MOSH & MOAH IN FOOD:
STATE-OF-THE-ART AND RECENT ADVANCEMENTS

Giorgia Purcaro, , Aleksandra Gorska, Grégory Bauwens

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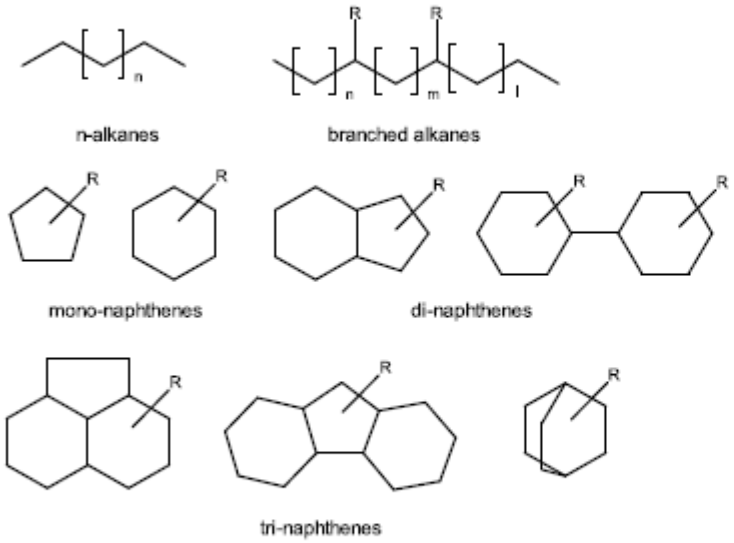
MINERAL OIL HYDROCARBONS (MOH): DEFINITION*

a wide range of products deriving from petroleum distillation fractions

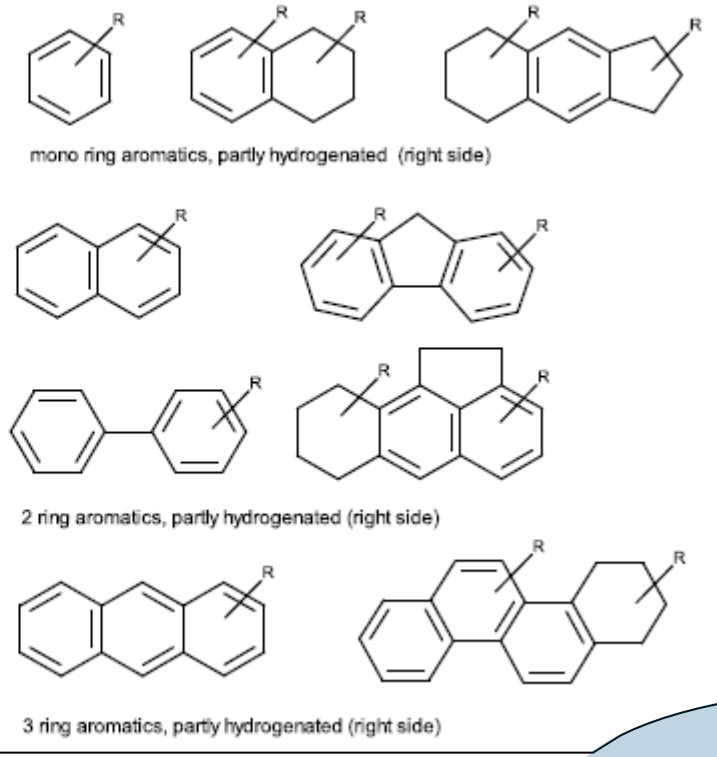
MOSH

Mineral oil saturated hydrocarbons



MOAH

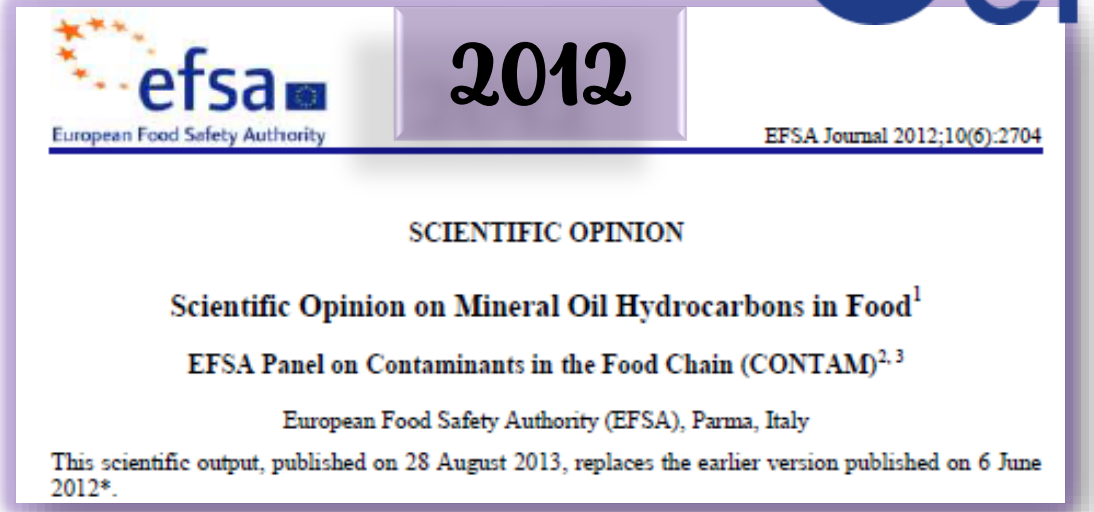
Mineral oil aromatic hydrocarbons



-n-alkane
- isoalkane
- cycloalkane

Aromatic hydrocarbons, mainly alkylated

MOSH & MOAH: STATE-OF-THE ART



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2012

EFSA Journal 2012;10(6):2704

SCIENTIFIC OPINION

Scientific Opinion on Mineral Oil Hydrocarbons in Food[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 28 August 2013, replaces the earlier version published on 6 June 2012*.

SCIENTIFIC OPINION

2023

efsa JOURNAL

ADOPTED: 12 July 2023

doi: 10.2903/j.efsa.2023.8215

Update of the risk assessment of mineral oil hydrocarbons in food

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MOSH & MOAH: STATE-OF-THE ART


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MOAH

- Generally considered of **no concern** at the concentration found, although accumulate in human body.

- **genotoxicity** of **MOAH with ≥3 aromatic rings**
- in the absence of reliable toxicity data, the dietary exposure to **1–2 ring MOAH might raise a concern**

Generation of further data for the refinement of the risk assessment is needed

MOSH & MOAH: STATE-OF-THE ART


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MOAH

Generation of further data for the refinement of the risk assessment is needed

- Better investigation of the **sources**
- Investigation on the **MOSH/MOAH structures and occurrence**

MOSH & MOAH: STATE-OF-THE ART


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European Food Safety Authority

2012

EFSA Journal 2012;10(6):2704

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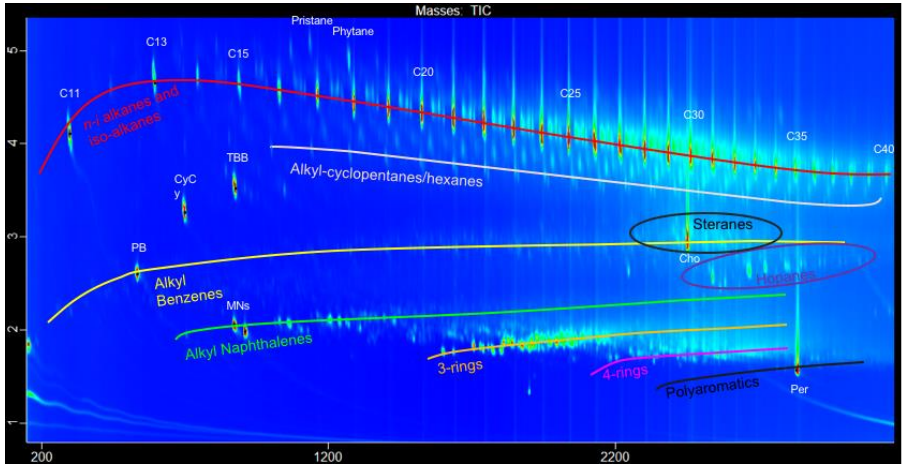
MOAH

Generation of further data for the refinement of the risk assessment is needed

➢ **Improvement of analytical methodology**

for better characterisation of MOSH&MOAH and **consistency in reporting**

MOSH & MOAH: STATE-OF-THE ART



Currently, the most efficient methods for analysis of MOSH and MOAH in food and feed comprise extraction followed by pre-separation by **high performance liquid chromatography (HPLC) on-line coupled to GC with flame ionisation detection (FID)**. Detection limits depend on the mass distribution, the sample matrix and any prior enrichment, and can be as low as 0.1 mg/kg. **Comprehensive GC×GC-FID** enables a rough separation and quantification of paraffins and naphthenes in the MOSH fraction, but it is of limited practicality for routine analysis. Contamination with polyolefin oligomeric saturated hydrocarbons (POSH), e.g. from plastic bags, heat sealable layers or adhesives, may interfere with MOSH analysis. Analytical capacity to distinguish the different MOAH subclasses in food is limited. For this purpose, **GC×GC appears to be the most effective method**. Due to the complexity and the variable composition of MOH mixtures, it is not possible to define certified standards of general applicability.

Quantitative advantages of the GC×GC over the GC

GC×GC's higher se … **MOSH and MOAH** and …

Green Analytical Chemistry 4 (2023) 100047

Contents lists available at ScienceDirect

Green Analytical Chemistry

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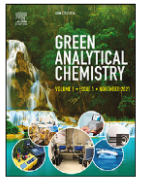

Validation of the liquid chromatography-comprehensive multidimensional gas chromatography-time-of-flight mass spectrometer/flame ionization detector platform for mineral oil analysis exploiting interlaboratory comparison data

Grégory Bauwens[a], Laura Barp[b,*], Giorgia Purcaro[a,*]

[a] Analytical Chemistry Lab, Gembloux Agro-Bio Tech, University of Liège, Gembloux, 5030, Belgium
[b] Department of Agri-Food, Environmental and Animal Science, University of Udine, via delle Scienze 206, Udine 33100, Italy

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Mineral oil saturated and aromatic hy… Mono- and two-dimensional approach…

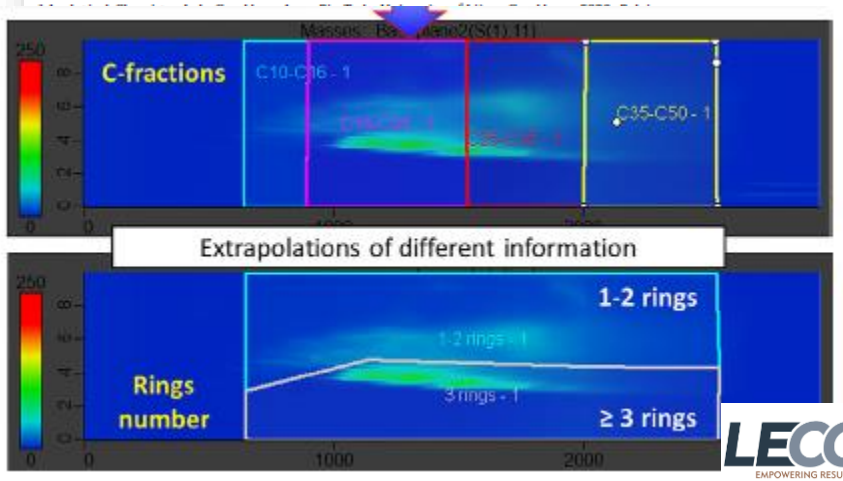
Grégory Bauwens[a], Sebastiano Pantó[b], Giorgia Purcaro[a,*]

…aphy Open 2 (2022) 100072 … available at ScienceDirect … …omatography Open … www.elsevier.com/locate/jcoa

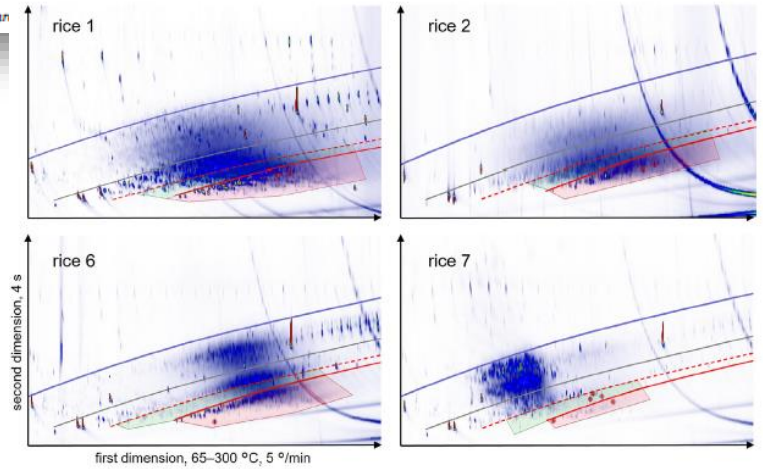
…rocarbons by number of …ensional gas chromatography: First results in food

