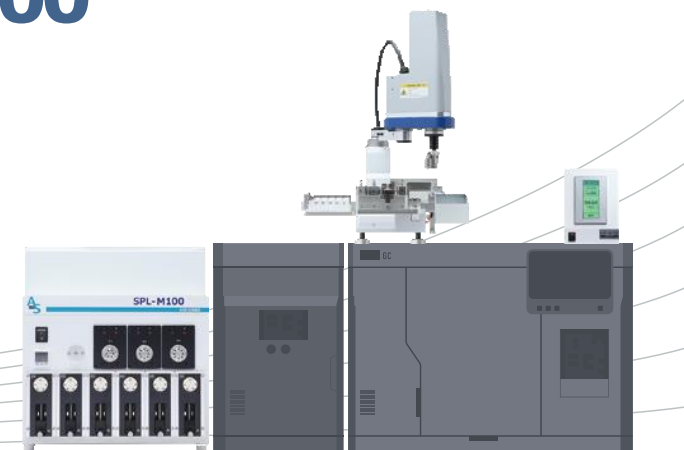




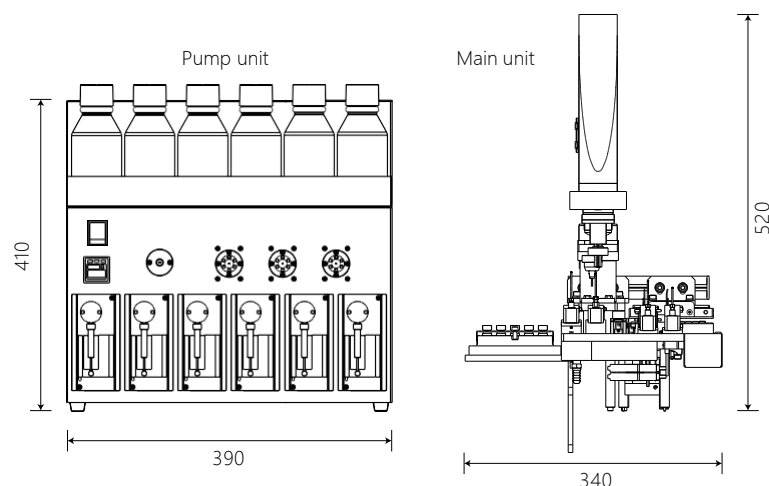
Guidebook of solid-phase derivatization

Online SPE-GC system for metabolome analysis

SPL-M100



Dimensional drawing (units : mm)



SPL-M100 specifications

Size	Main unit: Width=340 mm, Depth=560 mm, Height=520 mm (from installation surface) Pump unit: Width=390 mm, Depth=570 mm, Height=410 mm
Power supply	100 V
Power consumption	500 VA
Computer OS	Windows 7 or later
Number of samples	Maximum 50 samples
Liquid handling	Syringe
Gas type	Nitrogen gas or inert gas
Installation environment	Temperature: 18-28°C Humidity: 40-70%RH (no condensation). Other: An environment with minimal disturbances factors such as dust, vibration, spatial noise, and corrosive gases is preferable. A GC large volume injection port LVI-S250 (sold separately) is required. (If you have LVI-S200, this can be upgraded)

Product specifications, appearance, configuration, etc. may be changed without notice for improvement purposes.
Company names or product names listed in the catalog are registered trademarks or trademarks of the respective companies.

For product inquiries, please contact our company or agents.

AiSTI SCIENCE Co., Ltd.

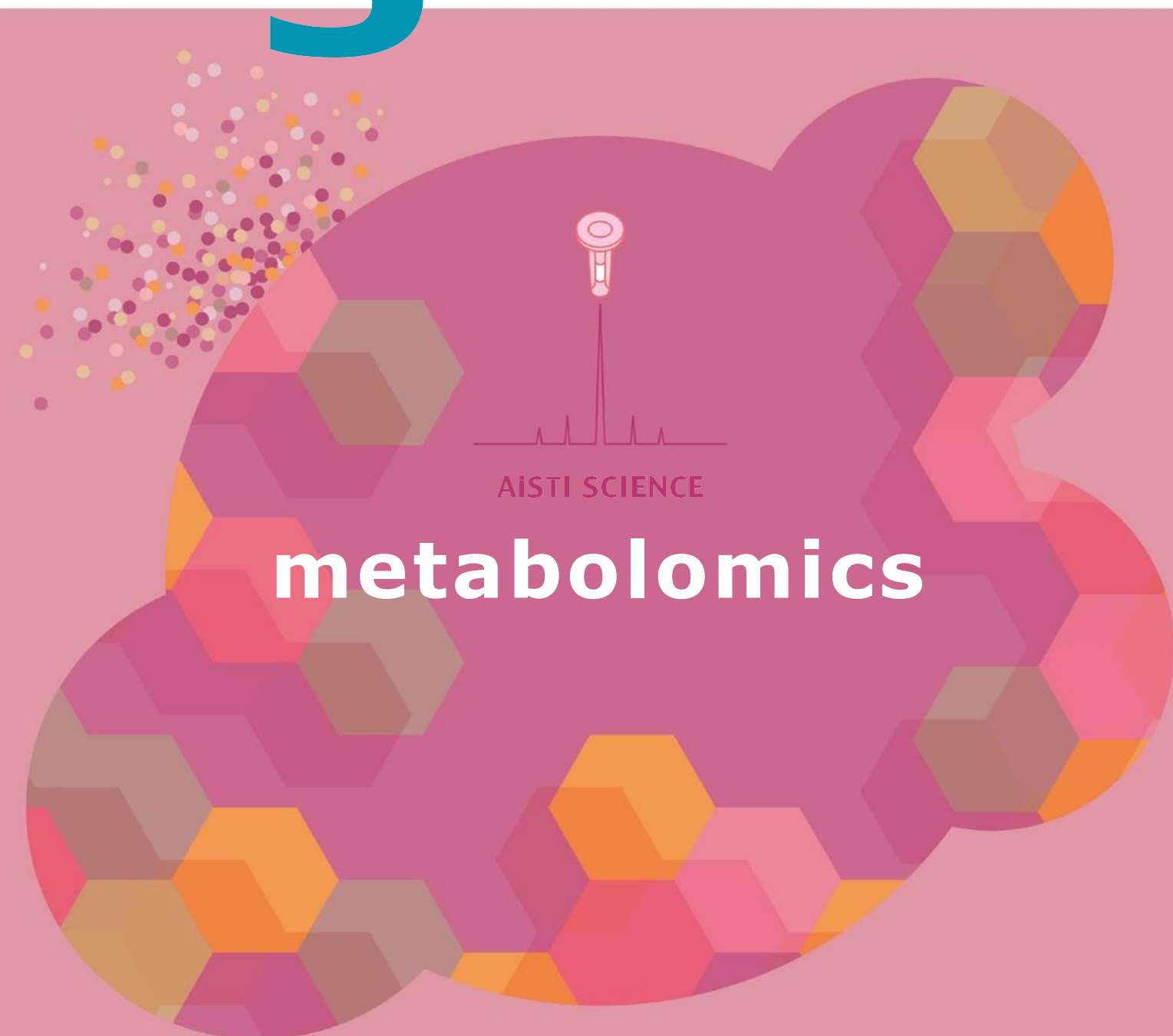
【Head Office】
18-3 Arimoto, Wakayama City,
Wakayama 640-8390 Japan
TEL: +81-73-475-0033 FAX: +81-73-497-5011

