Development of simultaneous analysis of short-chain fatty acids and organic acids by solid-phase derivatization method (Sample : Blue cheese)

Introduction

In conventional metabolome analysis, short-chain fatty acids are difficult to be pretreated using the same methods as in conventional metabolome analysis because of concerns about vaporization loss in the freeze-drying/centrifugal drying process, and methods such as diethyl ether extraction under hydrochloric acidic conditions are often used. Therefore, in this study, we developed a method for simultaneous analysis of short-chain fatty acids and organic acids by MTBSTFA derivatization reagents using an automated solid-phase derivatization on-line SPE/GC/MS system that does not require a lyophilization step.

Spiral Insert

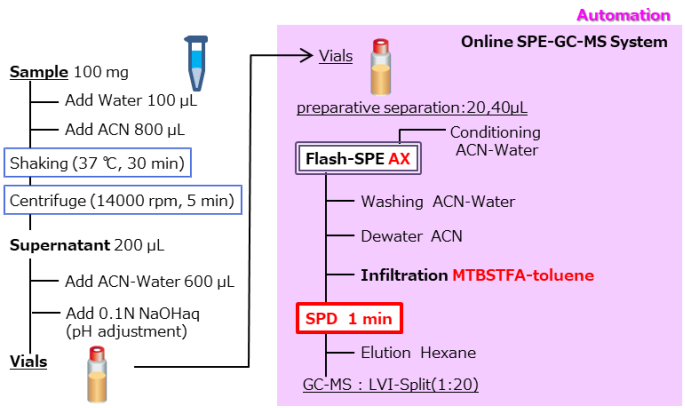
Flash-SPE

SPE-GC-MS system for metabolome analysis (Image)

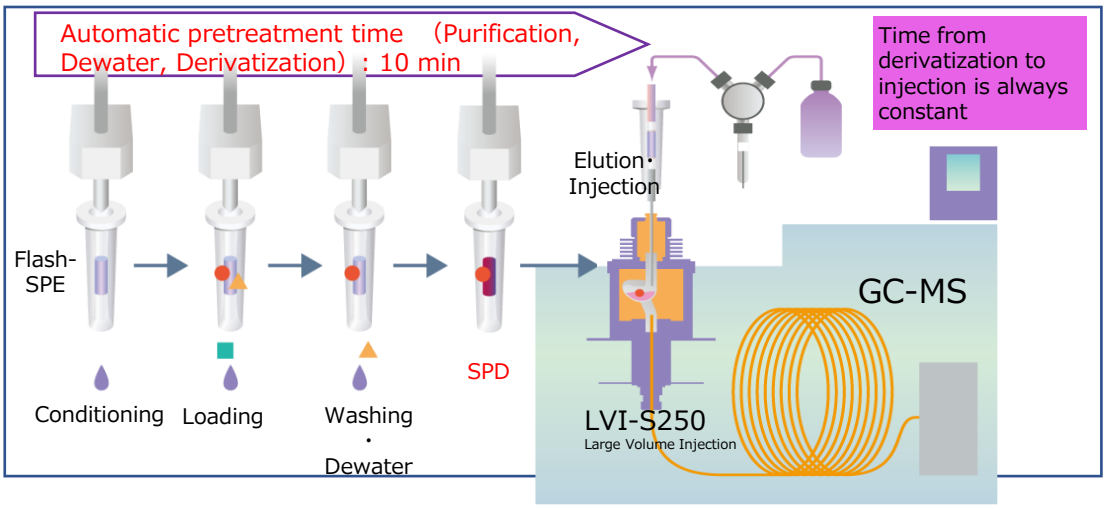
Analytical condition

SPE-GC Interface	**SGI-M100; AiSTI Science**
SPE Cartridge	Flash-SPE AX
PTV Injector	**LVI-S250(AiSTI Science)**
Insert Type	Spiral Insert
Injector Temp.	150℃(0.5min)-25℃/min-290℃
GC	**Agilent 7890B**
Inlet Mode	Splite
Splite Flow	20 mL/min
Flow Mode	Constant Flow, 1.1ml/min
Column	Vf-5ms, 0.25mm i.d. X 30m, df;0.25μm
Oven Temp.	60℃(3min)-10℃/min-100℃-20℃/min-310℃
Trans. Line Temp.	290℃
MS	**Agilent 5977B**
MS Method	SCAN, m/z;70-470