Maurus Biedermann*, Angela Eicher, Tanja Altherr, Gregor McCombie

Official Food Control Authority of the Canton of Zürich, Fehr…



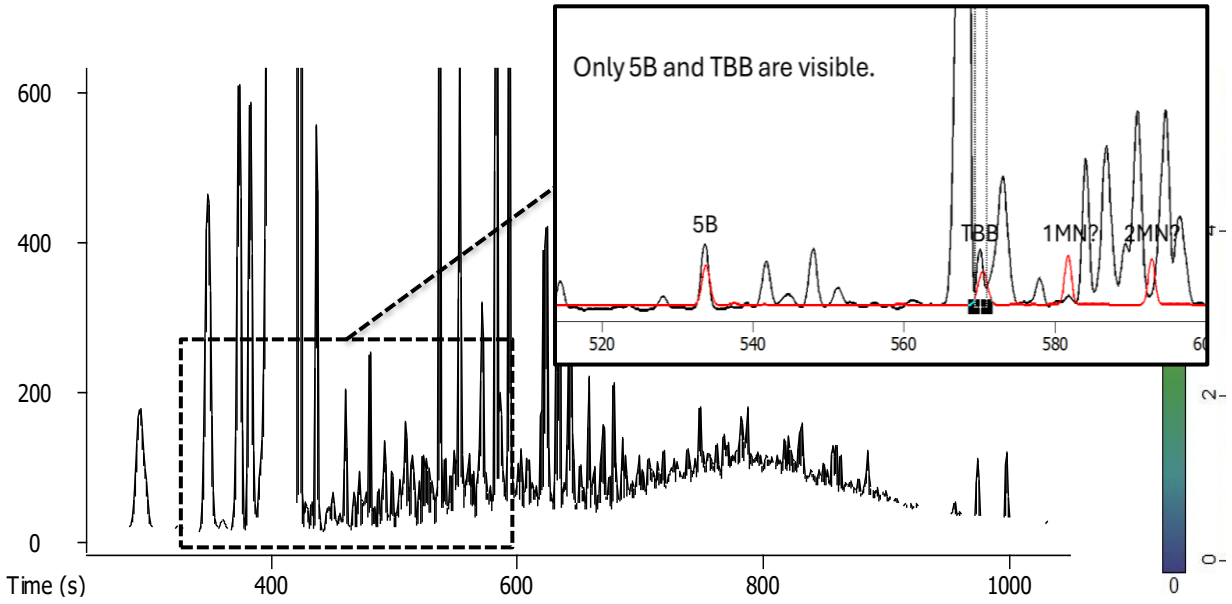
MOSH & MOAH and **sub-classes** quantification



Quantitative advantages of the GC×GC over the GC

GC×GC's higher separation power is useful for **more accurately determining MOSH and MOAH** and **sub-class determination**

MOAH analysis by GC-FID



Internal standards coeluted with interferences

→ impossible MOAH quantification

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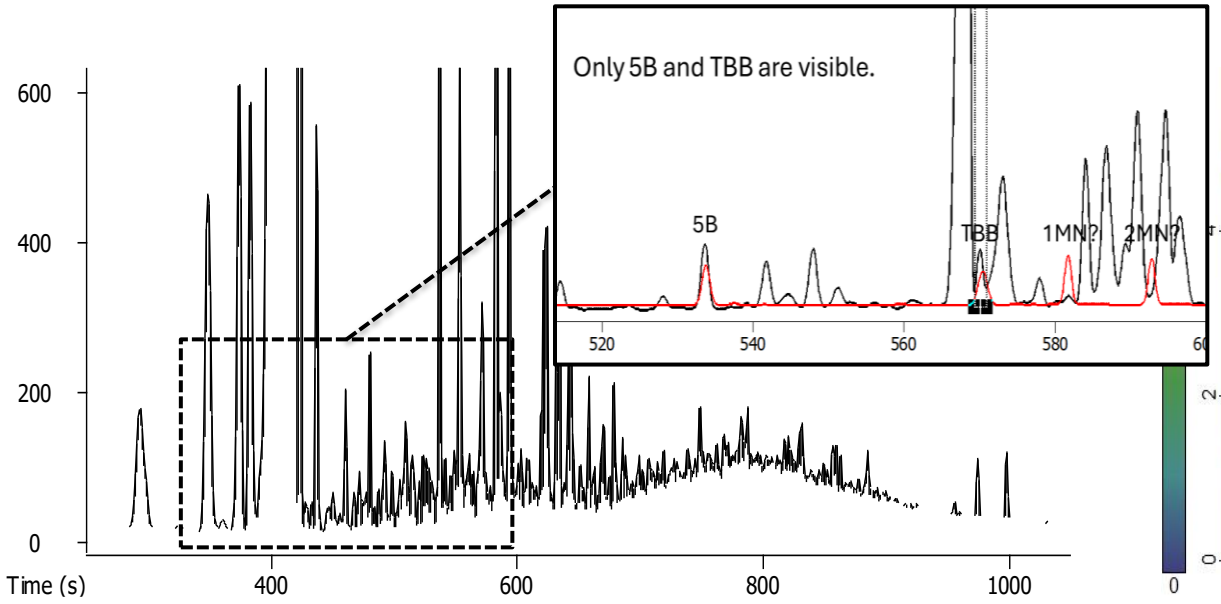

Internal standards coeluted with interferences
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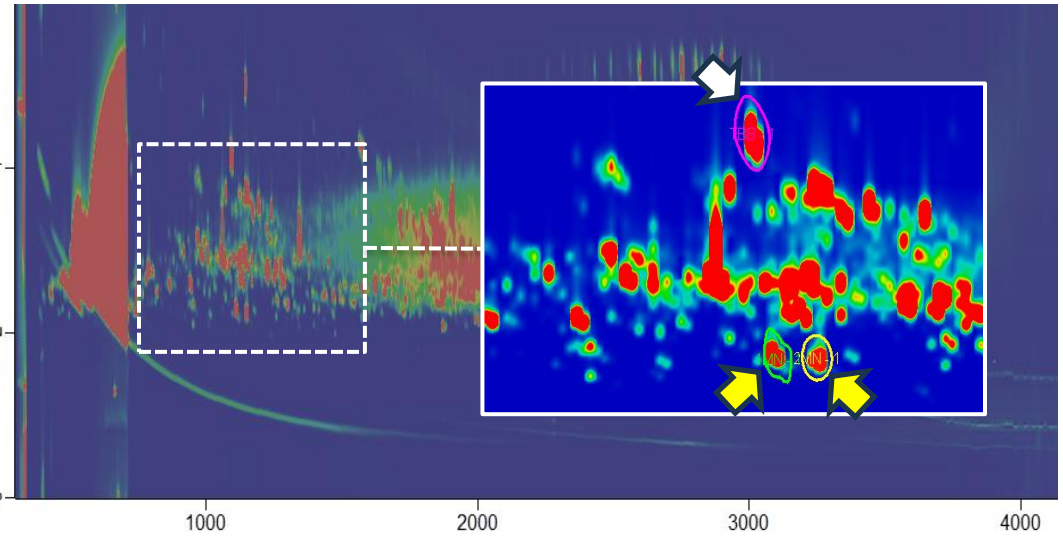
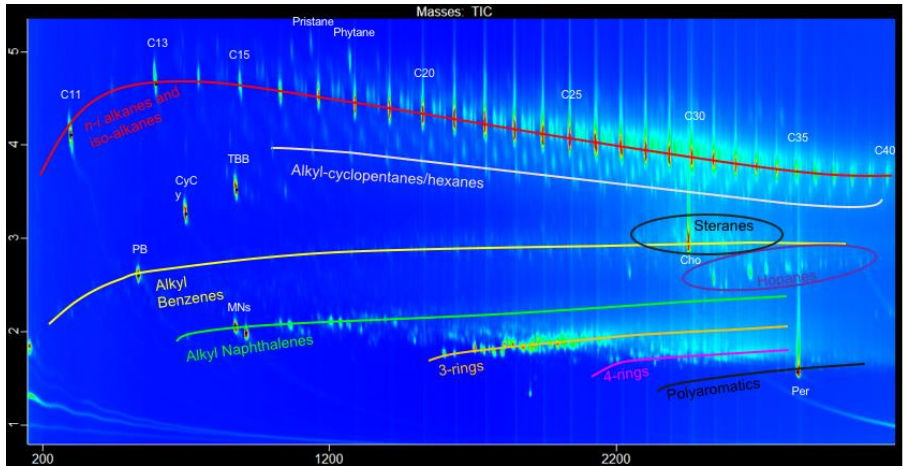
MOAH analysis by GC×GC-FID



Internal standards resolved from the interferences
→ quantification of MOAH is possible

ISs separation

MOSH & MOAH: STATE-OF-THE ART



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SCIENTIFIC OPINION

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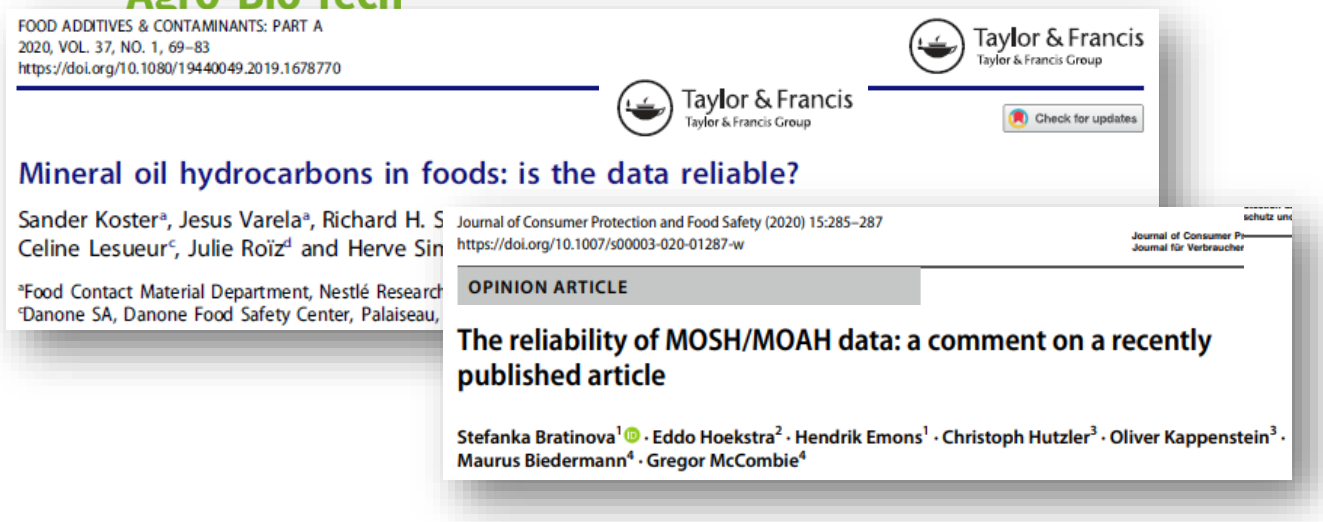
Update of the risk assessment of mineral oil hydrocarbons in food

EFSA Panel on Contaminants in the Food Chain (CONTAM),

"…Over the last 10 years, **progress in compositional analysis** has been achieved through comprehensive two-dimensional GC (GC × GC) with FID and MS. The main features of **GC × GC** are not only significantly **better separation** and **lower detection limits**, but also placing structurally related compounds in an order, e.g. MOAH according to the number of aromatic rings. In this way, GC × GC may **provide structural information** if just a single compound, or even no compound of the series could be identified owing to lacking standards or reference mass spectra. …"

MOSH & MOAH: STATE-OF-THE ART


FOOD ADDITIVES & CONTAMINANTS: PART A
2020, VOL. 37, NO. 1, 69–83
https://doi.org/10.1080/19440049.2019.1678770

Taylor & Francis
Taylor & Francis Group

Check for updates

Mineral oil hydrocarbons in foods: is the data reliable?

Sander Koster[a], Jesus Varela[a], Richard H. S
Celine Lesueur[c], Julie Roïz[d] and Herve Sin

[a]Food Contact Material Department, Nestlé Researc
[c]Danone SA, Danone Food Safety Center, Palaiseau,

Journal of Consumer Protection and Food Safety (2020) 15:285–287
https://doi.org/10.1007/s00003-020-01287-w

OPINION ARTICLE

The reliability of MOSH/MOAH data: a comment on a recently published article

Stefanka Bratinova[1] · Eddo Hoekstra[2] · Hendrik Emons[1] · Christoph Hutzler[3] · Oliver Kappenstein[3] · Maurus Biedermann[4] · Gregor McCombie[4]


2023

efsa JOURNAL

SCIENTIFIC OPINION

Update of the risk assessment of mineral oil hydrocarbons in food

EFSA Panel on Contaminants in the Food Chain (CONTAM),

"…There have been, and still are, **discussions about the reliability of the results,** particularly for measurements at low concentrations. The LC-GC-FID method can be considered as standard and reliable, validated by collaborative tests. Nonetheless, sometimes there were large differences in the results from different laboratories. There were several reasons for this."

(i) Blank and cross-contamination due to the **ubiquitous presence** of MOH.

(ii) Interference removal is a critical step ↔ at lower **limit of quantitation**

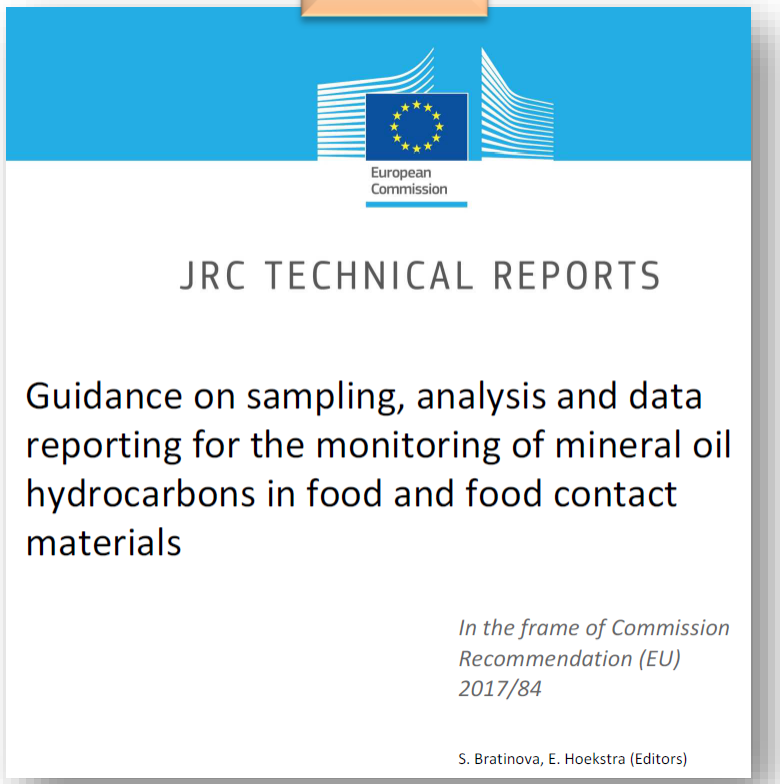
(iii) Chromatogram **interpretation** → needs experience in the interpretation

Consistency in reporting and data reliability

JRC guidance: **harmonised procedures**

- ✓ for sample prep (decision tree)/standardization
- ✓ C-fraction reported (extended to C50)

