,050	1,047,923	*992,562*	**6.3**
26	Adenine-2TMS	1,791,455	1,859,990	1,930,628	1,670,688	1,831,552	1,839,583	1,690,694	1,722,793	1,876,276	*1,801,518*	**5.0**
27	Lysine-4TMS	438,332	487,673	411,966	389,263	423,849	469,263	485,851	449,843	475,516	*447,951*	**7.8**
28	Tyrosine-3TMS	2,290,304	2,415,910	2,339,399	2,070,195	2,220,032	2,300,204	2,231,995	2,184,577	2,239,834	*2,254,717*	**4.4**
29	Guanine-3TMS	1,276,608	1,325,553	1,372,469	1,201,496	1,273,918	1,292,009	1,214,782	1,217,067	1,316,201	*1,276,678*	**4.5**

Evaluation with the mouse serum



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15. Proline-oxo-2TMS
18. Glutamic acid-3TMS
19. Phenylalanine-2TMS
20. Citric acid-4TMS
21. Ornithine-4TMS
23. Lysine-4TMS
24. Tyrosine-3TMS

Fig. 5. The SCAN total ion chromatogram of the mouse serum using SPE-GC-MS system with automated SPE-based derivatization method.

◆ Reproducibility

Table 2. Reproducibility of peak area with the mouse serum using SPE-GC-MS system.

No.	Compound	1	2	3	4	5	6	7	8	9	Ave.	RSD, %
1	Alanine-2TMS	5,541,262	5,554,574	5,750,943	5,697,229	5,588,344	5,721,789	5,850,742	5,681,853	5,706,369	*5,677,012*	**1.8**
2	Valine-2TMS	2,755,712	2,771,037	2,893,545	2,911,101	2,803,596	2,871,371	2,926,797	2,853,359	2,867,084	*2,850,400*	**2.1**
3	Phosphate (3:1)-3TMS	4,201,912	4,356,822	4,434,444	4,412,647	4,201,988	4,259,611	4,389,792	4,266,977	4,210,194	*4,303,821*	**2.2**
4	Norleucine-2TMS	4,056,211	4,080,919	4,276,463	4,306,038	4,157,663	4,270,224	4,312,737	4,223,433	4,227,870	*4,212,395*	**2.2**
5	Isoleucine-2TMS	1,078,007	1,080,660	1,131,523	1,141,625	1,094,518	1,124,087	1,139,671	1,121,838	1,118,968	*1,114,544*	**2.2**
6	Proline-2TMS	2,990,521	3,000,081	3,191,334	3,154,192	3,053,991	3,162,046	3,244,535	3,101,102	3,103,402	*3,111,245*	**2.8**
7	Glycine-3TMS	3,037,839	2,895,879	3,107,951	3,217,887	3,354,424	3,293,368	3,059,023	3,070,554	3,113,071	*3,127,777*	**4.5**
8	Succinic acid-2TMS	29,040	29,234	29,610	29,118	26,403	27,322	28,530	28,003	27,169	*28,270*	**3.9**
9	Fumaric acid-2TMS	14,400	13,605	14,528	13,757	13,469	13,211	12,847	12,142	11,996	*13,328*	**6.7**
10	Serine-3TMS	1,617,736	1,601,793	1,691,622	1,701,792	1,664,881	1,718,251	1,759,025	1,685,417	1,696,241	*1,681,862*	**2.9**
11	Threonine-3TMS	854,196	855,270	914,762	923,062	882,377	911,832	928,177	920,805	914,839	*900,591*	**3.2**
12	Malic acid-3TMS	20,457	20,641	21,151	21,808	19,071	20,283	20,751	19,941	19,094	*20,355*	**4.4**
13	Aspartic acid-3TMS	195,512	183,707	208,840	210,072	204,684	225,449	240,856	233,191	238,539	*215,650*	**9.3**
14	Methionine-2TMS	93,568	94,791	100,427	104,122	101,698	103,386	105,816	97,718	99,320	*100,094*	**4.2**
15	Proline-oxo-2TMS	719,078	784,164	740,721	869,323	610,496	641,127	674,841	868,861	839,473	*749,787*	**13.0**
16	*Cytosine-2TMS*	5,878	5,728	5,647	5,592	5,629	5,516	4,974	5,028	5,413	*5,489*	**5.6**
17	Aminobutyric acid-3TMS	87,027	83,832	70,197	83,924	75,857	76,002	85,355	82,175	87,546	*81,324*	**7.3**
18	Glutamic acid-3TMS	1,268,042	1,206,401	1,296,449	1,342,892	1,281,052	1,393,273	1,513,486	1,405,403	1,516,263	*1,358,140*	**8.0**
19	Phenylalanine-2TMS	699,562	714,084	762,498	773,628	724,391	758,118	786,916	753,925	773,827	*749,661*	**4.0**
20	Citric acid-4TMS	335,441	333,534	337,541	334,091	312,166	321,946	322,681	314,421	317,960	*325,531*	**3.0**
21	Ornithine-4TMS	663,367	673,444	787,729	789,718	771,470	808,655	771,975	652,659	737,745	*739,640*	**8.2**
22	*Adenine-2TMS*	4,077	3,243	3,358	3,410	3,217	2,885	3,216	2,978	3,005	*3,265*	**10.8**
23	Lysine-4TMS	384,118	400,505	466,485	466,911	449,562	483,398	450,203	364,748	433,653	*433,287*	**9.5**
24	Tyrosine-3TMS	739,551	763,670	838,110	848,309	773,737	850,307	875,807	790,095	852,336	*814,658*	**5.9**
25	*Guanine-3TMS*	3,773	3,789	3,207	3,438	2,485	2,720	2,968	3,136	2,457	*3,108*	**16.1**

