Expansion of Analyte Metabolites Using Online SPE-GC/MS System with Solid-phase Derivatization

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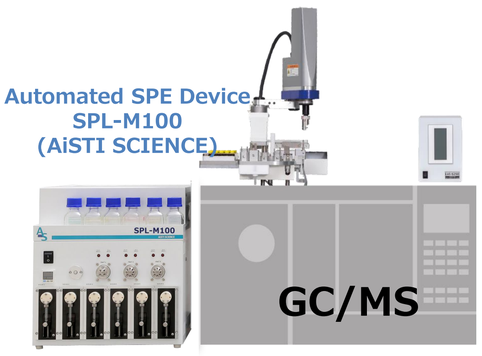
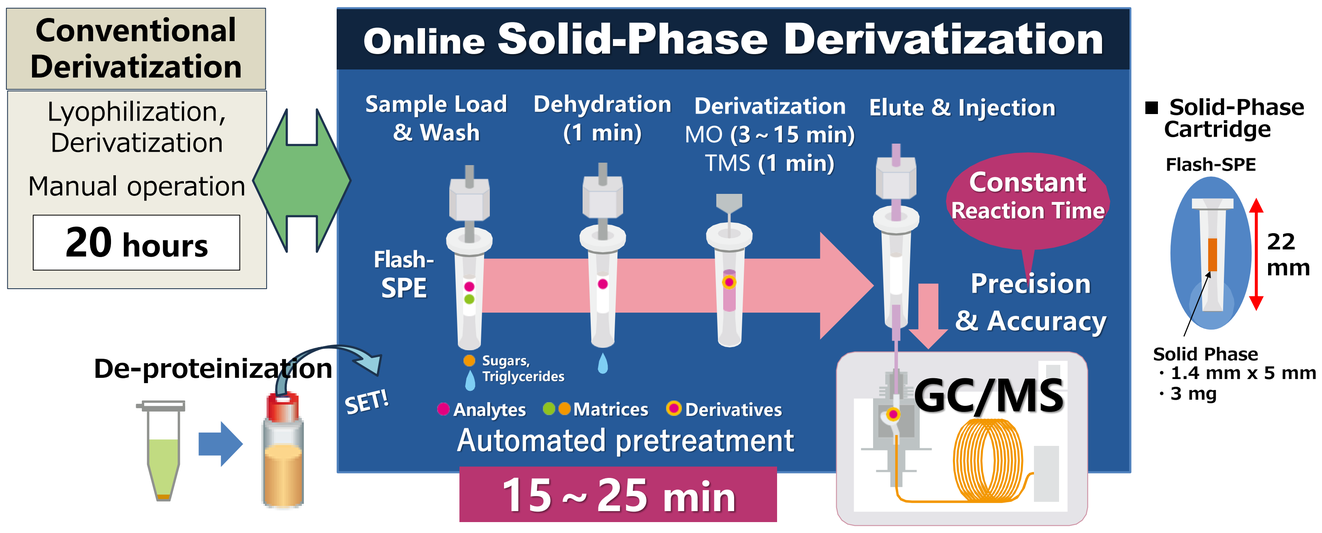
Abstract

We have developed an online SPE-GC/MS system with solid-phase derivatization in which a solid-phase retaining metabolite is dehydrated with acetonitrile, impregnated the solid-phase with a derivatization reagent (**MSTFA** or **MTBSTFA**) to derivatize the metabolites, and injected directly into GC while eluting the derivatized metabolites (TMS or tBDMS). The system has made it possible to shorten (15-25 minutes) and automate pre-processing, which used to be a lengthy (20 hours) and manual process. In addition, the time between derivatization and injection is constant, enabling stable analysis even in multiple samples. This study aimed to expand the number of target metabolites and optimize the solid-phase derivatization method by their types using the online SPE-GC/MS.

