

Application Data Sheet

No.87

GC-MS

Gas Chromatograph Mass Spectrometer

Analysis of Metabolites Extracted from Human Embryonic Stem Cells 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 extracted from human embryonic stem (ES) cells utilizing the GC/MS Metabolite Database Ver. 2, which contains metabolites detected in biological samples, such as serum, urine, and cells.

Analysis Conditions

Human ES cell lines (KhES-1) were cultivated using mTeSR1 on matrigel-coated 60 mm culture dishes. We consulted reference sources [1] and [2] regarding the extraction of metabolites from human ES cells. The extracted metabolites were subjected to methoxime and trimethylsilyl derivatization, and were then analyzed in Q3 scan mode with the triple quadrupole GCMS-TQ8030 using the conditions shown in Table 1.

Table 1: Analysis Conditions

GC-MS:	GCMS-TQ8030		
Column:	DB-5 (Length 30 m; 0.25 mm I.D.; df = 1.00 μ m)		
Glass insert:	Splitless insert with wool (P/N: 221-48876-03)		
[GC]		[MS]	
Sample injection unit temp.:	280 °C	Interface temp.:	280 °C
Column oven temp.:	100 °C (4 min) \rightarrow (4 °C/min) \rightarrow 320 °C (8 min)	Ion source temp.:	200 °C
Injection mode:	Splitless	Data acquisition time:	4 min to 67 min
Carrier gas control:	Linear velocity (39.0 cm/sec)	Measurement mode:	Scan
Injection volume:	1 μ L	Mass range:	<i>m/z</i> 45-600
		Event time:	0.3 sec

Analysis Results

Figure 1 shows the resulting total ion current chromatogram (TIC), and Table 2 lists the metabolites identified. From the human ES cell extract, it was possible to identify 104 metabolites including 2-isopropylmalic acid, added as an internal standard.

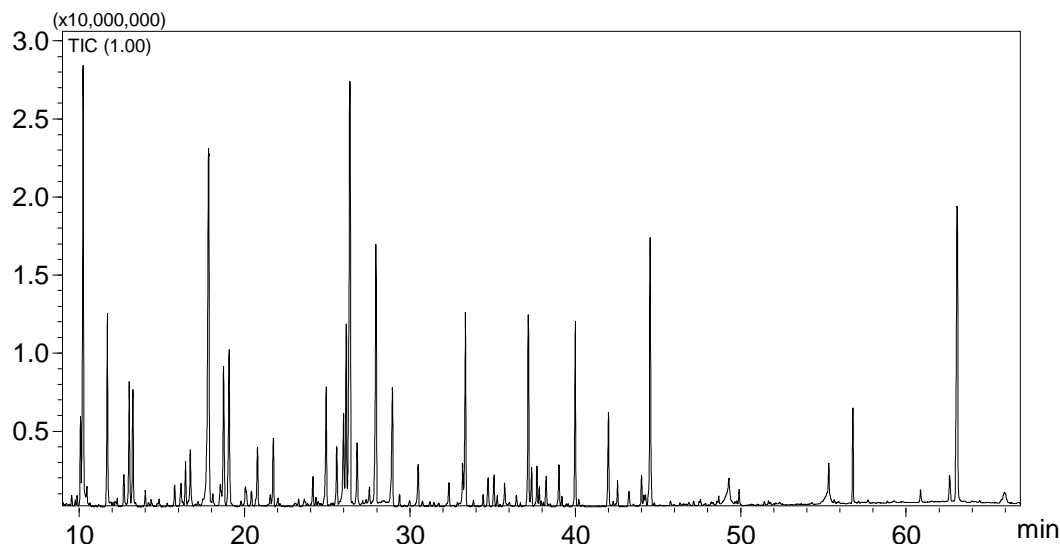


Fig. 1: Total Ion Current Chromatogram (TIC) for Metabolic Components Contained in Human ES Cell Extract

Table 2: List of Metabolites Identified

1	Adenine	36	Glycerol 2-phosphate	71	Myristic acid
2	Adenosine monophosphate	37	Glycerol 3-phosphate	72	N-Acetylaspartic acid
3	Adenosine	38	Glycine	73	Niacinamide
4	Alanine	39	Glycolic acid	74	Nonanoic acid
5	2-Amino adipic acid	40	Glycyl-Glycine	75	Octadecanol
6	4-Aminobutyric acid	41	1-Hexadecanol	76	Octanoic acid
7	3-Aminopropanoic acid	42	Histidine	77	Oleic acid
8	1,5-Anhydro-glucitol	43	Homocysteine	78	O-Phosphoethanolamine
9	Arachidonic acid	44	2-Hydroxyadipic acid	79	O-Phospho-Serine
10	Arginine	45	3-Hydroxybutyric acid	80	Ornithine
11	Ascorbic acid	46	2-Hydroxyglutaric acid	81	Oxalic acid
12	Asparagine	47	3-Hydroxyisobutyric acid	82	5-Oxoproline
13	Aspartic acid	48	3-Hydroxyisovaleric acid	83	Palmitic acid
14	Cadaverine	49	3-Hydroxypropionic acid	84	Palmitoleic acid
15	Cholesterol	50	Hypotaurine	85	Pantothenic acid
16	Citric acid	51	Inositol	86	Phenylalanine
17	Cystathionine	52	Isocitric acid	87	Phosphoenolpyruvic acid
18	Cysteine	53	Isoleucine	88	3-Phosphoglyceric acid
19	Dihydroxyacetone phosphate	54	Isomaltose	89	Phosphoric acid
20	Elaidic acid	55	2-Isopropylmalic acid	90	Proline
21	Fructose 1-phosphate	56	2-Ketoglutaric acid	91	Putrescine
22	Fructose 6-phosphate	57	Kynurenic acid	92	Pyruvic acid
23	Fructose	58	Kynurenine	93	Ribose 5-phosphate
24	Fumaric acid	59	Lactic acid	94	Ribose
25	Glucaric acid	60	Lauric acid	95	Ribulose
26	Glucosamine	61	Leucine	96	Sedoheptulose 7-phosphate
27	Glucose 6-phosphate	62	Lysine	97	Serine
28	Glucose	63	Maleic acid	98	Sorbitol
29	Glucuronic acid lactone	64	Malic acid	99	Stearic acid
30	Glucuronic acid	65	Maltose	100	Threonic acid
31	Glutamic acid	66	Margaric acid	101	Threonine
32	Glutamine	67	Methionine	102	Tyrosine
33	Glutaric acid	68	7-Methylguanane	103	Valine
34	Glyceraldehyde	69	3-Methyl-2-oxovaleric acid	104	Xylitol
35	Glyceric acid	70	Monostearin		

Note: The human ES cells were provided by Dr. Kazuhiro Aiba and Prof. Norio Nakatsuji, Institute for Integrated Cell-Material Sciences, Kyoto University.

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