



UNIVERSITÀ  
DI TORINO



LIÈGE université  
Gembloux  
Agro-Bio Tech



ACCESS

Academic Center of Excellence in Separation Science and Sensing



# ONE-STEP MICROWAVE-ASSISTED EXTRACTION AND DERIVATIZATION FOLLOWED BY COMPREHENSIVE TWO-DIMENSIONAL CHROMATOGRAPHY COUPLED WITH FLAME IONIZATION DETECTOR TO FAMES ANALYSIS IN COMPLEX FOOD MATRICES.

Donatella Ferrara<sup>1,2</sup>, Giulia Giacoppo<sup>3</sup>, Alberto Cavazzini<sup>3</sup>, Luisa Pasti<sup>3</sup>, Marco Beccaria<sup>3</sup>, Chiara Emilia Irma Cordero<sup>2</sup>, Giorgia Purcaro<sup>1</sup>

<sup>1</sup>University of Liege, Gembloux Agro-Bio Tech, Belgium., Gembloux, Belgium.

<sup>2</sup>University of Turin, Dipartimento di Scienza e Tecnologia del Farmaco, Turin, Italy.

<sup>3</sup>University of Ferrara, Department of Chemical, pharmaceutical and agricultural sciences, Ferrara, Italy

# PRESENTATION TIMELINE

01

INTRODUCTION



# PRESENTATION TIMELINE

01

INTRODUCTION



02

OBJECTIVE



# PRESENTATION TIMELINE

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OBJECTIVE



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RESULTS



# PRESENTATION TIMELINE

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OBJECTIVE



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RESULTS



04

CONCLUSIONS



# PRESENTATION TIMELINE

01

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02

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03

RESULTS

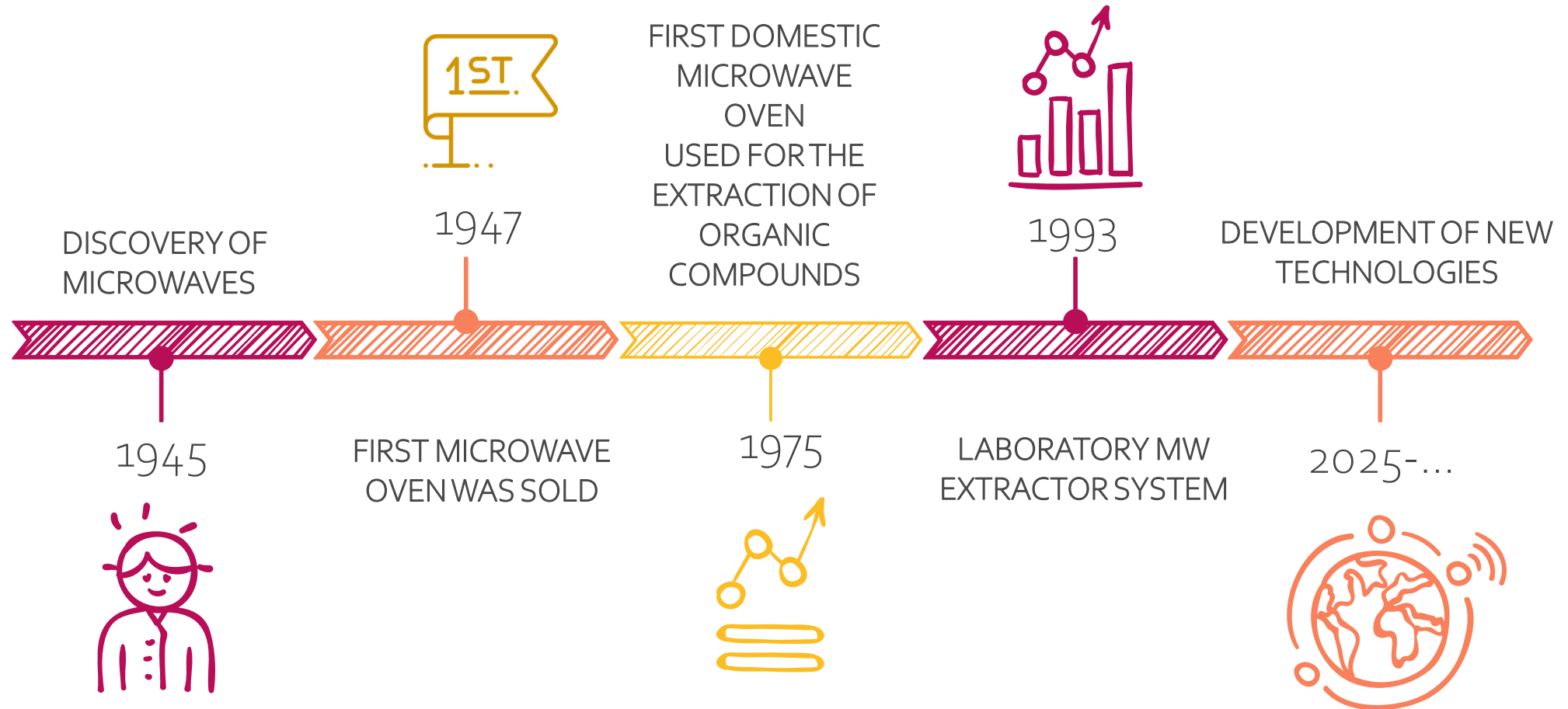


04

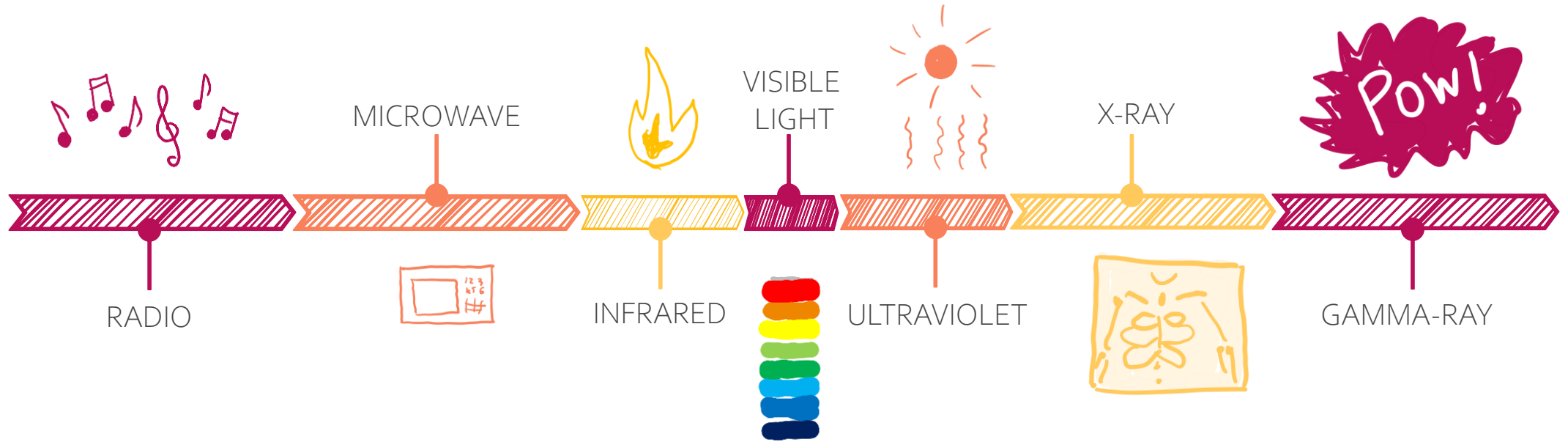
CONCLUSIONS



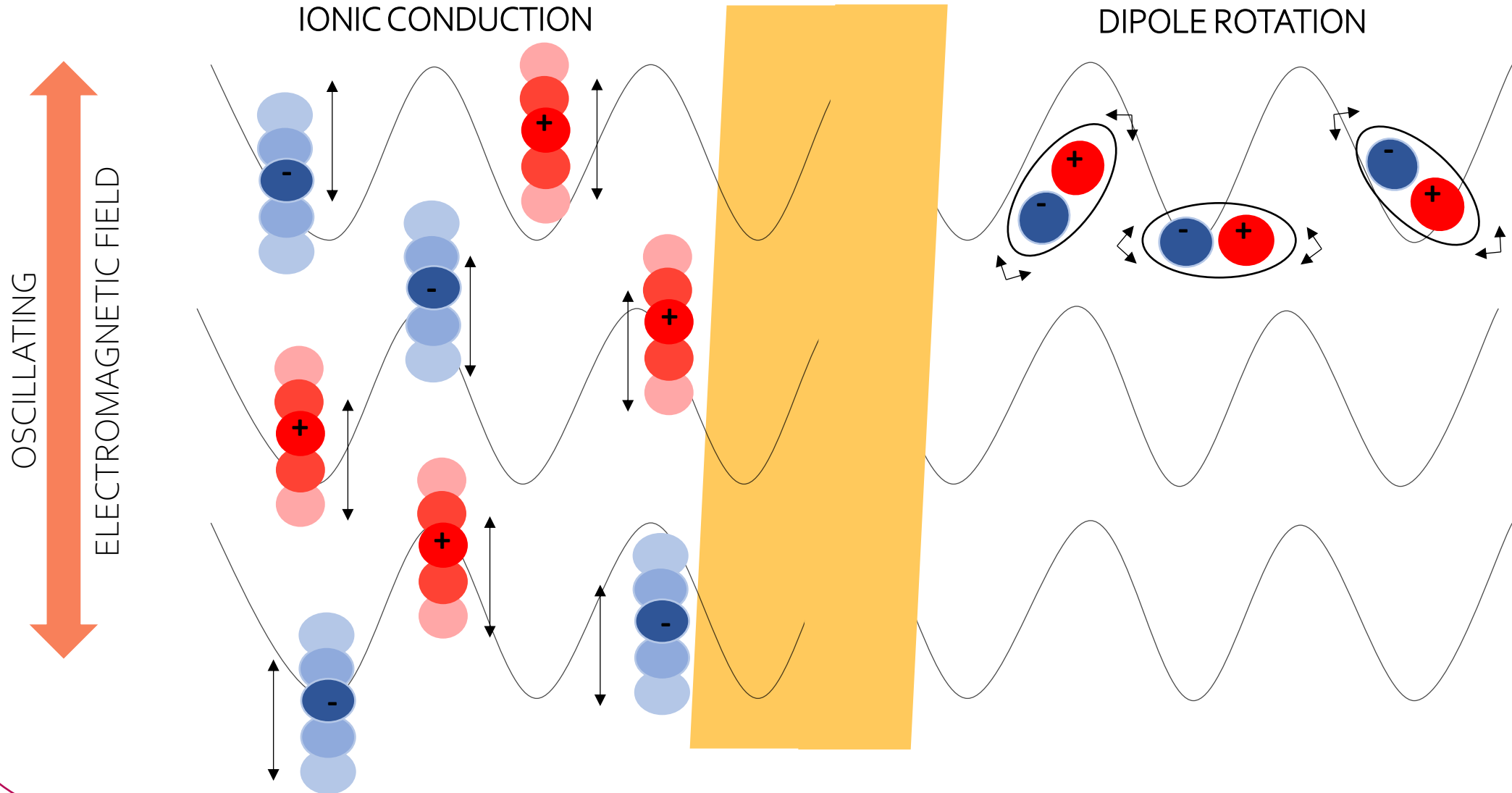
# MICROWAVE HISTORY



# WHAT MICROWAVES ARE?



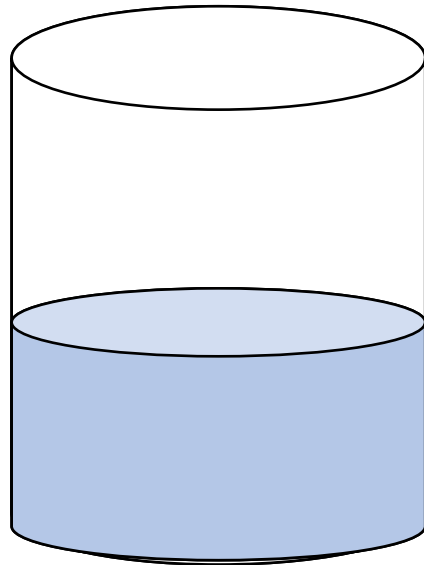
# HOW MICROWAVES HEATING WORKS?



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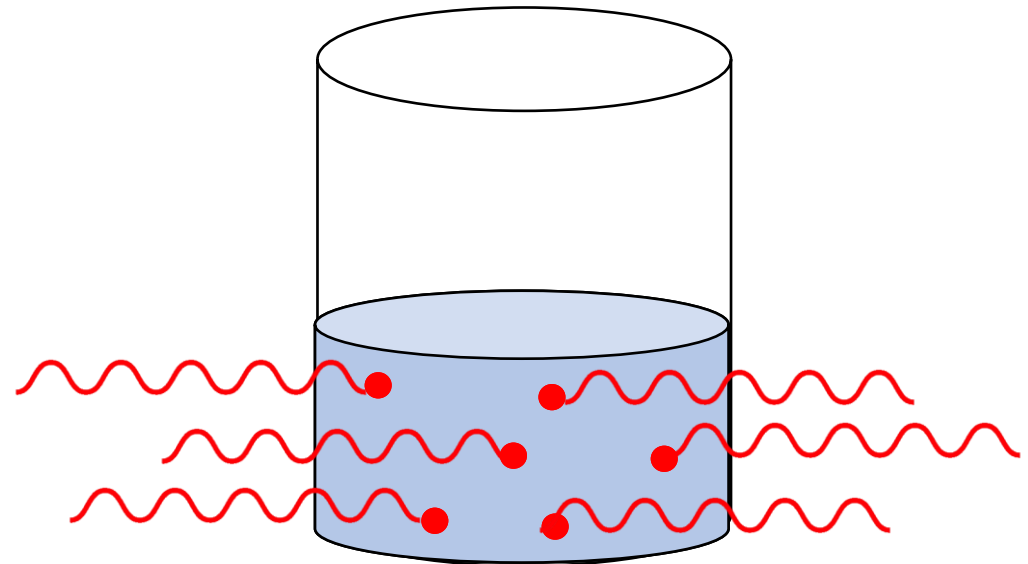
Conductive Heating



VS



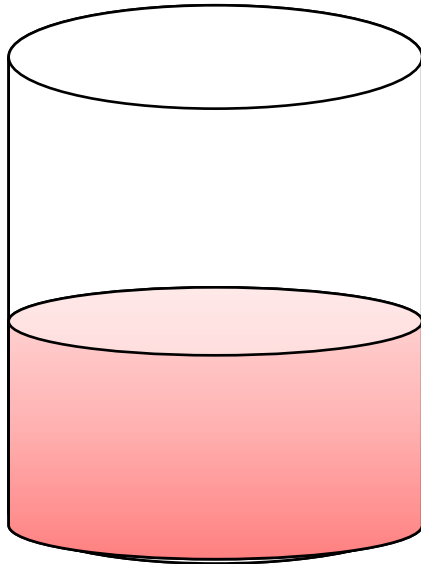
Microwave Heating



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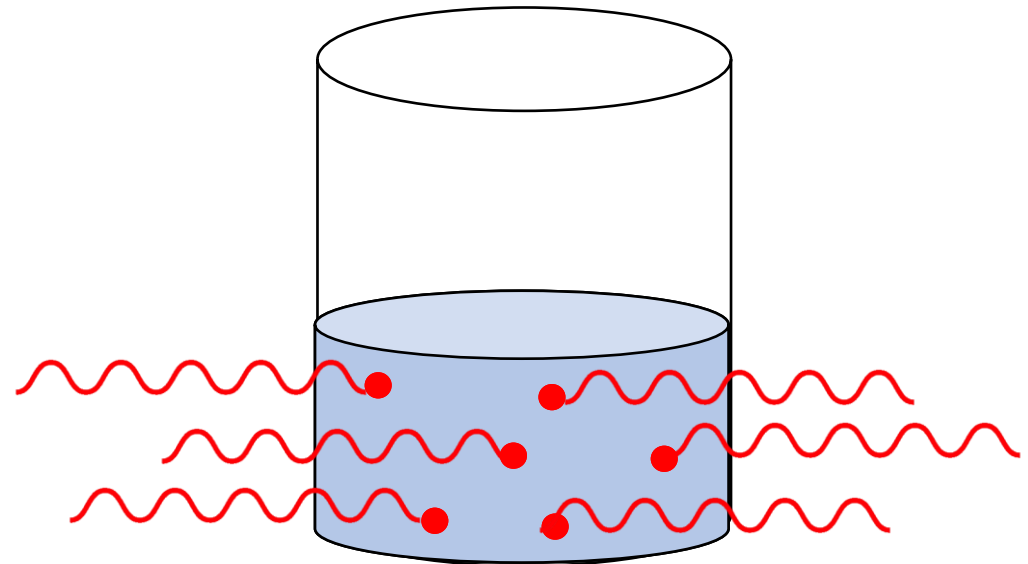
Conductive Heating



