GC×GC an opportunity for simplifying sample preparation or an extra difficulty in food analysis?

Giorgia Purcaro , Damien Eggermont, Aleksandra Gorska

Gembloux Agro Bio-Tech, University of Liège, Belgium

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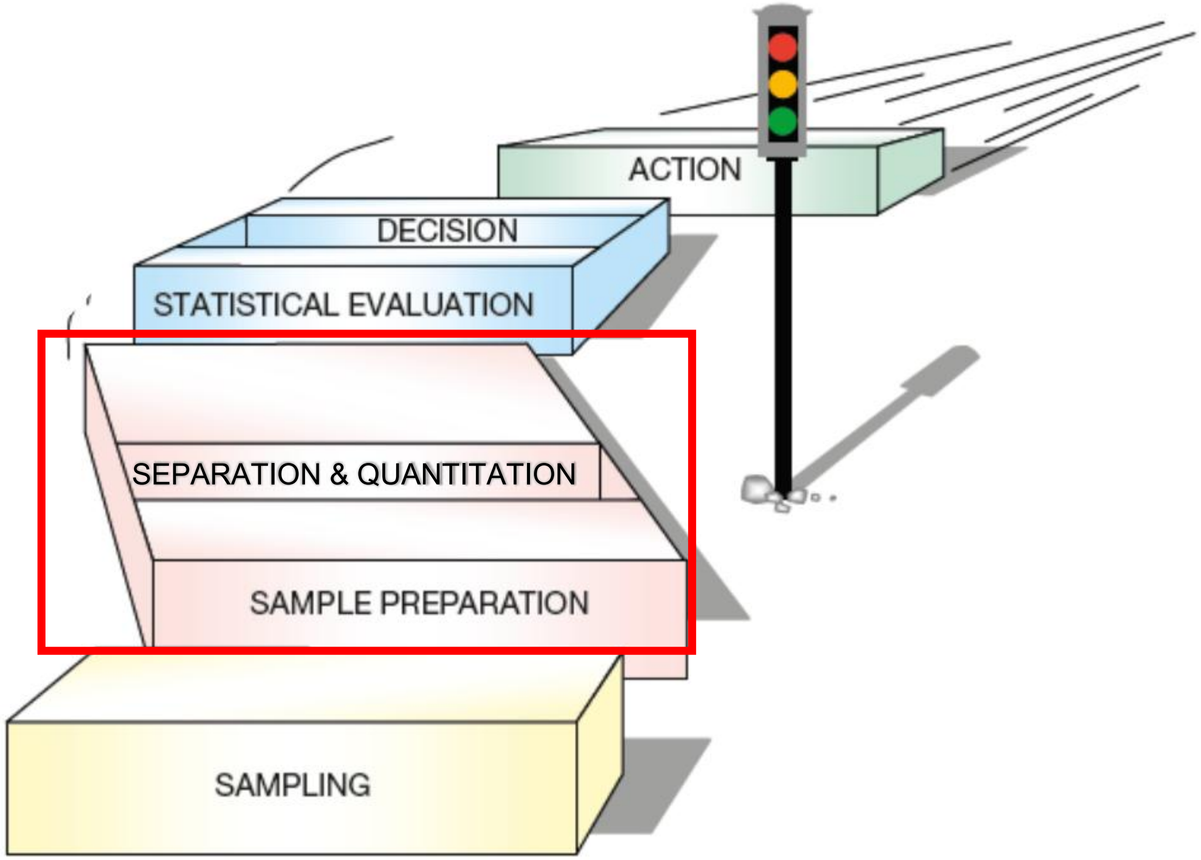
The analytical chemistry Workflow



Sample preparation:

- Handle Matrix complexity
  - Selectivity (e.g., targeted)
  - Representativeness (e.g., untargeted)
  - Greenness (e.g., miniaturization)
  - Automation
  - Sustainability
  - …

Chromatography:

- Maximize separation resolution

J. Pawliszyn, Comprehensive Sampling and sample preparation, Elsevier, 2012

Evolution of CHROMATOGRAPHY

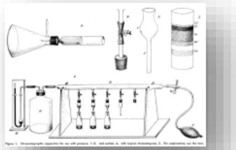
"reductionist approach"

"interactionism" and -omics sciences"

1800s **Wet chemistry era** main components

1900s **Instrumental era** minor and trace components

1906



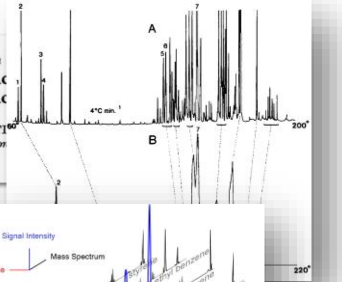
1934 Introduction of the "Acidimeter" (automatic pHmeter)

1941 LC

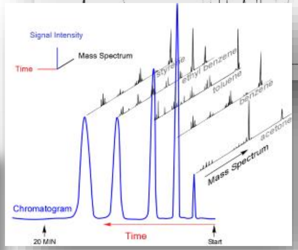
151. A NEW FORM OF CHROMATOGRAM EMPLOYING TWO LIQUID PHASES
I. A THEORY OF CHROMATOGRAPHY
2. APPLICATION T... OF THE HIGHER M...
By A. J. P. ...
From the Wool Industries R...

1951 GC

Gas-liquid Partition Chromatography: the Micro-estimation of Volatile Fatty Ac... Formic Acid to Dodecanoic Ac...
By A. T. JAMES AND A. J. P. MARTI...
National Institute for Medical Research, Mill Hill, Lon...
(*Received 5 June 1951*)



1958 *Capillary column*



1959 GC-MS

Time-of-Flight Mass Spectrometry and Gas-Liquid Partition Chromatography

1980 LC-GC

Journal of Chromatographic Science, Vol. 18, October 1980
Multidimensional High Performance Liquid Chromatography*

1991 GC×GC

Journal of Chromatographic Science, Vol. 29, June 1991
Comprehensive Two-Dimensional Gas Chromatography using an On-Column Thermal Modulator Interface
Zaiyou Liu and John B. Phillips*
Department of Chemistry & Biochemistry, Southern Illinois University, Carbondale, Illinois 62901

2000 LC×GC

Comprehensive LC × GC for Enhanced Headspace Analysis
Wes W. C. Quigl...
Department of Chemistry, ... University o...

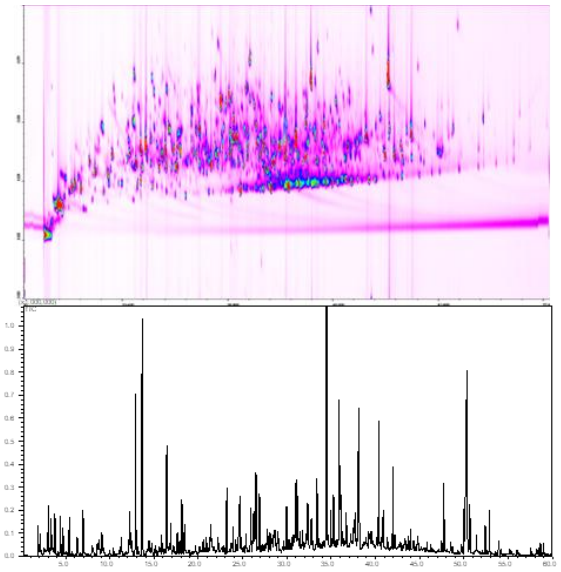
2005 LC-GC×GC

Journal of Chromatography A, 1086 (2005) 12–20
Comprehensive multi-dimensional chromatographic studies on the separation of saturated hydrocarbon ring structures in petrochemical samples
R. Edam[a], J. Blomberg[b,*], H.-G. Janssen[c], P.J. Schoenmakers[a]

Chromatography:

➤ Maximize separation resolution

Multidimensional Chromatography



PRINCIPLE OF GC×GC

- **1991** GC×GC

Journal of Chromatographic Science, Vol. 29, June 1991

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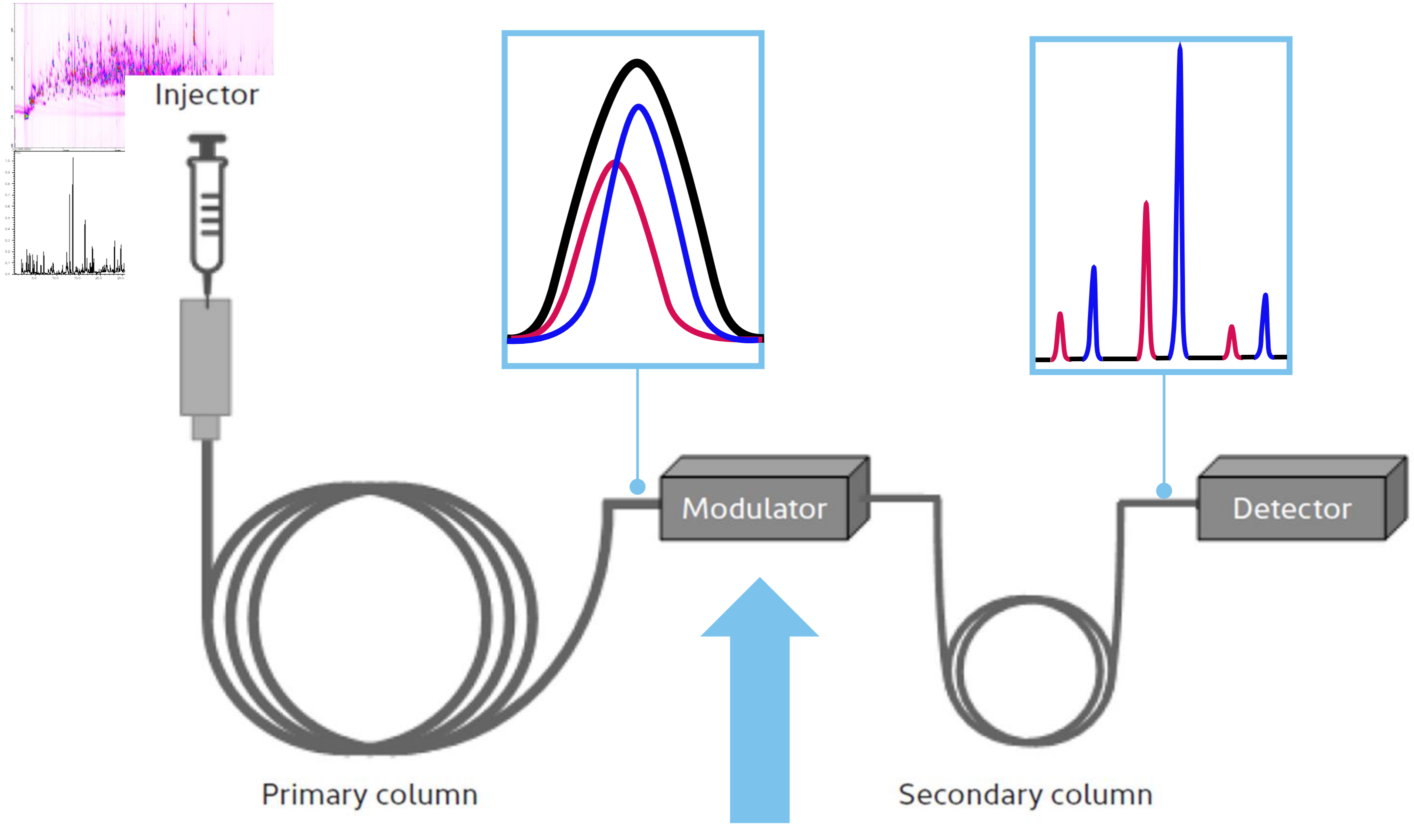
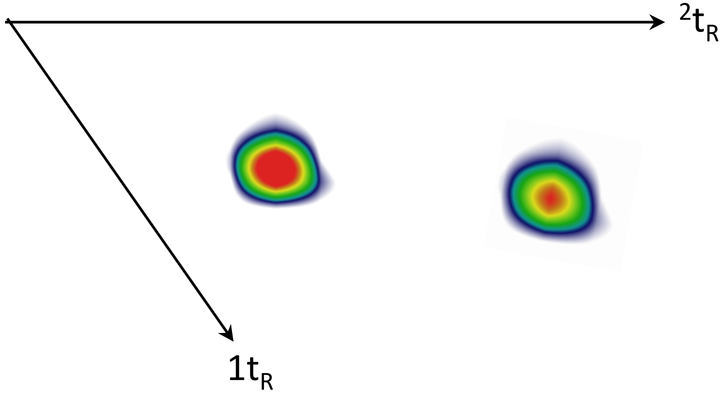
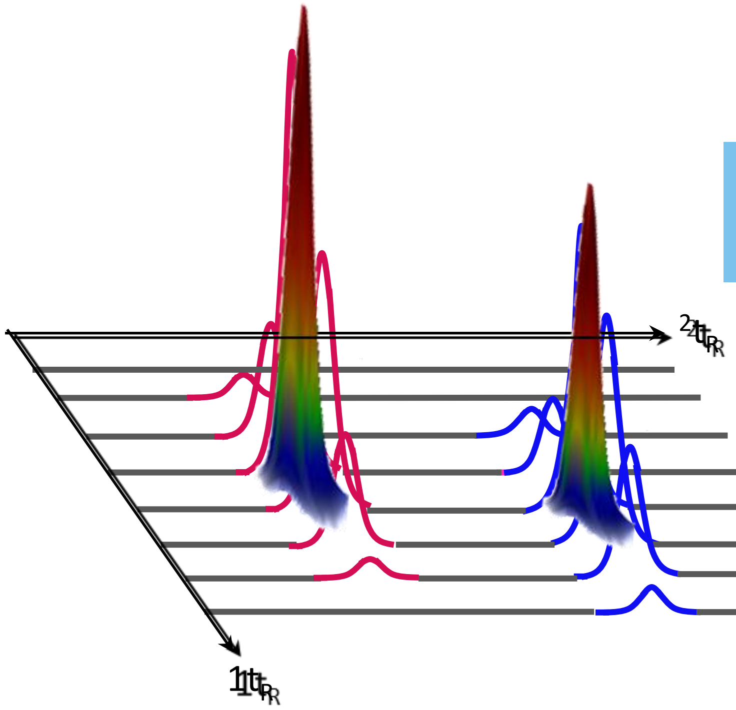


The "beating heart" of GC×GC



Colour plot

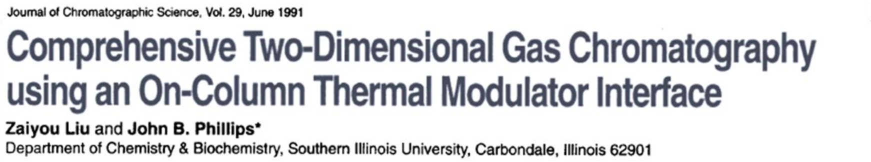


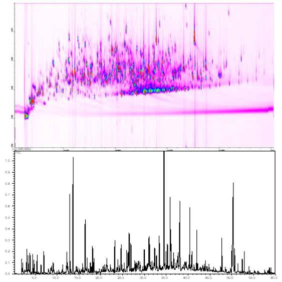
3D surface chart

Kindly provided by Laura Mc Gregor, Sepsolve

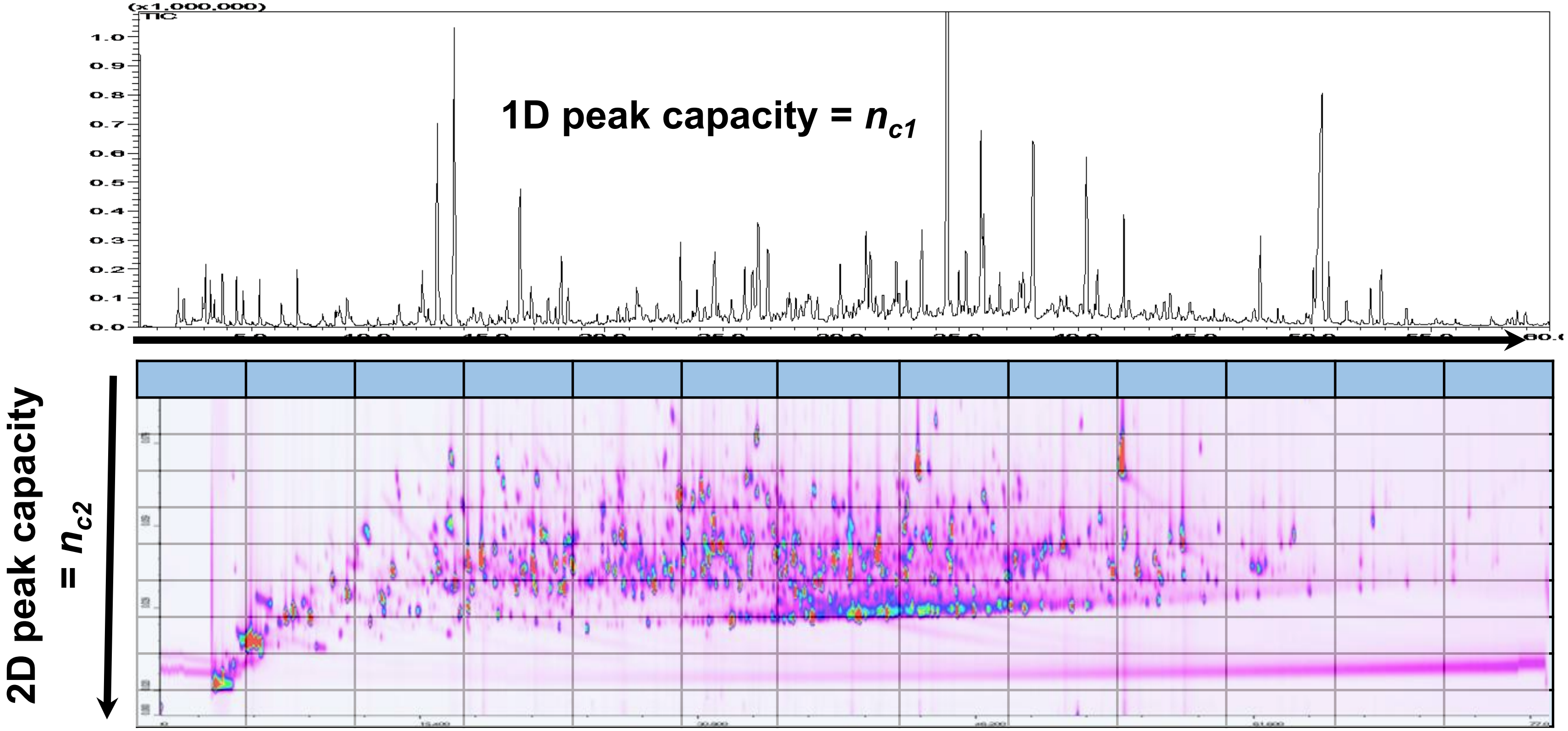
PRINCIPLE OF GC×GC

- **1991** GC×GC



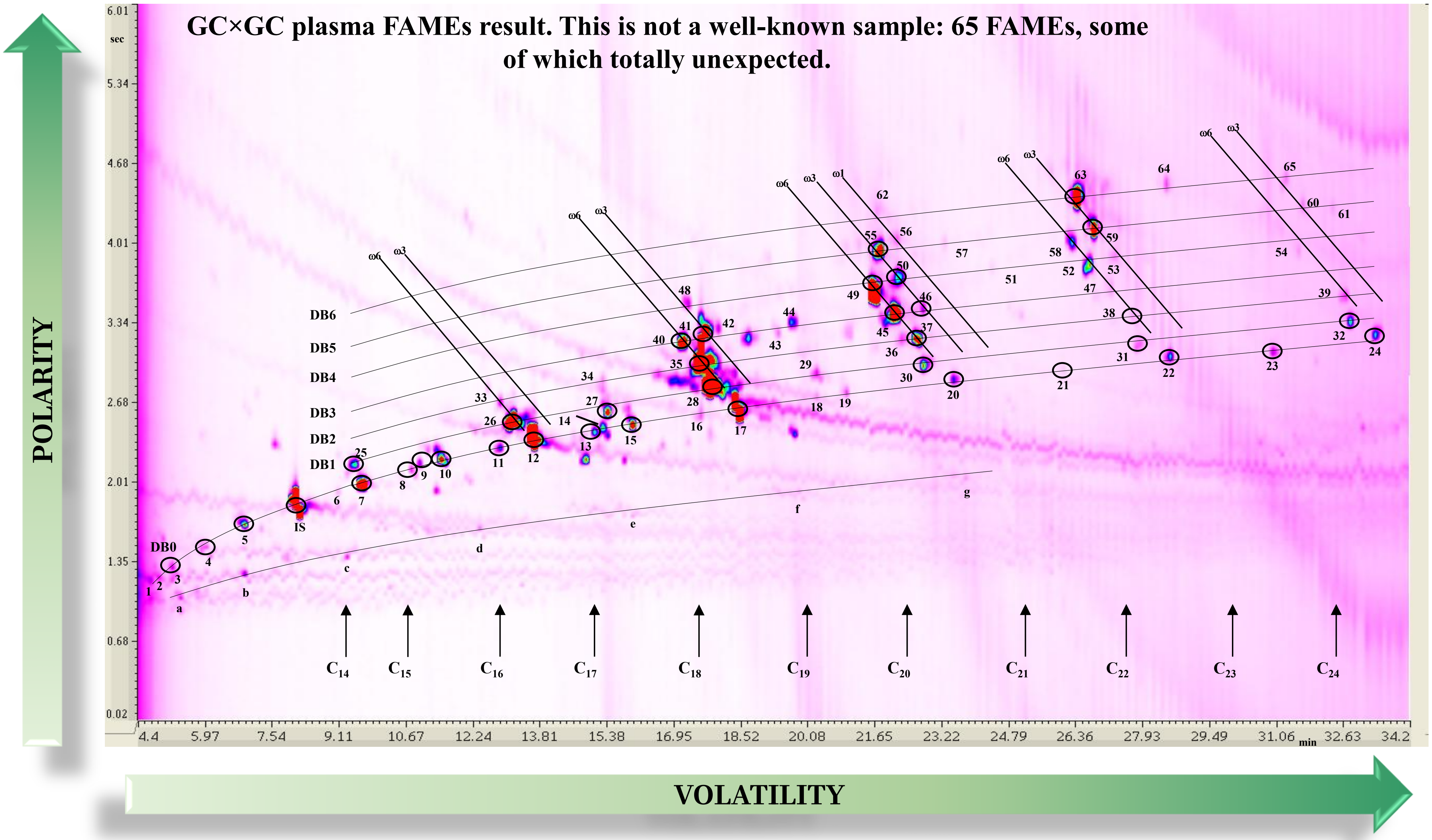


Comprehensive: CC × CC



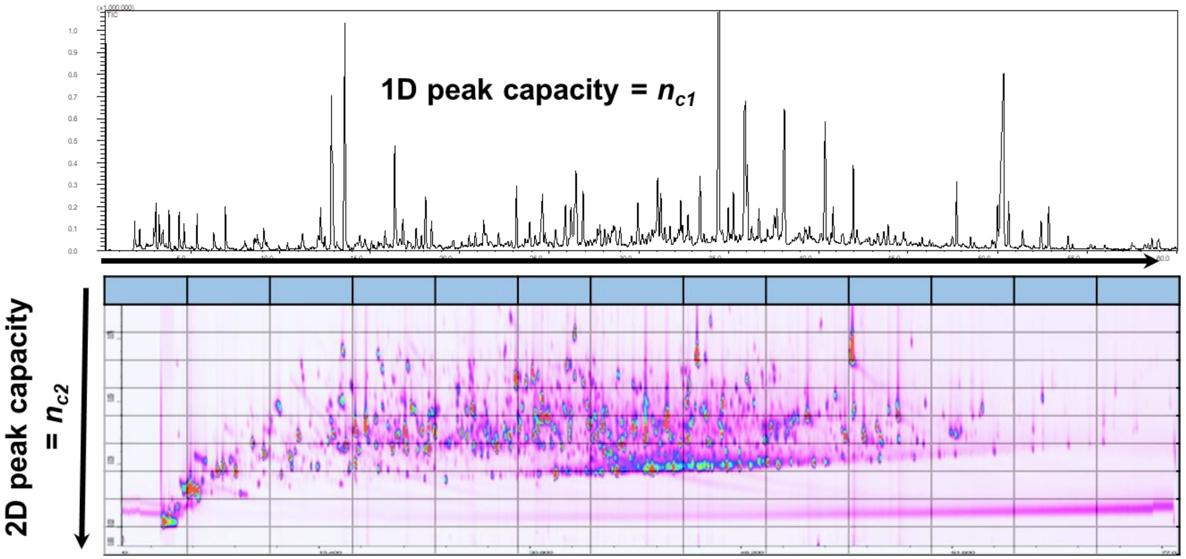
$(n_{c1} \times n_{c2})$.

GC×GC structured chromatogram



GC×GC in food analysis

Comprehensive: CC × CC

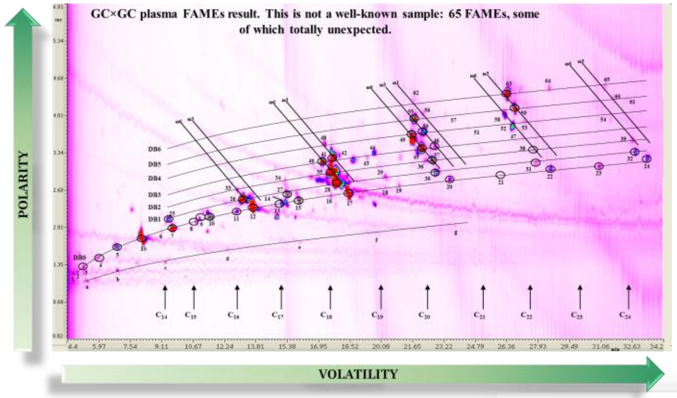


$(n_{c1} \times n_{c2})$.

