Evaluation of a New Microwave assisted Saponification/Extraction Method for Mineral Oil Analysis in Food

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Introduction

Considered to be toxic for human, especially the MOAH fraction, Mineral oil hydrocarbons (MOHs) are food contaminants coming from petrogenic sources. Their analytical determination is associated with a huge variability, caused mainly by the interpretation of the chromatograms and the sample preparation steps for difficult matrices consisting of enrichment and purification. Software development has taken place and is still being improved to address the former point. Regarding the latter point, 2 interlaboratory trials conducted in 2021-22 concluded at a poor reproducibility at low level of contamination in fat samples, resulting in a LOQ of 2 mg/ml. Interestingly, both trials observed a discrepancy in the recovery of the internal standards (IS). In fact, the mean ratio of the TBB area divided to the MN standards area stands at 1.15 and 1.20 instead of 1. More importantly, in one trial, the recovery calculated with TBB was delivering better results while the use of 2MN resulted in better recoveries in another, indicating that the recovery of contamination could vary according to its specific composition, more similar to TBB or 2MN, and sum up to 20 % of variability. In this work, specific attention was paid to the IS ratio while developing a microwave-assisted saponification (MAS) method tackling this issue.

Material & Methods

Sample Preparation

Table 1. *Comparison of saponification methods used for MOHs analysis*

draft update DGF EN-16995:2017 [2]	JRC IF ILC	JRC IF ILC final [1]	Olive oil microwave [3]	Proposed method
3 g of sample into 30 mL C6/EtOH (1:1)	5 g of sample + 5 mL H2O at 35°C	5 g of sample + 10 mL H2O at 35°C	1 g of sample + 10 mL KOH 1.5M in MeOH + 10 mL C6	1 g of sample + 10 mL KOH 2M in EtOH/H2O + 10 mL C6
Heat 30 min at 60°C	Heat for 5 min to 60°C and shake at 120 rpm			
10 mL of the upper phase + 3 mL of KOH in water (500g/L) (3.3M EtOH/H2O 3/5)	Add 10 mL of KOH in EtOH/water (1/1) at 270 g/L (3.3M EtOH/H2O 1/2)	Add 10 mL of KOH in water at 500 g/L and 5 mL of EtOH 3.5M EtOH/H2O 1/4	1.5M MeOH/H2O 1/1	2M EtOH/H2O 1/1
Saponification for 30 min at 60°C	Heat at 60°C for 30 min, 120 rpm (water bath)	Saponification for 30 min at 60°C	MAS 120°C x 20 min	MAS 60°C x 30 min
Cool down + 5 mL C6 + 5 mL EtOH/H2O (1:1) (double extraction)	Cool down the solution and Add 15 mL C6 + shake	Cool down + 15 mL C6 + 15 mL EtOH/H2O (1:1) (double extraction)	Add 40 mL H2O + 3 mL MeOH	Add 20 mL H2O Fridge x 20 min 1 mL EtOH second extraction 5 mL C6
Transfer the upper phase on column	Take the 14 mL of the upper phase + transfer on column + elution with 15 mL DCM.	Evaporate to 2 mL and transfer the upper phase on column	-18°C x 30 min Evaporation to 4 ml wash with 3 ml of MeOH/H2O(2/1)	transfer on column + elution with 15 mL DCM.
Evap to 1 mL	Evap to 1 mL	Evap to 1 mL	Evap to 700 ul	Evap to 1 mL
Go to epoxidation	Go to epoxidation	Go to epoxidation	Go to epoxidation	Go to epoxidation

LC-GC×GC-ToFMS/FID system

LC: column: 250mm × 2.1mm i.d. × 5µm dp Allure silica (Restek). *Solvents A*: hexane; *B*: CH_2Cl_2. *Gradient*: 0' 100% A; 1.5'-6' 65 % A; 0.3 mL/min.

LC-GC Interface:CHRONECT LC-GC (Axel Semrau).

