

Metabolomics 2017

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

Ryoichi SASANO¹, Koji MACHITANI², Shusuke OSAKI², Masahiro FURUNO³, Eiichiro FUKUSAKI³

1; Aisti Science co., Ltd., 2; Industrial Technology Center of Wakayama prefecture,
3; Osaka University



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」**

AiSTI SCIENCE

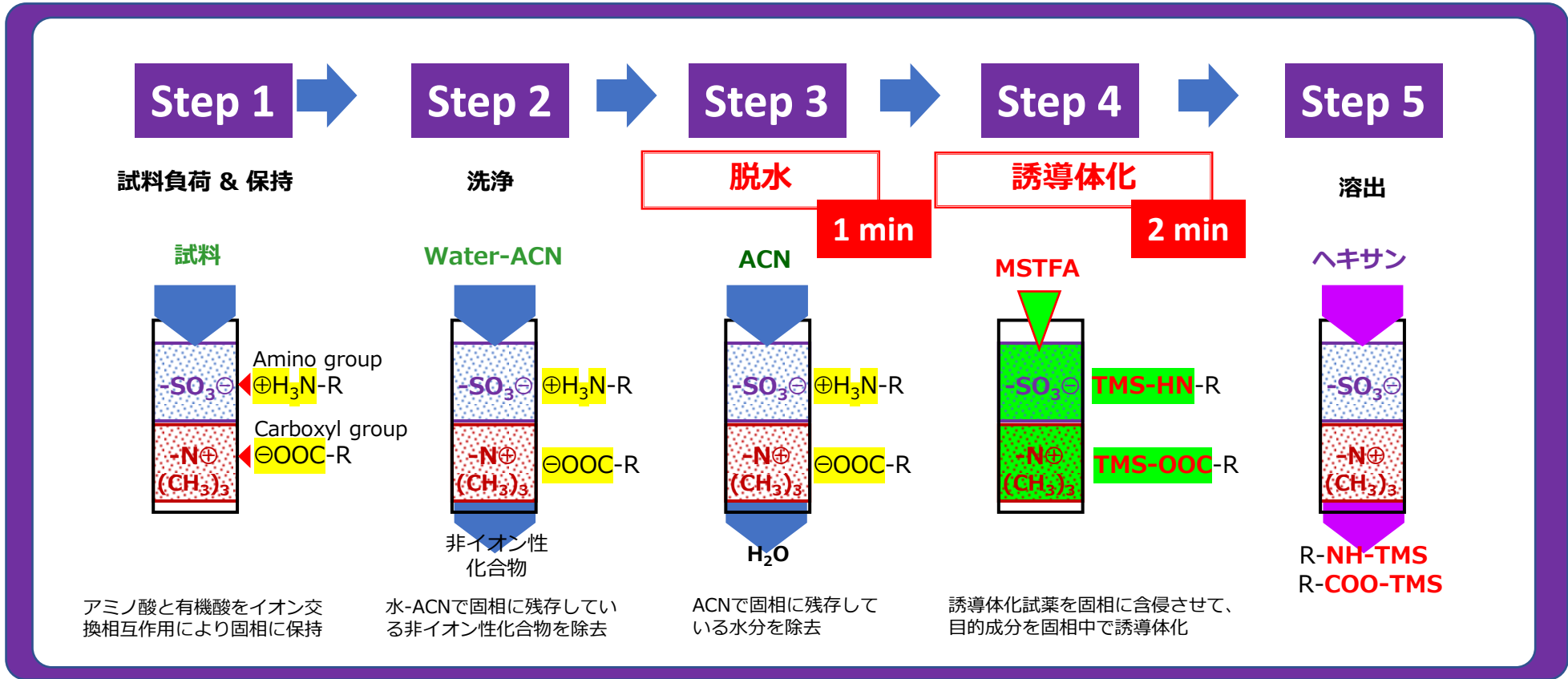
目的

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。

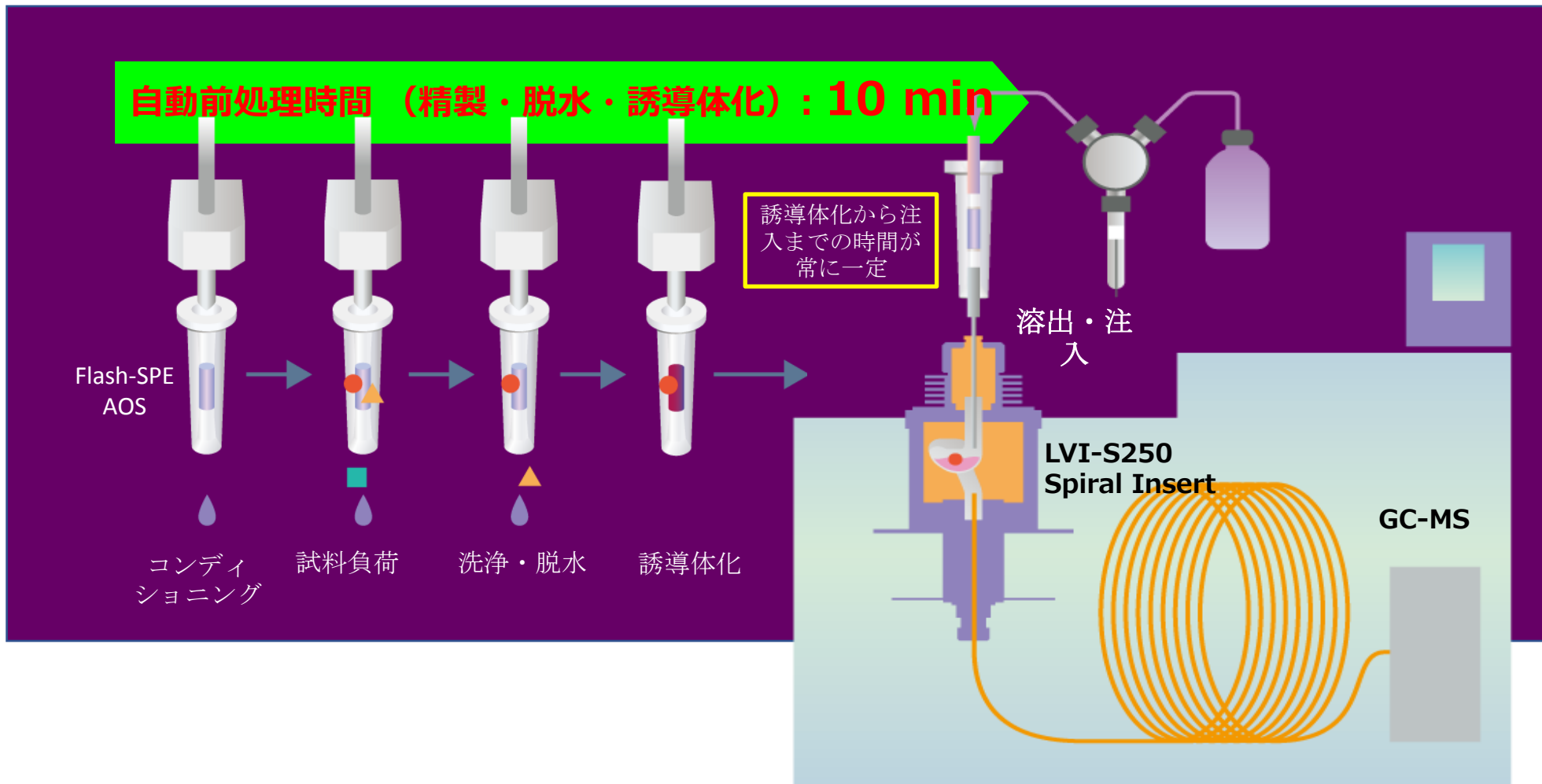
また、多検体の前処理をバッチ処理した場合、GC/MSでの測定はそれらの検体を順次測定していくため、それらの検体において誘導体化後から測定までの時間が異なってしまふ。これらは分析バッチ間の系統誤差の要因となり、多サンプルのメタボローム解析を遂行する上で致命的な短所である。

