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Application Data Sheet

No.104

GC-MS
Gas Chromatograph Mass Spectrometer

Multicomponent Analysis of Metabolites in Human Plasma using GC-MS/MS

The analysis of metabolomes, such as when searching for disease biomarkers, is performed in many areas in the medical field, whether it be for fundamental research or for clinical studies. Single quadrupole GC-MS provides excellent chromatographic resolution and enables stable measurements, and is therefore widely utilized for metabolome analyses involving the comprehensive analysis of in vivo metabolites. However, biological samples contain many metabolites and various matrices, so separation with single quadrupole GC-MS can be difficult. With triple quadrupole GC-MS/MS MRM, MS separation is performed twice, with Q1 and Q3. This helps remove the impact of overlapping peaks due to interfering components in comparison with scan mode and SIM mode, in which MS separation is performed with a single quadrupole, and thus enables the acquisition of accurate quantitative results with high-sensitivity detection.
Smart Metabolites Database registers MRM information of 475 metabolites mainly contained in biological samples such as blood, urine and cells. It enables simultaneous measurement of 475 metabolites using MRM mode. This application data sheet presents an analysis of metabolites in standard human plasma using the scan and MRM methods included in the Smart Metabolites Database, as well as a comparison of the results.

Analysis Conditions

In the pretreatment process, 2-isopropylmalic acid was added as an internal standard to 50 μL of standard human plasma, after which metabolites were extracted with a methanol/water/chloroform (2.5:1:1) solution. Methoxime and trimethylsilyl derivatives were then formed to obtain the samples[1]. The respective samples were measured each in scan and MRM modes using methods included in the Smart Metabolites Database. Table 1 shows the analysis conditions.

Table 1: Analysis Conditions

GC-MS:	GCMS-TQ8040		
Column:	BPX-5 (Length 30 m; 0.25 mm I.D.; df = 0.25 μm) (SGE, P/N:054101)		
Glass insert:	Split insert with wool (P/N: 225-20803-01)	[MS]	
		Interface temp.:	280 °C
[GC]		Ion source temp.:	200 °C
Sample injection unit temp.:	250 °C	Measurement mode:	Scan
Column oven temp.:	60 °C (2 min) → (15 °C/min) → 330 °C (3 min)	Mass range:	*m/z* 45-600
Injection mode:	Split	Event time:	0.2 sec
Split ratio:	30		
Carrier gas control:	Linear velocity (39.0 cm/sec)	Measurement mode:	MRM
Injection volume:	1 μL	Loop time:	0.25 sec

Analysis Results

Fig.1 shows the resulting total ion current chromatogram (TIC) of MRM measurement.

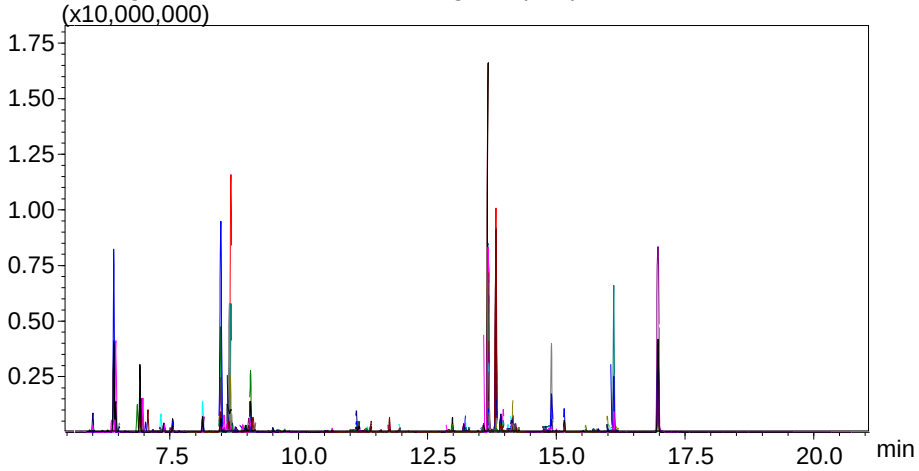

Fig. 1: Total Ion Current Chromatogram (TIC) of MRM measurement for Metabolites in Standard Human Plasma