Amino acids, amines, polyamines, nucleobases, nucleosides, catecholamines, polyvalent carboxylic acids, oxocarboxylic acids, hydroxycarboxylic acids, aromatic fatty acids, short-chain fatty acids, long-chain fatty acids, polyunsaturated fatty acids, steroids, bile acids, isoflavonoids, sugars, sugar acids, sugar phosphates, etc., were selected based on commonly reported metabolites and metabolic circuits. They were then measured with this system. The solid phases were **ion-exchange resins for ionic compounds with carboxyl and amino groups**, **HILIC for non-ionic hydrophilic compounds**, and **ODS for non-ionic hydrophobic compounds**. As a result, derivatization of 250 metabolites was confirmed. Those metabolites were grouped by type, good reproducibility was obtained by optimizing each process such as solid phase type, amount of derivatization reagent added, elution volume, and elution flow rate. Volatile components such as short-chain fatty acids were likely to be lost by freeze-drying in conventional methods, however, in this method, they were held in the solid phase and dehydrated so that they could be analyzed without loss. In addition, for metabolites such as sterols and isoflavonoids, high-sensitivity analysis was possible using the large-volume injection method.

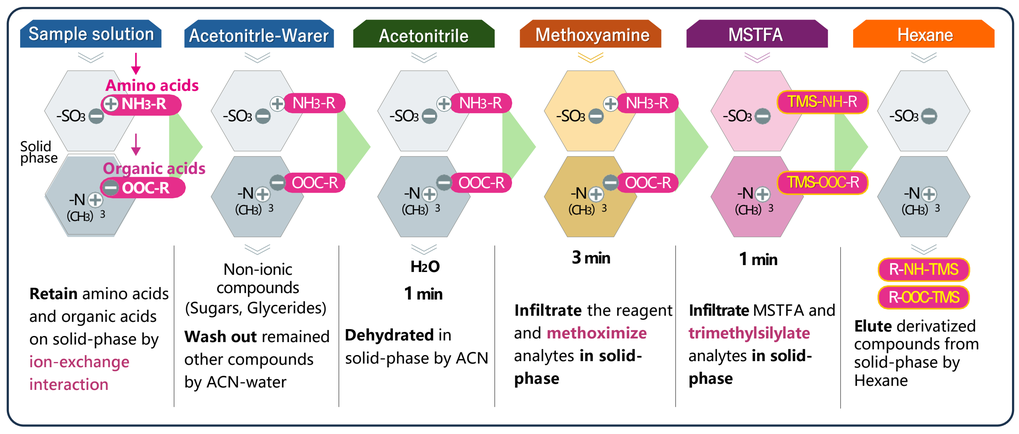


Online SPE-GC/MS system

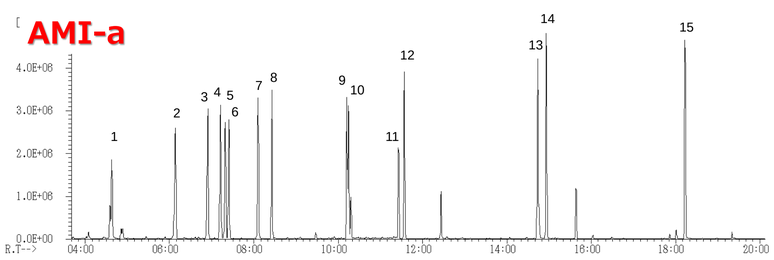


■ Solid-Phase Cartridge

■ Solid-Phase Derivatization

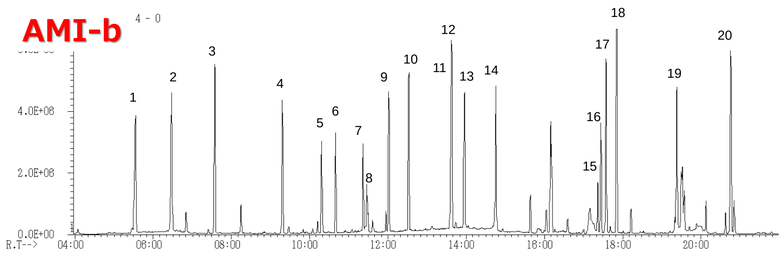


AMI-a



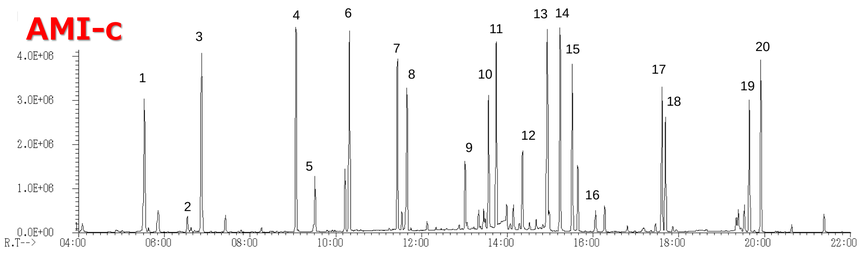
