

# LC-HRMS-based metabolomics as a tool to develop analytical methods: How to choose the best extraction protocol when it comes to untargeted analysis?

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*16<sup>èmes</sup> Journées Scientifiques du RFMF*

*Saint-Malo, France*

*04-06/06/2024*

Il y a 7 ans...



**À Saint-Malo,  
début du voyage avec la Métabolomique...**

**Avec Adrián Schwarzenberg**



Ghossou et al. *Plant Methods* (2018) 14:62  
<https://doi.org/10.1186/s13007-018-0329-0>

Plant Methods

**METHODOLOGY**

**Open Access**



Simultaneous untargeted and targeted metabolomics profiling of underivatized primary metabolites in sulfur-deficient barley by ultra-high performance liquid chromatography-quadrupole/time-of-flight mass spectrometry

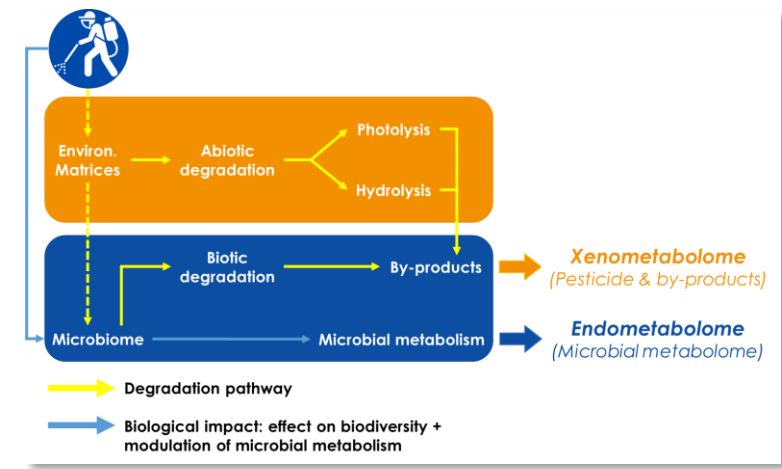
Hikmat Ghossou<sup>1,2</sup>, Adrián Schwarzenberg<sup>1\*</sup>, Frank Jamois<sup>1</sup> and Jean-Claude Yvin<sup>1</sup>

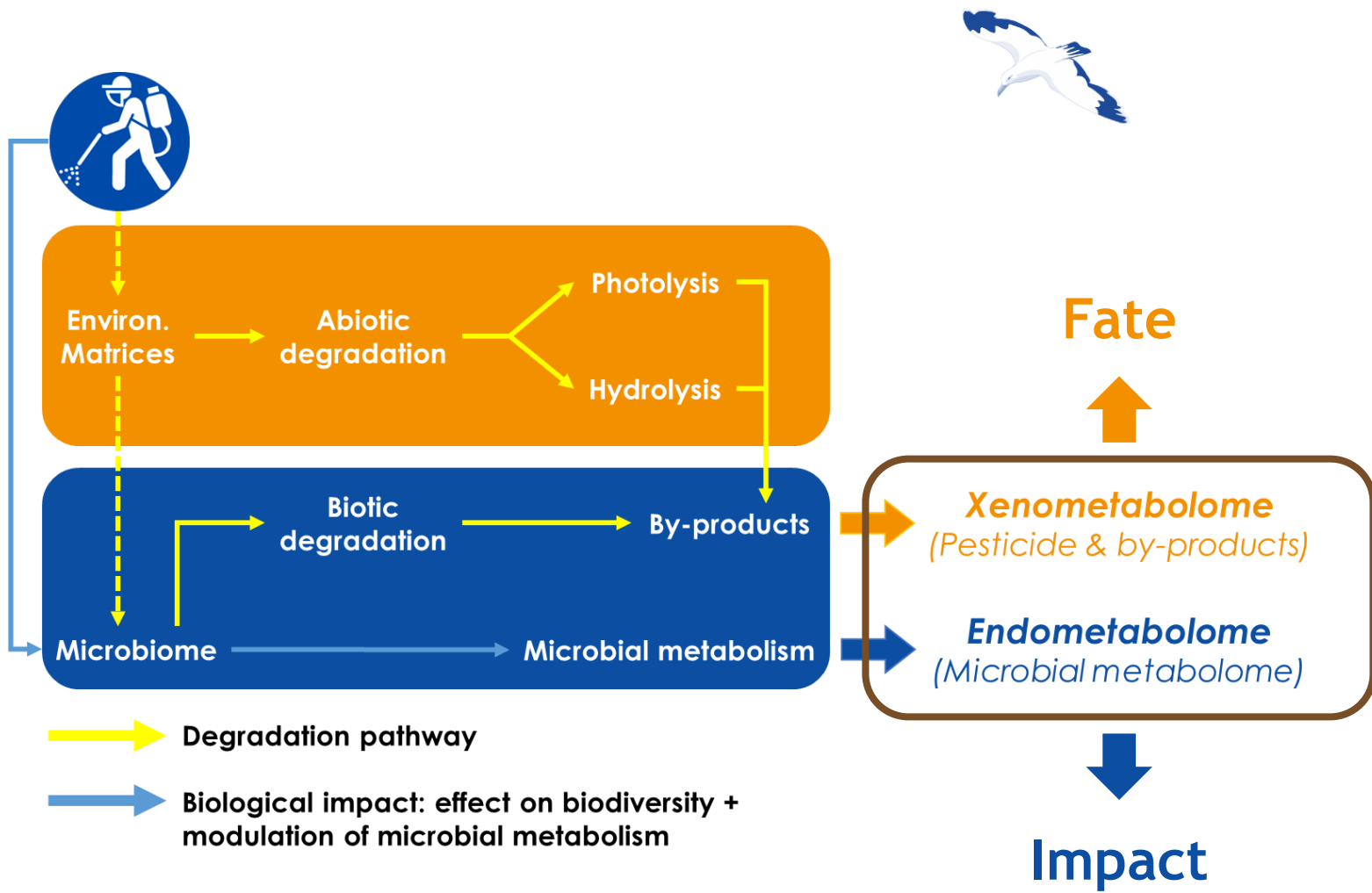
# Et puis à Perpignan...



