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Development of an analytical method for human blood triglycerides using triple quadrupole mass spectrometer

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1. Overview

A method for the analysis of triglycerides in human blood was developed using liquid chromatograph - triple quadrupole mass spectrometer (LC-MS/MS).

2. Introduction

Triglycerides (TGs) are important energy storage molecules in animals. They play an important role in transporting energy in blood, but excess triglycerides are thought to promote atherosclerosis or other circulatory diseases. Conventional blood tests estimate the total amount of triglycerides but do not provide quantitative information about the fatty acids bound to the various triglycerides.

This research describes an LC/MS/MS method developed for the analysis of triglycerides in blood. The developed method enables to analyze the 47 kinds of triglycerides with different molecular weights in 11 minutes (130 analysis / day). Two commercially available human plasma and serum were analyzed, and the slight differences of triglyceride could be captured by principal component analysis (PCA).

3. Methods and Pretreatment

The Nexera™ UHPLC system and LCMS™-8060 (Shimadzu Corporation, Kyoto, Japan) were used to make measurements (Fig 1). LC and MS conditions are shown in Table 1. Two human plasma samples (Plasma 1, 2) and human serum samples (Serum) were purchased from Kohjin Bio Co., Ltd. These were pretreated as shown in Fig 2. Glyceryl trilinolenate (SIGMA-ALDRICH) was used as an internal standard. After extraction, each sample was analyzed three times.

Fig 1. LC-MS/MS system (Nexera ™ X2 + LCMS ™ -8060)

Table 1 Analytical Conditions

[HPLC Conditions] (Nexera X2)	
Column:	Shim-pack Velox™, C18 (50 mm × 2.1 mm I.D., 2.7 μm)
Column Oven:	45 °C
Solvent A:	20 mM Ammonium formate - water
Solvent B:	2-Propanol/Acetonitrile (80/20, v/v)
Flowrate:	0.4 mL/min
Injection Volume:	3 μL
[MS Conditions] (LCMS-8060)	
Ionization:	ESI, Positive
Mode:	MRM
Nebulizing Gas :	2.5 L/min
Drying Gas	10.0 L/min
Heating Gas	10.0 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	150 °C
CID Gas Pressure:	230 kPa

Fig 2 Sample Pretreatment

1. Plasma or Serum 20 μL
   +
   Methanol/butanol (1/1) 960 μL
   +
   Internal standard (5 μg/mL) 20 μL in methanol
   ↓
2. Stirring (3 minutes)
   ↓
3. Centrifugation (15 minutes)
   ↓
4. Dilute the supernatant with methanol/butanol (1/1)
   ↓
5. LC/MS/MS analysis 3 μL

4. Analysis of triglycerides

Triglycerides have a structure in which three fatty acid molecules are esterified to glycerol (Fig. 3).

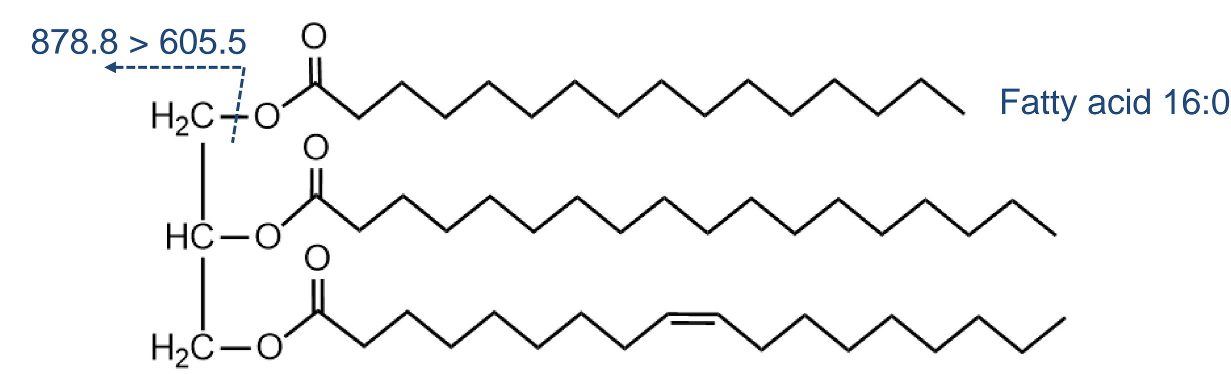


Fig. 3 Chemical Structure of a Triglyceride (TG 16:0/18:0/18:1)