1. Alanine_2TMS
2. Valine_2TMS
3. Leucine_2TMS
4. Isoleucine_2TMS
5. Proline_2TMS
6. Glycine_3TMS
7. Serine_3TMS
8. Threonine_3TMS
9. Aspartic acid_3TMS
10. Methionine_2TMS
11. Glutamic acid_3TMS
12. Phenylalanine_2TMS
13. Lysine_4TMS
14. Tyrosine_3TMS
15. Cystine_4TMS

AMI-b



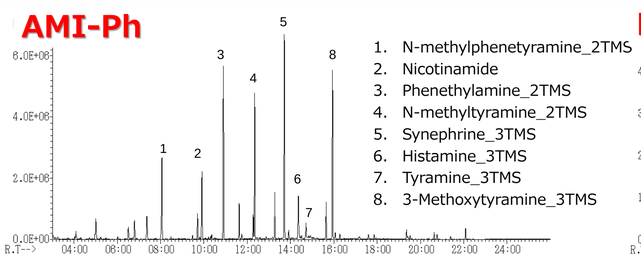
1. 2-Aminobutyric acid_2TMS
2. Norvaline_2TMS
3. Norleucine_2TMS
4. Homoserine_3TMS
5. Pyroglutamic acid_2TMS
6. Cysteine_3TMS
7. Ornithine_3TMS
8. Asparagine_4TMS
9. Asparagine_3TMS
10. 2-Aminoadipic acid_3TMS
11. 1-Methylhistidine_2TMS
12. Ornithine_4TMS
13. Hydroxylysine_4TMS
14. Histidine_3TMS
15. Kynurenine_3TMS
16. Tryptophan_2TMS
17. Tryptophan_3TMS
18. Monoiodotyrosine_3TMS
19. 5-Hydroxytryptophan_4TMS
20. Diiodotyrosine_3TMS

AMI-c



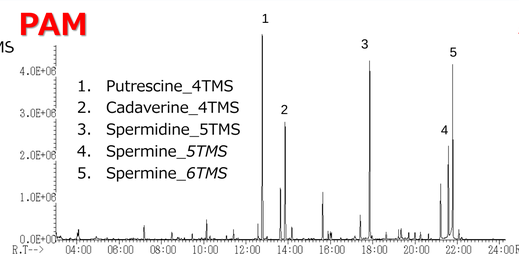
1. N-Methylalanine_2TMS
2. Urea_2TMS
3. Ethanolamine_3TMS
4. beta-Alanine_3TMS
5. Cysteamine_2TMS
6. Pyroglutamic acid_2TMS
7. Anthranilic acid_2TMS
8. Alanyl-glycine_2TMS
9. N-Acetylglutamine_3TMS
10. Glycylglycine_4TMS
11. Glycylglycine_3TMS
12. Allantoic acid_4TMS from Allantoin
13. Carbamoylaspartic acid_3TMS?
14. N2-acetylornithine_3TMS
15. N2-acetylornithine_4TMS
16. *Cysteinylglycine_3TMS from Glutathione*
17. Tryptamine_2TMS
18. Tryptamine_3TMS
19. Serotonin_4TMS
20. Serotonin_3TMS

AMI-Ph



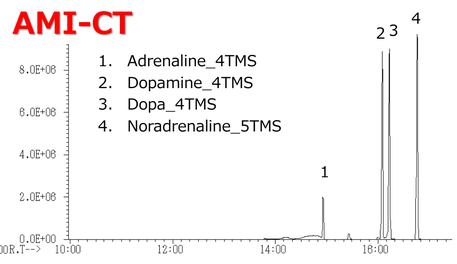
1. N-methylphenetyramine_2TMS
2. Nicotinamide
3. Phenethylamine_2TMS
4. N-methyltyramine_2TMS
5. Synephrine_3TMS
6. Histamine_3TMS
7. Tyramine_3TMS
8. 3-Methoxytyramine_3TMS

