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Examination of extraction method for metabolites appropriate to solid-phase derivatization by online SPE-GC system

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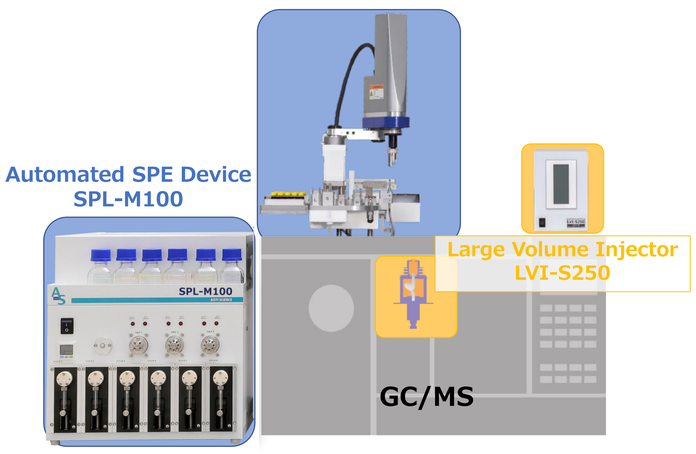
【Introduction】

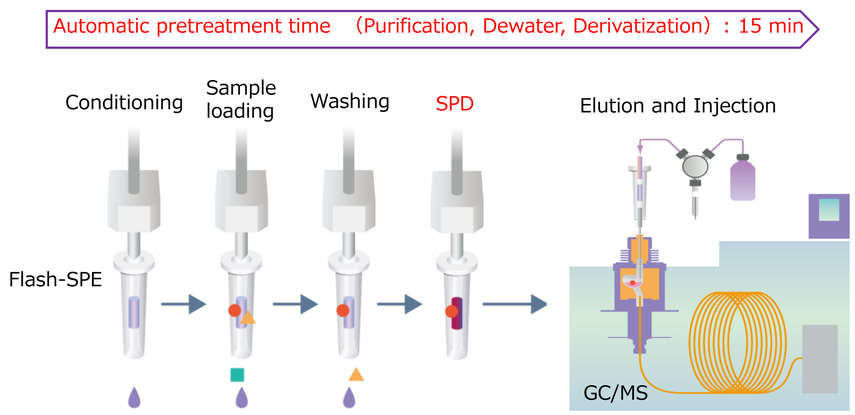
Overview of Solid Phase Derivatization (SPD) method by Online SPE-GC system

Online SPE-GC system



- Online SPE-GC equipped with automatic solid phase extraction device and large volume injection port device
- The process from solid phase extraction to GC/MS measurement can be fully automated.

SPD pretreatment process

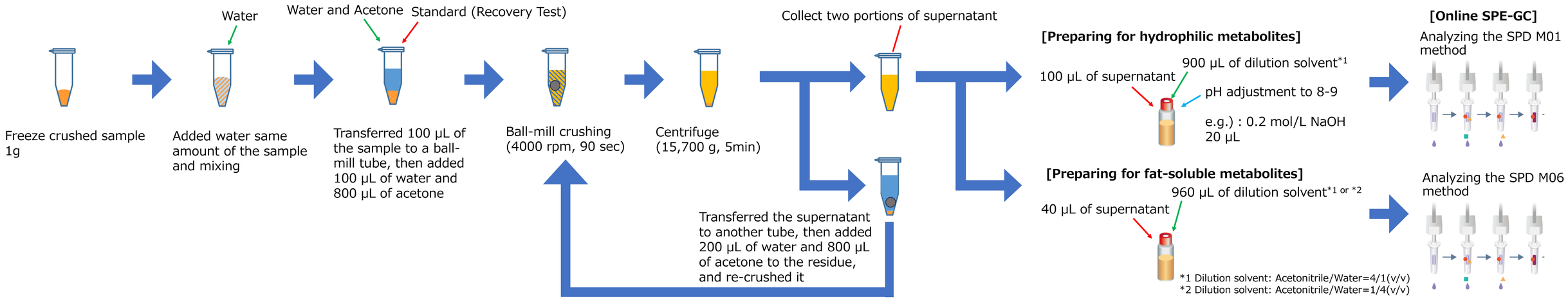


- Dehydration is achieved by passing acetonitrile or nitrogen gas through the solid phase while the metabolite is retained on the solid phase, and derivatization can then be quickly performed by reacting with a reagent on the solid phase after dehydration.
- After reacting, the target compounds retained on the solid phase with a derivatization reagent, they can be eluted with a solvent and directly injected into a GC.