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。

固相誘導体化法

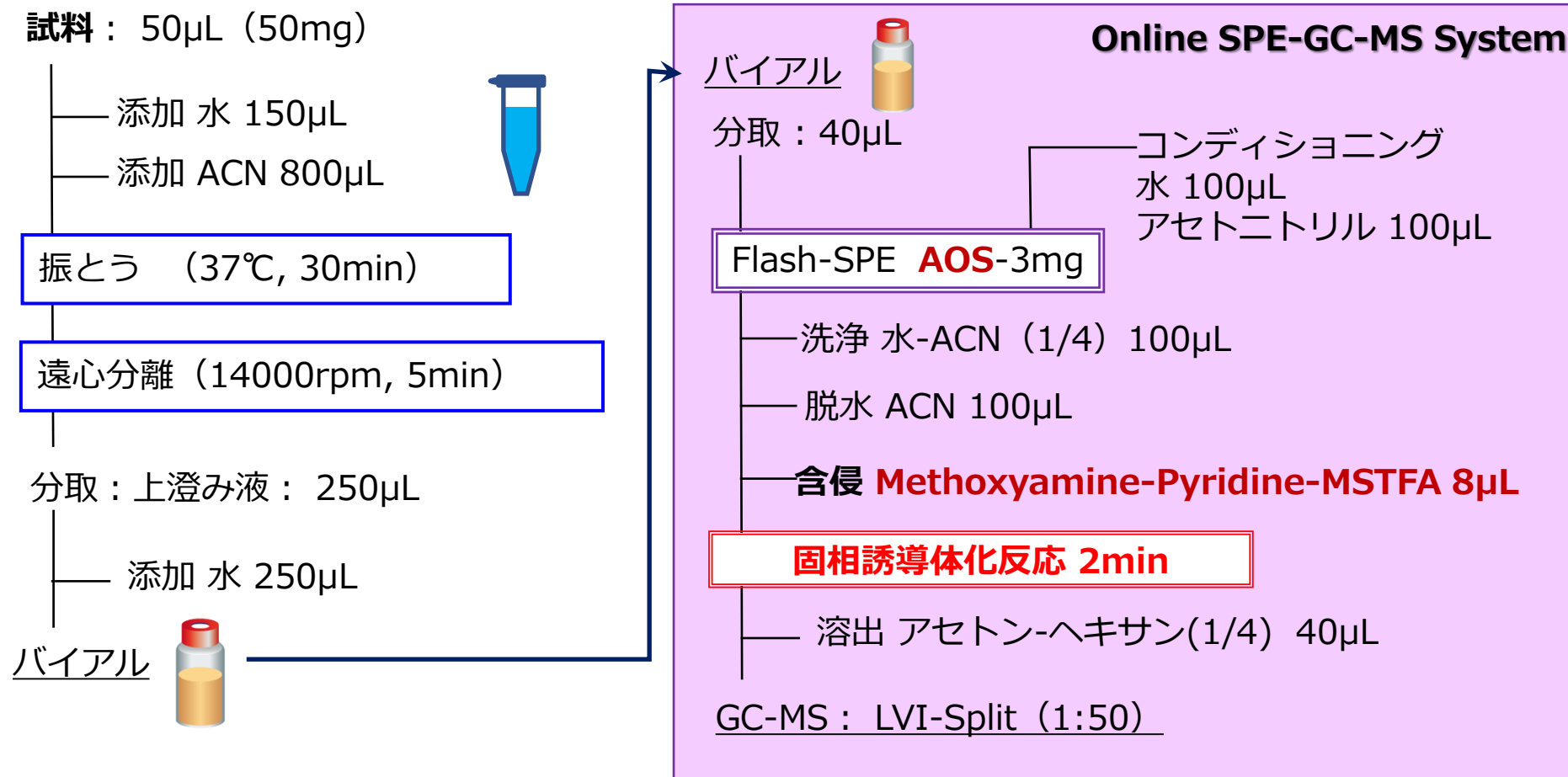


自動固相誘導体化オンラインSPE-GC-MSシステム

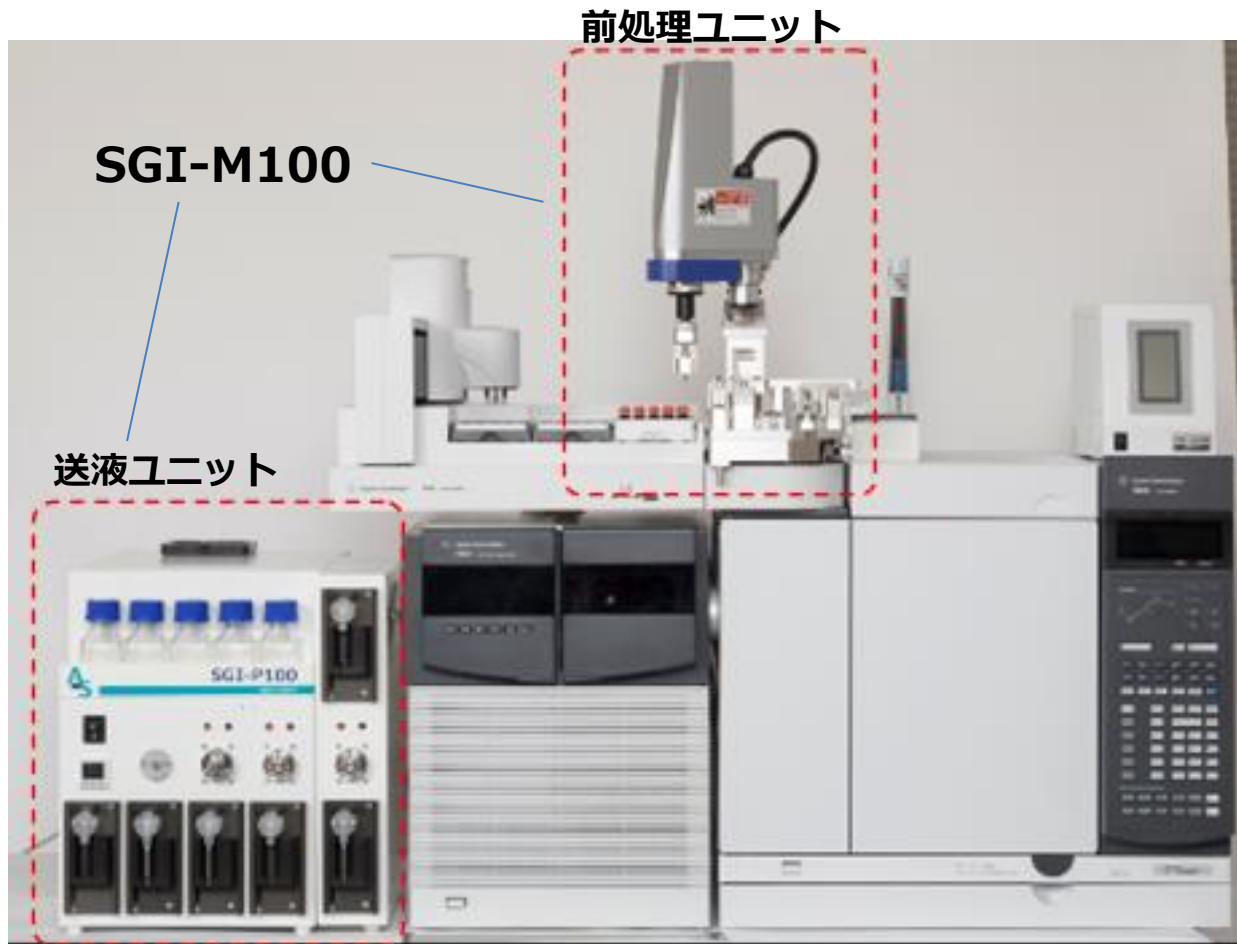


前処理フロー

Automation



オンラインSPE-GC-MSシステム



Sampling