【East Japan Sales Office】
Advanec 610, 1-1-31 Hamasaki, Asaka City,
Saitama 351-0033 Japan
TEL: +81-48-424-8384

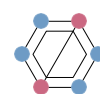
www.aisti.co.jp



SPL-M100

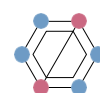


AiSTI SCIENCE



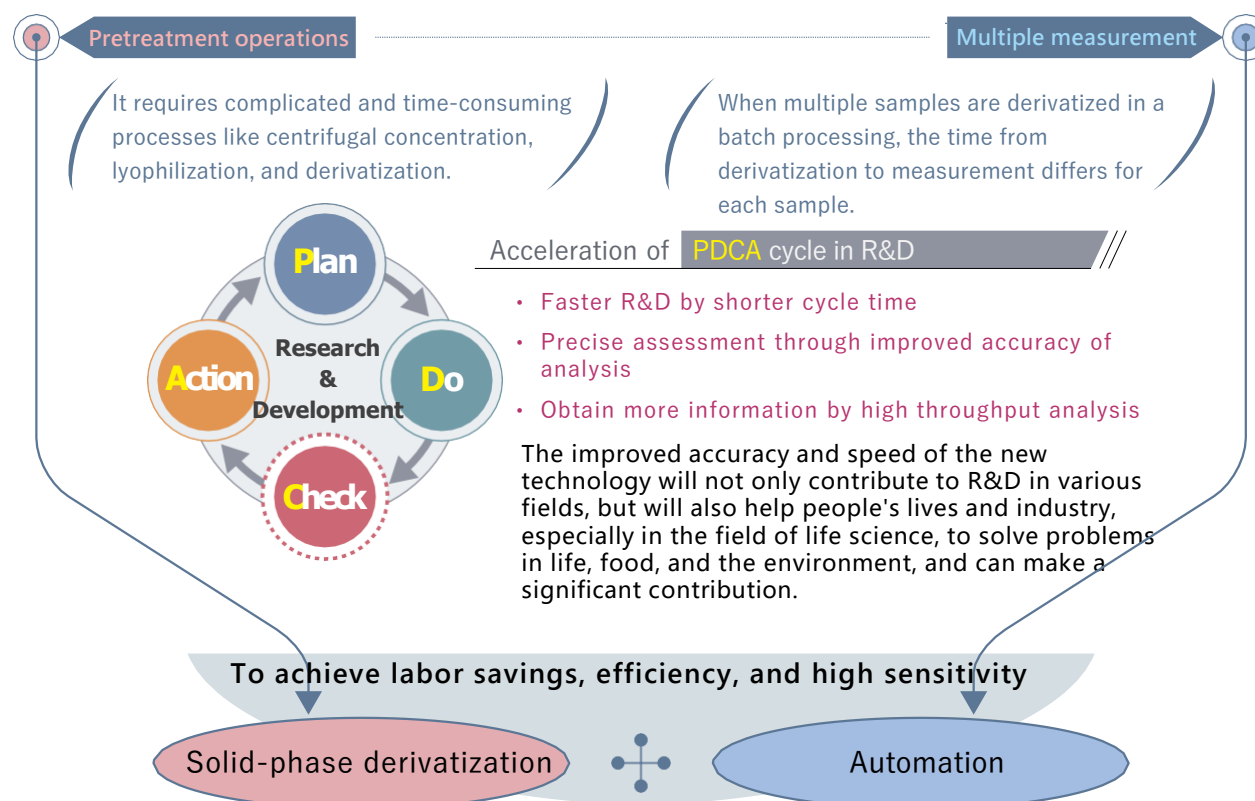
What is Metabolomics?

Metabolomics is a term for the comprehensive analysis of metabolites (metabolome) synthesized and consumed in living organisms (omics), and refers to the analytical techniques used in its study. By researching how metabolites, such as low-molecular-weight organic acids and amino acids, are metabolized in vivo, it is applied to early detection of specific diseases and investigation of drug effects in biological samples such as plasma and serum, and to quality control of food samples.



Challenges and Objectives of Metabolomics

Pretreatment for metabolome analysis is difficult and takes time to master analytical techniques. In metabolomics, it is preferable to comprehensively measure many compounds at once in order to understand the metabolism. Typical metabolites, such as amino acids, organic acids, and sugars, are non-volatile substances with high polarity and are usually measured by LC, but since they have different physical properties, it is difficult to analyze them all at once in LC.



SPE-GC enables online operation from derivatization to measurement

In solid-phase derivatization, simultaneous analysis by GC can be conducted to alter non-volatiles to volatiles. This is important because amino acids, organic acids, and sugars can be analyzed comprehensively.

