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Method development and Identification of Triacylglycerols species with Supercritical Fluid Chromatography and Method Scouting Software

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1. Introduction

Triacylglycerols (TAGs), which are the main constituents of natural oils and fats, compose of three fatty acids and glycerol connected by ether bonds. Analyzing TAGs is essential to evaluate the quality and functionality of oils and fats. However, chromatographic separation by typical methods that include GC and HPLC might be challenging due to a large number of TAGs species. Supercritical Fluid Chromatography (SFC) is one chromatography method that uses supercritical fluid (i.e. supercritical carbon dioxide) as a mobile phase. SFC is known to show similar separation to normal phase LC and by changing some parameters, has the potential to show superior performance for the analysis of TAGs as compared to the typical method.

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1. Introduction

1-1. Triacylglycerols analysis with MS/MS

For MRM of triacyl glycerides, 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. Figure 1 shows an example the MRM transition.

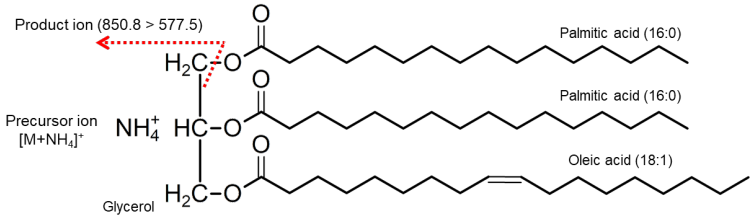


Figure1. MRM transition of Triacylglycerols species by MS/MS (Example. TAG 16:0/16:0/18:1)

1-2. Supercritical fluid chromatography

Figure 2 shows the system configuration. SFC is known to show superior performance relative to HPLC and has the potential to reduce consumption of organic solvents.

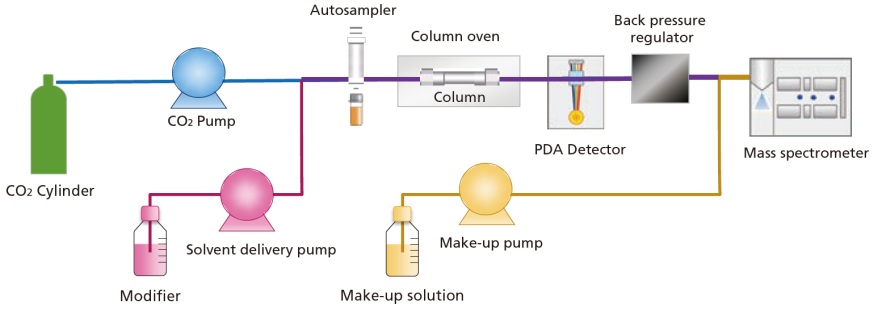


Figure 2. System Configuration of SFC/MS/MS

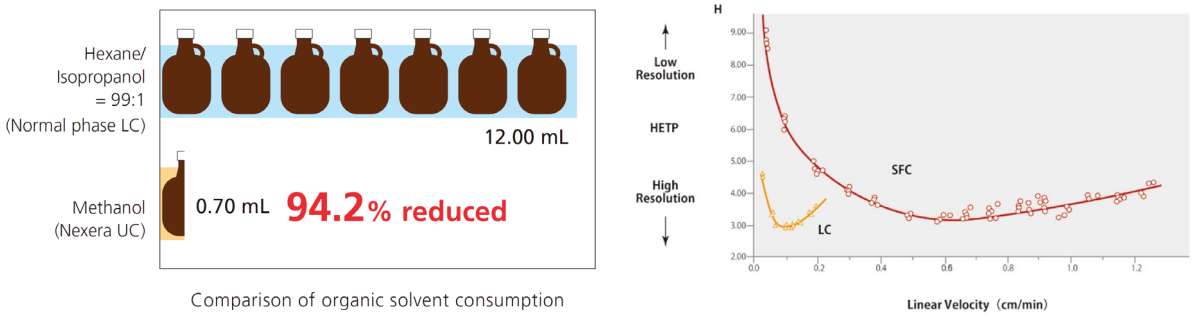


Figure 3. Comparison between typical HPLC and SFC

2. Method

2-1. Analytical conditions

In SFC analysis, an organic solvent, called as “modifier” is used to modulate the retention time and/or selectivity. The back pressure regulator and column oven are also used to manage the separation. Table 1 shows the analytical conditions in this article.

