## **Application** News

### Supercritical Fluid Chromatography

## Analysis of Triglycerides Using the Nexera™ UC Supercritical Fluid Chromatograph

# No. C189

Triglycerides are a kind of neutral lipids that are stored in adipose tissue in the bodies of animals, and are broken down to supply energy to cells when they need it. They are known to be the main component of vegetable oils etc., which are included in a lot of food. Triglycerides are constituted of glycerol (glycerin) bonded to three fatty acids (acyl groups) (Fig. 1) and are highly hydrophobic; there are very many molecular species depending on the composition of the acyl groups and their bonding positions. In vegetable oils, the types of acyl groups that constitute the triglycerides differ depending on the ingredients. Here, we introduce examples of the analysis of triglycerides using supercritical fluid chromatography (SFC).

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Fig. 1 Triglyceride Structure

#### ■ Target Components and Analysis Conditions

The targeted triglycerides and their detection conditions are listed in Table 1. The analysis conditions are summarized in Table 2, and a chromatogram obtained by analysis of a standard sample in which the concentration of each triglyceride was 10 µg/L is shown in Fig. 2. Because an ODS column is used, the retention time increases as the carbon chain of the acyl group lengthens, and for carbon chains of the same length, the retention time is shorter for the chain that has the higher number of double bonds.

Table 1 Target Components and MRM

G 36:0	C12:0/C12:0/C12:0		MRM
	C12:0/C12:0/C12:0	FSI(nositive)	
G 42·0		L3I(positive)	656.60>439.40
0 12.0	C14:0/C14:0/C14:0	ESI(positive)	740.70>495.45
G 48:3	C16:1/C16:1/C16:1	ESI(positive)	824.75>551.50
G 48:0	C16:0/C16:0/C16:0	ESI(positive)	818.70>547.45
G 54:9	C18:3/C18:3/C18:3	ESI(positive)	908.85>607.55
G 54:6	C18:2/C18:2/C18:2	ESI(positive)	902.80>603.55
G 54:3	C18:1/C18:1/C18:1	ESI(positive)	896.75>599.50
G 54:0	C18:0/C18:0/C18:0	ESI(positive)	890.70>595.45
G 60:3	C20:1/C20:1/C20:1	ESI(positive)	986.90>659.60
G 60:0	C20:0/C20:0/C20:0	ESI(positive)	992.95>663.65
G 66:3	C22:1/C22:1/C22:1	ESI(positive)	1071.00>715.65
G 66:0	C22:0/C22:0/C22:0	ESI(positive)	1077.05>719.70
	6 48:3 6 48:0 6 54:9 6 54:6 6 54:3 6 60:3 6 60:0 6 66:3	6 48:3 C16:1/C16:1/C16:1 6 48:0 C16:0/C16:0/C16:0 6 54:9 C18:3/C18:3/C18:3 6 54:6 C18:2/C18:2/C18:2 6 54:3 C18:1/C18:1/C18:1 6 54:0 C18:0/C18:0/C18:0 6 60:3 C20:1/C20:1/C20:1 6 66:3 C22:1/C22:1/C22:1	6 48:3 C16:1/C16:1/C16:1 ESI(positive) 6 48:0 C16:0/C16:0/C16:0 ESI(positive) 6 54:9 C18:3/C18:3/C18:3 ESI(positive) 6 54:6 C18:2/C18:2/C18:2 ESI(positive) 6 54:3 C18:1/C18:1/C18:1 ESI(positive) 6 60:3 C20:1/C20:1/C20:1 ESI(positive) 6 60:0 C20:0/C20:0/C20:0 ESI(positive) 6 66:0 C22:1/C22:1/C22:1 ESI(positive) 6 66:0 C22:0/C22:0/C22:0 ESI(positive)

#### **Table 2 Analysis Conditions**

Column Shim-pack™ UC-GIS II  $(150 \text{ mm L.} \times 2.1 \text{ mm I.D., 3 } \mu\text{m})$ À; CO<sub>2</sub> Mobile phase

B; 0.1 % (w/v) Ammonium formate in

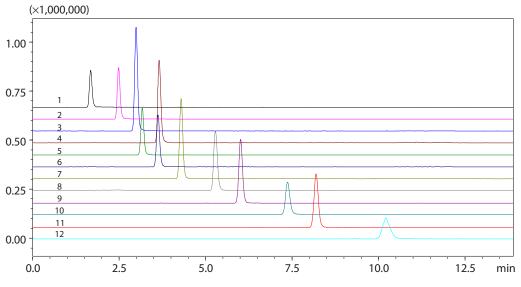
methanol/IPA = 3/7 (v/v) B.conc. 5 % (0 min) - 25 % (7 min) -Gradient 80 % (12-15 min) -5 % (15.1-18 min)

Flow rate 1.0 mL/min Column temp.  $40\,^{\circ}C$ BPR 10 MPa

Detector LCMS™-8050 (FSL MRM mode)

0.1 % (w/v) Ammonium formate in methanol Makeup

Makeup flow rate 0.05 mL/min Injection vol.