Synergic to Sample Prep

Unveil and explored sample multidimensionality

GC×GC: Guide sample prep optimization



Selectivity of different fibers

Journal of Chromatography A, 1251 (2012) 208–218

Contents lists available at SciVerse ScienceDirect

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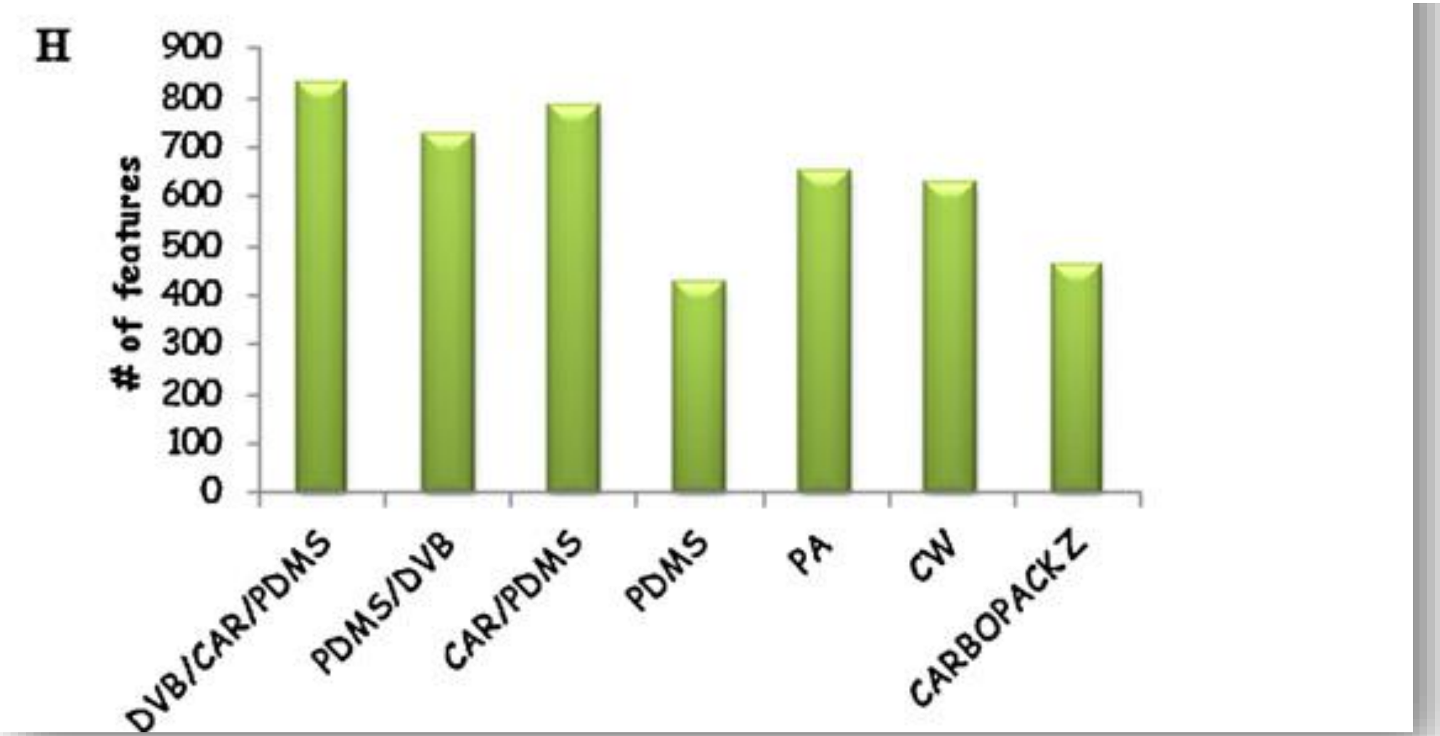
ELSEVIER

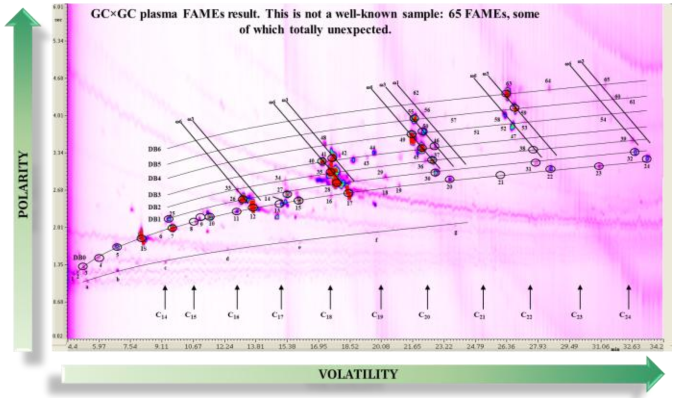
Solid phase microextraction coupled with comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for high-resolution metabolite profiling in apples: Implementation of structured separations for optimization of sample preparation procedure in complex samples☆

Sanja Risticevic[a], Jennifer R. DeEll[b], Janusz Pawliszyn[a,*]

[a] Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada
[b] Agriculture Development Branch, Ontario Ministry of Agriculture, Food and Rural Affairs, Simcoe, Ontario, Canada



GC×GC: Guide sample prep optimization



Qualitative

Selectivity of different fibers

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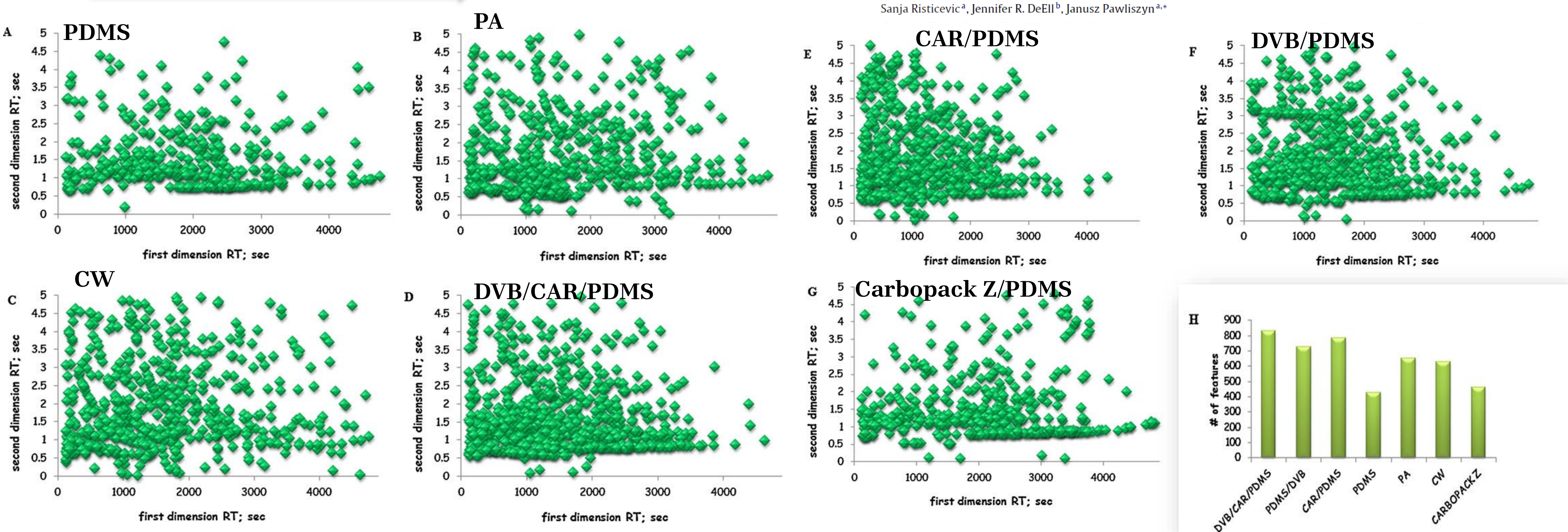

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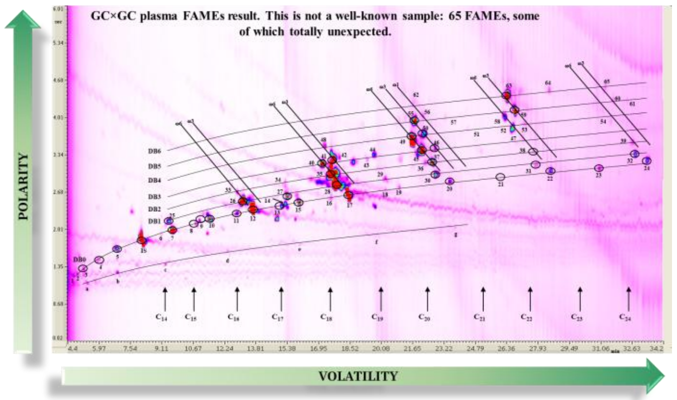
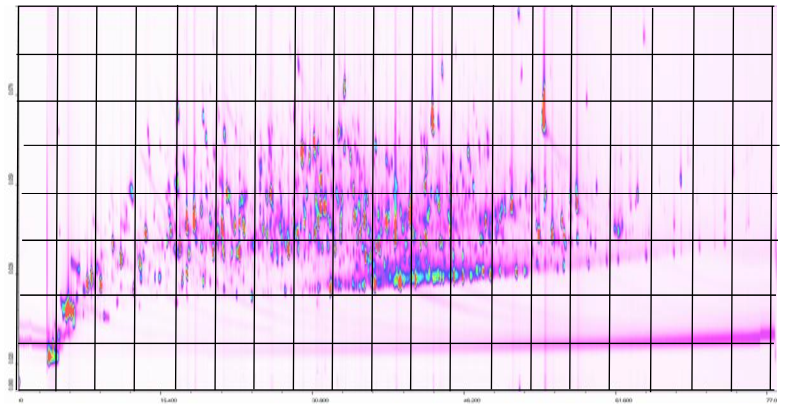


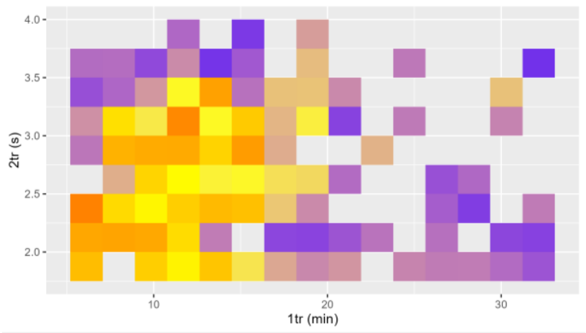
Quantitative



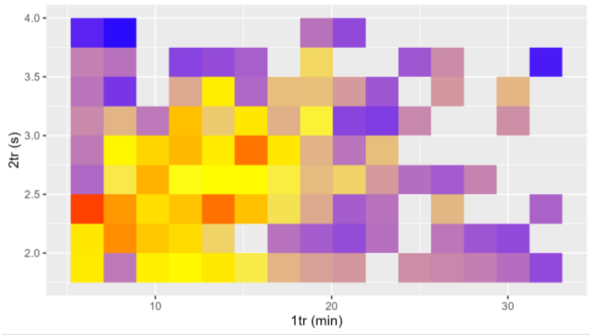
TIME PROFILE

Area

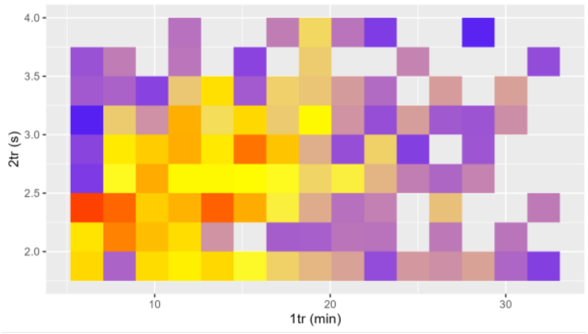
10 min



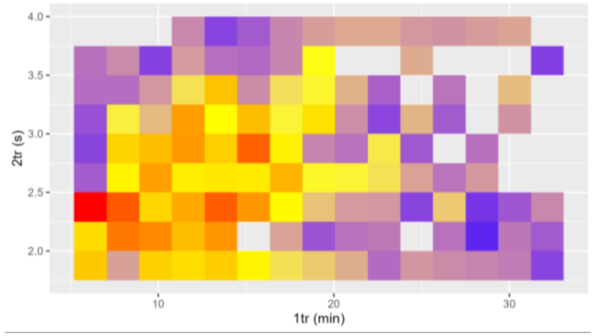
25 min



35 min



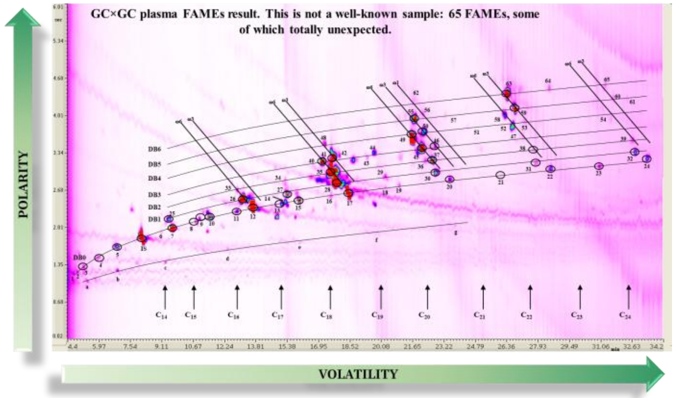
50 min



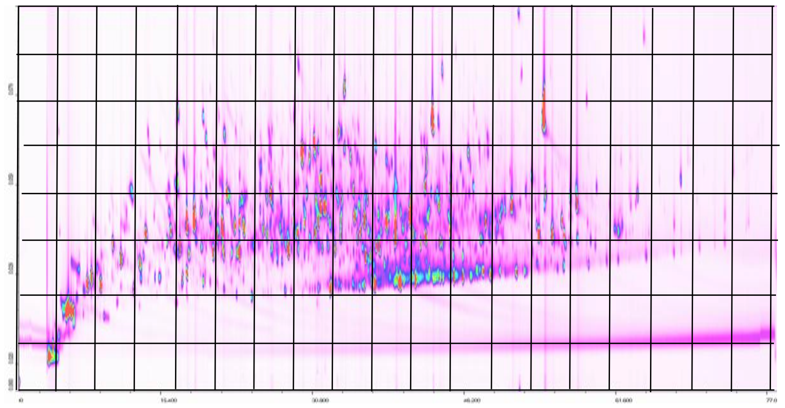
Chocolate aroma profile

Cozzolino R. , Eggermont D., Malorni L., Purcaro G., Talanta (submit)

GC×GC: Guide sample prep optimization



Quantitative



TIME PROFILE

Area

Ratio over 10 min

10 min

25 min

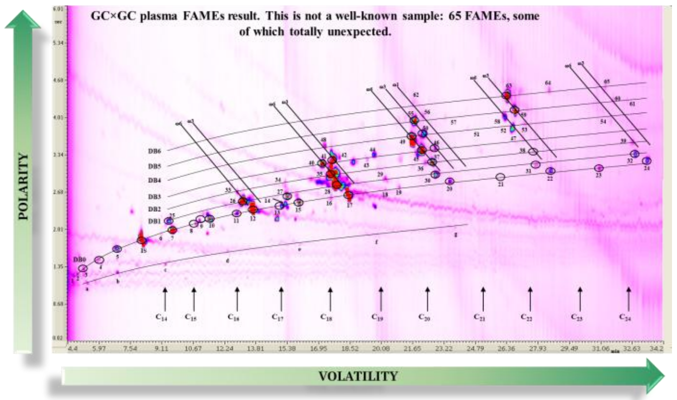
35 min

50 min

“improvement plots”

Cozzolino R. , Eggermont D., Malorni L., Purcaro G., Talanta (submit)

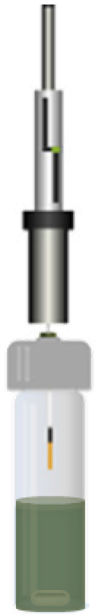
GC×GC: Guide sample prep optimization



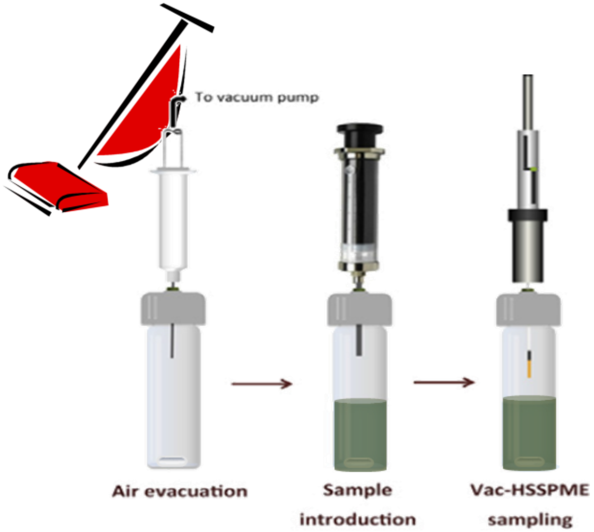
Selectivity of different techniques

HS-SPME

1cm fiber 100µm :
0.6 µL



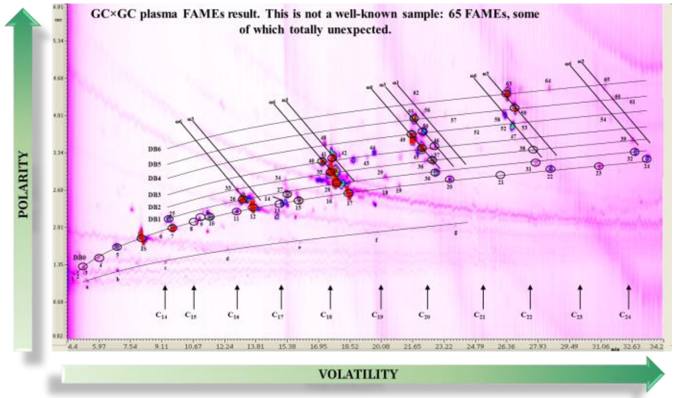
Vac-HS-SPME



HiSorb®

~10.5mm x 0.8 mm:
~ 63 µL

GC×GC: Guide sample prep optimization



Selectivity of different techniques

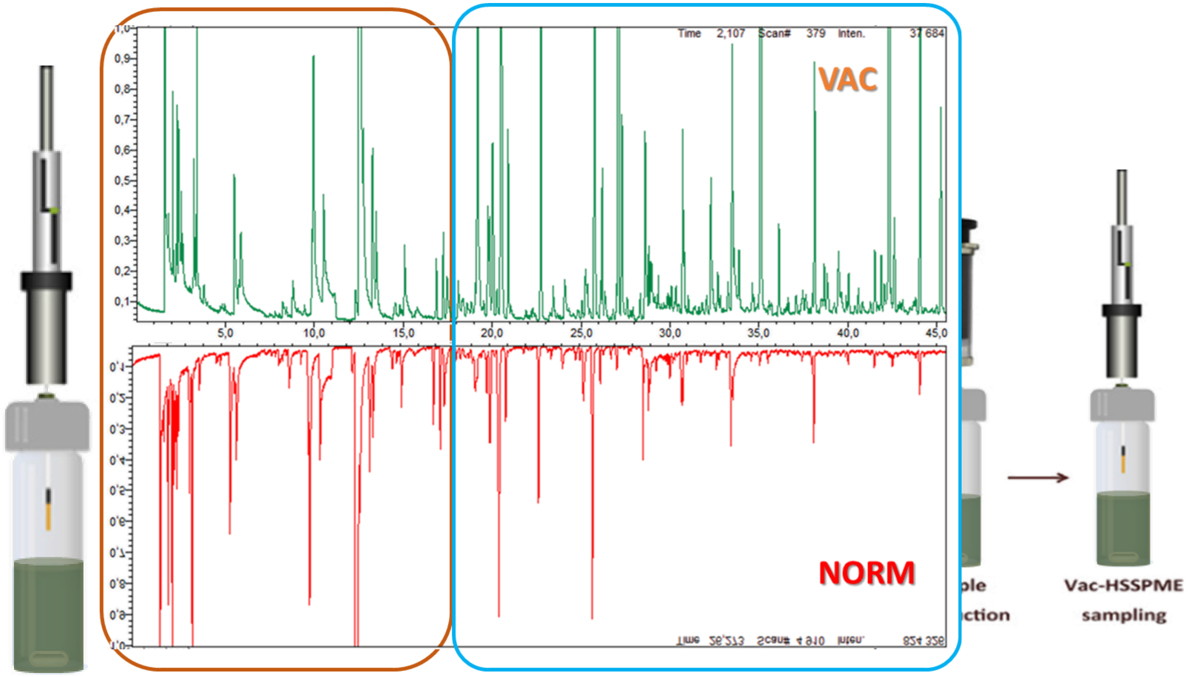
HS-SPME

Vac-HS-SPME

HiSorb®

1cm fiber 100µm :

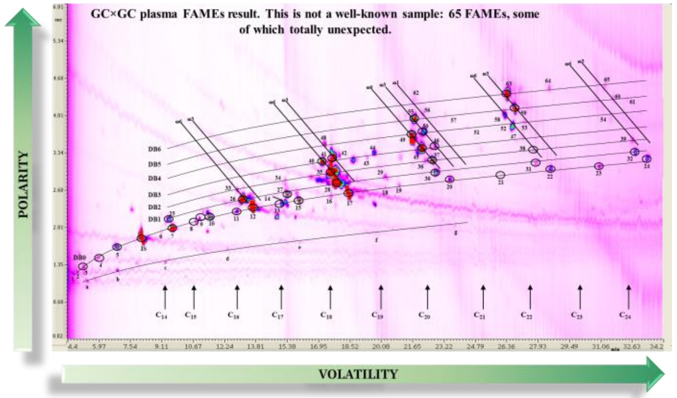
0.6 µL



~10.5mm x 0.8 mm:

~ 63 µL

GC×GC: Guide sample prep optimization

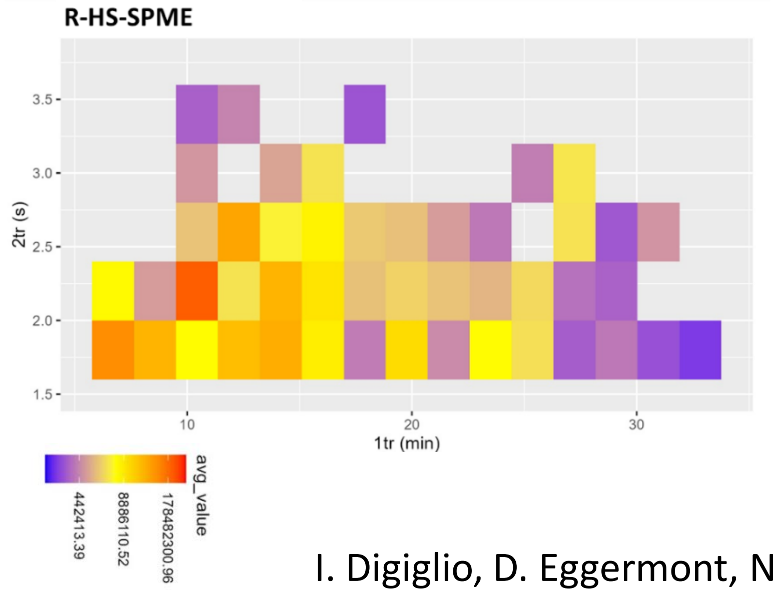


Selectivity of different techniques

HS-SPME

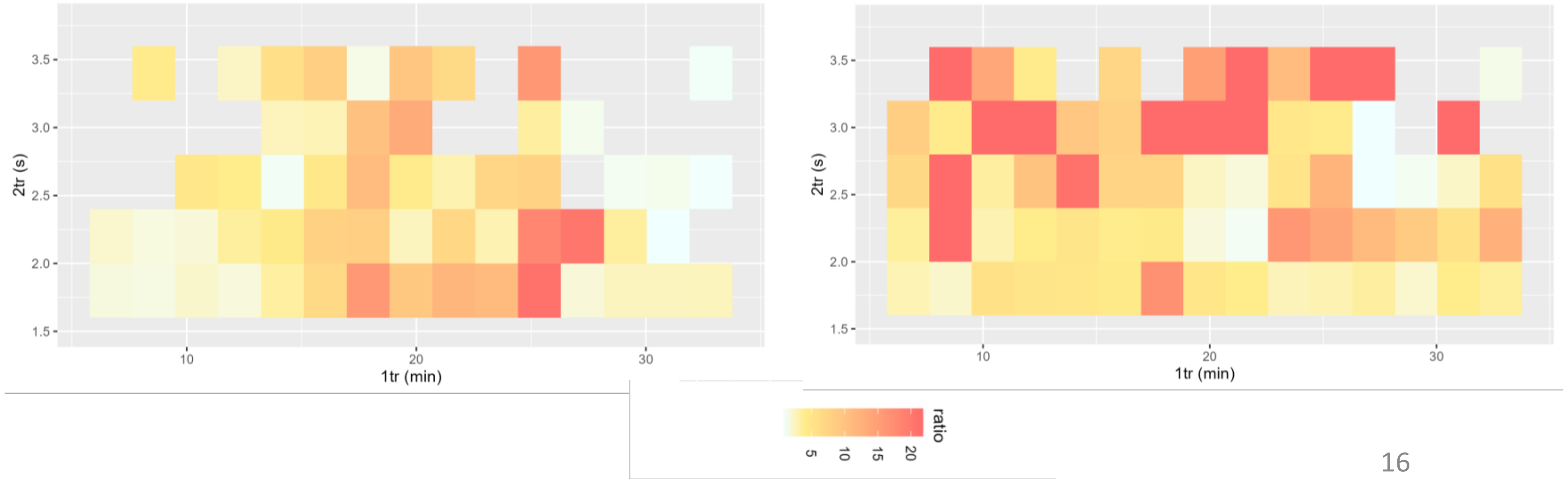
Vac-HS-SPME

HiSorb®



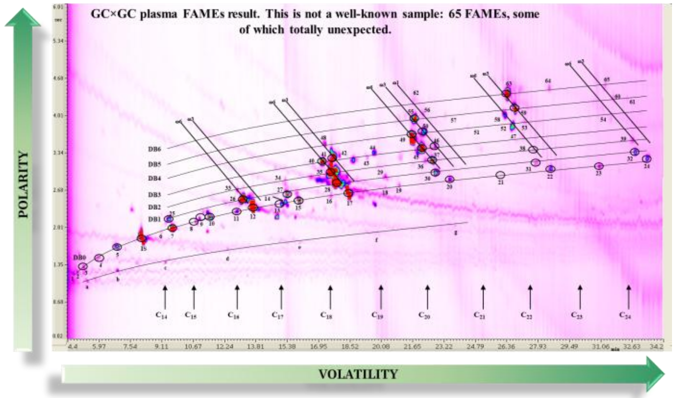
Vac-HS-SPME/HS-SPME

HiSorb/HS-SPME



I. Digiglio, D. Eggermont, N. D. Spadafora, T. Ugolini, L. Cecchi, N. Mulinacci, G. Purcaro, in preparation

GC×GC: Guide sample prep optimization



Selectivity of different techniques

Vacuum-assisted extraction

HS-SPME

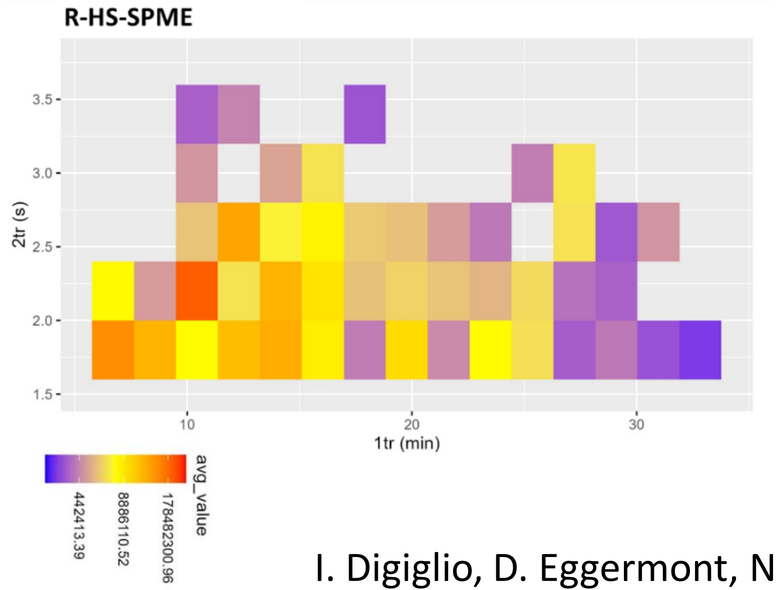
(Vac)-HS-SPME

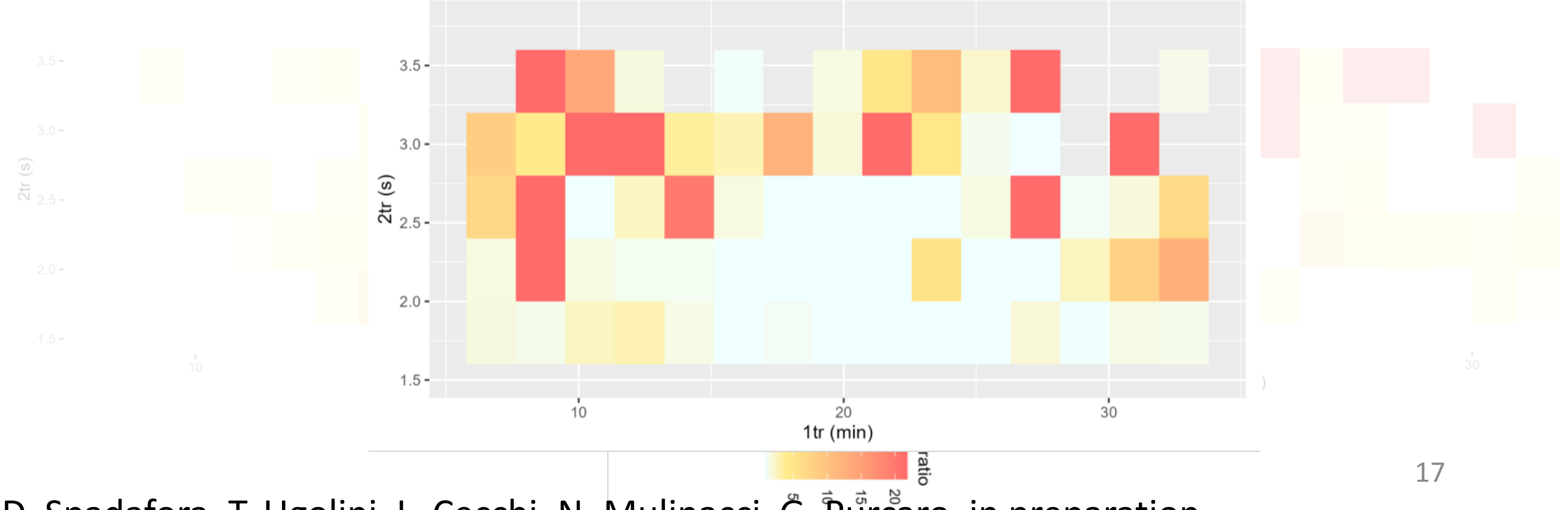
HiSorb®

Vac-HS-SPME/HS-SPME

HiSorb/Vac-HS-SPME

HiSorb/HS-SPME





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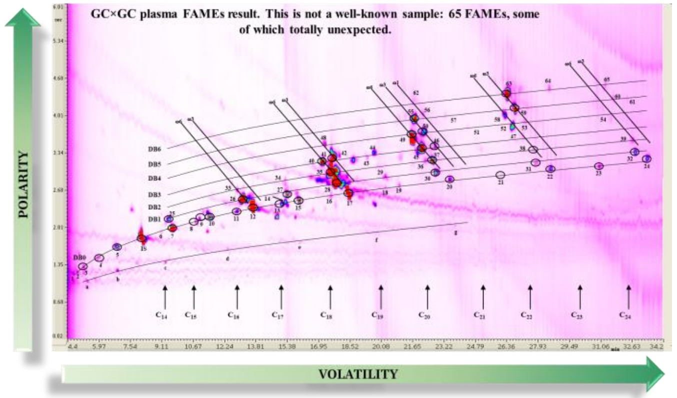
GC×GC: Guide sample prep optimization



Selectivity of different techniques

Vacuum-assisted extraction

HS-SPME

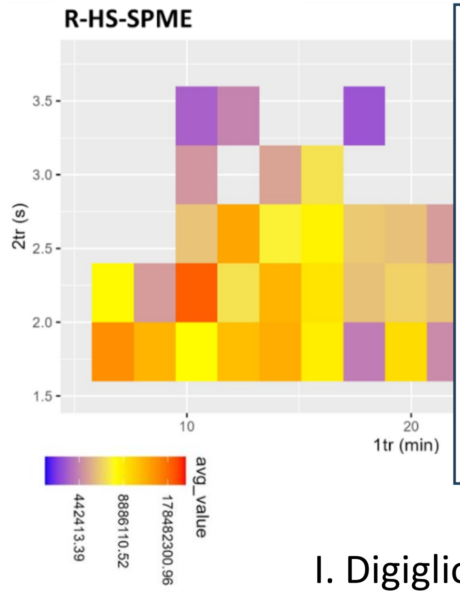
(Vac)-HS-SPME

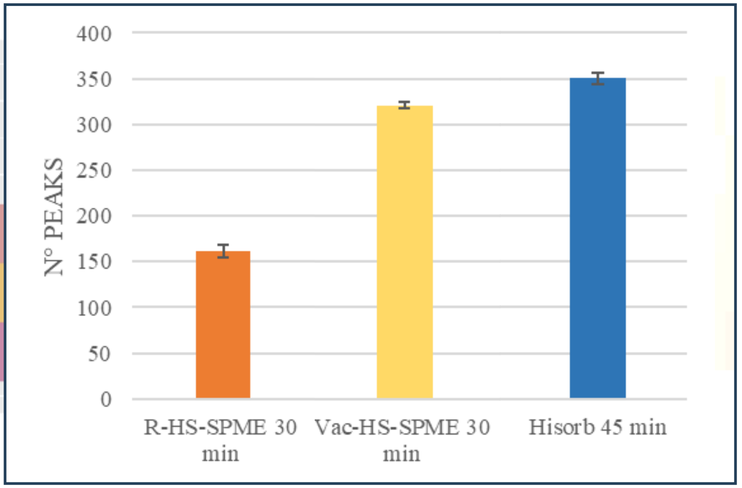
HiSorb®

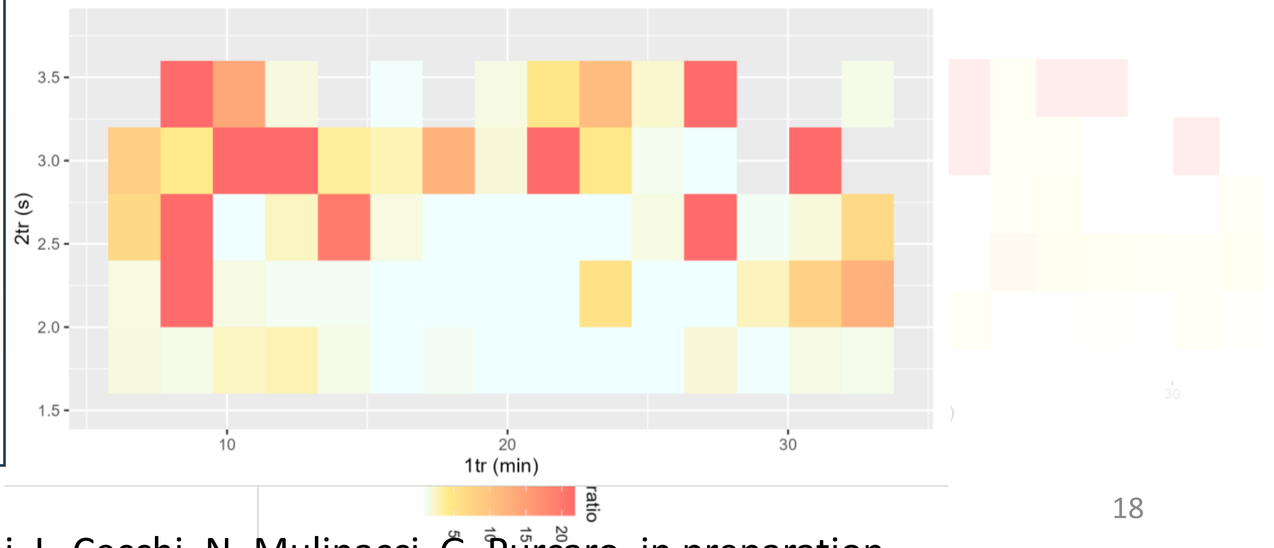
Vac-HS-SPME/HS-SPME

HiSorb/Vac-HS-SPME

HiSorb/HS-SPME







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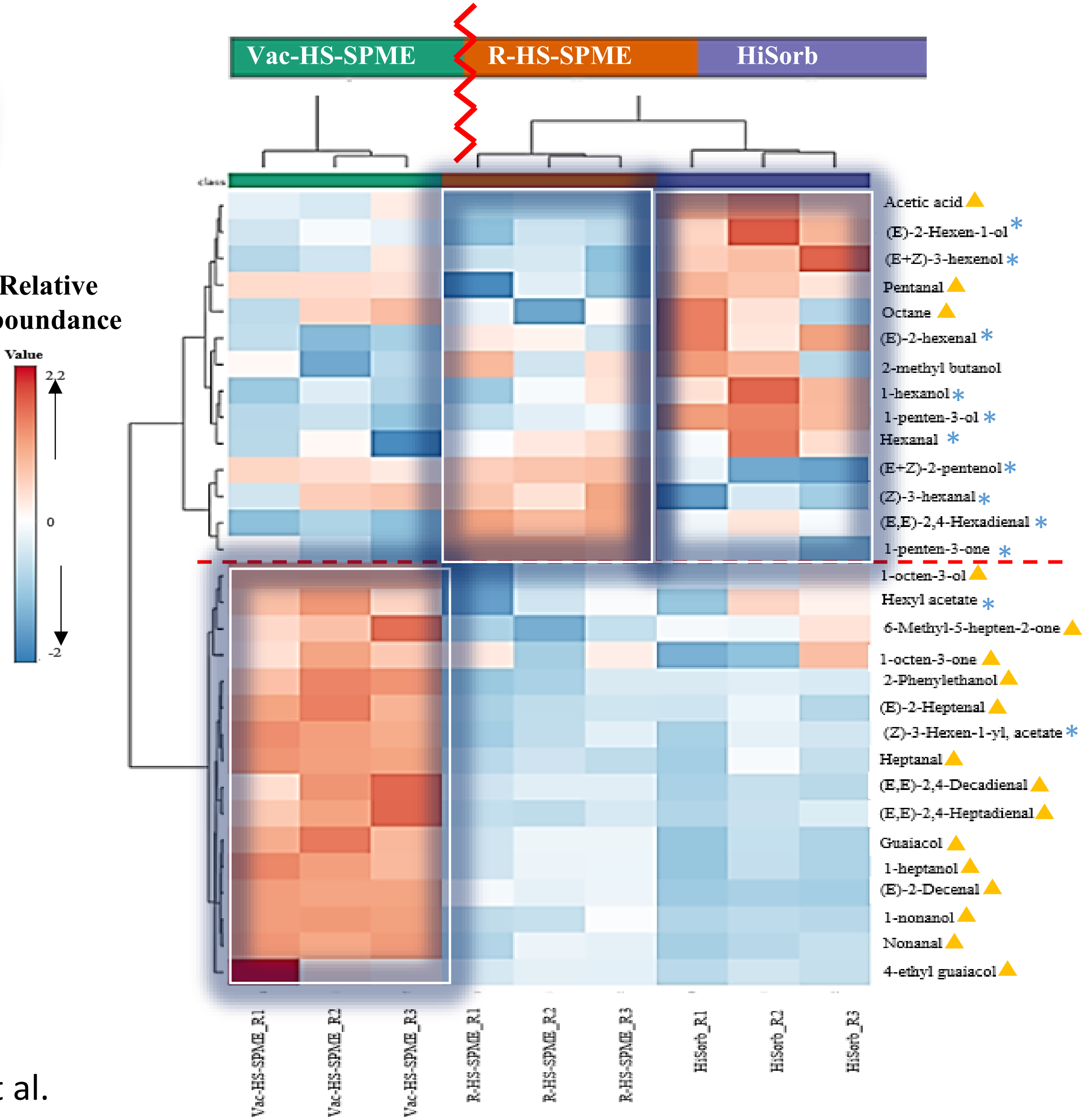
GC×GC: Guide sample prep optimization



Selectivity of different techniques

Higher volatility

Lipoxygenase (LOX) Pathway *

Series of biochemical reactions that lead to the formation of C5–C6 aldehydes, alcohols, and their corresponding esters, which are responsible for green and fruity aroma notes.

Lower volatility

C7-C12 VOCs ▲

Compounds typically associated with oxidative and microbiological defects, often linked to poor quality or improper preservation of the raw material and inadequate storage conditions.

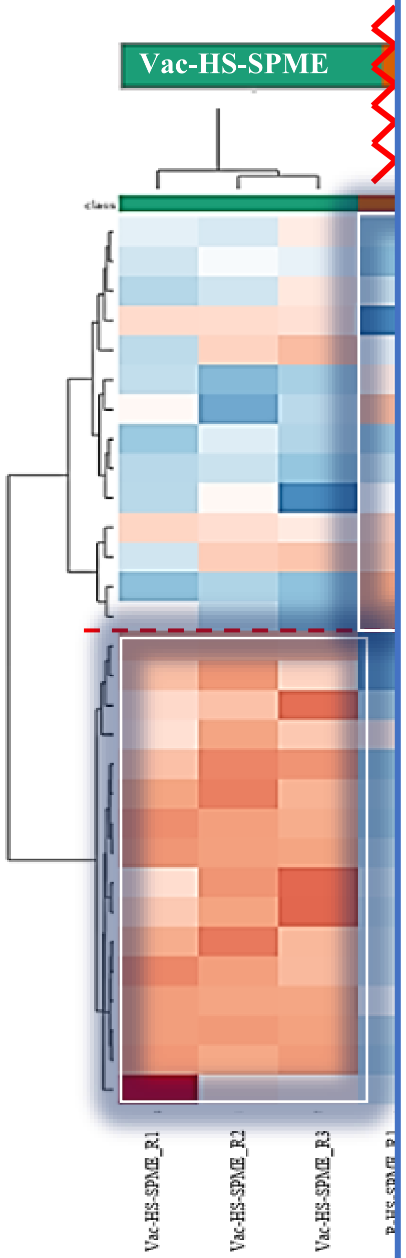
I. Digiglio et al.

LIÈGE université Gembloux Agro-Bio Tech

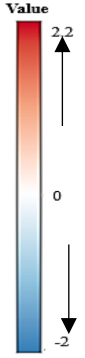
GC×GC: ...ization

ACESSS Academic Center of Excellence in Separation Science and Sensing

Vac-HS-SPME



Relative abundance



LIÈGE université Gembloux Agro-Bio Tech

ExTech 2025 Mülheim an der Ruhr

ACESSS Academic Center of Excellence in Separation Science and Sensing

Enhancing headspace sorbent analysis for target analysis in food by vacuum assisted headspace and/or multi-cumulative trapping

Damien Eggermont, Steven Mascrez, Giorgia Purcaro

Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium

INTRODUCTION

Headspace (HS) sorbent analysis refers to the techniques used to sample HS using a sorbent with varying volumes and surfac... significant enrichment factor of the analytes of interest. Solid-phase microextraction (SPME) is the most well-known and wide... fingerprint of the volatiles in the HS. HS-SPME offers significant advantages by minimizing matrix-related interferences, enhancing s... lifespan of both the extraction material and the analytical instrument. Nevertheless, in HS-SPME, the extraction of less volatile ... kinetics and thermodynamic limitations. To improve the overall extraction, several strategies can be used. Stirring the sample in... while adjusting the extraction temperature impacts both the kinetics and thermodynamics, but it can be critical for the creat... alternatives are the use of reduced pressure, called vacuum-assisted HS extraction (Vac-HS-SPME), which significantly favors the m... thus increasing the extraction of the less volatile, and the use of multiple extractions from the same vial, which allows for first ext... then remodulate the equilibrium and favors the extraction of the less volatile. The two fractions are desorbed and trapped together ... MS or GC×GC-MS.

Vac-HS-SPME

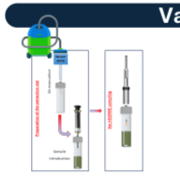


Fig. 1: Scheme of Vac-HS-SPME extraction

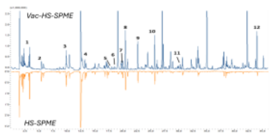


Fig. 2: Chromatogram obtained during Vac-HS-SPME and regular HS-SPME.

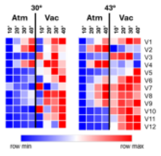


Fig. 3: Heatmaps showing the extraction response obtained using regular and Vac-HS-SPME at 30 and 43 °C for different extraction times.

A special cap, provided by ExtraTECH (Greece) is needed to guarantee the sealing and maintain the vacuum in the vial (**Fig.1**).

For the most volatile compounds, the same performance can be observed between regular and Vac-HS-SPME (**Fig. 2**). The kinetics of these compounds is rapid; thus, the effect of using reduced pressure conditions is limited or even non-existent. For the rest of the compounds, the effect of VAC and T° is instead synergic, significantly improving the extraction efficiency at an earlier sampling time (**Fig. 3**).

MCT-HS-SPME

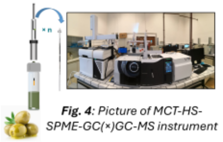
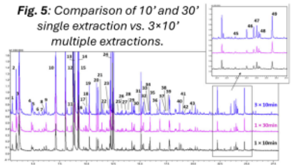


Fig. 4: Picture of MCT-HS-SPME-GC(×)GC-MS instrument

In single-vial multi-cumulative trapping (SV-MCT-)HS-SPME extraction, the same vial is extracted several times, and the extracts are collected on a focusing trap that rapidly desorbs the entire shot at once into the GC system (**Fig.4**).

Extraction yield is higher for MCT extraction compared to an equivalent but single extraction time (**Fig. 5**). This effect is more pronounced for VOCs with less affinity to air compared to oil (high K_{OA}) (**Fig. 6**).

Fig. 5: Comparison of 10' and 30' single extraction vs. 3×10' multiple extractions.



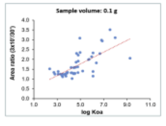


Fig. 6: Improvement ratio against log K(octanol-air)

MCT-HS-SPME increases the extraction yield of the less volatile compounds.

Vac-MCT-HS-SPME

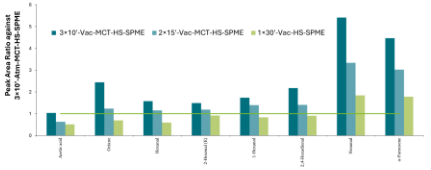
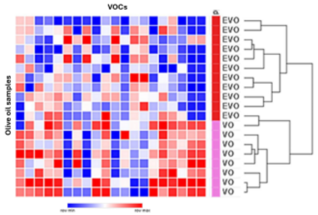


Fig. 7: Ratio of improvement of different Vac-MCT-HS-SPME extractions (modalities in legend) of 0.1 g of olive oil extracted at 43 °C

When Vac and MCT extractions are combined, a synergistic effect is observed, resulting in increased extraction yield, especially for low-volatile compounds (**Fig. 7**). Notably, long Vac extractions show close to similar performances to Atm-MCT but significantly less than Vac-MCT for the same total time.

APPLICATION: Extra-Virgin / Virgin Olive Oil Classification



Eighteen olive oil samples – 10 Extra Virgin Oil (EVO) and 8 Virgin Oil (VO) – were analysed by Vac-MCT-HS-SPME. Hierarchical cluster analysis based on distance of Pearson's correlation of selected VOCs allows to correctly cluster EVO from VO (**Fig. 8**).

Fig. 8: Heatmap of selected VOCs of EVO/VO samples analysed by 3×10' Vac-MCT-HS-SPME (0.1 g of olive oil extracted at 43 °C)

Conclusion

The use of vacuum or multi-cumulative trapping separately increase the extraction of low-volatile compounds. Their combination benefits from the advantages of both techniques to give a significantly enhanced extraction. This synergistic approach has been successfully tested for the classification of extra virgin and virgin olive oils in the context of commercial quality assessment.

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Mascrez et al., Analytica Chimica Acta 1103 (2020) 106e114

ACKNOWLEDGMENTS

The authors would like to thank Markes, ExtraTECH, Sepsolve, Shimadzu and Supelco for their support. This work is supported by ACESSS (Academic Center of Excellence for Separation Science and Sensing).

...of different techniques

...ty

...ty

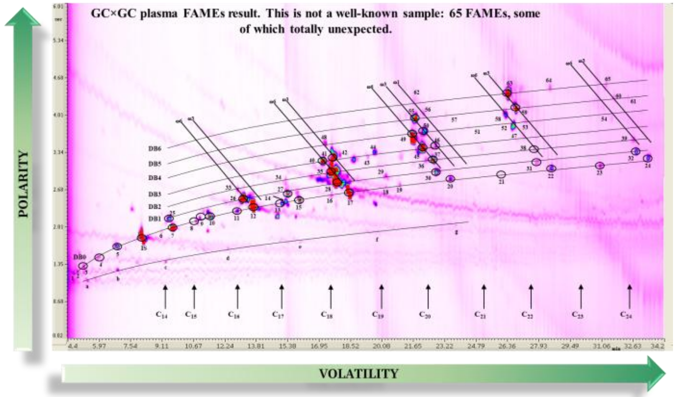
Lipoxygenase (LOX) Pathway *

Series of biochemical reactions that lead to the formation of C5–C6 aldehydes, alcohols, and their corresponding esters, which are responsible for green and fruity aroma notes.

C7-C12 VOCs

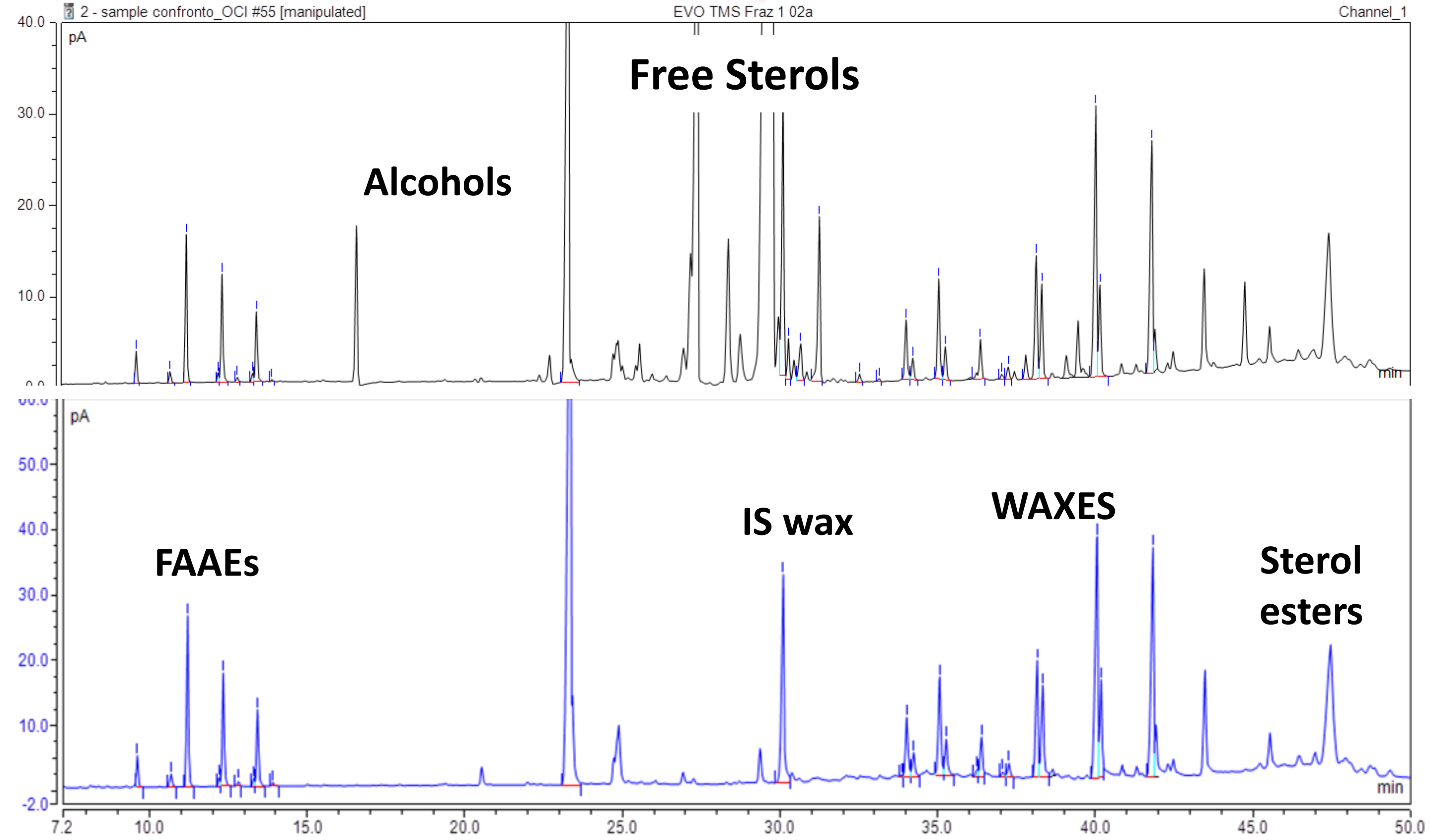
Compounds typically associated with oxidative and microbiological defects, often linked to poor quality or improper preservation of the raw material and inadequate storage conditions.

