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Application News

Supercritical Fluid Chromatograph – Nexera™ UC

Method Development and Identification of Triacylglycerols Species with Supercritical Fluid Chromatography and Method Scouting Software

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User Benefits

- Analytical SFC may provide shorter analytical time than conventional normal phase HPLC.
- Method optimization for various analytical parameters can be achieved by Method Scouting Solutions.

■ Introduction

Triacylglycerols (TAGs), which are the main constituents of natural oils and fats, compose of three fatty acids and glycerol linked with ester 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 known to show similar separation to normal phase LC and by changing some parameters, has the potential capability to show superior performance for the analysis of TAGs as compared to the typical method.

■ 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. Fig.1 shows an example of the MRM transition.

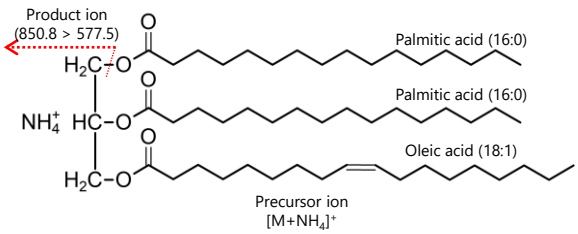

Fig. 1 MRM Transition of Triacylglycerols Species by MS/MS
(Example. TAG 16:0/16:0/18:1)

■ Supercritical Fluid Chromatography

SFC is one of the chromatography with supercritical fluid (i.e., supercritical carbon dioxide) used as the mobile phase. Supercritical CO_2 has a higher diffusion coefficient than HPLC solvents and can penetrate packing material pores more easily. That makes SFC an analytical technique that can achieve ultra-fast analysis without sacrificing resolution even at high linear velocities. (Fig. 2)

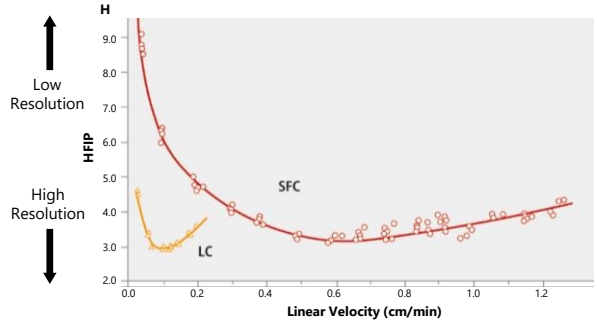

Fig. 2 Van-Deemter plot Comparison for LC and SFC

■ Analytical Conditions

In SFC analysis, an organic solvent called "modifier" is utilized on order 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

Nexera UC		
Column	:	Shim-pack™ XR-ODSIII, (150 × 2.0 mm I.D., 2.2 um P/N S228-59910-91)
Flow Rate	:	0.8 mL/min
Modifier (Pump B)	:	Acetonitrile (10, 15, 20%)
Back Pressure Regulator	:	10, 15, 20 MPa
Oven Temperature	:	25, 30, 35 ℃
Injection Volume	:	0.5 µL
LCMS-8050		
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

■ Method Scouting Solutions

Method Scouting Solution, a dedicated software for method scouting, can create multiple methods for optimization using different analytical conditions. (Fig. 3)