9. TG 60:3 (Trieicosenoin) 10.TG 60:0 (Triarachidin)

7. TG 54:3 (Triolein)

8. TG 54:0 (Tristearin)

1. TG 36:0 (Trilaurin) 2. TG 42:0 (Trimvristin) 3. TG 48:3 (Tripalmitolein)

4. TG 48:0 (Tripalmitin) 5. TG 54:9 (Trilinolenin) 6. TG 54:6 (Trilinolein)

11.TG 66:3 (Trierucin) 12.TG 66:0 (Tribehenin)

Fig. 2 Chromatogram of the Standard Solution (10 µg/L Each)

#### Linearity

Table 3 lists the coefficients of determination,  $R^2$ , for the calibration curves obtained by analyzing standard samples with concentrations from 0.1  $\mu$ g/L to 1000  $\mu$ g/L for each triglyceride.

Very good linearity was observed for all the components.

#### **Table 3 Linearity**

		•		
Compounds	Slope	Intercept	R <sup>2</sup>	
Trilaurin	4.98E-05	-0.16	0.9999	
Trimyristin	2.63E-05	3.37	0.9993	
Tripalmitolein	1.40E-05	5.71	0.9993	
Tripalmitin	1.20E-05	5.98	0.9993	
Trilinolenin	1.83E-05	5.42	0.9979	
Trilinolein	1.85E-05	0.45	0.9995	
Triolein	2.57E-05	3.08	0.9998	
Tristearin	2.83E-05	5.94	0.9986	
Trieicosenoin	1.96E-05	4.39	0.9974	
Triarachidin	3.39E-05	3.71	0.9979	
Trierucin	1.88E-05	5.4	0.9983	
Tribehenin	2.61E-05	4.06	0.9995	

#### Repeatability

Table 4 lists repeatability of the retention time and peak area obtained on analyzing standard samples in which the concentration of each triglyceride was 10  $\mu$ g/L five times successively.

**Table 4 Repeatability** 

Compounds	Retention	Time	Peak Area	
	Average (min)	RSD (%)	Average	RSD (%)
Trilaurin	1.677	0.14	224599	8.25
Trimyristin	2.484	0.08	328876	5.15
Tripalmitolein	3.659	0.09	524514	4.27
Tripalmitin	2.987	0.06	629534	4.61
Trilinolenin	5.285	0.09	454456	4.98
Trilinolein	4.406	0.04	445476	2.75
Triolein	3.622	0.06	312026	2.28
Tristearin	3.17	0.07	274512	3.14
Trieicosenoin	6.011	0.07	464821	2.67
Triarachidin	7.382	0.05	271482	3.59
Trierucin	8.194	0.04	408751	4.87
Tribehenin	10.22	0.22	315842	5.77

### Application to the Comparison of Edible Oils

Fig. 3 shows a chromatogram obtained by analyzing a sample of fish oil diluted to 1 in 10,000 with hexane.

Similarly, Fig. 4 shows the chromatogram for a sample that was a 1 in 10,000 dilution of sesame oil. Edible oils contain a large number of triglycerides, and the types of acyl groups that constitute the triglycerides differ depending on the ingredients; therefore, there exist very many different types of molecular species. In these figures, only the mass chromatograms of the components analyzed in the standard samples are shown, but in actuality it is possible to detect many molecular species.

 1. TG 36:0 (Trilaurin)
 7. TG 54:3 (Triolein)

 2. TG 42:0 (Trimyristin)
 8. TG 54:0 (Tristearin)

 3. TG 48:3 (Tripalmitolein)
 9. TG 60:3 (Trieicosenoin)

 4. TG 48:0 (Tripalmitin)
 10. TG 60:0 (Triarachidin)

 5. TG 54:9 (Trilinolenin)
 11. TG 66:3 (Trierucin)

 6. TG 54:6 (Trilinolein)
 12. TG 66:0 (Tribehenin)

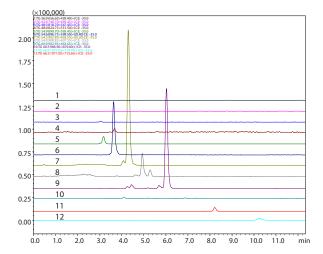


Fig. 3 Chromatogram of Fish Oil

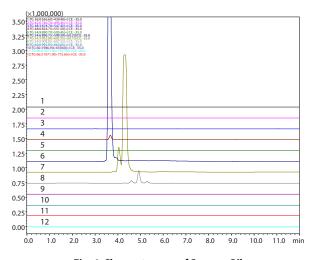


Fig. 4 Chromatogram of Sesame Oil

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