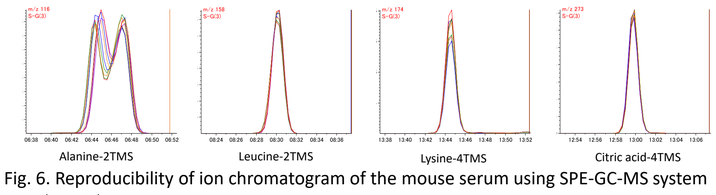

Fig. 6. Reproducibility of ion chromatogram of the mouse serum using SPE-GC-MS system (n = 9).

◆ Recovery

Table 3. Recoveries of metabolites added to mouse serum

No.	Compound	Standard ST	Mause M	Mause +ST A	REC, % (A-M)/ST
1	Alanine-2TMS	*2,395,769*	*5,539,074*	*7,703,074*	**90**
2	Valine-2TMS	*2,857,898*	*2,759,114*	*5,744,538*	**104**
3	Phosphate (3:1)-3TMS	*1,691,961*	*4,279,367*	*6,787,045*	**148**
4	Norleucine-2TMS	*3,402,608*	*4,067,823*	*7,424,476*	**99**
5	Isoleucine-2TMS	*2,801,029*	*1,086,415*	*4,096,438*	**107**
6	Proline-2TMS	*3,037,141*	*2,998,133*	*6,301,408*	**109**
7	Glycine-3TMS	*1,800,878*	*2,966,859*	*4,603,320*	**91**
8	Succinic acid-2TMS	*2,958,640*	*224,256*	*3,375,441*	**107**
9	Fumaric acid-2TMS	*1,676,379*	*13,946*	*1,869,217*	**111**
10	Serine-3TMS	*1,769,642*	*1,608,041*	*3,632,037*	**114**
11	Threonine-3TMS	*900,834*	*852,911*	*1,865,424*	**112**
12	Malic acid-3TMS	*475,088*	*20,319*	*551,360*	**112**
13	Aspartic acid-3TMS	*440,006*	*190,845*	*795,480*	**137**
14	Methionine-2TMS	*1,186,002*	*93,758*	*1,417,378*	**112**
15	Proline-oxo-2TMS	*1,684,508*	*740,135*	*3,085,018*	**139**
16	Cytosine-2TMS	*1,069,383*	*5,948*	*1,182,673*	**110**
17	Aminobutyric acid-3TMS	*1,425,778*	*82,936*	*1,774,441*	**119**
18	Ketoglutaric acid-3TMS	*162,941*		*163,614*	**100**
19	Glutamic acid-3TMS	*381,767*	*1,237,713*	*1,524,345*	**75**
20	Phenylalanine-2TMS	*1,361,973*	*705,325*	*2,285,882*	**116**
21	Asparagine-3TMS	*212,637*		*291,982*	**137**
22	Putrescine-4TMS	*928,721*	*35,714*	*1,124,501*	**117**
23	Aconitic acid-3TMS	*482,874*	*3,083*	*544,525*	**112**
24	Citric acid-4TMS	*2,365,829*	*334,488*	*3,143,411*	**119**
25	Ornithine-4TMS	*741,337*	*668,406*	*1,295,449*	**85**
26	Adenine-2TMS	*1,710,221*	*3,660*	*1,966,746*	**115**
27	Lysine-4TMS	*344,472*	*389,645*	*680,111*	**84**
28	Tyrosine-3TMS	*2,171,566*	*748,544*	*3,535,152*	**128**
29	Guanine-3TMS	*1,112,060*	*3,595*	*1,479,513*	**133**

＊Sample were spiked at 0.01 nmol/µL of each metabolites.

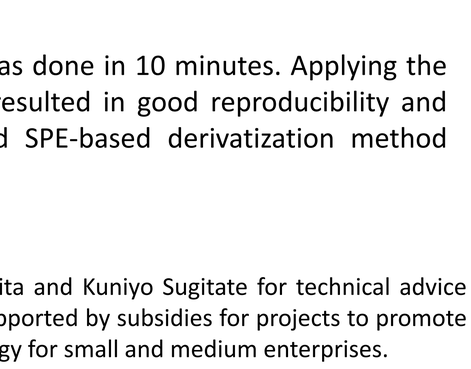

Fig. 7. Relations of sample loading volume and the peak area.

【Conclusion】

Pre-treatment, dehydration and derivatization of the sample was done in 10 minutes. Applying the system to metabolite standard solutions and mouse serums resulted in good reproducibility and robustness. Thus, online SPE-GC-MS system with automated SPE-based derivatization method enables the rapid and reliable analysis of metabolites.

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