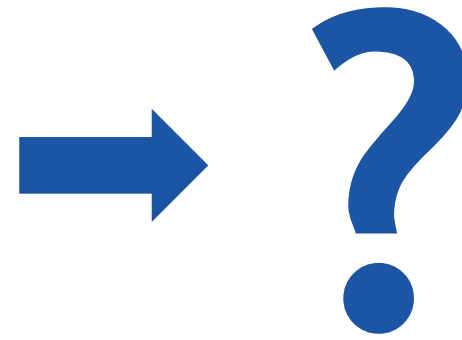
*Avec Cédric Bertrand & Marie-Virginie Salvia*

**Suite du voyage,  
Pour découvrir quelques obstacles...**





How to extract maximum number of metabolites?



Patil et al. *Sci Total Environ* (2016), 566-567:552–558  
 Salvia et al. *Environ Sci Pollut Res Int* (2017), 25(30):29841–29847  
 Ghosson, Ph.D. Thesis (2020)



## Factor #1

### ◇ 5 extraction protocols to be tested

- **E1:** Former in-house protocol (EtOAc + HCl ; 1-step)
- **E2:** Reference protocol “QuPPE” (MeOH ; 1-step)
- **E3:** Reference protocol “QuPPE” (MeOH + 1% FA ; 1-step)
- **E4:** Novel in-house protocol (ACN/iPA + H<sub>2</sub>O/MeOH ; 2-steps)
- **E5:** Novel in-house protocol (ACN/iPA + H<sub>2</sub>O/MeOH + 1% FA ; 2-steps)



5 Protocols

Romdhane et al. *Front Microbiol* (2016), 7:775  
Anastassiades et al. *EURL-SRM* (2016), V9.1  
Ghossou et al. *Anal Chem* (2024), 96(7):2810-2821

## Factor #2

### ◇ 2 types of soil

**SP:**

**Soil from Perpignan**  
*Rich in Organic Matter*



**ST:**

**Soil from Torreilles**  
*Poor in Organic Matter*

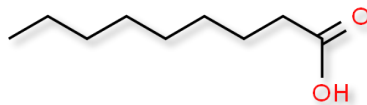


**5 Protocols**  
**2 Soils**

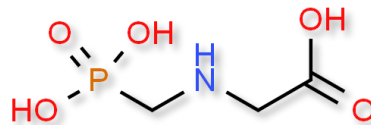
## Factor #3

◆ 2 types of pesticides (+ 1 control)