2019



2022

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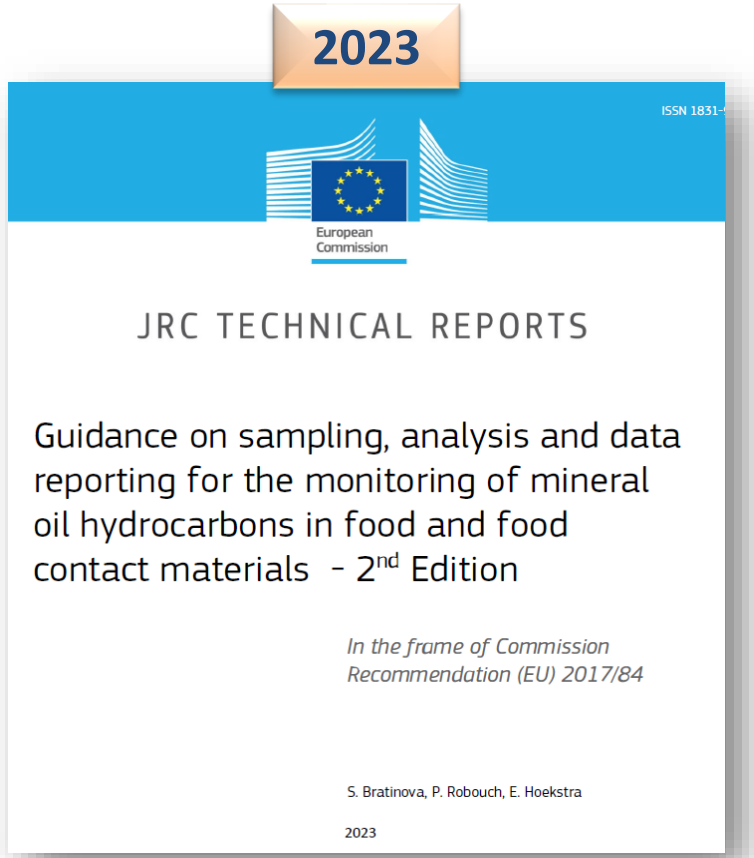

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Outline of the Analytical approach

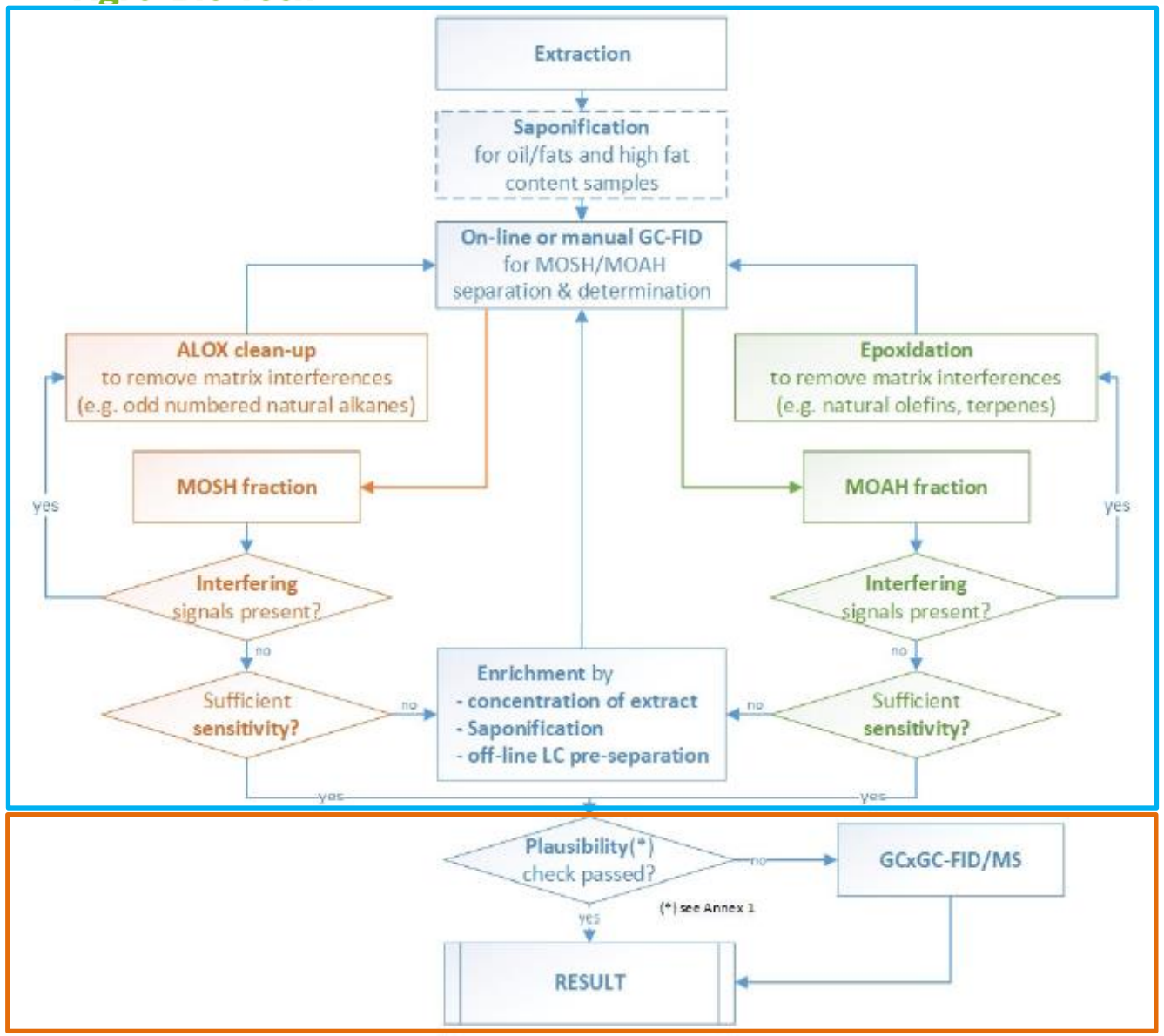
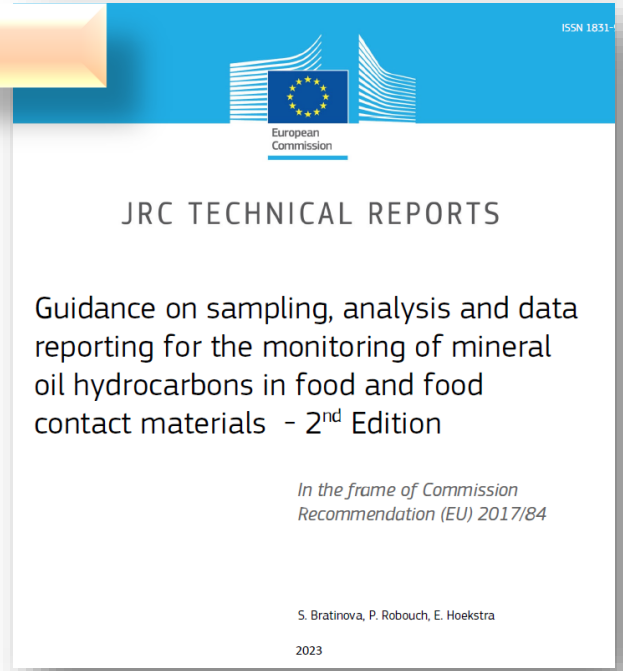


Figure 5 Decision tree on the use of auxiliary methods.

Sample Preparation



✓*Need for matrix-tailored sample prep protocols*

> 20% of uncertainty!

Data Interpretation

Data Integration

Consistency in reporting and data reliability

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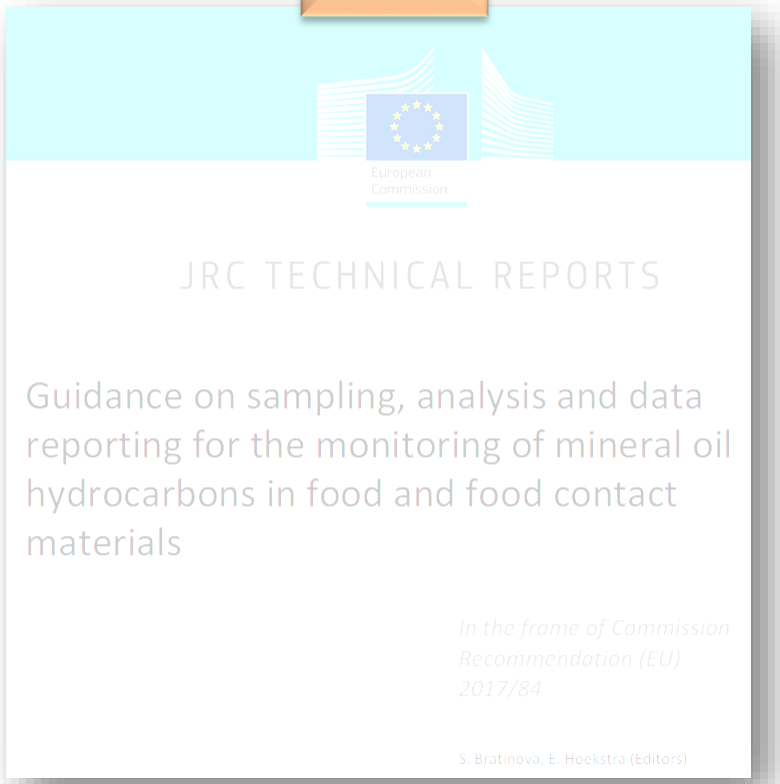

2022

2023

Consistency in reporting and data reliability

> 20% of uncertainty!

Data Interpretation

Data Integration

- **Baseline**
- **Riding peaks subtraction**

Consistency in reporting and data reliability

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Data Integration

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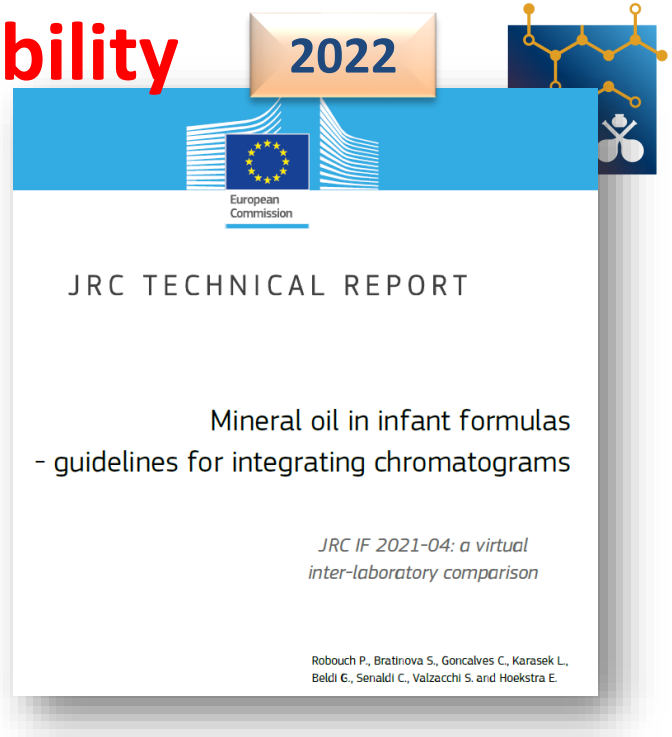
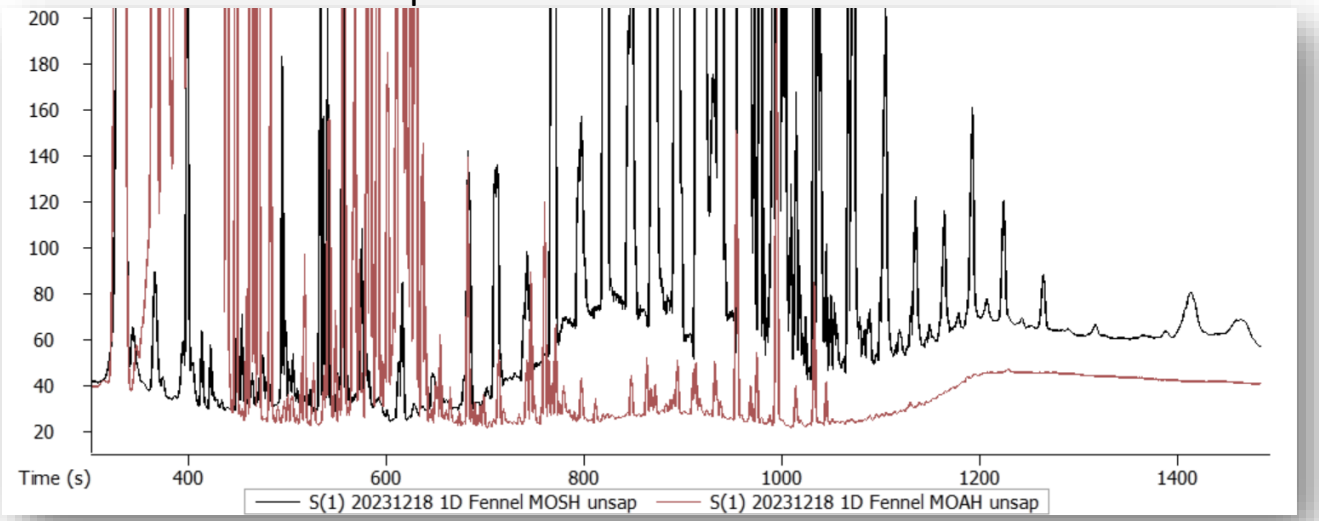