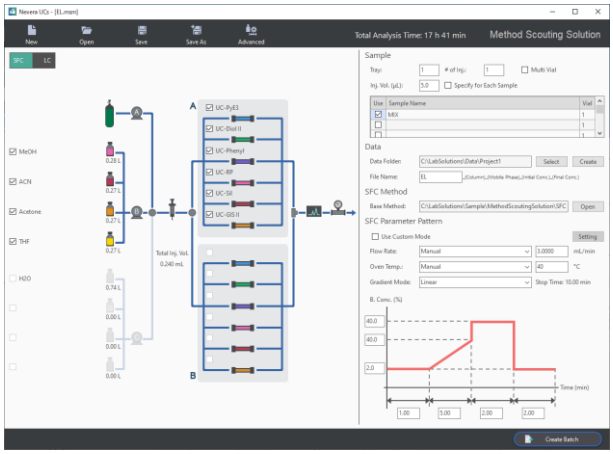

Fig. 3 User Interface of Method Scouting Solution

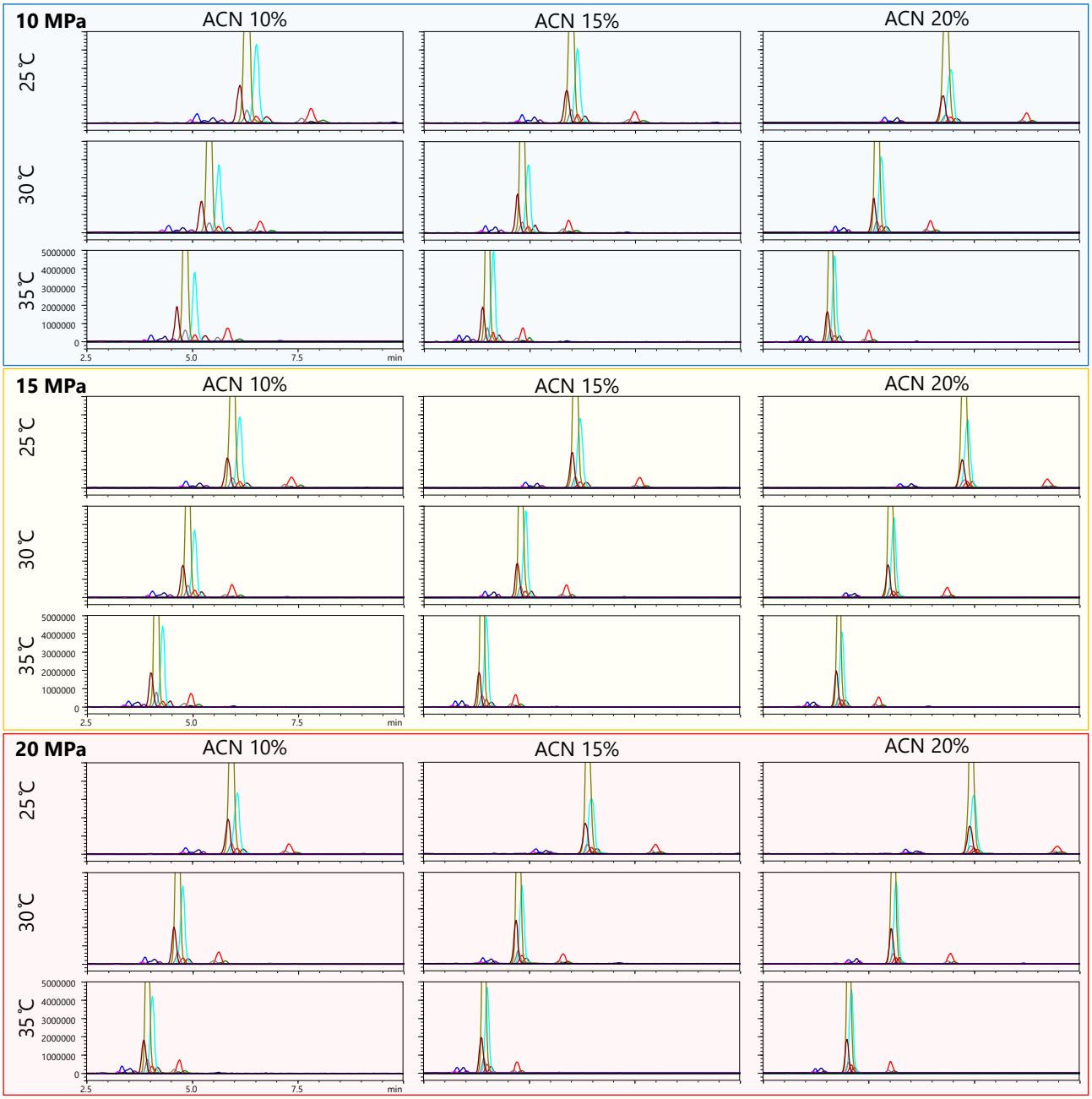

Fig. 4 Chromatograms of Palm Oil Analysis using Method Scouting Solution

■ Method Scouting Results

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

■ 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. (Fig. 5)