For MRM of triglycerides, ammonium adduct ion was set as the precursor ion, and the ion detected by neutral loss (NL) of fatty acids was set as the product ion. For example, for the compound in Fig 3, the MRM transition of 878.8 > 605.5 measures TG 16:0_36:1, which is neutral loss of fatty acid 16:0 from TG 52:1. The notation of triglycerides was decided according to Reference. Briefly, the fatty acids detected in triglycerides are shown in front of the underscore, followed by the sum of the remaining two acyl residues.

In this method, fatty acids which have a carbon number from C14 to C22 and a degree of unsaturation from 0 to 6 are considered. In addition to 195 MRM transitions targeting 47 triglycerides in blood, an MRM transition of Glyceryl trilinolenate (TG 54:9) was prepared as an internal standard.

5. Results

Representative MRM Chromatograms

An MRM chromatogram obtained by plasma analysis is shown in Fig 4. TG 52:2 was detected as the highest peak. TG 54:9 added as an internal standard eluted in 2.2 min, and all target compounds eluted within 8 min. Triglycerides with the same carbon number tended to elute faster as the number of double bonds increased.

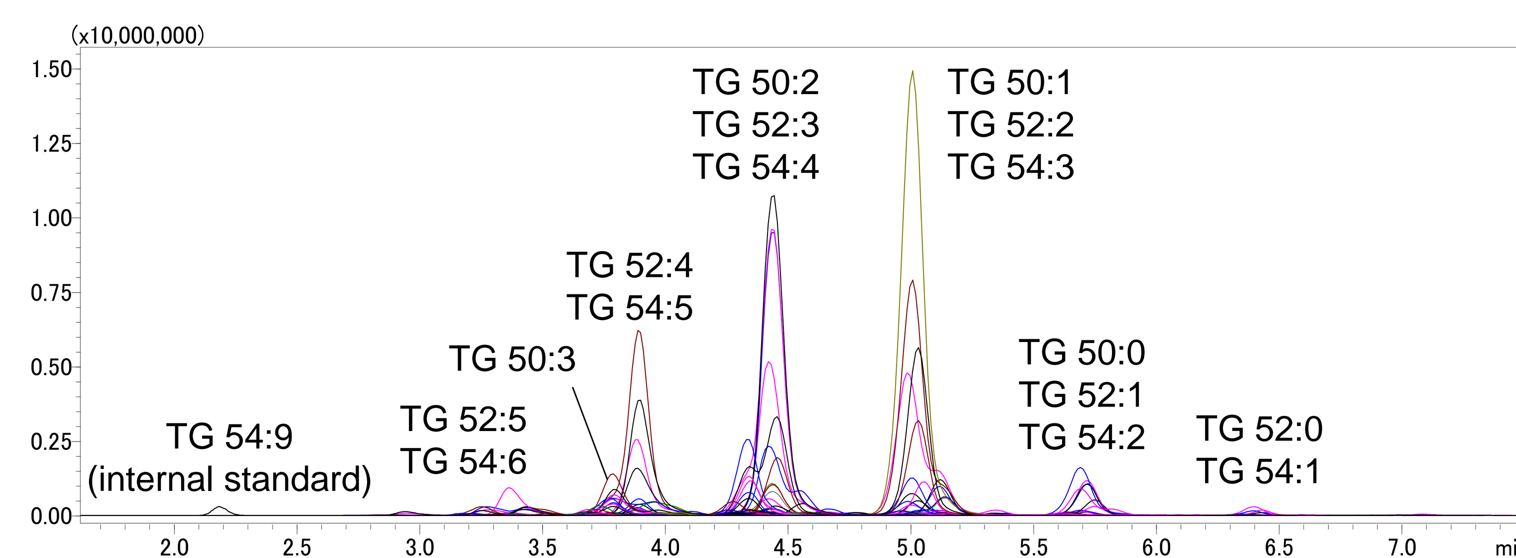
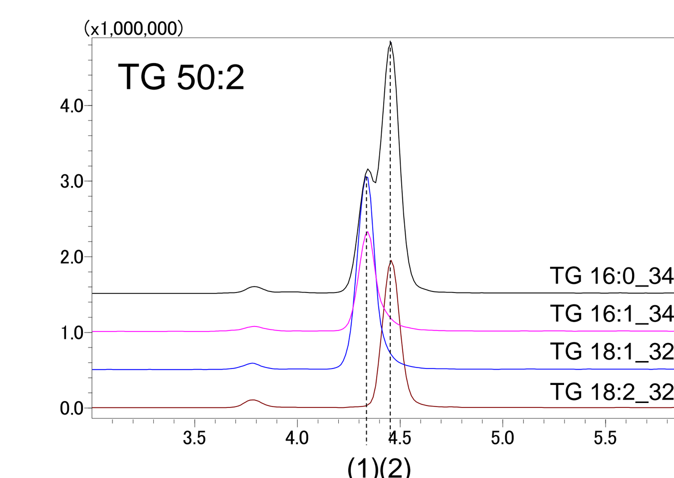
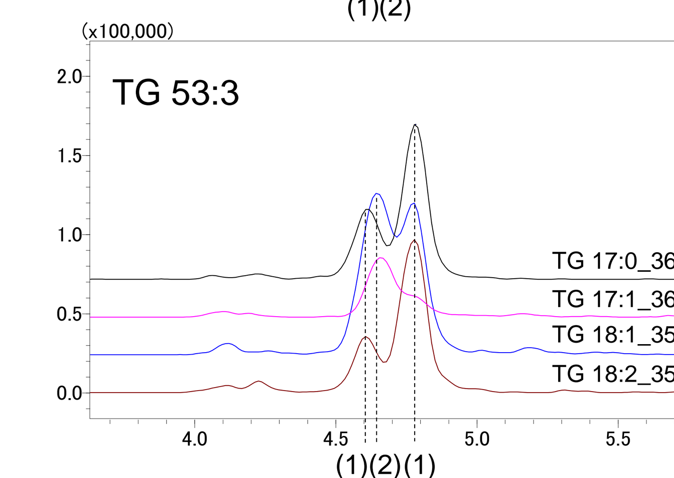