VS



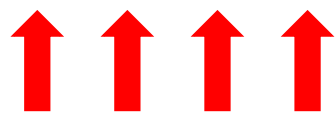
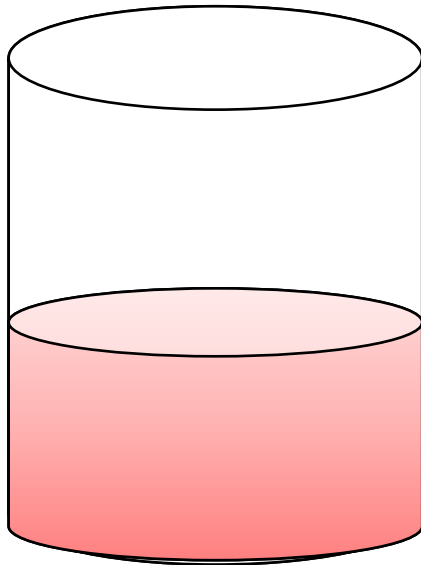
Microwave Heating



# HOW MICROWAVES HEATING WORKS?



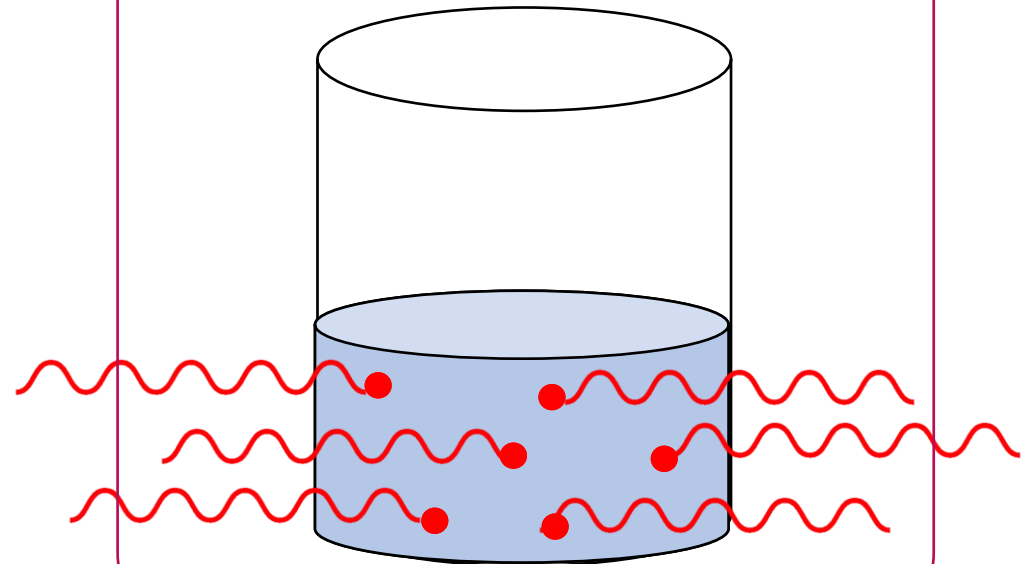
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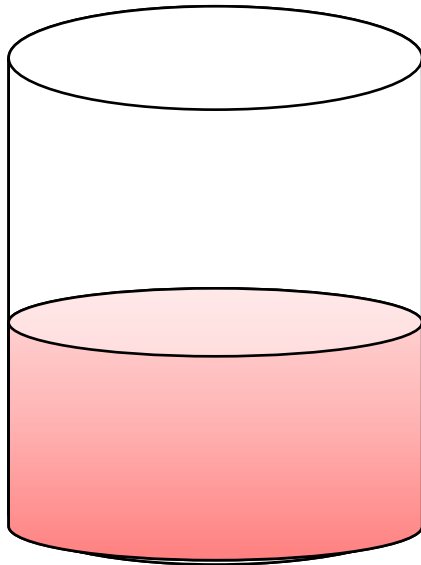
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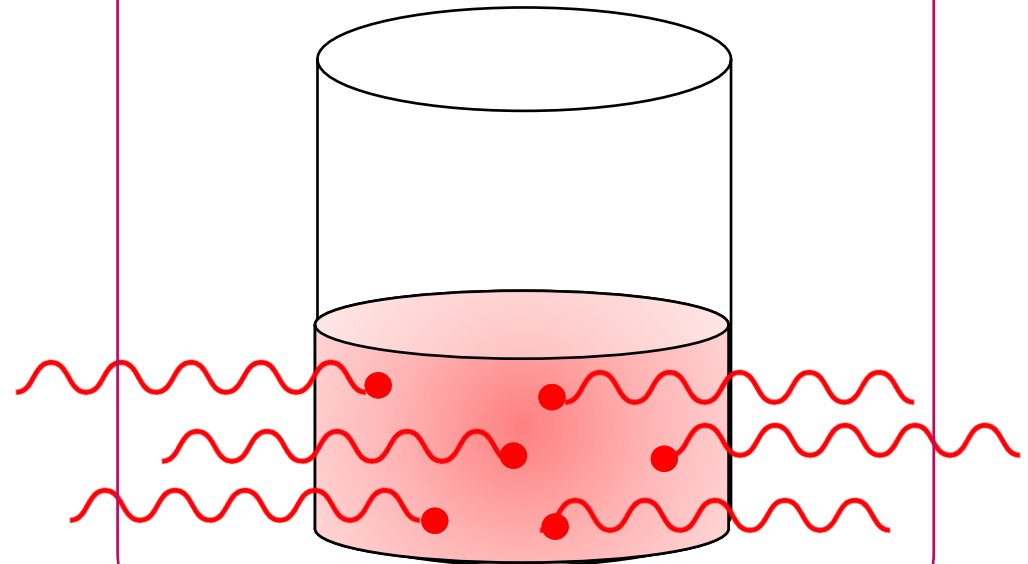
Conductive Heating



VS



Microwave Heating



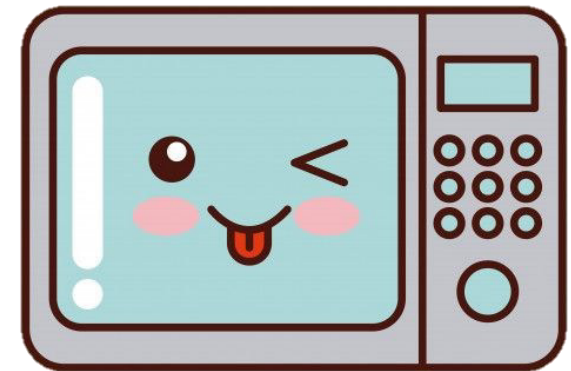
# ADVANTAGES OF MICROWAVES HEATING

DIRECT HEATING

HIGH EFFICIENCY

SHORT PROCESSING TIMES  
AND GREEN PROCESS

LESS HEATING EXPOSURE



# MICROWAVES FOR FAMEs ANALYSIS

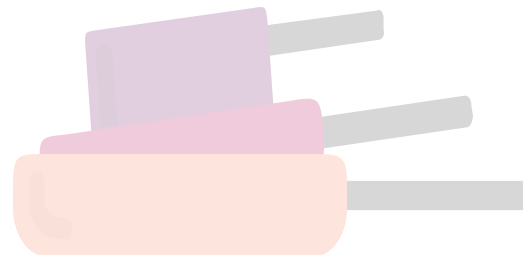
## CHARACTERIZATION

CHARACTERIZATION AND DETERMINATION OF TOTAL FAT CONTENT IN FOODS



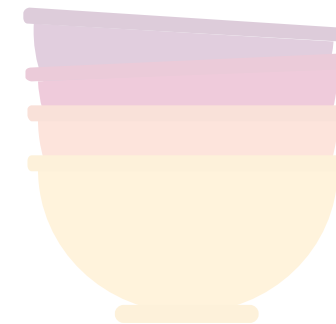
## LABELING

FOR NUTRITIONAL LABELING AND TO UNDERSTAND THE AVAILABILITY OF FAT



## HEALTH IMPACT

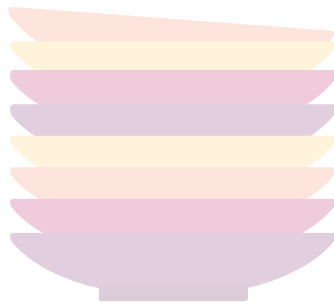
HEALTH IMPACT AND CORRELATION WITH SOME DISEASES



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## CHARACTERIZATION

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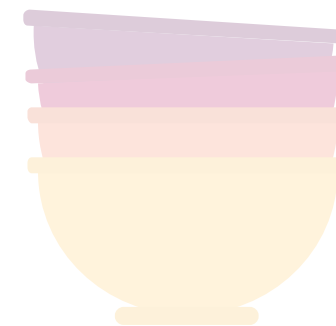
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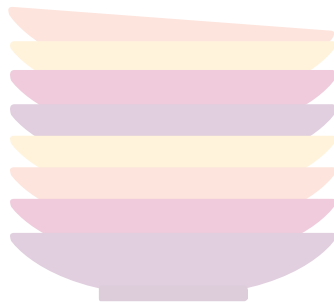
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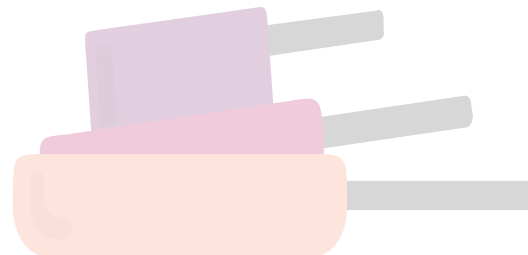
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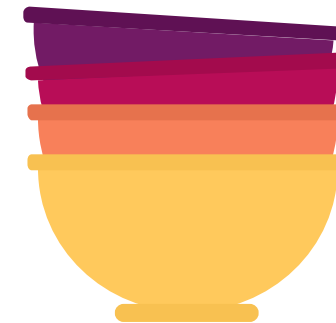
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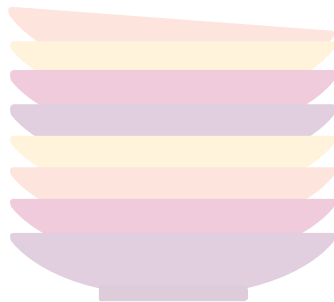
HEALTH IMPACT AND CORRELATION WITH SOME DISEASES



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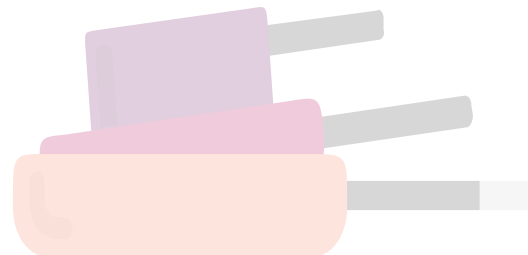
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CHARACTERIZATION AND DETERMINATION OF TOTAL FAT CONTENT IN FOODS



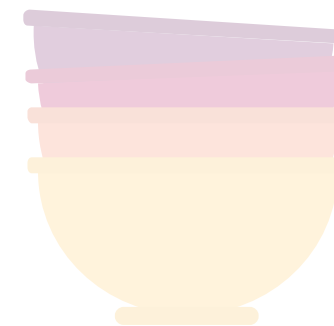
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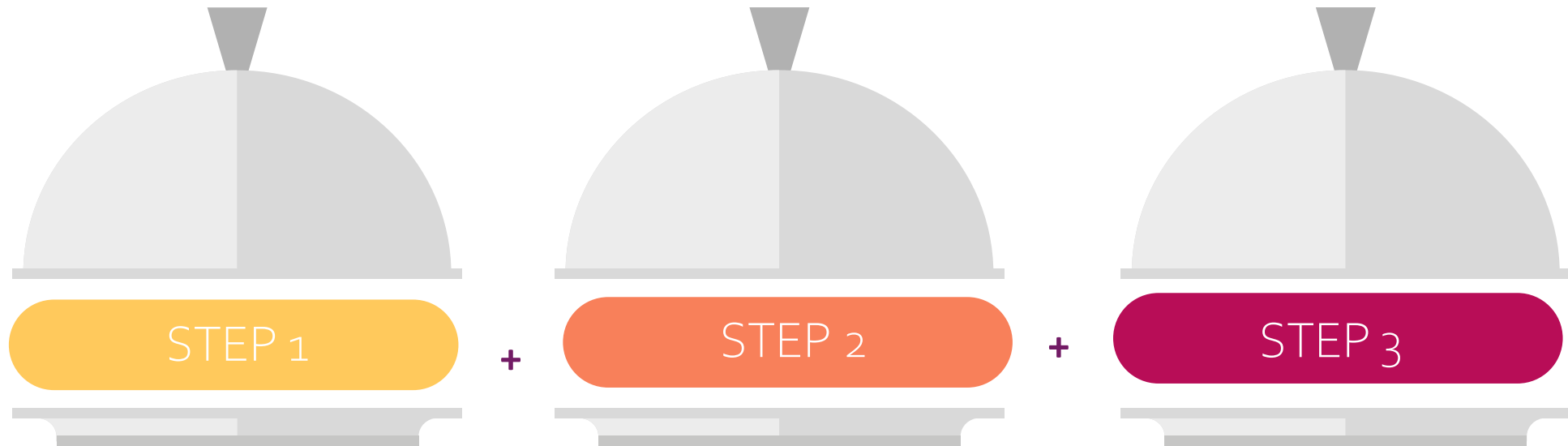
HEALTH IMPACT AND CORRELATION WITH SOME DISEASES



INTRODUCTION

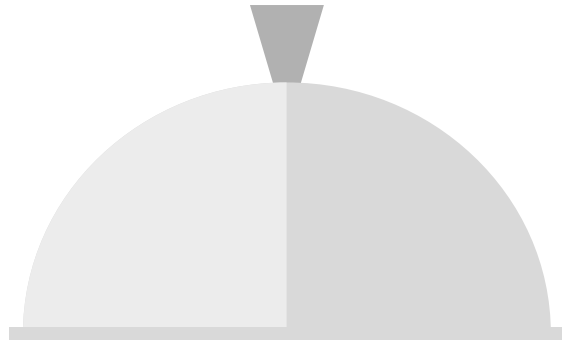
# MICROWAVES FOR FAMEs ANALYSIS

## GENERAL PROCEDURE



# MICROWAVES FOR FAMEs ANALYSIS

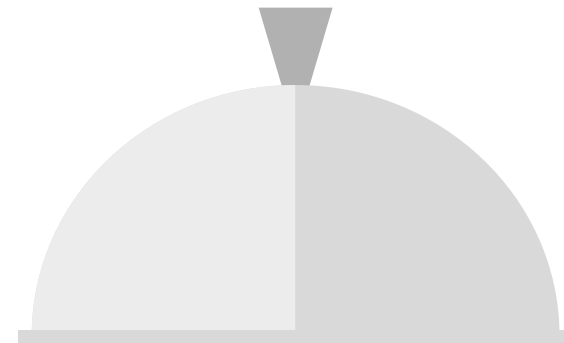
## GENERAL PROCEDURE



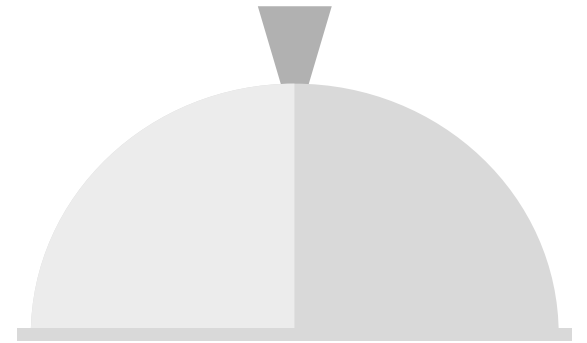
EXTRACTION



+

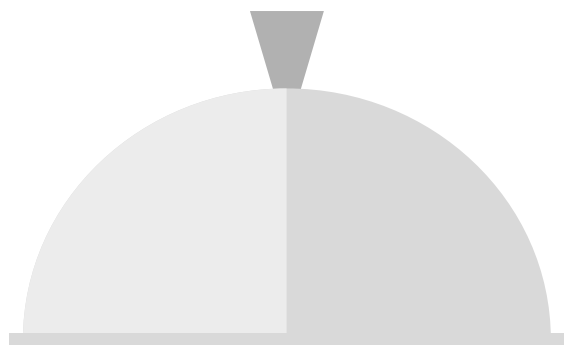


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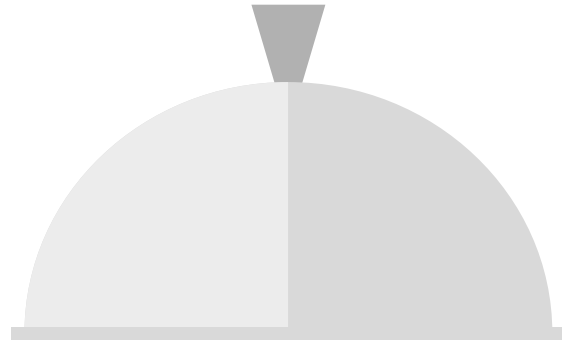
## GENERAL PROCEDURE