**Bel:**  
**Beloukha®**  
*Natural Nonpolar*



**Gly:**  
**Tartan Super 360**  
*Synthetic Polar*

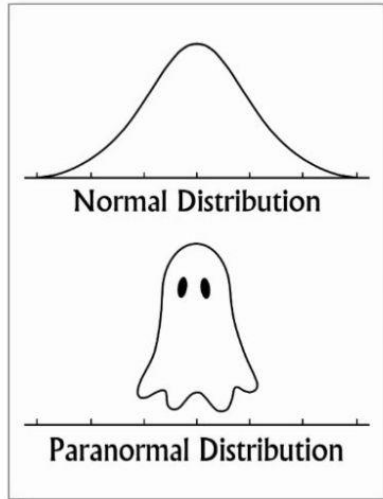


**Ctr:**  
**Untreated control**  
*Ultrapure Water*

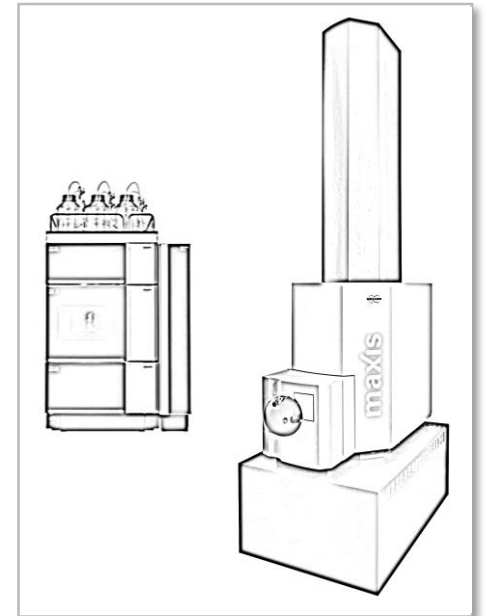


**5 Protocols**  
**2 Soils**  
**3 Conditions**

## Complex Tri-factorial Experimental Design for LC-Q/ToF analysis



**5 Protocols  
2 Soils  
3 Conditions  
5 Replicates / Group**



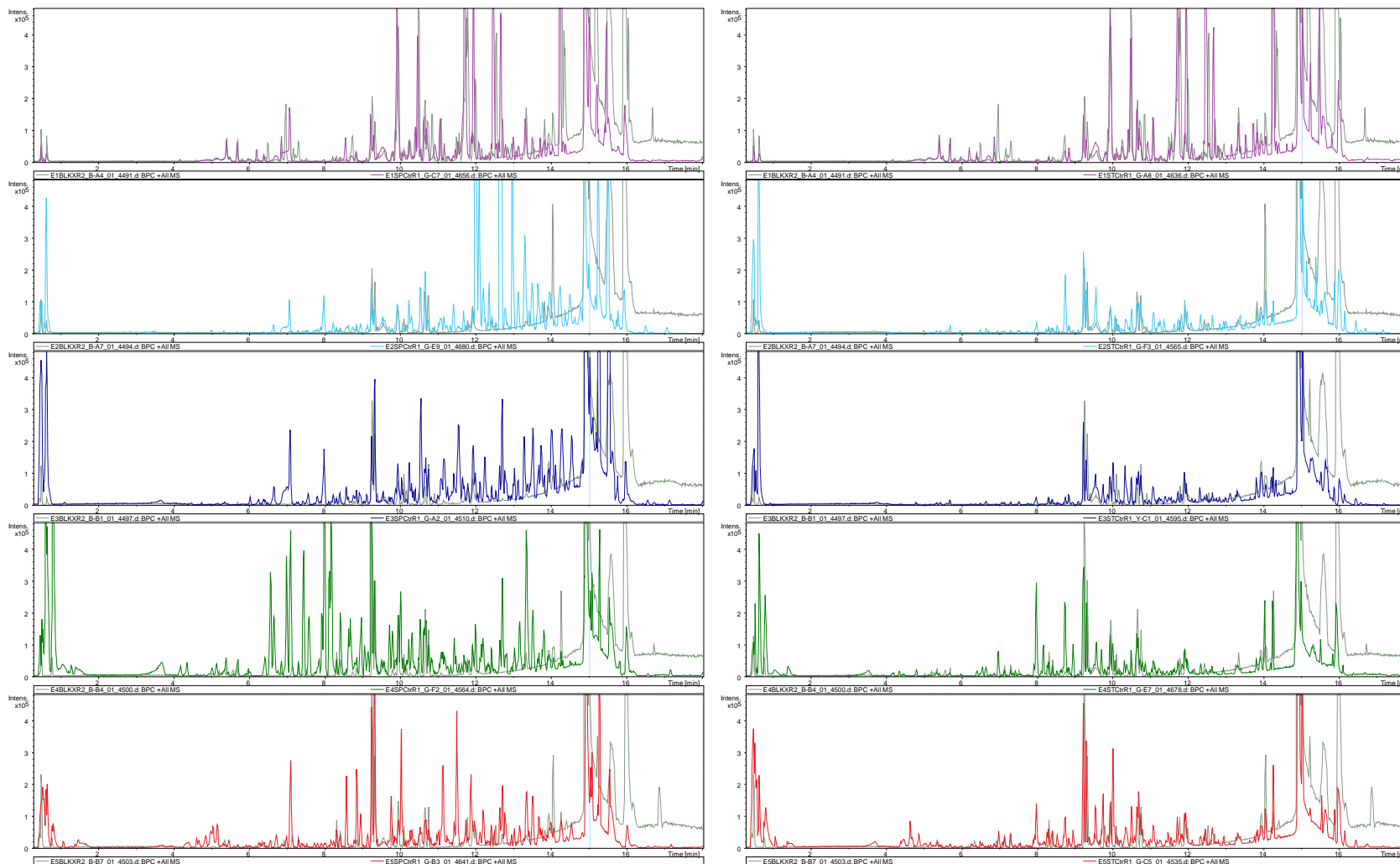
**Total Number of Samples:  
 $5 \times 2 \times 3 \times 5 = 150$**