[1] S. Nishiumi, M. Shinohara, A. Ikeda, T. Yoshie, N. Hatano, S. Kakuyama, S. Mizuno, T. Sanuki, H. Kutsumi, E. Fukusaki, T. Azuma, T. Takenawa, M. Yoshida, Metabolomics 6 (2010) 518-528

Fig. 2 shows mass chromatograms for plasma metabolites obtained in scan and MRM modes. In scan mode, some of the metabolites shown were not detected due to interfering components and insufficient sensitivity. In contrast, favorable results were obtained with MRM, which eliminated the impact of interfering components, enabling high-sensitivity measurements

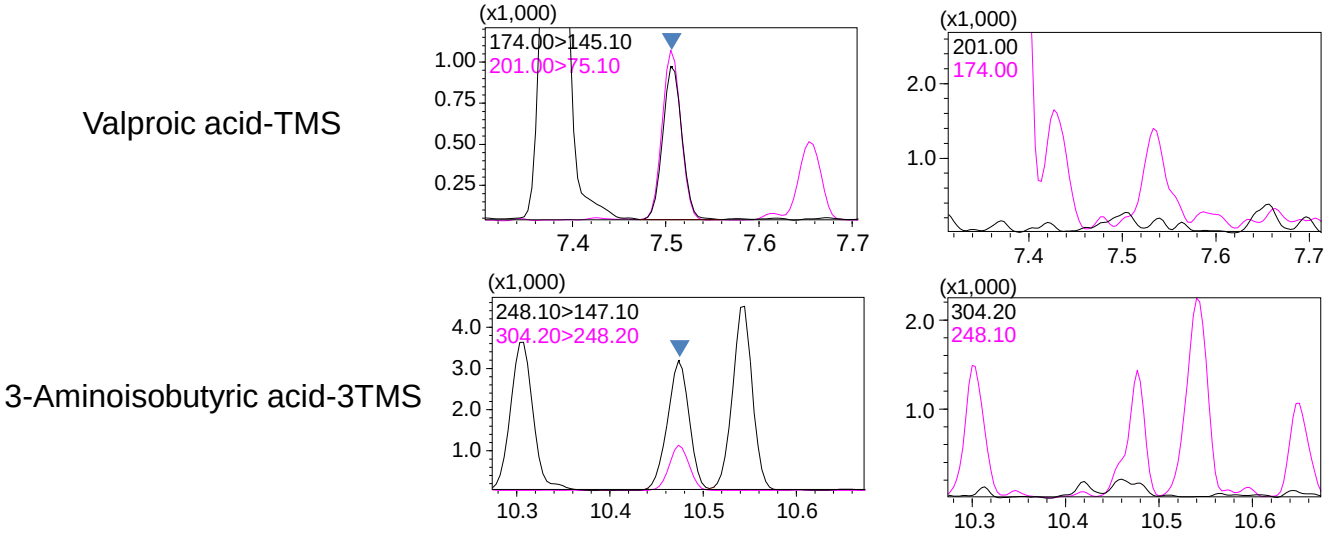

Fig. 2: Comparison of MRM (Left) and Scan (Right) Mass Chromatograms for Metabolites in Standard Human Plasma

Table 2 lists the metabolites detected using MRM measurement. From the standard human plasma, it was possible to detect 221 TMS derivatized metabolites, including 2-isopropylmalic acid, added as an internal standard.