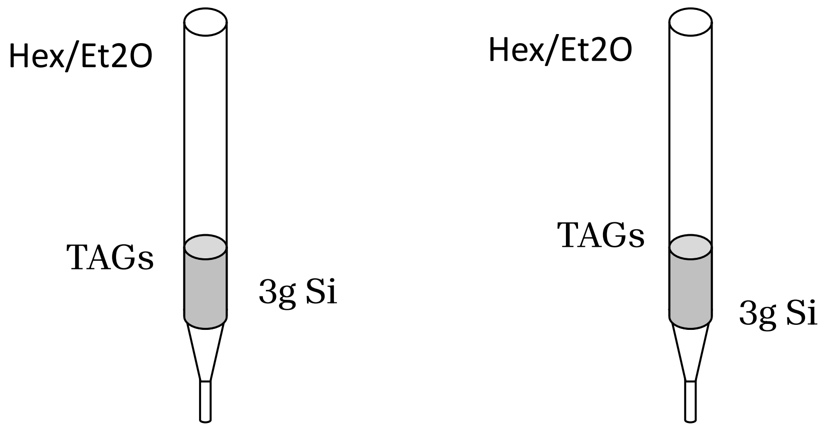
GC×GC: Simplify sample prep



- Merge workflows

Silylation before loading the oil





FAAEs + Waxes + Esterified Sterols

Official method

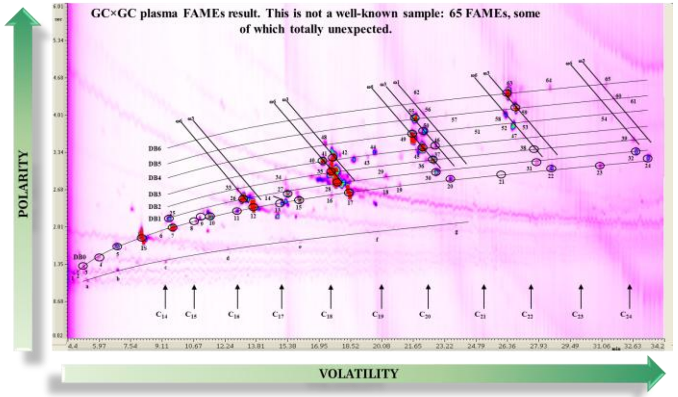
FAAEs + Waxes + Esterified Sterols

Alcohols, Sterols-TMS

LIÈGE université Gembloux Agro-Bio Tech

GC×GC: Simplify sample prep

ACESSS Academic Center of Excellence in Separation Science and Sensing



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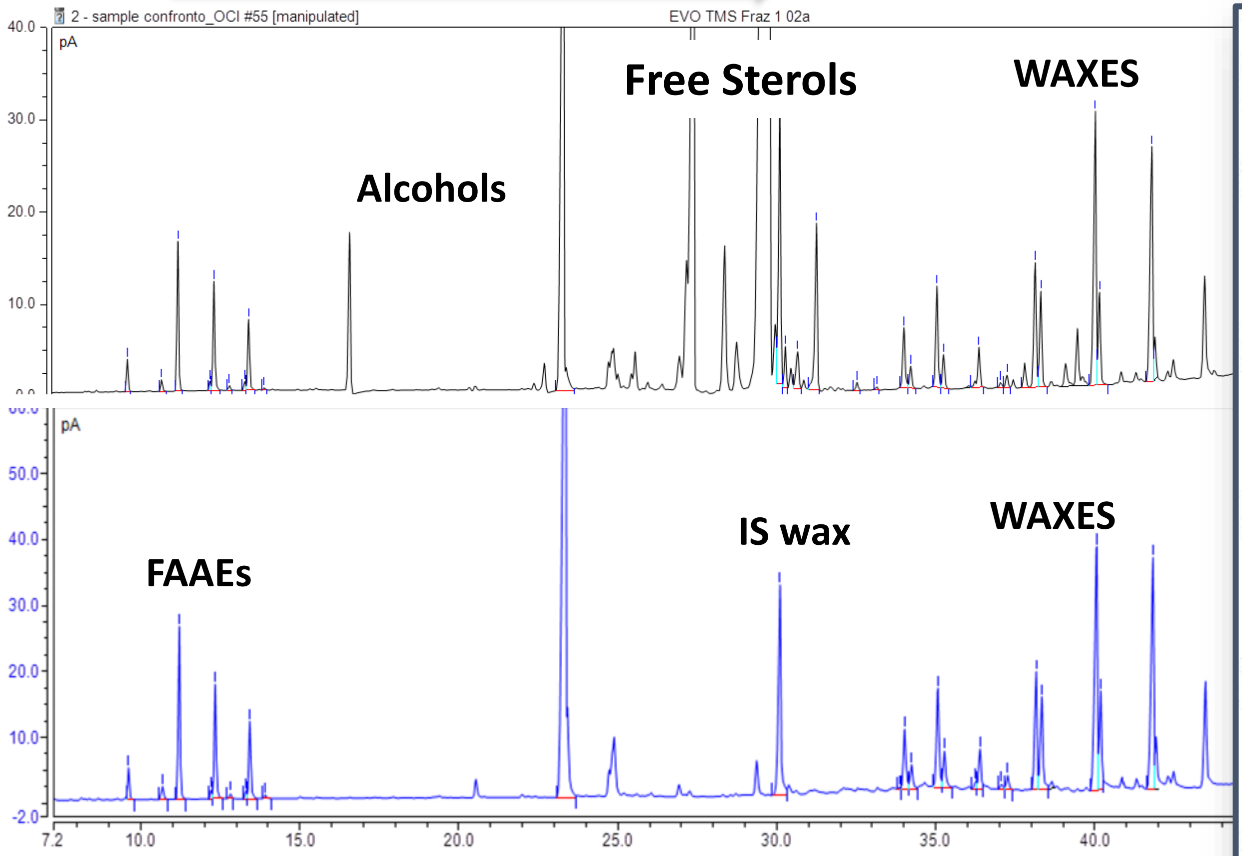

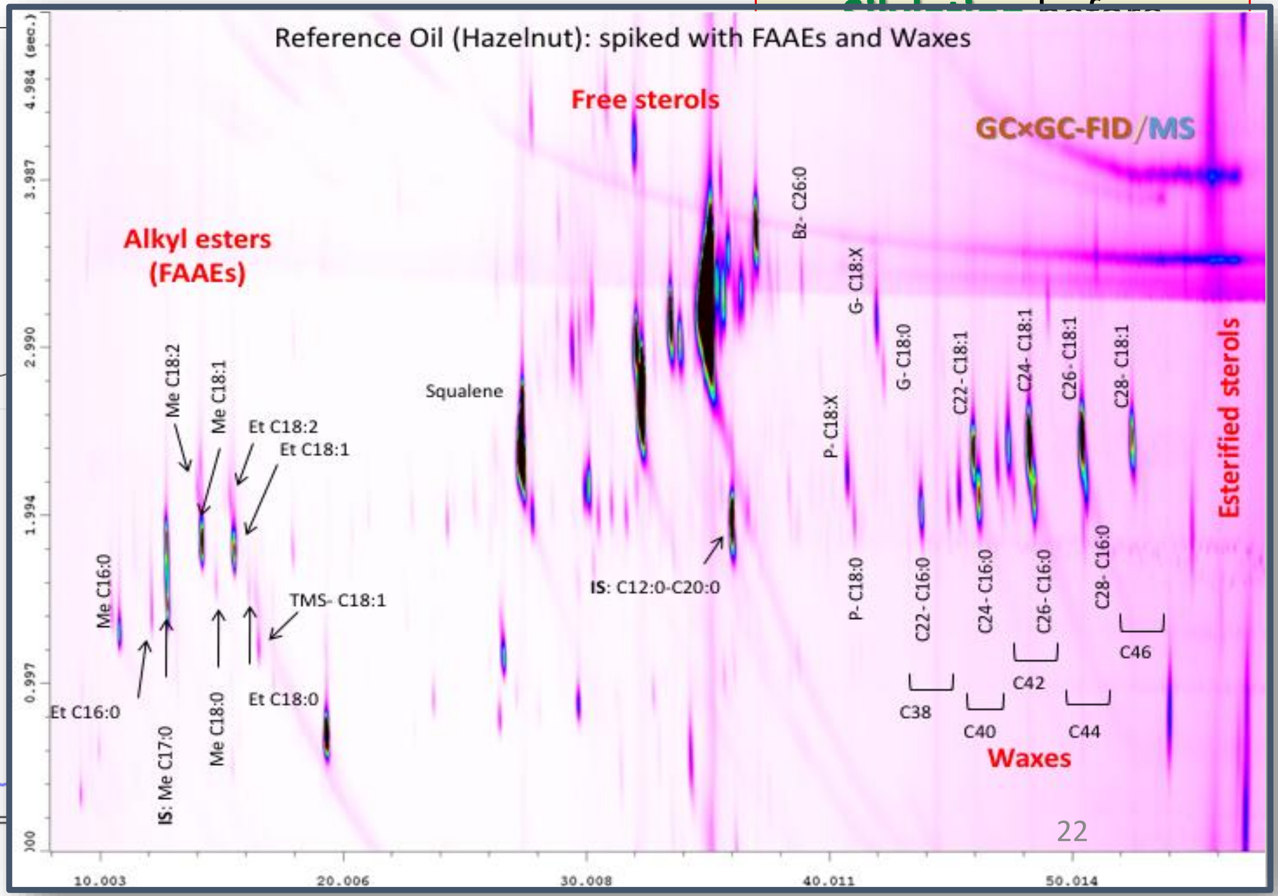

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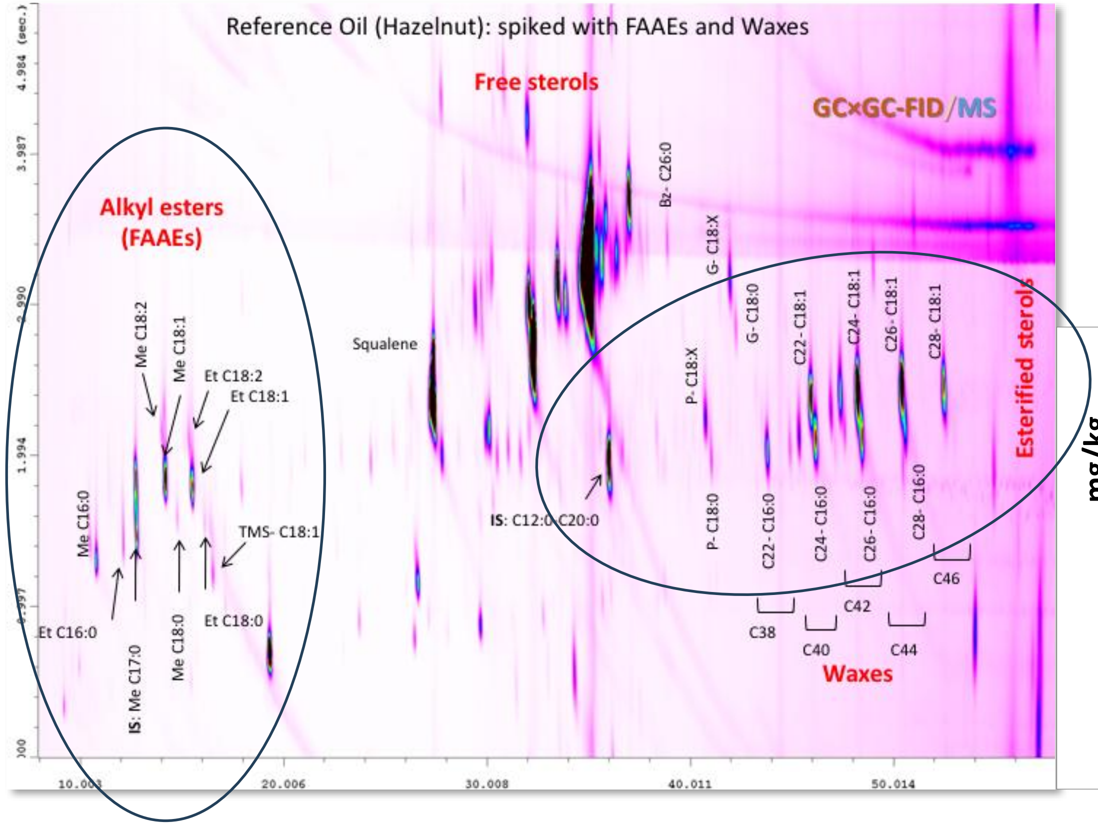
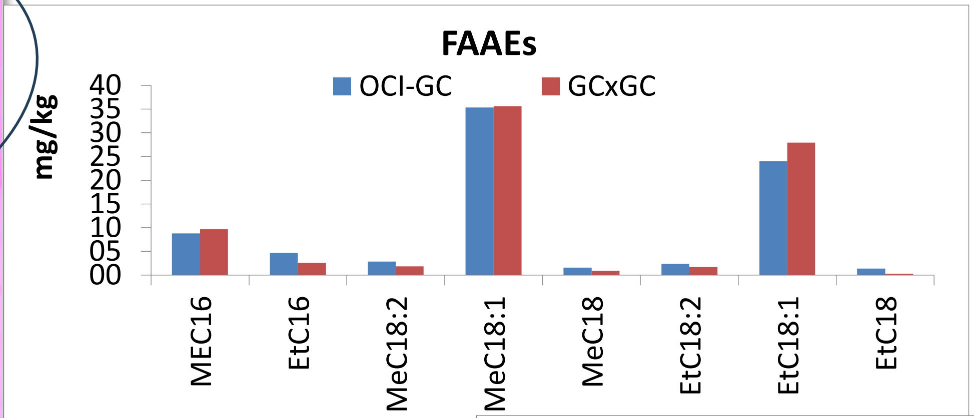
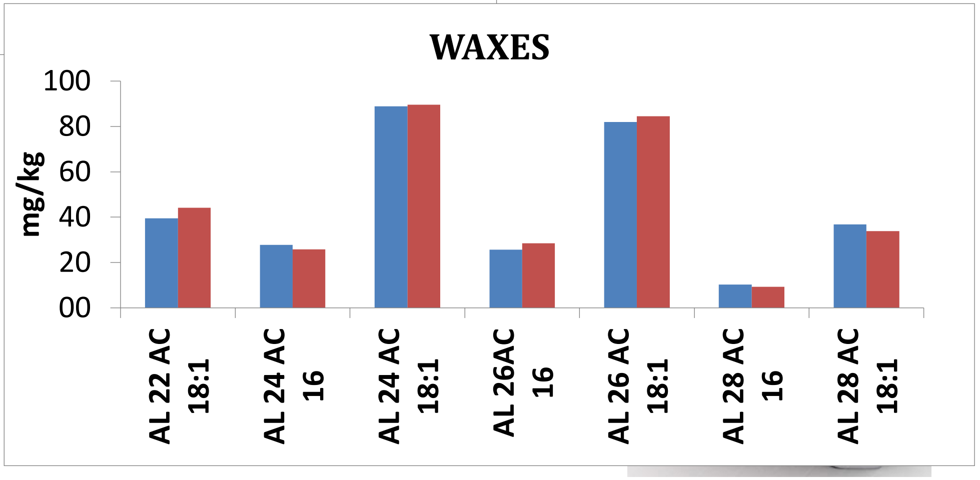


comparison with the Official method

(FAAEs and Wax) GC×GC-FID/MS





G. Purcaro*, L. Barp, L. Conte, **J. Sep. Sci.** 38 (2015) 2278-2285

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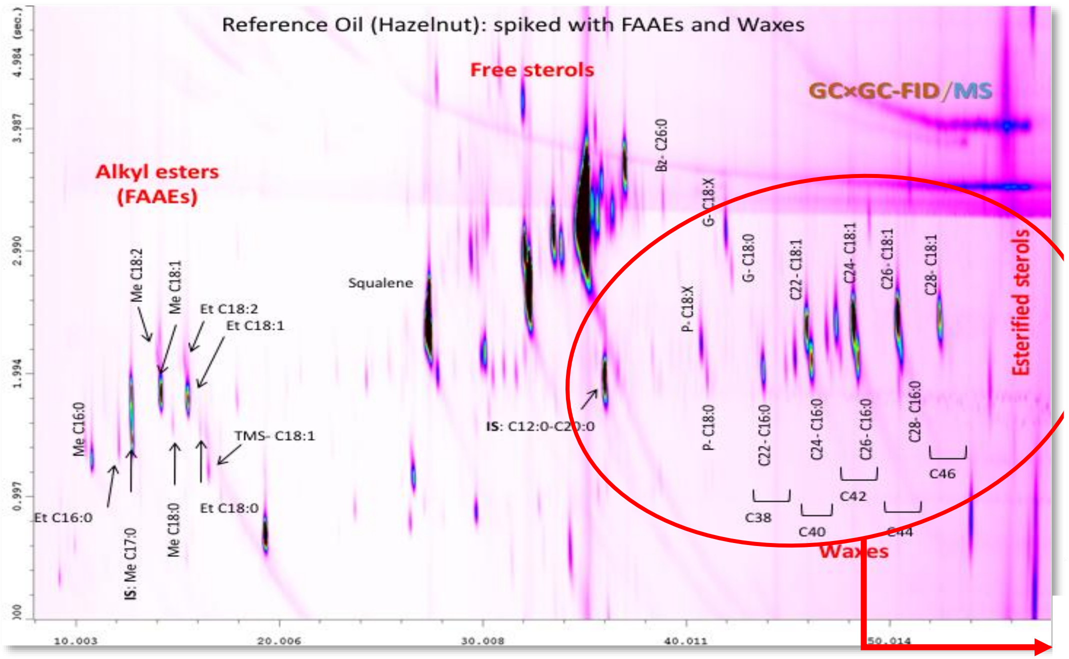
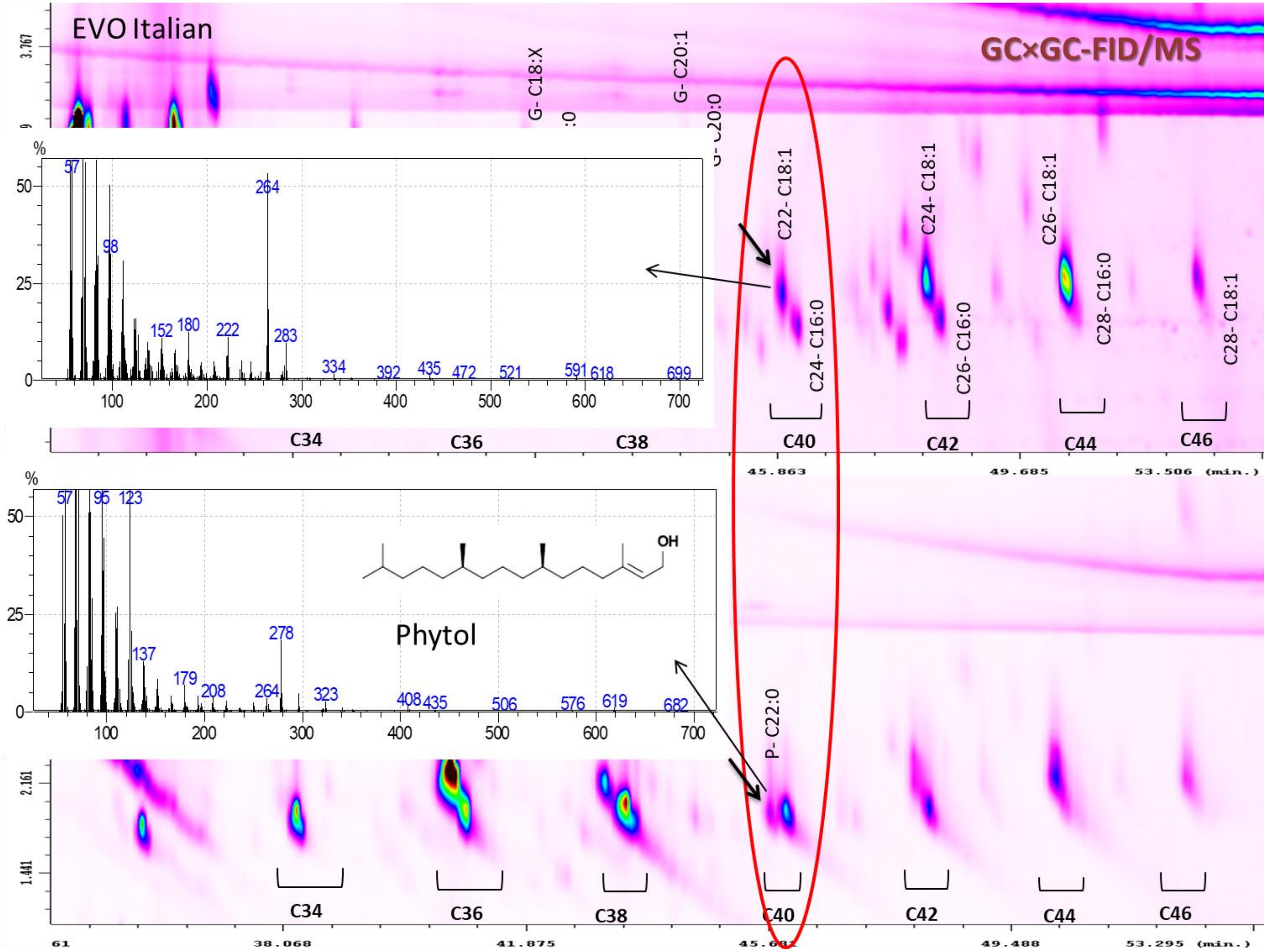
L. Barp, F. A. Franchina, **G. Purcaro***, P. Q. Tranchida, L. Mondello, **Talanta** 165 (2017) 598–603.