Flash-SPE



Injection

Online SPE-GC-MS System

SPE-GC-MS条件

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

大量注入-スプリット モード



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。

固相誘導体化時間

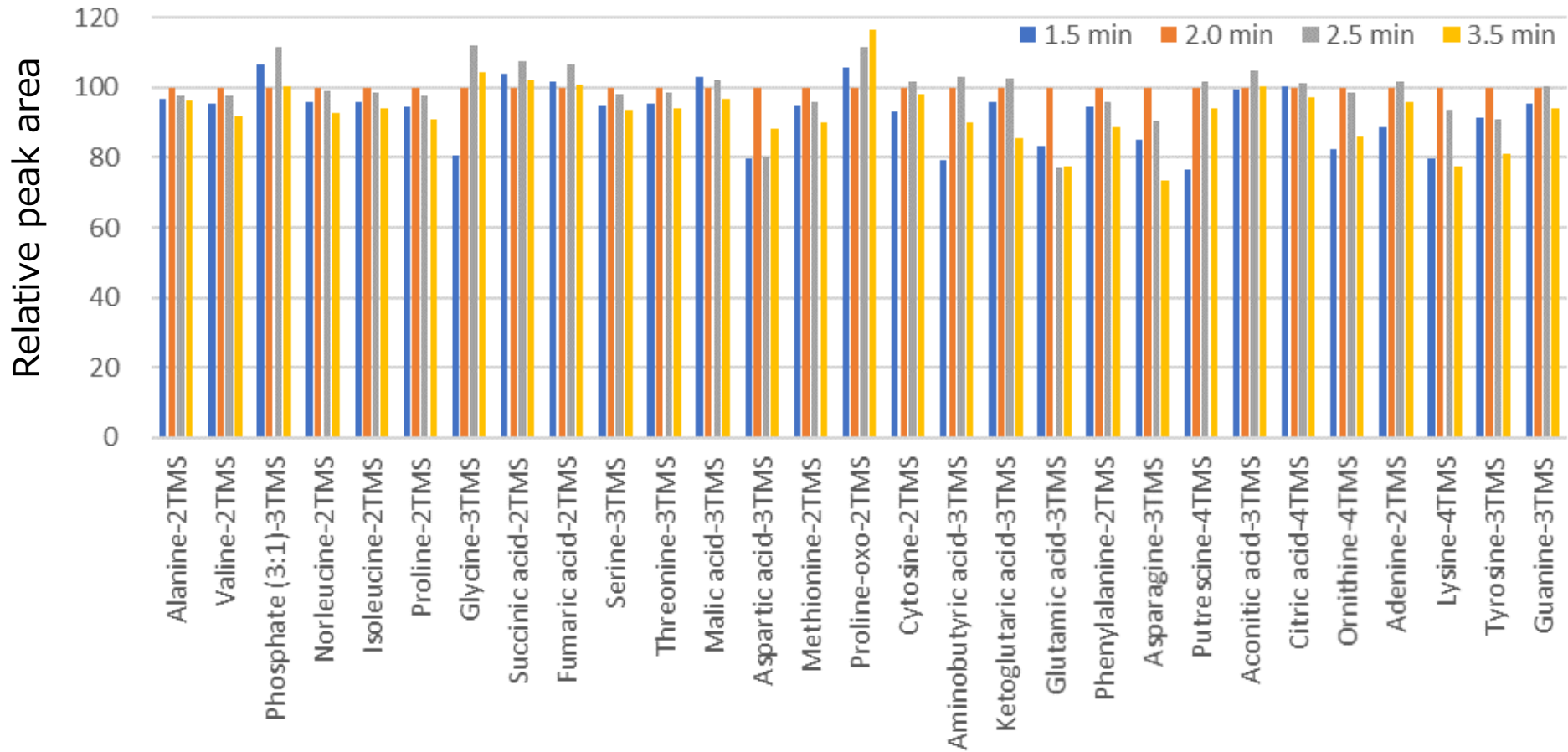


