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Application News

LCMS™-8060 High-Performance Liquid Chromatograph Mass Spectrometer

Development of an Analytical Method for Blood Triglycerides Using Triple Quadrupole Mass Spectrometer

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User Benefits

- The method allows the measurement of 47 triglycerides of different molecular weights in 11 minutes.
- Enables estimation of the combination of fatty acids in triglycerides.
- Useful for biomarker research by high-throughput screening.

■ 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 article describes an LC/MS/MS method developed for the analysis of triglycerides in blood. The method uses 195 multiple reaction monitoring (MRM) transitions to allow the measurement of 47 triglycerides with different molecular weights in 11 minutes (130 analyses/day). Using this method, we analyzed two commercially available human plasma samples and one human serum sample. The slight differences between samples were captured by multivariate analysis. This method is available as the "LC/MS/MS MRM Library for Triglycerides."

■ Analysis of Triglycerides

Triglycerides have a structure in which three fatty acid molecules are esterified to glycerol (Fig. 1).

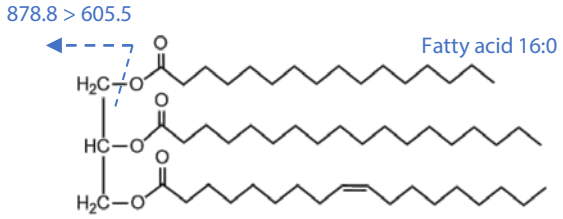


Fig. 1 Chemical Structure of a Triglyceride (TG 16:0/18:0/18:1)

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 1, 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 Ref 1. 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.

■ Analytical Conditions

The Nexera™ UHPLC system and LCMS-8060 were used to make measurements.

Table 1 Analytical Conditions

[HPLC Conditions] (Nexera)	
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 Flow:	2.5 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
DL Temp.:	250 °C
Block Heater Temp.:	400 °C
Interface Temp.:	150 °C
CID Gas Pressure:	230 kPa

* P/N: 227-32009-02

■ Sample Pretreatment

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.

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

Fig. 2 Sample Pretreatment

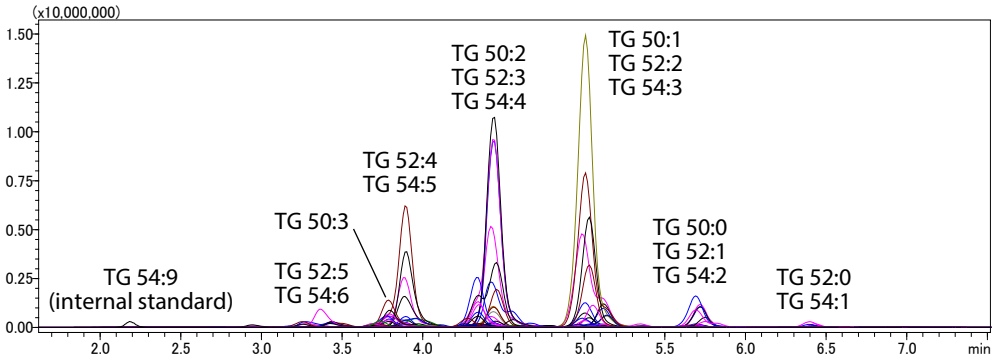


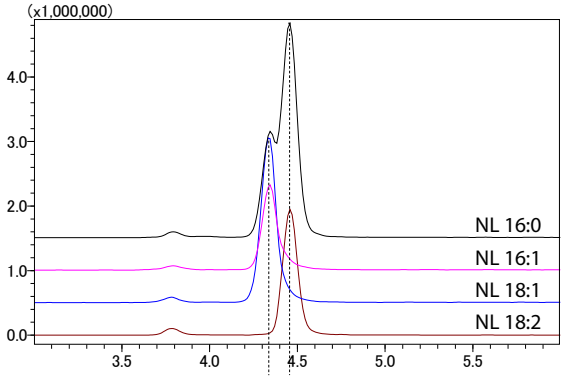
Fig. 3 196 MRM Chromatogram Obtained by Analyzing Triglycerides in Plasma (Overlaid)
Some Triglycerides with Large Area Values are Labeled.