PAM



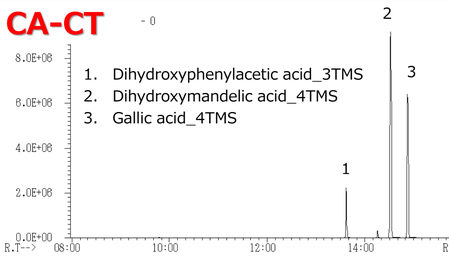
1. Putrescine_4TMS
2. Cadaverine_4TMS
3. Spermidine_5TMS
4. Spermine_*5TMS*
5. Spermine_*6TMS*

AMI-CT



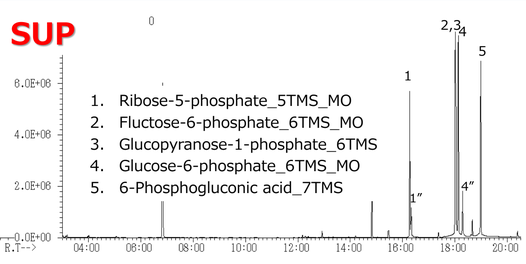
1. Adrenaline_4TMS
2. Dopamine_4TMS
3. Dopa_4TMS
4. Noradrenaline_5TMS

CA-CT



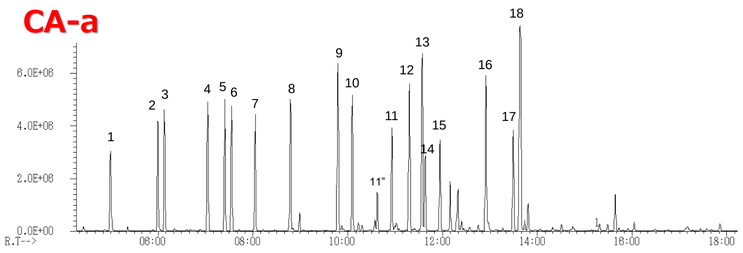
1. Dihydroxyphenylacetic acid_3TMS
2. Dihydroxymandelic acid_4TMS
3. Gallic acid_4TMS

SUP



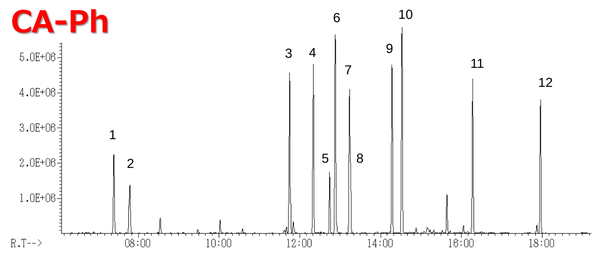
1. Ribose-5-phosphate_5TMS_MO
2. Fluctose-6-phosphate_6TMS_MO
3. Glucopyranose-1-phosphate_6TMS
4. Glucose-6-phosphate_6TMS_MO
5. 6-Phosphogluconic acid_7TMS

CA-a



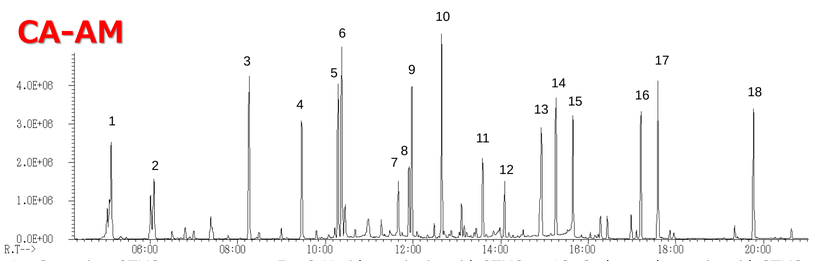
1. Oxalic acid_2TMS
2. Malonic acid_2TMS
3. Methylmalonic acid_2TMS
4. Ethylmalonic acid_2TMS
5. Succinic acid_2TMS
6. Maleic acid_2TMS
7. Fumaric acid_2TMS
8. Glutaric acid_2TMS
9. Malic acid_3TMS
10. Adipic acid_2TMS
11. α-Ketoglutaric acid_MO_2TMS
12. Pimelic acid_2TMS
13. Tartaric acid_4TMS
14. 2-Oxoadipic acid_MO_2TMS_1
15. 2-Oxoadipic acid_MO_2TMS_2
16. cis-Aconitic acid_3TMS
17. Azelaic acid_2TMS
18. Citric acid_4TMS
19. Isocitric acid_4TMS