Medical/Pharmaceutical

Food/Beverage

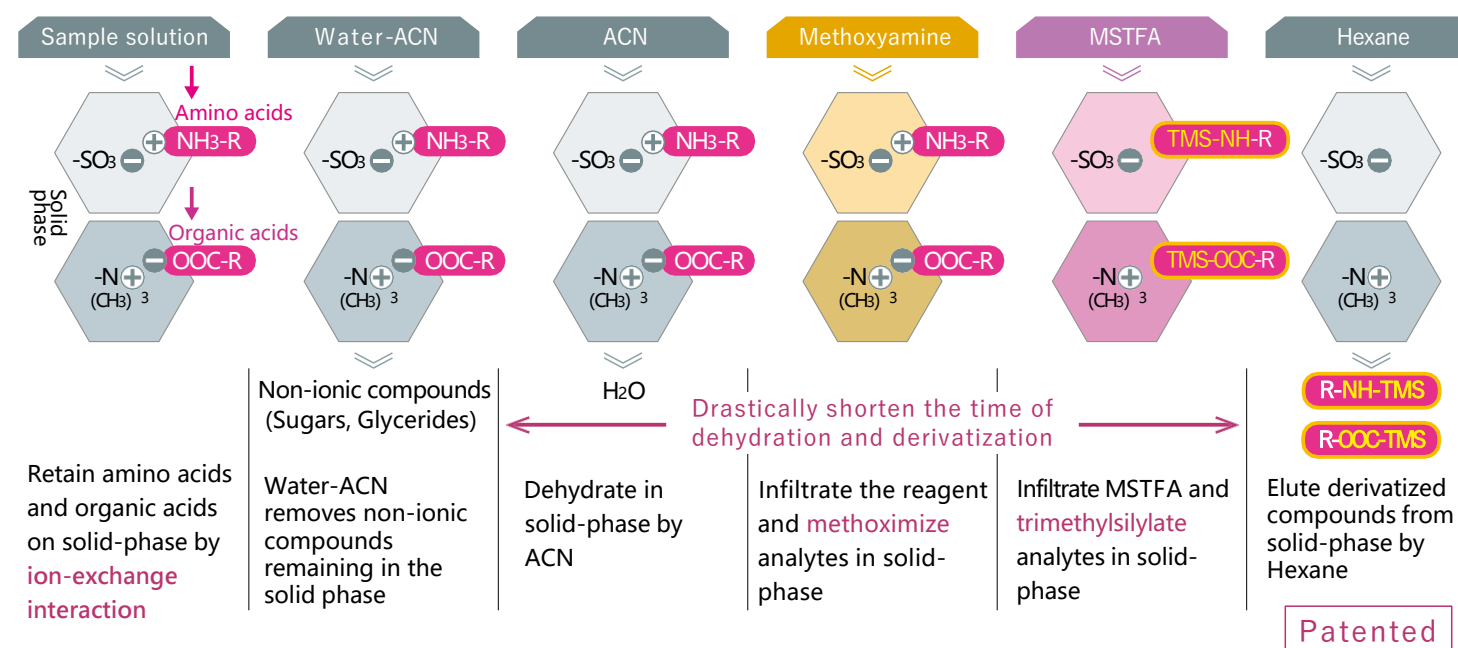
Agriculture/Stockbreeding



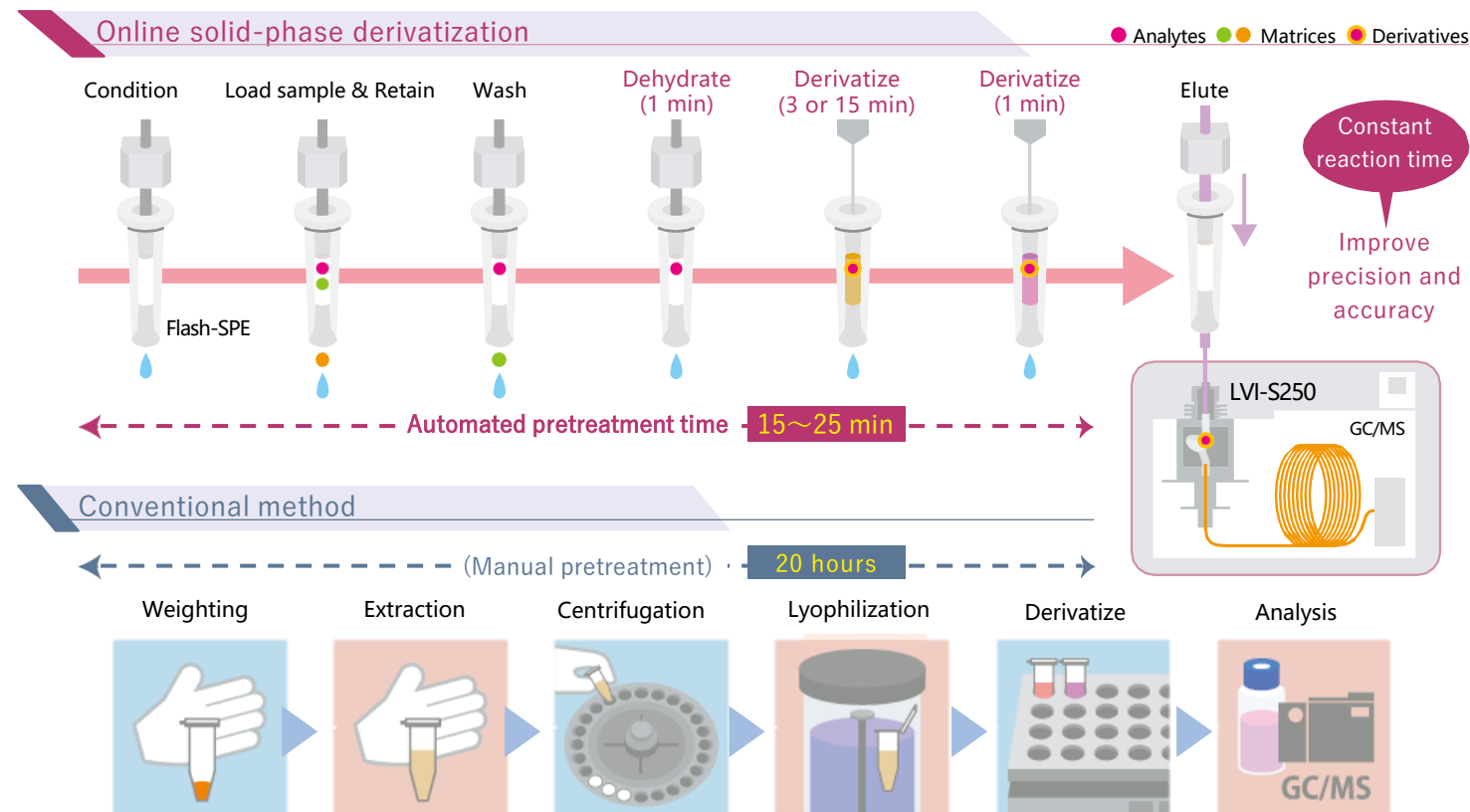
Principle of online solid-phase derivatization

Trimethylsilylation of amino acids and organic acid

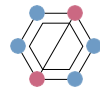
Trimethylsilylation enables analysis by GC/MS, altering non-volatile analytes to easily vaporized ones.



Comparison of conventional method and this method



Conventional method is complicated and time-consuming. Additionally, when samples are derivatized in a batch, the time from derivatization to measurement varies for each sample.



Target compounds by solid-phase derivatization

250 metabolites or more

Amino acid: AMI-a

Alanine, Serine, Valine, Glycine, Tyrosine, Leucine, Proline, Lysine, Isoleucine, Threonine, Cystine, Methionine, Aspartic acid, Phenylalanine, Glutamic acid

Amino acid: AMI-b

Asparagine, N-Acetylglycine, Cysteine, Norleucine, Diiodotyrosine, Norvaline, Histidine, Ornithine, Homocystine, Tryptophan, Homoserine, Hydroxylysine, Kynurenine, 2-Aminoadipic acid, 1-Methylhistidine, 2-Aminobutyric acid, Monoiodotyrosine, 5-Hydroxytryptophan

Amino acid: AMI-c

Alanyl-glycine, Glycylglycine, Cysteamine, Homocystine, Cysteinylglycine, N2-acetylmethionine, Ethanolamine, N-Acetylglutamine, Dihydrobiopterin, Urea, Carbamoylaspartic acid

Phenylamine: AMI-Ph

Histamine, Synephrine, Phenethylamine, Tyramine, N-methyltyramine, 3-Methoxytyramine, N-Methylphenethylamine

Polyamine: PAM

Cadaverine, Spermidine, Putrescine, Spermine

Catecol: CT

Adrenaline, Dopamine, Noradrenaline, Dopa, Gallic acid, Dihydroxymandelic acid, Dihydroxyphenylacetic acid

Organophosphate: PE

Pyrophosphoric acid, Phosphoserine, Pyridoxal phosphate, Phosphohydroxyphenylpyruvic acid

Sugar phosphate: SUP

Fluctose-6-phosphate, Glucose-6-phosphate, Glucopyranose-1-phosphate, Ribose-5-phosphate, Glucopyranose-6-phosphate, 6-Phosphogluconic acid

Sugar acid: SUC

Galacturonic acid, Glyceric acid, Glucaric acid, Glucuronic acid, Threonic acid, Glucuronic acid, N-Acetylmuramic acid, N-Acetylneuraminic acid, Glucaric acid-g-lactone