■ Representative MRM Chromatograms

An MRM chromatogram obtained by plasma analysis is shown in Fig 3. TG 52:2 was detected as the highest peak.

The MRM chromatogram of TG 50:2 is shown in Fig 4. 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.

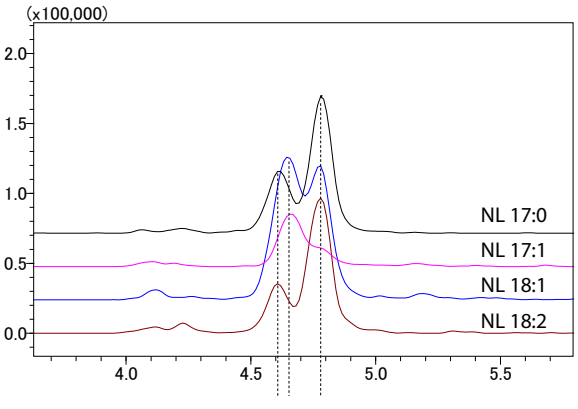


(1) (2)

Fig. 4 MRM Chromatogram of TG 50:2

The MRM chromatogram of TG 53:3 is shown in Fig 5. Peaks were detected in the MRM chromatogram to monitor neutral loss of fatty acids 17:0, 17:1, 18:1, and 18:2. Odd-chain fatty acids, such as 17:0 and 17:1, were also detected in plasma.

As with TG 50:2, the fatty acid combinations were estimated to be (1) TG 17:0_18:1_18:2 and (2) TG 17:1_18:1_18:1. Regarding (1), two peaks were detected probably because they contain isomers with different fatty acid binding positions or *cis-trans* isomers.

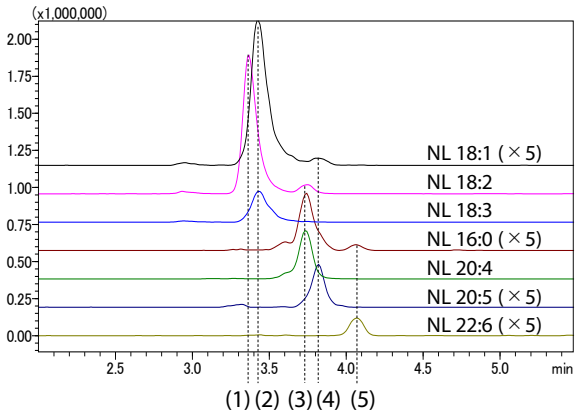


(1) (2) (1)

Fig. 5 MRM Chromatogram of TG 53:3

The MRM chromatogram of TG 54:6 is shown in Fig 6. Peaks were detected in the MRM chromatogram to monitor neutral loss of fatty acids 18:1, 18:2, 18:3, 16:0, 20:4, 20:5, and 22:6. Polyunsaturated fatty acids such as 18:2, 18:3, 20:4, 20:5, and 22:6 were also detected in plasma.

As with TG 50:2, the fatty acid combinations were estimated to be (1) TG 18:2_18:2_18:2, (2) TG 18:1_18:2_18:3, (3) TG 16:0_18:2_20:4, (4) TG 16:0_18:1_20:5, and (5) TG 16:0_16:0_22:6.



(1) (2) (3) (4) (5)

Fig. 6 MRM Chromatogram of TG 54:6
For MRM Chromatograms Derived from Fatty Acids 18:1, 16:0, 20:5, and 22:6, the Vertical Axis is Magnified 5 Times.

As described above, 107 isomers with different fatty acid binding positions 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 (Table 2).