CA-Ph



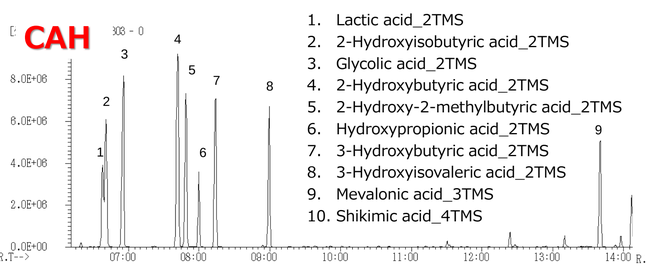
1. Nicotinic acid_1TMS
2. Picolinic acid_1TMS
3. p-Hydroxyphenylactic acid_2TMS
4. Phthalic acid_2TMS
5. Quinolinic acid_2TMS
6. Orotic acid_3TMS
7. Homovanilic acid_2TMS
8. 4-Hydroxyphenylpyruvic acid_3TMS
9. Vanillylmandelic acid_3TMS
10. Hydroxyphenyllactic acid_3TMS
11. Kynurenic acid_2TMS
12. Xanthurenic acid_3TMS

CA-AM



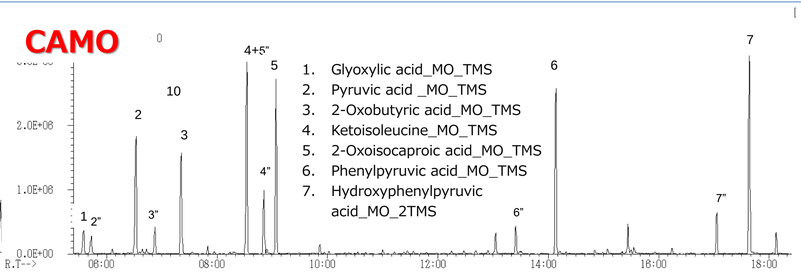
1. Sarcosine_2TMS
2. 3-Aminoisobutyric acid_2TMS
3. Pipecolic acid_2TMS
4. 3-Aminoisobutyric acid_3TMS
5. Hydroxyproline_3TMS
6. γ-Aminoisobutyric acid_3TMS
7. 3-Ureidopropionic acid_3TMS
8. N-Acetylaspartic acid_2TMS
9. N-Acetylaspartic acid_3TMS
10. 5-Aminolevulinic acid_3TMS_2
11. Hippuric Acid_2TMS
12. Hippuric Acid_TMS
13. Carbamoylaspartic acid_3TMS
14. 3-Indoleacetic acid-2TMS
15. trans-Urocanic acid_2TMS
16. 5-Methoxyindoleacetic acid_2TMS
17. 5-Hydroxyinlodeacetic acid_3TMS
18. Biotin_3TMS

CAH



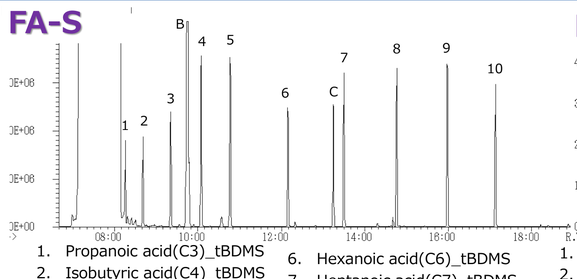
1. Lactic acid_2TMS
2. 2-Hydroxyisobutyric acid_2TMS
3. Glycolic acid_2TMS
4. 2-Hydroxybutyric acid_2TMS
5. 2-Hydroxy-2-methylbutyric acid_2TMS
6. Hydroxypropionic acid_2TMS
7. 3-Hydroxybutyric acid_2TMS
8. 3-Hydroxyisovaleric acid_2TMS
9. Mevalonic acid_3TMS
10. Shikimic acid_4TMS