Carboxylic acid: CA-a

α-Aconitic acid, Malic acid, Citric acid, Malonic acid, Ethylmalonic acid, Methylmalonic acid, Fumaric acid, Oxalic acid, Glutaric acid, Oxoglutaric acid, Isocitric acid, Succinic acid, Maleic acid, Tartaric acid

Aminocarboxylate: CA-AM

Creatinine, Urocanic acid, Hippuric acid, Sarcosine, Hydroxyproline, N-Acetylaspartate, Pipecolic acid, N-Carbamoylaspartate, 3-Aminoisobutyric acid, Carbamoylaspartic acid, 4-Aminobutyric acid, 5-Aminolevulinic acid

Phenylcarboxylate: CA-Ph

Homovanillic acid, Phthalic acid, Xanthurenic acid, Picolinic acid, Kynurenine acid, Quinolinic acid, Nicotinic acid, Orotic acid, Vanillylmandelic acid, Hydroxyphenyllactic acid

Hydroxycarboxylate: CAH

Glycolic acid, Lactic acid, Mevalonic acid, Hydroxypropionic acid, 2-Hydroxybutyric acid, 2-Hydroxyisobutyric acid, 3-Hydroxybutyric acid, 3-Hydroxyisovaleric acid, 2-Hydroxy-2-methylbutyric acid

Keto acid: CAMO

Glyoxylic acid, Pyruvic acid, Ketoisoleucine, Phenylpyruvic acid, 2-Oxobutyric acid, 2-Oxoisocaproic acid, 4-Hydroxyphenylpyruvic acid

Short chain fatty acid: FAS

Propanoic acid, Hexanoic acid, Isobutyric acid, Heptanoic acid, Butyric acid, Octanoic acid, Isovaleric acid, Nonanoic acid, Valeric acid, Decanoic acid

Long chain fatty acid: FAL

Undecanoic acid (C11), Dodecanoic acid (C12), Tridecanoic acid (C13), Myristic acid (C14), Pentadecanoic acid (C15), Palmitic acid (C16), Palmitoleic acid (C16:1n-7), Heptadecanoic acid (C17), Stearic acid (C18), Oleic acid (C18:1, n-9), Linoleic acid (C18:2, n-6), α-Linolenic acid (C18:3, n-3), γ-Linolenic acid (C18:3n-6), Nonadecanoic acid (C19), Arachidic acid (C20), Dihomo-γ-linolenic acid (C20:3n-6), Arachidonic acid (C20:4n-6), Eicosapentaenoic acid (C20:5, n-3), Heneicosanoic acid (C21), Docosapentaenoic acid (C22n-3), Docosahexaenoic acid (C22n-3)

Nucleobase: NUB

Adenine, Guanine, Cytosine, Xanthine

Nucleoside: NUS

Adenosine, Deoxyinosine, Deoxyguanosine, Xanthosine

Monosaccharide: SUG1

Arabinose, Mannitol, Fructose, Mannose, Fucose, myo-Inositol, Galactitol, Rhamnose, Galactose, Ribitol, Glucitol, Ribose, Glucopyranose, Xylitol, Glucose, Xylose, Lyxose, 2-Deoxyglucose

Disaccharide: SUG2

Galactinol, Melibiose, Lactose, Sucrose, Maltose, Trehalose

Bile acid: BA

Cholic acid, Deoxycholic acid, Lithocholic acid, Hyodeoxycholic acid, Ursodeoxycholic acid, Chenodeoxycholic acid

Sterol: ST1

Androstenedione, Estrinol, Androsterone, Estrone, Testosterone, Estradiol, Progesterone, Pregnenolone, Dihydrotestosterone, Dehydroepiandrosterone

Sterol: ST2

β-Sitosterol, Dihydrostanosterol, Brassicasterol, Ergosterol, Campesterol, Lanosterol, Cholecalciferol, Stigmasterol, Cholesterol, 7-Dehydrocholesterol

Flavonoid: FLV

Daidzein, Glycitein, Formononetin, Quercetin, Genistein

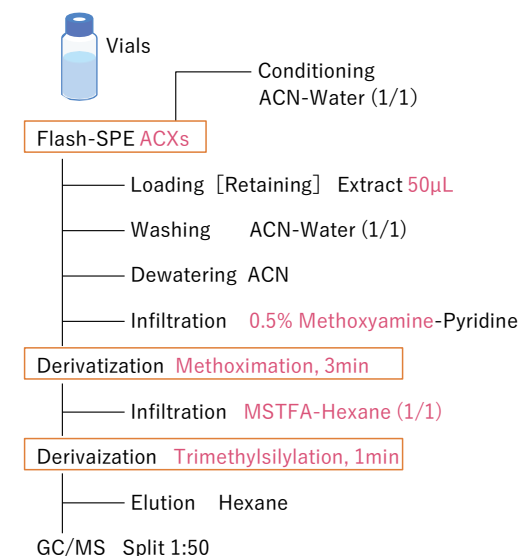
Hydrophobic vitamin: V-DK

Phytonadione, γ-Tocopherol, α-Tocopherol, δ-Tocopherol



Amino acids & Organic acids analysis

Pretreatment workflow



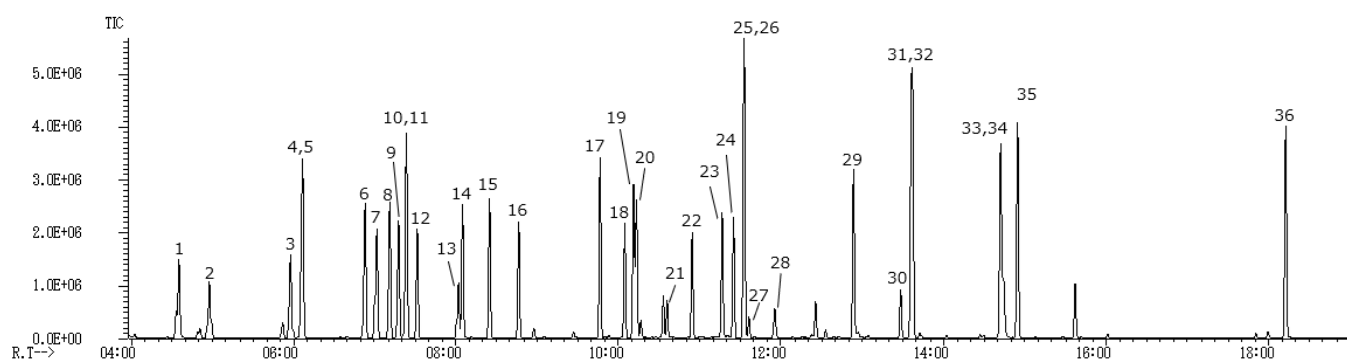
Injection condition

SPE-GC Interface: SPE-M100 (AiSTI SCIENCE)
SPE Cartridge: Flash-SPE ACXs
Sampling Volume: 50μL
PTV Injector: LVI-S250 (AiSTI Science)
Insert Type: Spiral Insert
Injector Temp.: 220°C(0.5min)-50°C/min-290°C(16min)

