Analysis of enantiomers and regioisomers of triacylglycerols using supercritical fluid chromatography

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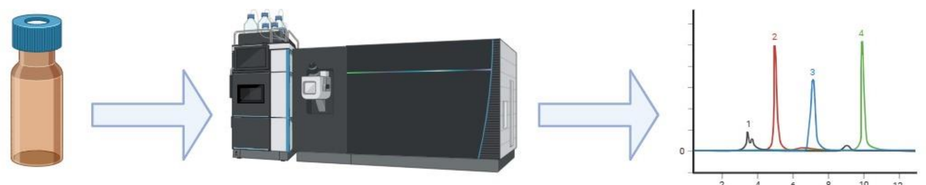
Introduction

Triacylglycerols (TAGs) can have various isomeric structures, including enantiomers and regioisomers, determined by the fatty acids' structures and position on the glycerol backbone. These also influence their metabolic pathways, affecting absorption, distribution, and metabolism in biological systems. Analyzing TAGs accurately is essential for understanding their nutritional, biological, and technological implications. Supercritical fluid chromatography (SFC) has recently gained attention for lipid analysis due to its cost-effectiveness, environmental friendliness, and ability to identify also isomeric lipid species.

The overall aim of this study was to familiarize oneself with the operating principles of SFC instrumentation. More specifically, this study aimed to apply and optimize a method to simultaneously analyze regioisomers and enantiomers of TAG isomers. Subcritical fluid chromatography using mass spectrometric (MS) detection was applied for that.

Method

Method optimization

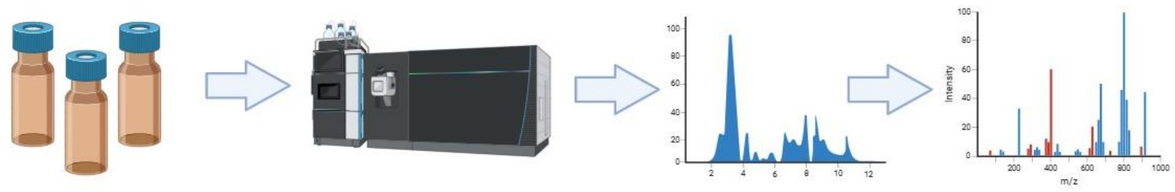


Preparation of reference TAG

Experimentation of different methods with different SFC conditions (SFC MS/MS)

Selection of optimum conditions based on the resolution

Analysis of TAGs and samples



Preparations of oil samples and TAG mixtures

Analysis of TAG isomers by applying the optimized method (SFC MS/MS)

Identification of TAG isomers

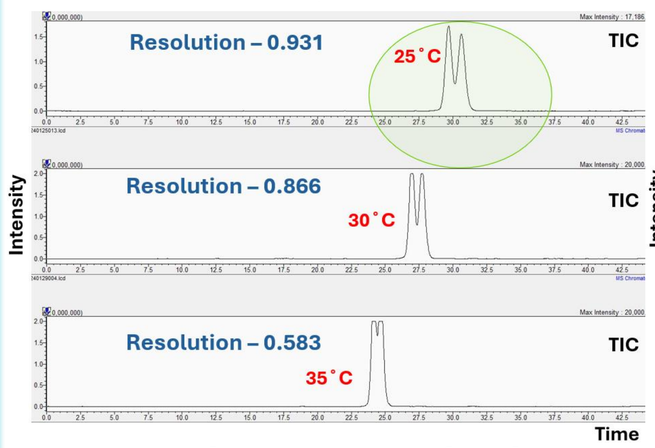
Calculation of isomer ratios using mass intensities

Results

Optimal SFC conditions

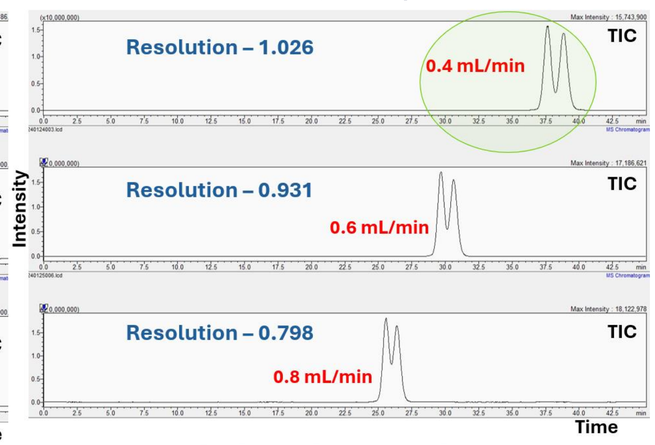
Column oven temperature

BP=10 MPa, FR=0.6 mL/min



Flow rate (FR)

BP=10 MPa, Temp=25º C



Back pressure (BP)

Temp=25ºC, FR=0.6 mL/min

Injection volume

BP=12 MPa, FR=0.4 mL/min, Temp=25ºC

Figure 1. MS chromatograms comparing the SFC conditions

Two similar chiral columns were used in series with Acetonitrile/methanol (90/10, v/v) as the modifier. Methanol with 0.1% ammonium formate (w/v) was used as the makeup solvent. The optimized modifier gradient was as follows: 5–35% (10 min), 35–40% (25 min), 40-5% (0.01 min), and 5% (3 min).