EXTRACTION



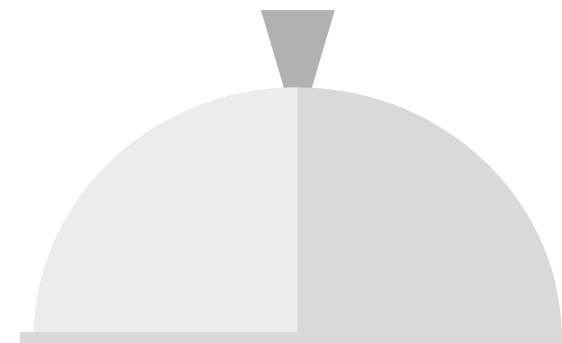
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DERIVATIZATION

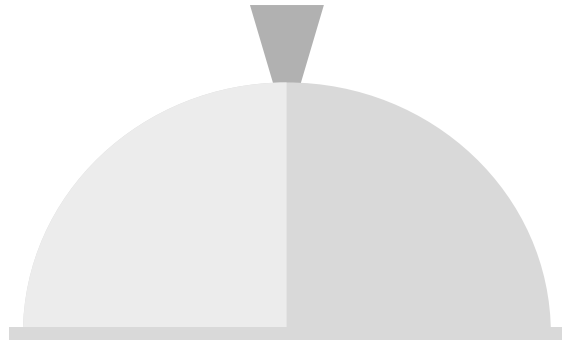


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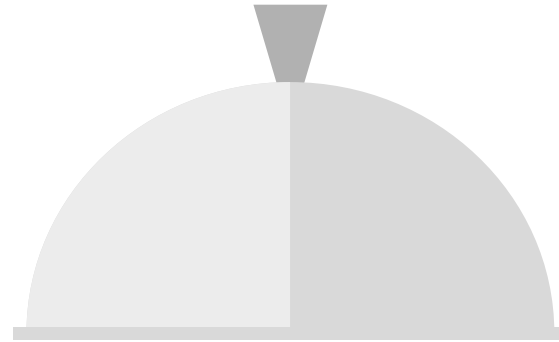
## GENERAL PROCEDURE



EXTRACTION



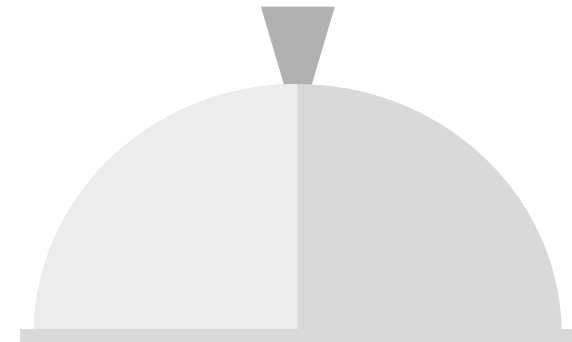
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DERIVATIZATION



+



GC ANALYSIS

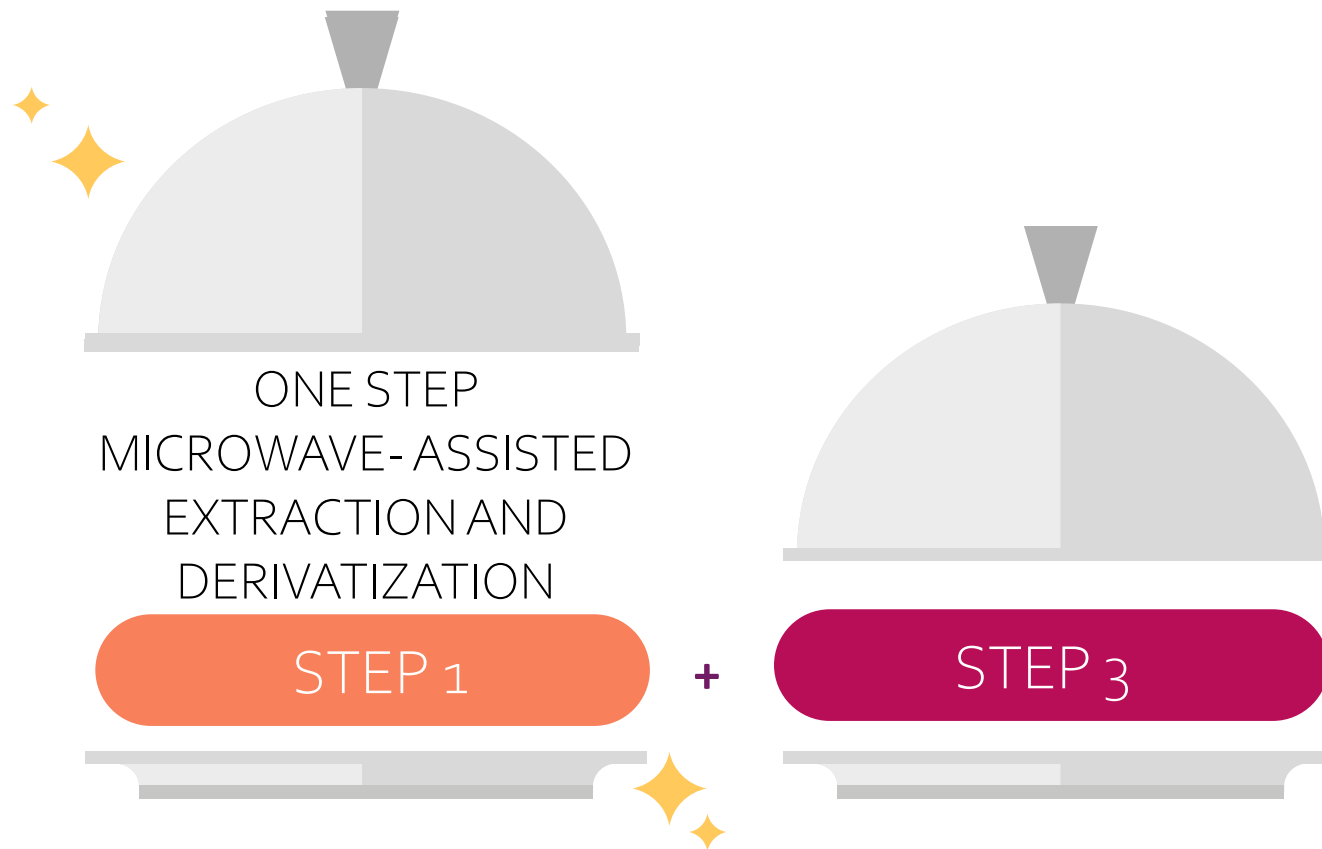


# MICROWAVES FOR FAMEs ANALYSIS

## NEW PROCEDURE



0.5 g of sample  
+ 10 mL HCl/MeOH  
+25 mL Cyclohexane  
15 minutes at 120 °C

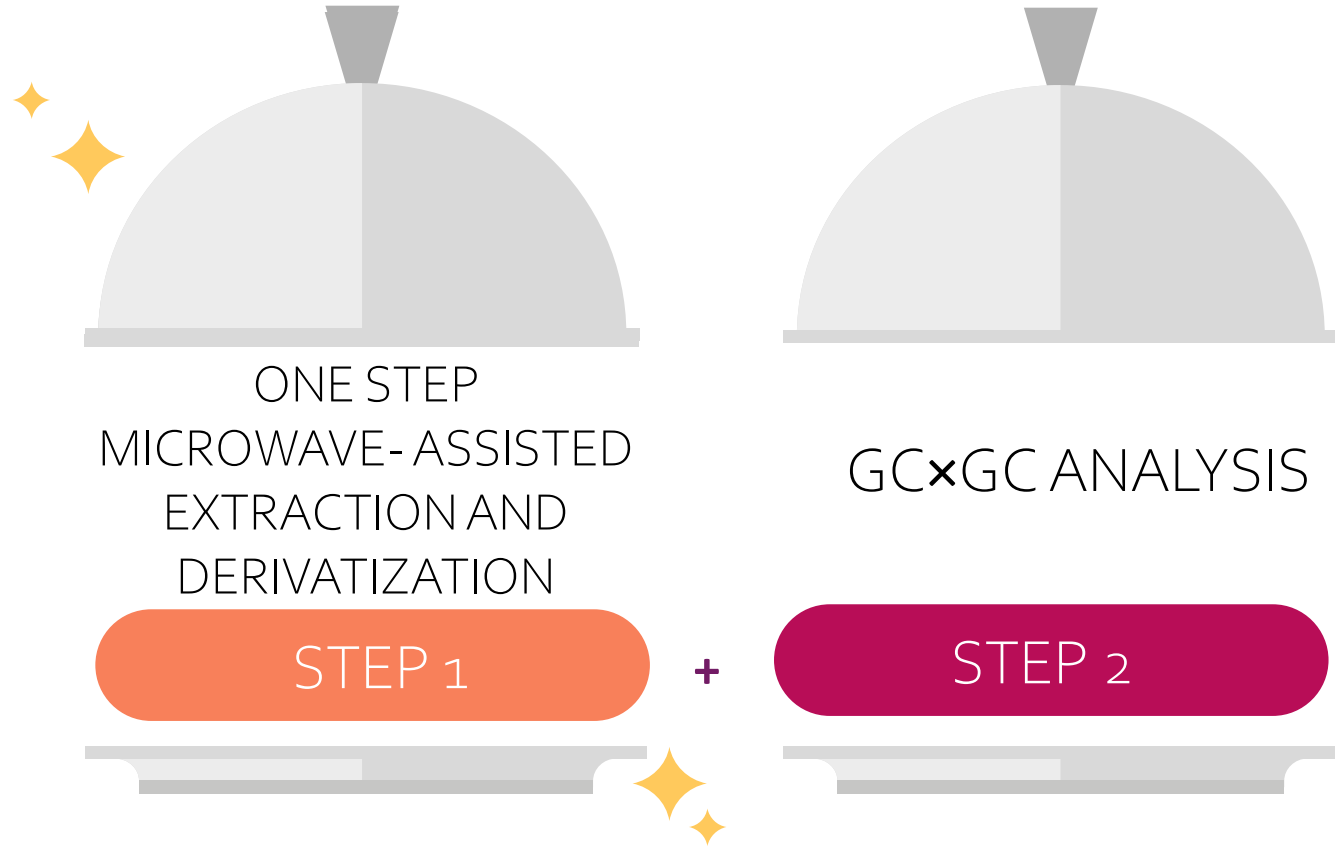


# MICROWAVES FOR FAMEs ANALYSIS

## NEW PROCEDURE

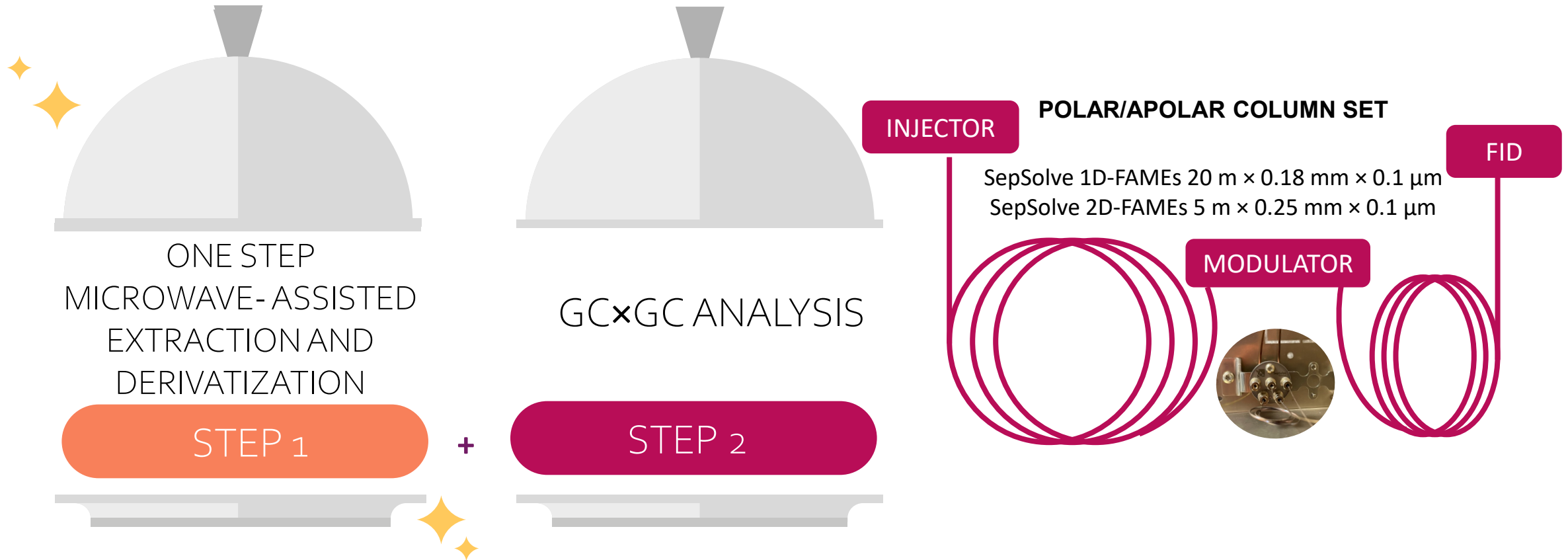


0.5 g of sample  
+ 10 mL HCl/MeOH  
+25 mL Cyclohexane  
15 minutes at 120 °C



# MICROWAVES FOR FAMEs ANALYSIS

## NEW PROCEDURE



# CHROMATOGRAPHIC ANALYSIS

1D limitations:



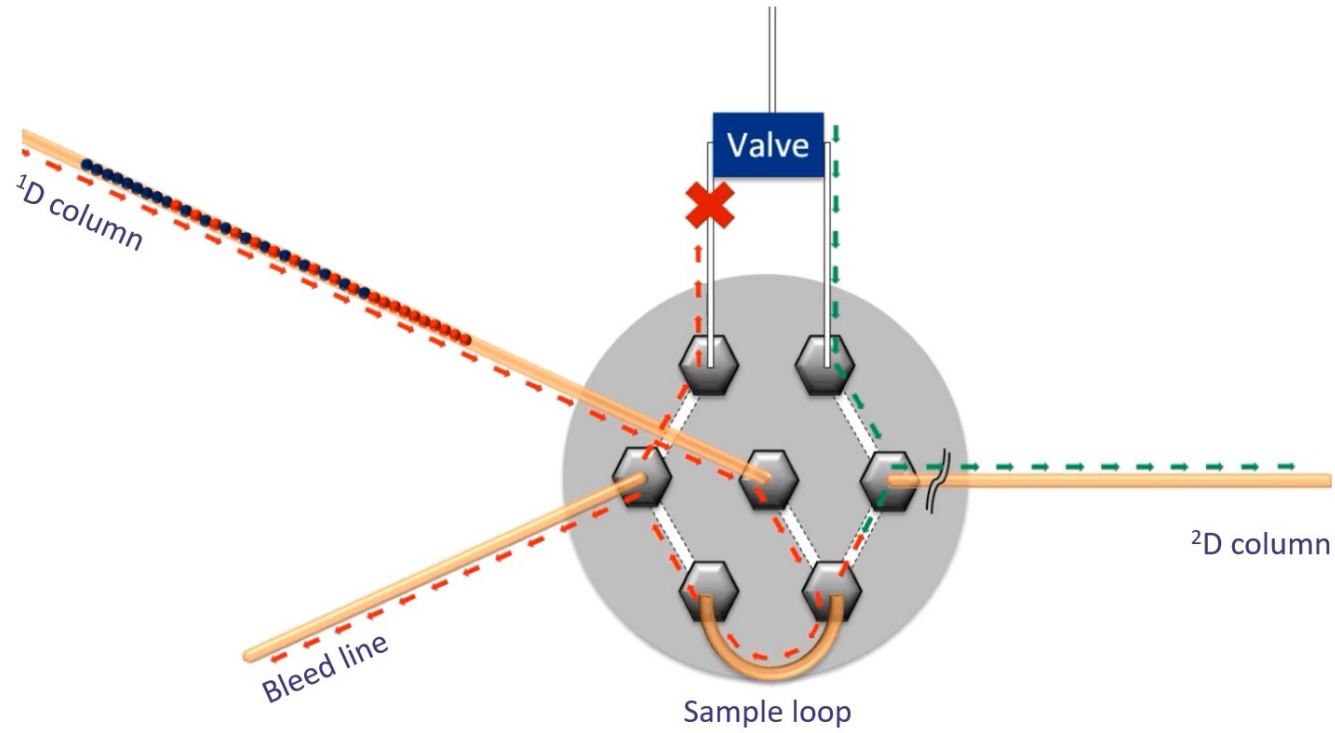
COMPLEX  
MATRIX

- Difficult and/or time-consuming to resolve all isomers
  - High polarity columns:
    - ☹ Polyunsaturated FAMES coelute with higher-chain-length saturated FAMES
    - ☹ Stability of columns
  - Use long columns (up to 200 m in some cases)
    - ☹ Expensive, with longer run times
  - Multiple analyses
    - ☹ Decreased productivity