MOSH vs MOAH – No purification



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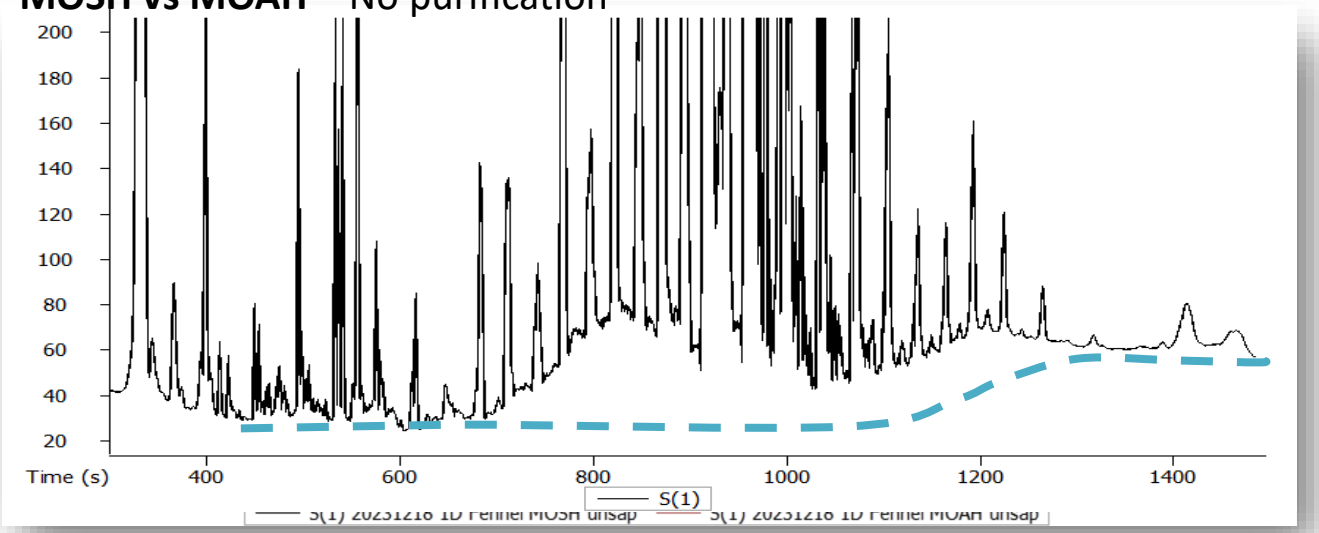

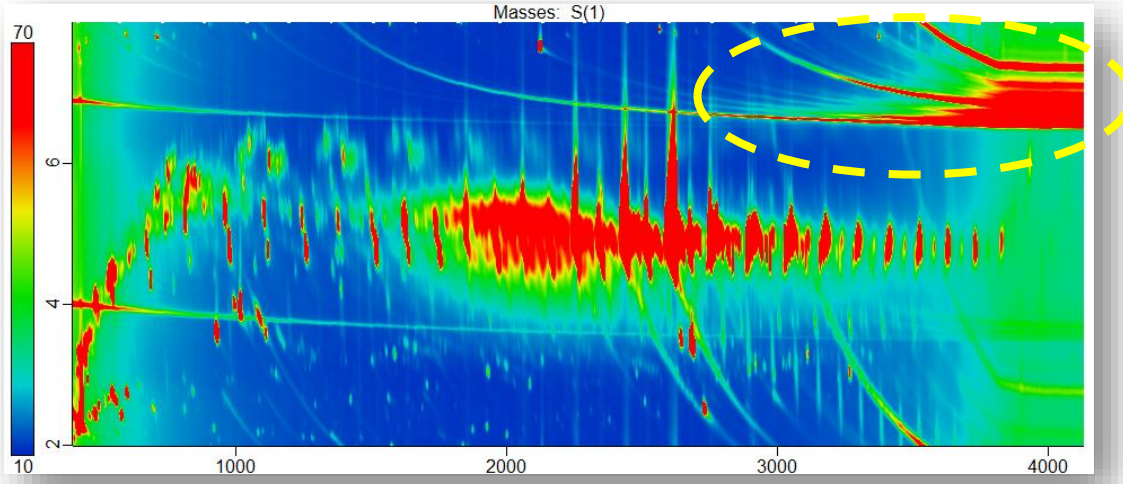

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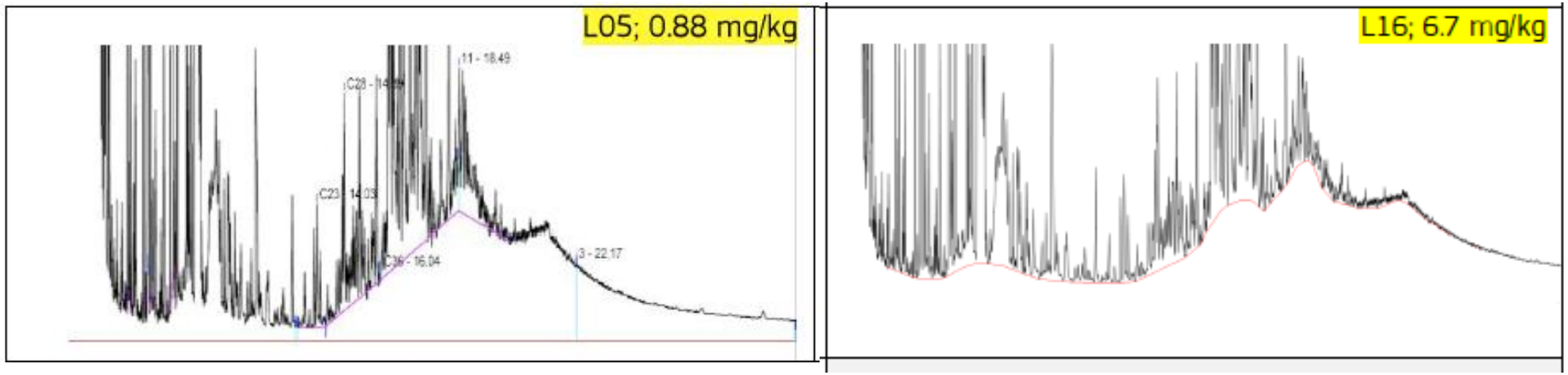

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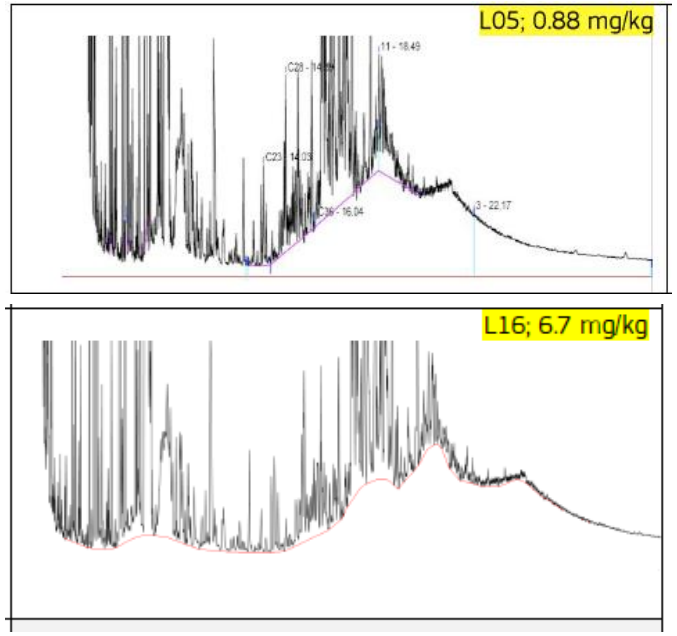


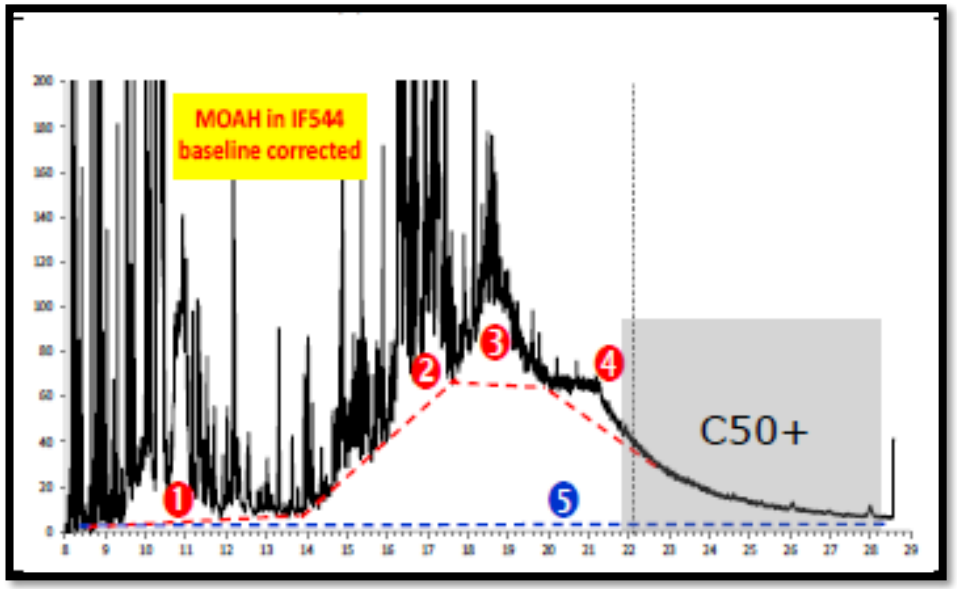


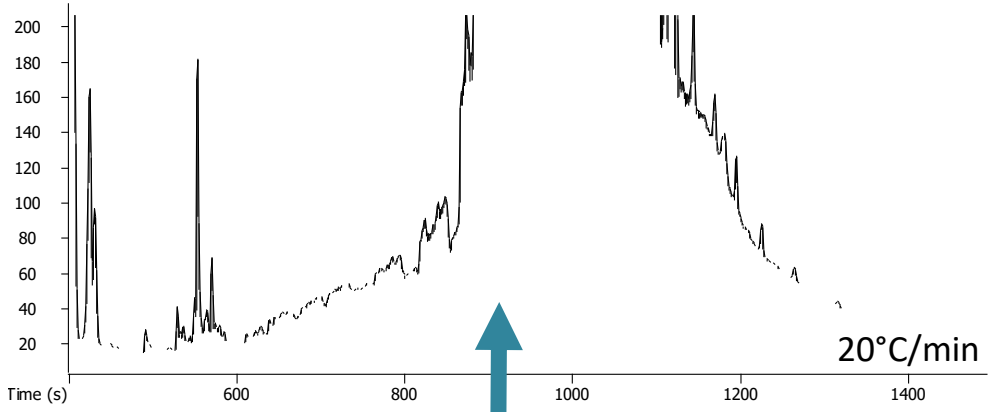
Figure 3: MOAH in IF 544 chromatograms - Comparison of different integration approaches presented by laboratories having reported total mass fractions of MOAH (C10-C50) ranging from 0.88 to 6.7 mg/kg.

← The last chromatogram (on the left) highlights various RT regions to be considered (1 to 5), while the table below summarises the riding peaks/humps included (yes, no, or partially) by the laboratories.

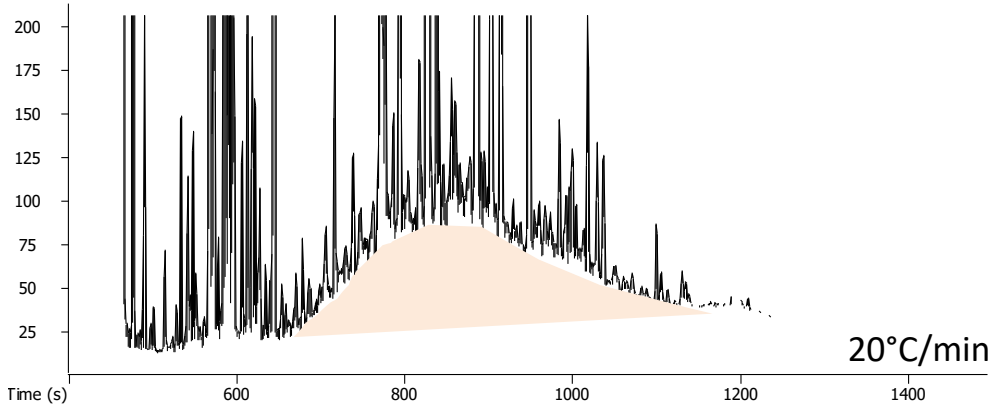
Consistency in reporting and data reliability

Is the 1D enough?

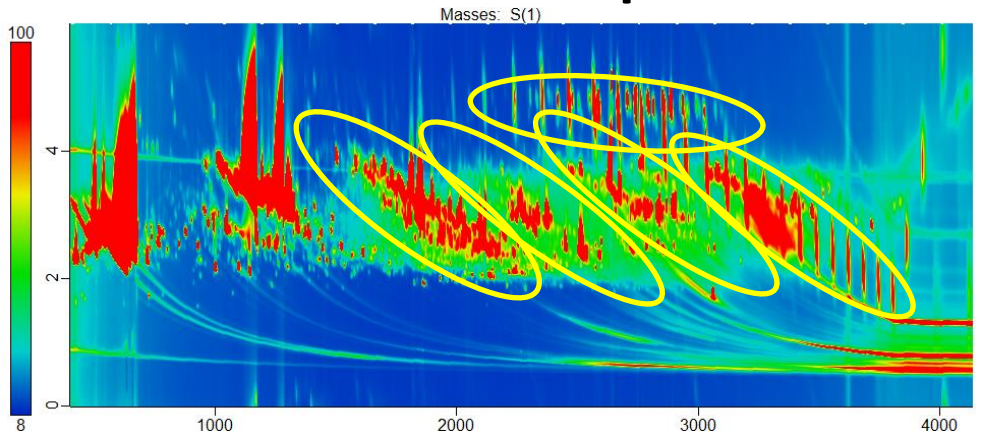
MOAH – no epox



MOAH – with epox



MOAH – no epox



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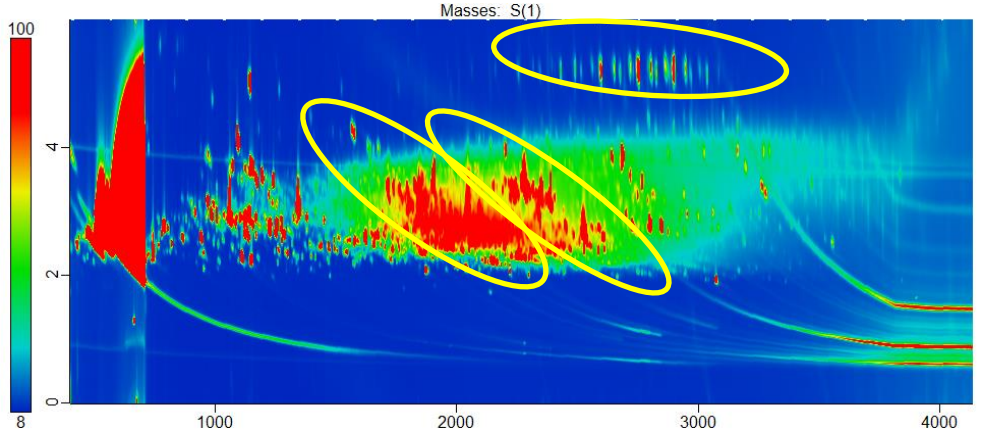


