Automatable analytical workflow for characterization of fatty acids in animal-based matrix by GC×GC-TOFMS

Kinjal Bhatt *, Thibaut Dejong , Pierre-Hugues Stefanuto, and Jean- François Focant

University of Liège, Molecular Systems, Organic & Biological Analytical Chemistry Group, 11 Allée du Six Août, 4000 Liège, Belgium

Background

- Animal fats are a by-product of the meat industry, and can be used for biodiesel production, reducing waste and promoting sustainability.
- Its production process invloves the conversion of triglycerides into fatty acid methyl esters (FAMEs) through transesterification.
- FAMEs can be divided into three categories: Saturated Fatty acids (SFAs), Monounsaturated FAs (MUFAs), Polyunsaturated FAs (PUFAs).
- PUFAs are not preferred in biodiesel production due to their susceptibility to oxidation, which can lead to instability in the final product due to increased no of double bonds.

Challenges

- Derivatization is a critical step in FAMEs analysis while using GC, as it converts non-volatile fatty acids into volatile compounds suitable for GC analysis.
- To have a derivatization protocol that does not discriminate towards any specific class of FAMEs in order to accurately monitor samples is critical.
- Derivatization can be time-consuming and labour-intensive, making automation of the process is desirable for high-throughput analysis.

Aim of study

- Developing automatable sample preparation protocol without creating bias towards any classes of FAMEs.

Results

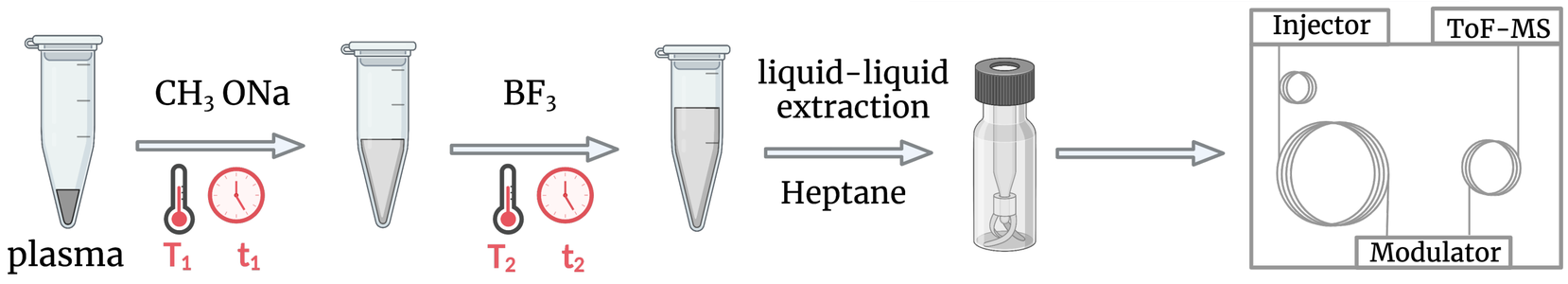

Figure 1: Schematic diagram of two-stage chemical derivatization and extraction approach

Full Factorial Design:(4,16)
Center point : 3

Factors	-1	0	+1
T_1 (°C)	**85**	95	105
t_1 (min)	5	15	**25**
T_2 (°C)	**85**	95	105
t_2 (min)	5	15	**25**

Table 1: Factors and levels tested for optimization using DoE (The optimized conditions are in bold)

Instrumental parameters

Column Configuration:
Rxi-5sil (30m,0.25mm ID,1.0 µm)
VF-17ms (2m,0.25mm ID,0.5 µm)

GC Method: 69.33 mints
Modulation time: 8 s
Acquisition rate: 150 spectra/s

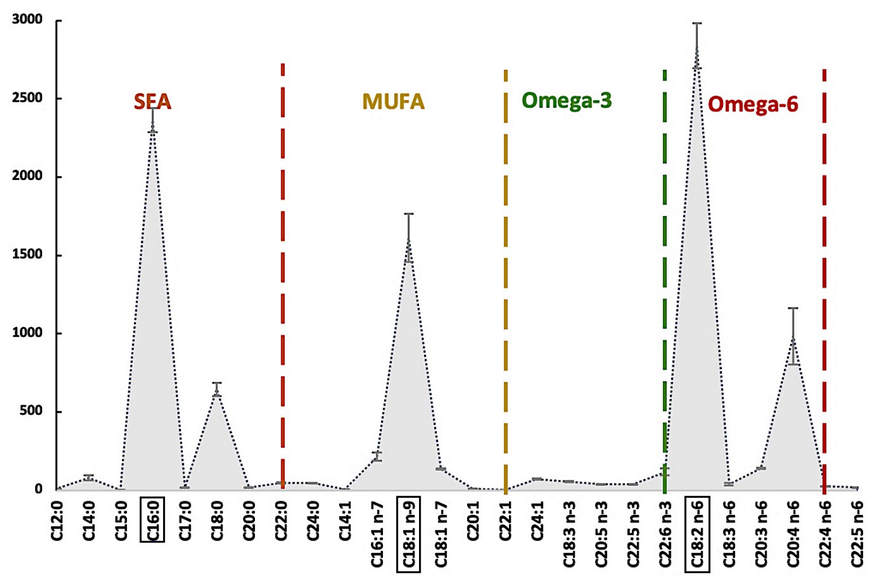

Figure 2: Concentration range of lipids in human plasma as per NIST certificate of Analysis

