Development of in-SPE derivatization method for GC-MS metabolome analysis

Ryoichi SASANO[1], Yoko KOJIMA[1], Shusuke OSAKI[2], Yoshie TANAKA[2], Akihiro MATSUMOTO[2], Toshiyuki YAMASHITA[3], Takeshi BAMBA[3], Eiichiro FUKUSAKI[3]

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

【Introduction】

Due to complex and long-time sample preparations, including solvent extraction, centrifugal concentration, freeze-drying and derivatization, technical skills have been required in a conventional metabolome analysis for GC-MS. It causes discrepancies in the acquired data from multiple experiments, especially between in analysis batches. Furthermore, it causes a critical problem when the statistical analysis is done because a lot of samples should be handled in the metabolome analysis. The purpose of this study was to develop a rapid and robust method of sample preparation for metabolomics using solid phase extraction (SPE).

【Methods】

Extracted samples with acetonitrile were loaded on an ion-exchange cartridge SPE. Ion-exchange SPEs were used depending on what kind of chemical functional group the compounds have. The target compounds were retained in the SPE, then the cartridge was washed by acetonitrile for dehydration. Derivatization was done by methoxyamine/pridine and MSTFA which were directly added sequentially on the SPE. After derivatization, the object substances were eluted by acetone/n-hexane. The 10μL of derivatized compounds were injected in the GC-MS with a Large Volume Injection system equipped with a spiral shaped liner.

【Preliminary Results】

A cation-exchange column, CXi, was used for amino acids which have cationic amino group, and an anion-exchange column, AXi, was applied for the compounds which have carbonyl and hydroxyl group, such as organic acids and sugars. Washing with acetonitrile after loading the target compounds enabled not only to eliminate other interferences but also to dehydrate at the same time. It took several hours to dry the samples in the conventional method, while dehydration was done in a few minutes using SPE. In this method, target metabolites which were retained in the SPEs by ion-exchange interaction were methoxyaminated by methoxyamine hydrochlolide in pyridine followed by trimethlysilylation effectively. Derivatized metabolites were changed into less-polar compounds and easily eluted using organic solvent, acetone/n-hexane. The total preparation time from sample loading on the column to derivatization was within 10 minutes, and good reproducibility was obtained.

The conventional derivatization method

The derivatization processing 8～19 Hour

Extract fractionation： 400μL

Vacuum concentration（ **1～2 Hour**）

Freeze-drying（**5～16Hour**）

Added 2%Methoxyamin/pyridine 100uL

Incubation（30℃, **90min**） : Derivatization

Added MSTFA 50uL

Incubation（37℃, **30min**） : Derivatization

Centrifugal separation（16000 rpm, 4℃, 3min）

Vial

GC/MS : Injection 1μL（Split 25:1）

CX-SPE derivatization method for Amino acid

The derivatization processing 6 min

Extract fractionation： 50μL

Hybrid-SPE **CXi3**-2mg ⊖

Wash with ACN 100μL（**Dehydration**）

Added MSTFA 20μL in SPE

Derivatization in SPE

Elute Hexane 100μL

Added Hexane 400μL

Vial

GC/MS : Injection 10μL (LVI)

AX-SPE derivatization method for Organic acid

The derivatization processing 6 min

Extract fractionation： 50μL

Hybrid-SPE **AXi3**-2mg ⊕

Wash with ACN 100μL（**Dehydration**）

Added MSTFA 20μL in SPE

Derivatization in SPE

Elute Hexane 100μL

Added Hexane 400μL

Vial

GC/MS : Injection 10μL (LVI)

AX-SPE derivatization method for saccharides

The derivatization processing 10 min

Extract fractionation： 20μL

Added ACN 100μL

Hybrid-SPE **AXi3**-2mg ⊕

Wash with ACN 100μL（**Dehydration**）

Added 20%Methoxyamin/pyridine 5uL in SPE

Derivatization in SPE 3 min

Added MSTFA 25μL in SPE

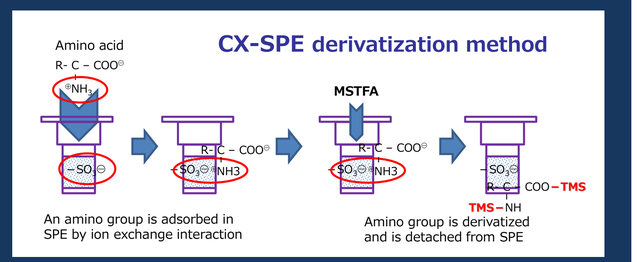
Derivatization in SPE

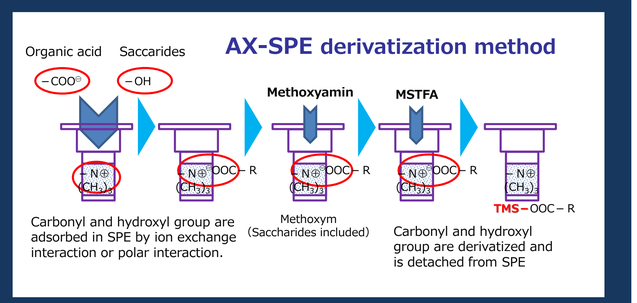
Elute Hexane 100μL

Added Hexane 400μL

Vial

GC/MS : Injection 10μL (LVI)