Fig. 1. Relations of derivatization time in sorbent and the relative peak area.

誘導体化試薬について

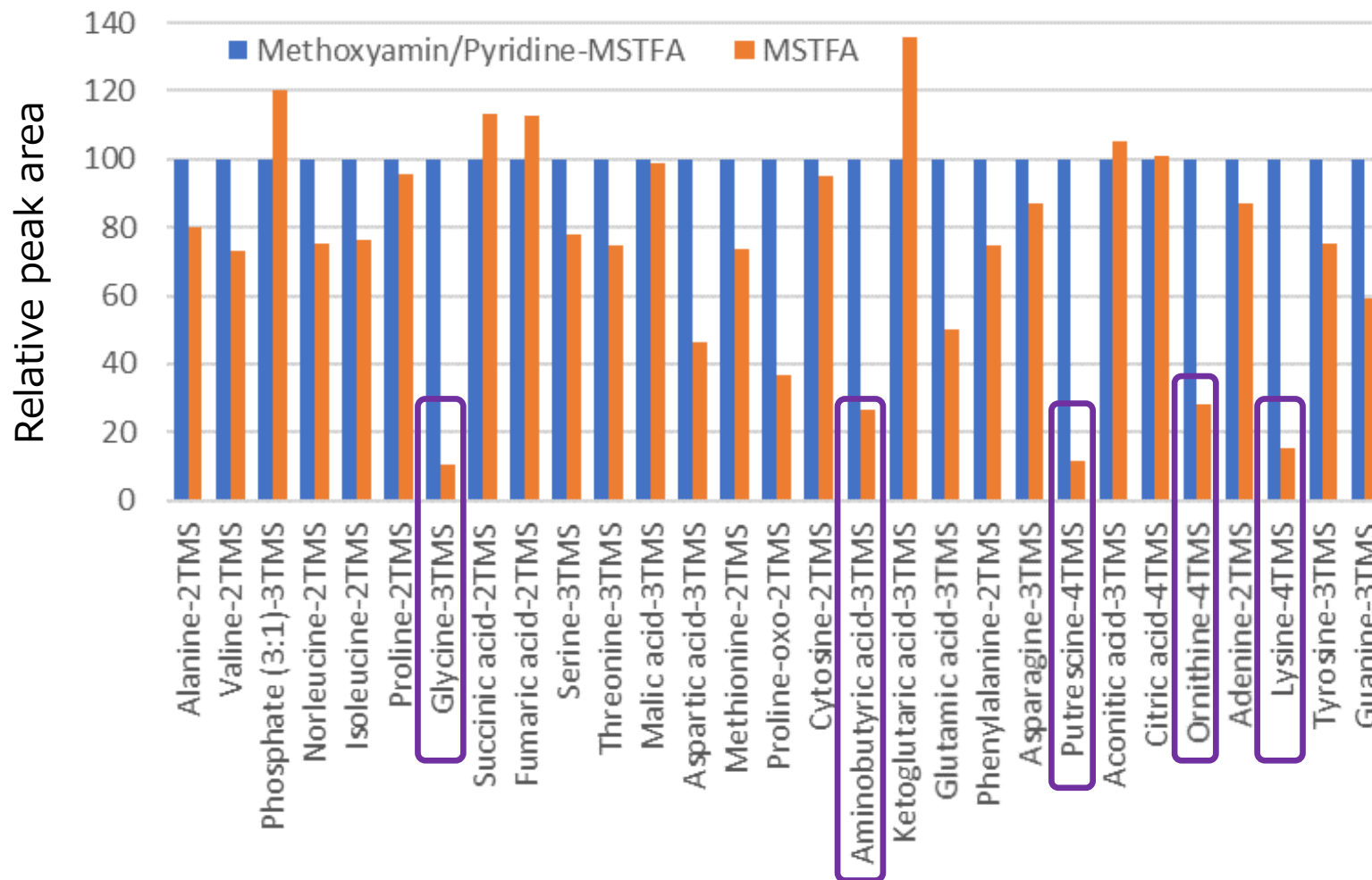
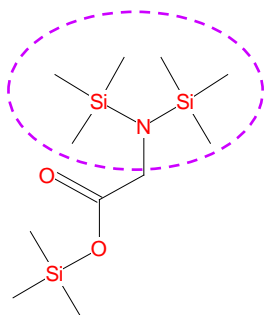
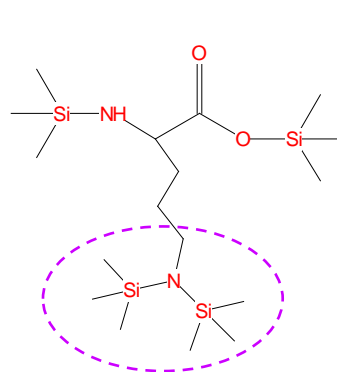