Purpose of this study
Examination of extraction methods suitable for hydrophilic metabolites (such as amino acids) and fat-soluble metabolites (such as steroids) for analysis using the SPD method.

【Material and Method】

Sample: Chicken Liver



SPD method M01 (for hydrophilic metabolites)

Flash-SPE ACXs
- Conditioning
  - ・Acetonitrile/Water=1/1 240 µL
- Loading [Retained], **Extract 50µL**
  - Washing, Acetonitrile/Water=1/1 240µL
  - Dewater, Acetonitrile 400µL
- Drying, N_2 purge 15 sec
  - Infiltration, 0.5% Methoxyamine-Pyridine 4 µL
- SPD reaction Methoximation, 3 min
- Drying, N_2 purge 15 sec
  - Infiltration, MSTFA/Hexane=1/1 7 µL
- SPD reaction Trimethylsilylation , 60 sec
  - Elution, Hexane 25 µL
- GC/MS

GC/MS method M01

Column:	DB-5MS [30 m x 0.25 mm (0.25 µm)]
Oven Temp.:	100°C(2 min)–10°C/min–320°C(2 min)
Inje. Mode:	Split (1:50)
Inje. Temp.:	220°C(0.5 min)–70°C/min–290°C(26min)
Flow rate:	1 mL/min (He)
MS:	Scan (*m/z* 70–470)

SPD method M06 (for fat-soluble metabolites)

Flash-SPE C18
- Conditioning
  - ・Hexane 100 µL
  - ・Acetone 230 µL
  - ・Water 220 µL of Water
- Loading [Retained], **Extract 100µL**
  - Washing, Water 50µL
- Drying, N_2 purge 2 min
  - Infiltration, MSTFA/Hexane=1/1 8 µL
- SPD reaction Trimethylsilylation, 1min sec
  - Elution, Hexane 35 µL
- GC/MS

GC/MS method M06

Column:	DB-5MS [30 m x 0.25 mm (0.25 µm)]
Oven Temp.:	170°C(4 min)–10°C/min–320°C(6 min)
Inje. Mode:	Solvent vent (150 mL/min(0.3 min)–splitless–50mL/min(4 min)
Inje. Temp.:	80°C(0.4 min)–120°C/min–300°C(20min)
Flow rate:	1 mL/min (He)
MS:	Scan (*m/z* 70–700)

Table 1 Recovery results of representative compounds

M01 SPD method

Compounds	Category	$LogP_{ow}$	Recovery%
Norleucine_2TMS	Amino acid	-1.53	124
Adipic acid_2TMS	Organig acid	0.08	118

M06 SPD method

Compounds	Category	$LogP_{ow}$	Dilution solvent	Recovery%
Daidzein_2TMS	Flavonoid	2.63	*2	105
Estradiol_2TMS	Steroid	4.01	*2	120
Chenodeoxycholic acid_3TMS	Bile acid	4.15	*2	94
Stigmasterol_1TMS	Steroid	6.95	*1	99
Phylloquinone	Vitamin (fat-soluble)	8.48	*1	85

The recovery rates of representative compounds in the extraction procedure using chicken liver samples are indicated. The hydrophilic components were measured using the M01 SPD method, while the fat-soluble components were measured using the M06 SPD method. Good recovery results were obtained for compounds with a wide range of polarities.