CX-SPE derivatization method for Amino acid

Examination of the optimum when a sample is loaded

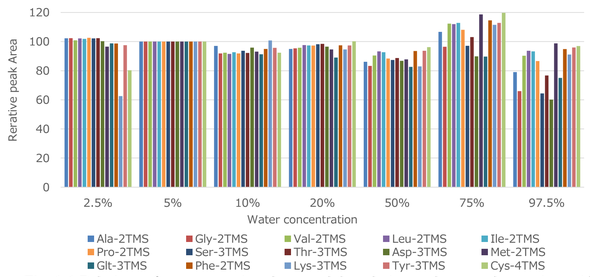


Fig. 1-1 Relations of water conc. in solvent and the relative peak area, when amino acids in solvent are loaded through the CX-SPE and adsorbed by the SPE.

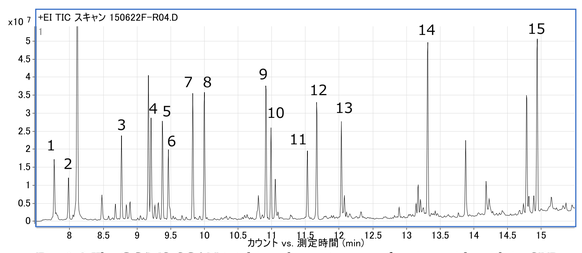


Fig. 1-2 The GC/MS-SCAN total ion chromatogram of amino acids with in-SPE derivatization method

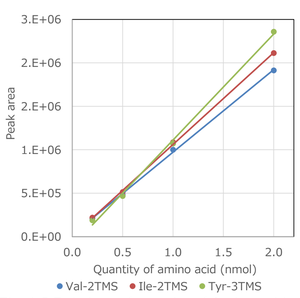


Fig. 1-3 Relations of quantity of Amino acids and the relative peak area with in-SPE derivatization method.

Table 1 : A coefficient of correlation and RSD

No.	Amino acid	R2	RSD n=6, %
1	Ala-2TMS	0.9996	7.4
2	Gly-2TMS	0.9958	13.1
3	Val-2TMS	0.9993	7.5
4	Leu-2TMS	0.9992	6.7
5	Ile-2TMS	0.9995	6.2
6	Pro-2TMS	0.9982	12.5
7	Ser-3TMS	0.9988	5.5
8	Thr-3TMS	0.9990	4.7
9	Asp-3TMS	0.9989	4.9
10	Met-2TMS	0.9991	7.5
11	Glt-3TMS	0.9911	8.8
12	Phe-2TMS	0.9996	5.6
13	Lys-3TMS	0.9927	32.2
14	Tyr-3TMS	0.9977	4.8
15	Cys-4TMS	0.9950	29.2

AX-SPE derivatization method for Organic acid

Examination of the optimum when a sample is loaded

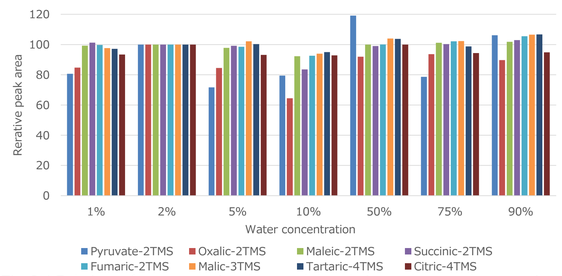


Fig. 2-1 Relations of water conc. in solvent and the relative peak area, when organic acids in solvent are loaded through the AX-SPE and adsorbed by the SPE.

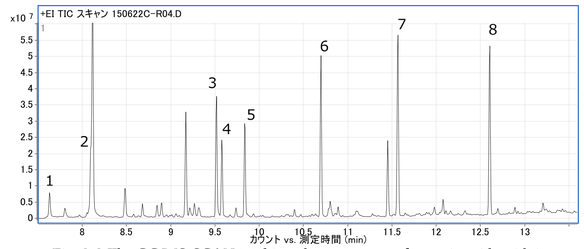


Fig. 2-2 The GC/MS-SCAN total ion chromatogram of organic acids with in-SPE derivatization method

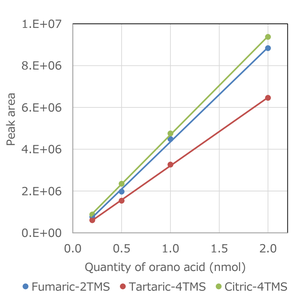


Fig. 2-3 Relations of quantity of Organic acids and the relative peak area with in-SPE derivatization method.