Fig. 4 196 MRM Chromatogram Obtained by Analyzing Triglycerides in Plasma (Overlaid)
Some Triglycerides with Large Area Values are Labeled.





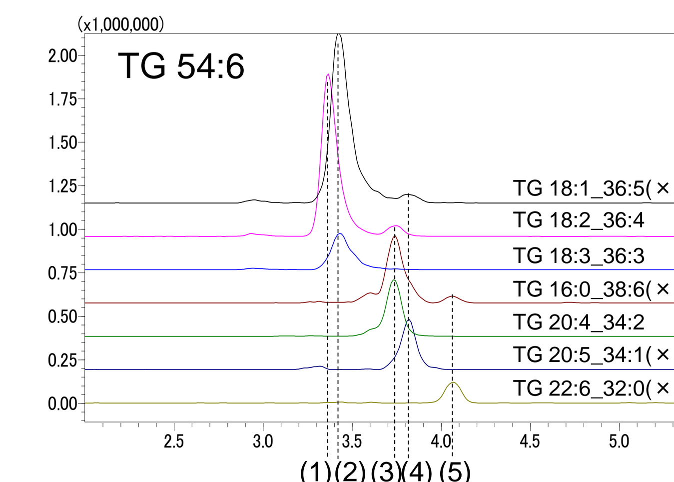


Fig 5. MRM Chromatogram of TG 50:2, TG 53:3 and TG 54:6

The representative MRM chromatograms of TG 50:2, TG 53:3 and TG 54:6 are shown in Fig 5. In TG 50:2, peaks were detected in the MRM chromatogram to monitor neutral loss of fatty acids 16:0, 16:1, 18:1, and 18:2. As neutral loss-derived peaks of fatty acids 16:0, 16:1, and 18:1 were detected at the retention time 4.33 min, the fatty acid combination was estimated to be (1) TG 16:0_16:1_18:1. Similarly, as neutral loss-derived peaks of fatty acids 16:0 and 18:2 were detected at 4.46 min, the fatty acid combination was estimated to be (2) TG 16:0_16:0_18:2. The binding sites of fatty acids and glycerol are not considered. As with TG 50:2, isomers of TG 53:3 and TG 54:6 were also estimated.