Example of solid-phase derivatization pretreatment flow



Solid phase extraction to GC-MS injection process (fully automated process)



SPL-M100
for SPE-GC system

Sample

Blue cheese

Information

AiSTI SCIENCE

Product

SPE-GC system
SGI-M100

Solid phase cartridge
Flash-SPE

GC Lage Volume Injection
LVI-S250

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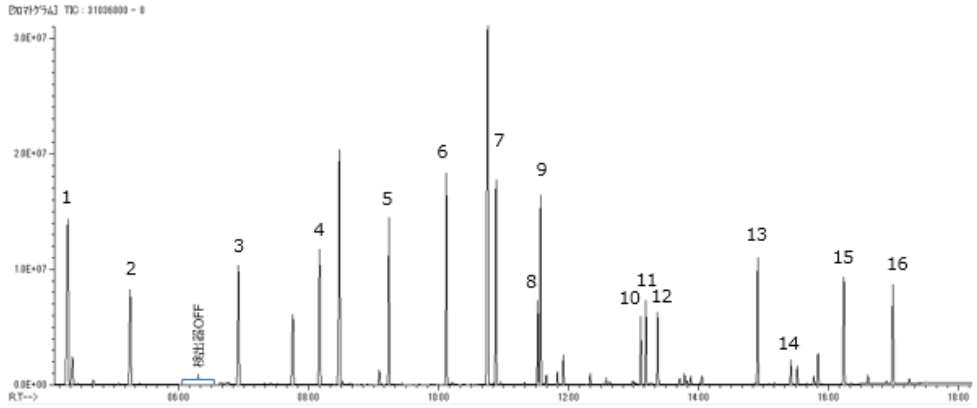
HP : www.aisti.co.jp

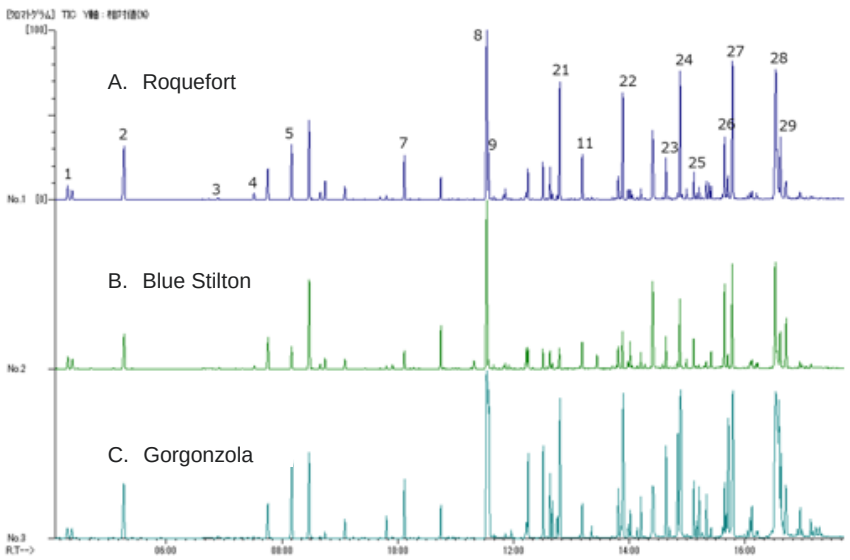
SCAN total ion chromatograms and reproducibility of standard solutions by this method



1. Methanoic acid-tBDMS
2. Ethanoic acid-tBDMS
3. Propanoic acid-tBDMS
4. Butanoic acid-tBDMS
5. Pentanoic acid-tBDMS
6. Hexanoic acid-tBDMS
7. Heptanoic acid-tBDMS
8. Lactic acid-2tBDMS
9. Octanoic acid-tBDMS
10. Maleic acid-2tBDMS
11. Succinic acid-2tBDMS
12. Fumaric acid-2tBDMS
13. Malic acid-3tBDMS
14. a-Ketoglutaric acid-3tBDMS
15. Tartaric acid-4tBDMS
16. Citric acid-4tBDMS

