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Application News

No. C189

Supercritical Fluid Chromatography

Analysis of Triglycerides Using the Nexera™ UC Supercritical Fluid Chromatograph

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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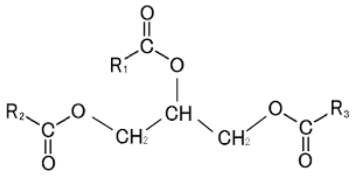

Fig. 1 Triglyceride Structure

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

Compounds	Acyl Composition		MRM	
Trilaurin	TG 36:0	C12:0/C12:0/C12:0	ESI(positive)	656.60>439.40
Trimyristin	TG 42:0	C14:0/C14:0/C14:0	ESI(positive)	740.70>495.45
Tripalmitolein	TG 48:3	C16:1/C16:1/C16:1	ESI(positive)	824.75>551.50
Tripalmitin	TG 48:0	C16:0/C16:0/C16:0	ESI(positive)	818.70>547.45
Trilinolenin	TG 54:9	C18:3/C18:3/C18:3	ESI(positive)	908.85>607.55
Trilinolein	TG 54:6	C18:2/C18:2/C18:2	ESI(positive)	902.80>603.55
Triolein	TG 54:3	C18:1/C18:1/C18:1	ESI(positive)	896.75>599.50
Tristearin	TG 54:0	C18:0/C18:0/C18:0	ESI(positive)	890.70>595.45
Trieicosenoin	TG 60:3	C20:1/C20:1/C20:1	ESI(positive)	986.90>659.60
Triarachidin	TG 60:0	C20:0/C20:0/C20:0	ESI(positive)	992.95>663.65
Trierucin	TG 66:3	C22:1/C22:1/C22:1	ESI(positive)	1071.00>715.65
Tribehenin	TG 66:0	C22:0/C22:0/C22:0	ESI(positive)	1077.05>719.70

Table 2 Analysis Conditions

Column	:	Shim-pack™ UC-GIS II (150 mm L. × 2.1 mm I.D., 3 µm)
Mobile phase	:	A; CO_2 B; 0.1 % (w/v) Ammonium formate in methanol/IPA = 3/7 (v/v)
Gradient	:	B.conc. 5 % (0 min) - 25 % (7 min) – 80 % (12-15 min) –5 % (15.1-18 min)
Flow rate	:	1.0 mL/min
Column temp.	:	40 °C
BPR	:	10 MPa
Detector	:	LCMS™-8050 (ESI, MRM mode)
Makeup	:	0.1 % (w/v) Ammonium formate in methanol
Makeup flow rate	:	0.05 mL/min
Injection vol.	:	1 µL

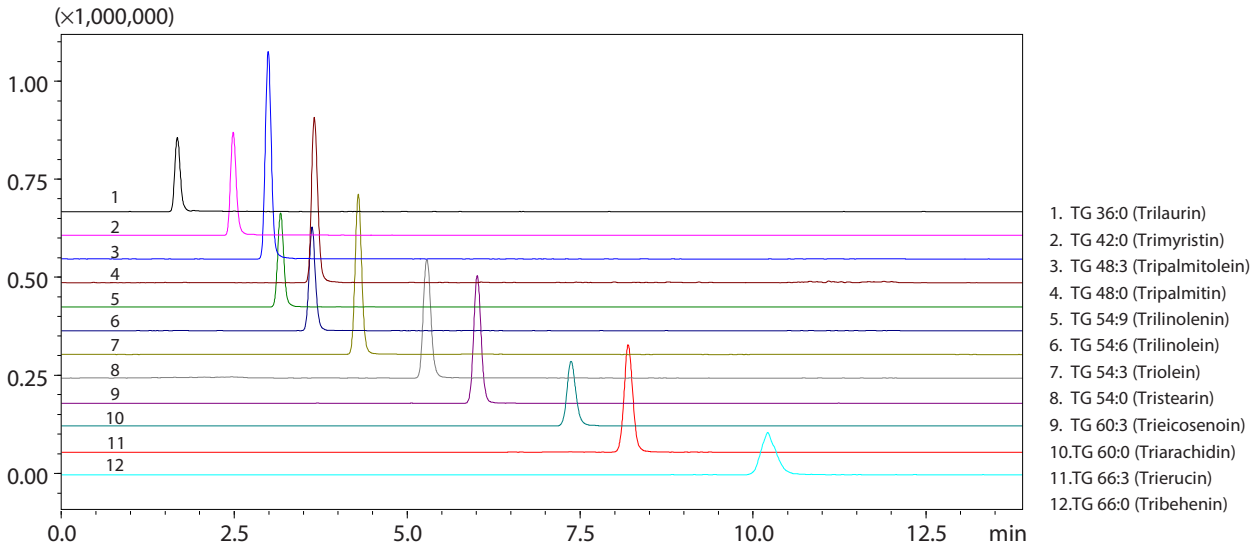

Fig. 2 Chromatogram of the Standard Solution (10 µg/L Each)

Linearity

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

Very good linearity was observed for all the components.

Table 3 Linearity

Compounds	Slope	Intercept	R^2
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 µg/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)
2. TG 42:0 (Trimyristin)
3. TG 48:3 (Tripalmitolein)
4. TG 48:0 (Tripalmitin)
5. TG 54:9 (Trilinolenin)
6. TG 54:6 (Trilinolein)
7. TG 54:3 (Triolein)
8. TG 54:0 (Tristearin)
9. TG 60:3 (Trieicosenoin)
10. TG 60:0 (Triarachidin)
11. TG 66:3 (Trierucin)
12. TG 66:0 (Tribehenin)

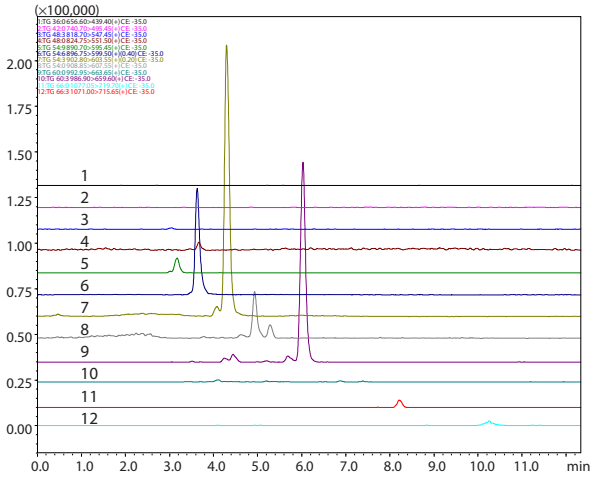

Fig. 3 Chromatogram of Fish Oil

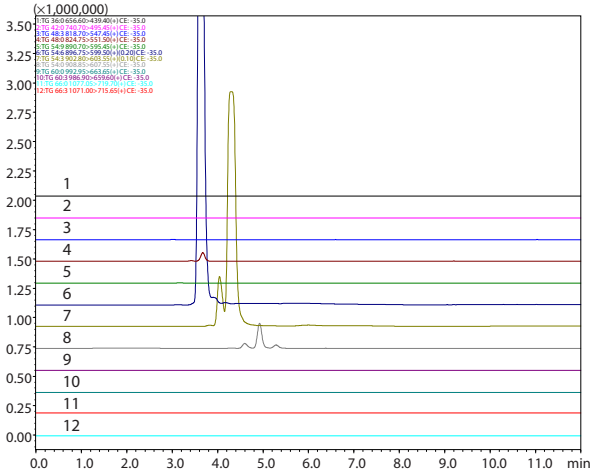

Fig. 4 Chromatogram of Sesame Oil

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