Outline of the Analytical approach

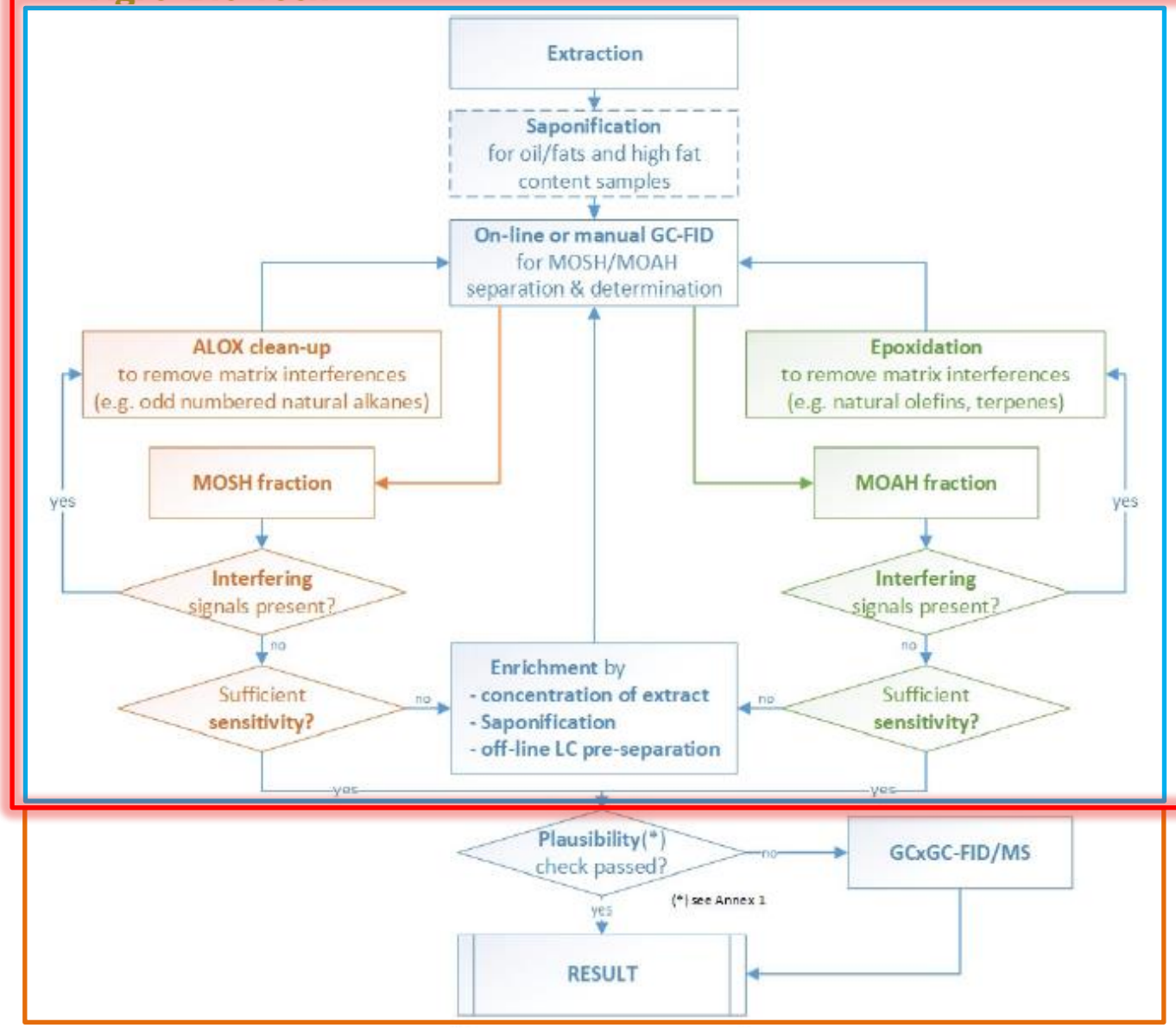
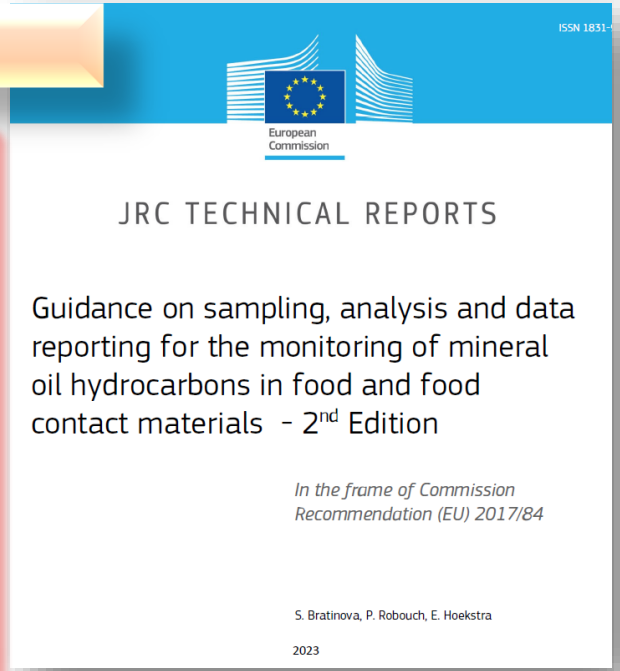


Figure 5 Decision tree on the use of auxiliary methods.

Sample Preparation

Data Interpretation

Data Integration



✓*Need for matrix-tailored sample prep protocols*

> 20% of uncertainty!

Consistency in reporting and data reliability

ISO 20122:2024

Vegetable oils

Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with online-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID) analysis

Method for low limit of quantification

Status : **Published**

ISSN 1831-9424

European Commission

JRC TECHNICAL REPORT

Determination of MOAH in infant formula

JRC IF 2022-05 – the ring trial validation study

Consistency in reporting and data reliability

ISO 20122:2024

In order to ensure correct signal evaluation, especially at the level of the LOQ, the offset of the baseline in the area of integration of MOSH and MOAH must not exceed one quarter of the height of the signal to be integrated (see Figure 18, Annex A). In such cases the chromatographic separation performance of the system must be improved or the LOQ must be increased.

In summary, the following conditions should be examined:

- appropriate straight baseline, blank level ≤ 1/3 of the LOQ,
- peaks are symmetrical and do not show a significant tailing,
- complete separation of solvent and C10,
- discrimination C10/C20 and C50/C20 between 80 % ≤ x ≤ 120 %,
- peak ratio between TBB and 2MN ≤1,25,
- Check the LOQ of a matrix by adding a suitable mineral oil product before sample preparation. The signal of the hump at the LOQ should have a relative standard deviation ≤ 20 % and a signal to noise ratio ≥ 10. In addition, the LOQ should be tested with a mineral oil product, whose signal width from start to end is comparable to or higher than the boiling range of the MOH in the sample to be evaluated.

peak ratio between TBB and 2MN **≤1.25**

peak ratio between TBB and 2MN **= 1.00**

Infant Formula

Even if statistically negligible, the mass fraction of total MOAH referred to TBB was constantly 13 % lower than the one obtained using 2MN. It is therefore recommended, in order to ensure proper comparability of results, to report the MOAH in IF using TBB as the internal standard.

Still, users should be aware that this experimental protocol does not fully remove all the interfering compounds present in some challenging matrices, which may require a cautious interpretation and integration of the recorded chromatograms. Further characterisation with two-dimensional GC-GC techniques may be required.

total MOAH referred to **TBB** was constantly **13 %** lower than the one obtained using **2MN**.

Consistency in reporting and data reliability

ISO 20122:2024

TBB/2MN ≤1.25

Infant Formula

TBB/2MN =1.13

ISSN 1831-

European Commission

JRC TECHNICAL REPORTS

Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials - 2nd Edition

2023

In the frame of Commission Recommendation (EU) 2017/84

S. Bratinova, P. Robouch, E. Hoekstra

2023

When complete saponification is applied and the extraction of mineral oils is performed in hexane in the absence of fat layers, the ratio TBB vs 1- or 2-MN is above 1 with a mean value around 1.15, even after a second hexane extraction of the aqueous saponification solution. This was confirmed during the two ring trial validation studies for the determination of MOSH/MOAH in edible oil and fats by CEN and in infant formula by the JRC organised in 2021/22. It might be attributed to the difference in distribution between the aqueous and hexane phases of the two IS belonging to two different classes of substances (2-ring non-branched and single ring branched aromatic hydrocarbons). In such cases, the quantification is performed *vs* TBB as the efficiency of the extraction of 1-MN and 2-MN in hexane is lower. Consequently, when saponification is applied, the results reported *vs* TBB should be reported with uncertainty reflecting the contribution of the saponification step.

TBB/2MN =1.15

Consistency in reporting and data reliability

ISO 20122:2024

TBB/2MN ≤1.25

Infant Formula

TBB/2MN =1.13

European Commission

JRC TECHNICAL REPORTS

Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials - 2nd Edition

2023

In the frame of Commission Recommendation (EU) 2017/84

S. Bratinova, P. Robouch, E. Hoekstra

2023

When complete saponification is applied and the extraction of mineral oils is performed in hexane in the absence of fat layers, the ratio TBB vs 1- or 2-MN is above 1 with a mean value around 1.15, even after a second hexane extraction of the aqueous saponification solution. This was confirmed during the two ring trial validation studies for the determination of MOSH/MOAH in edible oil and fats by CEN and in infant formula by the JRC organised in 2021/22. It might be attributed to the difference in distribution between the aqueous and hexane phases of the two IS belonging to two different classes of substances (2-ring non-branched and single ring branched aromatic hydrocarbons). In such cases, the quantification is performed *vs* TBB as the efficiency of the extraction of 1-MN and 2-MN in hexane is lower. Consequently, when saponification is applied, the results reported *vs* TBB should be reported with uncertainty reflecting the contribution of the saponification step.

Use **TBB**

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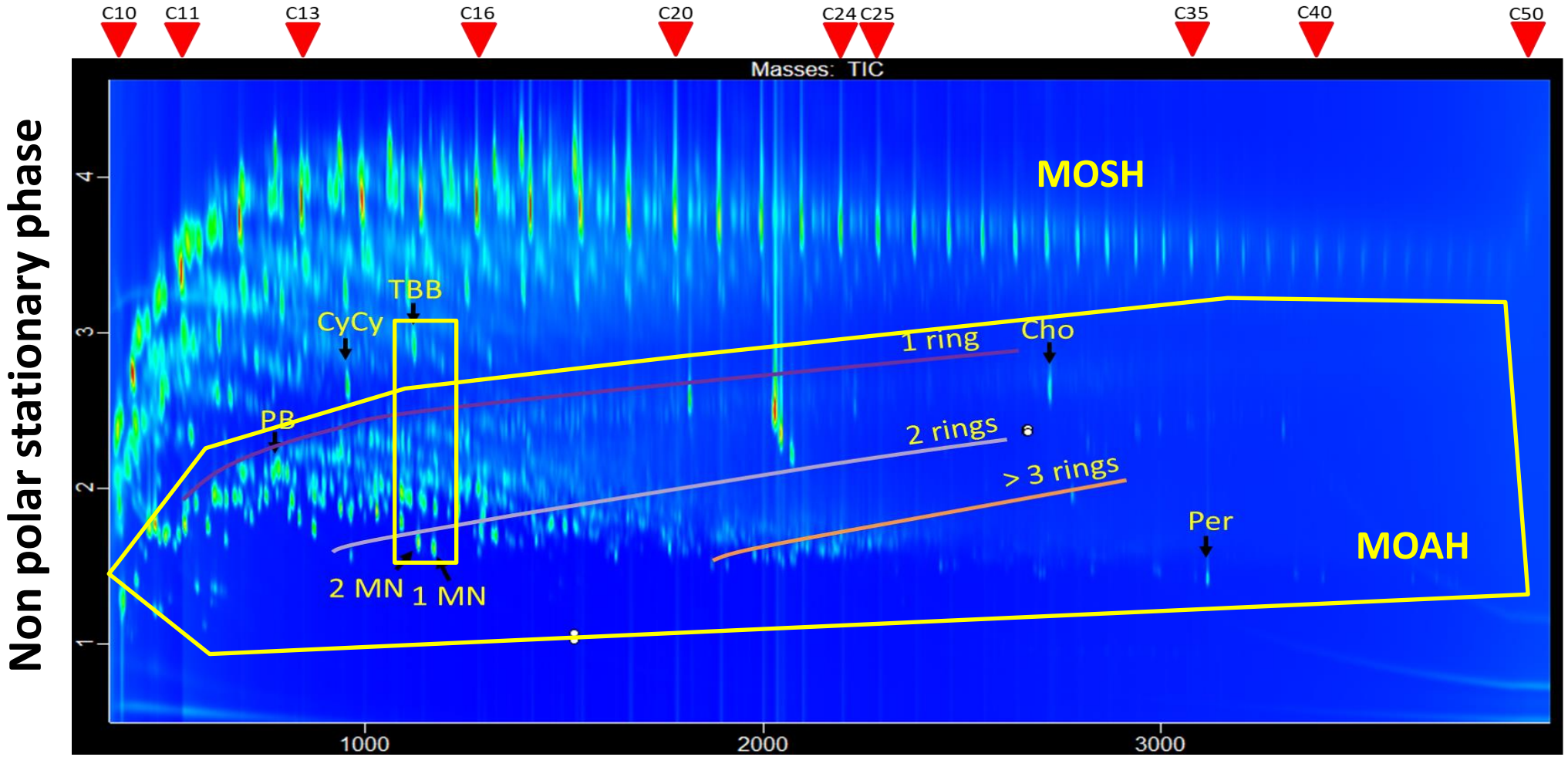
GC: a partition process

BUT an internal standard should be representative!