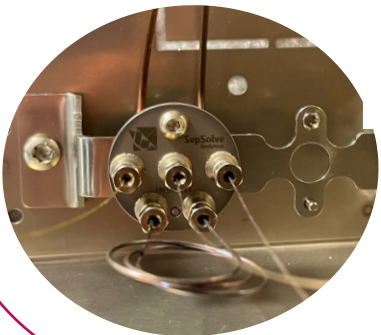


# CHROMATOGRAPHIC ANALYSIS

## FLOW MODULATOR



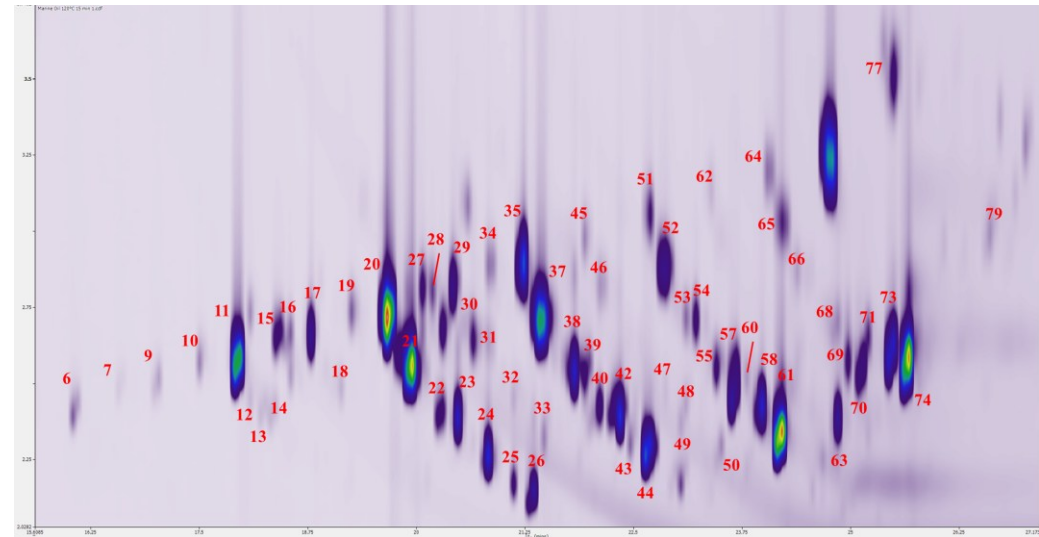
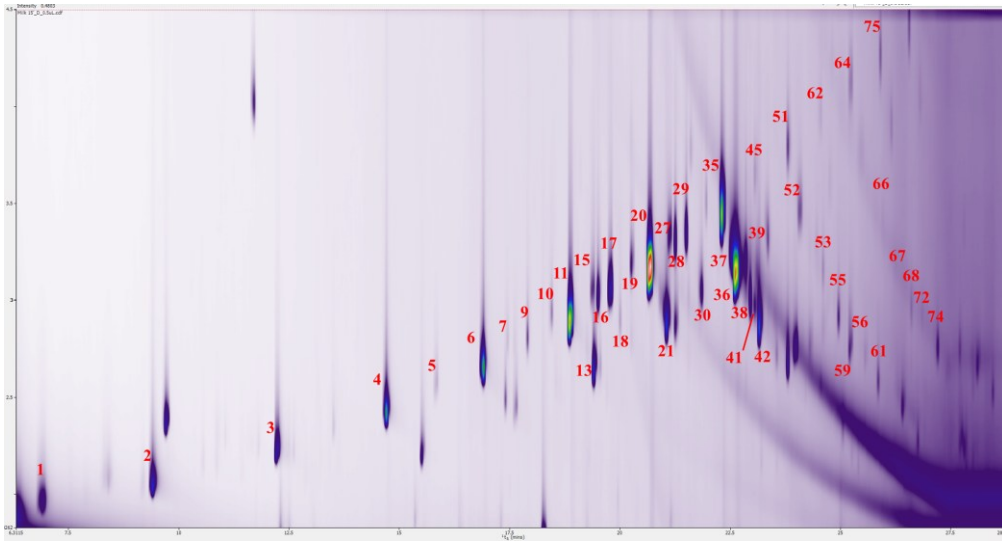
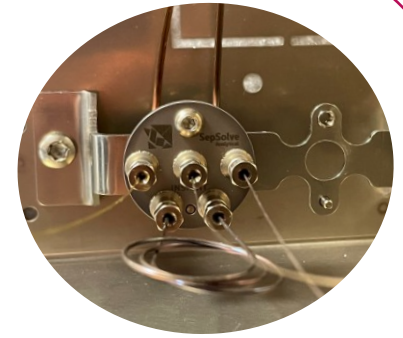
Reversed fill/flush  
Flow modulation



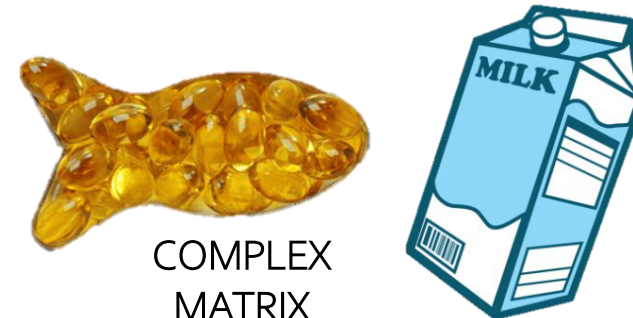
COMPLEX  
MATRIX

# CHROMATOGRAPHIC ANALYSIS

## FLOW MODULATOR

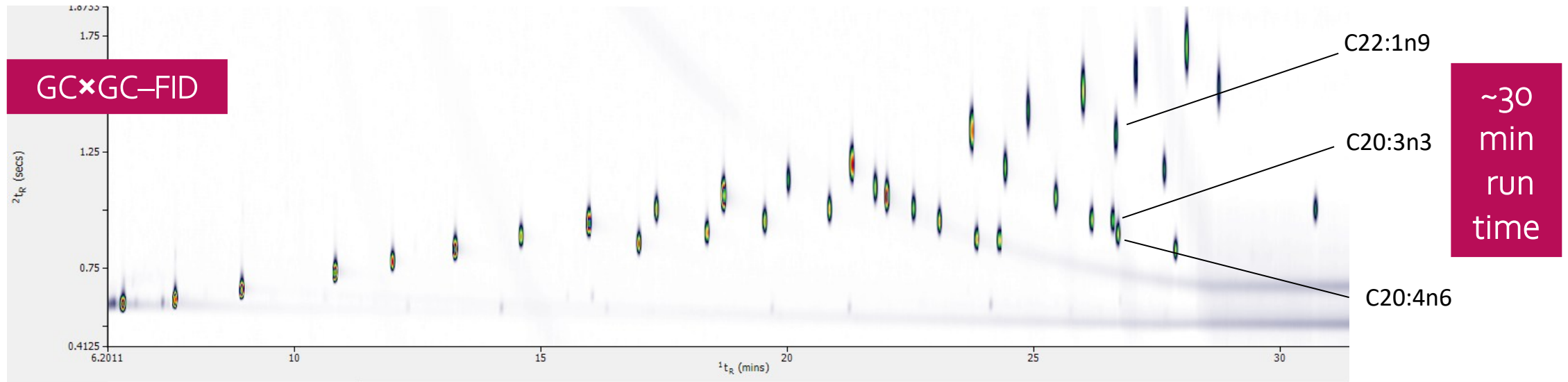


Same number of compounds separated compared to cryogenic modulation



# CHROMATOGRAPHIC ANALYSIS

## ADVANTAGES OF GC×GC



ENHANCED  
SEPARATION

REDUCED  
CO-ELUTION

INCREASED  
SENSITIVITY

STRUCTURED  
ANALYSIS

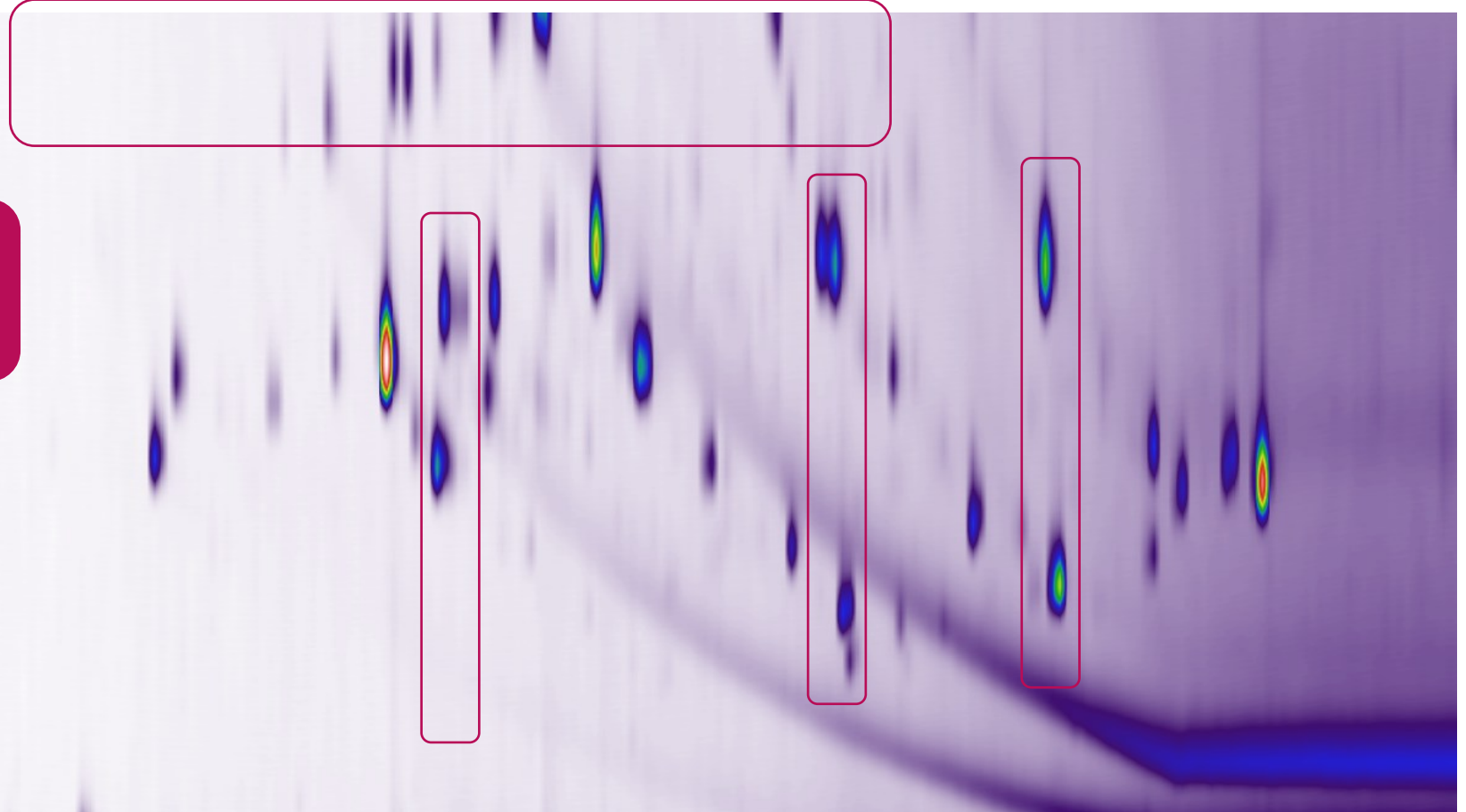
# CHROMATOGRAPHIC ANALYSIS

## ADVANTAGES OF GCxGC

NO FAMES

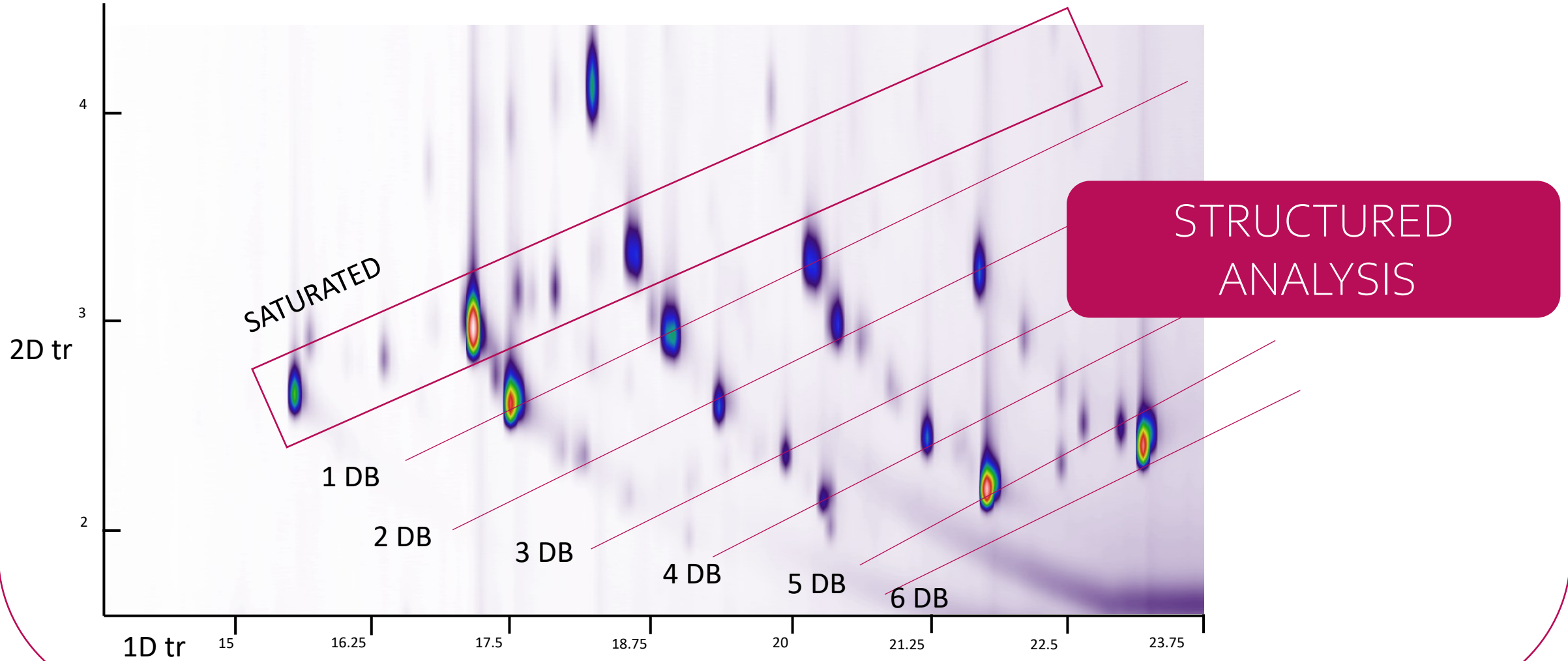
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CO-ELUTION