GC/MS condition

GC
Inlet Mode: Split 1:50
Flow Mode: Constant Flow, 1mL/min
Pre-Column: 0.25mm i.d. x 1m
Column: Vt-5ms, 0.25mm i.d. x 30m, df, 0.25μm
Oven Temp.: 100°C(2min) - 10°C/min - 320°C(2min)
Trans. Line Temp.: 290°C
MS
MS Method: SCAN, m/z:70-600

Chromatogram



1. Alanine_2TMS
2. Oxalic acid_2TMS
3. Malonic acid_2TMS
4. Valine_2TMS
5. Methylmalonic acid_2TMS
6. Leucine_2TMS
7. Ethylmalonic acid_2TMS
8. Isoleucine_2TMS
9. Proline_2TMS
10. maleic acid_2TMS
11. Glycine_3TMS
12. Succinic acid_2TMS
13. Fumaric acid_2TMS
14. Serine_3TMS
15. Threonine_3TMS
16. Glutaric acid_2TMS
17. Malic acid_3TMS
18. Adipic acid_2TMS
19. Aspartic acid_3TMS
20. Methionine_2TMS
21. Oxoglutaric acid_1MO_2TMS_1
22. Oxoglutaric acid_1MO_2TMS_2
23. Pimelic acid_2TMS
24. Glutamic acid_3TMS
25. Tartaric acid_4TMS
26. Phenylalanine_2TMS
27. 2-Oxoalipic acid_1MeOX_2TMS_1
28. 2-Oxoalipic acid_1MeOX_2TMS_2
29. cis-Aconitic acid_3TMS
30. Azelaic acid_2TMS
31. Citric acid_4TMS
32. Isocitric acid_4TMS
33. Lysine_4TMS
34. Histidine_4TMS
35. Tyrosine_3TMS
36. Cystine_4TMS

Repeatability

No.	Compound name	RT min	Solid phase derivatization of amino acids/organic acids, peak area and RSD (n=6%)						
			1	2	3	4	5	6	Ave. RSD, %
1	Alanine_2TMS	4.59	2,796,457	2,740,757	2,908,211	2,987,506	3,004,500	2,819,434	2,876,144 3.7
2	Oxalic acid_2TMS	4.97	1,433,569	1,467,446	1,612,087	1,621,832	1,673,343	1,574,603	1,563,813 6.0
3	Malonic acid_2TMS	5.97	2,885,096	2,856,364	3,118,479	2,949,609	3,159,205	2,819,550	2,964,717 4.8
4	Valine_2TMS	6.11	579,229	575,750	600,898	615,348	618,040	579,793	594,843 3.2
5	Methylmalonic acid_2TMS	6.11	3,246,706	3,269,490	3,432,962	3,315,929	3,491,980	3,233,253	3,331,720 3.2
6	Leucine_2TMS	6.88	3,881,185	3,822,130	4,066,306	4,153,562	4,171,868	3,904,054	3,999,851 3.7
7	Ethylmalonic acid_2TMS	7.02	3,047,504	3,084,252	3,211,158	3,076,174	3,326,485	2,989,030	3,122,434 4.0
8	Isoleucine_2TMS	7.18	3,663,868	3,606,025	3,827,550	3,904,952	3,902,472	3,669,337	3,762,367 3.5
9	Proline_2TMS	7.30	3,976,149	4,021,867	4,173,805	4,297,605	4,400,630	4,076,584	4,157,773 4.0
10	maleic acid_2TMS	7.38	187,564	182,938	190,539	186,127	189,306	175,356	185,305 3.0
11	Glycine_3TMS	7.38	441,766	411,880	435,644	485,230	418,364	473,858	444,457 6.6
12	Succinic acid_2TMS	7.52	3,249,928	3,318,058	3,425,971	3,252,991	3,559,463	3,175,138	3,330,258 4.2
13	Fumaric acid_2TMS	8.02	873,768	883,647	922,253	879,591	957,324	838,431	892,502 4.6
14	Serine_3TMS	8.07	1,674,335	1,752,221	1,772,483	1,788,152	1,917,059	1,683,341	1,764,599 5.0
15	Threonine_3TMS	8.40	846,598	878,908	879,263	886,326	957,675	835,527	880,716 4.9
16	Glutaric acid_2TMS	8.76	1,892,273	1,943,032	1,972,436	1,872,551	2,070,120	1,826,859	1,929,545 4.5
17	Malic acid_3TMS	9.77	1,898,685	1,902,816	1,950,773	1,871,083	2,025,701	1,832,427	1,913,581 3.5
18	Adipic acid_2TMS	10.07	267,676	270,067	276,389	252,531	288,724	255,179	268,428 5.0

No.	Compound name	RT min	Solid phase derivatization of amino acids/organic acids, peak area and RSD (n=6%)						
			1	2	3	4	5	6	Ave. RSD, %
19	Aspartic acid_3TMS	10.18	2,073,616	2,057,755	2,105,653	2,047,118	2,253,268	1,976,365	2,085,629 4.4
20	Methionine_2TMS	10.22	2,102,155	2,045,739	2,134,686	2,138,921	2,209,420	2,017,018	2,107,990 3.3
21	Oxoglutaric acid_1MO_2TMS	10.60	194,061	171,180	181,426	186,296	188,760	190,227	185,325 4.4
22	Oxoglutaric acid_1MO_2TMS	10.90	563,402	506,070	600,138	562,836	628,315	545,493	567,709 7.5
23	Pimelic acid_2TMS	11.27	949,060	950,102	992,660	923,898	1,027,899	931,097	962,453 4.2
24	Glutamic acid_3TMS	11.40	1,616,891	1,512,525	1,623,064	1,572,957	1,667,557	1,493,032	1,581,004 4.3
25	Tartaric acid_4TMS	11.54	1,150,168	1,136,548	1,166,379	1,131,077	1,175,842	1,099,986	1,143,333 2.4
26	L-Phenylalanine_2TMS	11.54	1,784,337	1,722,138	1,807,079	1,793,271	1,845,791	1,718,739	1,778,559 2.8
27	2-Oxoalipic acid_1MeOX_2TMS	11.60	86,824	80,582	99,366	91,980	104,581	93,235	92,761 9.2
28	2-Oxoalipic acid_1MeOX_2TMS	11.92	141,037	128,203	152,500	147,414	169,723	147,440	147,720 9.2
29	cis-Aconitic acid_3TMS	12.88	948,599	933,617	989,232	924,903	1,012,133	905,734	952,370 4.3
30	Azelaic acid_2TMS	13.46	89,252	93,415	96,004	86,490	101,419	87,494	92,346 6.2
31	Citric acid_4TMS	13.60	347,136	343,898	350,436	343,549	347,426	332,581	344,171 1.8
32	Isocitric acid_4TMS	13.62	1,164,276	1,166,799	1,204,285	1,162,976	1,183,800	1,127,241	1,168,230 2.2
33	Lysine_4TMS	14.70	384,160	355,654	386,336	424,543	372,317	422,343	390,892 7.0
34	Histidine_3TMS	14.73	453,526	420,048	489,843	519,435	423,086	484,064	465,000 8.5
35	Tyrosine_3TMS	14.91	4,586,160	4,691,735	4,737,119	4,663,853	4,957,822	4,691,567	4,721,376 2.7
36	Cystine_4TMS	18.20	2,406,871	2,663,673	2,525,474	2,669,877	2,754,298	2,836,693	2,642,854 5.9