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Outline of the Analytical approach

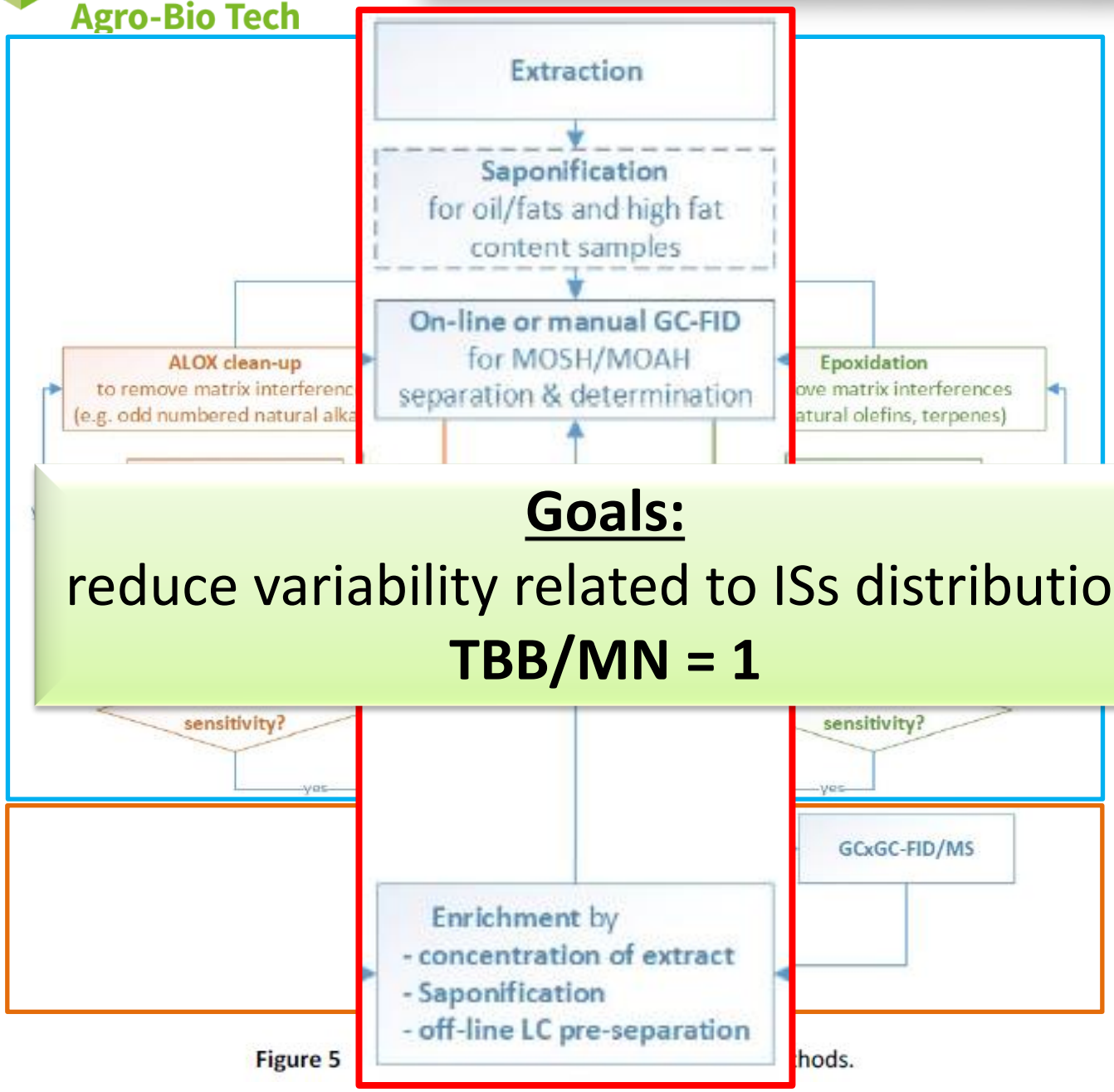


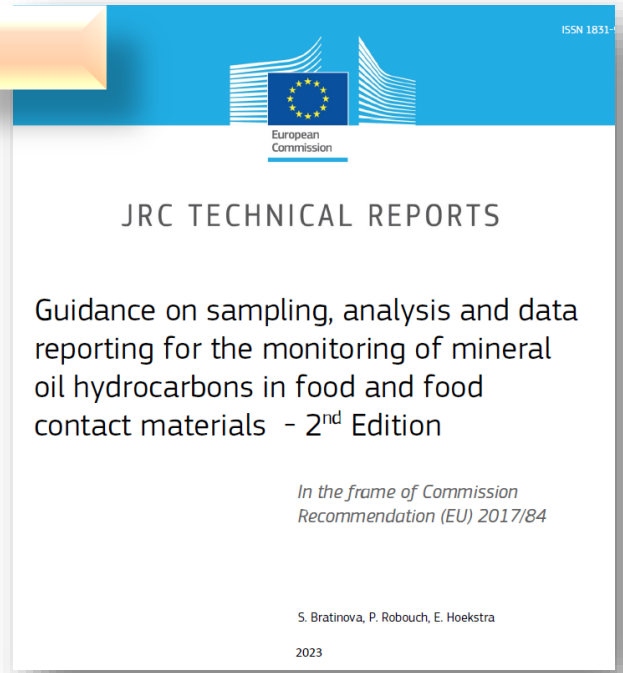
Figure 5 hods.

Goals:

reduce variability related to ISs distribution

TBB/MN = 1

Sample Preparation



✓*Need for matrix-tailored sample prep protocols*

> 20% of uncertainty!

Data Interpretation

Data Integration

Extraction: saponification step

MOAH

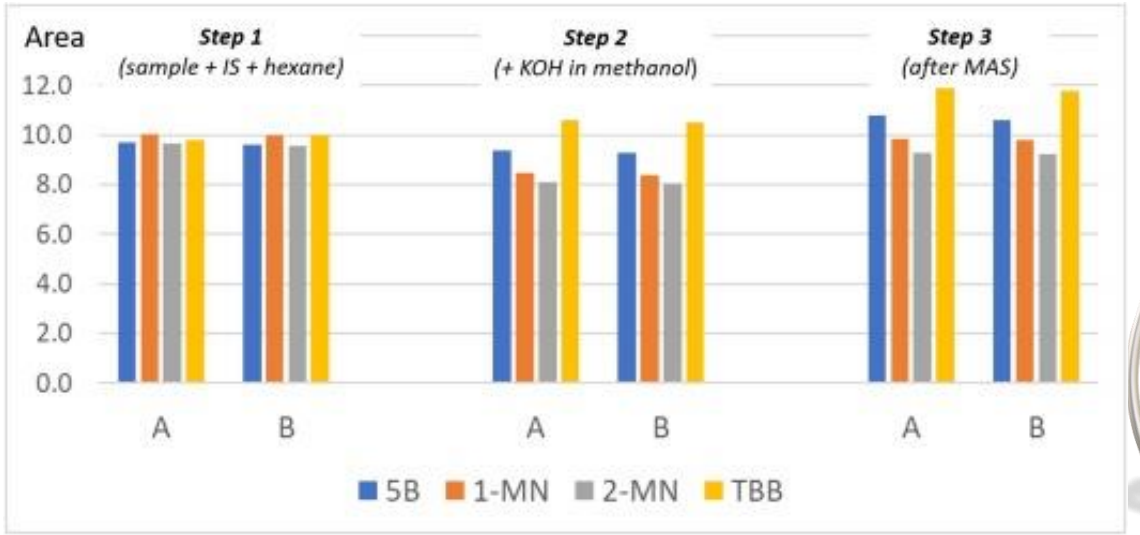
Enrichment by **concentration** of extracts and/or **saponification** and/or off-line LC pre-separation

Saponification

- Traditional saponification
  ISO 20122:2024
- Microwave assisted saponification-MAS

TBB/2MN **≤1.25**

TBB/MN= 1.15-1.2



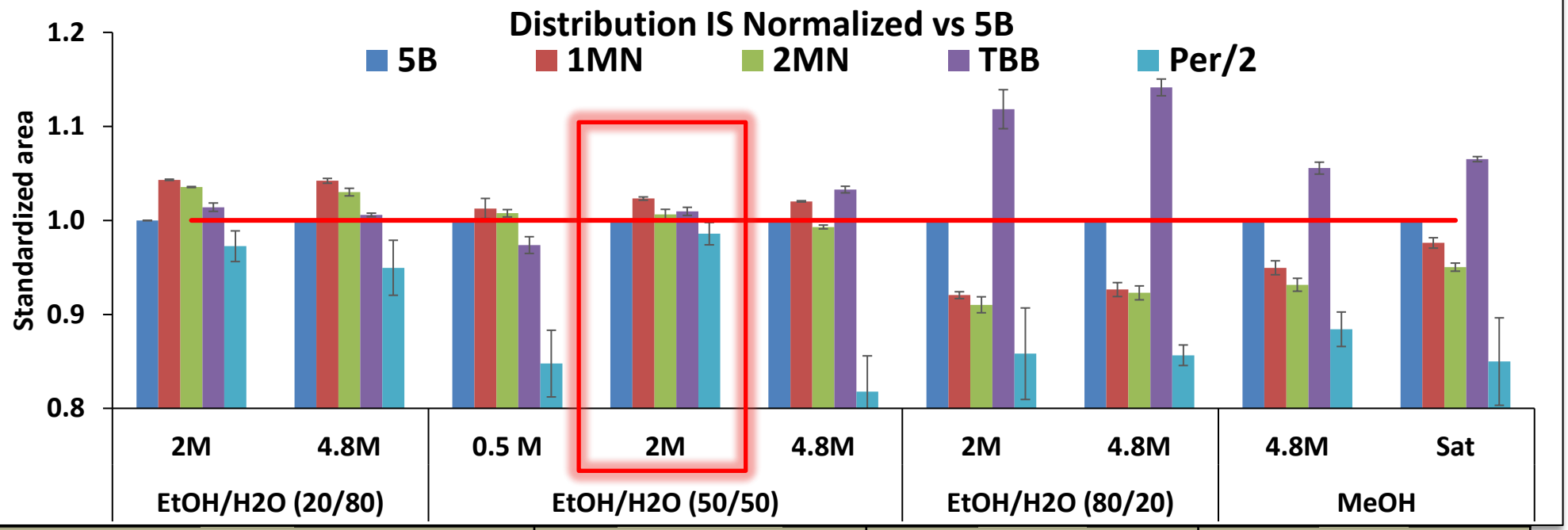
Menegoz Ursol et al., Food Chemistry, 2022, 370, 13096

Re-optimize the saponification step

MOAH		*Menegoz-Ursol et al 2022 [22]*	*DGF/ISO- 20122 [18]*	*JRC-IF [19]*
Brief description	***Saponification***	1g oil + 10 mL 1.5 M KOH (1.5 M)/MeOH + 10 mL C6. Saponification at 120 °C for 20 min.	3 g oil in 30 mL C6/EtOH (1:1). 10 mL of this added with 3 mL KOH in H2O (0.5 g/mL). Saponification at 60 °C for 30min	5 g infant formula + 10 mL H2O. Add 10 mL of KOH (4.8 M) in EtOH/water (1:1) + 5mL EtOH. Saponification at 60°C for 30 min
	washing	40 mL of H2O + 3 mL of MeOH added in the vessel. Store at -18 °C for 30 min. Collected the C6 lyer, concentrate to 4 mL and wash with 3 mL MeOH/H2O (2/1 v/v)	Add 5mL C6 + 5 mL EtOH/H2O (1/1 v/v). Repeat twice and combine the C6 phases.	15 mL C6 + 2.5 mL EtOH twice. Wash C6 with 15 mL of EtOH/ H2O (1/1 v/v)
Mass sample		1g oil	1 g oil in 10 mL C6/EtOH (1:1)	5 g IF + 5 mL H2O
[KOH]		1.5 M KOH	~3.4 M	4.8 M
Solvent mixture Sap		MeOH (+ 10 mL C6)	EtOH/H2O (5/3 v/v) + (5mL C6)	EtOH/H2O (6/4 v/v)
Apparatus		MAS	Water Bath	Water Bath
Temperature		120°C	60 °C	60 °C
Time		20min	30 min	30 min

Re-optimize the saponification step

MOAH	*Menegoz-Ursol et al 2022 [22]*	*DGF/ISO- 20122 [18]*	*JRC-IF [19]*



[KOH]	1.5 M KOH	~3.4 M	4.8 M
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Apparatus	MAS	Water Bath	Water Bath
Temperature	120°C	60 °C	60 °C
Time	20min	30 min	30 min

Re-optimize the saponification step

MOAH

Enrichment by **concentration** of extracts and/or **saponification** and/or off-line LC pre-separation

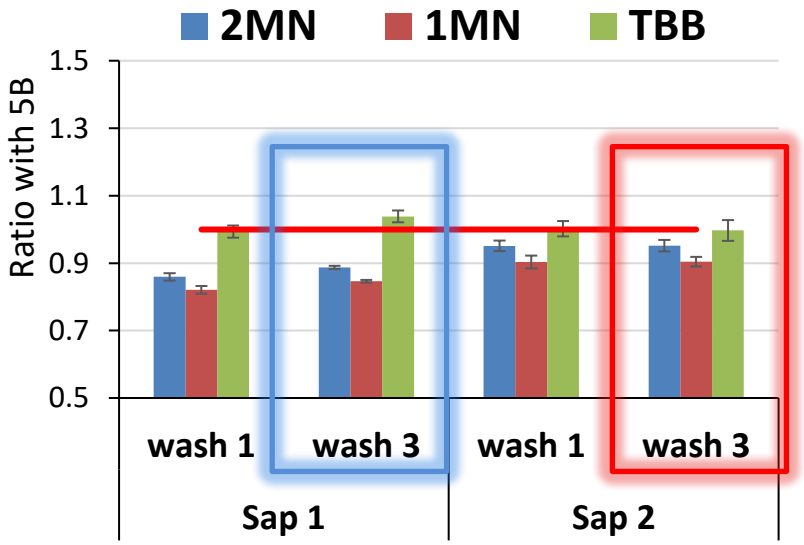
Saponification

- Microwave assisted saponification-MAS

with the conditions used in *ISO 20122:2024*.