Table 2: List of TMS Derivatized Metabolites Detected using MRM measurement *

1	Acetylglycine-TMS	33	Cystamine-nTMS	65	Glutamine-3TMS
2	Aconitic acid-3TMS	34	Cysteine-3TMS	66	Glutamine-4TMS
3	Adipic acid-2TMS	35	Cystine-4TMS	67	Glutaric acid-2TMS
4	Alanine-2TMS	36	Decanoic acid-TMS	68	Glyceric acid-3TMS
5	Allantoin-4TMS	37	5-Dehydroquinic acid-5TMS	69	Glycerol 2-phosphate-4TMS
6	Allose-meto-5TMS(1)	38	5-Dehydroquinic acid-meto-4TMS	70	Glycerol 3-phosphate-4TMS
7	2-Aminoadipic acid-3TMS	39	2-Deoxy-glucose-4TMS(1)	71	Glycerol-3TMS
8	2-Aminobutyric acid-2TMS	40	2-Deoxy-glucose-4TMS(2)	72	Glycine-2TMS
9	2-Aminoethanol-2TMS	41	2'-Deoxyuridine-3TMS	73	Glycine-3TMS
10	2-Aminoethanol-3TMS	42	Dihydrouracil-TMS	74	Glycolic acid-2TMS
11	3-Aminoisobutyric acid-3TMS	43	Dihydroxyacetone phosphate-meto-3TMS(1)	75	Glycyl-Glycine-4TMS
12	2-Aminopimelic acid-3TMS	44	Dihydroxyacetone phosphate-meto-3TMS(2)	76	Glyoxylic acid-meto-TMS
13	3-Aminopropanoic acid-3TMS	45	Dihydroxyacetone-2TMS	77	1-Hexadecanol-TMS
14	5-Aminovaleric acid-3TMS	46	Dimethylglycine-TMS	78	Histidine-3TMS
15	1,5-Anhydro-glucitol-4TMS	47	Dopamine-4TMS	79	Homocysteine-3TMS
16	1,6-Anhydroglucose-3TMS	48	Elaidic acid-TMS	80	Homoserine-2TMS
17	Arabitol-5TMS	49	Erythrulose-meto-3TMS(2)	81	Hydroquinone-2TMS
18	Arginine-3TMS	50	Ethylmalonic acid-2TMS	82	3-Hydroxyanthranilic acid-3TMS
19	Ascorbic acid-4TMS	51	Fructose-meto-5TMS(2)	83	2-Hydroxybutyric acid-2TMS
20	Asparagine-3TMS	52	Fucose-meto-4TMS(2)	84	3-Hydroxybutyric acid-2TMS
21	Asparagine-4TMS	53	Fumaric acid-2TMS	85	2-Hydroxyglutaric acid-3TMS
22	Aspartic acid-3TMS	54	Galactitol-6TMS	86	3-Hydroxyglutaric acid-3TMS
23	Azelaic acid-2TMS	55	Galacturonic acid-meto-5TMS(2)	87	2-Hydroxyhippuric acid-2TMS
24	Benzoic acid-TMS	56	Glucaric acid-6TMS	88	2-Hydroxyisobutyric acid-2TMS
25	Cadaverine-3TMS	57	Gluconic acid-6TMS	89	3-Hydroxyisobutyric acid-2TMS
26	Caproic acid-TMS	58	Glucosamine-5TMS(1)	90	2-Hydroxyisocaproic acid-2TMS
27	Catechol-2TMS	59	Glucose 6-phosphate-meto-6TMS(1)	91	2-Hydroxyisovaleric acid-2TMS
28	Cholesterol-TMS	60	Glucose-meto-5TMS(1)	92	3-Hydroxyisovaleric acid-2TMS
29	Citramalic acid-3TMS	61	Glucose-meto-5TMS(2)	93	Hydroxylamine-3TMS
30	Citric acid-4TMS	62	Glucuronic acid-meto-5TMS(1)	94	5-Hydroxymethyl-2-furoic acid-2TMS
31	Citrulline-3TMS	63	Glucuronic acid-meto-5TMS(2)	95	4-Hydroxyphenylacetic acid-2TMS
32	Creatinine-3TMS	64	Glutamic acid-3TMS	96	4-Hydroxyphenyllactic acid-3TMS

Table 2: List of TMS Derivatized Metabolites Detected using MRM measurement (continued)