Table 1. Analytical conditions

SFC conditions	
Column	Shim-pack XR-ODSIII, 150 × 2.0 mm I.D., 2.2 um
Flow Rate	0.8 mL/min
Modifier (Pump B)	Acetonitrile (10 ~ 20%)
Back Pressure Regulator	10 ~ 20 MPa
Oven Temperature	25 ~ 35 ℃
Injection Volume	0.5 µL

LCMS-8050 and Interface conditions	
Make-up solvent	0.2 mL/min (Methanol with 10 mmol/L ammonium acetate)
Interface	DUIS
MS Mode	Positive mode
Block Temperature	40 ℃
DL Temperature	235 ℃
Nebulizing Gas Flow	2 L/min
Drying Gas Flow	10 L/min
Heating Gas Flow	10 L/min

2-2. Method Scouting Solutions

Method Scouting Solution, a dedicated software for method scouting, can create multiple methods for optimization using different analytical conditions.

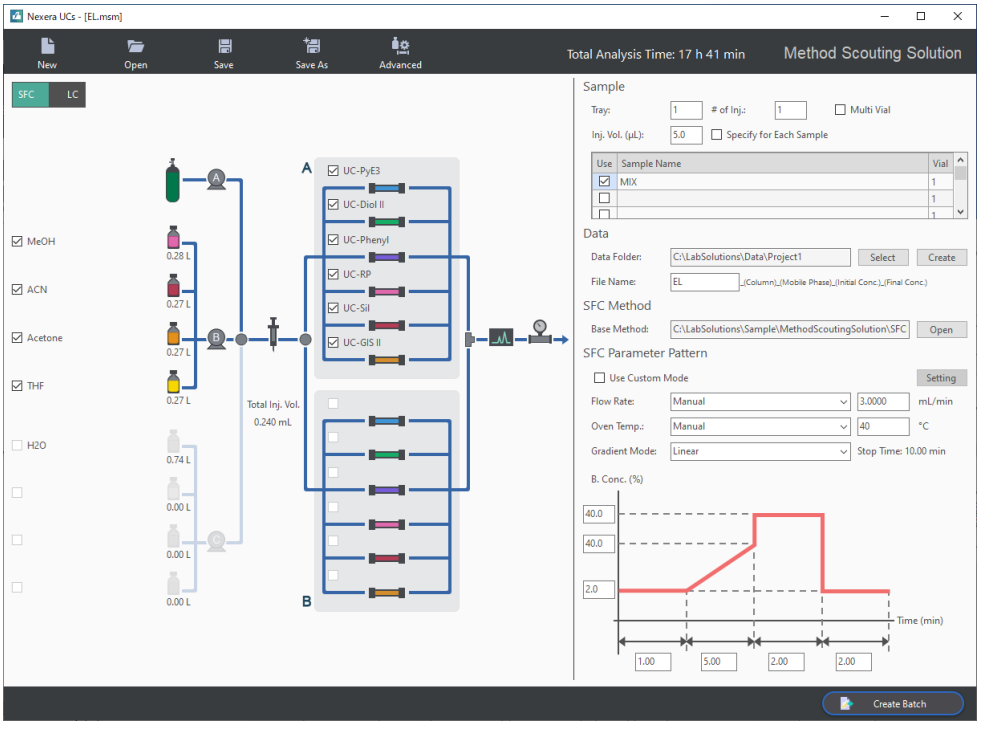


Figure 4. User interface of Method Scouting Solutions

3. Results and Discussion

3-1. Method scouting results

In this study, 27 analytical conditions using three different oven temperatures, modifier concentrations, and back pressure settings were investigated. Figure 4 shows the obtained results by method scouting.