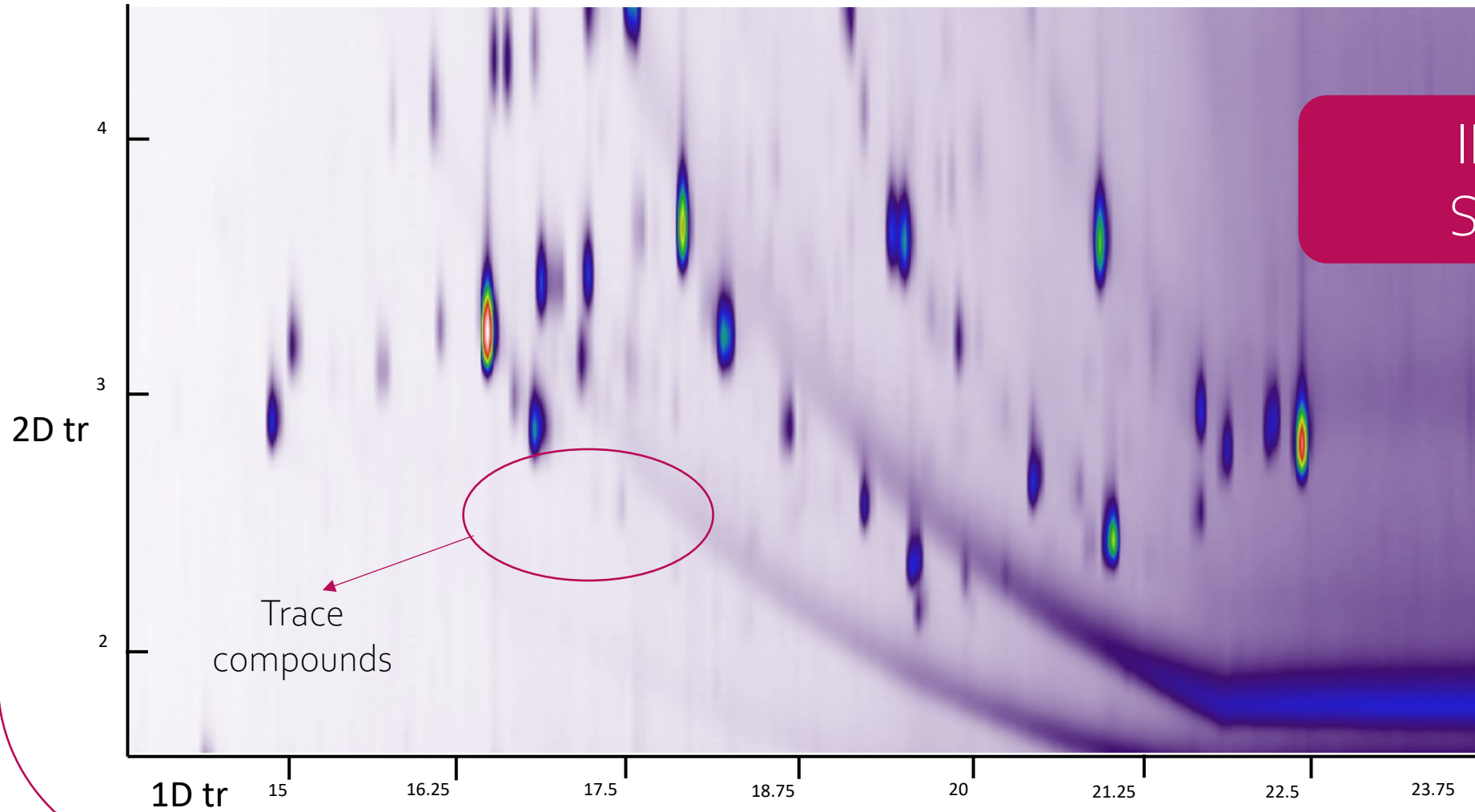
# CHROMATOGRAPHIC ANALYSIS

## ADVANTAGES OF GCxGC



# CHROMATOGRAPHIC ANALYSIS

## ADVANTAGES OF GC×GC



INCREASED SENSITIVITY

# CHROMATOGRAPHIC ANALYSIS

## INSTRUMENTAL PERFORMANCES

### INTRA-DAY

Average C37 FAMES  
standard (n=6)

RSD%  $tr_1 < 0.02\%$

RSD%  $tr_2 < 1.2\%$

RSD% Area  $< 1.15\%$

### INTER-DAY

Average C37 FAMES standard  
(n=16 consecutive days)

RSD%  $tr_1 < 0.12\%$

RSD%  $tr_2 < 3.1\%$

RSD% Area  $< 8\%$

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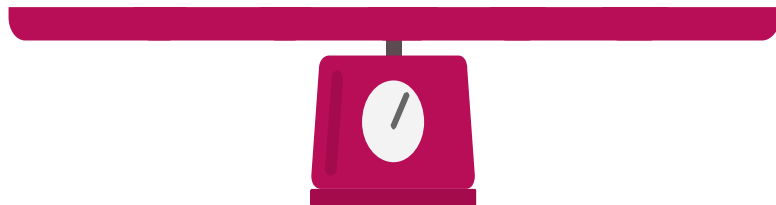
CONCLUSIONS



# PART 1

SAMPLE PREPARATION

IS MAE A RELIABLE ALTERNATIVE TO OFFICIAL METHODS?



# PART 2

CHROMATOGRAPHIC ANALYSIS

IS THE QUANTIFICATION IN 2D RELIABLE?  
COMPARISON 1D VS 2D TO FIND THE ANSWER



OBJECTIVE

# COMPARISON OF MAED AND OFFICIAL METHODS AOCS Ce 2b-11 & AOCS Ce 2c-11

BEFORE



NOW



OBJECTIVE  
ALL METHODS APPLIED

COMPARISON OFFICIAL METHODS AND MAED



AOCS Ce2c-11

Direct Methylation by  
Acid- Alkaly hydrolysis  
AcMeOH + NaOH +  $\text{BF}_3$   
+ Hexane



AOCS Ce2b-11

Direct Methylation by  
Alkaly hydrolysis  
NaOH +  $\text{BF}_3$  + Hexane



MAED

One-Step Microwave-  
assisted extraction and  
derivatization  
HCl/MeOH + Cyclohexane

RESULTS

# COMPARISON OFFICIAL METHODS AND MAED



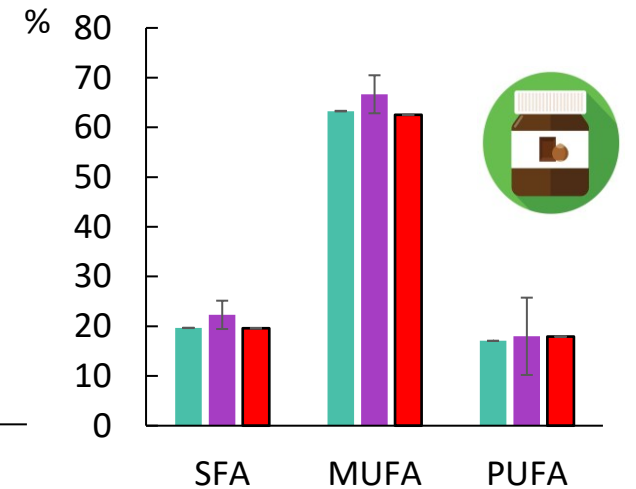
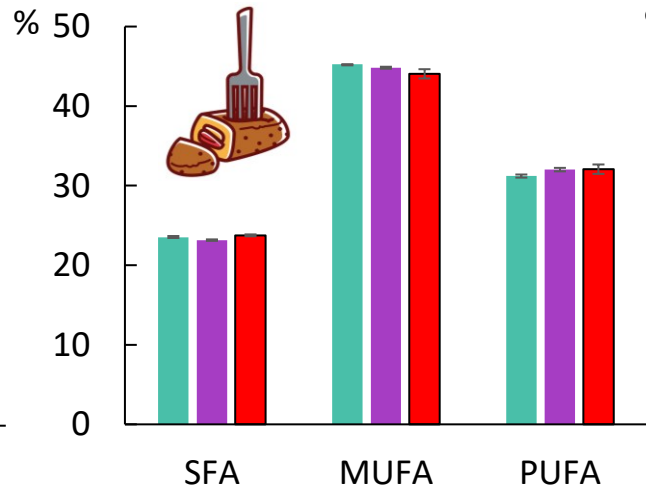
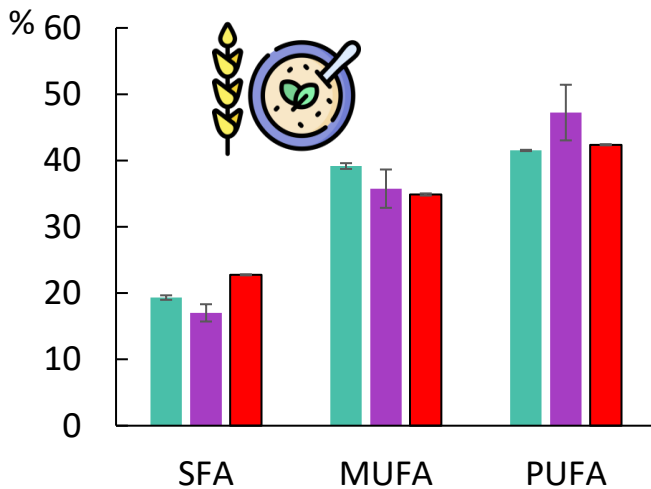
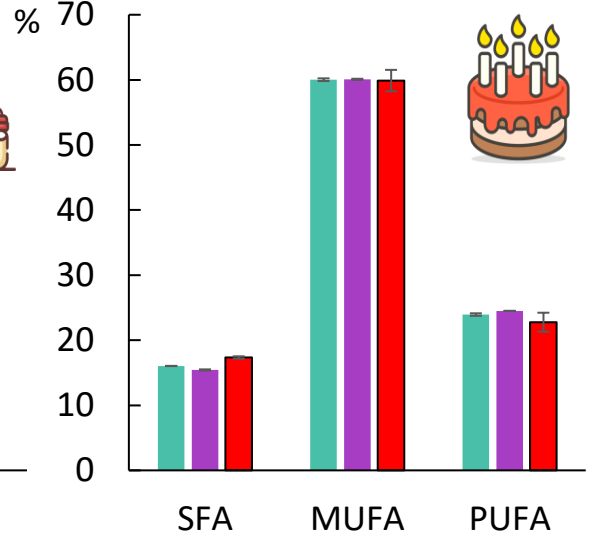
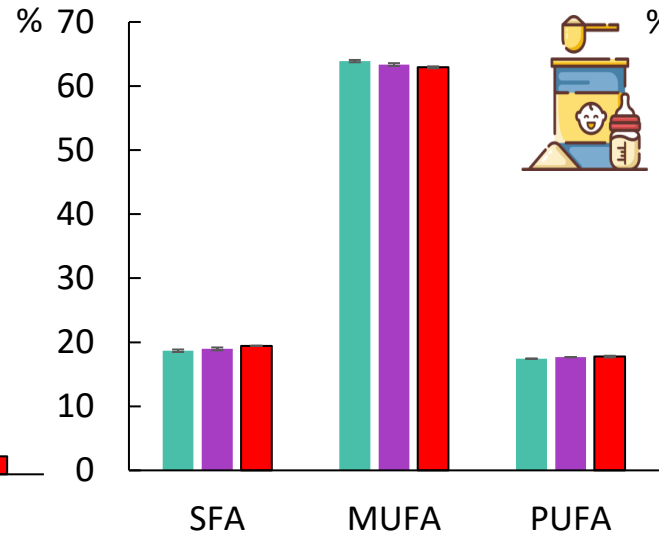
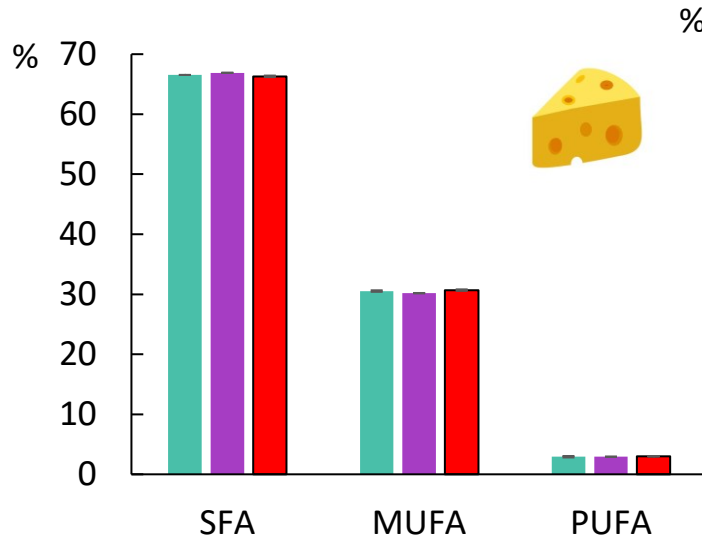
AOCS Ce2c-11



AOCS Ce2b-11



MAED

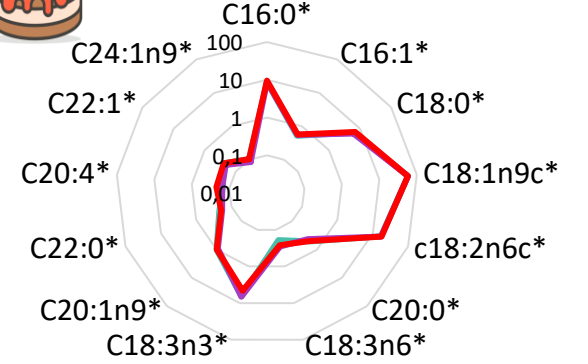
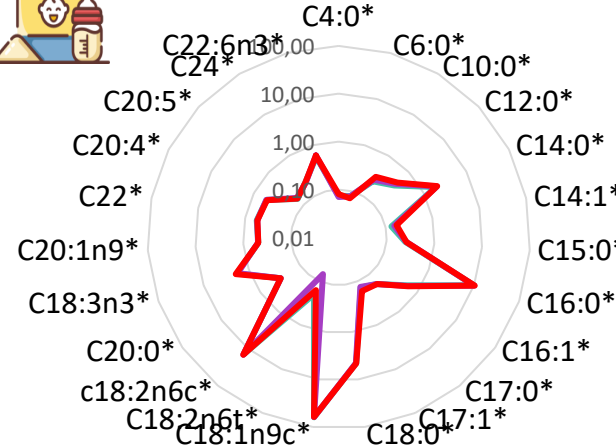
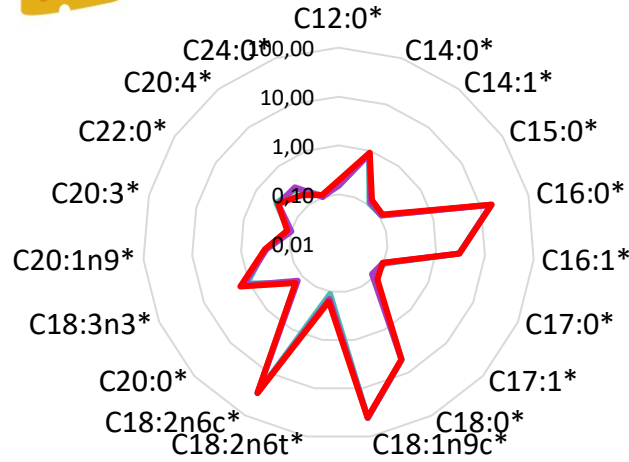


RESULTS

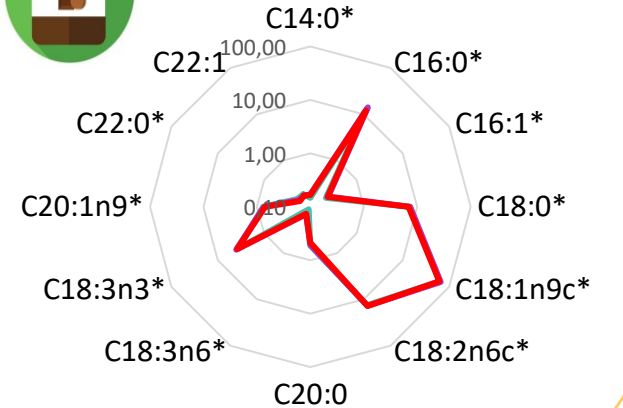
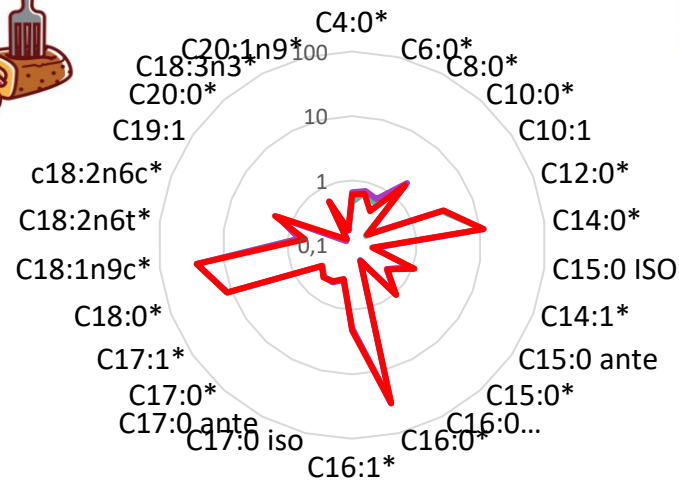
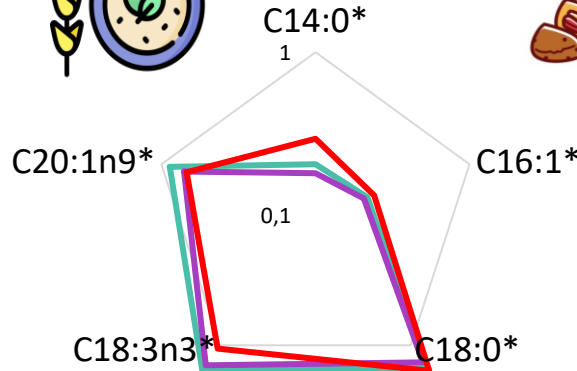
# COMPARISON OFFICIAL METHODS AND MAED