As described above, 107 isomers with different fatty acid could be estimated among 47 triglycerides.

Data analysis

The area values of the peaks in 195 MRM chromatograms were used for data analysis. When the peak tops were divided into two or more peaks, multiple peaks were processed in a batch, and the total area value was used for analysis. Corrections were made by dividing each area value by the area value of the internal standard.

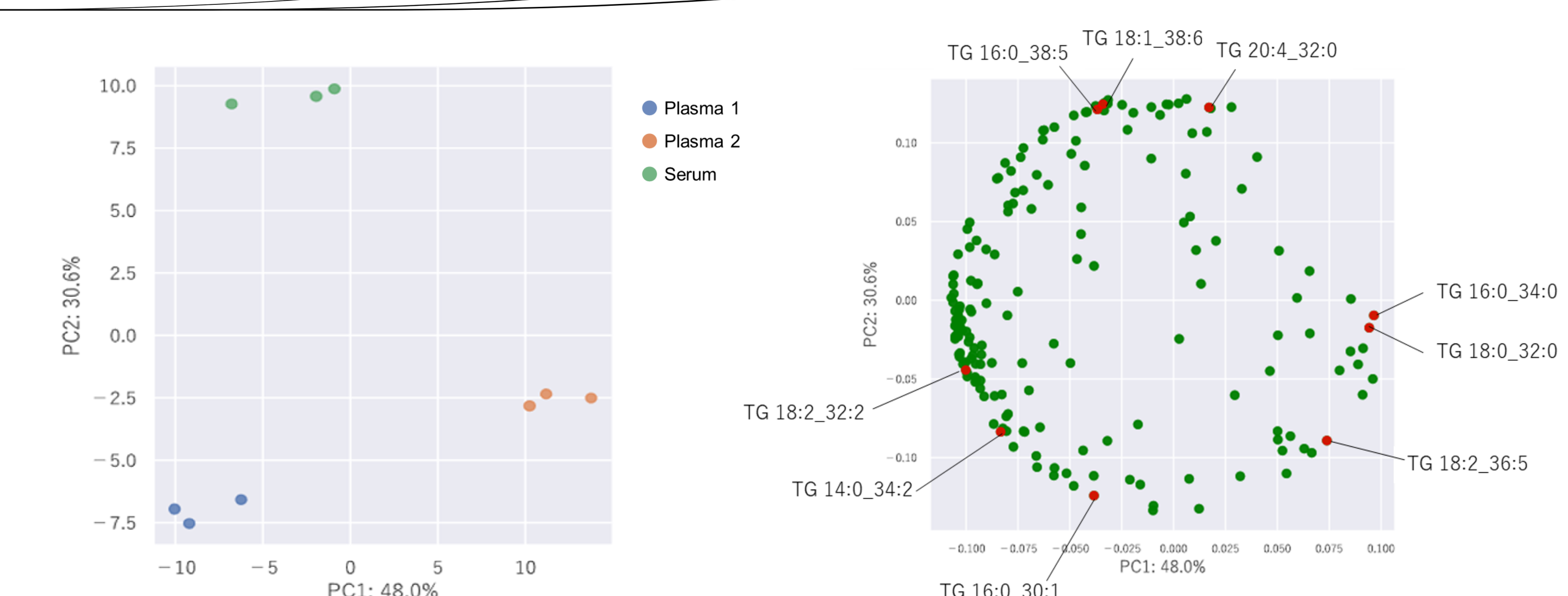


Fig. 7 Principal Component Analysis
(a) Score Plot, (b) Loading Plot

Principal component analysis was performed on the two human plasma and one human serum samples. As a result, they were divided into three groups on the score plot (Fig 7, a). One-way ANOVA showed significant differences ($p < 0.01$) for 109 of the 181 components. Some components that showed significant differences are highlighted in red on the loading plot (Fig 7, b).