Raw data  
“.d” format

BPCs:  
m/z 90-1000

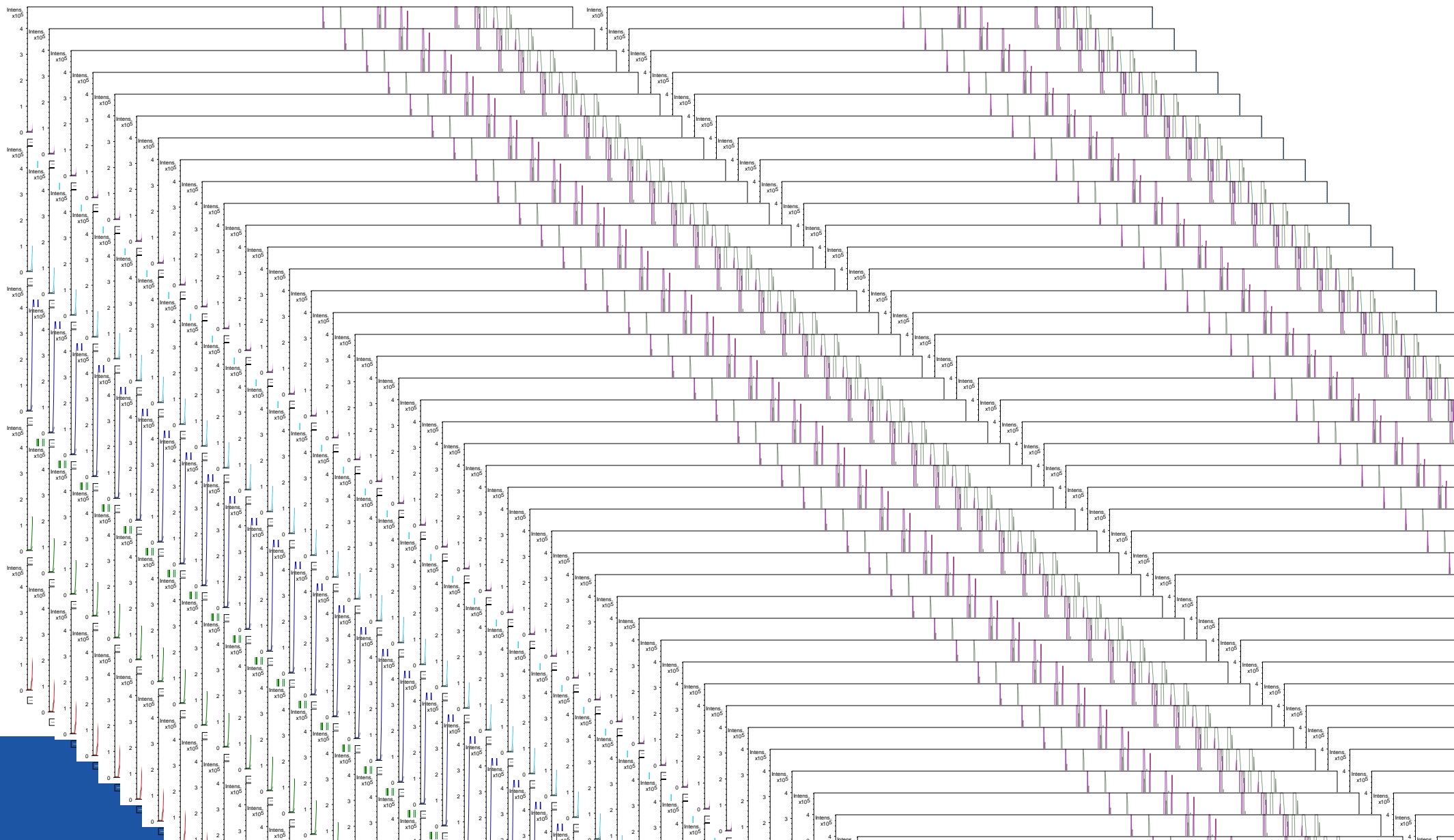
Fixed intensity:  
4.80E5



Raw data  
“.d” format

BPCs:  
 $m/z$  90-1000

Fixed intensity:  
 $4.80E5$



# Solution!



## *Use Metabolomics for Metabolomics!*



*Hegel is happy  
about it...*



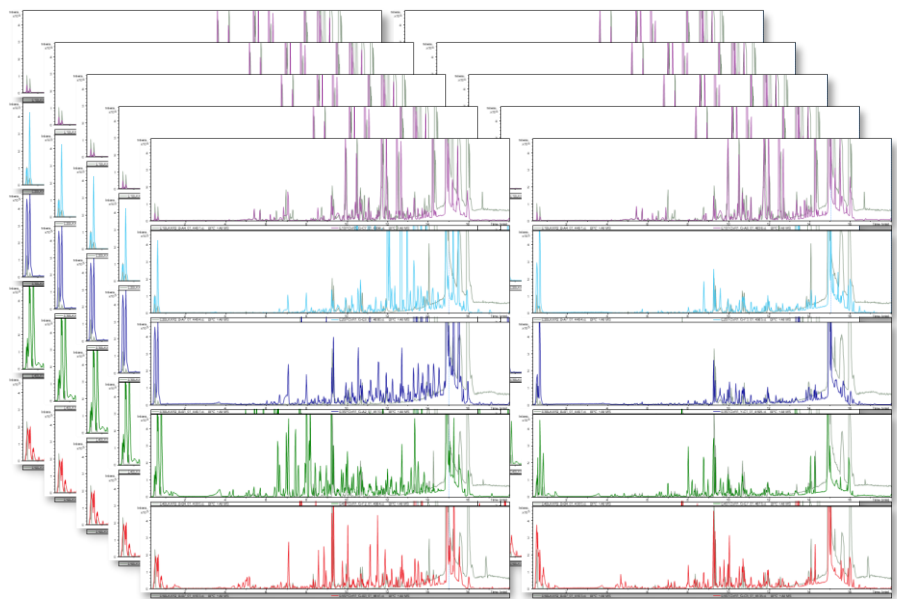
Prof. Coral Barbas' Plenary Session (P1) at the 12JSRFMF  
May 21, 2019, Clermont-Ferrand, France



21 AU 23 MAI 2019  
POLYDOME, CLERMONT-FERRAND

## Matrix #1 "861V"

861 ions for 314 compounds



4  
Wm



Transformation



## Matrix #2 "314V"

The 314 compounds without redundancies

**LC-HRMS raw data**  
150 Samples  
30 QC  
30 Blanc Extractions  
10 MS/HRMS Analyses  
4 Standards mix Analyses

Giacomini et al. *Bioinformatics* (2015), 31(9):1493-1495  
Guillon et al. *Int J Biochem Cell Biol* (2017), 93:89-101