Fig. 2. Relations of the derivatization reagent and the relative peak area.

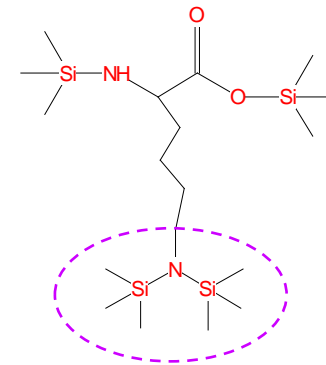
Methoxyamin / Pyridine-MSTFAの効果



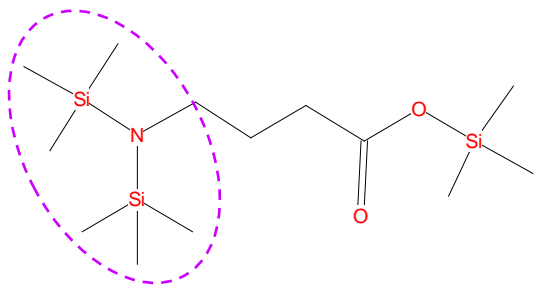
Glycine-3TMS



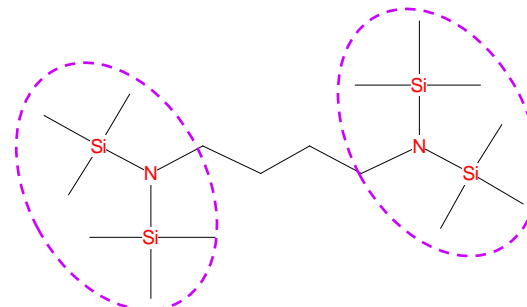
Ornithine-4TMS



Lysine-4TMS



Aminobutyric acid-3TMS



Putrescine-4TMS

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

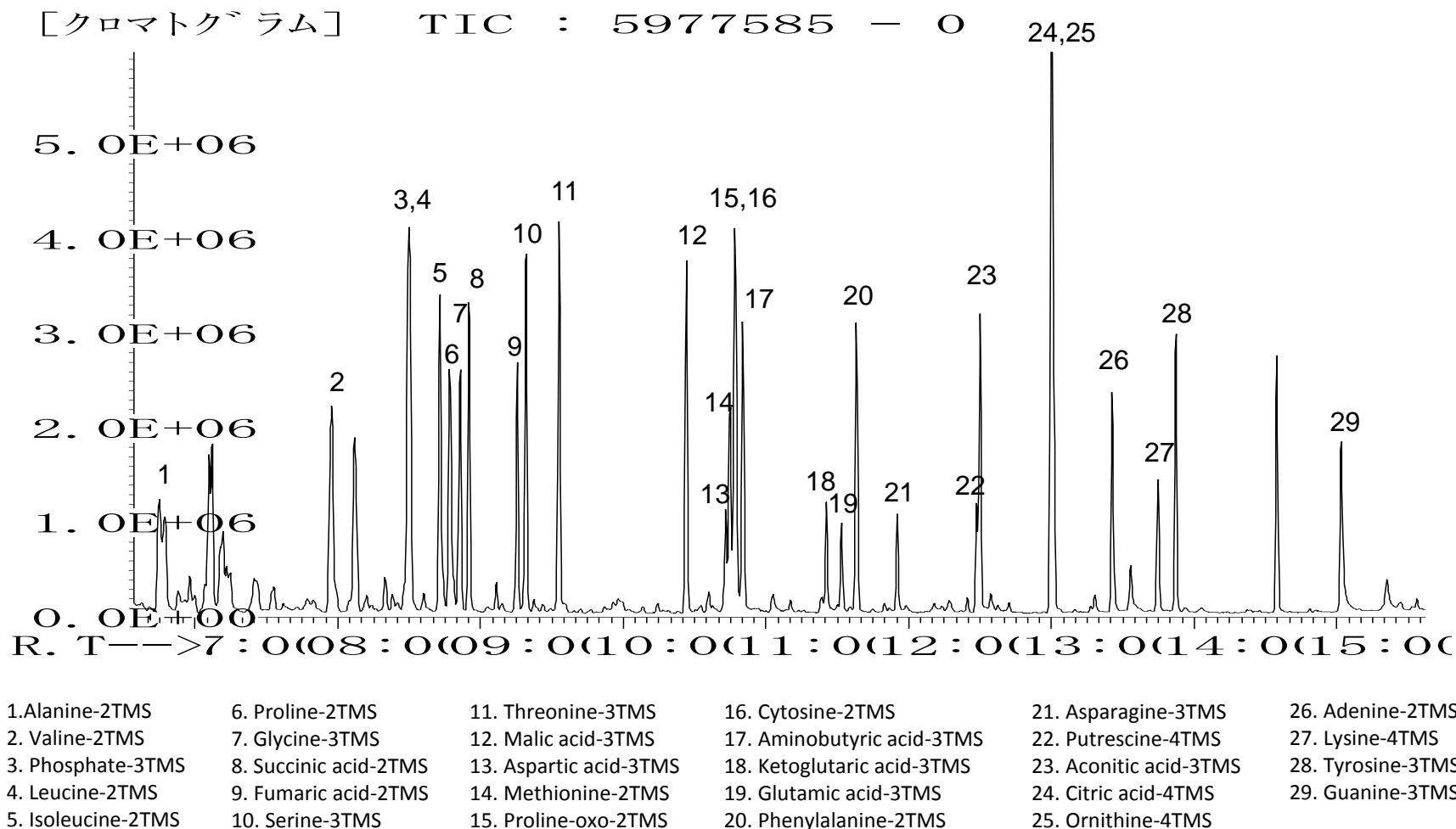


Fig. 3. The SCAN total ion chromatogram of standard solution using SPE-GC-MS system with automated SPE-based derivatization method.

* 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.	Compound	1	2	3	4	5	6	7	8	9	Ave.	RSD, %