97	4-Hydroxyproline-3TMS	139	2-Methyl-3-hydroxybutyric acid-2TMS(2)	181	Quinolinic acid-2TMS
98	3-Hydroxypropionic acid-2TMS	140	3-Methylglutaric acid-2TMS	182	Rhamnose-meto-4TMS(2)
99	Hypotaurine-3TMS	141	7-Methylguanine-2TMS	183	Ribonolactone-3TMS
100	Hypoxanthine-2TMS	142	Methylsuccinic acid-2TMS	184	Ribose-meto-4TMS
101	Indol-3-acetic acid-2TMS	143	Monostearin-2TMS	185	Ribulose-meto-4TMS
102	Inositol-6TMS(2)	144	Myristic acid-TMS	186	Sarcosine-2TMS
103	Isocitric acid-4TMS	145	N6-Acetyllysine-2TMS	187	Sebacic acid-2TMS
104	Isoleucine-2TMS	146	N-Acetylglutamine-3TMS	188	Serine-2TMS
105	Isoleucine-TMS	147	N-Acetylmannosamine-meto-4TMS(1)	189	Serine-3TMS
106	Isomaltose-meto-8TMS(2)	148	N-Acetylneuraminic acid-6TMS	190	Sorbitol-6TMS
107	2-Isopropylmalic acid-3TMS	149	N-Acetyl-Ornithine-4TMS	191	Sorbose-meto-5TMS(2)
108	Isovalerylglycine-TMS	150	N-Acetylserine-2TMS	192	Stearic acid-TMS
109	2-Ketobutyric acid-meto-TMS(1)	151	Niacinamide-TMS	193	Suberic acid-2TMS
110	2-Ketoglutaric acid-3TMS	152	Nicotinic acid-TMS	194	Succinic acid-2TMS
111	2-Ketoglutaric acid-meto-2TMS	153	Nonanoic acid-TMS	195	Sucrose-8TMS
112	2-Ketoisocaproic acid-meto-TMS(1)	154	O-Acetylserine-2TMS	196	3-Sulfinoalanine-3TMS
113	2-Ketoisocaproic acid-meto-TMS(2)	155	Octadecanol-TMS	197	Tagatose-meto-5TMS(1)
114	2-Keto-isovaleric acid-meto-TMS	156	Octanoic acid-TMS	198	Threitol-4TMS
115	Kynurenine-3TMS	157	Oleamide-TMS	199	Threonic acid-4TMS
116	Lactic acid-2TMS	158	Oleic acid-TMS	200	Threonine-3TMS
117	Lactitol-9TMS	159	O-Phosphoethanolamine-4TMS	201	Trehalose-8TMS
118	Lactose-meto-8TMS(1)	160	Ornithine-3TMS	202	Triethanolamine-3TMS
119	Lactose-meto-8TMS(2)	161	Ornithine-4TMS	203	Tryptamine-2TMS
120	Lauric acid-TMS	162	Oxalic acid-2TMS	204	Tryptophan-3TMS
121	Leucine-2TMS	163	5-Oxoproline-2TMS	205	Tyramine-3TMS
122	Linoleic acid-TMS	164	Palmitic acid-TMS	206	Tyrosine-3TMS
123	Lysine-4TMS	165	Palmitoleic acid-TMS	207	Uracil-2TMS
124	Maleic acid-2TMS	166	Pantothenic acid-3TMS	208	Urea-2TMS
125	Malic acid-3TMS	167	ParaXanthine-TMS	209	Uric acid-4TMS
126	Maltose-meto-8TMS(1)	168	Phenylacetic acid-TMS	210	Uridine-3TMS
127	Mannitol-6TMS	169	Phenylalanine-2TMS	211	Uridine-4TMS
128	Mannose-meto-5TMS(2)	170	3-Phenyllactic acid-2TMS	212	Urocanic acid-2TMS
129	Margaric acid-TMS	171	3-Phosphoglyceric acid-4TMS	213	Valine-2TMS
130	Mesaconic acid-2TMS	172	Phosphoric acid-3TMS	214	Valproic acid-TMS
131	meso-Erythritol-4TMS	173	Proline-2TMS	215	Vanilmandelic acid-3TMS
132	Methionine sulfone-2TMS	174	2-Propyl-5-hydroxy-pentanoic acid-2TMS	216	Xanthine-3TMS
133	Methionine-2TMS	175	Psicose-meto-5TMS(2)	217	Xanthosine monophosphate-6TMS
134	3-Methoxy-4-hydroxybenzoic acid-2TMS	176	Putrescine-4TMS	218	Xylitol-5TMS
135	5-Methoxytryptamine-2TMS	177	Pyridoxal-meto-2TMS(1)	219	Xylose-meto-4TMS(1)
136	3-Methyl-2-oxovaleric acid-meto-TMS(1)	178	Pyridoxamine-4TMS	220	Xylose-meto-4TMS(2)
137	3-Methyl-2-oxovaleric acid-meto-TMS(2)	179	Pyrogallol-3TMS	221	Xylulose-meto-4TMS
138	2-Methyl-3-hydroxybutyric acid-2TMS(1)	180	Pyruvic acid-meto-TMS		

∗ : TMS and meto indicate trimethylsilylation and methoximation, respectively.

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