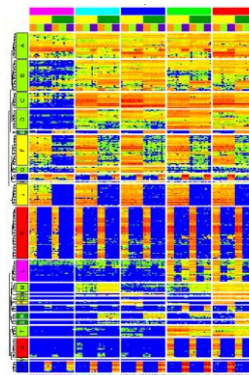


## Performance assessment

- Metabolome coverage
- Quantitative capacity

314V

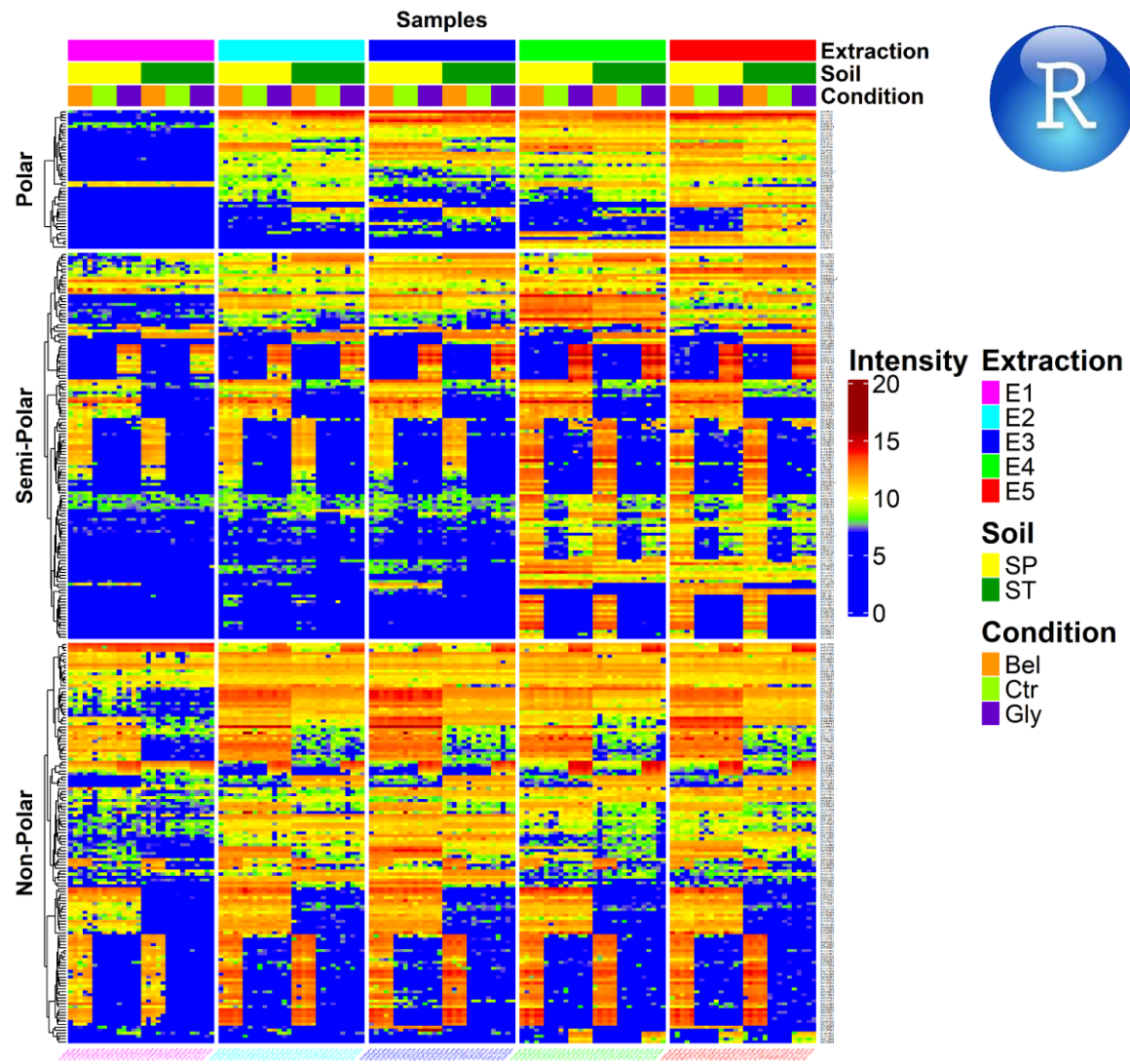
861V



Polar compounds: **E5** ←

Semi-polar compounds: **E4** ←

Non-polar compounds: **E3** ←

