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Application Data Sheet

No.89

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of metabolites in human serum using GC-MS

Metabolome analysis, a comprehensive analysis of the various metabolites generated as biological functions are maintained, is widely used in disease biomarker searches and other investigations. To conduct these investigations, it is necessary to identify the metabolites contained in biological samples.
This application data sheet introduces the results of identifying metabolites in human serum utilizing the GC/MS Metabolite Database Ver. 2, which contains metabolites detected in biological samples, such as serum, urine, and cells.

Experimental

In the pretreatment process, 2-isopropylmalic acid was added as an internal standard to 50 µL of standard human serum, 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] and were analyzed in Q3 scan mode with the triple quadrupole GCMS-TQ8030 using the conditions shown in Table 1.

Table 1: Analytical Conditions

GC-MS:	GCMS-TQ8030
Column:	DB-5 (Length 30m, 0.25 mm I.D., df=1.00 µm)
Glass Liner :	Splitless insert with wool (PN:221-48876-03)

[GC]	
Injection temp.:	280 °C
Column oven temp.:	100 °C (4 min)→(4 °C /min)→320 °C (8 min)
Injection mode:	Splitless
Flow control mode:	Linear velocity (39.0 cm/sec)
Injection volume:	1 µL

[MS]	
Interface temp.:	280 °C
Ion source temp.:	200 °C
Data acquisition time:	4 to 67 min
Acquisition mode:	Scan
Mass range:	*m/z* 45-600
Event time:	0.3 sec

Results

Fig. 1 shows the resulting total ion current chromatogram (TIC), and Table 2 lists the metabolites identified. From the human serum, it was possible to identify 107 metabolites including 2-isopropylmalic acid, added as an internal standard.

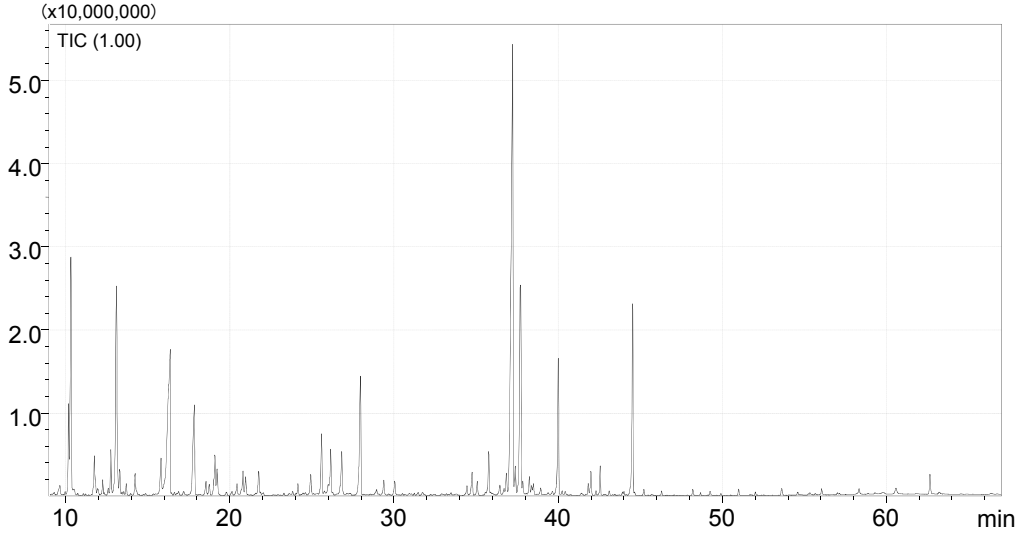

Fig. 1: Total Ion Current Chromatogram (TIC) for Metabolic Components Contained in Human Serum

[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

Table 2: List of Metabolites Identified

1	Acetoacetic acid	41	Glycyl-Glycine	81	5-Oxoproline
2	Adipic acid	42	Hippuric acid	82	Palmitic acid
3	Alanine	43	Homocysteine	83	Palmitoleic acid
4	2-Aminobutyric acid	44	2-Hydroxybutyric acid	84	Phenol
5	1,5-Anhydro-glucitol	45	3-Hydroxybutyric acid	85	Phenylalanine
6	1,6-Anhydroglucose	46	3-Hydroxyisobutyric acid	86	Phosphoric acid
7	Arabinose	47	2-Hydroxyisovaleric acid	87	Proline
8	Arabitol	48	3-Hydroxyisovaleric acid	88	Psicose
9	Arachidonic acid	49	4-Hydroxyphenyllactic acid	89	Putrescine
10	Arginine	50	4-Hydroxyproline	90	Pyruvic acid
11	Ascorbic acid	51	3-Hydroxypropionic acid	91	Ribitol
12	Asparagine	52	Hypoxanthine	92	Ribose
13	Aspartic acid	53	Indol-3-acetic acid	93	Ribulose
14	Azelaic acid	54	Inositol	94	Serine
15	Benzoic acid	55	Isocitric acid	95	Sorbitol
16	Citramalic acid	56	Isoleucine	96	Stearic acid
17	Citric acid	57	2-Isopropylmalic acid	97	Sucrose
18	Coniferyl aldehyde	58	Isovalerylglycine	98	Taurine
19	4-Cresol	59	2-Keto-isovaleric acid	99	Threonic acid
20	Cysteine	60	Kynurenic acid	100	Threonine
21	Cystine	61	Lactic acid	101	Tryptophan
22	2-Deoxytetronic acid	62	Lactose	102	Tyrosine
23	Elaidic acid	63	Lauric acid	103	Urea
24	Erythrulose	64	Leucine	104	Uric acid
25	Fructose	65	Linoleic acid	105	Valine
26	Fucose	66	Lysine	106	Xylitol
27	Fumaric acid	67	Maleic acid	107	Xylose
28	Galacturonic acid	68	Malic acid		
29	Glucaric acid	69	Maltose		
30	Gluconic acid	70	Mannitol		
31	Glucose	71	Mannose		
32	Glucuronic acid	72	Margaric acid		
33	Glutamic acid	73	Monostearin		
34	Glutaric acid	74	Myristic acid		
35	Glyceraldehyde	75	Naproxen		
36	Glyceric acid	76	Octadecanol		
37	Glycerol	77	Octanoic acid		
38	Glycerol 3-phosphate	78	Oleic acid		
39	Glycine	79	Ornithine		
40	Glycolic acid	80	Oxalic acid		

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