GC×GC: Simplify sample prep

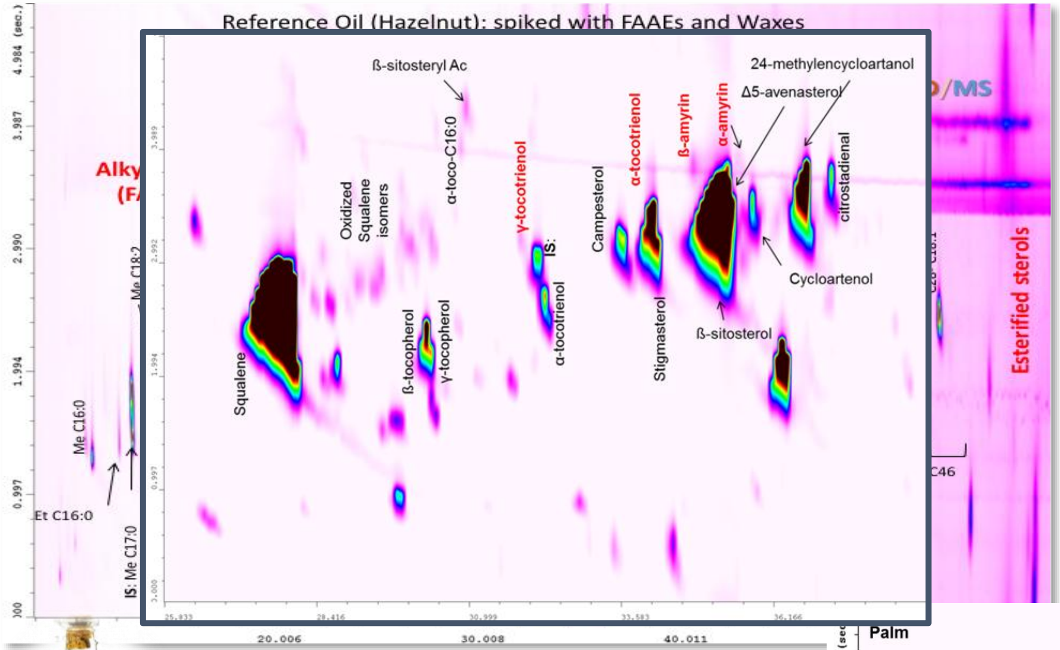


Support data interpretation



More information

GC×GC: Simplify sample prep

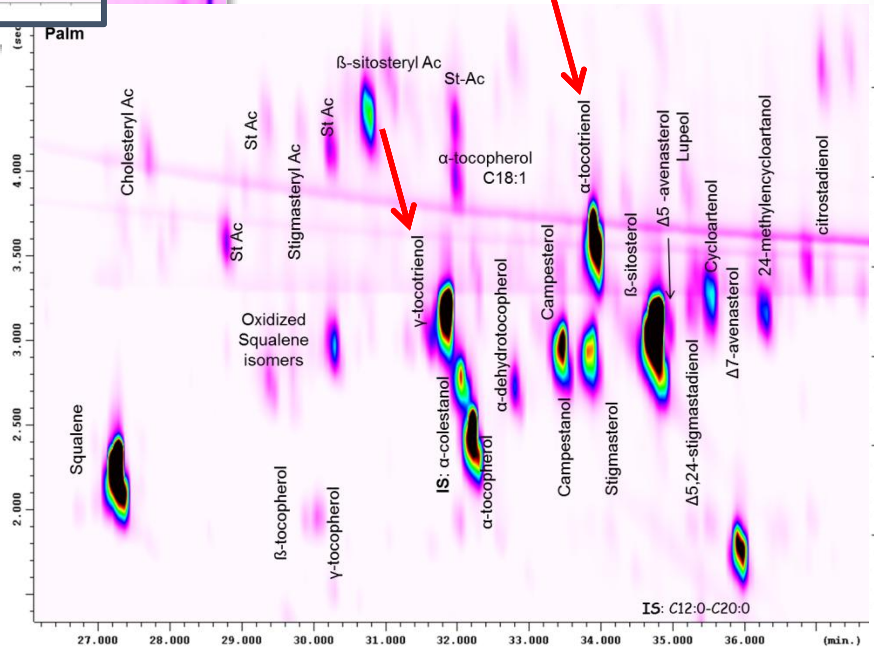


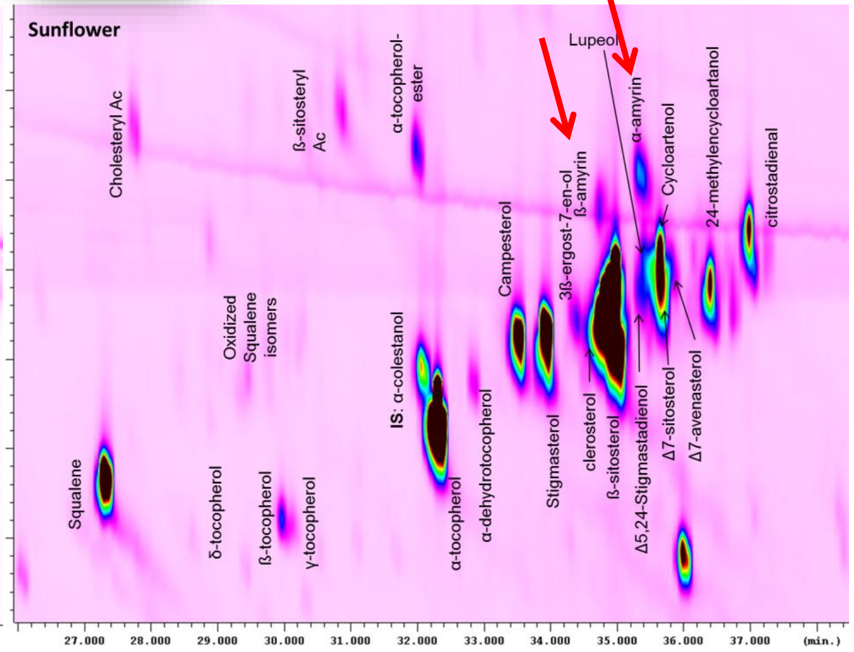
It was clearly highlighted the presence of the following sterols, not present in EVO

α- and γ-tocotrienol → Palm

α- and β-amyrin. → Sunflower

More information





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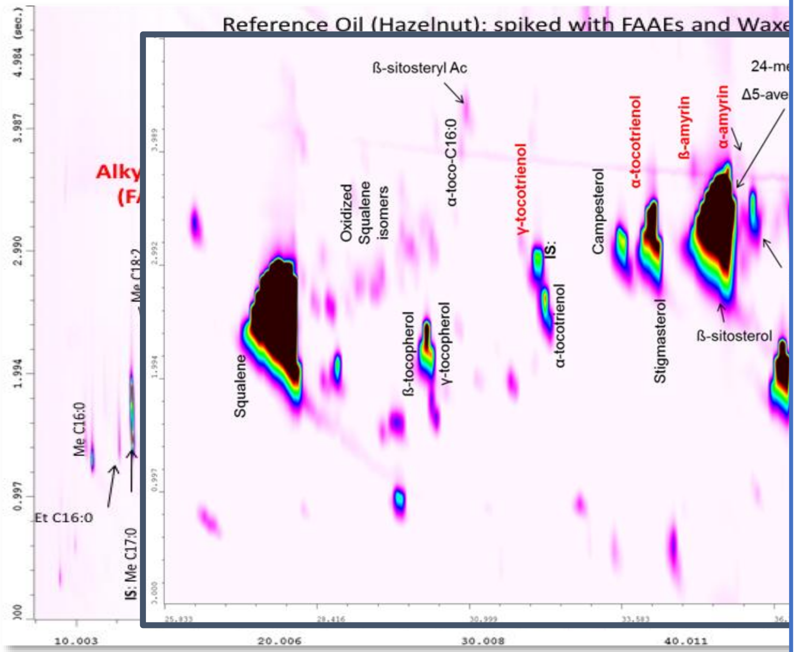
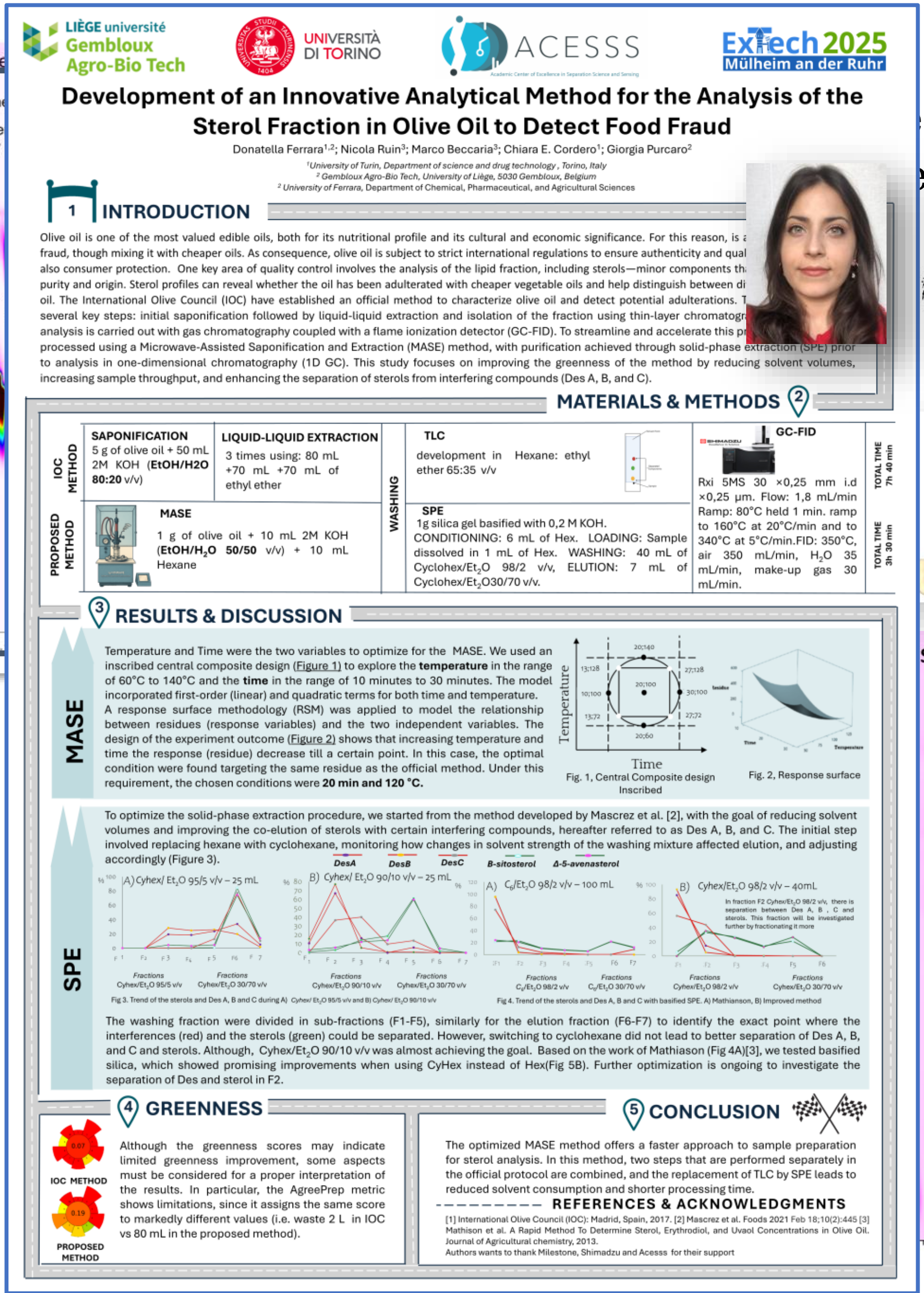




Development of an Innovative Analytical Method for the Analysis of the Sterol Fraction in Olive Oil to Detect Food Fraud

Donatella Ferrara[1,2]; Nicola Ruin[3]; Marco Beccaria[3]; Chiara E. Cordero[1]; Giorgia Purcaro[2]

[1]University of Turin, Department of science and drug technology, Torino, Italy
[2] Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium
[2] University of Ferrara, Department of Chemical, Pharmaceutical, and Agricultural Sciences

1 INTRODUCTION

Olive oil is one of the most valued edible oils, both for its nutritional profile and its cultural and economic significance. For this reason, is a fraud, though mixing it with cheaper oils. As consequence, olive oil is subject to strict international regulations to ensure authenticity and qual also consumer protection. One key area of quality control involves the analysis of the lipid fraction, including sterols—minor components th purity and origin. Sterol profiles can reveal whether the oil has been adulterated with cheaper vegetable oils and help distinguish between di oil. The International Olive Council (IOC) have established an official method to characterize olive oil and detect potential adulterations. T several key steps: initial saponification followed by liquid-liquid extraction and isolation of the fraction using thin-layer chromatogr analysis is carried out with gas chromatography coupled with a flame ionization detector (GC-FID). To streamline and accelerate this pr processed using a Microwave-Assisted Saponification and Extraction (MASE) method, with purification achieved through solid-phase extraction (SPE) prior to analysis in one-dimensional chromatography (1D GC). This study focuses on improving the greenness of the method by reducing solvent volumes, increasing sample throughput, and enhancing the separation of sterols from interfering compounds (Des A, B, and C).

MATERIALS & METHODS 2

IOC METHOD	SAPONIFICATION 5 g of olive oil + 50 mL 2M KOH (EtOH/H2O 80:20 v/v)	LIQUID-LIQUID EXTRACTION 3 times using: 80 mL +70 mL +70 mL of ethyl ether	WASHING	TLC development in Hexane: ethyl ether 65:35 v/v	TOTAL TIME 7h 40 min
PROPOSED METHOD	MASE 1 g of olive oil + 10 mL 2M KOH ($EtOH/H_2O$ 50/50 v/v) + 10 mL Hexane			SPE 1g silica gel basified with 0,2 M KOH. CONDITIONING: 6 mL of Hex. LOADING: Sample dissolved in 1 mL of Hex. WASHING: 40 mL of Cyclohex/Et_2O 98/2 v/v, ELUTION: 7 mL of Cyclohex/Et_2O30/70 v/v.	TOTAL TIME 3h 30 min

GC-FID: Rxi 5MS 30 ×0,25 mm i.d ×0,25 µm. Flow: 1,8 mL/min Ramp: 80°C held 1 min. ramp to 160°C at 20°C/min and to 340°C at 5°C/min.FID: 350°C, air 350 mL/min, H_2O 35 mL/min, make-up gas 30 mL/min.

3 RESULTS & DISCUSSION

MASE

Temperature and Time were the two variables to optimize for the MASE. We used an inscribed central composite design (Figure 1) to explore the **temperature** in the range of 60°C to 140°C and the **time** in the range of 10 minutes to 30 minutes. The model incorporated first-order (linear) and quadratic terms for both time and temperature. A response surface methodology (RSM) was applied to model the relationship between residues (response variables) and the two independent variables. The design of the experiment outcome (Figure 2) shows that increasing temperature and time the response (residue) decrease till a certain point. In this case, the optimal condition were found targeting the same residue as the official method. Under this requirement, the chosen conditions were **20 min and 120 °C.**

Fig. 1, Central Composite design Inscribed

Fig. 2, Response surface

SPE

To optimize the solid-phase extraction procedure, we started from the method developed by Mascrez et al. [2], with the goal of reducing solvent volumes and improving the co-elution of sterols with certain interfering compounds, hereafter referred to as Des A, B, and C. The initial step involved replacing hexane with cyclohexane, monitoring how changes in solvent strength of the washing mixture affected elution, and adjusting accordingly (Figure 3).

Fig 3. Trend of the sterols and Des A, B and C during A) Cyhex/ Et_2O 95/5 v/v and B) Cyhex/ Et_2O 90/10 v/v

Fig 4. Trend of the sterols and Des A, B and C with basified SPE. A) Mathianson, B) Improved method

The washing fraction were divided in sub-fractions (F1-F5), similarly for the elution fraction (F6-F7) to identify the exact point where the interferences (red) and the sterols (green) could be separated. However, switching to cyclohexane did not lead to better separation of Des A, B, and C and sterols. Although, Cyhex/Et_2O 90/10 v/v was almost achieving the goal. Based on the work of Mathiason (Fig 4A)[3], we tested basified silica, which showed promising improvements when using CyHex instead of Hex(Fig 5B). Further optimization is ongoing to investigate the separation of Des and sterol in F2.

4 GREENNESS

IOC METHOD 0.07

PROPOSED METHOD 0.19

Although the greenness scores may indicate limited greenness improvement, some aspects must be considered for a proper interpretation of the results. In particular, the AgreePrep metric shows limitations, since it assigns the same score to markedly different values (i.e. waste 2 L in IOC vs 80 mL in the proposed method).

5 CONCLUSION

The optimized MASE method offers a faster approach to sample preparation for sterol analysis. In this method, two steps that are performed separately in the official protocol are combined, and the replacement of TLC by SPE leads to reduced solvent consumption and shorter processing time.

REFERENCES & ACKNOWLEDGMENTS

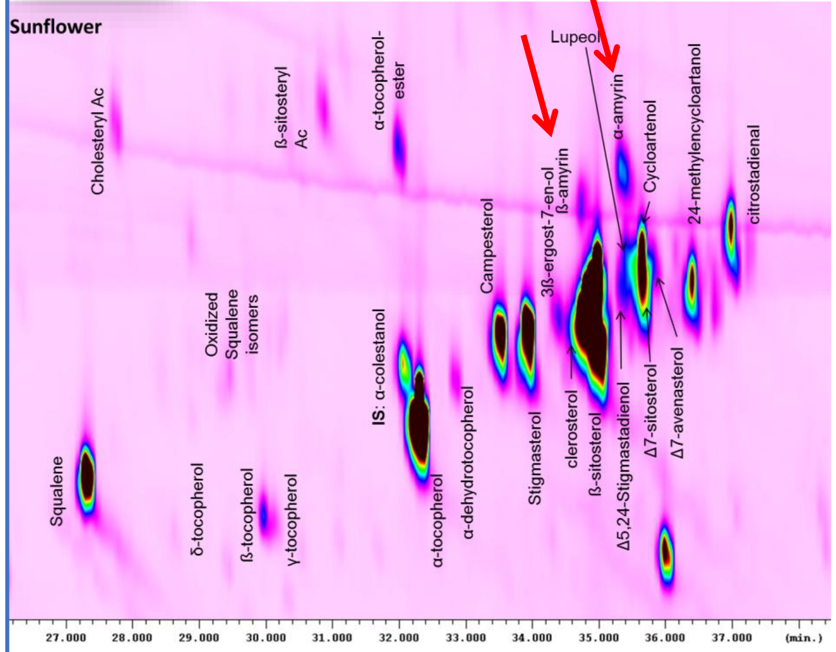
[1] International Olive Council (IOC): Madrid, Spain, 2017. [2] Mascrez et al. Foods 2021 Feb 18;10(2):445 [3] Mathison et al. A Rapid Method To Determine Sterol, Erythrodiol, and Uvaol Concentrations in Olive Oil. Journal of Agricultural chemistry, 2013.

Authors wants to thank Milestone, Shimadzu and Acesss for their support

presence of the following sterols, esent in EVO

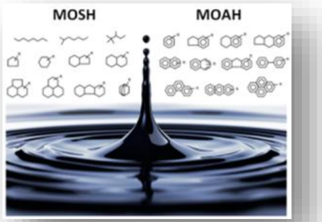
α- and β-amyrin.

Sunflower



More information

GC×GC: Support sample prep & data interpretation

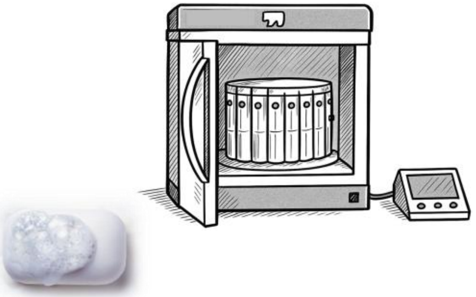


Sample Preparation

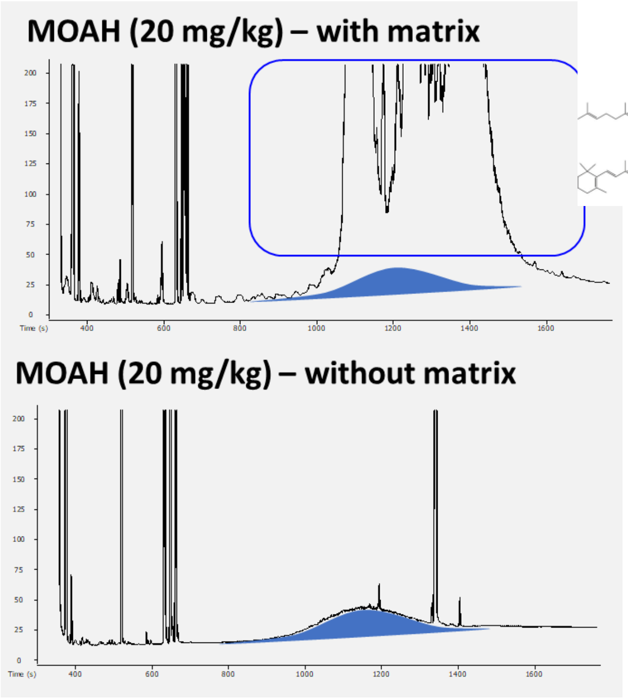
Data Interpretation

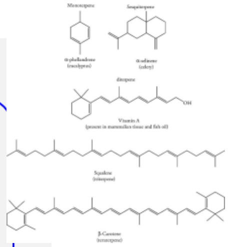
SAPONIFICATION → PURIFICATION → LC-GC-FID

Data Integration

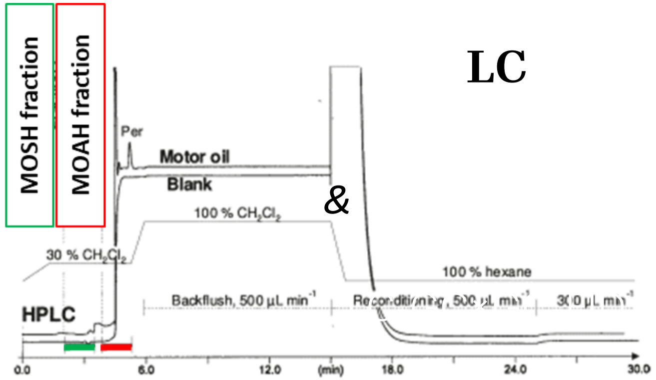


Microwave-assisted saponification and extraction (*MASE*) method



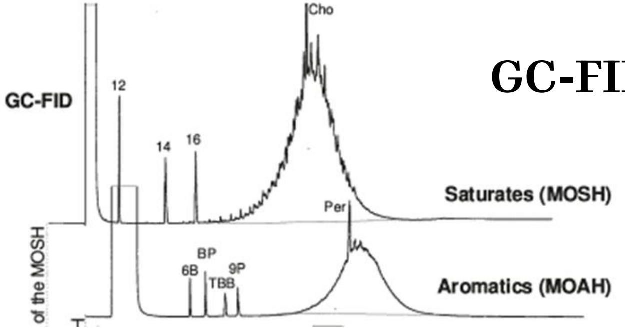


LC



&

GC-FID



Outline of the Analytical approach

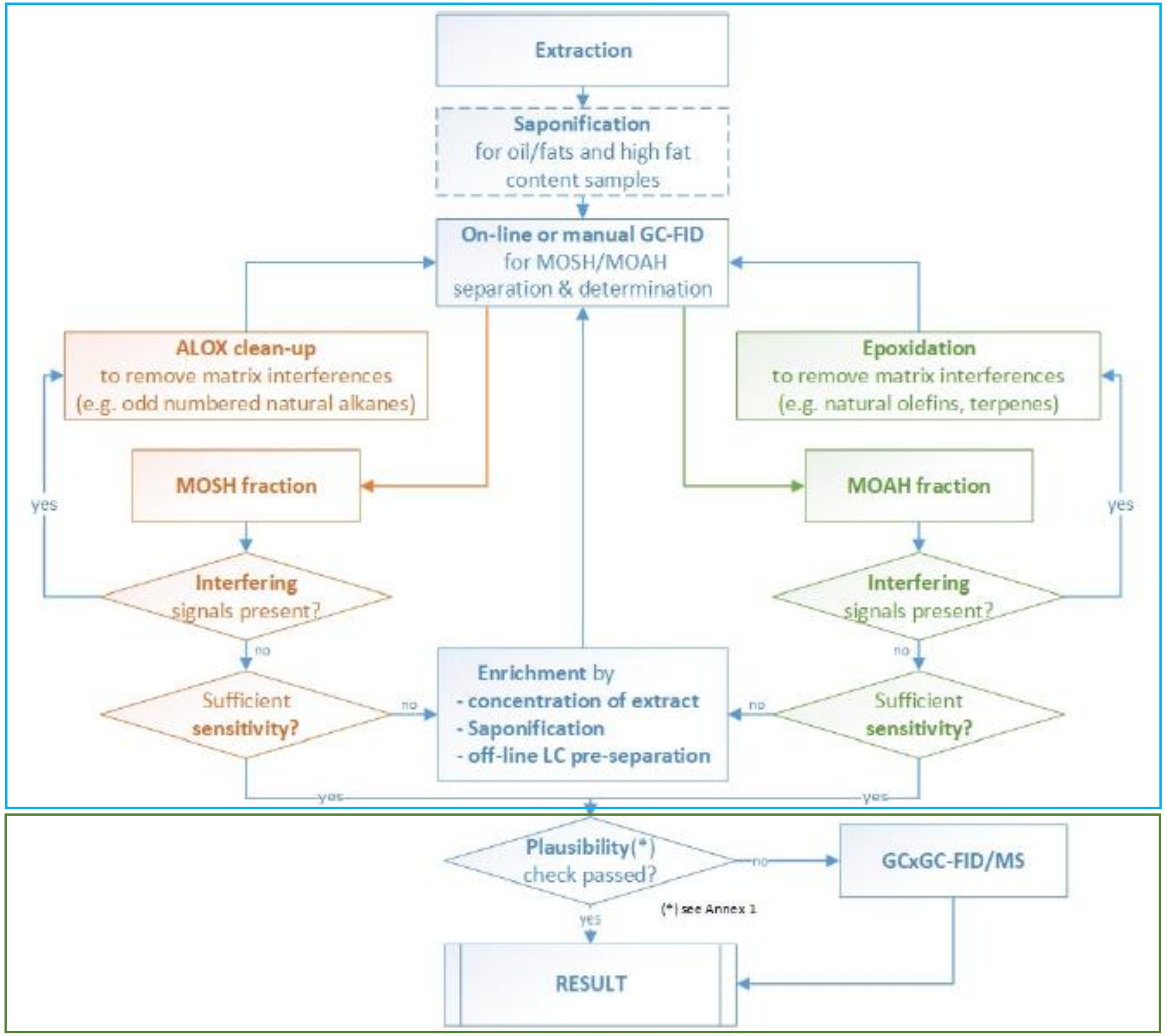


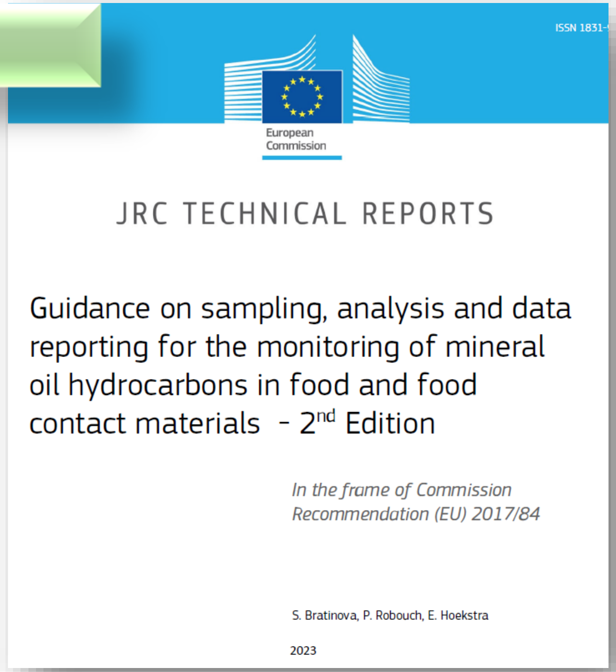
Figure 5 Decision tree on the use of auxiliary methods.

