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Application Data Sheet

No.102

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Glycolysis Metabolites in Human Embryonic Stem Cells 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 systems are widely used for the analysis of metabolomes, since they are capable of superior chromatographic separations and offer stable analysis. On the other hand, biological samples include numerous metabolites and complex matrices, which can sometimes make separations using single quadrupole GC-MS difficult. Since triple quadrupole GC-MS/MS performs MS separation two times, the effects of the interfering components can be eliminated, thus making the analysis of a variety of metabolites possible.

This Application Data Sheet presents the results of the analysis of glycolysis metabolites extracted from human embryonic stem cells using an MRM method file included in the Smart Metabolites Database.

Analysis Conditions

Embryonic stem cell extracts collected respectively from four dishes (60 mm) were subjected to trimethylsilylation (TMS). For detailed procedures regarding the extraction and derivatization of metabolites from cells, refer to Shimadzu Journal vol.2.

The analysis conditions are shown in Table 1.

Table 1: Analysis Conditions

GC-MS/MS:	GCMS-TQ8040		
Column:	DB-5 (Length 30 m; 0.25 mm I.D.; df = 1.00 μm)		
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) → (4 °C/min) → 320 °C (8 min)	Ion source temp.:	200 °C
Injection mode:	Splitless	Measurement mode:	MRM
Carrier gas control:	Linear velocity (39.0 cm/sec)	Loop time:	0.3 sec
Injection volume:	1 μL		

Table 2: MRM Conditions

Compound Name	Retention Time (min)	Retention Index	MRM Monitoring *m/z* Precursor>Product
Pyruvic acid-meto-TMS	9.415	1047	174.00>74.00
Lactic acid-2TMS	9.873	1061	219.00>147.10
Phosphoenolpyruvic acid-3TMS	28.119	1611	369.10>147.10
Glyceraldehyde 3-phosphate-meto-3TMS(2)	31.624	1734	328.10>298.10
Dihydroxyacetone phosphate-meto-3TMS(1)	32.302	1760	315.20>299.10
2-Phosphoglyceric acid -4TMS	33.410	1799	459.10>299.10
3-Phosphoglyceric acid-4TMS	34.092	1825	387.20>73.00
Glucose-meto-5TMS(1)	36.799	1930	319.10>129.10
2,3-Bisphosphoglyceric acid-5TMS	43.741	2225	315.10>73.00
Fructose 6-phosphate-meto-6TMS	46.507	2354	459.20>315.10
Glucose 6-phosphate-meto-6TMS(1)	46.799	2368	387.20>73.00
2-Isopropylmalic acid-3TMS(I.S.)	27.589	1593	349.10>259.10

Analysis Results

By means of analysis with MRM mode using a GC-MS/MS system, components that were difficult to detect using single quadrupole GC-MS were successfully detected (Fig. 1). (For the results obtained using single quadrupole GC-MS, refer to LAAN-J-MS-E103 "Analysis of metabolites in an extract of human embryonic stem cells using GC-MS.") Moreover, good precision of analysis repeatability could be obtained for a great number of compounds (Table 3). Thus, as explained above, by using GC-MS/MS, the analysis of a broad range of metabolites becomes possible.

