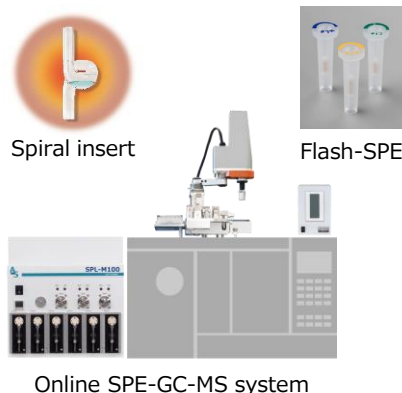


オンライン固相誘導体化-GC-MSシステムによる マウス糞便中メタボローム分析

Introduction

固相誘導体化法（Solid-phase derivatization: SPD）は、分析対象成分を固相に保持し溶媒通液によって脱水処理を行うことで時間のかかる遠心乾固や凍結乾燥をすることなく誘導体化反応を行う手法です。

本アプリケーションではマウス糞便中メタボローム分析における前処理方法および分析条件を紹介します。



SPD pretreatment workflow

Sample 10 mg

ACN-Water (1/1) 500 μ L
Crashing (4000 rpm, 1 min $\times$ 2)

Centrifuge (14000 rpm, 3 min)

Supernatant 500 μ L

ACN 500 μ L

Shaking (37 $^{\circ}$ C, 30 min)

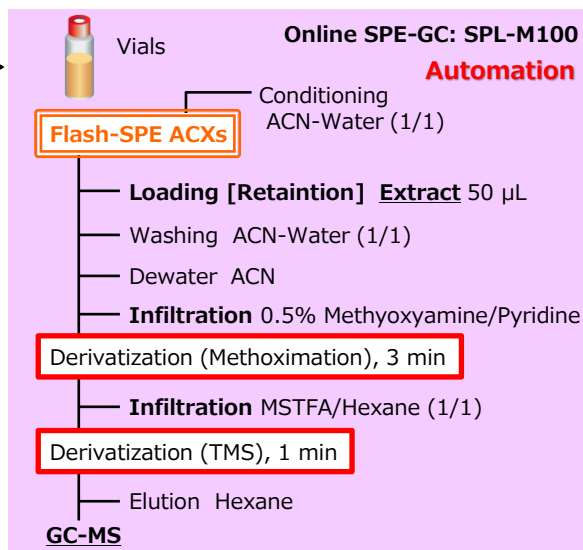
Centrifuge (14000 rpm, 3 min)

Supernatant 500 μ L

ACN-Water (1/1) 500 μ L

Analytical solution

pH adjustment (8-9)



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. $\times$ 0.5 m

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



SPL-M100
for SPE-GC system

Sample



マウス糞便

Information

AiSTI SCIENCE

Product

オンライン SPE-GC
SPL-M100

固相カートリッジ
Flash-SPE

大量注入口装置
LVI-S250



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Results

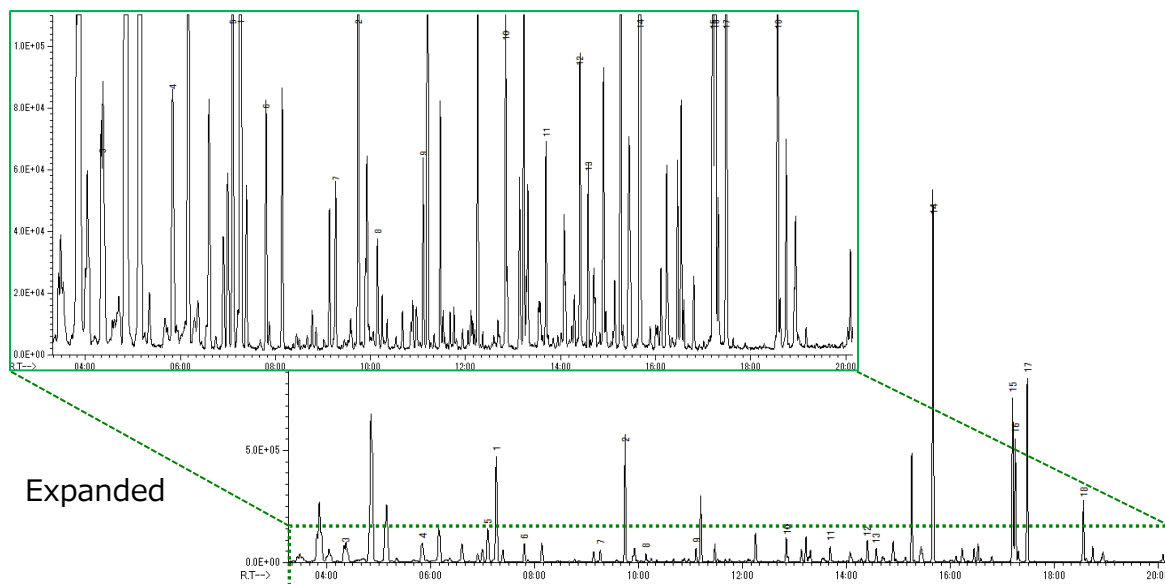


Figure: Total ion current chromatogram

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:	S2	1,409,000	80,230
20 μ M in vials	S3	1,371,000	79,830
	S4	1,327,000	74,290
	S5	1,292,000	75,900
	Ave.	1,366,000	78,752
	RSD, %	4.2	4.7

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

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

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