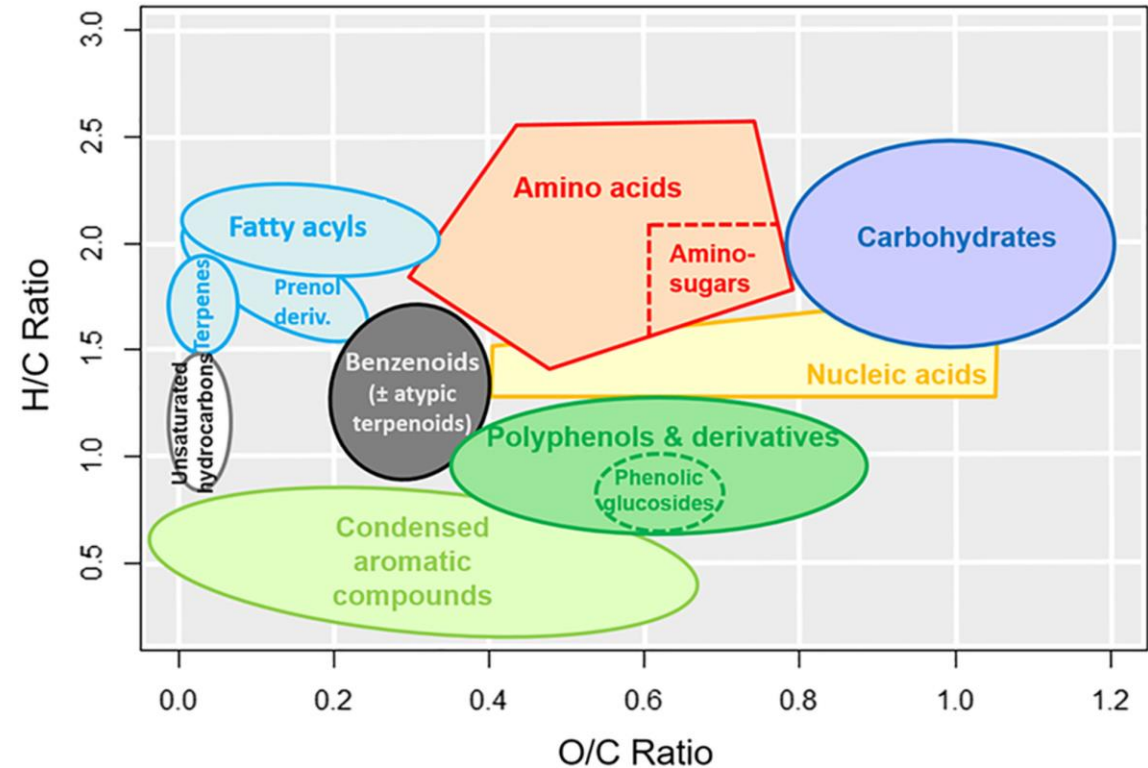


Ghosson et al. Anal Chem (2024), 96(7):2810-2821

## Chemical families coverage and detection ability

Van Krevelen diagram:

- H/C vs. O/C
- Idea about (bio)chemical families
- Level 4-annotation necessary
- Non-exhaustive cartography