AOCS Ce2c-11



AOCS Ce2b-11



MAED

## COMPARISON OFFICIAL METHODS AND MAED



AOCS Ce2c-11



AOCS Ce2b-11

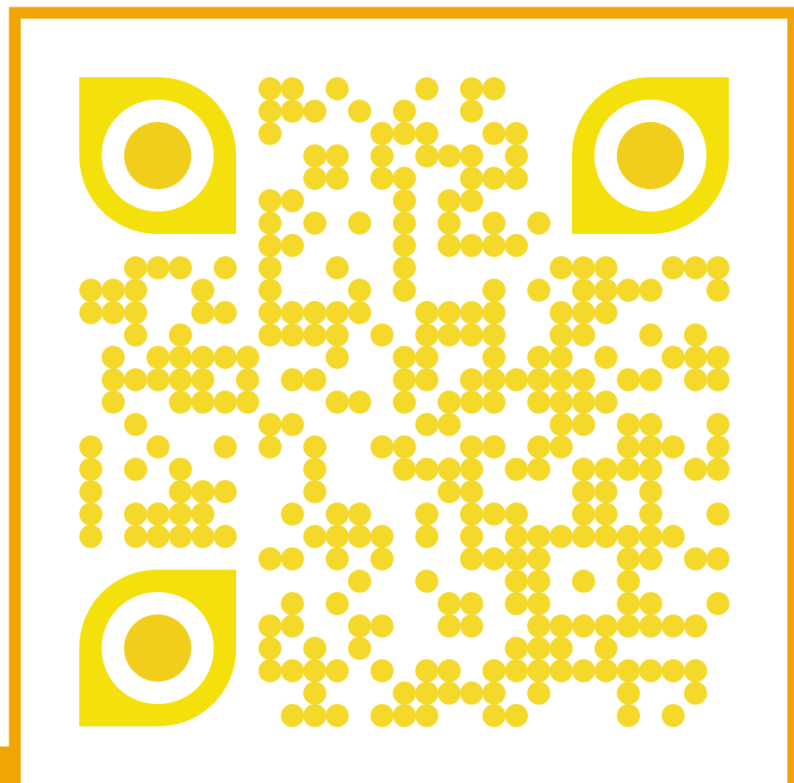


MAED

MAED IS CONFIRMED A  
RELIABLE ALTERNATIVE TO  
THE OFFICIAL METHOD

RESULTS

## IF YOU WANT MORE DETAIL



SCAN ME

Comprehensive comparison of fatty acid methyl ester profile in different food matrices using microwave-assisted extraction and derivatization methods and comprehensive two-dimensional gas chromatography coupled with flame ionization detection.

Donatella Ferrara, Marco Beccaria, Chiara E. Cordero, Giorgia Purcaro

<https://doi.org/10.1016/j.sampre.2024.100124>

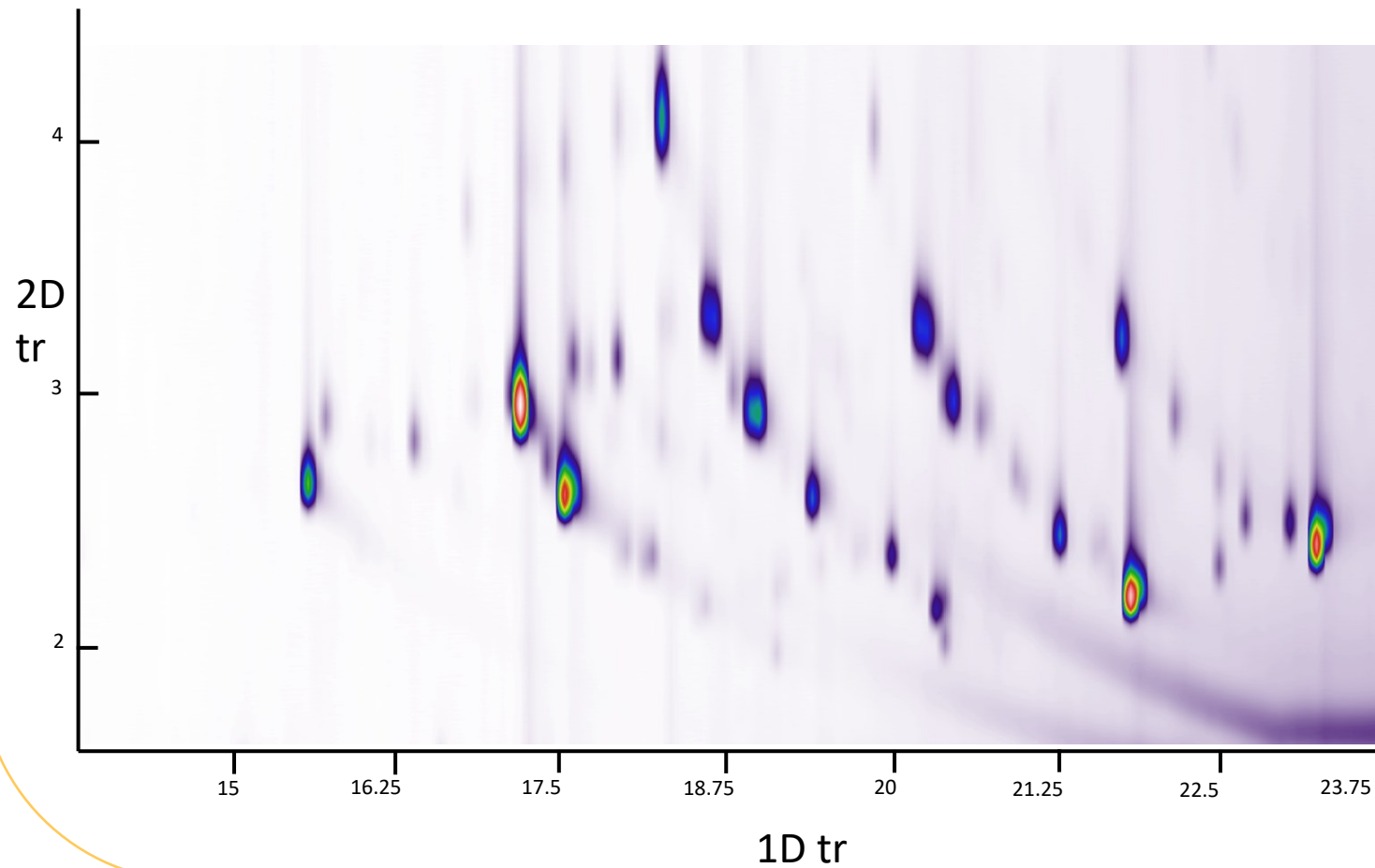
## CONTACTS

[donatella.ferrara@unito.it](mailto:donatella.ferrara@unito.it)

[gpurcaro@uliege.be](mailto:gpurcaro@uliege.be)

## RESULTS

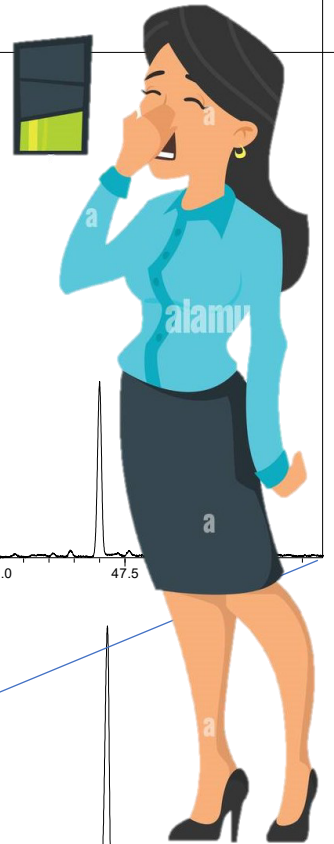
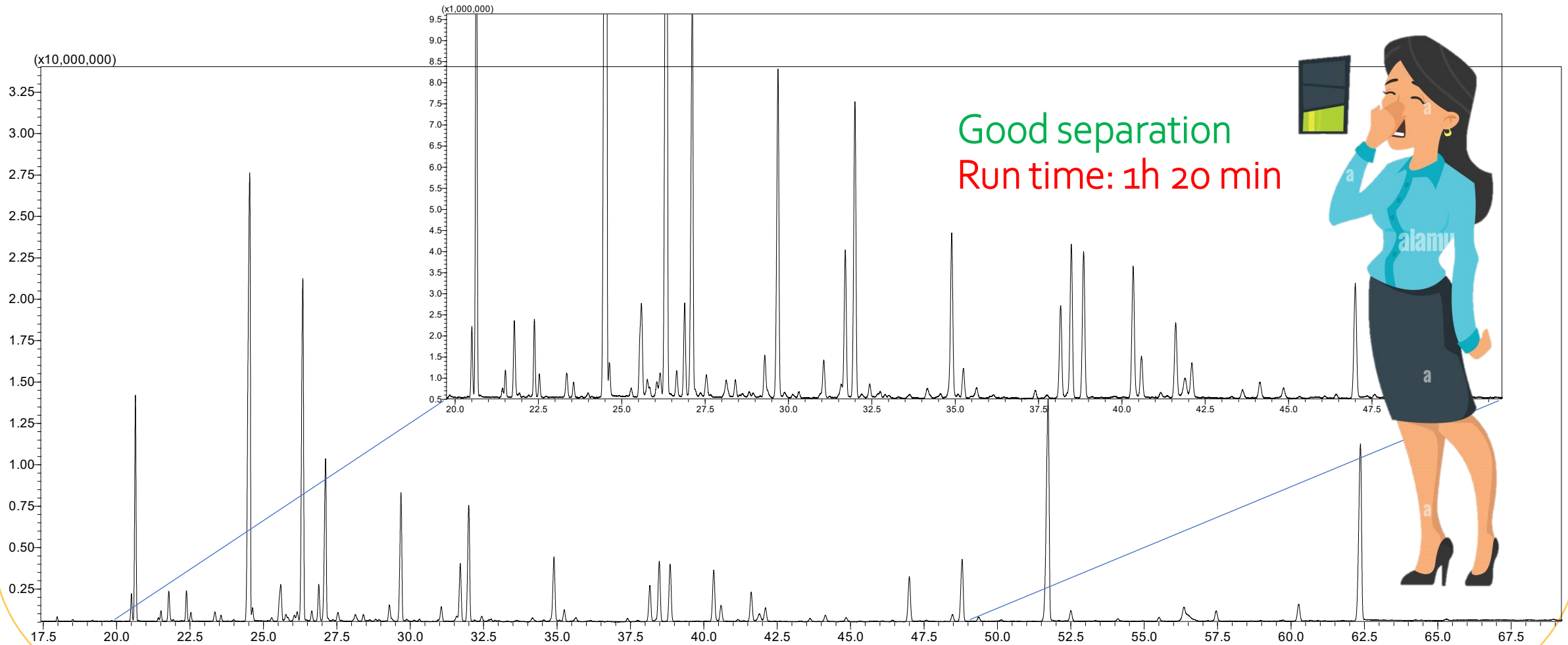
# COMPARISON OF 1D AND 2D FOR FAME<sub>s</sub> PROFILE IN A COMPLEX MATRIX MUSSELE SAMPLE



RESULTS

# COMPARISON OF 1D AND 2D FOR FAMEs PROFILE IN A COMPLEX MATRIX

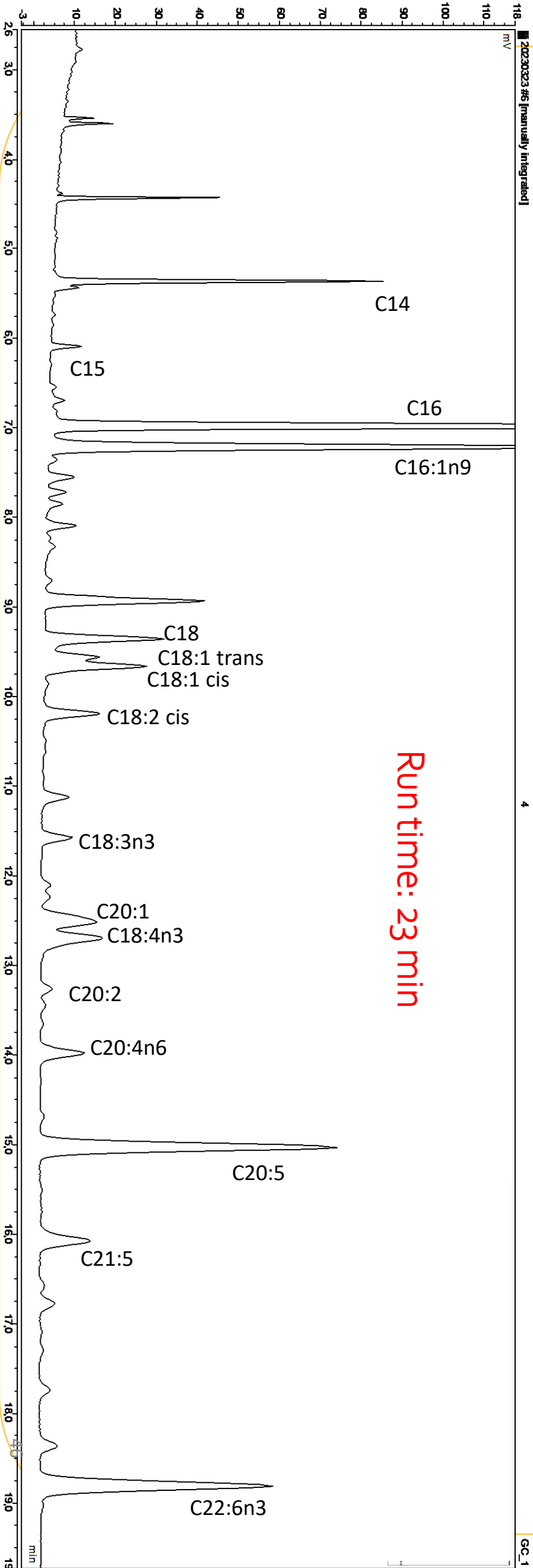
1D SEPARATION ON **SP2560** 100m x 0.25 mm d x 0.25 μm



