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 silvlated metabolites are unstable and the time required for complete silulation 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 silulation to GC-MS injection constant.

[Experimental]





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.

Insert

MS Method



Condition	on of SPE-GC-MS Systen
SPE-GC Interface	SGI-M100 ·AiSTI Science

Flash-SPE AOS ;AiSTI Science SPE Cartridge

LVI-S250 ;AiSTI Science PTV Injecto Spiral Insert ; AiSTI Science 80℃(0.2min)-50℃/min-290℃(38min) Injector Temp

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°C

SCAN; 70 - 500 m/z

Sampling	Flash-SPE	Injection

Pre-t	reatment Unit	9	
Pump Unit			
SCI-P100			

	Large Volume	Injection-Split m	node
1st Stage	2nd Stage	3rd Stage	4th Stage
Injection	Concentration	Transfer	Elimination
The injector is kept at a temperature lower than the boiling point of sample solvent.	While the evaporated sample solvent is exhausted, the sample is concentrated.	Target compounds are transferred to the column at an elevated temperature.	Matrix compounds a eliminated from the insert at further elevated temperatur

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

Online SPE-GC-MS System

(Results)





75.857

699,562 714,084 762,498 773,628 724,391 758,118 786,916 753,925 773,827

335,441 333,534 337,541 334,091 312,166 321,946 322,681 314,421 317,960

1.281.052 1.393.273

1 296 449 1 342 892

76.002

85.355

1.405.403

1.513.486

Recovery

7.3

8.0

4.0

3.0

81.324

1,358,140

749,661

325,531

Table 3. Recoveries of metabolites added to mouse serum

No	Compound	Standard	Mause	Mause +ST	REC, %
NO.	Compound	ST	Μ	А	(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	lsoleucine-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	<i>93,758</i>	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	<i>1,112,0</i> 60	<i>3,595</i>	<i>1,479,5</i> 13	133
					1

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

1.E+07 Alanine-2TMS	6.E+05 Succinic acid-2TMS
1.E+07 R ² = 0.9985	5.E+05 R ² = 0.9985
1.E+07	4.E+05
8.E+06	Lea
≪ × 6 F+06	≪ 3.E+05 ¥
4.E+06	2.E+05
2.E+06	1.E+05
0.E+00	0.E+00
0 20 40 60 80 Sample loading volume (μL)	0 20 40 60 80 Sample loading volume (μL)
3.E+05 Methionine-2TMS	1.E+06 Lysine-4TMS
3.E+05	1.E+06
2.E+05	8.E+05
Ž 2.E+05	Č6.E+05
۵ 1.E+05	۵́4.E+05
	2 5+05

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	<i>1,276,6</i> 78	4.5



.516.263

(Conclusion)

17 Aminobutyric acid-3TMS

18 Glutamic acid-3TMS

19 Phenylalanine-2TMS

20 Citric acid-4TMS

87.027

1.206.401

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

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Beyond your Imagination

「メタボローム分析のための自動固相誘導体化法 を用いたオンラインSPE-GC-MSシステムの開発」

目的

従来のメタボローム分析は、採取した試料を溶媒抽出、遠心濃縮、凍結乾燥、誘導体化等の前処理工程が煩雑であるため、長時間を要するとともに熟練された技術が必須となる.

また、多検体の前処理をバッチ処理した場合、GC/MSでの測定はそれらの 検体を順次測定していくため、それらの検体において誘導体化後から測定ま での時間が異なってしまう。これらは分析バッチ間の系統誤差の要因となり、 多サンプルのメタボローム解析を遂行する上で致命的な短所である.

そこで、本研究では、迅速な自動誘導体化とその誘導体化からGCMSへの直接試料導入を目指して、固相誘導体化法を用いたオンラインSPE-GC/MSシステムの開発を行ったので報告する。













Automation









Sampling



Flash-SPE



Injection

Online SPE-GC-MS System







スパイラルインサートの形状は固相カートリッジからの溶出液全量を一時的に受け止 めることができる。





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

誘導体化試薬について



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



Methoxyamin/pyridineをMSTFAに混合することで誘導体化が促進された物質

-CH2-NH2の基に対してしっかりと-CH2-N-2TMSに、グリシンの立体障害を受けない-C(-NH2)-についても-C(-N-2TMS)-になりました。

本法による標準溶液のSCANトータルイオンクロマトグラム



Fig. 3. The SCAN total ion chromatogram of standard solution using SPE-GC-MS system with automated SPE-based derivatization method. *標準溶液バイアル中濃度: 0.01nmol/µL

本法による標準溶液の再現性

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

No.	Com pound	1	2	3	4	5	6	7	8	9	Ave.	RSD,%
1	A lan ine-2TM S	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-2TM S	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)-3TM S	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-2TM S	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	lsoleucine−2TM S	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-2TM S	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 – 2TM S	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	Fum aric acid-2TM S	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-3TM S	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-3TM S	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	Malicacid-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-3TM S	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-2TM S	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	Am inobutyric acid-3TM S	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-3TM S	179,954	187,293	167,292	138,034	169,160	156,600	151,045	147,256	178,112	163,861	10.1
19	G lutam ic acid-3TM S	486,088	482,193	528,880	375,004	494,429	585,775	510,207	483,426	320,272	474,030	16.8
20	Phenylalanine-2TM S	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-3TM S	264,587	293,568	269,785	215,342	260,091	264,791	258,735	249,746	263,337	259,998	7.9
22	Putrescine–4TM S	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	A con itic ac id-3TM S	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-4TM S	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	0 mithine–4TM S	948,801	1,074,181	928,962	889,675	963,445	1,025,487	1,049,537	1,005,050	1,047,923	<i>992,562</i>	6.3
26	Adenine–21M S	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—4TM S	438,332	487,673	411,966	389,263	423,849	469,263	485,851	449,843	475,516	447,951	7.8
28	Tyrosine-3TM S	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-3TM S	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