Response optimization

Structured Chromatographic Separation

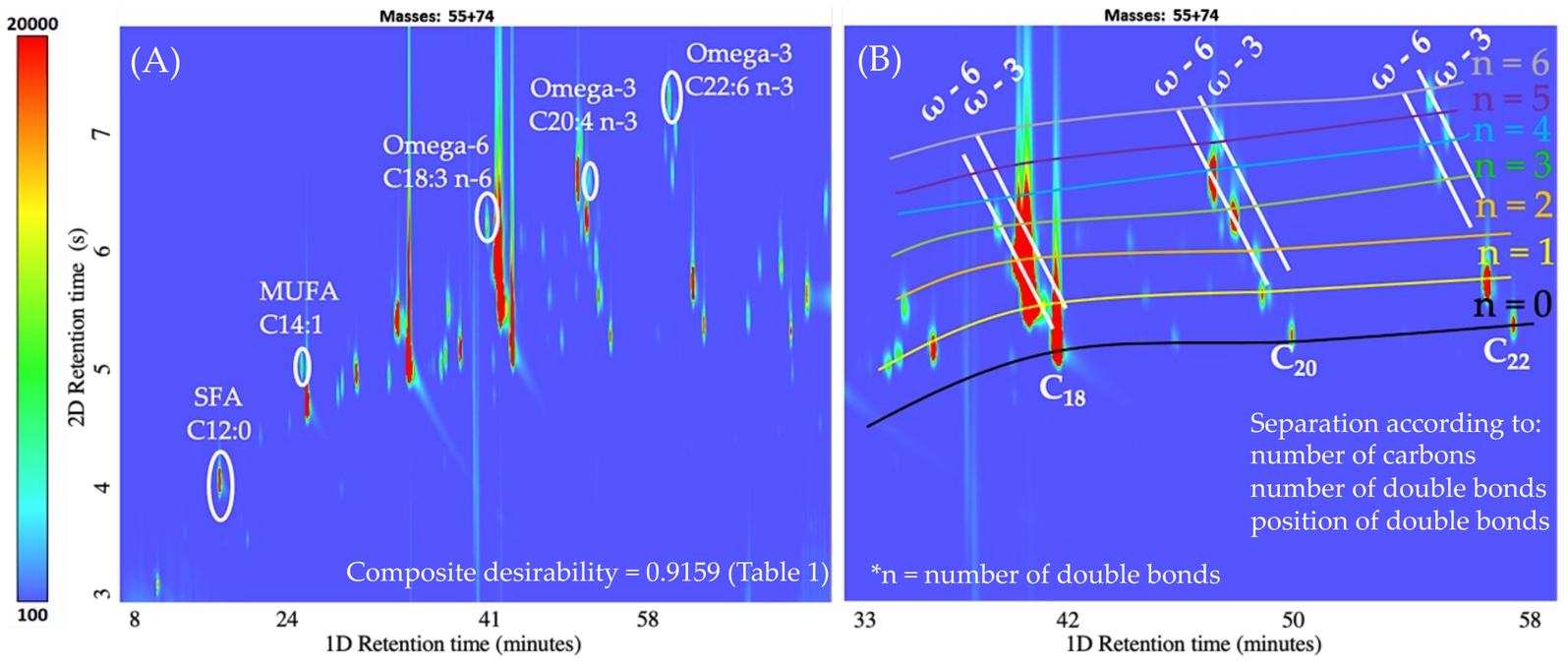

Figure 3: (A) Representative of each class selected for response optimization: contour plot of pooled human plasma (B) Zoomed in contour plot of pooled human plasma

- Analytes covering the major range of ^1t_R (min), ^2t_R (sec), and representative of all classes of FAMEs were selected for response optimization (Figure 3(A)).
- Monitoring FAMEs at selective m/z makes the data processing automatable: 74, 55, 67 for zero to two double bonds, respectively, while for three to six double bonds at 79.

Conclusion

- Statistically optimized sample preparation protocol to maintain a wide selectivity towards multiple classes of FAMEs [SFA, MUFA, and PUFA (ω-3 and ω-6)] is fully automatized with dual head autosampler. After optimization of biodiesel production process with GC×GC, the method can be easily transferred to 1D GC-MS, making it more economical and scalable.
- Developed on human plasma, verified with NIST plasma applied on pig plasma makes it most suitable for the analysis of animal fat feedstock and biodiesel testing[1].

Reference: [1] Bhatt, K.; Dejong, T.; Dubois, L.M.; Markey, A.; Gengler, N.; Wavreille, J.; Stefanuto, P.-H.; Focant, J.-F. Lipid Serum Profiling of Boar-Tainted and Untainted Pigs Using GC×GC–TOFMS: An Exploratory Study. Metabolites 2022, 12, 1111. https://doi.org/10.3390/metabo12111111

Kinjal Bhatt
kinjal.bhatt@uliege.be
ikinjalbhatt@gmail.com