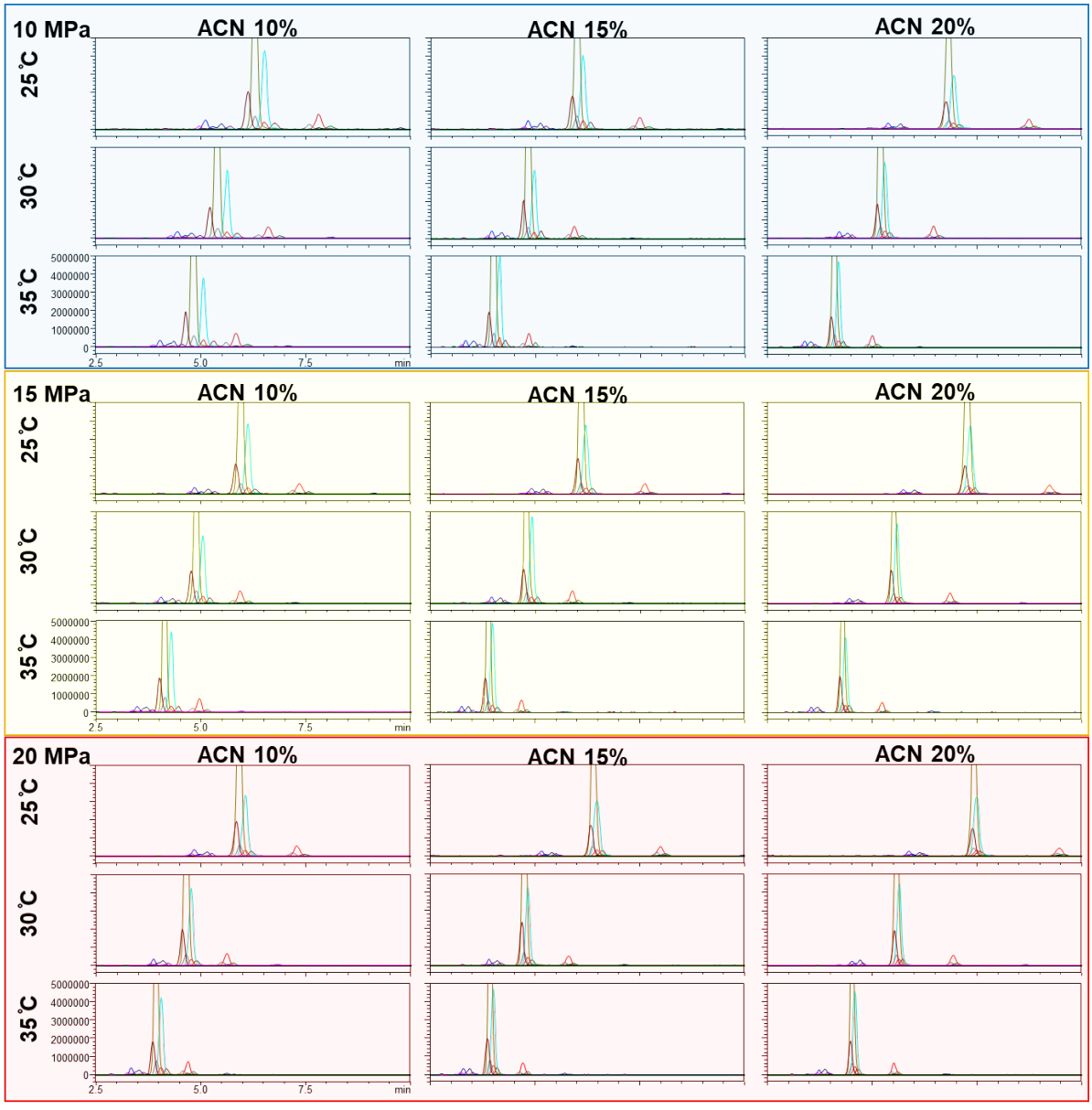


Figure 5. Chromatograms of palm oil analysis using Method Scouting Solutions

3. Results

3-2. Comparison of each analytical parameter

Based on the results of method scouting, we found the effect of each parameter on the retention times and separation of each of the TAG elution peaks.