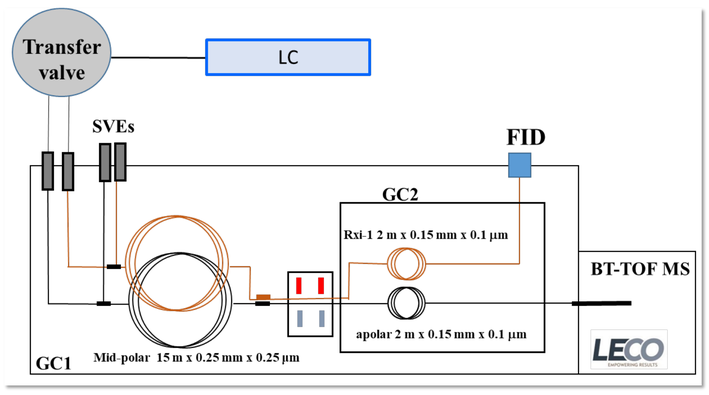

Fig 1. *Scheme of the instrument*

GC×GC: Pegasus BT 4D GC×GC ToFMS (LECO) Oven prog: 60 °C (8 min) to 350 °C (5 min) at 8 °C min^{-1}. The column set consisted of a MXT-1 15 m × 0.25 mm i.d. × 0.1 µm df connected to a Rxi-PAHs 1.2 m × 0.15 mm i.d. × 0.1 µm df (both from Restek). Second oven had a 5°C offset. Modulation time: 5 s.

Matrices abbreviations

SFO: Sunflower oil
CCNO: Coconut oil
RSO: Rapeseed oil
Palm: Palm oil
EVOO: Extra virgin olive oil

Results & discussion

Internal standard distribution

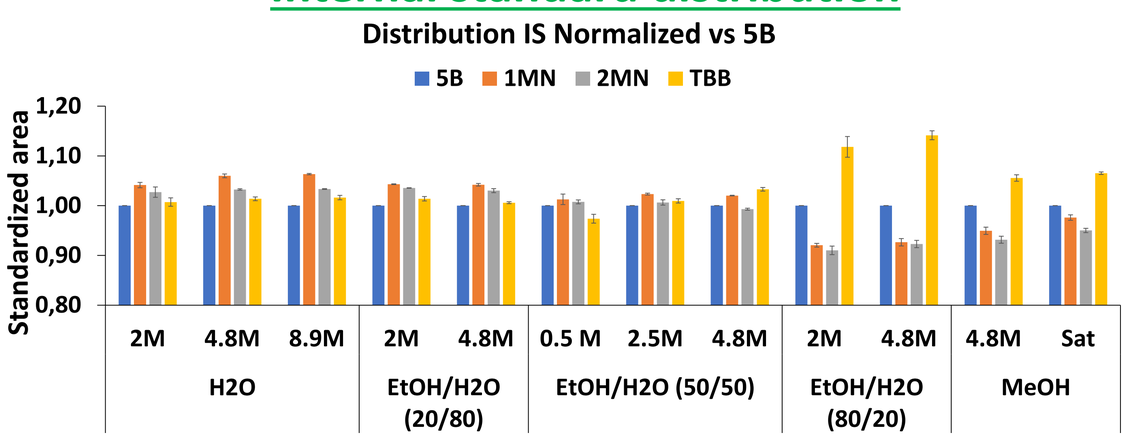

Fig 2. *Comparison of the MOAH IS ratio in different KOH solution*

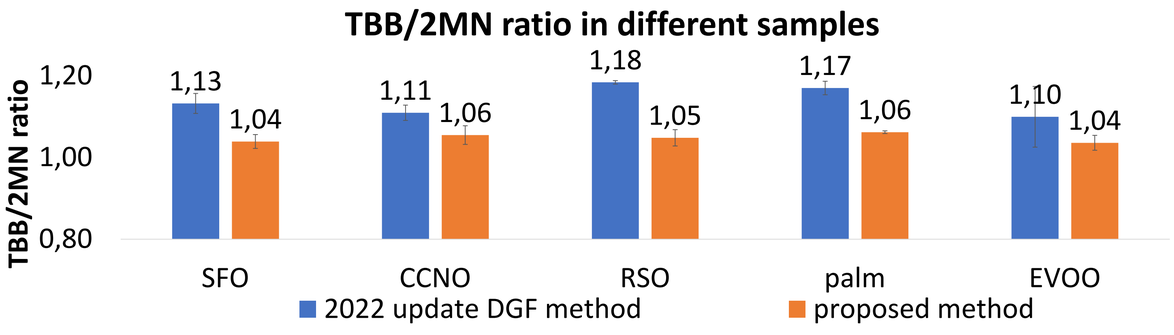

Fig 3. *MOAH IS ratios performing the 2022 update of the DGF method and the proposed one.*

The partition of the MOAH IS extracted with hexane from different KOH solutions was studied without the impact of the matrix (**Fig 2**). The TBB/2MN ratio of the method proposed at the end of development showed better results compared to the DGF-ILC method in different matrices (**Fig 3**).

