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Technical Report

Determination of Fatty-acid Composition of Lipids in Human Embryonic Stem Cells Using GC-MS

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Abstract:

The cellular lipids were extracted from frozen pellets of human ES (embryonic stem) cells, and the constituent fatty acids were esterified to form fatty acid methyl esters. GC/MS analysis of the methylated fatty acids resulted in identification of 19 types of fatty acids. This report introduces the pretreatment protocol used to extract the lipids from the cells, and the fatty acids that can be analyzed by this process.

Keywords: human ES cells, fatty acids, gas chromatography, quadrupole mass spectrometer, GC/MS

1. Introduction

Lipids are one of the major components that make up the living body. In addition to the roles they play in the storage of energy and as a component of the lipid bilayer of the cell membrane, lipids are very important biological molecules involved in signal transduction and membrane transport.

Lipids typically exist in a state in which various fatty acids are bound to a backbone such as glycerol, sphingosine, cholesterol, etc. With recent advances in analytical techniques, especially those associated with mass spectrometry, identification of lipid components, including their molecular species, is becoming possible. However, it is still difficult to identify all the components of lipids. Fatty acids, as mentioned above, are a major component of lipids, and obtaining information on the fatty acid composition of all the lipids contained in a biological sample is a critical step in comprehensive lipid analysis. An effective approach to analyzing fatty acid content is to apply gas chromatography/mass spectrometry (GC/MS) to the analysis of the constituent fatty acids of derivatized methyl esters.

The above method was examined to investigate its applicability to fatty acid analysis of human ES cells.

2. Experiment

2-1. Reagents

The Supelco® 37 Component FAME Mix and the Methyl all-*cis*-7,10,13,16,19-docosapentaenoate (Sigma-Aldrich Japan) products were used as the methylated fatty acid standards. For the internal standard, heptadecanoic-17,17,17-d3 acid (CDN Isotopes Inc.) was dissolved in methanol at a concentration of 0.2 mg/mL.

2-2. Preparation of Human ES Cell Sample

Culturing of the human ES cell line (KhES-1), was conducted at the Kyoto University Institute for Frontier Medical Sciences. The cells were collected in Eppendorf tubes by the trypsin-EDTA method (2 × 10^6 cells/tube), and were stored at –80°C until use.

2-3. Extraction from Cells

Extraction from the frozen cell pellet was conducted according to the cited literature. A vortex mixer was used to prepare a suspension after adding 600 µL of cold acetone to the cell pellet, and the cells were frozen with liquid nitrogen. The samples were then dissolved and treated for five minutes using ultrasonication. After repeating the above operation three times, the suspended cells were incubated for 1 hour at –20°C. Centrifuge separation was then conducted (15,000 rpm, 15 min), and the supernatant was collected in an autosampler vial. Then, 400 µL of extract solution (methanol : MilliQ water : formic acid = 86.5 : 12.5 : 1.0) was added to the cell pellet. After stirring for 1 minute in a vortex mixer, the mixture was ultrasonicated, and the cell suspension was then incubated for one hour at –20°C. Then, following centrifugation (15,000 rpm, 15 min), the supernatant was collected in the abovementioned vial. The organic solvent was then removed from the extract solution through exposure to flowing nitrogen gas, and the resulting mixture was freeze-dried.

The above extraction operation is shown in the flow chart of Fig. 1.

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2-4. Methyl Esterification of Fatty Acids

25 µL of 0.2 mg/mL Heptadecanoic-17,17,17-d3 acid (methanol solution) was added to the dried sample. Methyl-esterification of the fatty acids and purification of the methylated fatty acids were conducted using the Nacalai Tesque fatty acid methylation kit (P/N: 06482-04) and the methylated fatty acid purification kit (P/N: 06483-94). The methyl-derivatized fatty acid sample was concentrated to approximately 0.5 mL through exposure to flowing nitrogen gas, and this was used as the GC/MS sample.