- **60 °C for 30 min**
- Comparing **Sap: 1**- 2 M KOH in EtOH/H2O (**5/3 v/v**) *ISO 20122:2024*
  2- 2 M KOH in EtOH/H2O (**1/1 v/v**).
- Comparing **Washing : 1-** Add 5 mL of C6 + 5 mL EtOH/H2O (1/1 v/v). *ISO 20122:2024*
  3- 20 mL of H2O

EVO



Re-optimize the saponification step

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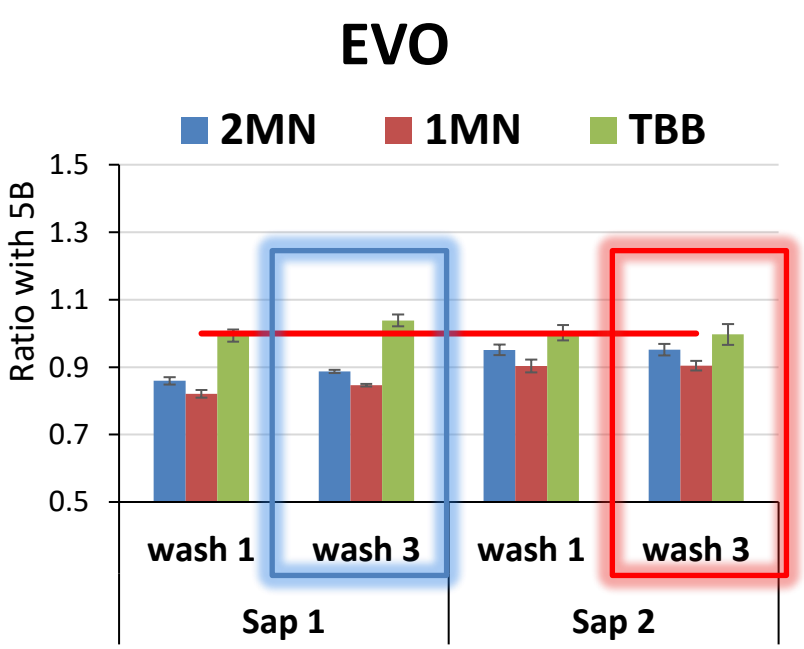

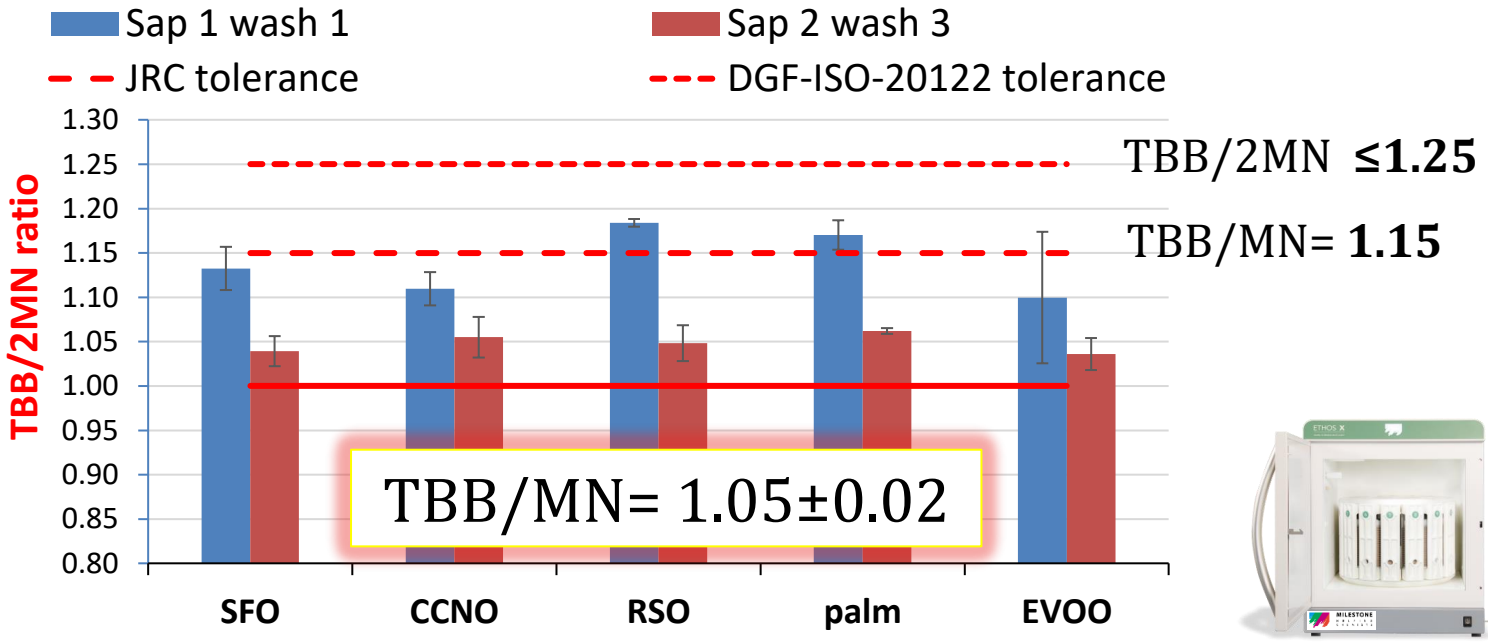

Re-optimize the saponification step

MOAH

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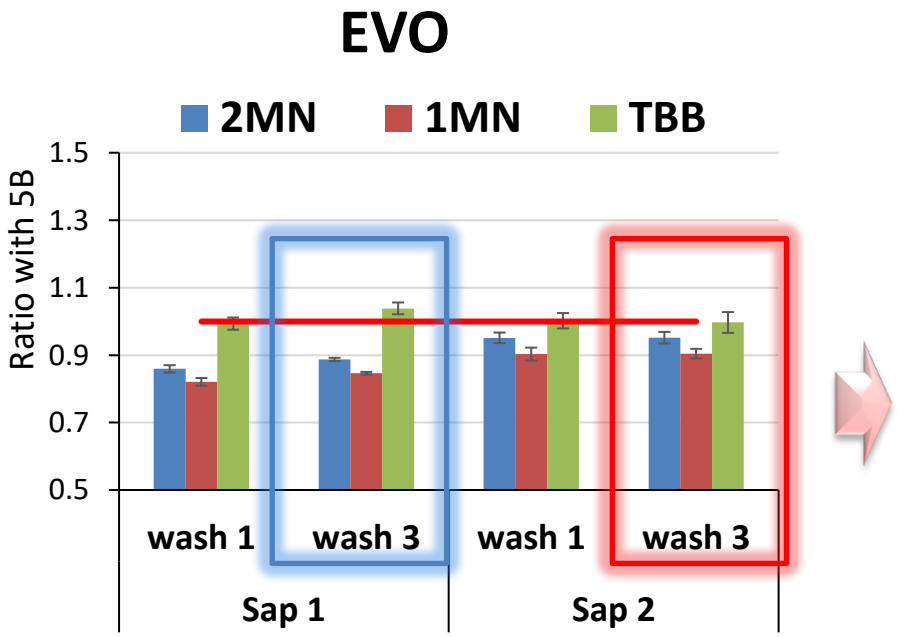

TBB/2MN ratio

2024 Posters | Analytical

Session: Analytical Poster Session

Reducing the variability of the saponification step for MOSH&MOAH analysis using microwave-assisted extraction

Monday, April 29, 2024 5:00 PM – 6:00 PM EDT

Presenting Author(s)

Aleksandra Gorska, Ir (she/her/hers)
Teaching Assistant and PhD candidate
Gembloux Agro-Bio Tech (University of Liège)
Gembloux, Namur, Belgium

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Outline of the Analytical approach

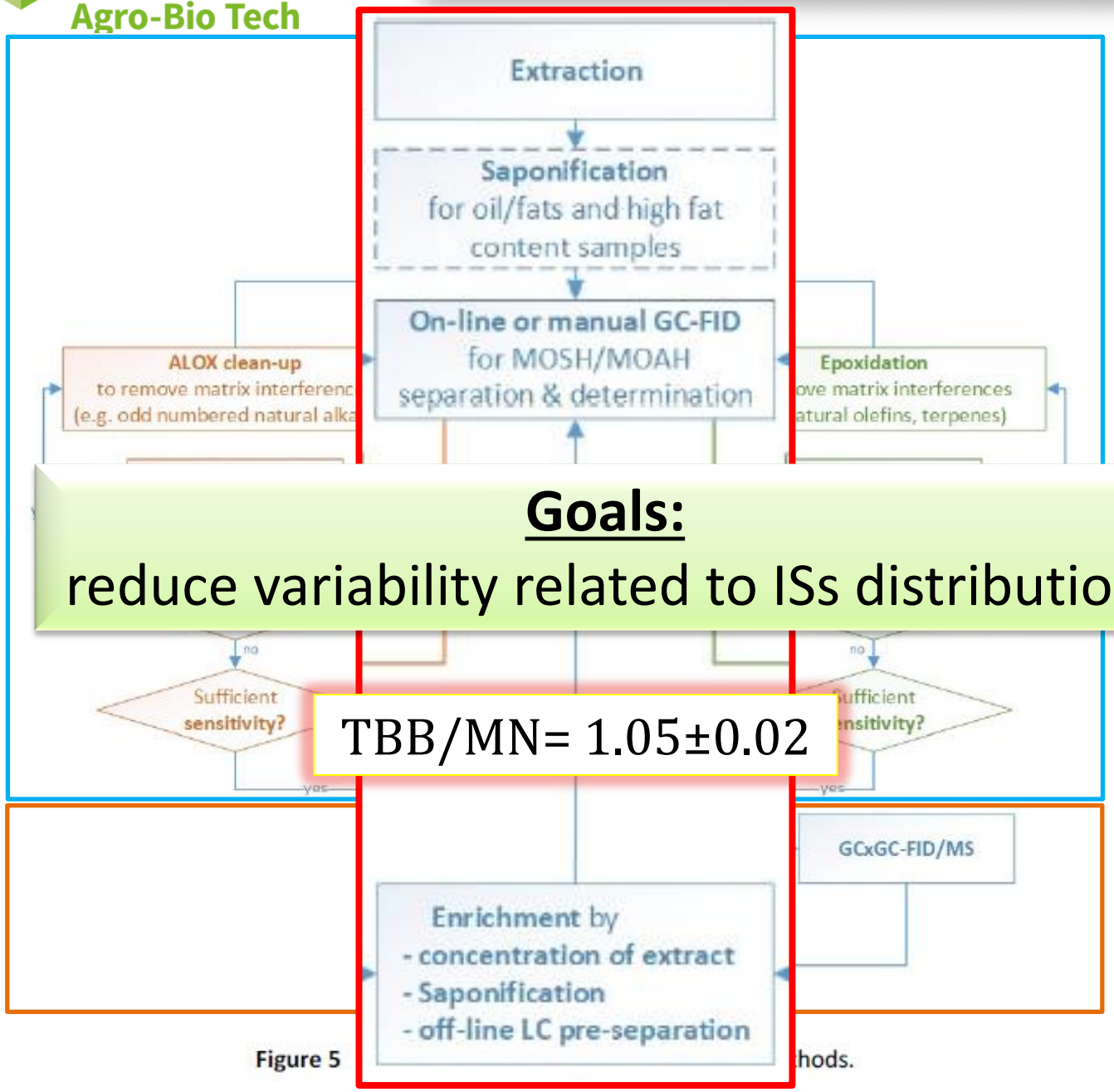


Figure 5 hods.

Goals:

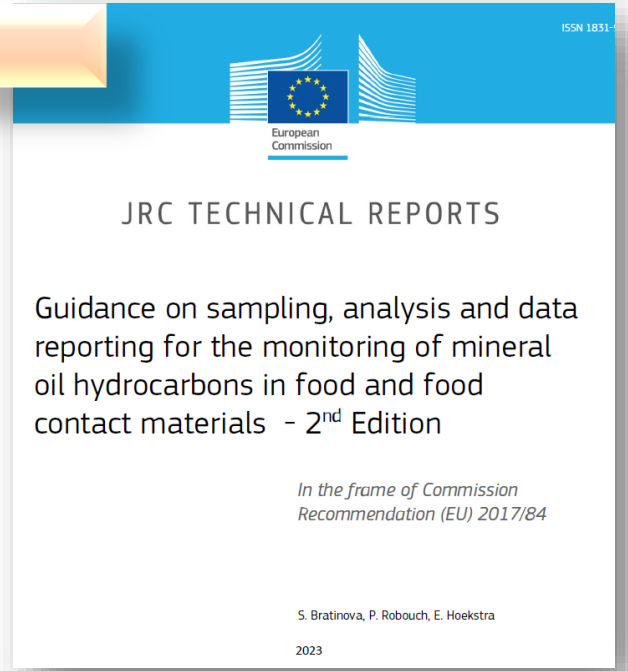
reduce variability related to ISs distribution