*標準溶液バイアル中濃度: 0.01nmol/µL

濃度とピーク面積値の関係(直線性)



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

本法によるマウス血清のトータルイオンクロマトグラム



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

本法によるマウス血清のピーク面積値の再現性

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	A ve.	RSD,%
1	A lan ine-2TM S	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-2TM S	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)-3TM S	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-2TM S	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−2TM S	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-2TM S	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-2TM S	29,040	29,234	29,610	29,118	26,403	27,322	28,530	28,003	27,169	28,270	3.9
9	Fum aric acid-2TM S	14,400	13,605	14,528	13,757	13,469	13,211	12,847	12,142	11,996	13,328	6.7
10	Serine-3TM S	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–3TM S	854,196	855,270	914,762	923,062	882,377	911,832	928,177	920,805	914,839	900,591	3.2
12	Malicacid-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-3TM S	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-2TM S	5,878	5,728	5,647	5,592	5,629	5,516	4,974	5,028	5,413	5,489	5.6
17	Am inobutyric acid-3TM S	87,027	83,832	70,197	83,924	75,857	76,002	85,355	82,175	87,546	81,324	7.3
18	G lutam ic acid-3TM S	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-2TM S	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-4TM S	335,441	333,534	337,541	334,091	312,166	321,946	322,681	314,421	317,960	325,531	3.0
21	0 m ith in e-4TM S	663,367	673,444	787,729	789,718	771,470	808,655	771,975	652,659	737,745	739,640	8.2
22	Adenine-2TM S	4,077	3,243	3,358	3,410	3,217	2,885	3,216	2,978	3,005	3,265	10.8
23	Lysine—4TM S	384,118	400,505	466,485	466,911	449,562	483,398	450,203	364,748	433,653	<i>433,287</i>	9.5
24	Tyrosine—3TM S	739,551	763,670	838,110	848,309	773,737	850,307	875,807	790,095	852,336	814,658	5.9
25	Guanine-3TM S	3,773	3,789	3,207	3,438	2,485	2,720	2,968	3,136	2,457	3,108	16.1

本法によるマウス血清の添加回収試験

AISTI SCIENCE

No		Standard	M ause	M ause + ST	REC,%
N 0.		ST	М	Α	(A-M)/ST
1	A lan in e-2TM S	2,395,769	5,539,074	7,703,074	90
2	Valine-2TM S	2,857,898	2,759,114	5,744,538	104
3	Phosphate (3:1)-3TM S	1,691,961	4,279,367	6,787,045	148
4	Norleucine-2TM S	<i>3,402,608</i>	4,067,823	7,424,476	99
5	Isoleucine−2TM S	2,801,029	1,086,415	4,096,438	107
6	Proline-2TM S	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-2TM S	2,958,640	224,256	3,375,441	107
9	Fum aric ac id-2TM S	1,676,379	13,946	1,869,217	111
10	Serine-3TM S	1,769,642	1,608,041	3,632,037	114
11	Threonine-3TM S	900,834	852,911	1,865,424	112
12	Malicacid-3TMS	475,088	20,319	551,360	112
13	Aspartic ac id–3TM S	440,006	190,845	795,480	137
14	Methionine-2TMS	1,186,002	<i>93,758</i>	1,417,378	112
15	Proline-oxo-2TMS	1,684,508	740,135	3,085,018	139
16	Cytosine–2TM S	1,069,383	5,948	1,182,673	110
17	Am inobutyric acid-3TM S	1,425,778	<i>82,936</i>	1,774,441	119
18	Ketoglutaric acid-3TM S	<i>162,941</i>		163,614	100
19	G lutam ic ac id-3TM S	381,767	1,237,713	1,524,345	75
20	Phenylalanine-2TM S	1,361,973	705,325	2,285,882	116
21	Asparagine-3TM S	212,637		291,982	137
22	Putrescine–4TM S	<i>928,721</i>	35,714	1,124,501	117
23	Acon itic ac id-3TM S	<i>482,874</i>	3,083	544,525	112
24	C itric ac id-4TM S	2,365,829	334,488	3,143,411	119
25	0 m ith in e-4TM S	741,337	668,406	1,295,449	85
26	Adenine–2TM S	1,710,221	3,660	1,966,746	115
27	Lysine-4TM S	344,472	389,645	680,111	84
28	Tyrosine—3TM S	2,171,566	748,544	3,535,152	128
29	Guanine-3TM S	1,112,060	3,595	1,479,513	133

Table 3. Recoveries of metabolites added to mouse serum

*添加濃度: 0.01nmol/µL(バイアル中換算)

サンプル抽出液の負荷量とピーク面積値



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

本法によるマウス血清の定量イオンクロマトグラム の再現性(重ね描き, n=9)



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



- 抽出後の本システムによる誘導体化を含めた前処理時間は10分で、かつ、 その前処理からGC-MSによる測定まで完全に自動で行うことができた。
- 誘導体化してから注入するまでの時間が常に一定であるため、多検体においても安定した結果を得られると考えられる。
- 本システムを用いて、スタンダードおよびマウス血清による再現性、直線 性を評価したところ、共に良好な結果を得た。
- 開発したオンライン自動固相誘導体化SPE-GC/MSシステムはメタボローム 分析に有効であることがわかった。