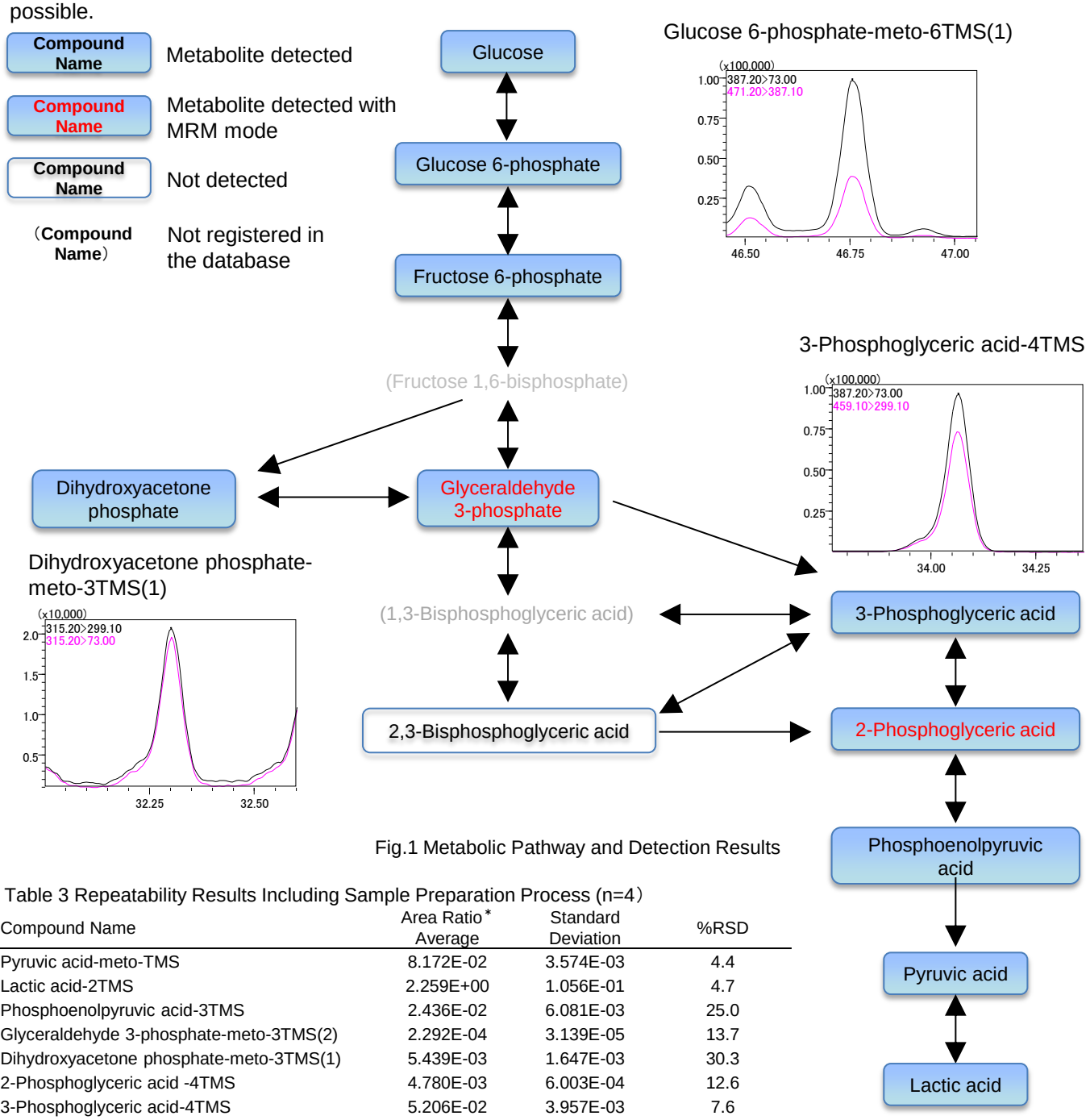

Fig.1 Metabolic Pathway and Detection Results

Table 3 Repeatability Results Including Sample Preparation Process (n=4)

Compound Name	Area Ratio* Average	Standard Deviation	%RSD
Pyruvic acid-meto-TMS	8.172E-02	3.574E-03	4.4
Lactic acid-2TMS	2.259E+00	1.056E-01	4.7
Phosphoenolpyruvic acid-3TMS	2.436E-02	6.081E-03	25.0
Glyceraldehyde 3-phosphate-meto-3TMS(2)	2.292E-04	3.139E-05	13.7
Dihydroxyacetone phosphate-meto-3TMS(1)	5.439E-03	1.647E-03	30.3
2-Phosphoglyceric acid -4TMS	4.780E-03	6.003E-04	12.6
3-Phosphoglyceric acid-4TMS	5.206E-02	3.957E-03	7.6
Glucose-meto-5TMS(1)	4.596E+00	6.496E-01	14.1
2,3-Bisphosphoglyceric acid-5TMS	0.000E+00	0.000E+00	N/A
Fructose 6-phosphate-meto-6TMS	5.395E-03	2.992E-04	5.5
Glucose 6-phosphate-meto-6TMS(1)	4.907E-02	1.832E-03	3.7

* : Value divided by the area value for 2-isopropylmalic acid-3TMS

Note: The human embryonic stem cell samples were provided by Dr. Kazuhiro Aiba and Prof. Norio Nakatsuji of the Institute for Integrated Cell-Material Sciences, Kyoto University.

First Edition: February, 2015

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