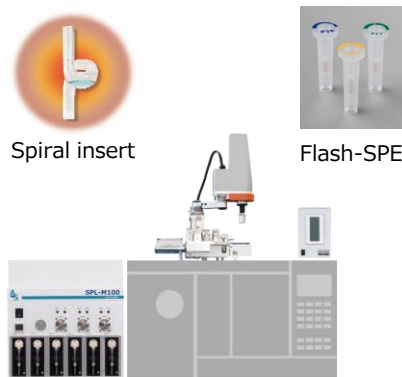


# Metabolome analysis of mouse feces

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 mouse feces are shown below.



Online SPE-GC-MS system



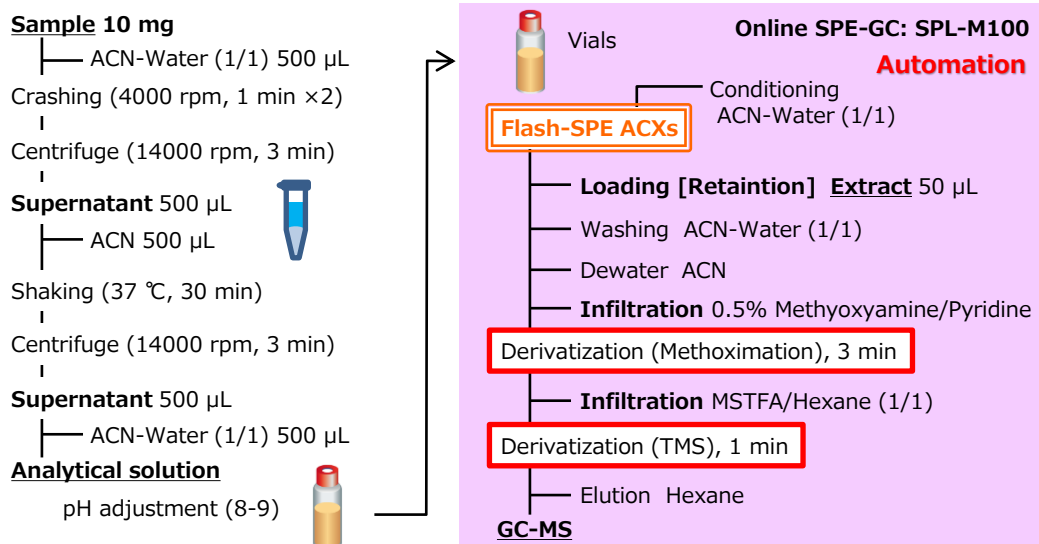
**SPL-M100**  
for SPE-GC system

## Sample



Feces (mouse)

## SPD pretreatment workflow



## Analytical condition

### SPE-GC interface

SPE cartridge

### PTV injection port

Insert type

Temp.

### Gas chromatograph

Inlet mode

Flow mode

Pre-column

Column

Oven Temp.

Transfer line Temp.

### Mass spectrometer

Acquisition mode

Data acquisition

### SPL-M100 (AiSTI SCIENCE)

Flash-SPE

### LVI-S250 (AiSTI SCIENCE)

Spiral insert

220 $^{\circ}$ C(0.5 min)-50 $^{\circ}$ C/min-290 $^{\circ}$ C(23 min)

Split 1:50

Constant flow, 1.0 mL/min

0.25 mm i.d. x 0.5 m

Vf-5ms, 0.25 mm i.d. x 30 m, df=0.25  $\mu$ m

100 $^{\circ}$ C(2 min)-10 $^{\circ}$ C/min-320 $^{\circ}$ C(2min)

290 $^{\circ}$ C

Scan ( $m/z$  70-600)