CAMO



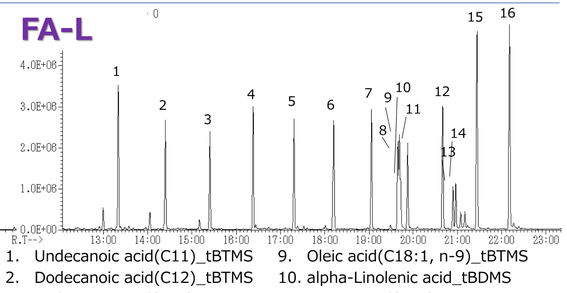
1. Glyoxylic acid_MO_TMS
2. Pyruvic acid _MO_TMS
3. 2-Oxobutyric acid_MO_TMS
4. Ketoisoleucine_MO_TMS
5. 2-Oxoisocaproic acid_MO_TMS
6. Phenylpyruvic acid_MO_TMS
7. Hydroxyphenylpyruvic acid_MO_2TMS

FA-S



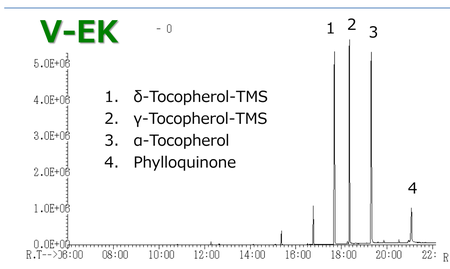
1. Propanoic acid(C3)_tBDMS
2. Isobutyric acid(C4)_tBDMS
3. Butyric acid(C4)_tBDMS
4. Isovaleric acid(C5)_tBDMS
5. Valeric acid_tBDMS
6. Hexanoic acid(C6)_tBDMS
7. Heptanoic acid(C7)_tBDMS
8. Octanoic acid(C8)_tBDMS
9. Nonanoic acid(C9)_tBDMS
10. Decanoic acid(C10)_tBDMS

FA-L



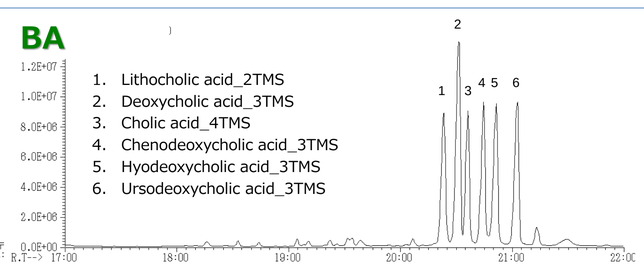
1. Undecanoic acid(C11)_tBTMS
2. Dodecanoic acid(C12)_tBTMS
3. Tridecanoic acid(C13)_tBTMS
4. Myristic acid(C14)_tBTMS
5. Pentadecanoic acid(C15)_tBTMS
6. Palmitic acid(C16)_tBTMS
7. Heptadecanoic acid(C17)_tBTMS
8. Linoleic acid(C18:2, n-6)_tBTMS
9. Oleic acid(C18:1, n-9)_tBTMS
10. alpha-Linolenic acid_tBDMS
11. Stearic acid(C18)_tBTMS
12. Nonadecanoic acid(C19)_tBTMS
13. Arachidonic acid_tBTMS
14. Eicosapentaeoic acid_tBDMS
15. Arachidic acid(C20)_tBTMS
16. Heneicosanoic acid(C21)_tBTMS

V-EK



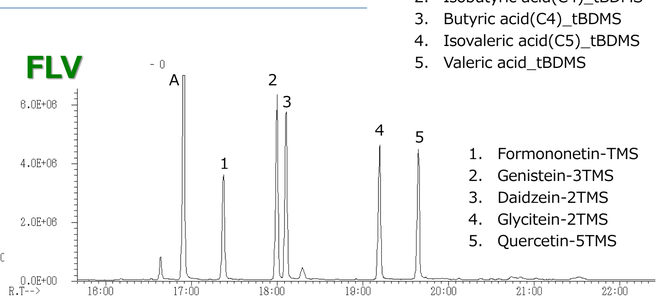
1. δ-Tocopherol-TMS
2. γ-Tocopherol-TMS
3. α-Tocopherol
4. Phylloquinone