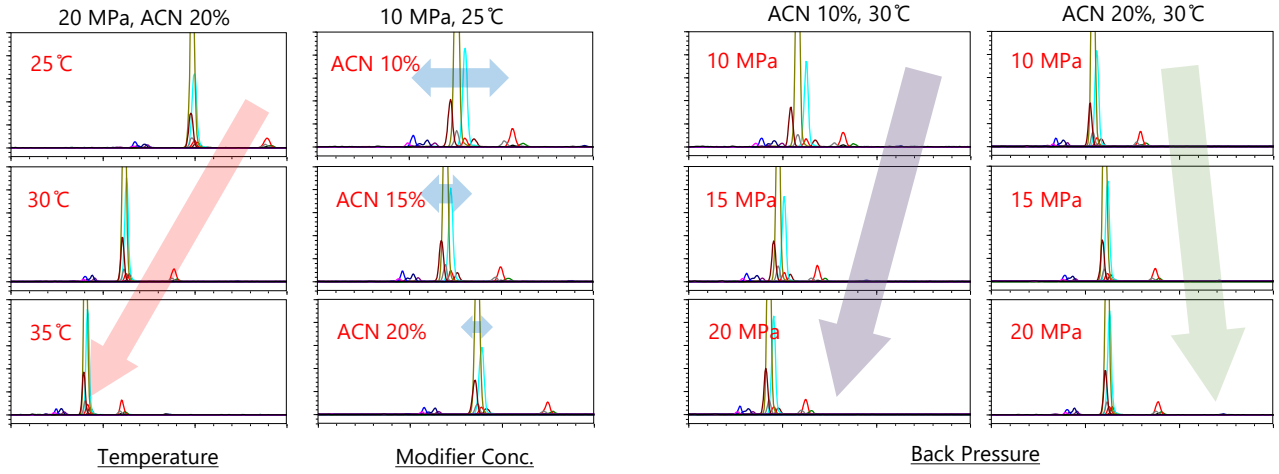

Fig. 5 Chromatograms Comparing the Effect of Each Analytical Parameter

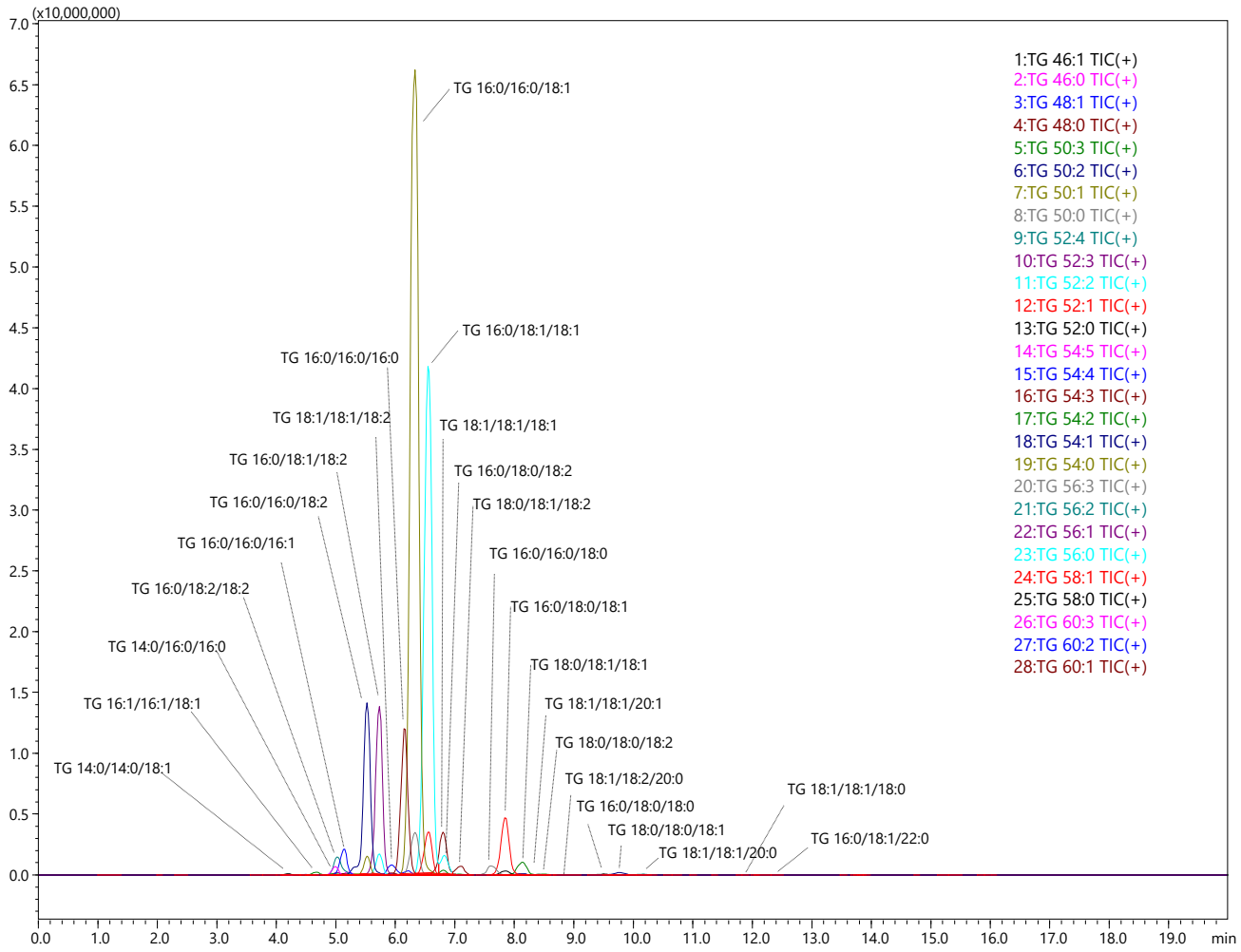

Fig. 6 Obtained MS Chromatogram of Palm Oil
(Oven temp. 25℃, Acetonitrile 10%, Back Pressure 10 MPa)

Table 3 TAG Composition Ratio in Palm Oil

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

■ TAG Composition Analysis in Palm Oil

We analyzed palm oil samples using optimized analytical conditions. (Oven temp. 25℃, ACN 10%, Back pressure 10 MPa) Fig. 6 shows the MS chromatogram and Table 3 shows the TAG composition ratio table that is calculated by MS results.

■ 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.

■ Acknowledgements

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