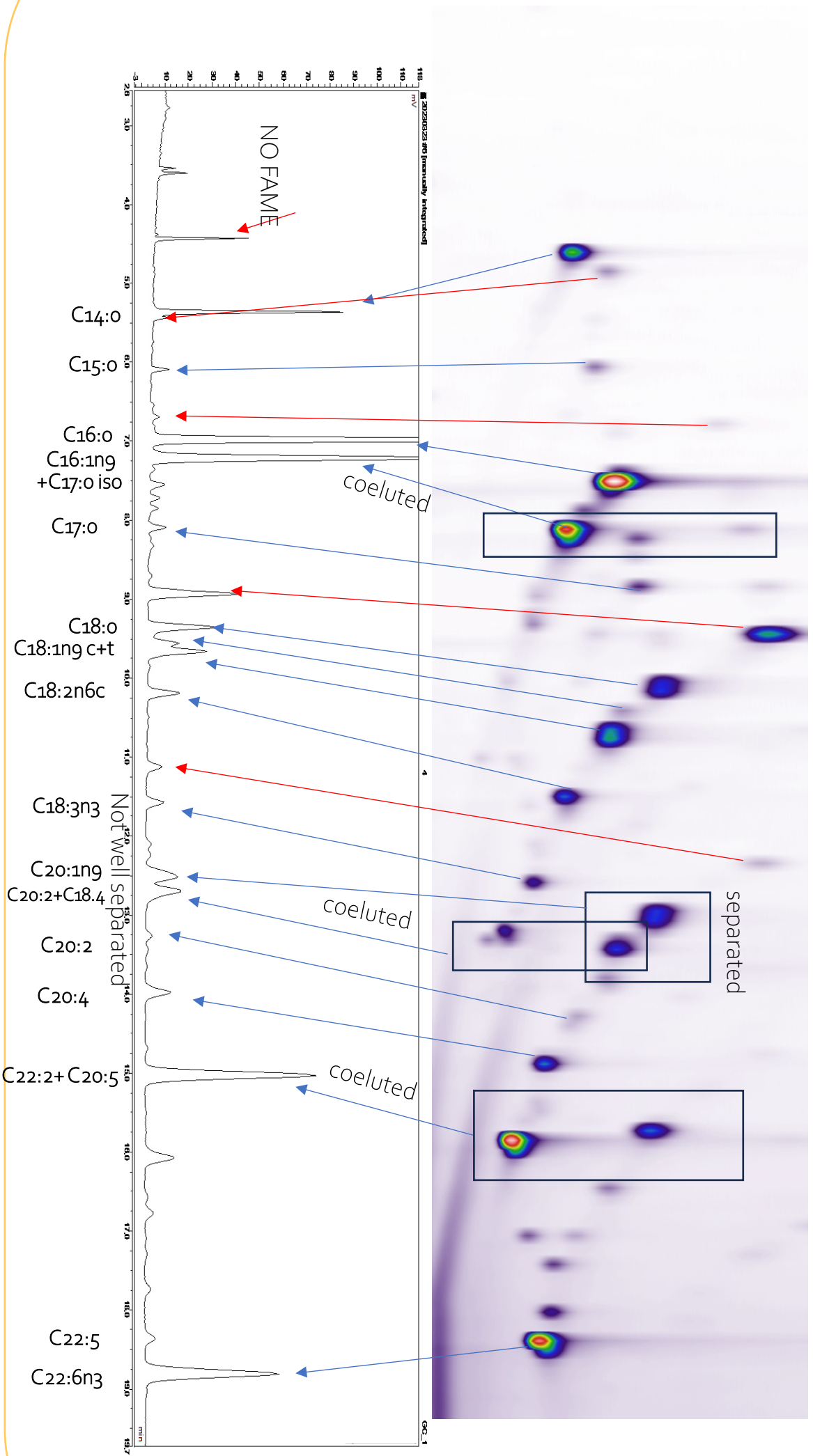
RESULTS

COMPARISON OF 1D AND 2D FOR FAMES PROFILE IN A COMPLEX MATRIX  
1D SEPARATION ON Stabilwax-DA 30m x 0.25 mm d x 0.25 µm

Run time: 23 min



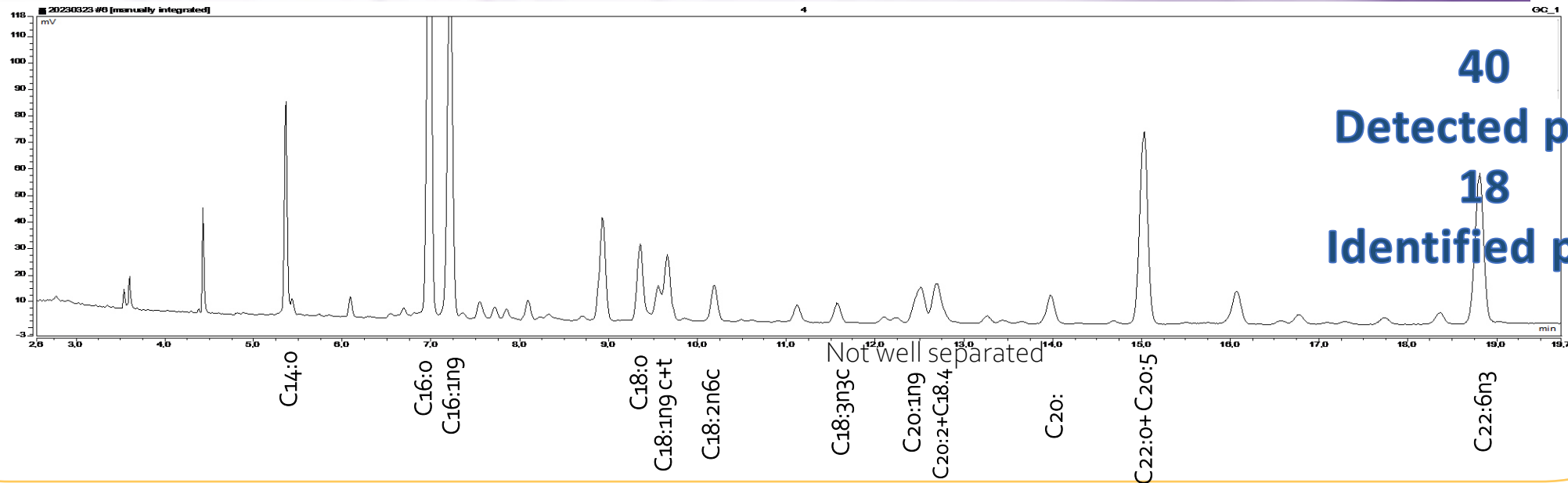
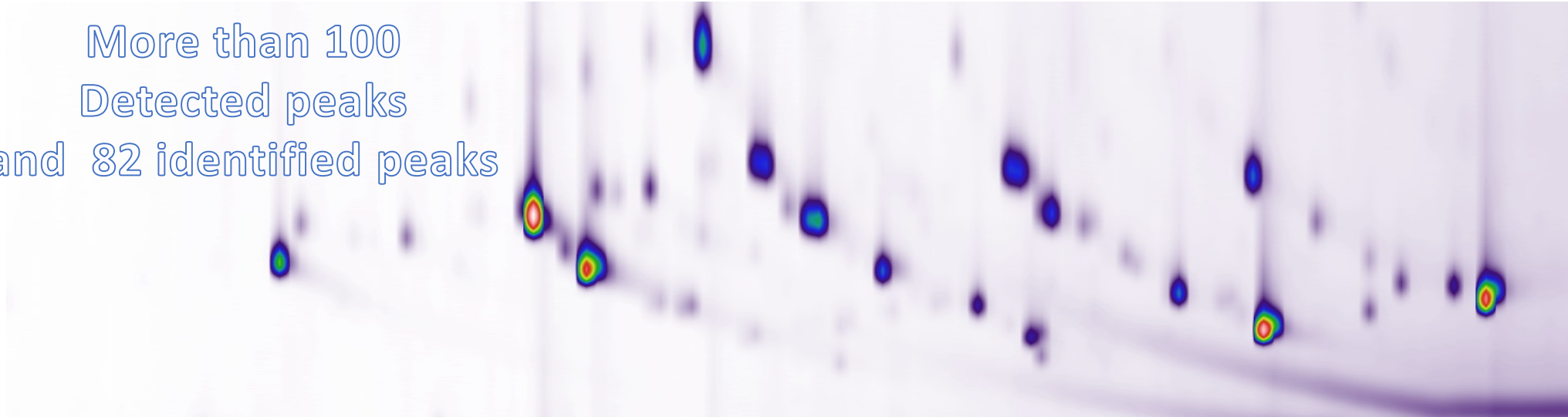
# COMPARISON OF 1D AND 2D FOR FAMES PROFILE IN A COMPLEX MATRIX



RESULTS

COMPARISON OF 1D AND 2D FOR FAMEs PROFILE IN A COMPLEX MATRIX

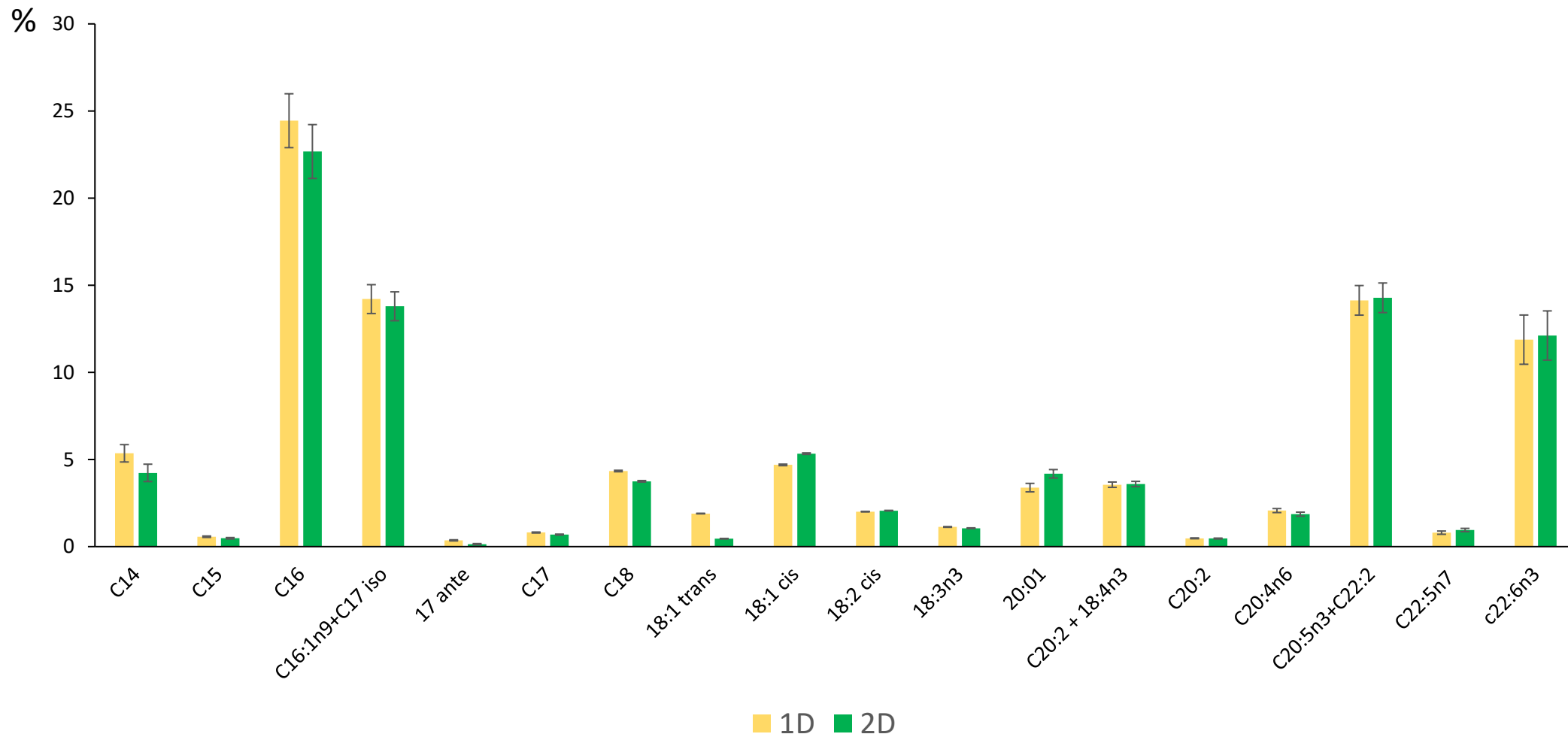
More than 100  
Detected peaks  
and 82 identified peaks



40  
Detected peak  
18  
Identified peak

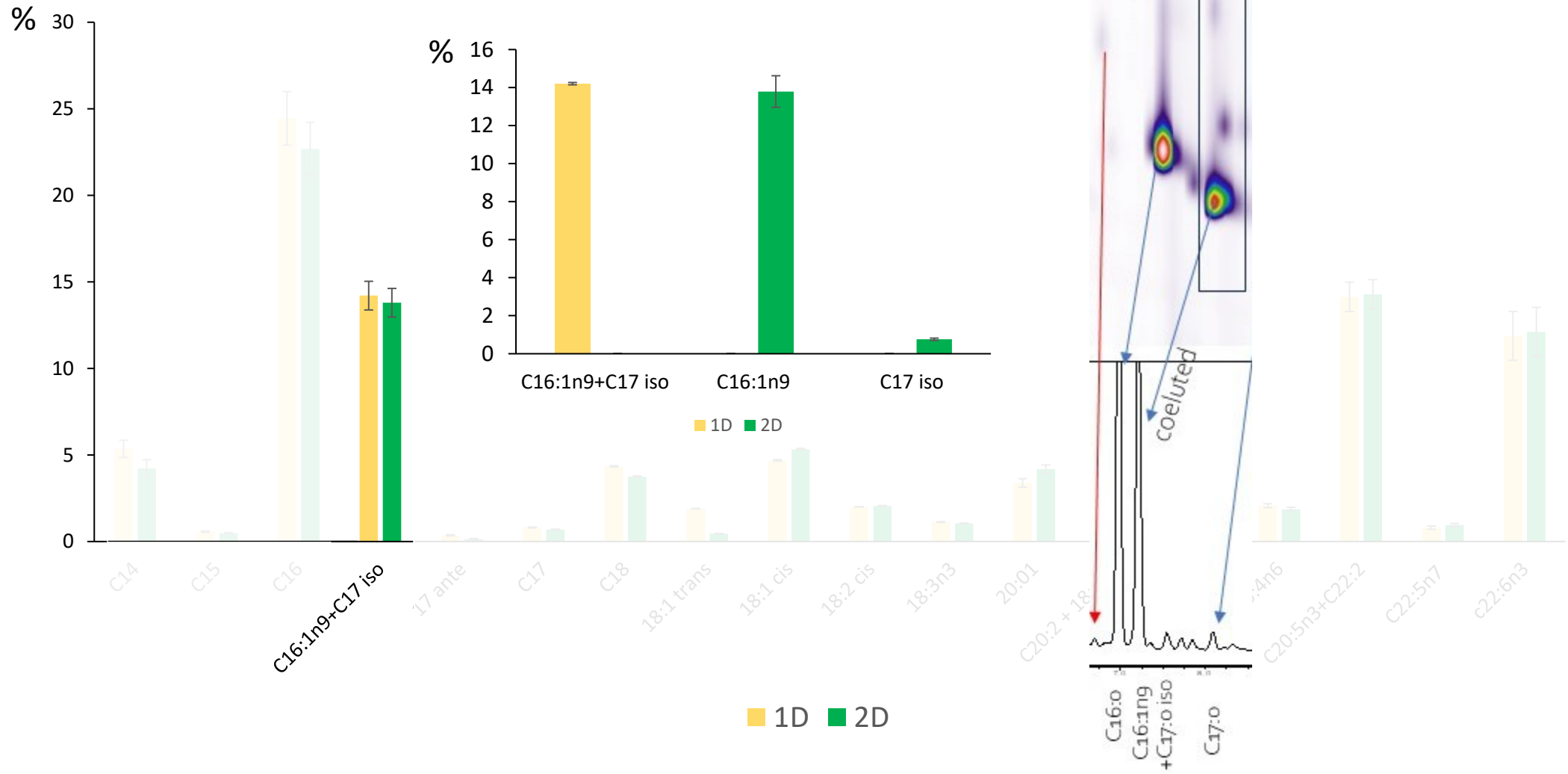
## RESULTS

# COMPARISON OF 1D AND 2D FOR FAMEs PROFILE IN A COMPLEX MATRIX



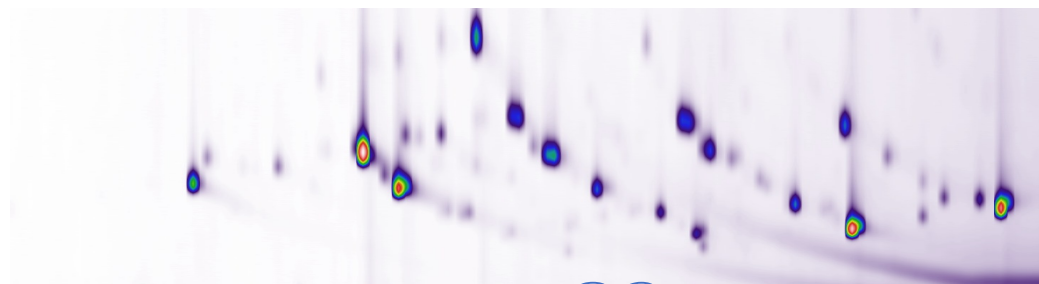
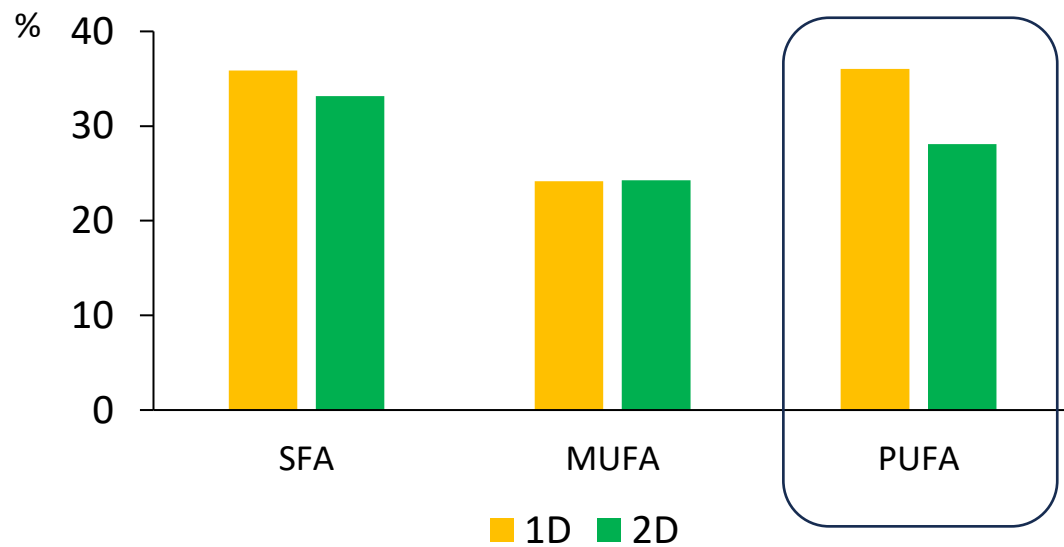
# RESULTS