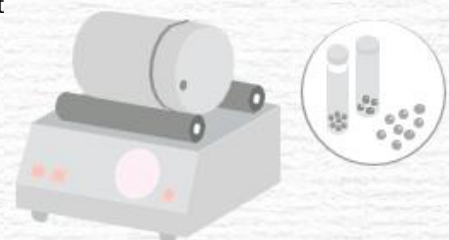


Grinding and Extraction

One of the important points common to food analysis is "sample uniformity". For example, in residual pesticide analysis, if pesticide residues in food are unevenly distributed or scattered, poor sample uniformity leads directly to poor quantitation. Even if the same sample is aliquoted, which may give different quantitative values in each analysis, it becomes difficult to compare maximum residual limits. In addition, in recent years, the amount of analytical samples has decreased along with the achievement of higher sensitivity by analytical instruments. Therefore making sample uniformity even more important

Beads grinding

- For small samples
- Crushes hard tissue by impact as beads
- Capable of processing multiple samples



Pre-cooled freeze grinder

- Processing with water in samples
- High capacity for whole sample
- Suitable for analyzing component concentrations containing water



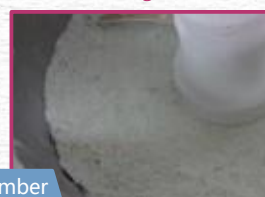
Even samples with a large amount of sinew or fibrous material can be ground to a powder form. Freeze grinder suppresses enzyme activity in the sample and prevents the decomposition of metabolites.

Room temp. processing



Cucumber

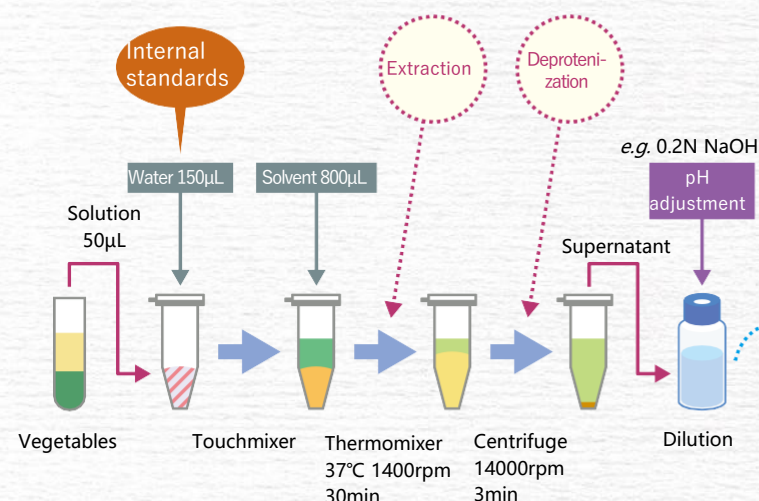
Freeze grinder



Chicken breast tender

Pretreatment flow for analysis of ionic compounds

Extraction and Deproteinization

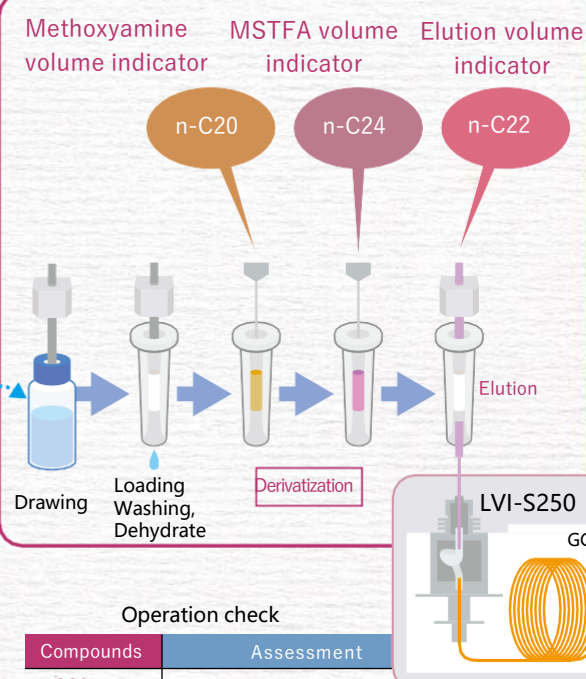


Validation reagents

*The table above is one example of monitoring indicators for process control.

Internal standards*	
Compounds	Targets
Norleucine	Amino acids
Adipic acid	Organic acids
Ribitol	Sugars
Hexanoic acid-d11	SCFAs

Automated

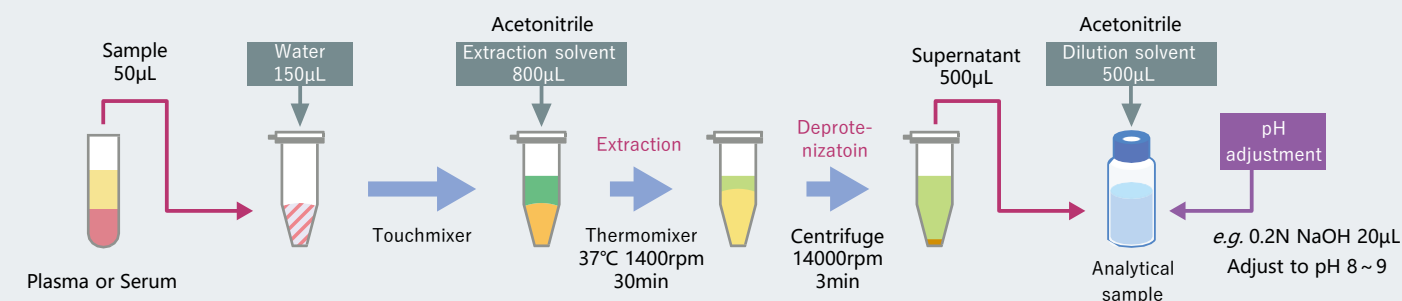


Operation check	
Compounds	Assessment
n-C20	Methoxyamine
n-C22	Hexane (elution solvent)
n-C24	MSTFA-Hexane (1/1)