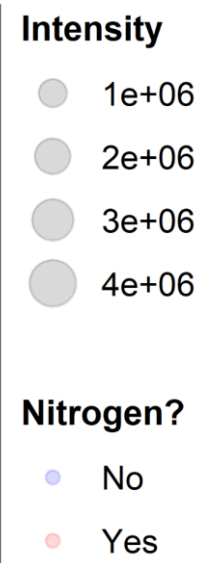
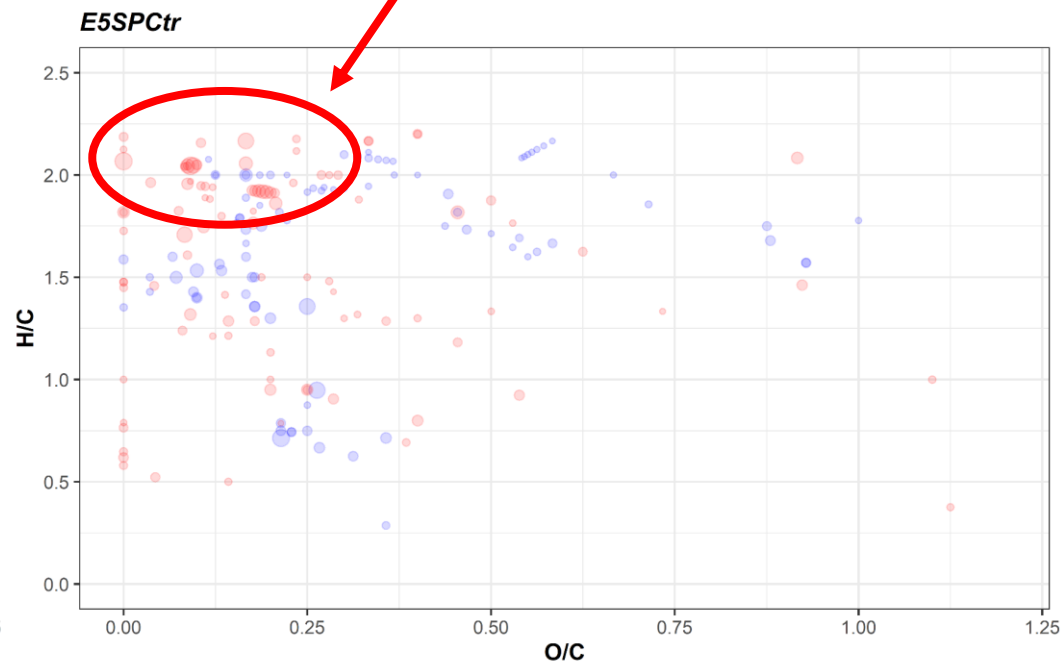
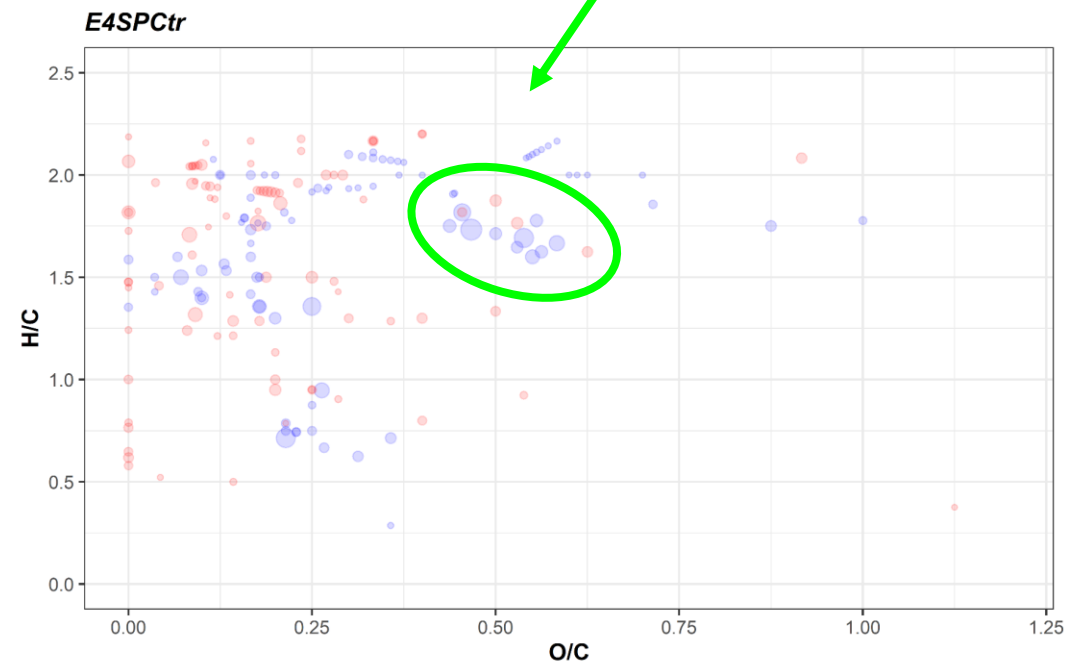
Ollivier et al. *Phytochem Anal* (2022), 33(7):1111–1120