1	Alanine-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	Isoleucine-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-3TM S	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	Fumaric 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	Malic acid-3TM S	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-2TM S	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-2TM S	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	Glutam 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	Aconitic acid-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	Omithine-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	992,562	6.3
26	Adenine-2TM 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）

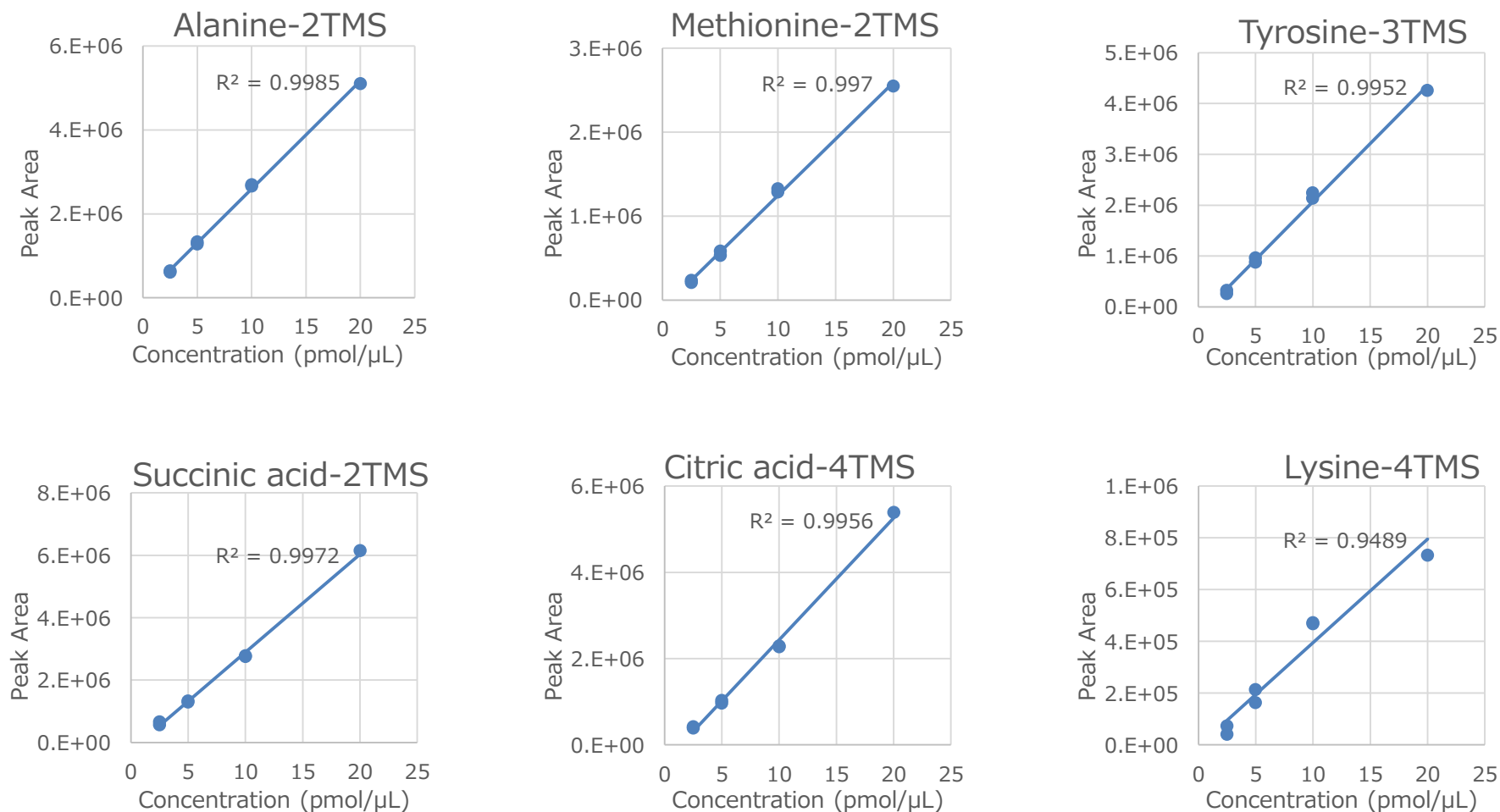


Fig. 4. Relations of concentration in vial and the peak area.