Analysis of isomers in TAG mixtures

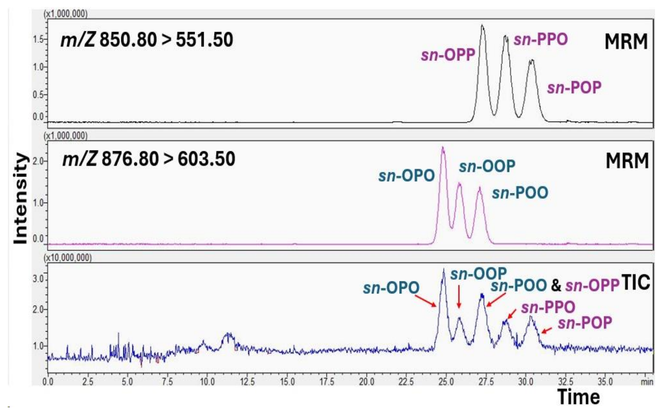


Figure 2. MS chromatogram of studied TAG isomers (*sn*-OOP+OPO 60+40, *sn*-PPO+POP 60+40)

With the developed method TAG isomers of OOP, OPP, MPO and OPS were separated (Figures 2,3 and 4). All the separations were completed within 35 minutes.

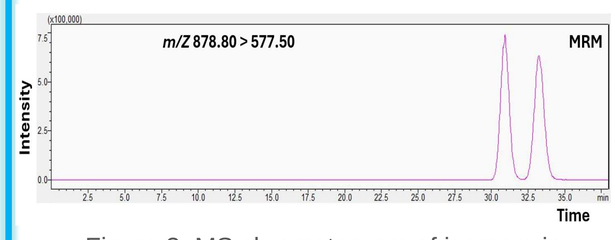


Figure 3. MS chromatogram of isomers in *rac*-OPS

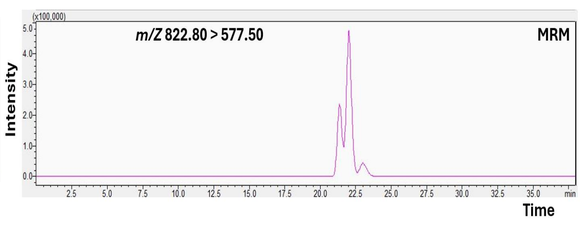


Figure 4. MS chromatogram of isomers in OPM/MPO 40/60

Analysis of TAG percentages in oil samples

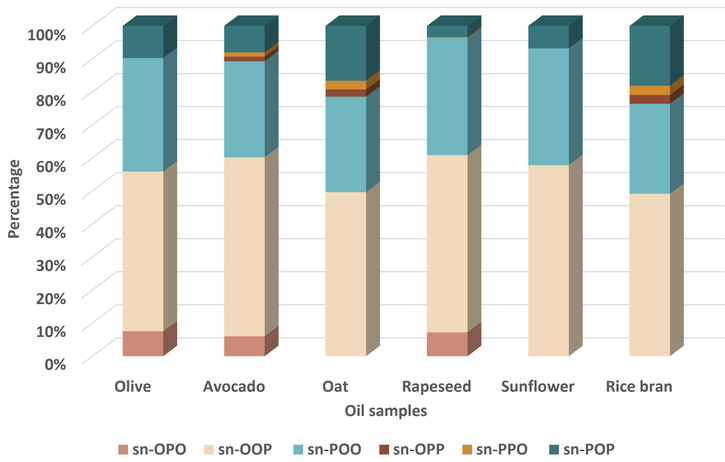


Figure 5. Percentages of selected TAG isomers in oil samples

The enantiomers of OOP and PPO present in vegetable oils are not racemates (Figure 5).

Conclusion

The developed novel chiral SFC method was able to separate TAG regioisomers and enantiomers. The analysis time was 35 minutes. Further experiments are necessary to determine the elution order of some enantiomers and concentrations of TAG isomers in selected plant oils.

References

Masuda, K., Abe, K., & Murano, Y. (2021). A Practical Method for Analysis of Triacylglycerol Isomers Using Supercritical Fluid Chromatography. *Journal of the American Oil Chemists' Society*, 98(1), 21–29. https://doi.org/10.1002/aocs.12432

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