Sample Preparation

↓

Data Interpretation

Data Integration


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

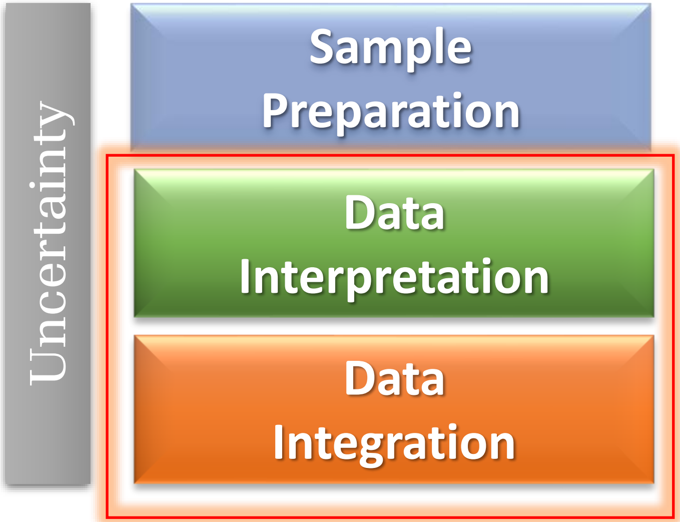
In the frame of Commission Recommendation (EU) 2017/84

S. Bratinova, P. Robouch, E. Hoekstra

2023

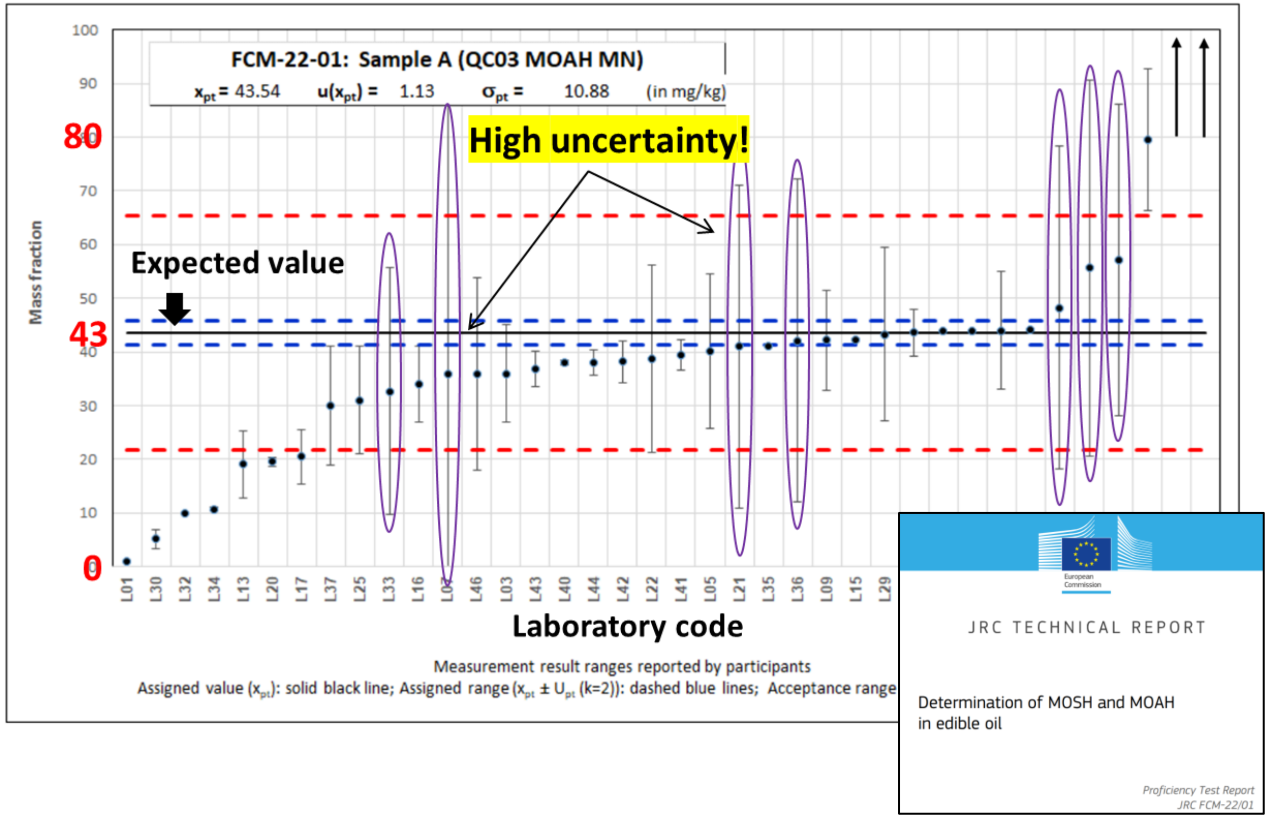
✓Need for matrix-tailored sample prep protocols

Uncertainty in MOSH and MOAH determination



> 20% of uncertainty!

JRC proficiency test 2023 on olive oil



Uncertainty in MOSH and MOAH determination

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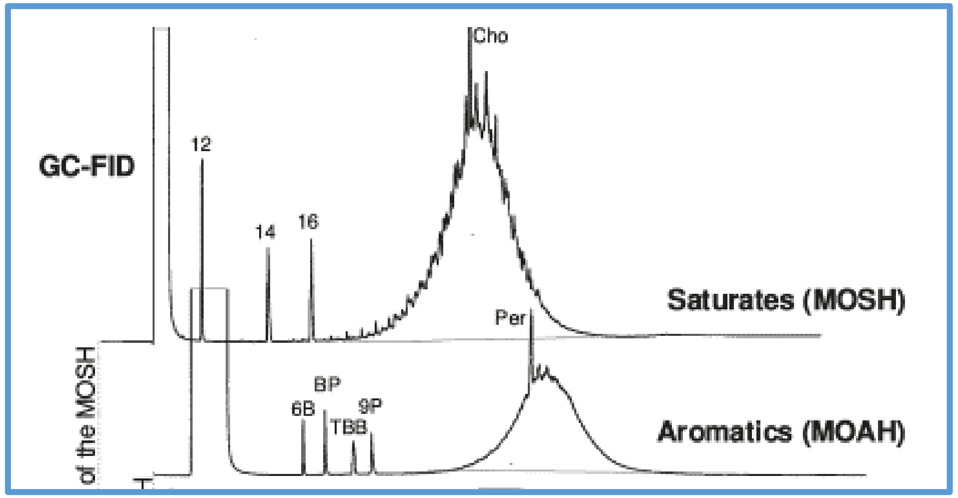
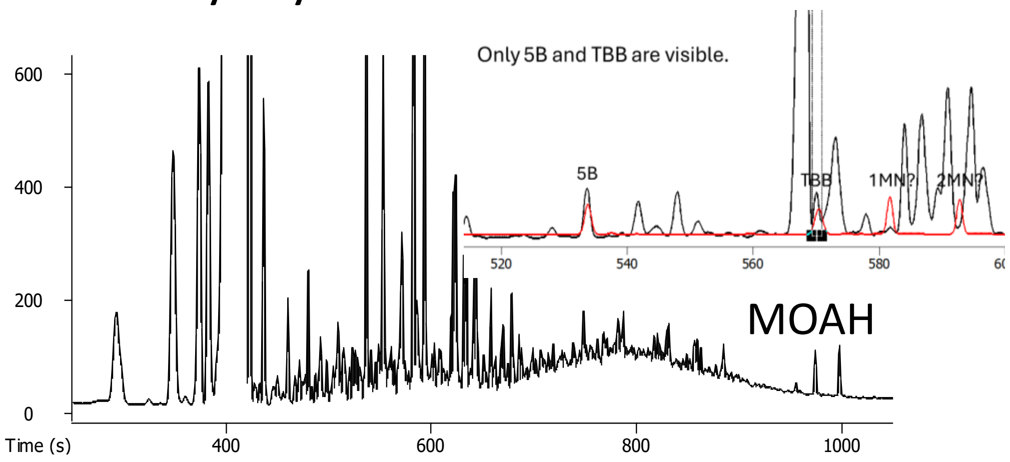
ACESSS Academic Center of Excellence in Separation Science and Sensing

Uncertainty
- Sample Preparation
- Data Interpretation
- Data Integration

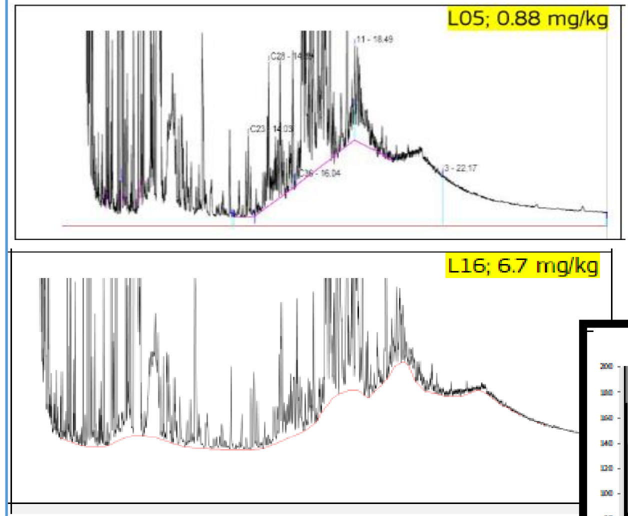


MOAH analysis by GC-FID

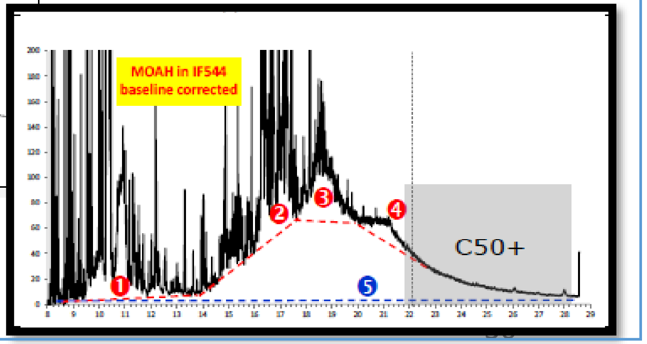
IS for quantification



Internal standards coeluted with interferences
→ impossible MOAH quantification



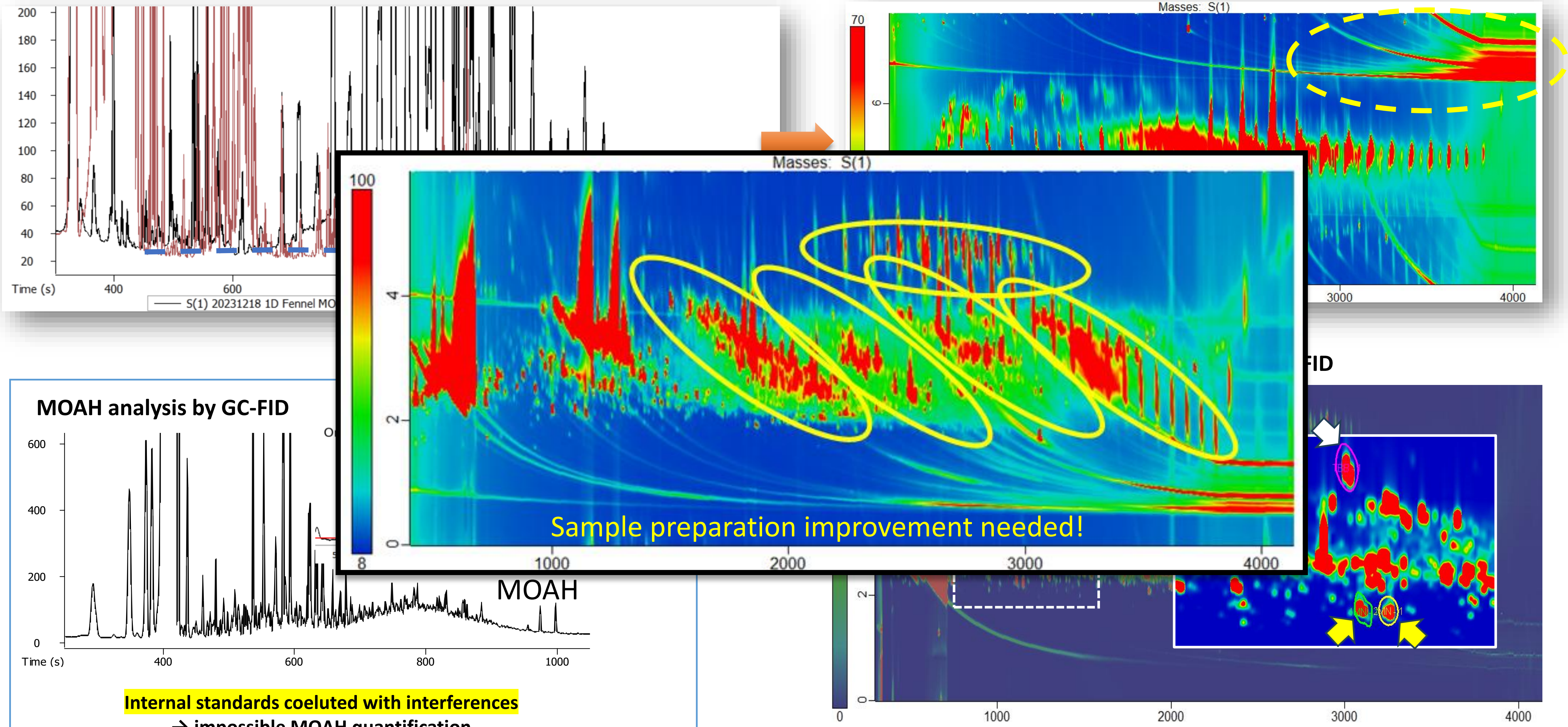
Coeluted interferences to be removed
→ High uncertainty of the result



GC×GC: Support sample prep & data interpretation

MOSH vs MOAH – No purification

More accurate quantification!



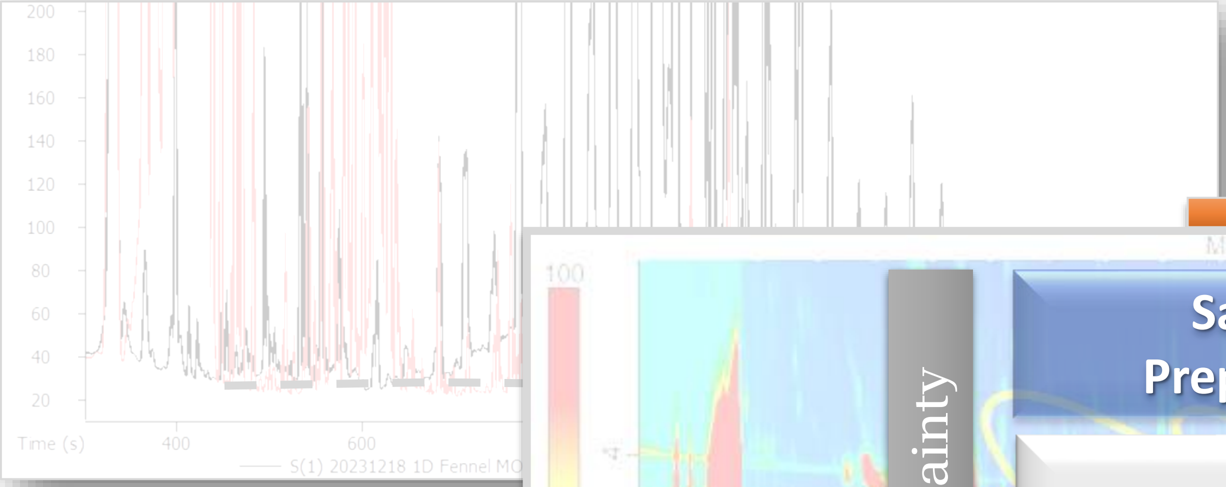
Internal standards coeluted with interferences
→ impossible MOAH quantification

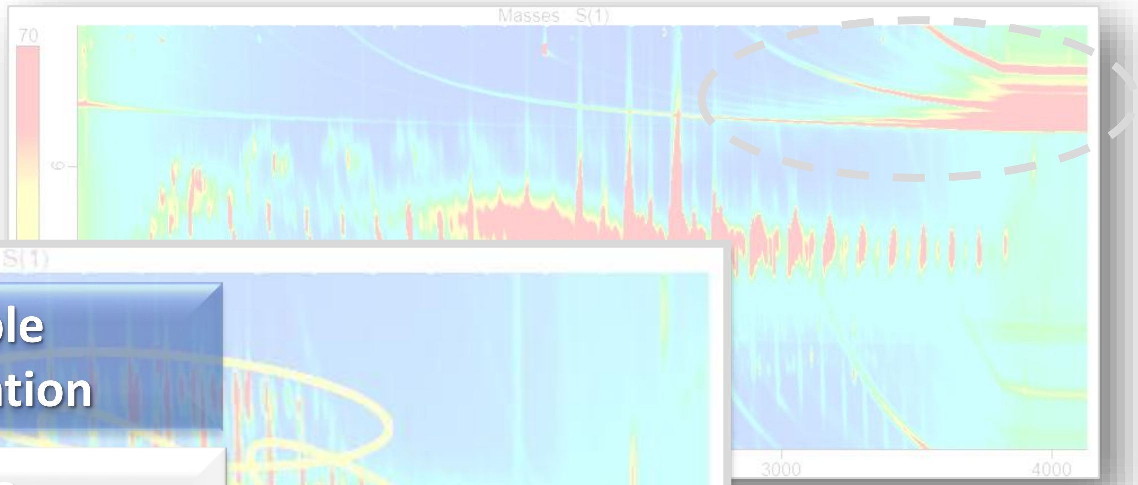
Internal standards resolved from the interferences
→ quantification of MOAH is possible

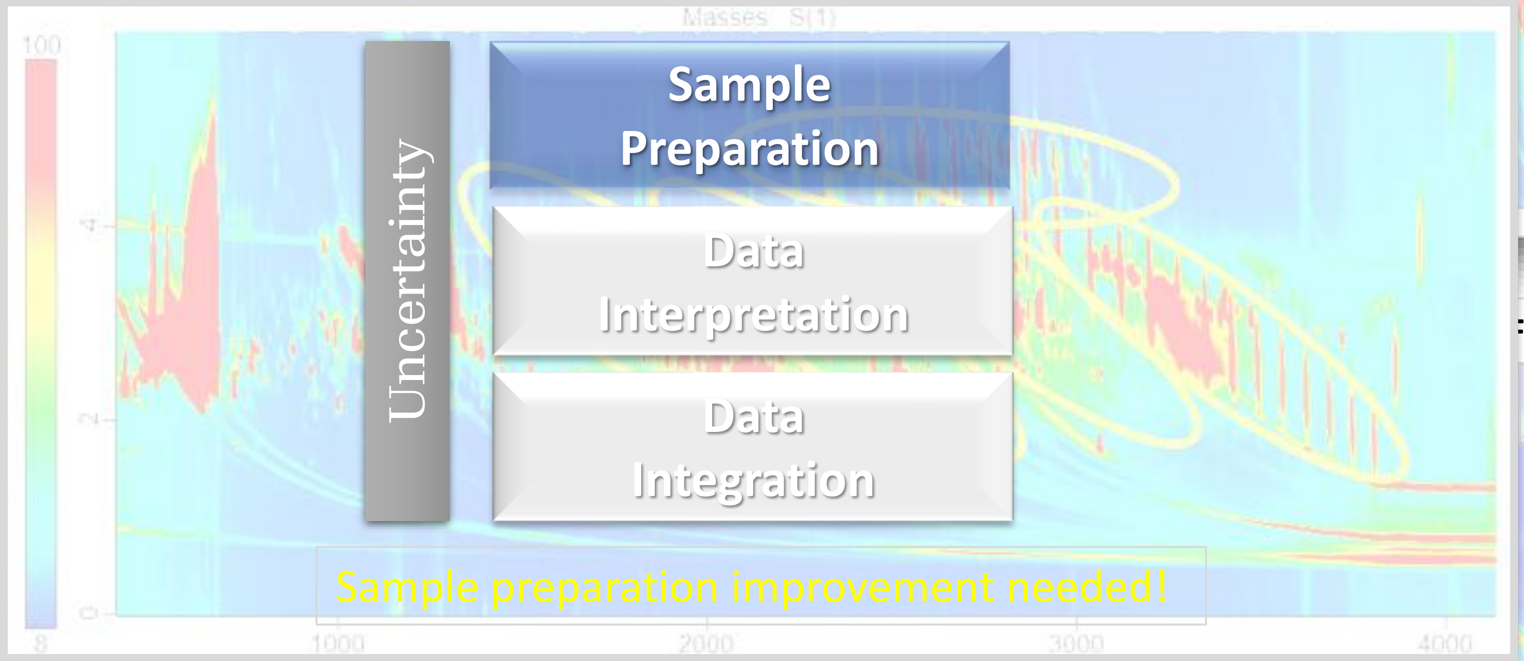
GC×GC: Support sample prep & data interpretation

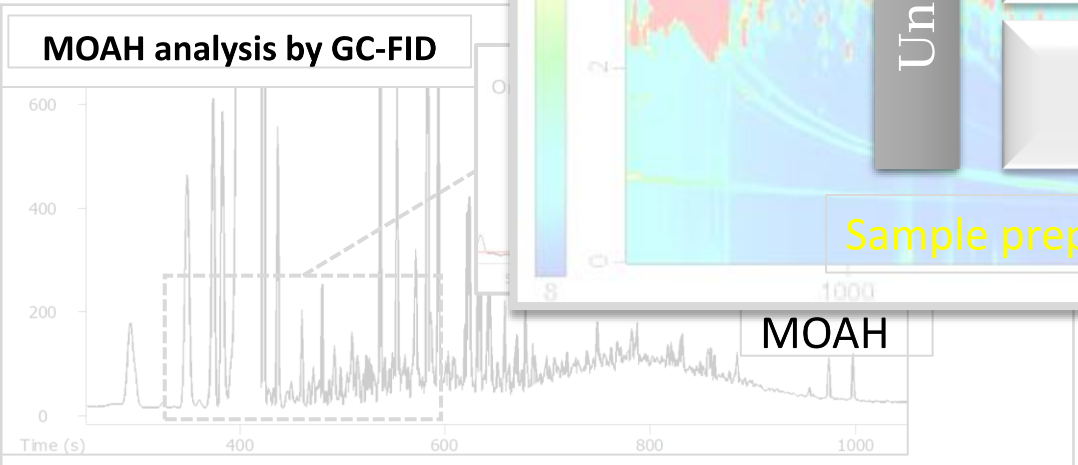
MOSH vs MOAH – No purification

More accurate quantification!









Internal standards coeluted with interferences
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GC×GC: Support sample prep & data interpretation

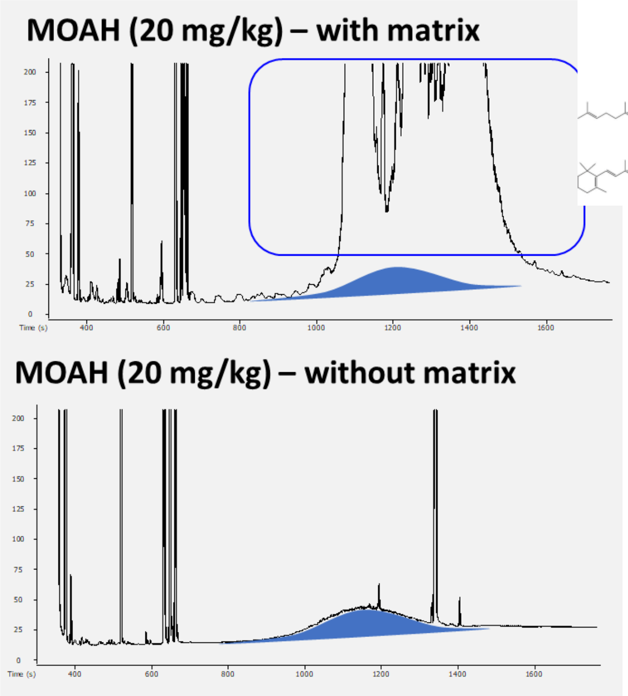
ACESSS – Academic Center of Excellence in Separation Science and Sensing

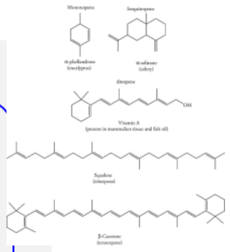
Uncertainty
- **Sample Preparation**
- Data Interpretation
- Data Integration

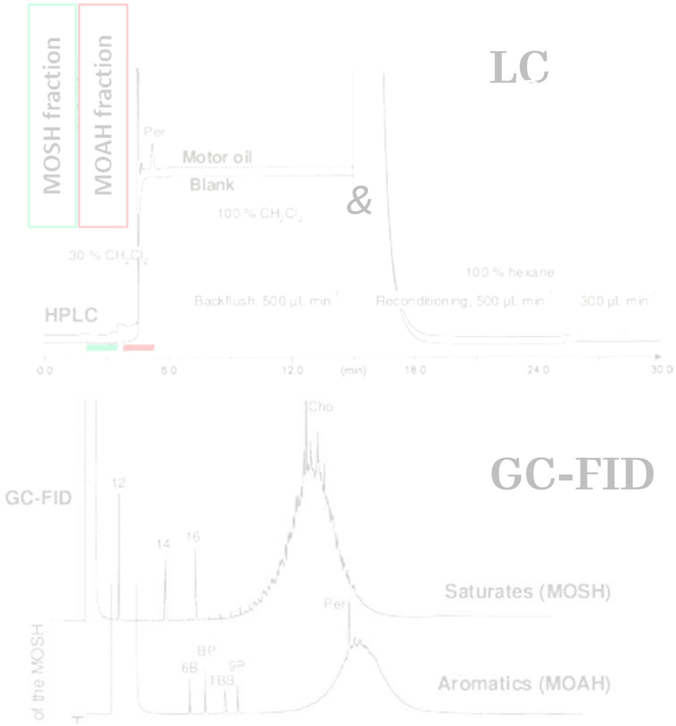
SAPONIFICATION | **PURIFICATION** → **LC-GCxGC-FID**