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

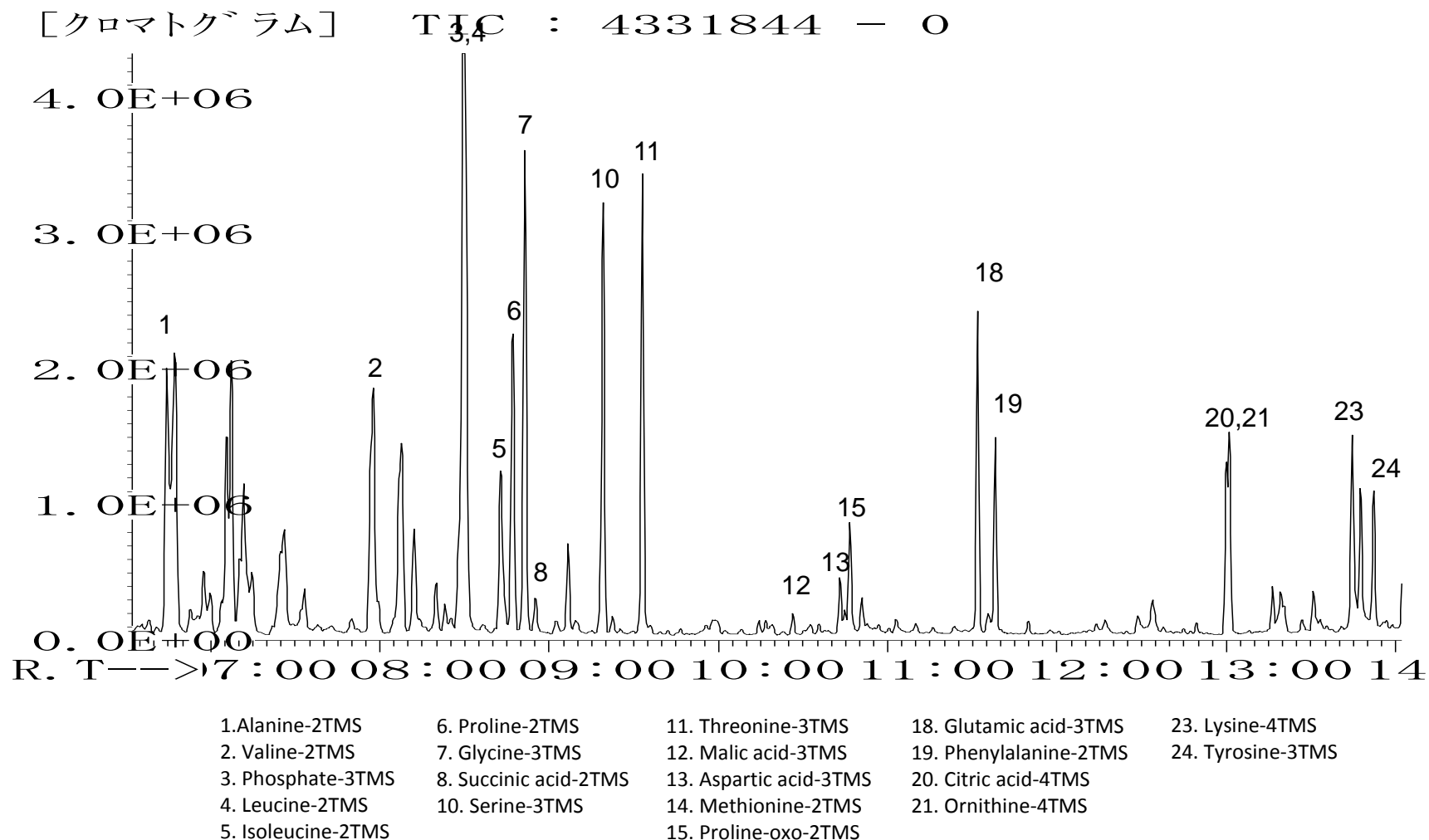


Fig. 5. The SCAN total ion chromatogram of the mouse serum using SPE-GC-MS system with automated SPE-based derivatization method.

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	Ave.	RSD, %
1	Alanine-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-3TM S	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	Fumaric 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	Malic acid-3TM S	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-2TM S	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-2TM S	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	C itric 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	O m ith ine-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	433,287	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

No.	Compound	Standard	M a use	M a use + ST	REC, % (A-M)/ST
		ST	M	A	
1	Alanine-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	3,402,608	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	6,307,408	109
7	Glycine-3TM S	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	Fumaric acid-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	Malic acid-3TM S	475,088	20,319	551,360	112
13	Aspartic acid-3TM S	440,006	190,845	795,480	137
14	Methionine-2TM S	1,186,002	93,758	1,417,378	112
15	Proline-oxo-2TM S	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	82,936	1,774,441	119
18	Ketoglutaric acid-3TM S	162,941		163,614	100
19	Glutam ic acid-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	928,721	35,714	1,124,501	117
23	Aconitic acid-3TM S	482,874	3,083	544,525	112
24	Citric acid-4TM S	2,365,829	334,488	3,143,411	119
25	Omithine-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