Table 2: A coefficient of correlation and RSD

No.	Organic acid	R2	RSD n=6, %
1	Pyruvate-2TMS	0.9998	15.1
2	Oxalic-2TMS	0.9946	6.2
3	Maleic-2TMS	0.9991	1.8
4	Succinic-2TMS	0.9588	22.0
5	Fumaric-2TMS	0.9991	2.2
6	Malic-3TMS	0.9997	2.1
7	Tartaric-4TMS	0.9998	1.9
8	Citric-4TMS	0.9998	2.5

RSD;Reproducibility parameters for the peak area with in-SPE derivatization method

AX-SPE derivatization method for saccharides

Examination of the optimum when a sample is loaded

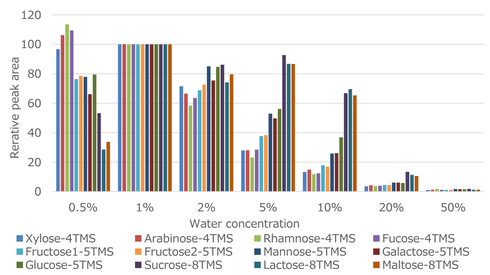


Fig. 3-1 Relations of water conc. in solvent and the relative peak area, when saccharides in solvent are loaded through the AX-SPE and are adsorbed by the SPE.

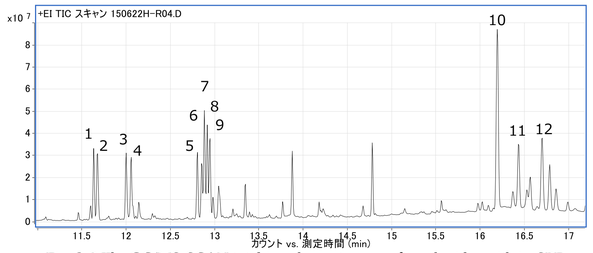


Fig. 3-2 The GC/MS-SCAN total ion chromatogram of saccharides with in-SPE derivatization method

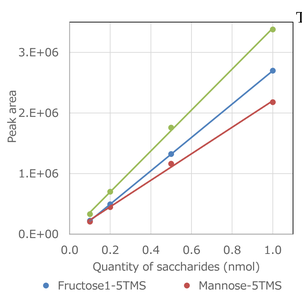


Fig. 3-3 Relations of quantity of Saccharides and the relative peak area with in-SPE derivatization method.

Table 3: A coefficient of correlation and RSD

No.	Saccharides	R2	RSD n=6, %
1	Xylose-4TMS	0.9998	5.4
2	Arabinose-4TMS	0.9996	7.5
3	Rhamnose-4TMS	0.9974	10.8
4	Fucose-4TMS	0.9988	9.8
5	Fructose1-5TMS	1.0000	3.3
6	Fructose2-5TMS	0.9998	3.2
7	Mannose-5TMS	0.9982	2.5
8	Galactose-5TMS	0.9995	1.8
9	Glucose-5TMS	0.9778	2.3
10	Sucrose-8TMS	0.9993	2.9
11	Lactose-8TMS	0.9997	7.2
12	Maltose-8TMS	0.9999	6.5

RSD;Reproducibility parameters for the peak area with in-SPE derivatization method

Examination of the optimum for methoxym derivatization

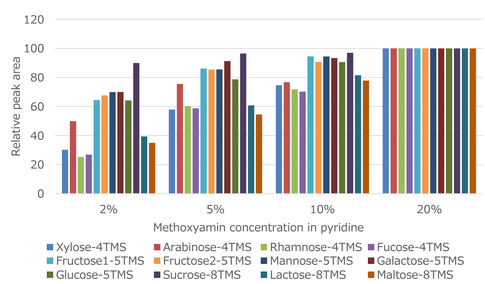


Fig. 3-4 Relations of Methoxyamin conc. in pyridine and the relative peak area.

Table 4: GC/MS condition

PTV Injector	**LVI-S200 (AiSTI Science) : Spiral Insert**
Injectoin Temp.	**70℃(0.3min)-120℃/min-240℃(0.5min)-50℃/min-280℃(35min)**
Solvent Purge Time	**0.25min**
Injection Volume	**10, 25μL**
GC	**Agilent 7890N**
Pre-column	**Deactivated silica capillary tube 0.25mm×0.3m**
Column	**BPX5, 0.25mm i.d.× 30m, df0.25μm (SGE)**
Column Oven Temp.	**60℃(4min)-20℃/min-310℃(4min)**
Splitpurge Flow	**150mL/min(0.25min)-0mL/mion(3.75min)-50mL/min(6min)-20mL/min**
Splitless Time	**4min**
MS	**Agilent 5975C**
Detector Temp.	**280℃**
MS Method	**SCAN : 50-450**

【Conclusion】

By means of development of in-SPE derivarization method, we can achieve rapid and robust method of sample preparation for GC/MS metabolome analysis.

By using this solid phase derivatization ,

1. With holding targeted compounds into solid phase and washing with ACN, we could achieve dehydration process become quite easy and quickly.
2. As Methoxyamine and MSTFA directly added into the strong ionic exchange solid phase which hold the targeted compounds, the derivatization reaction was proceeding promptly on the solid phase.
3. The operating time of dehydration and derivatization was greatly reduced from 19 hours (conventional method) to 10 minutes .

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