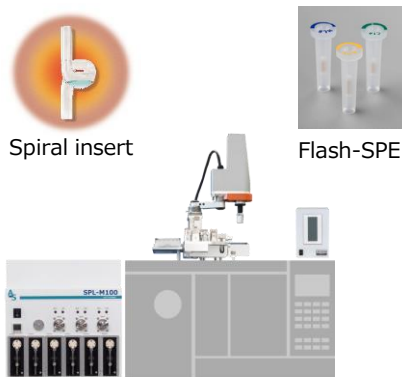


# Metabolome analysis of serum

by online solid-phase analytical derivatization GC-MS system

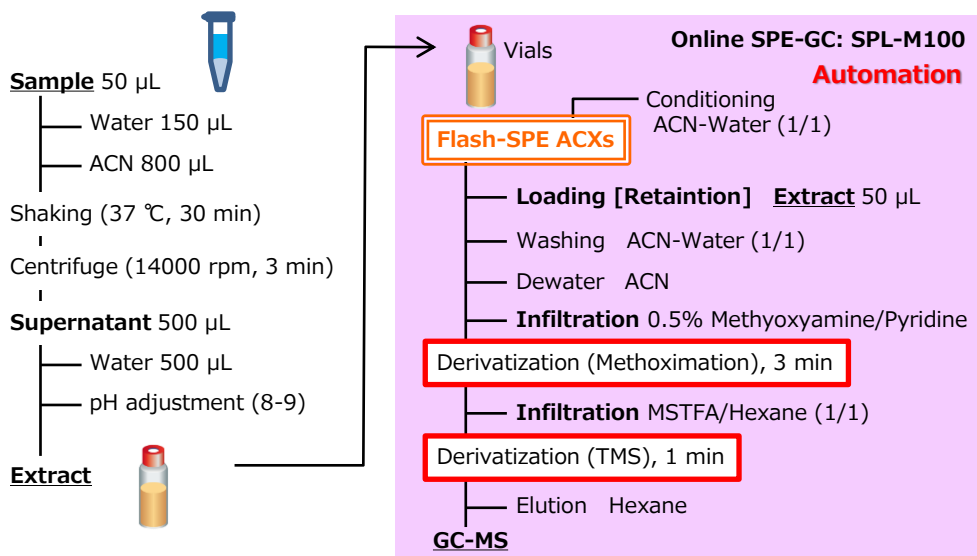
## Introduction

**Solid-phase derivatization (SPD)** is a technique of derivatization without the time-consuming centrifugal concentration and lyophilization, by retaining the target compounds on a solid phase and dewatering it by passing an organic solvent through it then infiltrating the derivatization reagent and performing the reaction on the solid phase. The example of pretreatment method and analytical condition for metabolome analysis of serum are shown below.



Online SPE-GC-MS system

## SPD pretreatment workflow



## Analytical condition

<b>SPE-GC interface</b>	<b>SPL-M100 (AiTI SCIENCE)</b>
SPE cartridge	Flash-SPE
<b>PTV injection port</b>	<b>LVI-S250 (AiTI SCIENCE)</b>
Insert type	Spiral insert
Temp.	220 $^{\circ}$ C(0.5 min)-50 $^{\circ}$ C/min-290 $^{\circ}$ C(23 min)
<b>Gas chromatograph</b>	
Inlet mode	Split 1:50
Flow mode	Constant flow, 1.0 mL/min
Pre-column	0.25 mm i.d. x 0.5 m
Column	Vf-5ms, 0.25 mm i.d. x 30 m, df=0.25 $\mu$ m
Oven Temp.	100 $^{\circ}$ C(2 min)-10 $^{\circ}$ C/min-320 $^{\circ}$ C(2min)
Transfer line Temp.	290 $^{\circ}$ C
<b>Mass spectrometer</b>	
Acquisition mode	Scan ( $m/z$ 70-600)
Data acquisition	3.0-26 min



**SPL-M100**  
for SPE-GC system

## Sample



## Information

**AiTI SCIENCE**

## Product

Online SPE-GC  
**SPL-M100**  
Solid-phase cartridge  
**Flash-SPE**  
GC large volume injection port  
**LVI-S250**