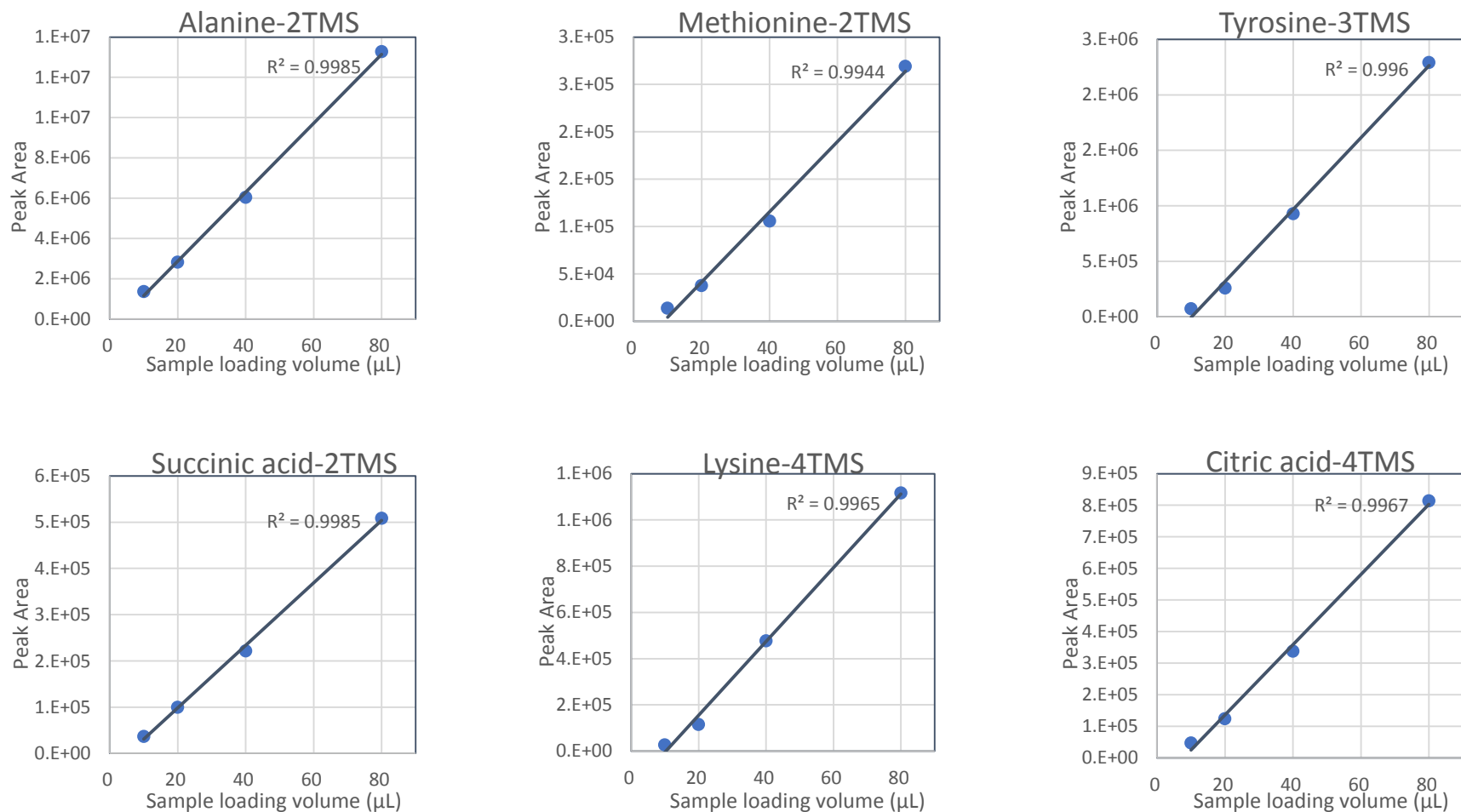


Fig. 7. Relations of sample loading volume and the peak area.

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)

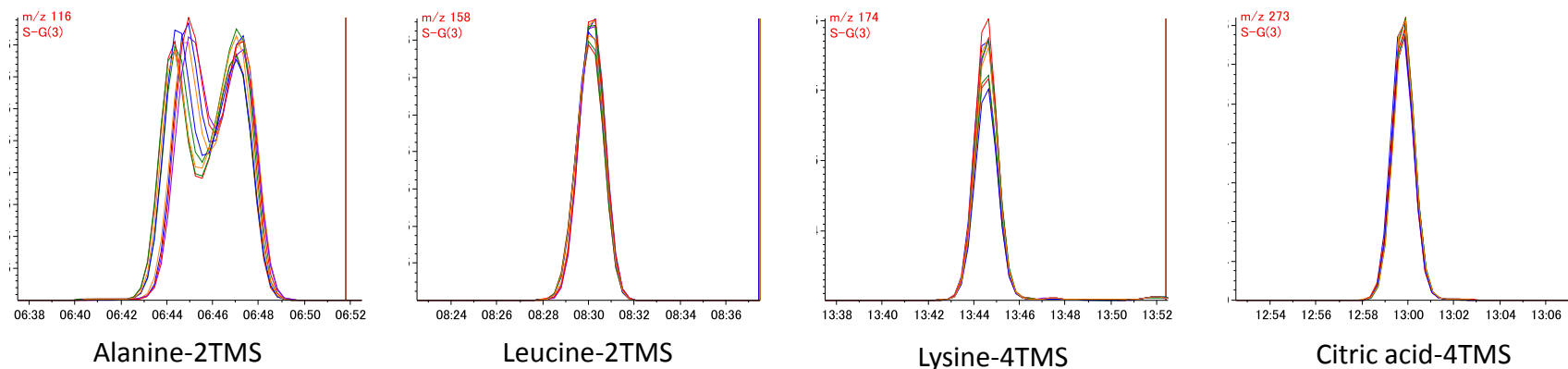


Fig. 6. Reproducibility of ion chromatogram of the mouse serum using SPE-GC-MS system (n = 9).

まとめ

- 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。