Microwave-assisted saponification and extraction (*MASE*) method

TBB/MN= 1.05±0.02

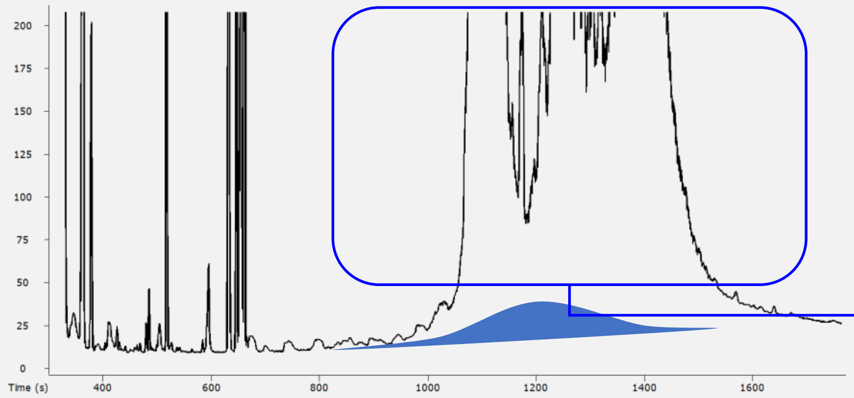




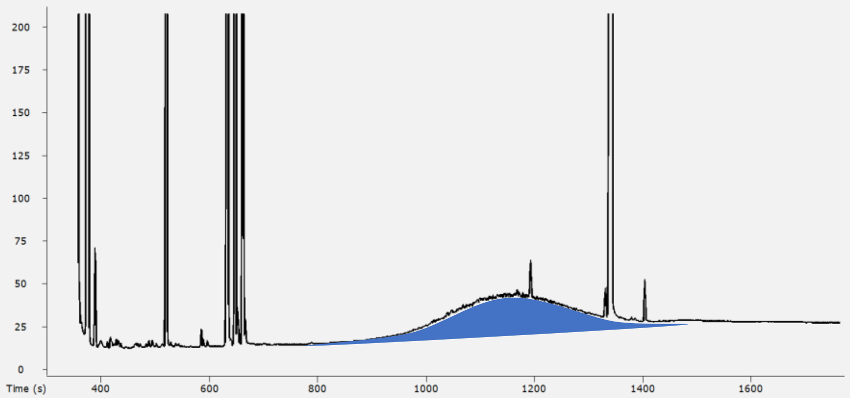


CHALLENGES in MOSH/MOAH analaysis

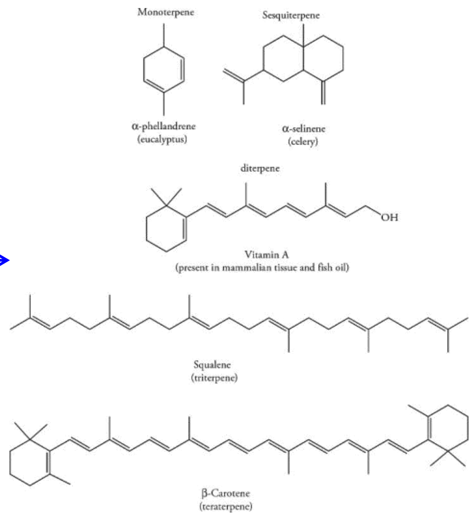
MOAH (20 mg/kg) – with matrix



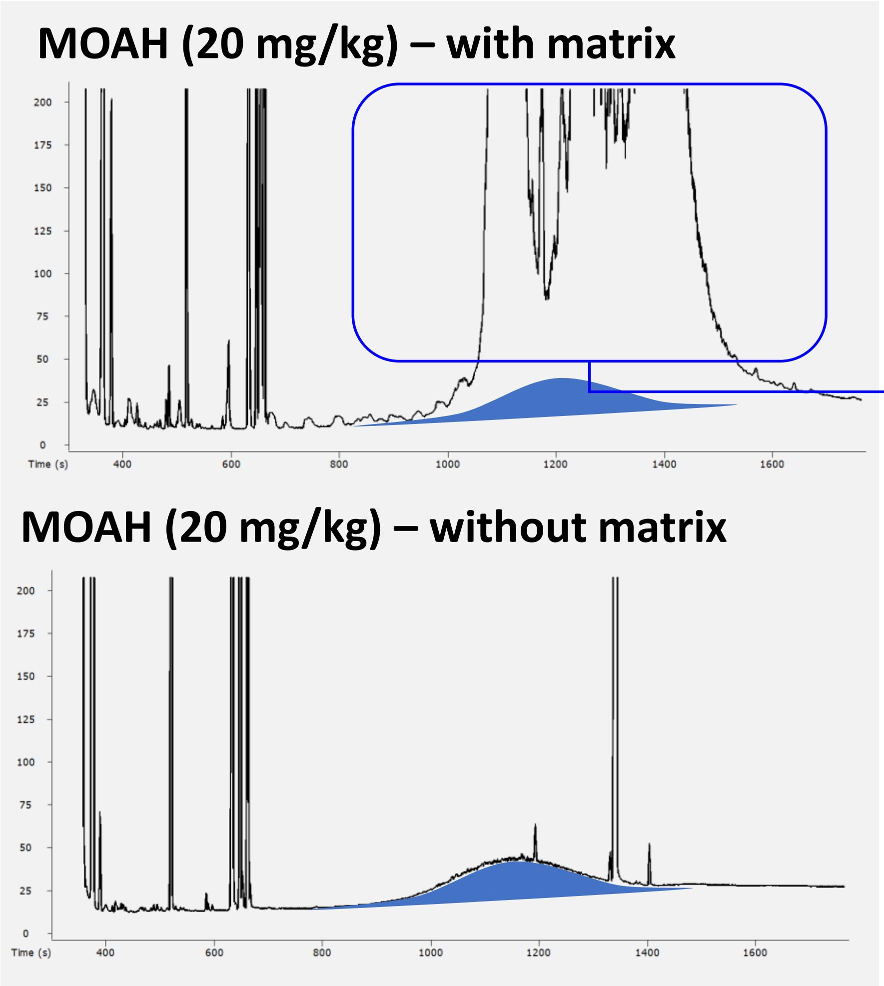
MOAH (20 mg/kg) – without matrix



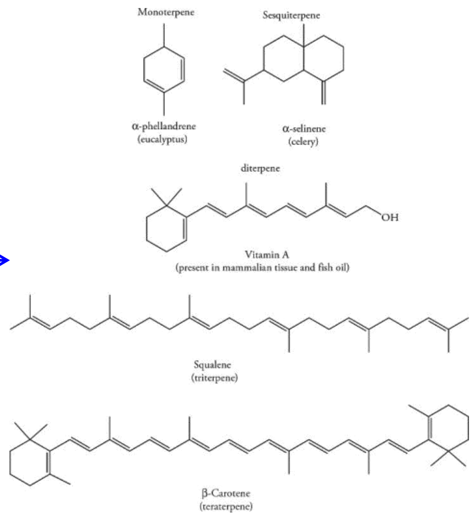
Food matrices can contain terpenes at concentrations that overload MOAH chromatograms

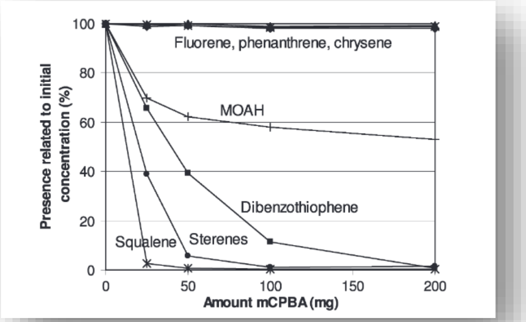


CHALLENGES in MOSH/MOAH analysis

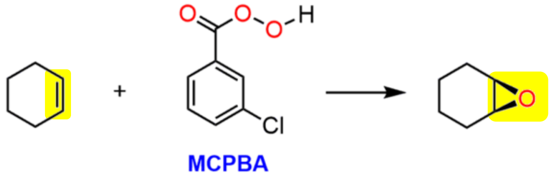


Food matrices can contain **terpenes** at concentrations that overload MOAH chromatograms





Epoxidation is used to remove these terpenes

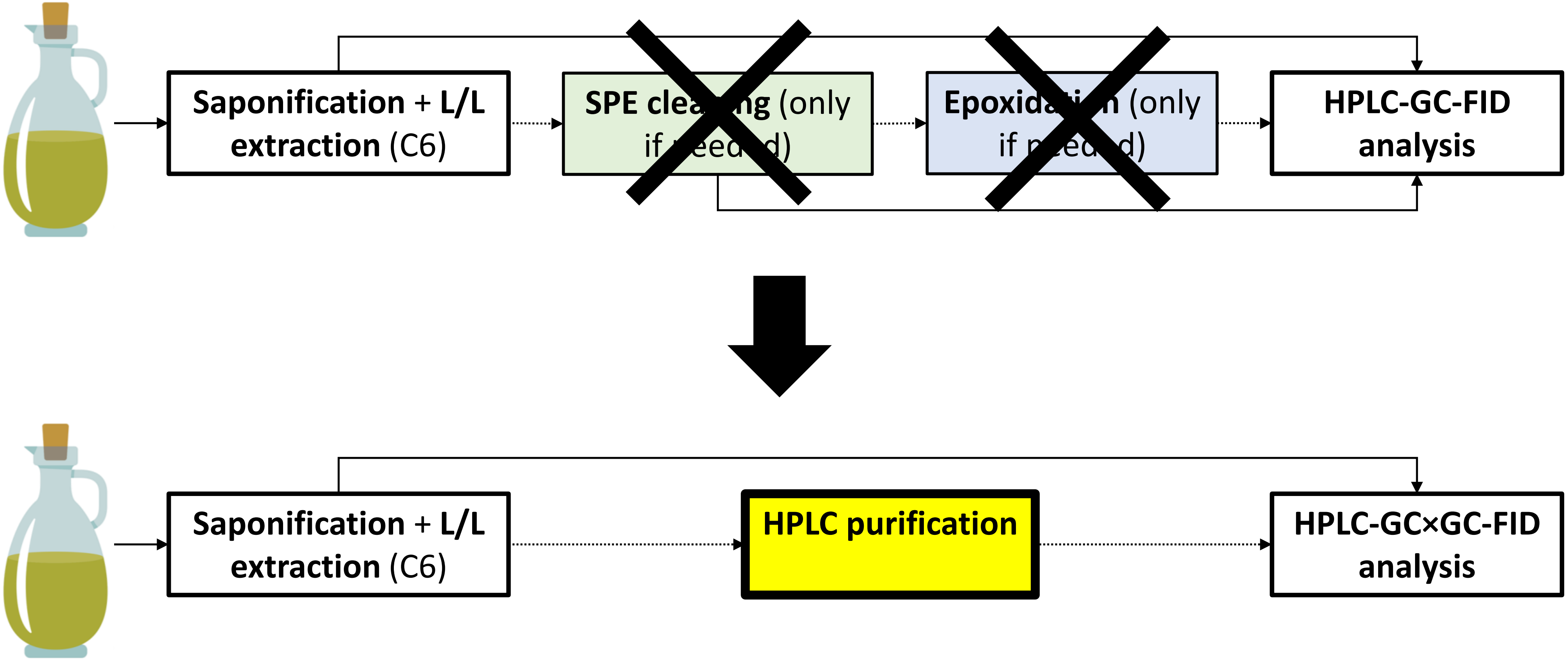


20-40% of MOAH can be also be lost (particularly those having many d.b.)

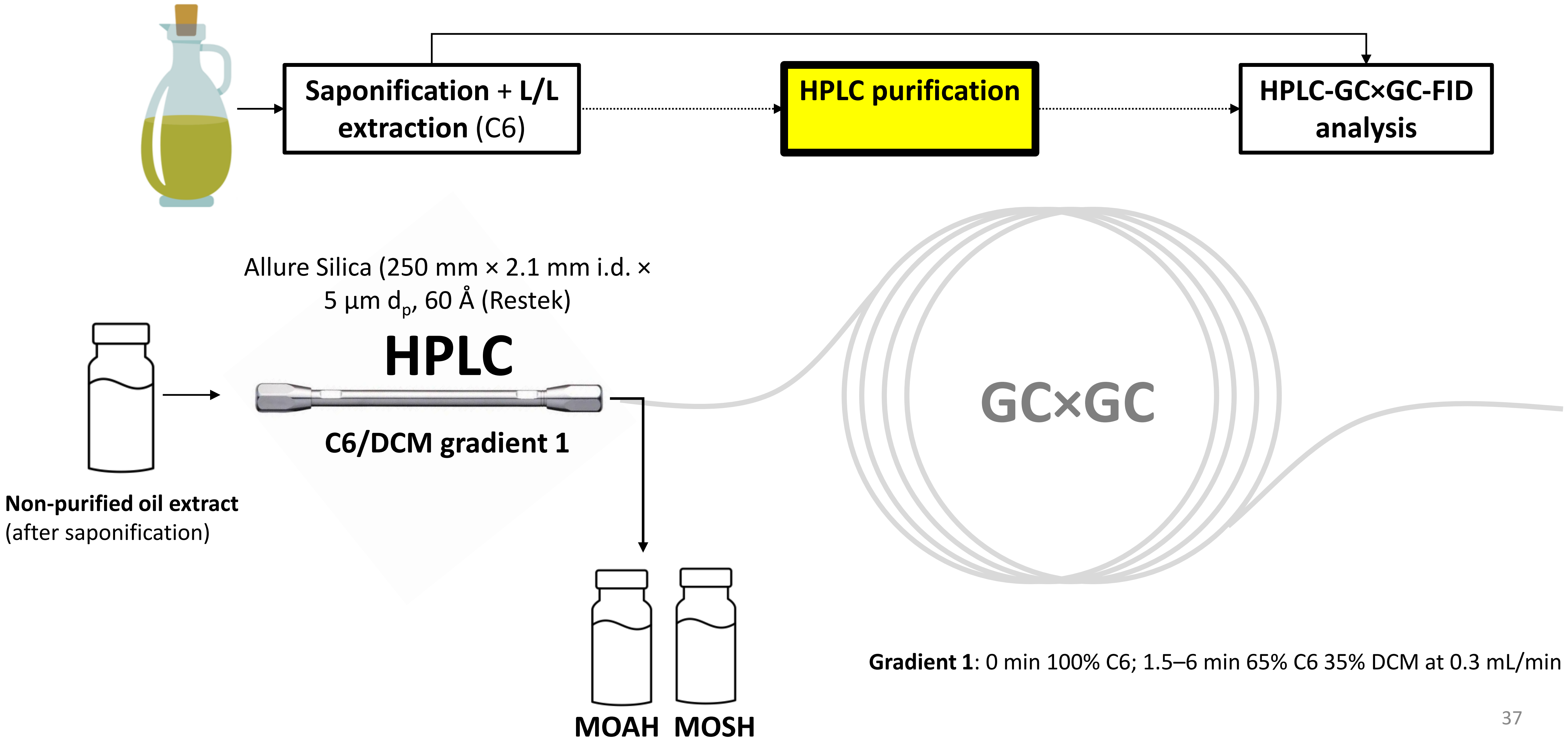
Another cause of uncertainty!

GC×GC: Simplify & support sample prep

ISO 20122:2024 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

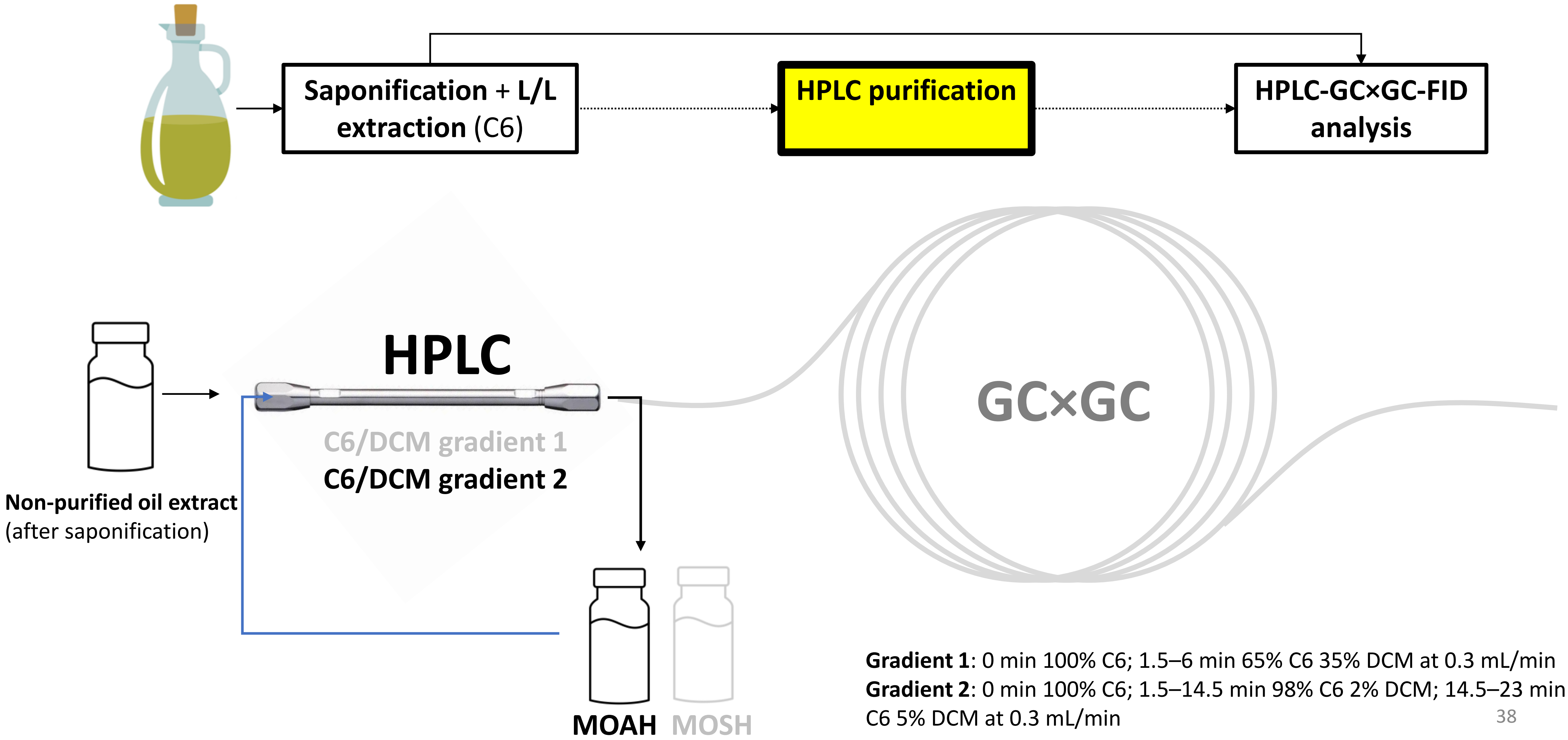


GC×GC: Simplify & support sample prep



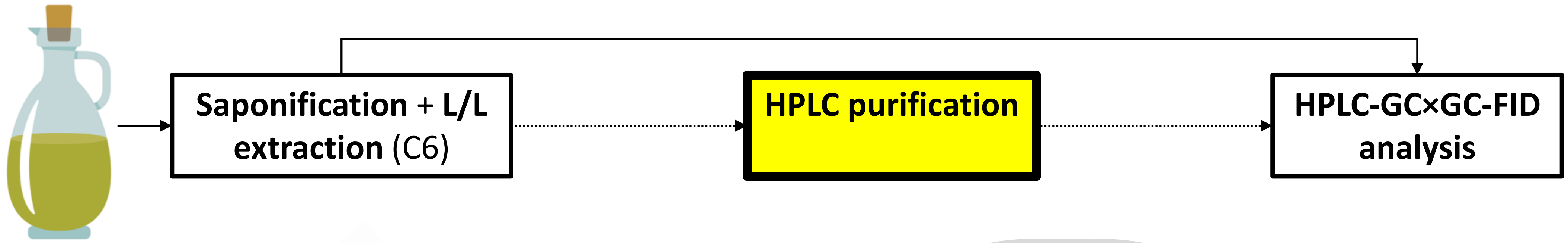
Gradient 1: 0 min 100% C6; 1.5–6 min 65% C6 35% DCM at 0.3 mL/min

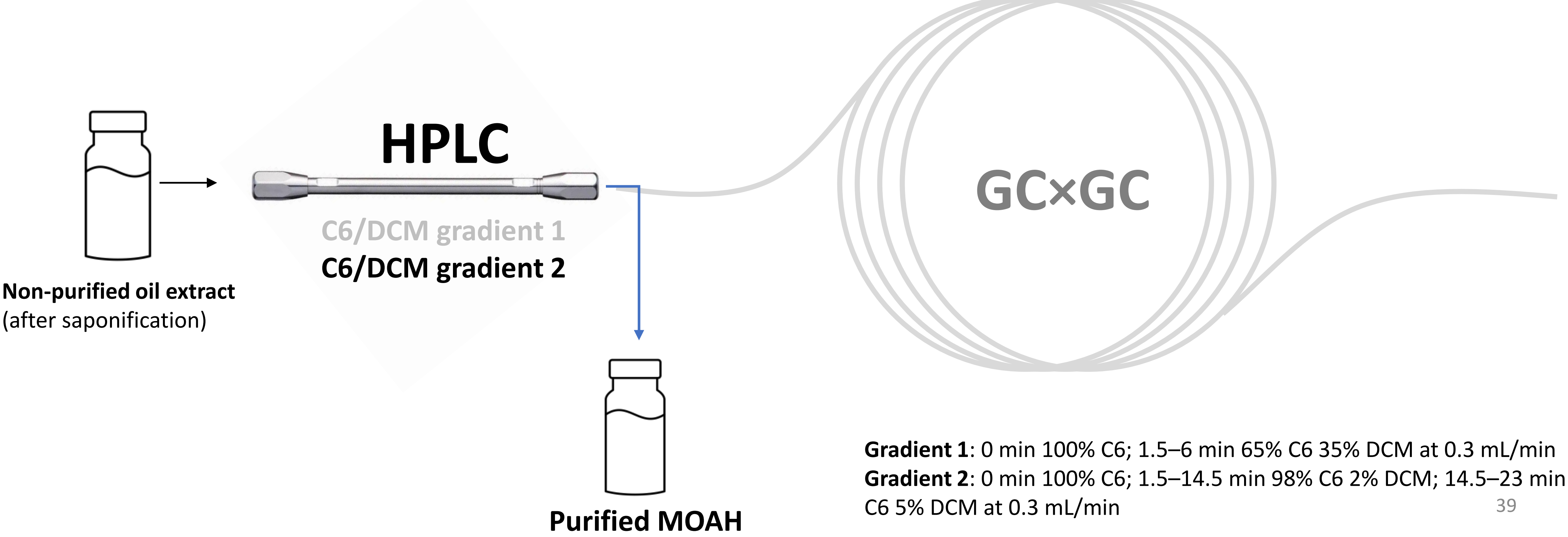
GC×GC: Simplify & support sample prep



Gradient 1: 0 min 100% C6; 1.5–6 min 65% C6 35% DCM at 0.3 mL/min
Gradient 2: 0 min 100% C6; 1.5–14.5 min 98% C6 2% DCM; 14.5–23 min 95% C6 5% DCM at 0.3 mL/min

GC×GC: Simplify & support sample prep





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GC×GC: Simplify & support sample prep

2025

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ELSEVIER

Purification of mineral oil aromatic hydrocarbons and separation based on the number of aromatic rings using a liquid chromatography silica column. An alternative to epoxidation

Aleksandra Gorska [a], Grégory Bauwens [a], Marco Beccaria [b], Giorgia Purcaro [a,*]