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## Results

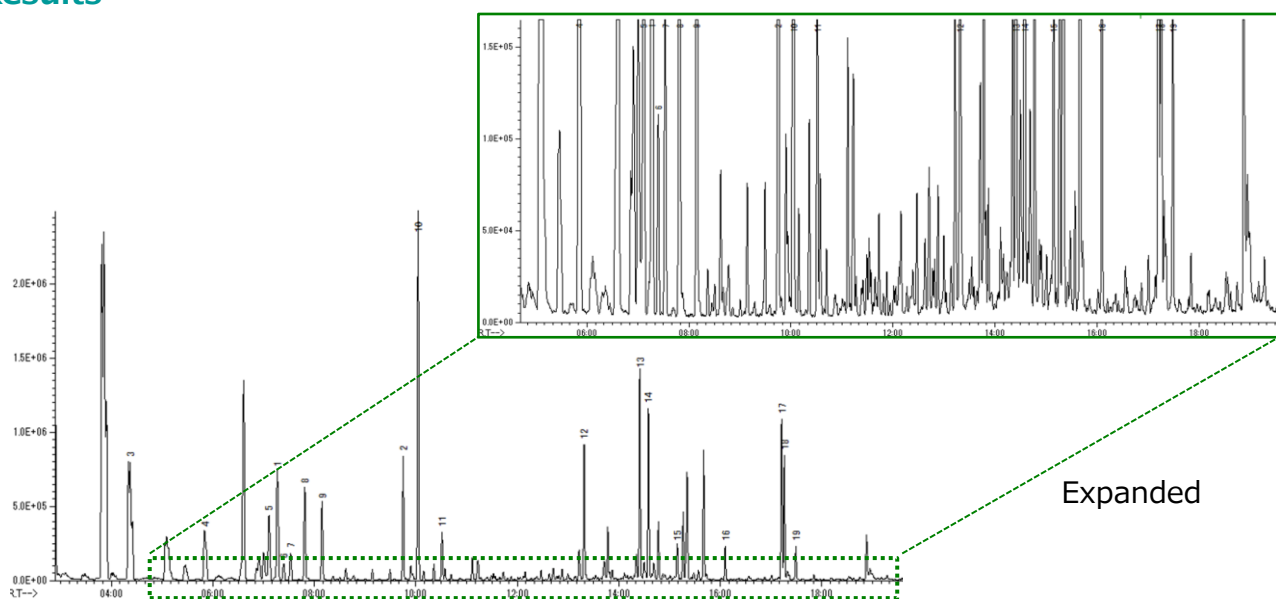


Figure: Total ion current chromatogram

Table: Result of recovery test (n=5)

Sample	No.	Norleucine_2TMS	Adipic acid_2TMS	Sample	No.	Norleucine_2TMS	Adipic acid_2TMS
Standard solution	S1	1,309,000	76,220	Serum	Serum_K1	1,158,000	86,250
IS concentrations:	S2	1,315,000	76,540	Dilution:	Serum_K2	1,220,000	85,480
20 $\mu$ M in vials	S3	1,331,000	75,590	40 times	Serum_K3	1,229,000	84,950
	S4	1,264,000	75,570	Spike period of IS:	Serum_K4	1,204,000	89,980
	S5	1,305,000	73,340	After deprotonization,	Serum_K5	1,186,000	91,570
	Ave.	1,304,800	75,452	20 $\mu$ M in vials	Ave.	1,199,400	87,646
	RSD, %	1.9	1.7		RSD, %	2.4	3.4
				(K/Sx100)	Recovery, %	92	116
				Serum	Serum_A1	1,064,000	66,510
				Dilution:	Serum_A2	1,010,000	66,890
				40 times	Serum_A3	1,023,000	63,600
				Spike period of IS:	Serum_A4	974,600	64,240
				Before extraction	Serum_A5	1,028,000	62,160
				800 $\mu$ M in serum	Ave.	1,019,920	64,680
				(20 $\mu$ M in vials)	RSD, %	3.2	3.1
				(A/Sx100)	Recovery, %	78	86
				(A/Kx100)	Recovery, %	85	74

Table: Result of repeatability test (RSD%, n=5)

No.	Metabolites	1	2	3	4	5	Ave.	RSD, %
1	Alanine_2TMS	334,600	350,200	355,900	354,900	346,900	348,500	2.5
2	Valine_2TMS	243,400	249,100	252,400	250,000	240,600	247,100	2.0
3	Glycine_3TMS	41,200	45,320	44,200	41,390	41,800	42,782	4.4
4	Serine_3TMS	43,120	45,950	45,350	42,740	44,340	44,300	3.1
5	Aspartic acid_3TMS	20,790	20,680	18,860	21,390	25,040	21,352	10.6
6	Threonic acid_4TMS	16,010	17,030	16,220	19,310	20,380	17,790	11.0
7	Glutamic acid_3TMS	56,340	51,980	52,690	51,270	54,720	53,400	3.9
8	L-Glutamine_3TMS	61,450	60,640	62,000	65,270	66,760	63,224	4.2
9	Myristic acid_TMS	25,350	25,520	25,690	25,130	25,660	25,470	0.9
10	L-Lysine_4TMS	19,610	22,540	21,750	21,620	22,150	21,534	5.3
11	Tyrosine_3TMS	73,360	72,320	75,530	71,580	78,300	74,218	3.7
12	Palmitate_TMS	653,500	659,600	653,400	639,400	647,500	650,680	1.2
13	Linoleic acid_TMS	103,700	107,100	107,000	108,100	101,300	105,440	2.7
14	Oleic acid_TMS	109,200	117,900	116,900	114,200	111,500	113,940	3.2
15	Stearic acid_TMS	308,600	310,500	307,700	305,100	312,100	308,800	0.9
16	Arachidonic acid_TMS	18,280	17,290	17,470	17,450	17,750	17,648	2.2