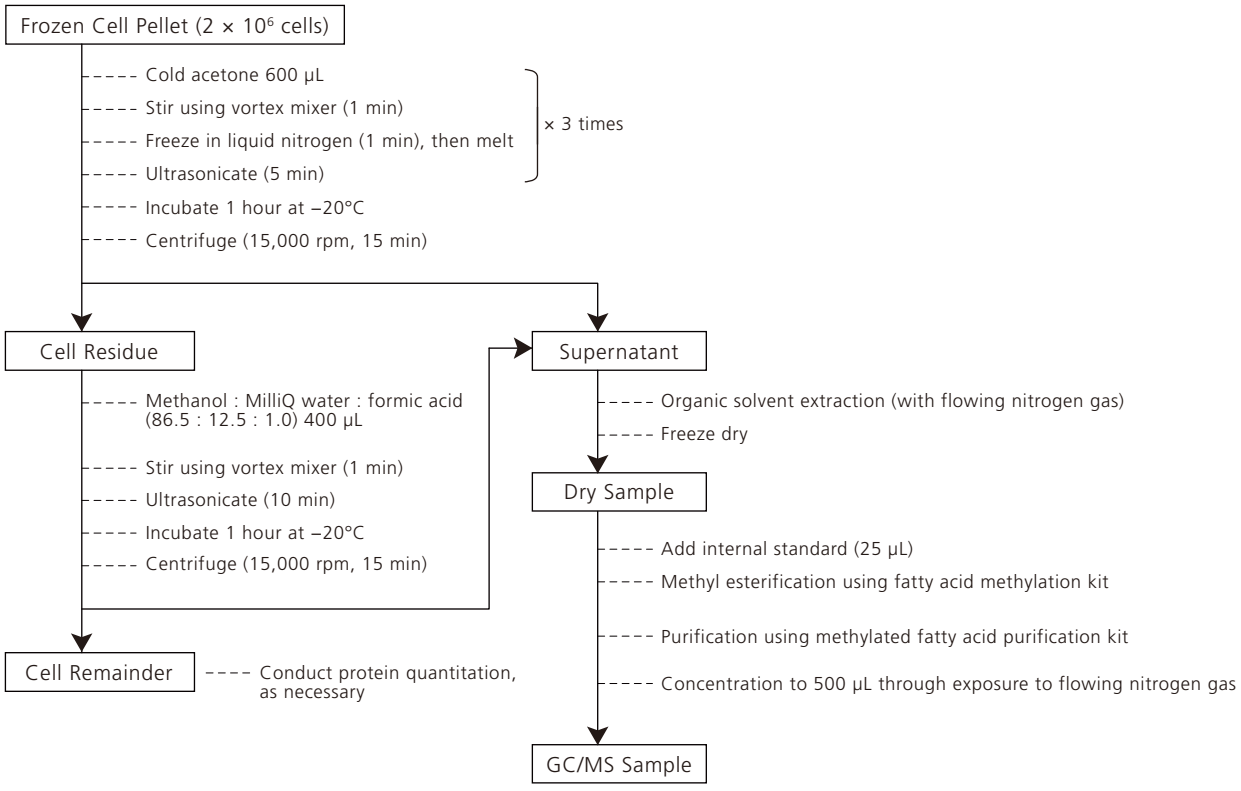

Fig. 1 Sample Preparation Procedure for GC/MS Analysis

2-5. Analytical Conditions

The GC/MS analytical conditions are shown in Table 1.

Table 1 GC/MS Analytical Conditions

Instrument	GCMS-QP2010 Ultra, AOC-20i + s (Auto injector)
[GC Conditions]	
Column:	SP™-2560 Sigma-Aldrich Japan (Length 100 m, I.D. 0.25 mm, film thickness 0.20 µm)
Injection temperature:	250°C
Column temperature:	40°C (2 min) - (4°C/min) - 240°C (15 min)
Injection mode:	Splitless
Carrier gas:	He (Constant Linear Velocity)
Linear velocity:	20.0 cm/sec
Injection volume:	1 µL
[MS Conditions]	
Ion source temperature:	200°C
Interface temperature:	250°C
Measurement range:	*m/z* 40–500
Event time:	0.3 sec
Scan speed:	1666 u/sec

3. Results

Fig. 2 shows the TIC (total ion chromatogram) obtained from analysis of the methyl ester derivatives of a human ES cell line (KhES-1) sample. In addition, Table 2 shows a list of fatty acid methyl esters that were detected. GC/MS analysis of these methyl ester derivatives from the human ES cell sample resulted in identification of 19 fatty acids.

