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Application News

Supercritical Fluid Chromatograph – Nexera™ UC

Chiral Separation of Triacyclglycerols Isomers by Supercritical Fluid Chromatography

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User Benefits

- Efficient separation of analogues and/or chiral compounds was achieved by high penetration mobile phase.
- The high-speed performance of SFC can enable to reduce the analytical time which is compatible for the method scouting.

■ Introduction

Triacylglycerols (TAGs), which are the main constituents of natural oils and fats, compose of three fatty acids and glycerol linked with ester bonds. TAGs have various isomers with differing physical and metabolic pathways.

In this article, we optimized the analytical conditions for TAGs isomer separation by SFC using Nexera UC Chiral Screening system.

■ Two Types of Triacylglycerides Isomers

TAGs have two different types of isomers. Positional isomers are the TAGs where different fatty acids are connected to the *sn*-2 position. Enantiomers are the isomers that have chiral centers at the *sn*-2 position.(Fig.1) These isomers require chromatographic separation due to the challenge of MS separation of isomers with identical molecular weights.

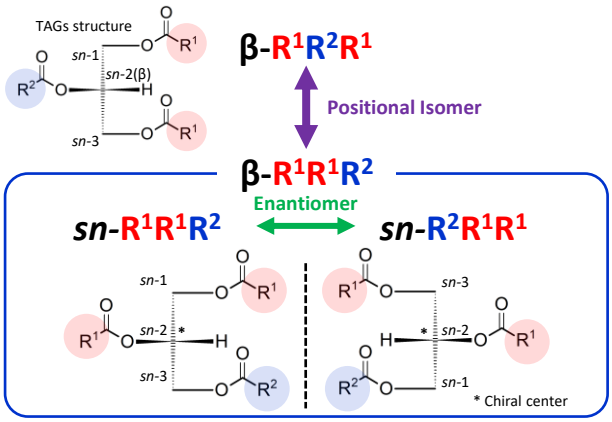

Fig. 1 Two Types of Triacylglycerides Isomers

■ Chiral Analysis with Nexera UC and CHIRALPAK Series

CHIRALPAK Series and CHIRALCEL Series columns (Daicel Corporation) for chiral analysis are capable of resolving a wide variety of compounds by showing complementary separation targets. The combination of the Nexera UC Chiral Screening System and these columns simplifies method scouting for chiral analysis.

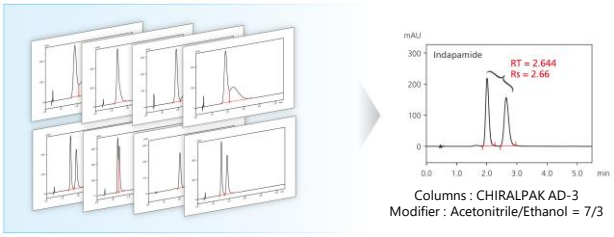

Fig. 2 Example of Chiral Screening with Nexera UC and CHIRALPAK

■ Analytical Conditions

In SFC analysis, an organic solvent called a "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

Nexera UC		
Column	:	Daicel CHIRALPAK IG-3 (250×4.6 mm I.D., 5 um)
Flow Rate	:	1.5 mL/min
Modifier	:	Acetonitrile / Methanol =9:1
Pump B Conc.	:	20, 30, 40%
Back Pressure Regulator	:	10, 15, 20 Mpa
Oven Temperature	:	20, 25, 30℃
Injection Volume	:	0.2 µL
LCMS-8050		
Make-up solvent	:	0.1 mL/min (Methanol with 0.1% ammonium acetate)
Interface	:	DUIS
MS Mode	:	Positive mode
Block Temperature	:	40℃
DL Temperature	:	250℃
Nebulizing Gas Flow	:	3 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)