No	Compound names	1	2	3	4	5	6	7	8	9	10	*Ave.*	RSD, %
1	Methanoic acid-tBDMS	30,227,430	30,889,896	30,429,839	30,948,441	31,244,577	31,557,736	32,493,298	32,124,585	32,670,291	31,126,554	*31,371,265*	**2.7**
2	Ethanoic acid-tBDMS	12,340,883	12,538,808	12,137,282	12,298,944	12,381,311	12,578,273	13,094,081	12,862,755	12,800,785	12,135,503	*12,516,863*	**2.6**
3	Propanoic acid-tBDMS	12,823,811	12,909,258	12,779,143	12,845,278	13,087,033	13,015,973	13,636,656	13,327,889	13,578,399	12,345,834	*13,034,927*	**3.0**
4	Butanoic acid-tBDMS	11,475,299	11,581,978	11,808,966	11,688,344	11,946,609	12,009,882	12,394,007	11,964,797	12,362,085	11,127,740	*11,835,971*	**3.3**
5	Pentanoic acid-tBDMS	11,588,243	11,720,223	12,243,830	11,920,575	12,135,019	12,354,232	12,670,558	12,084,388	12,720,821	11,576,139	*12,101,403*	**3.4**
6	Hexanoic acid-tBDMS	11,580,646	11,842,742	12,353,900	11,988,066	12,169,039	12,387,848	12,572,832	12,065,398	12,647,753	11,874,440	*12,148,266*	**2.8**
7	Heptanoic acid-tBDMS	11,606,883	11,805,299	12,464,553	12,009,352	12,146,574	12,496,200	12,573,604	11,959,907	12,691,060	12,106,993	*12,186,043*	**2.9**
8	Lactic acid-2tBDMS	1,077,185	1,230,088	1,131,334	1,076,805	1,162,595	1,154,103	1,154,143	1,145,913	1,187,630	1,112,992	*1,143,279*	**4.1**
9	Octanoic acid-tBDMS	10,608,992	10,881,088	11,527,448	11,111,574	11,200,905	11,651,375	11,407,126	10,950,090	11,613,534	11,248,975	*11,220,111*	**3.0**
10	Maleic acid-2tBDMS	1,867,779	1,969,324	2,040,317	1,900,232	2,065,959	2,002,221	1,949,168	1,898,625	2,022,759	2,113,877	*1,983,026*	**4.0**
11	Succinic acid-2tBDMS	2,406,066	2,438,793	2,495,130	2,401,336	2,499,333	2,510,922	2,491,212	2,377,200	2,506,791	2,573,343	*2,470,013*	**2.5**
12	Fumaric acid-2tBDMS	4,193,048	4,331,927	4,480,305	4,218,660	4,487,927	4,390,857	4,285,980	4,205,144	4,422,052	4,579,983	*4,359,588*	**3.1**
13	Malic acid-3tBDMS	1,570,355	1,544,780	1,523,746	1,576,209	1,573,185	1,590,439	1,612,939	1,578,652	1,571,389	1,623,141	*1,576,484*	**1.8**
14	a-Ketoglutaric acid-3tBDMS	553,488	530,143	534,318	509,953	582,938	521,569	534,178	552,656	537,514	609,245	*546,600*	**5.4**
15	Tartaric acid-4tBDMS	563,021	521,446	497,159	546,241	530,917	526,143	540,008	583,506	526,641	580,916	*541,600*	**5.1**
16	Citric acid-4tBDMS	924,916	849,805	796,506	911,965	892,992	862,443	834,170	973,466	842,453	1,025,026	*891,374*	**7.8**

SCAN total ion chromatogram comparison of three blue cheeses



Sample : Blue cheese 3

C B A

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9 Octanoic acid-tBDMS
11 Succinic acid-2tBDMS
21 Decanoic acid-tBDMS
22 Dodecanic acid-tBDMS
24 Tetradecanoic acid-tBDMS
25 Aspartic acid-3tBDMS
26 Glutamic acid-3tBDMS
27 Hexadecanoic acid-tBDMS
28 cis-9-Octadecenoic acid
29 Stearic acid-tBDMS

【Summary】

Good chromatograms were obtained for both short-chain fatty acids and organic acids, including formic and acetic acids. The derivatization reaction time was 2 minutes, and the automated pretreatment took about 10 minutes. Solid-phase derivatization with MBSTFA enabled automated and rapid analysis without a lyophilization step. This method was found to be effective for the simultaneous analysis of short-chain fatty acids and organic acids.