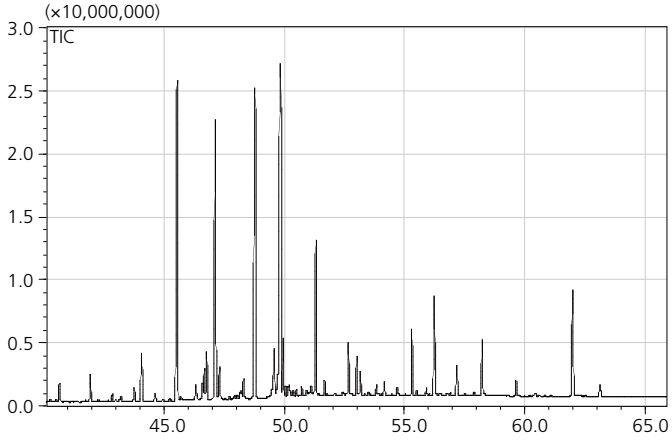

Fig. 2 TIC of Methyl Ester Derivatives from Human ES Cell Extract Sample

Table 2 List of Detected Fatty Acid Methyl Esters

Compound Name	Retention Time (min)	Retention Index	Similarity*	S/N
Methyl myristate	41.925	2204	98	380
Methyl pentadecanoate	43.755	2309	99	372
Methyl palmitate	45.505	2413	98	12739
Methyl palmitoleate	46.760	2490	95	143
Methyl margarate	47.150	2515	98	606
Methyl stearate	48.735	2619	98	29218
Methyl oleate	49.790	2689	96	96
Methyl linoleate	51.300	2793	99	1101
Methyl arachisate	51.690	2821	98	811
Methyl *cis*-11-icosenoate	52.675	2891	99	1602
Methyl linolenate	53.005	2913	98	122
Methyl *cis*-11,14-Icosadienoate	54.165	2991	98	124
Methyl eicosa-8,11,14-trienoate	55.315	3069	99	632
Methyl erucate	55.535	3083	98	93
Methyl *cis*-11,14,14-Icosatrienoate	55.910	3108	97	24
Methyl arachidonate	56.240	3130	99	457
Methyl *cis*-5,8,11,14,17-Eicosapentaenoate	58.230	3254	99	243
Methyl *cis*-7,10,13,16,19-docosapentaenoate	61.995	3478	98	617
Methyl *cis*-4,7,10,13,16,19-Docosahexaenoate	63.150	3547	99	62
Methyl margarate-d3	47.085	2511	97	19864

*Similarity reflects the results obtained in the NIST mass spectrum library search.

4. Summary

Identification of 19 fatty acids in human ES cells was possible using this method, demonstrating that GC/MS is effective for determination of fatty acids. It is believed that the method introduced here can be applied effectively to fatty acid composition analysis for various types of cultured cells.

References

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Fatty Acid Analysis in Total Lipids Using a Shimadzu GC/MS

GC-MS can be used to conduct fatty acid analysis in total lipids. The fatty acids that comprise the lipids are converted to methyl esters, which are then measured by GC-MS.

In addition to the MS electron ionization (EI) method, the positive ion chemical ionization (PCI) method is used when there are components present in the matrix that interfere with MS ionization. The EI method is typically used for ionization when conducting analysis of fatty acid methyl esters by GC-MS. However, due to the many fragment ions that are generated during EI ionization, even ions which are not adversely affected by interfering substances may display decreased intensity. In such instances, since mainly only molecular ions are formed when using the PCI method, using that ion permits separation of the target fatty acid methyl esters from any interfering substances, thereby permitting high-sensitivity analysis.

Separation of the interfering substances can be accomplished extremely effectively by using a triple quadrupole gas chromatograph mass spectrometer (GC-MS/MS) for conducting MS.

The Shimadzu GCMS-QP2010 Ultra offers the features and performance necessary for conducting fatty acid analysis in total lipids.

1. Features a wide dynamic range to permit measurement of fatty acids present at widely varying concentrations, from extremely high to low concentrations.
2. Effective measurement of highly contaminated samples is also possible using PCI (option).
3. The GC/MS metabolite database for the GCMS-QP2010 Series offers method files that include the optimal conditions and quantitation parameters for analysis of the fatty acids in total lipids.
4. Includes retention indices for effective identification of isomers of fatty acids.

Gas Chromatograph / Mass Spectrometer

GCMS-QP2010 Ultra

GCMS-QP2010 Ultra Features
1. Wide dynamic range
2. Various ionization options (EI, PCI*, NCI*)
3. Compound identification using retention indices

*Options

GC/MS Metabolite Database
(Amino Acids, Fatty Acids, Organic Acids)

The GC/MS Metabolite Database is a library designed for the GCMS-solution Workstation, the software package used with the GCMS-QP2010 Series gas chromatograph mass spectrometer.

The mass spectrum library with retention indices significantly reduces the number of candidate compounds, thereby improving the reliability of results.

This database contains four types of method files including information such as analytical conditions, mass spectra and retention indices, four types of libraries including compound information along with CAS numbers, mass spectra, and retention indices, as well as a handbook (published library information). The methods and libraries contain spectra for a wide range of metabolite-related substances, including amino acids, fatty acids, and organic acids. The database contains 261 spectra that were obtained using the electron ionization method, and 50 spectra that were obtained using the positive ion chemical ionization method.

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