3.0-26 min

**AiSTI SCIENCE**

## Product

Online SPE-GC  
**SPL-M100**

Solid-phase  
cartridge

**Flash-SPE**

GC large volume  
injection port  
**LVI-S250**



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## Results

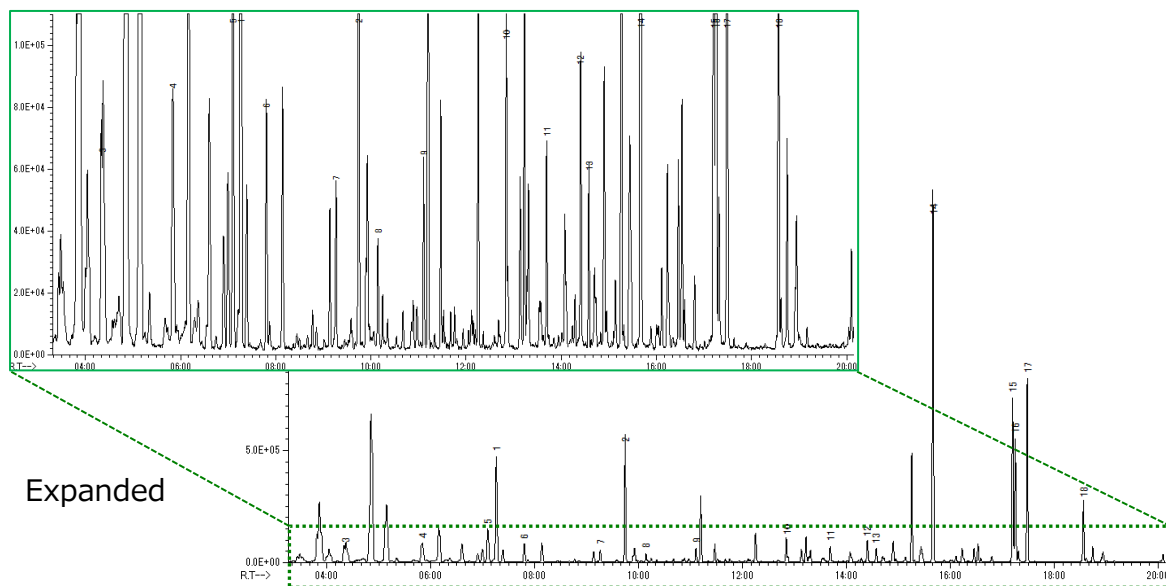


Figure: Total ion current chromatogram

Table 1: Result of recovery test (n=5)

Sample	No.	Norleucine_2TMS	Adipic acid_2TMS
Standard solution	S1	1,431,000	83,510
IS concentrations: 20 $\mu$ M in vials	S2	1,409,000	80,230
	S3	1,371,000	79,830
	S4	1,327,000	74,290
	S5	1,292,000	75,900
	<i>Ave.</i>	<i>1,366,000</i>	<i>78,752</i>
<i>RSD, %</i>	<i>4.2</i>	<i>4.7</i>	

Sample	No.	Norleucine_2TMS	Adipic acid_2TMS
Mouse Feces	Feces_K1	1,556,000	94,920
Dilution: 200 times	Feces_K2	1,597,000	93,000
	Feces_K3	1,607,000	95,600
Spike period of IS:	Feces_K4	1,638,000	96,670
After deprotonization, 20 $\mu$ M in vials	Feces_K5	1,612,000	97,420
	<i>Ave.</i>	<i>1,602,000</i>	<i>95,522</i>
	<i>RSD, %</i>	<i>1.9</i>	<i>1.8</i>
(K/Sx100)	<i>Recovery, %</i>	<i>117</i>	<i>121</i>
Mouse Feces	Feces_A1	1,864,000	95,250
Dilution: 200 times	Feces_A2	1,876,000	106,400
	Feces_A3	1,869,000	103,300
Spike period of IS:	Feces_A4	1,937,000	107,900
Before extraction 2 mM in samples (20 $\mu$ M in vials)	Feces_A5	1,926,000	107,700
	<i>Ave.</i>	<i>1,894,400</i>	<i>104,110</i>
	<i>RSD, %</i>	<i>1.8</i>	<i>5.1</i>
(A/Sx100)	<i>Recovery, %</i>	<i>139</i>	<i>132</i>
(A/Kx100)	<i>Recovery, %</i>	<i>118</i>	<i>109</i>

Table 2 : Result of repeatability test (n=5, RSD%)

No.	Metabolites	1	2	3	4	5	<i>Ave.</i>	<i>RSD, %</i>
1	Alanine_2TMS	3,665,000	3,692,000	3,688,000	3,774,000	3,788,000	<i>3,721,400</i>	<b>1.5</b>
2	Valine_2TMS	1,036,000	1,049,000	1,044,000	1,072,000	1,077,000	<i>1,055,600</i>	<b>1.7</b>
3	Glycine_3TMS	834,500	821,000	828,300	830,500	868,700	<i>836,600</i>	<b>2.2</b>
4	Glyceric acid_3TMS	46,900	55,450	59,530	66,660	67,390	<i>59,186</i>	<b>14.3</b>
5	Uracil_2TMS	170,700	186,000	175,700	181,500	182,500	<i>179,280</i>	<b>3.4</b>
6	Serine_3TMS	379,400	396,500	395,100	406,400	408,100	<i>397,100</i>	<b>2.9</b>
7	Threonine_3TMS	266,400	266,800	273,900	273,700	276,600	<i>271,480</i>	<b>1.7</b>
8	$\gamma$ -Aminobutyric acid_3TI	599,200	598,900	595,000	596,000	615,400	<i>600,900</i>	<b>1.4</b>
9	Phenethylamine_2TMS	424,000	444,700	430,100	430,200	438,600	<i>433,520</i>	<b>1.9</b>
10	Ornithine_4TMS	529,800	538,400	553,400	554,100	580,800	<i>551,300</i>	<b>3.5</b>
11	Lysine_4TMS	336,000	352,900	369,600	371,000	393,200	<i>364,540</i>	<b>5.9</b>
12	Gluconic acid_6TMS	44,270	47,430	47,080	50,950	49,490	<i>47,844</i>	<b>5.3</b>
13	Isofolic acid_2TMS	85,120	93,230	93,490	96,300	96,570	<i>92,942</i>	<b>5.0</b>
14	Stearic acid_TMS	156,300	170,600	168,000	167,200	163,500	<i>165,120</i>	<b>3.4</b>
15	Oleic acid_TMS	160,500	174,900	161,500	168,800	169,000	<i>166,940</i>	<b>3.6</b>
16	Stearic acid_TMS	81,640	96,580	90,460	89,640	89,500	<i>89,564</i>	<b>5.9</b>