Table 2 Reproducibility results of compounds detected in chicken liver

M01 SPD method

No.	Compounds	RSD% (n=5)	No.	Compounds	RSD% (n=5)
1	Alanin_2TMS	6.9	22	Aspartic acid_3TMS	6.9
2	3-Hydroxybutyric acid_2TMS	8.1	23	Pyroglutamic acid_2TMS	12.1
3	2-Aminobutyric acid_2TMS	6.1	24	Threonic acid_4TMS	11.3
4	beta-Alanine_2TMS	11.2	25	Glutamic acid_3TMS	12.5
5	Valine_2TMS	6.7	26	Phenylalanine_2TMS	5.0
6	Ethanolamine_3TMS	5.3	27	Tartaric acid_4TMS	8.4
7	Phosphoric acid_3TMS	6.5	28	Myristic acid(C14)_1TMS	3.1
8	Leucine_2TMS	6.6	29	Hydroxyphenyllactic acid_3TMS	5.9
9	Isoleucine_2TMS	7.2	30	Tyrosine_3TMS	10.7
10	Maleic acid_2TMS	6.9	31	Glucuronic acid_5TMS_1	9.4
11	Proline_2TMS	7.6	32	Galacturonic acid_1MO_5TMS	13.2
12	Glycine_3TMS	8.0	33	Gluconic acid_6TMS	11.0
13	Succinic acid_2TMS	12.7	34	Palmitic acid(C16)_1TMS	2.4
14	Glyceric acid_3TMS	10.8	35	Heptadecanoic acid(C17)_1TMS	4.6
15	Uracil_2TMS	4.5	36	gamma-Linolenic acid(C18:3, n-6)_1TMS	6.4
16	Fumaric acid_2TMS	5.7	37	Linoleic acid(C18:2, n-6)_1TMS	4.9
17	Serine_3TMS	7.4	38	Oleic acid(C18:1, n-9)_1TMS	1.5
18	Pipecolic acid_2TMS	4.4	39	Stearic acid(C18)_1TMS	4.5
19	Threonine_3TMS	7.3	40	Arachidonic acid(C20:4, n-6)_1TMS	6.2
20	beta-Alanine_3TMS	8.3	41	Eicosapentaenoic acid(C20:5, n-3)_1TMS	6.5
21	Malic acid_3TMS	6.2	42	Arachidic acid(C20)_1TMS	8.7

M06 SPD method

No.	Compounds	Dilution solvent	RSD% (n=5)
1	alpha-Tocopherol_1TMS	*1	12.0
2	Cholesterol_1TMS	*1	9.7
3	Campesterol_1TMS	*1	3.6
4	beta-Sitosterol_1TMS	*1	12.5

The reproducibility of extracting and measuring compounds detected in chicken liver was indicated. The M01 method provided stable and reproducible results for 42 compounds, including amino acids, organic acids, and fatty acids. The M06 method provided good reproducibility for four compounds, including steroids and fat-soluble vitamins.

【Results】

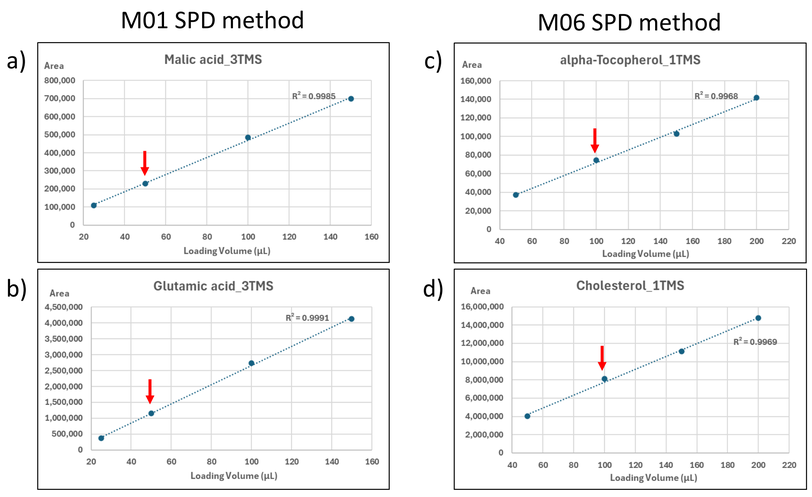


Fig. 1 Correlation between sample loading volume and peak area

Figure 1 shows the correlation between sample load and peak area value for representative compounds. a) and b) show data measured by the M01 SPD method, while c) and d) show data measured by the M06 SPD method. Each SPD method also demonstrated a good correlation between the loading amount and area value for compounds with varying abundances. Subsequent tests were performed at loading amounts indicated by red arrows that did not exceed the retention capacity of the solid phase.

【Discussion】

Under the extraction conditions examined this study, it was possible to detect compounds with a wide range of polarities, but the number of fat-soluble compounds detected was limited. This is because the amount of cholesterol present in chicken liver is high, making it difficult to detect compounds with low concentrations. Therefore, it was considered necessary to examine conditions that would allow for a good balance between measuring compounds with high and low amounts.

【Conclusion】

The extraction conditions examined in this study were adaptable to the SPD method, enabling high-throughput analysis from extraction to GC/MS measurement.

【Acknowledgment】

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