Recovery tests

Samples of coconut oil and rapeseed oil having a low initial MOAH content were chosen to perform the recoveries trials (2.2 mg/kg for RSO and 3.9 mg/kg for CCNO). They were spiked with a solution of gravex (low volatility) and a solution of QC-10 (high-boiling point compounds) as references. These standards were chosen in order to cover all the volatility range of the MOHs. The amount spiked was respectively at 2.8 mg/kg of MOAH for QC-10 and 4.3 mg/kg of MOAH for gravex resulting in a total MOAH spiked of 7.1 mg/kg with a total of MOSH spiked at 29.7 mg/kg.

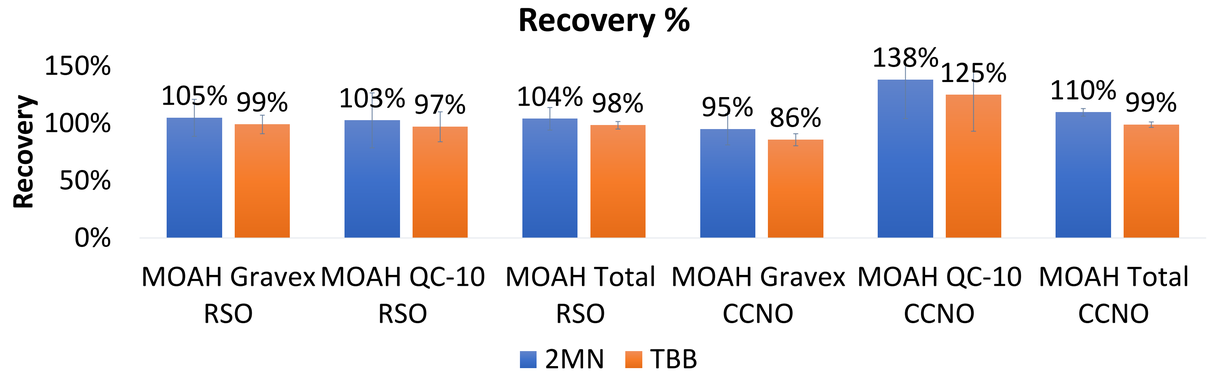

Fig 4. *Recoveries of MOH spiked with the developed method*

Conclusion

With the use of an optimized MAS method, it is possible to reduce the variability of the ratio between the TBB and 2MN IS compared with the reference methods for oils. The ratio is reduced from a range of 1.11 to 1.18 to a range of 1.02 to 1.08. The uncertainty linked with the choice of IS for quantification is then reduced by the use of this method. The novel method results in a more consistent extraction of the MOAH compounds regardless of the contamination profile.

References: [1] Bratinova, S., Robouch, P., Beldi, G., Senaldi, C., Karasek, L., Oliveira Goncalves, C., Valzacchi, S., Garcia Ruiz, S. and Hoekstra, E., Determination of MOAH in infant formula, EUR 31476 EN, Publications Office of the **European Union, Luxembourg, 2023, ISBN 978-92-68-01815-6**, doi:10.2760/606967, JRC133243. [2] Draft version for updating EN-16995:2017, October 2022-V5. Vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH) with online coupled HPLC-GC-FID analysis -Method for low limit of quantification [3] L. Menegoz Ursol, C. Conchione, A. Srbinovska, S. Moret, Optimization and validation of microwave assisted saponification (MAS) followed by epoxidation for high-sensitivity determination of mineral oil aromatic hydrocarbons (MOAH) in extra virgin olive oil, Food Chem. 370 (2022) 130966, doi:10.1016/j.foodchem.2021.130966..

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