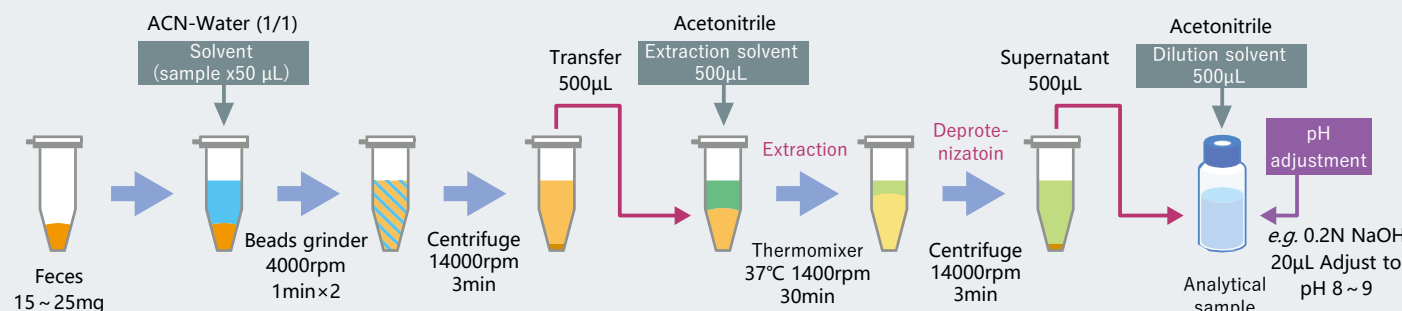


Examples of extraction procedure

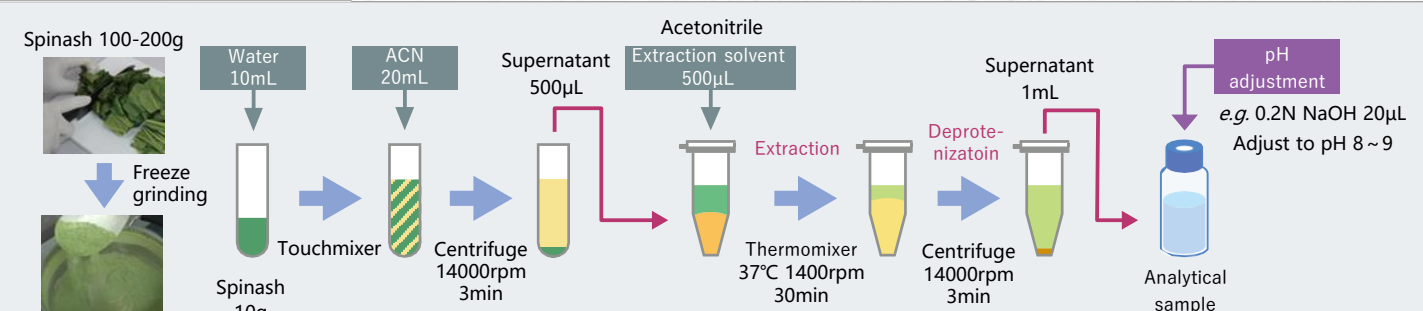
Plasma, Serum



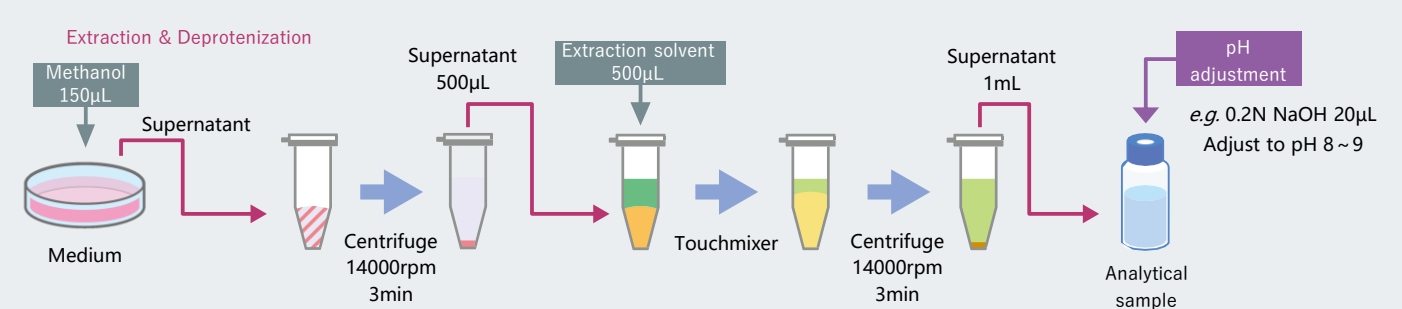
Feces (Mouse)



Spinach



Medium



Solid-phase derivatization



Analytes are derivatized on solid-phase, enabling online GC/MS analysis

From solid-phase extraction to GC analysis

Fully automated

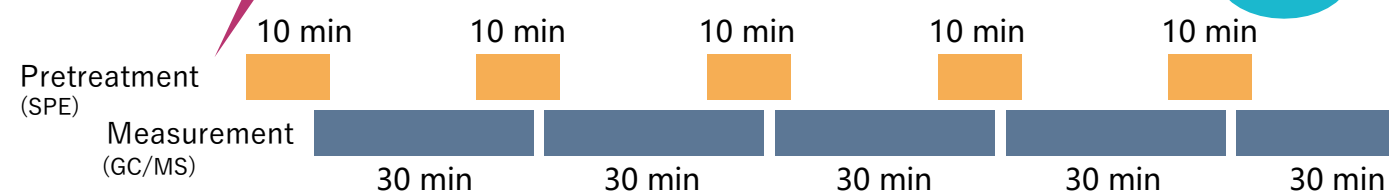
"Online" system

means it does not separate pretreatment and measurement, automatically injects directly into GC or LC.

Prepare solvents and SPE cartridges
Just **set samples**



START



Measurement and Pretreatment overlap

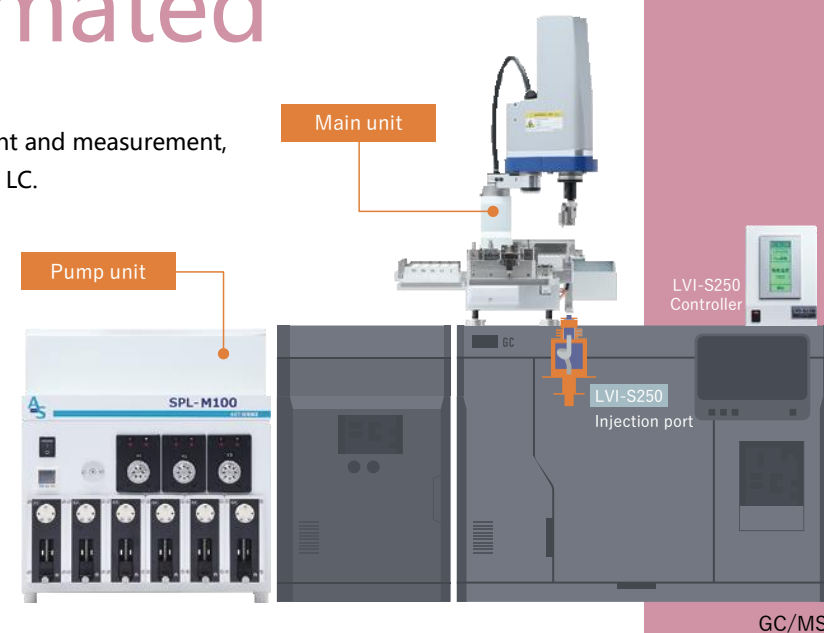
High throughput analysis

FINISH

Online SPE-GC system for metabolomics

SPL-M100

For Gas Chromatography



Main unit

Reagent holder

Sample tray

Robot arm

SPE tray

Flash-SPE
Up to 100

Washing cartridge holder

GC injection port

Discard box

Working table

Injection needle adaptor

Passing port

N nozzle
Drying (N₂ purge)

L nozzle
Conditioning,
Sample loading

S nozzle
Sample loading,
Reagent loading

E nozzle
Elution of analytes to GC with needle adaptor,
Elution of analytes to LC port

Reagents (Max. 5 vials)

D1 : MSTFA-Hexane (1/1)
D2 : 0.5mg/mL Methoxyamine-pyridine
D3 : 10mg/mL Methoxyamine-pyridine
D4 : MTBSTFA- Hexane (1/1)
D5 : n-Alkane mix. (Retention Indices)



Reagent holder



Each nozzle and passing port
Dedicated nozzles perform operations
(conditioning, sample loading, washing,
drying, elution).