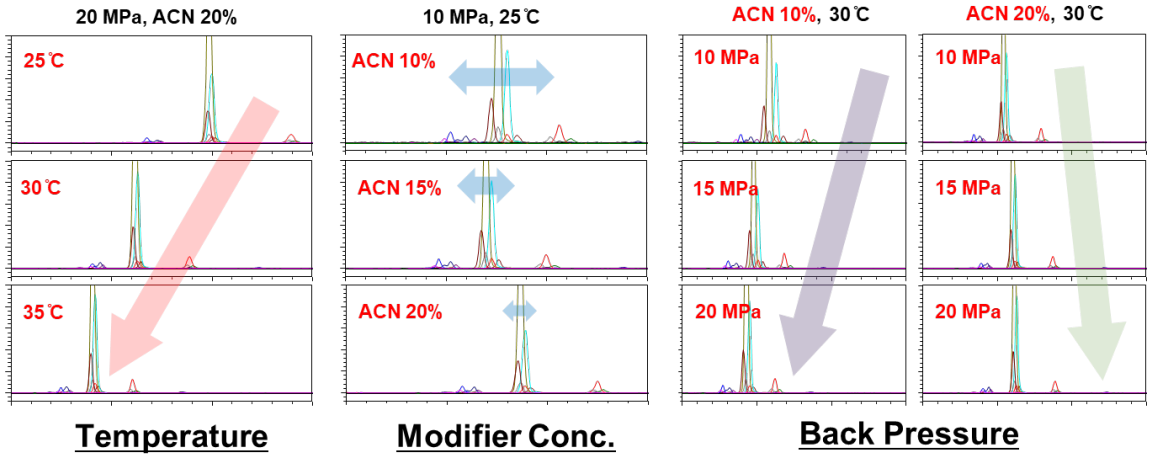
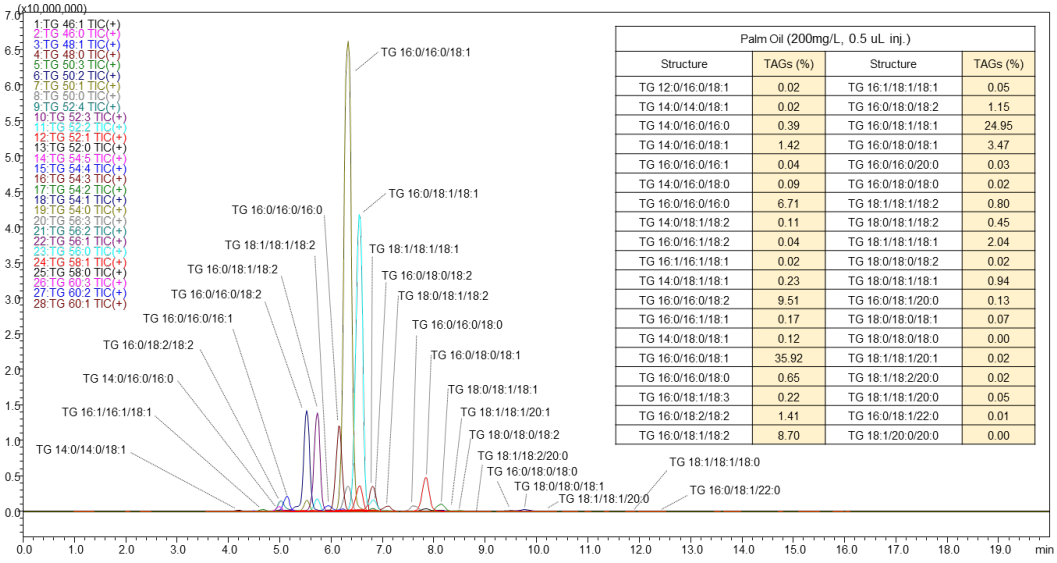


Figure 6. Comparison of each analytical parameter

3-3. TAG composition analysis in palm oil

We analyzed palm oil samples using optimized analytical conditions. (Oven temp. 25°C, ACN 10%, Back pressure 10 MPa) Figure 6 shows the MS chromatogram and TAG composition ratio table that we obtained.



Palm Oil (200mg/L, 0.5 uL inj.)