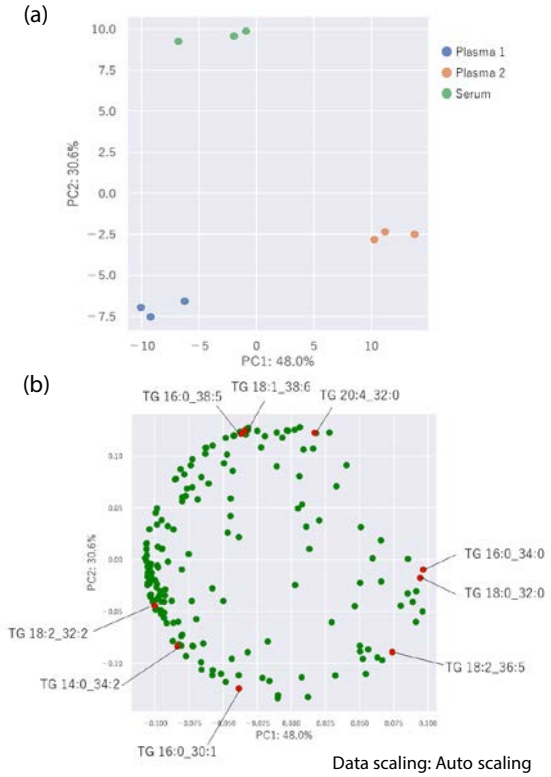
Table 2 Area Value of Internal Standards

	Area
Plasma 1	$(1.30 \pm 0.02) \times 10^6$
Plasma 2	$(1.21 \pm 0.07) \times 10^6$
Serum	$(1.18 \pm 0.07) \times 10^6$

(n = 3, Mean ± SD)

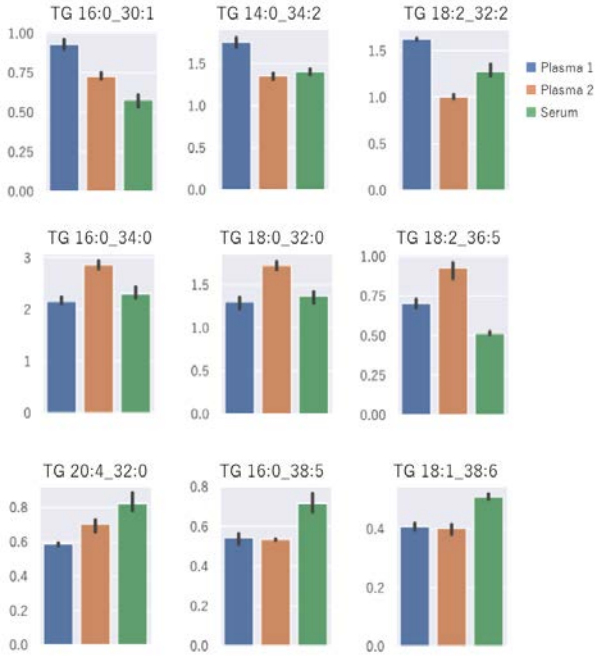
Each sample was analyzed 3 times, and 181 components with an RSD on the corrected area value within 15% were used for data analysis. Python 3.8 was used for data analysis.

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).



Data scaling: Auto scaling

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



Vertical axis: Area values corrected by internal standards
(n = 3, Mean ± SD)

Fig. 8 Triglycerides in Blood Samples

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 shows bar graphs for 9 components. TG 16:0_30:1, TG 14:0_34:2, TG 18:2_32:2 are more abundant in Plasma 1, TG 16:0_34:0, TG 18:0_32:0, TG 18:2_36:5 are more abundant in Plasma 2, and TG 20:4_32:0, TG 16:0_38:5, TG 18:1_38:6 are more abundant in Serum.

■ 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. It is also possible to quantitatively analyze the fatty acids bound to the triglycerides. Using this method, two commercially available human plasma samples and one human serum sample were analyzed, and the minute differences between samples were captured.

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

1. 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

01-00205-EN First Edition: Oct. 2021

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