TBB/MN= 1.05±0.02

Sample Preparation

Data Interpretation

Data Integration



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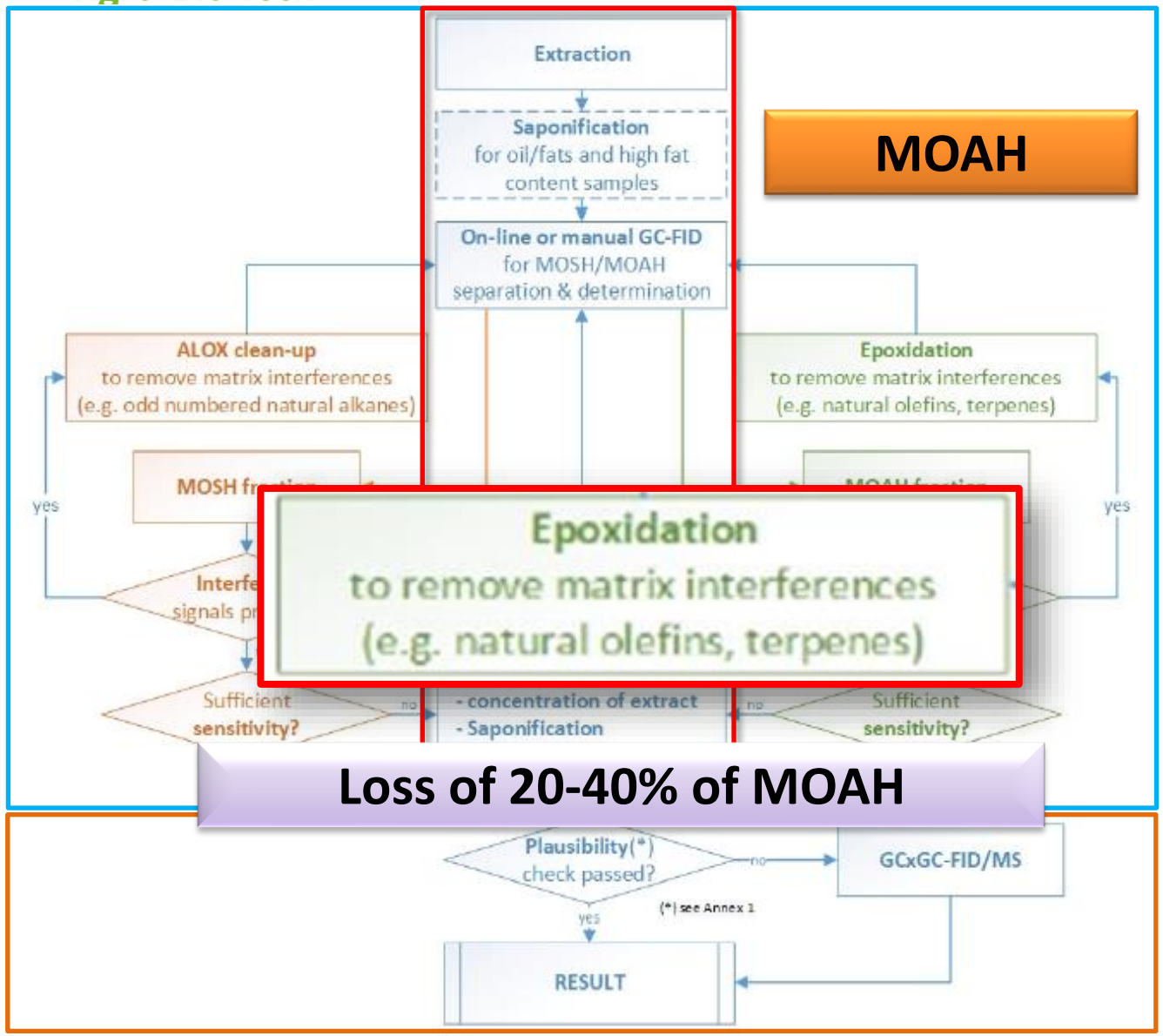
Outline of the Analytical approach



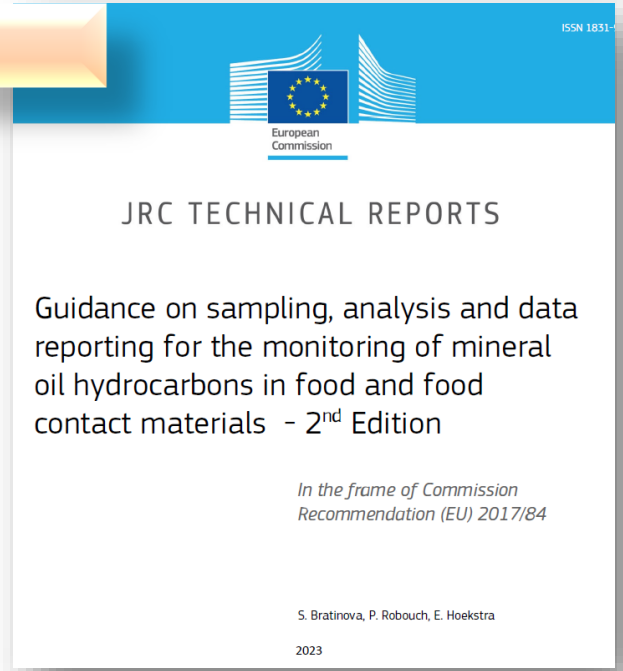
MOAH

Epoxidation
to remove matrix interferences
(e.g. natural olefins, terpenes)

Loss of 20-40% of MOAH

Figure 5 Decision tree on the use of auxiliary methods.

Sample Preparation



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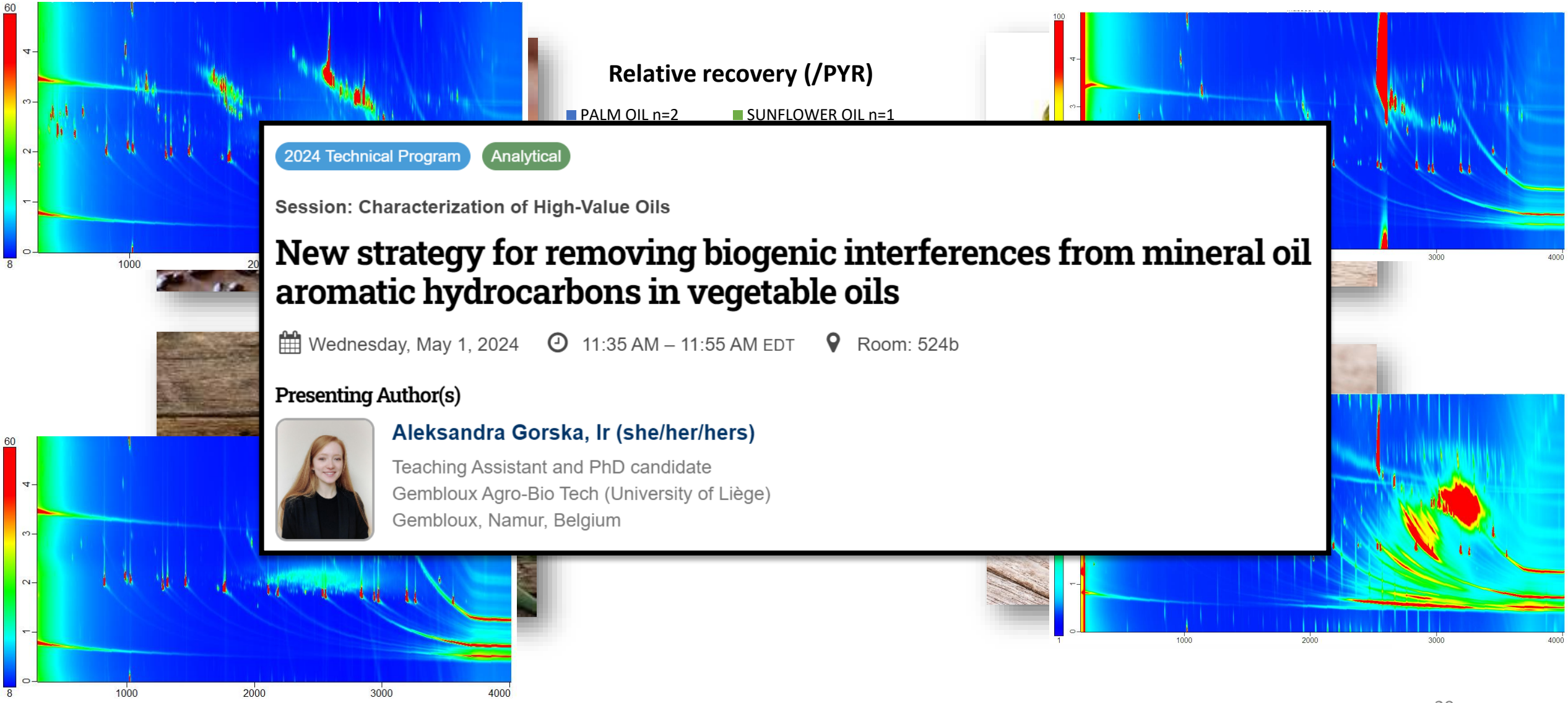
Data Interpretation

Data Integration

Alternative to Epoxidation

Alternative to Epoxidation



2024 Technical Program | Analytical

Session: Characterization of High-Value Oils

New strategy for removing biogenic interferences from mineral oil aromatic hydrocarbons in vegetable oils

Wednesday, May 1, 2024 | 11:35 AM – 11:55 AM EDT | Room: 524b

Presenting Author(s)

Aleksandra Gorska, Ir (she/her/hers)

Teaching Assistant and PhD candidate
Gembloux Agro-Bio Tech (University of Liège)
Gembloux, Namur, Belgium

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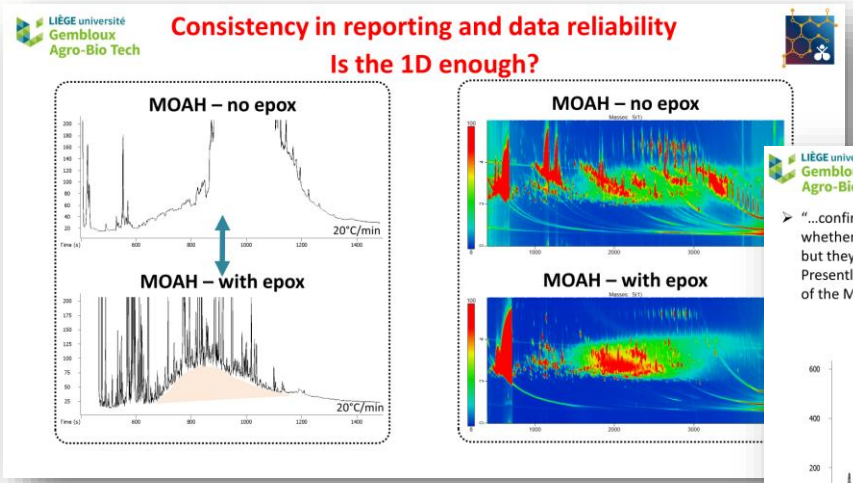
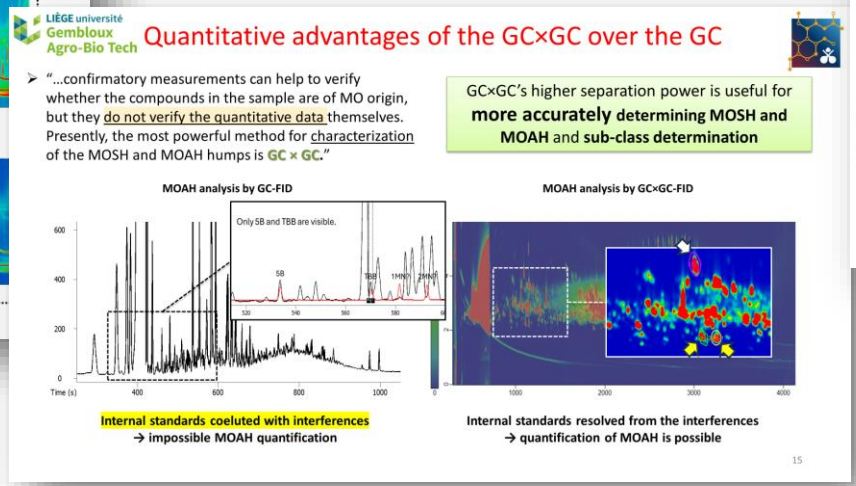
CONCLUSION AND FUTURE DIRECTIONS

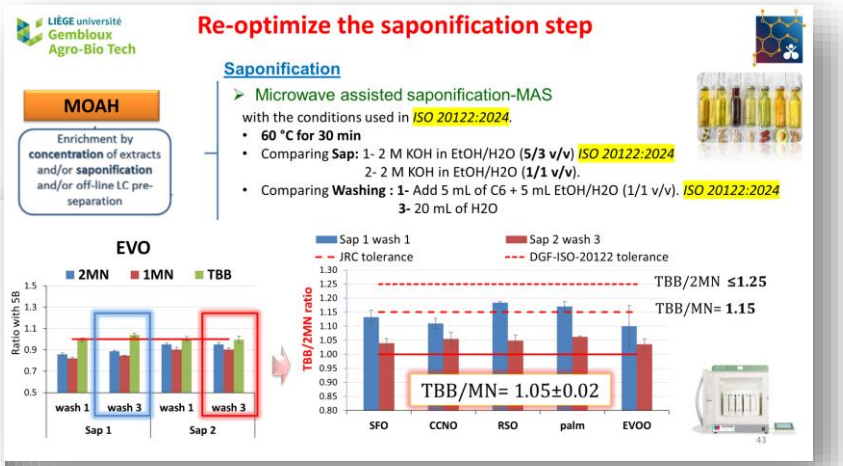
Reduction of uncertainty and analyst interpretation is fundamental to guarantee more reliability in the results

GC×GC & sample preparation

- Reduce operator interpretation
- Handle complex matrices (e.g., essential oils)
- Avoid artifacts and biased results







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My research group:

Sophie Vancraenenbroeck

Paula Albendea

Steven Mascrez

Damien Eggermont

Aleksandra Gorska

Donatella Ferrara

Damien Pierret

Grégory Bauwens

Visiting students:

Andrea Schincaglia

Silvia Pranteddu

Pedro Bahia

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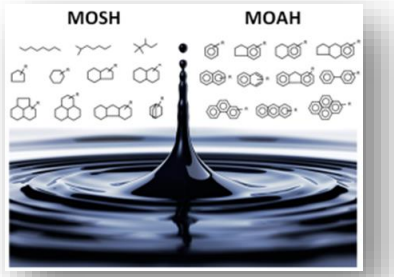

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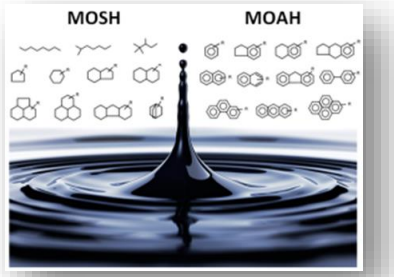

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