Eluate directly injected



Pump unit

Pumping by syringes

Efficient liquid transfer is enabled by using
independent syringe pump for each solvent

Switching flow path with valves

Valve position can be switch freely depending
on the method.



Syringe pumps and valves



Bottle rack

Use a normal injector

By removing the working table, a normal injector and injection port (front) can be used to perform analysis.



Shimadzu



Agilent





Maintenance

To keep analytical instruments running smoothly, it is necessary to perform daily and regular maintenance properly. Additionally, regularly replacing consumables prevents issues before they occur and leads to stable analysis. This ensures continuous, highly accurate results. Here is a list of the maintenance items required for the SPL-M100.

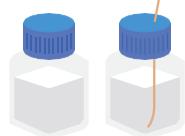


Daily maintenance

Please check the following items before operating the equipment.

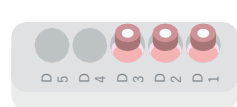
Checking the solvent

- Remaining volume
- Tube insertion depth
- Solvent replacement



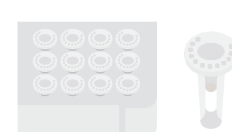
Checking the reagent

- Remaining volume
- The position on tray
- Reagent replacement



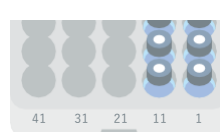
Checking SPE cartridges

- Filling SPE tray
- Discarding used cartridges



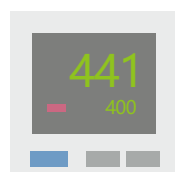
Setting samples

- Sample No. on tray
- Using septums with slit



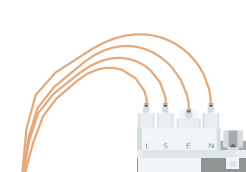
Checking the gas

- Pressure gauge value



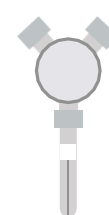
Fixing nozzle tubes

- No tangle and hooking



Solvent purging ※ Execute "Purge solvent" in maintenance menu

- Retightening the syringe connection
- Removing the air in the syringe barrel
- Dispensing solvents from the nozzle
- No leak from each connection

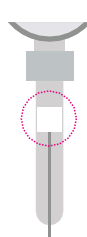


Regular maintenance

Please check the following items once a week.

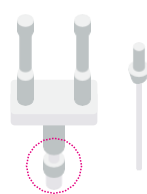
Syringes

- Wipe off dirt from the plunger and inside of the barrel.



Injection needle adaptor

- Wash the adapter part



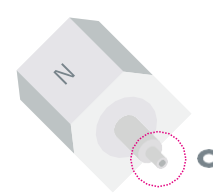
Washing cartridge

- Replace every 100 runs



Cleaning of N nozzle

- Wipe the tip of N nozzle

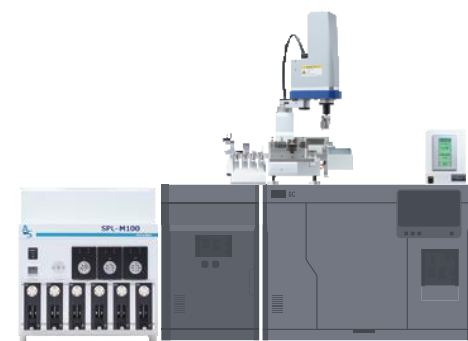


Consumables

Please replace according to recommended frequency or usage status.

- | | |
|--|---|
| • Spiral insert Every 100 injections | • Flash-SPE cartridge for washing..... Every 100 injections |
| • Advanced green septum Every 100 injections | • O-ring for N nozzle Every 6 months |
| • Precolumn Every 3 months | • Batteries for robot arm Every 2 years |
| • Trap filter Every 6 months | • Memory battery for robot Every 4 years |

Related products



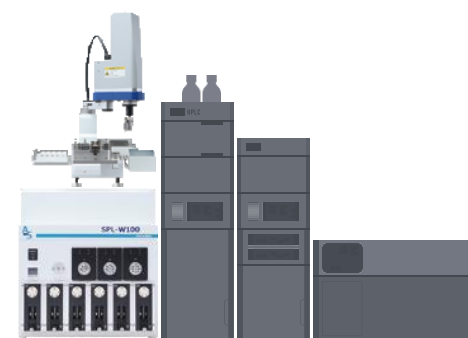
GC/MS

Headspace analysis by solid phase adsorption-solvent elution

Online SPE-GC system for volatiles **SPL-M100FE**

"Fully automated" from pretreatment to GC/MS analysis

- | | |
|---------|---------------------------------|
| Fields | Odor, strange smell, musty odor |
| Samples | Foods, beverages, etc. |
| Targets | Volatiles |



LC/MS

Online LC/MS with SPE interface

Online SPE-LC system **SPL-W100**

"Fully automated" from pretreatment to LC/MS analysis in various fields

- | | |
|---------|-----------------------------|
| Fields | SPE → HPLC, LC/MS |
| Samples | Liquid samples and extracts |
| Targets | Detectable compounds in LC |

SPE → Fraction collect by HPLC → LC/MS analysis

Online SPE-LC-LC system **SPL-X100L**

"Fully automated" targeted analysis in samples with many impurities

- | | |
|---------|--------------------------------|
| Fields | Isolation, purification |
| Samples | For complex matrix composition |
| Targets | Detectable compounds in LC |



HPLC

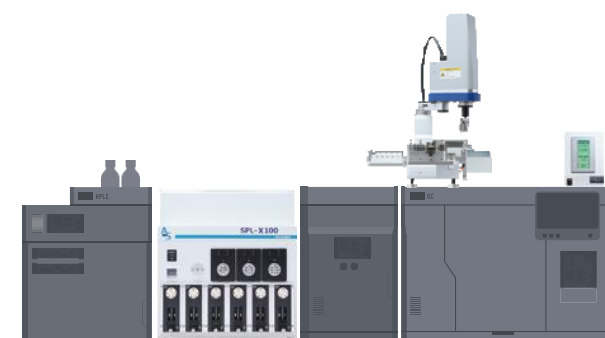
LC/MS

SPE → Fraction collect by HPLC → GC/MS analysis

Online SPE-LC-GC system **SPL-X100G**

"Fully automated" targeted analysis in samples with many impurities

- | | |
|---------|--------------------------------|
| Fields | Isolation, purification |
| Samples | For complex matrix composition |
| Targets | Detectable compounds in GC |



HPLC

GC/MS

Crushing uniformly from large amounts of sample

Freeze grinder Frestent **FST-4000**

Supports a variety of samples, including leaves, roots, fruits, meat, and processed foods

- | | |
|-------------|---|
| Main sets | Main unit, container, lid, cutter |
| Accessories | Dry ice storage box (up to 20 kg) |
| | Accessories set (scoop, pre-cooling container, 2 types of sample spoons x5) |



Samples into powder