BA



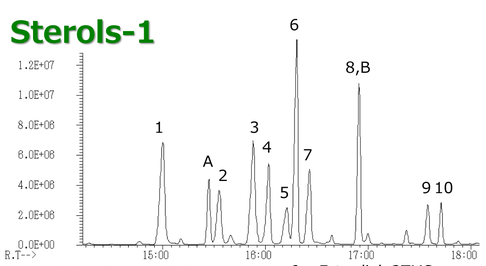
1. Lithocholic acid_2TMS
2. Deoxycholic acid_3TMS
3. Cholic acid_4TMS
4. Chenodeoxycholic acid_3TMS
5. Hyodeoxycholic acid_3TMS
6. Ursodeoxycholic acid_3TMS

FLV



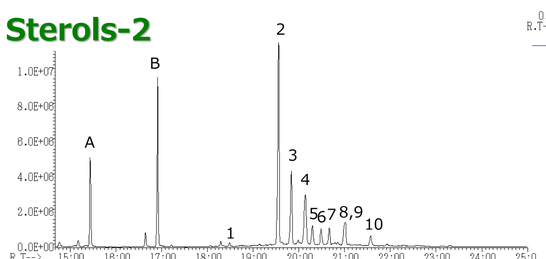
1. Formononetin-TMS
2. Genistein-3TMS
3. Daidzein-2TMS
4. Glycitein-2TMS
5. Quercetin-5TMS

Sterols-1



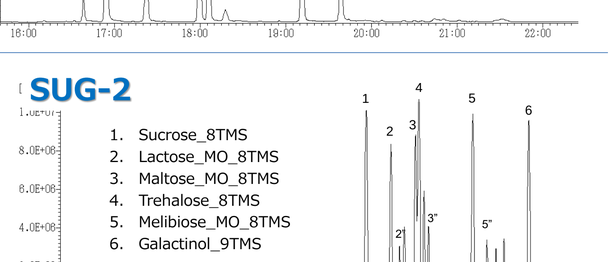
1. Androsterone_TMS
2. Dehydroepiandrosterone_MS
3. Dihydrotestosterone_TMS
4. Estrone_TMS
5. Androstenedione
6. Estradiol_2TMS
7. Testosterone_TMS
8. Pregnenolone_TMS
9. Progesterone
10. Estriol_3TMS

Sterols-2



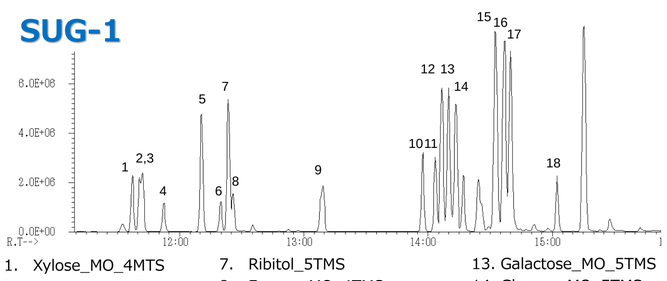
1. Cholecarciferol_TMS
2. Cholesterol_TMS
3. Brassicasterol_TMS
4. Ergosterol_TMS
5. Campesterol_TMS
6. Stigmasterol_TMS
7. Dihydrolanosterol_TMS
8. β-Sitosterol_TMS
9. Lanosterol_TMS
10. *7-Dehydrocholesterol_TMS*

SUG-2



1. Sucrose_8TMS
2. Lactose_MO_8TMS
3. Maltose_MO_8TMS
4. Trehalose_8TMS
5. Melibiose_MO_8TMS
6. Galactinol_9TMS

SUG-1



1. Xylose_MO_4MTS
2. Lyxose_MO_4TMS
3. Arabinose_MO_4TMS
4. Ribose_MO_4TMS
5. Xylitol_5TMS
6. Rhamnose_MO_4TMS
7. Ribitol_5TMS
8. Fucose_MO_4TMS
9. 2-Deoxyglucose_MO_4TMS
10. Fructose_MO_5TMS_1
11. Fructose_MO_5TMS_2
12. Mannose_MO_5TMS
13. Galactose_MO_5TMS
14. Glucose_MO_5TMS
15. Mannitol_6TMS
16. Glucitol_6TMS
17. Galactitol_6TMS
18. Glucopyranose_5TMS

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