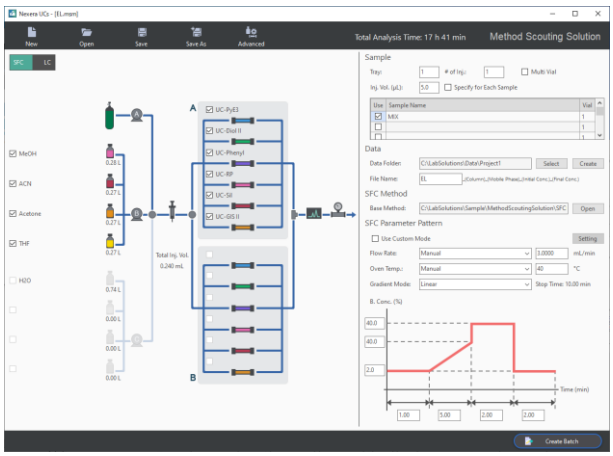

Fig. 3 User Interface of Method Scouting Solution

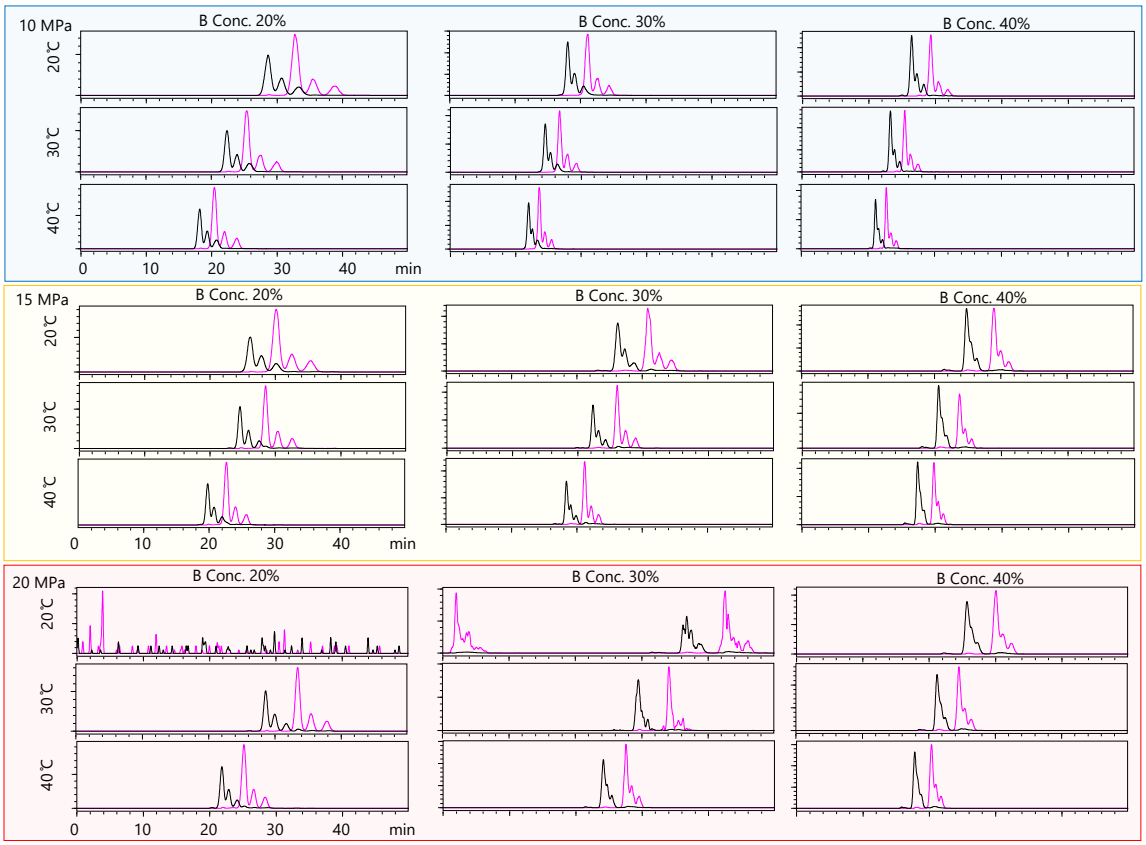

Fig. 4 Chromatograms of TAGs Isomers Analysis Using Method Scouting Solution
(*sn*-POO, *sn*-OOP, *sn*-OPO, *sn*-PPO, *sn*-OPP, *sn*-POP)

■ Method Scouting Results

In this study, 27 analytical conditions with three different oven temperatures, modifier concentrations, and back pressure settings were investigated. We analyzed standard solution mixtures that contain six type of TAGs isomers. (*sn*-POO, *sn*-OOP, *sn*-OPO, *sn*-PPO, *sn*-OPP, *sn*-POP)

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)

■ Obtained Chromatogram of TAGs Isomers

We analyzed sample solutions of TAGs isomer using optimized analytical conditions. (Oven temp. 30℃, Modifier 20%, Back pressure 20 MPa) Fig. 6 shows the MS overlapped chromatograms. All six peak were separated by SFC and every isomer has the potential to be analyzed with UV or ELSD.

■ Conclusion

We developed an analytical method for TAGs isomers using Nexera UC Chiral screening system and CHIRALPAK. As a result, we achieved method optimization for TAGs isomer analysis by Method Scouting Solution. The workflow of method scouting can be utilized for any method development, and Nexera UC will be a game changer for chiral separation.

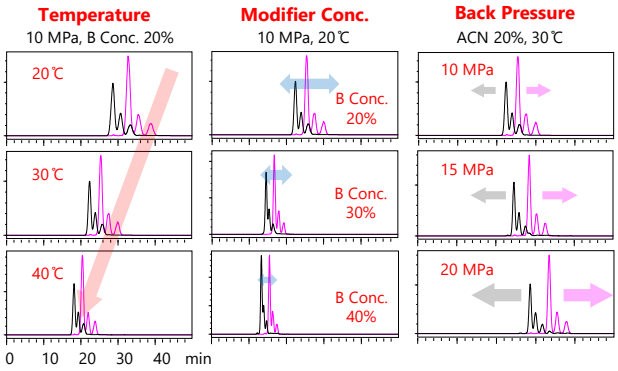

Fig. 5 Chromatograms Comparing the Effect of Each Analytical Parameter

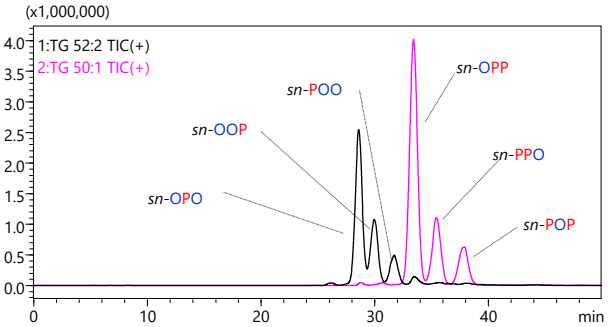

Fig. 6 Obtained MS Chromatogram of TAGs Isomers
(Oven temp. 30℃, Modifier 20%, Back Pressure 20 MPa)

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