Structure	TAGs (%)	Structure	TAGs (%)
TG 12:0/16:0/18:1	0.02	TG 16:1/18:1/18:1	0.05
TG 14:0/14:0/18:1	0.02	TG 16:0/18:0/18:2	1.15
TG 14:0/16:0/16:0	0.39	TG 16:0/18:1/18:1	24.95
TG 14:0/16:0/18:1	1.42	TG 16:0/18:0/18:1	3.47
TG 16:0/16:0/16:1	0.04	TG 16:0/16:0/20:0	0.03
TG 14:0/16:0/18:0	0.09	TG 16:0/18:0/18:0	0.02
TG 16:0/16:0/16:0	6.71	TG 18:1/18:1/18:2	0.80
TG 14:0/18:1/18:2	0.11	TG 18:0/18:1/18:2	0.45
TG 16:0/16:1/18:2	0.04	TG 18:1/18:1/18:1	2.04
TG 16:1/16:1/18:1	0.02	TG 18:0/18:0/18:2	0.02
TG 14:0/18:1/18:1	0.23	TG 18:0/18:1/18:1	0.94
TG 16:0/16:0/18:2	9.51	TG 16:0/18:1/20:0	0.13
TG 16:0/16:1/18:1	0.17	TG 18:0/18:0/18:1	0.07
TG 14:0/18:0/18:1	0.12	TG 18:0/18:0/18:0	0.00
TG 16:0/16:0/18:1	35.92	TG 18:1/18:1/20:1	0.02
TG 16:0/16:0/18:0	0.65	TG 18:1/18:2/20:0	0.02
TG 16:0/18:1/18:3	0.22	TG 18:1/18:1/20:0	0.05
TG 16:0/18:2/18:2	1.41	TG 16:0/18:1/22:0	0.01
TG 16:0/18:1/18:2	8.70	TG 18:1/20:0/20:0	0.00

Figure 7. MS chromatogram of Palm Oil and TAG composition table

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3. Results

3-4. Fully hydrogenated rapeseed oil analysis

TAGs, which consist long and fully hydrogenated fatty acid, takes long time to elute from column. We analyzed rapeseed oil which hydrogenated under high temperature and succeeded in reducing the elution time.

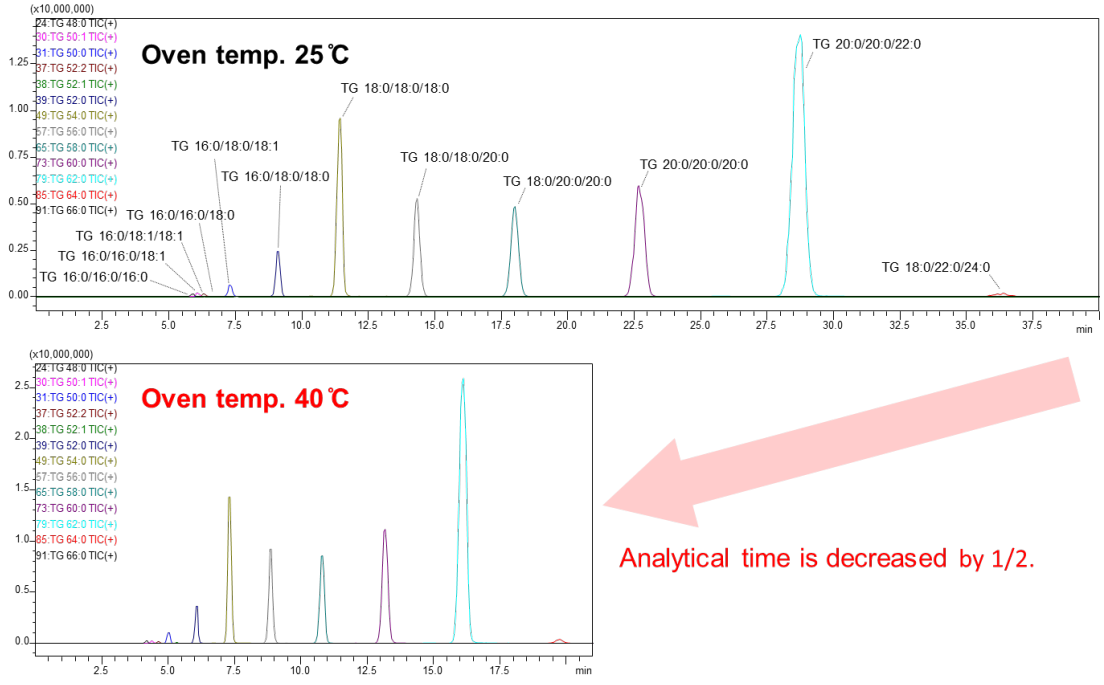

Figure 7. Chromatogram for preparative SFC

4. Conclusion

We developed an analytical method for TAGs in natural oils and fats using SFC/MS/MS. As a result, various TAGs species were found in palm oil and rapeseed oil, and the composition ratio of each TAGs was determined.

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