LC-GC×GC-FID/MS

Very good removal of carotenoids and squalene

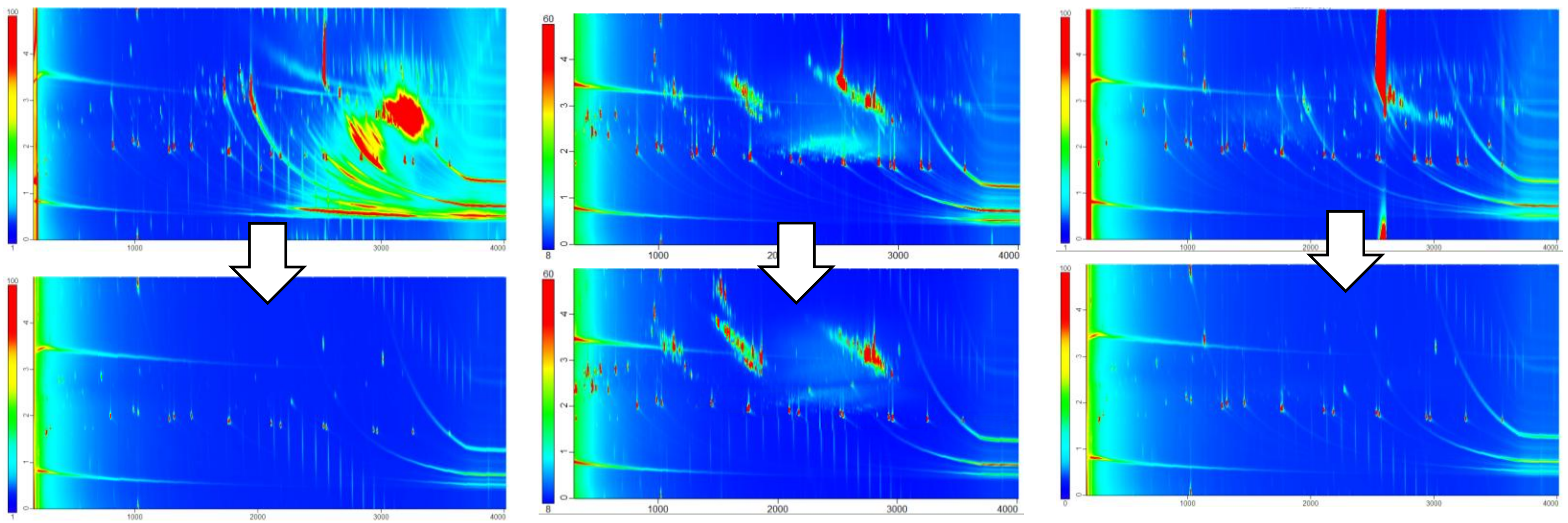
Other terpenoids are less well removed

Epoxidation

mCPBA
82%± 10%

performic acid
71 ± 16%

MOAH Recovery LC Purification:
94% ± 2%



GC×GC: Simplify & support sample prep

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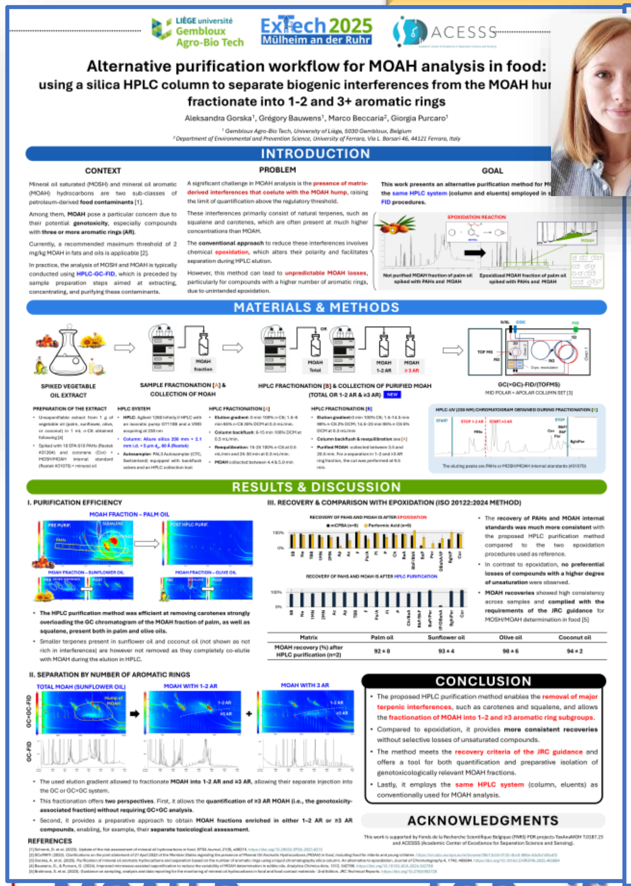
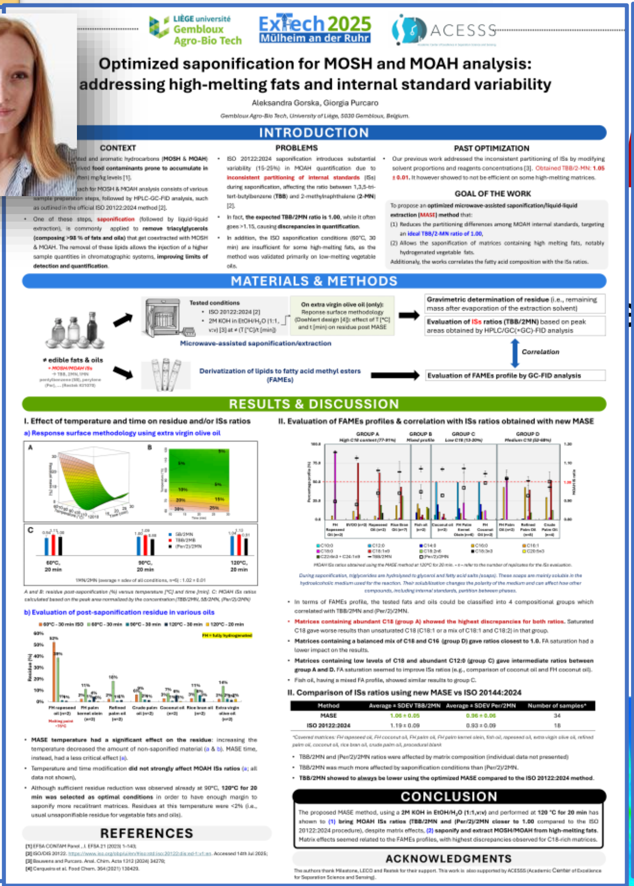
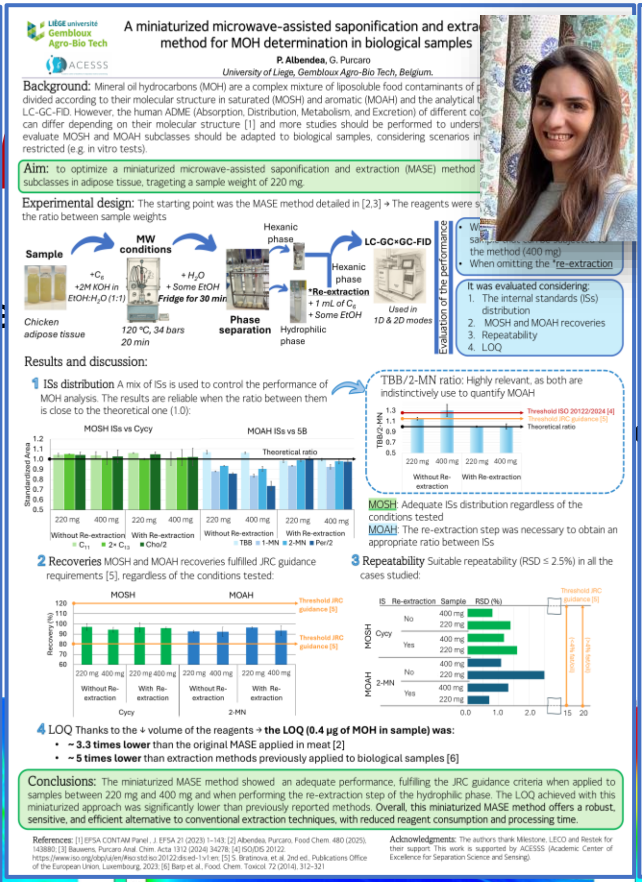
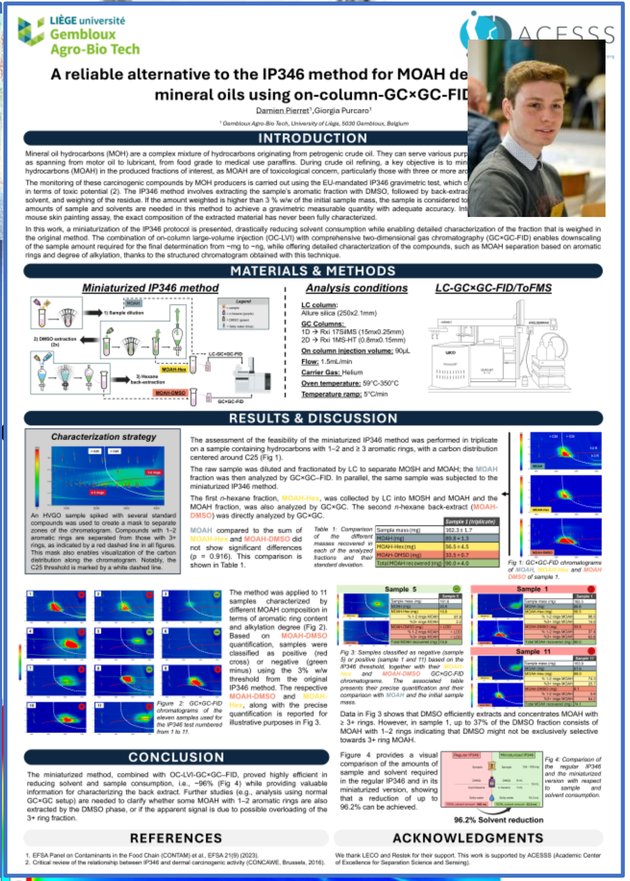


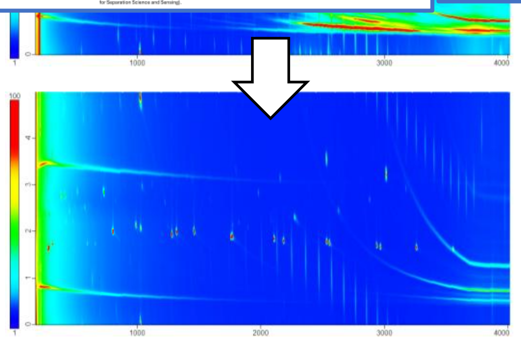


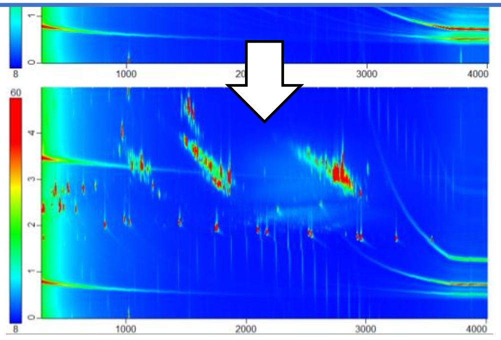


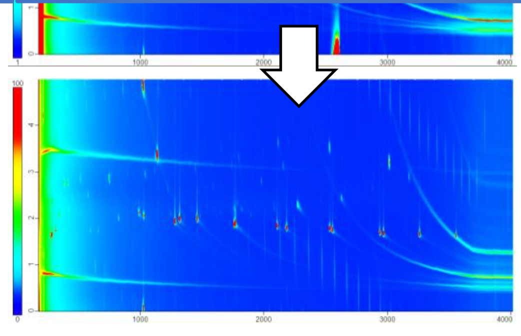


MOAH Recovery LC Purification:

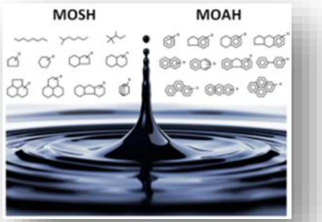
94% ± 2%







GC×GC: Support sample prep & data interpretation



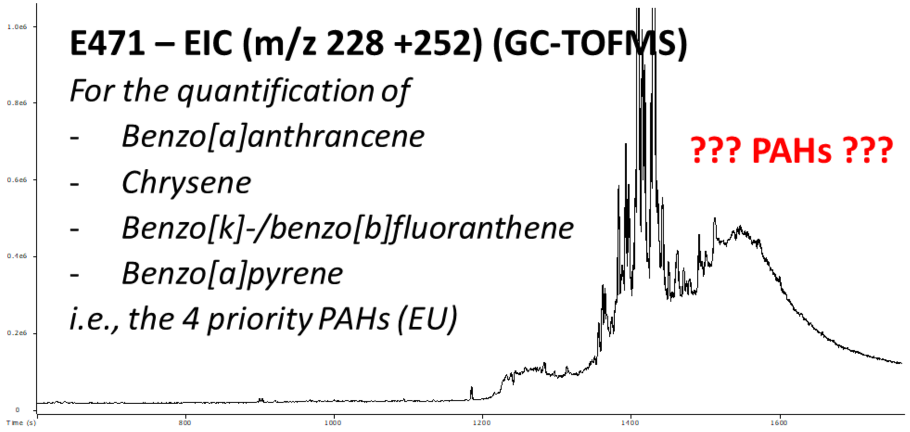
SAPONIFICATION

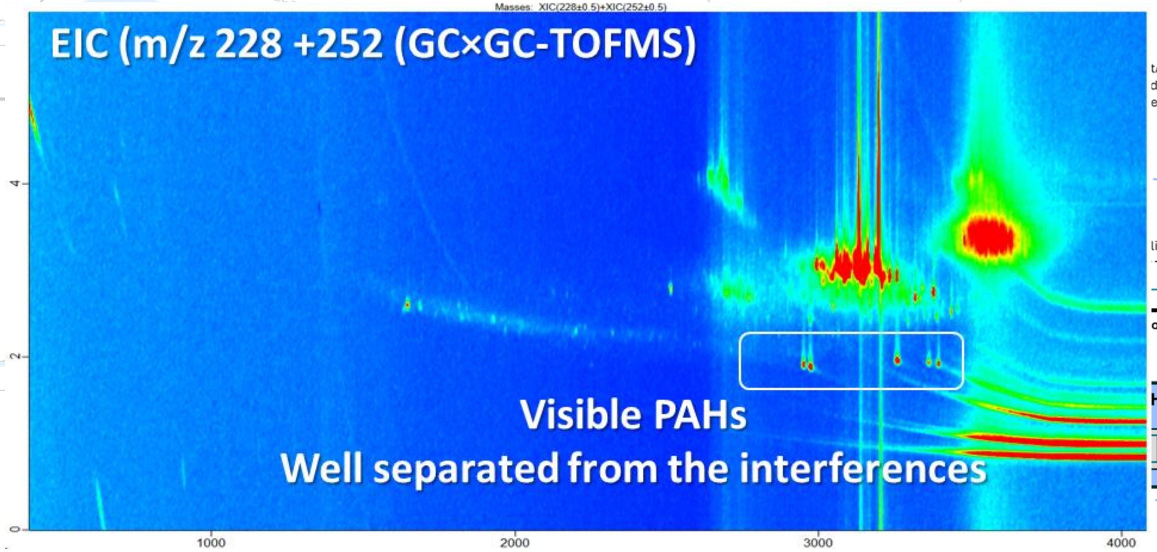
PURIFICATION → LC-GC×GC-FID/MS

→ MOAH & PAHs

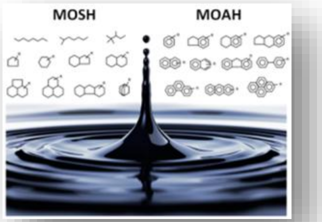
→ MOSH

Microwave-assisted

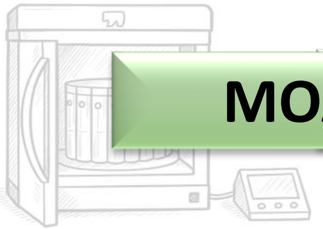




GC×GC: Support sample prep & data interpretation



SAPONIFICATION



MOAH

Microwave-assisted

Simultaneous analysis of MOSH/MOAH and PAHs in fat oils derived food additives using an adapted sample preparation workflow and HPLC/GC×GC-

Carlo Bellinghieri[1,2], Aleksandra Gorska[1], Marin Eugène[1], Flavio Franchina[3], Marco Beccaria[3], Giorgia Purcaro[1]

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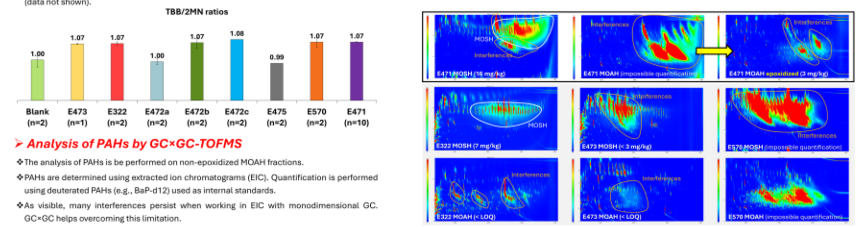
[2] Department of Environmental and Prevention Science, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy
[3] Department of Chemical, Pharmaceutical and Agricultural Science, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy

INTRODUCTION

Mineral oil hydrocarbons (MOHs) represent a broad class of compounds derived from petroleum distillation and refining processes. Based on their chemical structure, they are classified into mineral oil saturated hydrocarbons (**MOSH**) and mineral oil aromatic hydrocarbons (**MOAH**). The potential human health impact of MOH varies widely. MOSH, can accumulate in the human tissues like liver and lymphoid system, while **MOAH (3-7 ring)** may contain **genotoxic** substances [1].

In addition to MOHs, polycyclic aromatic hydrocarbons (PAHs) are contaminants that result from incomplete combustion of organic matter, and they have cancerogenic, genotoxic and mutagenic effects [2].

Both MOHs and PAHs can come in contact with food throug contamination of MOHs and PAHs in foods could be the incorporation are used to enhance flavor, appearance, texture, or shelf-life. **Usuall following separate workflows due to different analytical requireme developed for simultaneous analysis of both groups of contami** workflow was applied to some food additives as E471, E472, E322, substance added to food to help preserve them, improve taste, texture,



MATERIALS & METHODS

➢ *Analytical workflow*

Instrumental system	HPLC/GC×GC-FID/TOFMS	HPLC	GC×GC-FID/TOFMS
		Allure silica 250 mm × 2.1 mm i.d. × 5 µm d_p, 60 Å (Restek)	Pegasus BT 4D GC × GC ToFMS (LECO, St. Joseph, MI, USA) **FID line** (cold on-column) - Guard Column 10 m × 0.53 mm ; (1D) Rxi 17Sil MS 15m × 0.25 mm × 0.25 µm ; (2D) Rxi 1ms HT 0.8 m × 0.15 mm × 0.15 µm. **TOFMS line** (MMI) - (1D) Rxi 17Sil MS 15m × 0.25 mm × 0.25 µm ; (2D) Rxi 1ms HT 1.3 m × 0.15 mm × 0.15 µm. TOFMS acquisition range: 50-700 *m/z*

RESULTS & DISCUSSION

➢ *Evaluation of the sample preparation*

- All food additives tested were saponified efficiently
- **TBB/2MN** (i.e., two internal standards that can be used for MOAH quantification) **ratios were below the tolerance value of 1.15** defined in the JRC guidance for MOSH/MOAH[4]
- The applied workflow yielded repeatable peak areas for MOSH and MOAH standards, with coefficients of variation between 12% and 19% across all standards in the ten E471 samples (data not shown).

➢ *Analysis of PAHs by GC×GC-TOFMS*

- The analysis of PAHs is be performed on non-epoxidized MOAH fractions.
- PAHs are determined using extracted ion chromatograms (EIC). Quantification is performed using deuterated PAHs (e.g., BaP-d12) used as internal standards.
- As visible, many interferences persist when working in EIC with monodimensional GC. GC×GC helps overcoming this limitation.

➢ *HPLC/GC×GC-FID MOSH/MOAH profiles*

- Most of the tested food additives showed very high levels of interferences.
- Epoxidation was helpful but not 100% efficient for purification of MOAH.
- **In general, MOSH and MOAH levels were low**. MOAH were all below 2 mg/kg. Other tested additives were compliant, too, although it was not possible to determine the MOSH/MOAH of some specific samples, such as the E570 shown below.

CONCLUSION

The following analitical workflow was successfully applied for the determination of **MOSH/MOAH and PAHs in food additives** as E471, E322, E472, E473, E475 and 570. However, food additives proved to be a complex matrix due to a high abundance of coeluting interferences, particularly affecting MOAH identification. For this reason, **epoxidation step** facilitated MOAH analysis. In conclusion, GC×GC significantly improved the reliability of both MOSH/MOAH and PAH determination and **this workflow** could be adapted for the analysis of similar contaminants in other complex food matrices.

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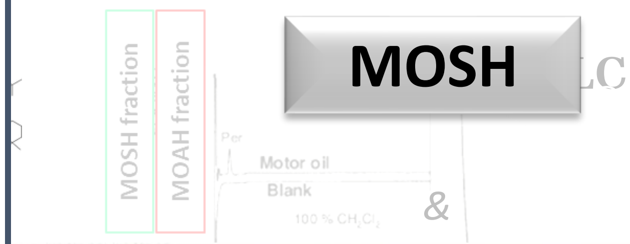
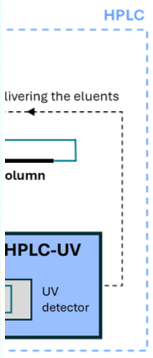
Poster Food 10

LC-GC×GC-FID



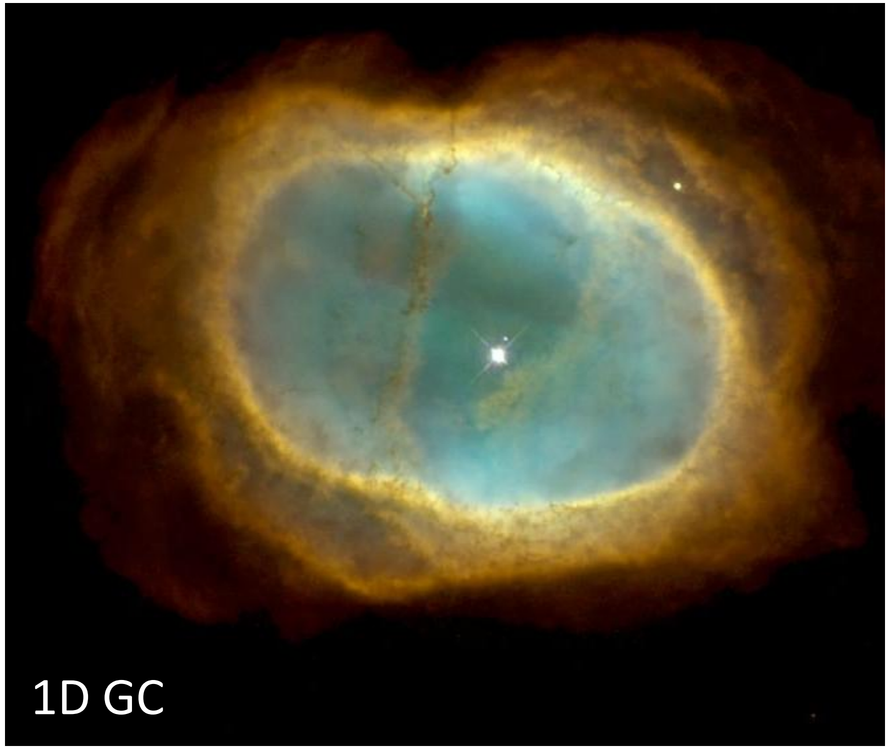
MOSH



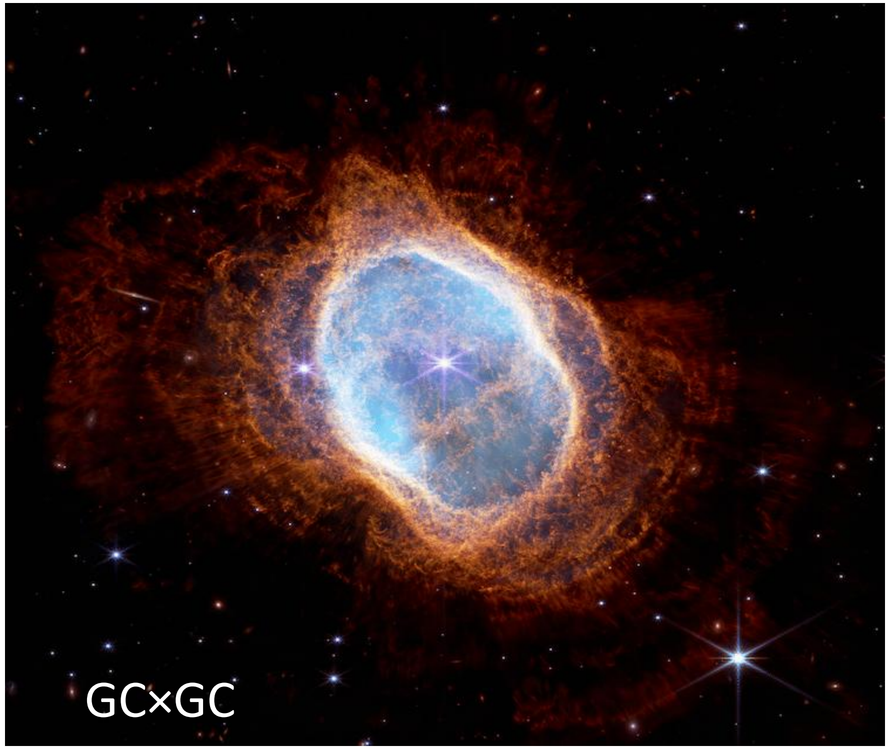
CONCLUSION

Is GC×GC worth the effort?

Southern Ring Nebula



1998 Hubble Telescope



Webb Space Telescope's view today

CONCLUSION


LIÈGE université Gembloux Agro-Bio Tech
ACESSS Academic Center of Excellence in Separation Science and Sensing
ExTech 2025 Mülheim an der Ruhr

GC×GC an opportunity for simplifying sample preparation or an extra difficulty in food analysis?

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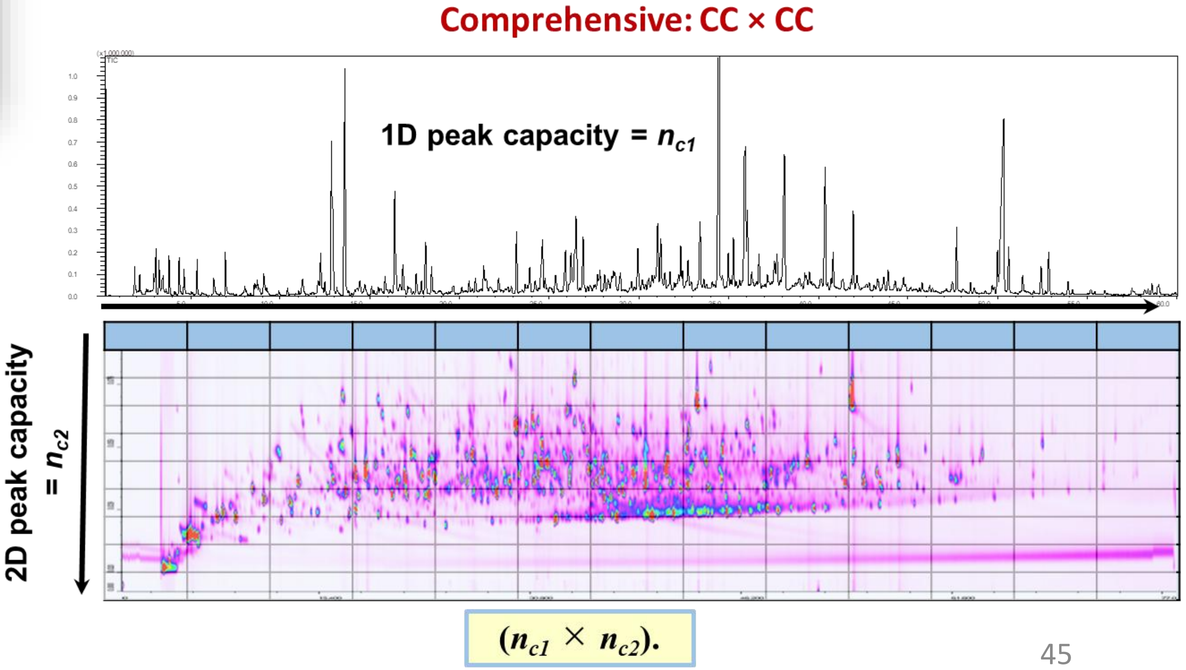
1

GC×GC

- ✓ Inform optimization
- ✓ maximizes the information
- ✓ Simplify sample preparation
- ✓ Support data interpretation



GC×GC doesn't replace **sample preparation** —
They provide ***reciprocal reinforcement***!

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