## COMPARISON OF 1D AND 2D FOR FAMES PROFILE IN A COMPLEX MATRIX



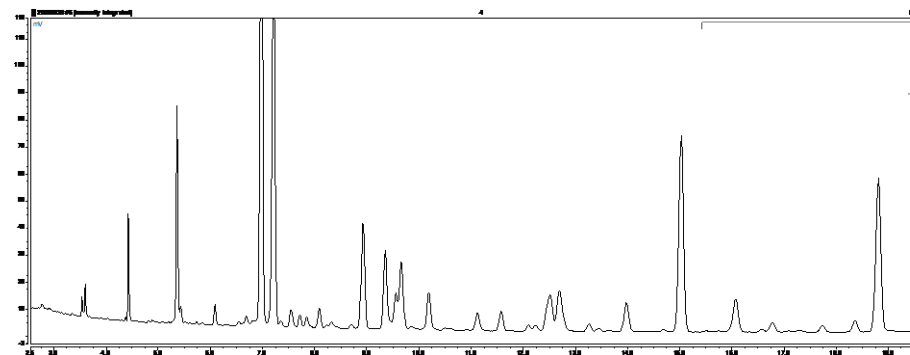
RESULTS

# COMPARISON OF 1D AND 2D FOR FAMES PROFILE IN A COMPLEX MATRIX



82

identified peaks

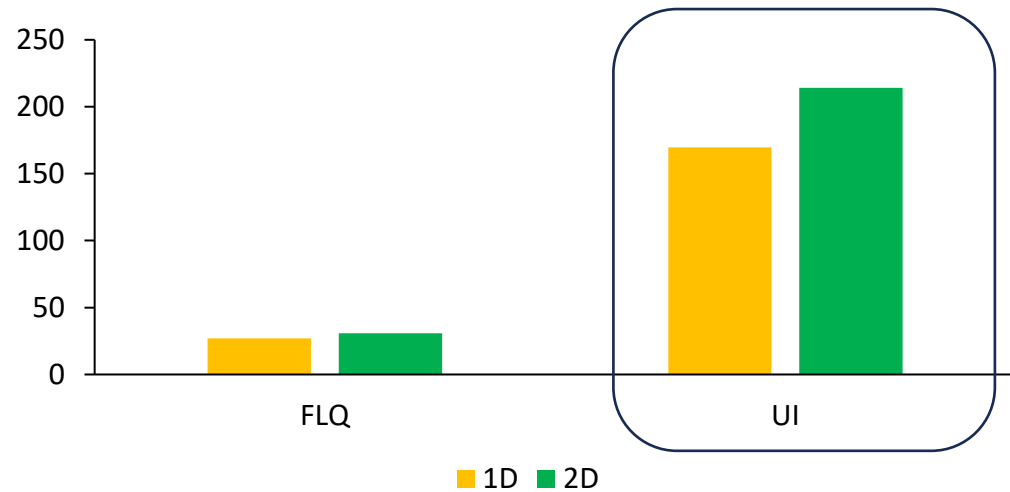
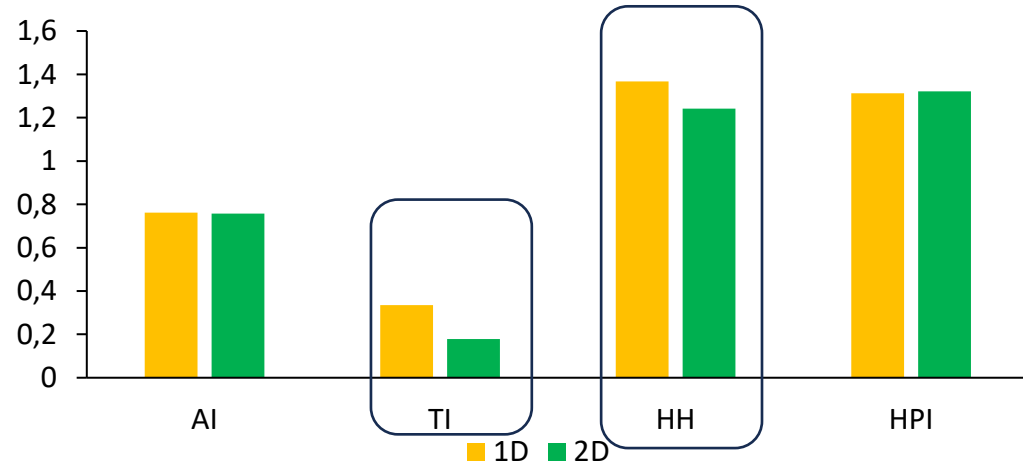


18

identified peaks

## RESULTS

# COMPARISON OF 1D AND 2D FOR FAMEs PROFILE IN A COMPLEX MATRIX



## Nutritional values

AI: index of atherogenicity

IT: index of thrombogenicity

HH: hypocholesterolemic/hypercholesterolemic ratio

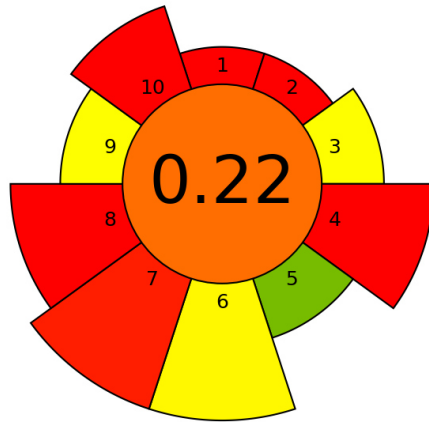
HPI: health-promoting index

FLO: fish lipid quality index

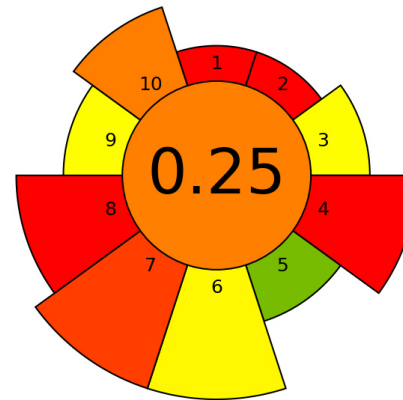
UI: unsaturation index

RESULTS

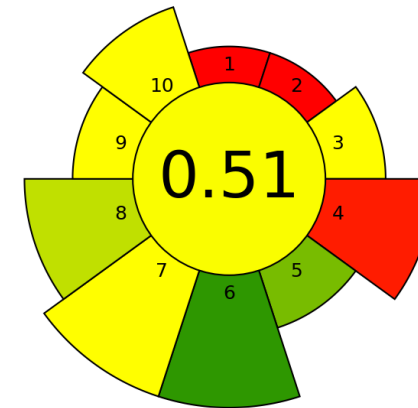
# EVALUATION OF GREENNES OF THE METHODS



AOCS Ce 2c-11



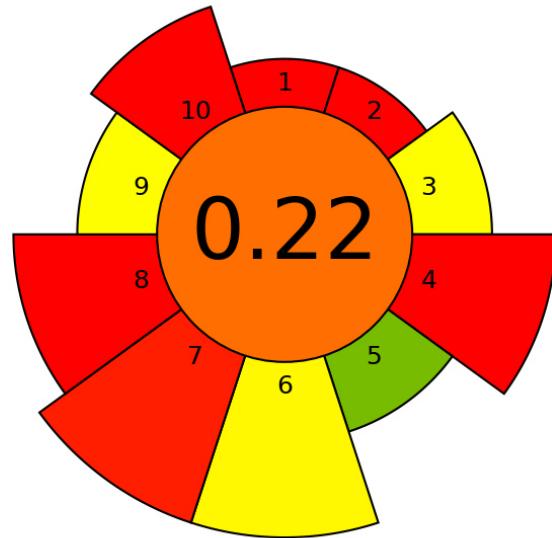
AOCS Ce 2b-11



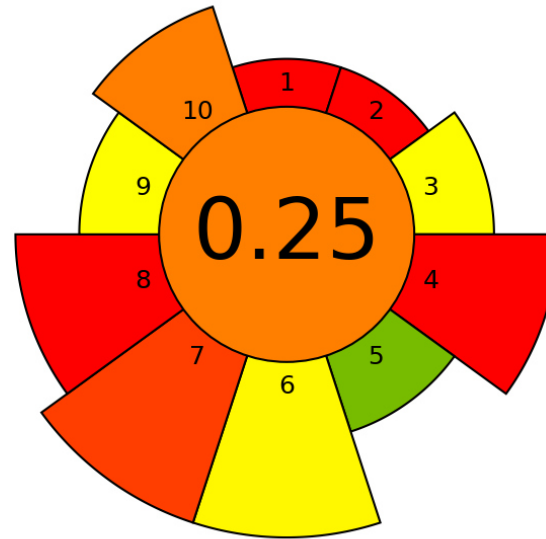
MAED

RESULTS

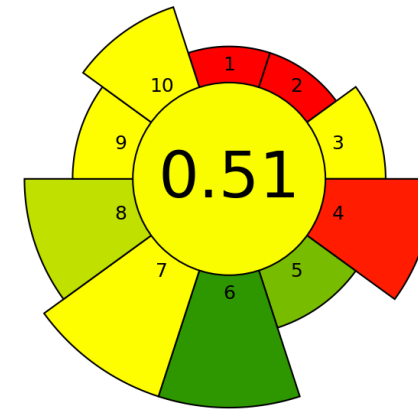
# EVALUATION OF GREENNES OF THE METHODS



AOCS Ce 2c-11



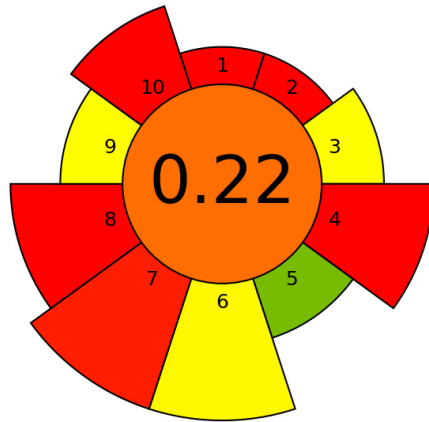
AOCS Ce 2b-11



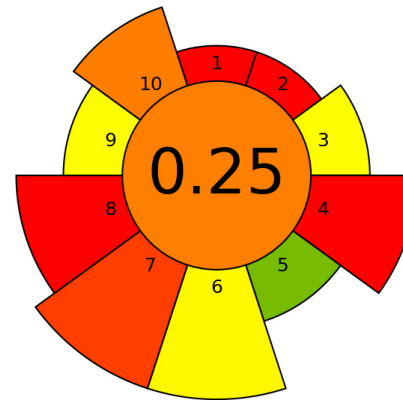
MAED

RESULTS

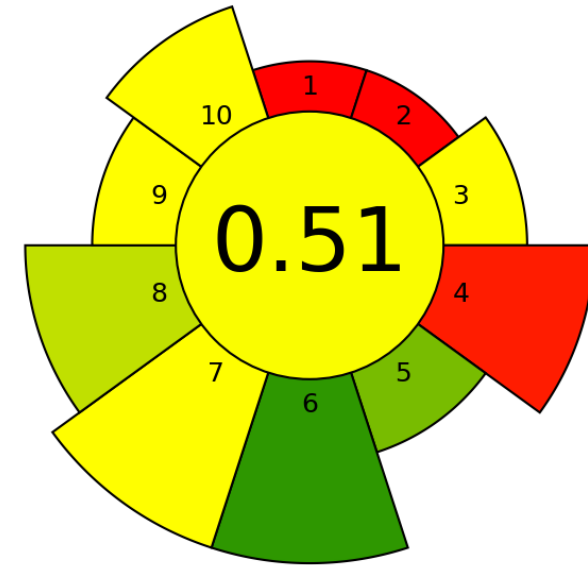
# EVALUATION OF GREENNES OF THE METHODS



AOCS Ce 2c-11



AOCS Ce 2b-11



MAED

RESULTS

# EVALUATION OF BLUENESS OF THE METHODS



AOCS Ce 2c-11  
AOCS Ce 2b-11



MAED

RESULTS

# EVALUATION OF BLUENESS OF THE METHODS



AOCS Ce 2c-11  
AOCS Ce 2b-11



MAED

# PRESENTATION TIMELINE

01

INTRODUCTION



02

OBJECTIVE



03

RESULTS



# PRESENTATION TIMELINE

01

INTRODUCTION



02

OBJECTIVE



03

RESULTS



04

CONCLUSIONS

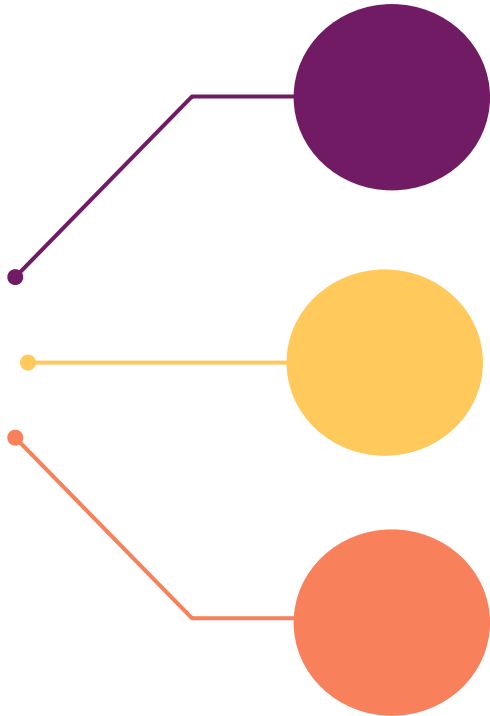
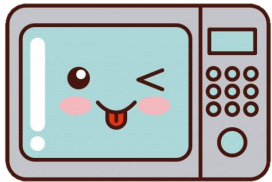


CONCLUSIONS

THE ONE-STEP MICROWAVE-ASSISTED EXTRACTION AND DERIVATIZATION  
IS COMPARABLE TO OFFICIAL METHODS



## MORE GREENER AND PRACTICAL



### HIGHTHROUGHPUT

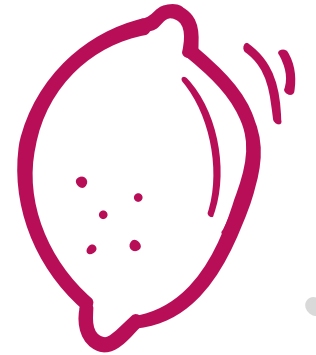
- 12 samples every 15 min so 48 per h (or even more if the rotor with 44 position is used)

### SUITABILITY

- It was demonstrated that with MAED a wide range of samples can be processed

### AUTOMATION & SECURITY

- minimizing energy consumption, less toxic solvents



## CONCLUSIONS

Confirmed that the quantification in 2D is comparable to the quantification in 1D, so is reliable.



## CONCLUSIONS

- Thanks to the structured chromatogram obtained in GCxGC, no need of an MS.
- Moreover the use of flow modulator is more energetically sustainable than the cryogenic modulator



**is better than**



# ADKNOWLEDGEMENTS



Giorgia Purcaro  
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Andrea Caratti  
Angelica Fina  
Fulvia Trapani





THANKYOU FOR THE ATTENTION

Do you have any questions?

Donatella Ferrara  
PhD student

# ADVANCES IN SEPARATION SCIENCE

*3<sup>th</sup> Edition*



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