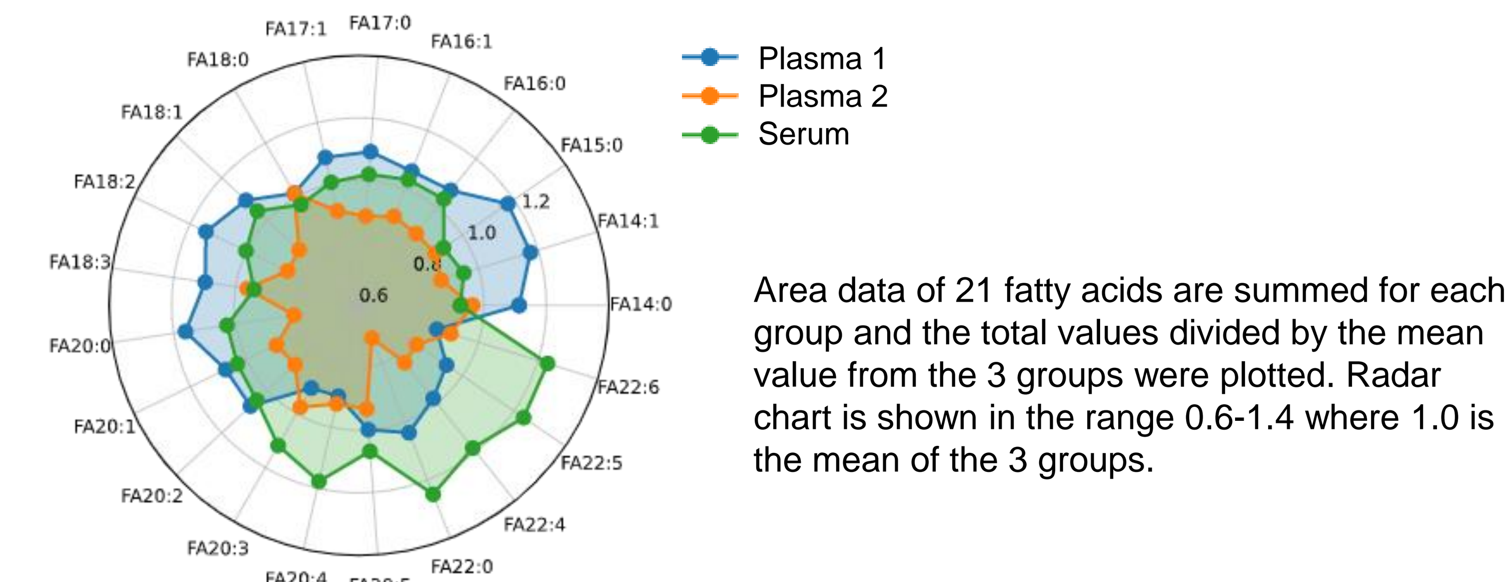


Fig. 8 Fatty Acids Constituting Triglycerides in Blood Samples

Area data of 21 fatty acids constituting triglycerides are displayed in a radar chart (Fig 8). All fatty acids except 18:0 were relatively low in Plasma 2. Polyunsaturated fatty acids (PUFA) such as FA 20:4, 22:4, 22:5, and 22:6 were found to be abundant in Serum.

6. Conclusion

A method for the analysis of triglycerides in human blood was developed. The method enables 47 triglycerides with different molecular weights to be analyzed in 11 minutes per cycle. Since this analysis method can detect the minute differences in blood triglycerides with high throughput (130 analysis/day), it is expected to be useful for disease biomarker discovery.

Reference

Liebisch G, Fahy E, Aoki J, *et al.* Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures. J Lipid Res. 2020;61(12):1539-1555. doi:10.1194/jlr.S120001025

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