# Chemical families coverage and detection ability

Primary metabolites

Lipidic hydroxyamides




Ghosson et al. *Anal Chem* (2024), 96(7):2810-2821



# Chemical families coverage and detection ability

## Characteristic signature of formulation ingredients (PEG)

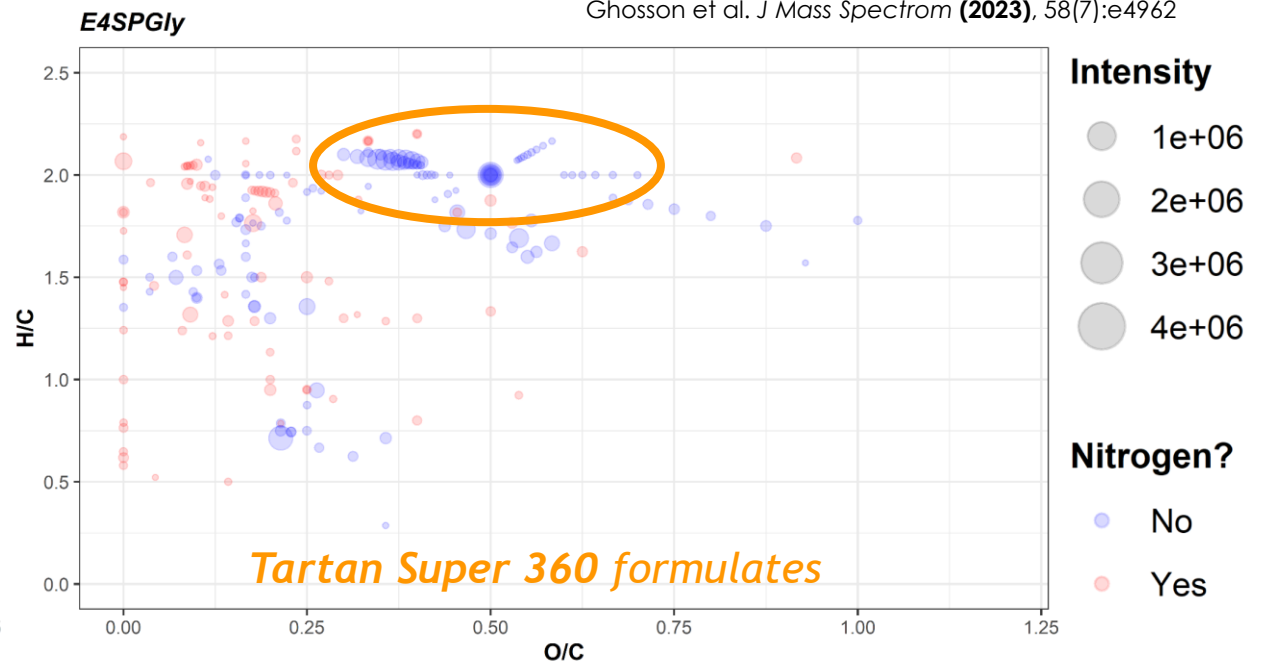
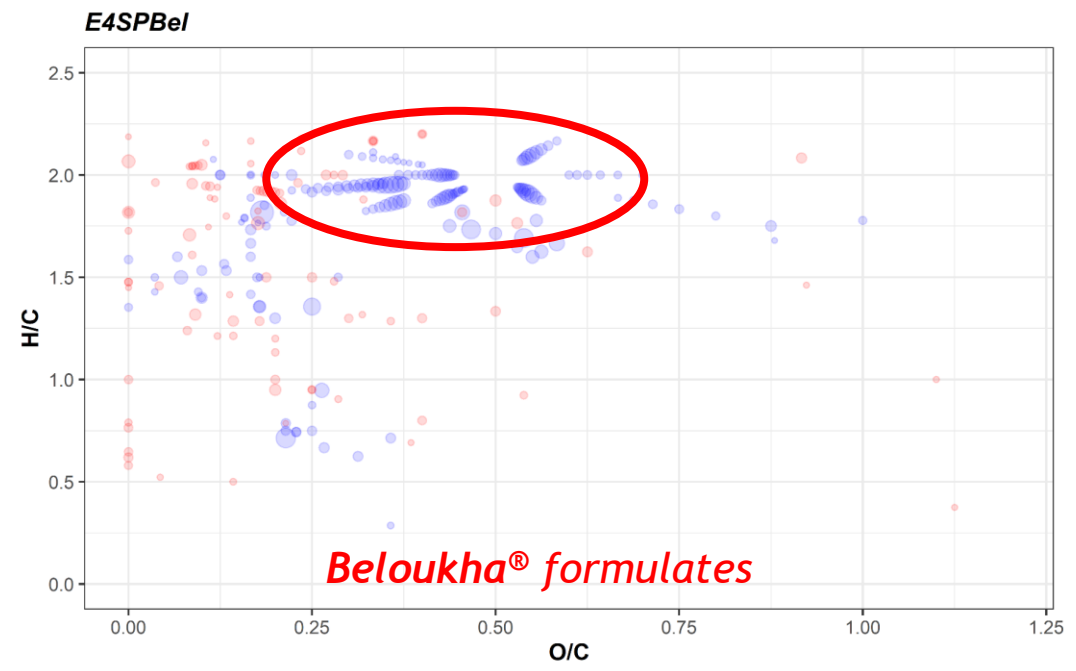
Received: 26 April 2023 | Revised: 31 May 2023 | Accepted: 22 June 2023  
 DOI: 10.1002/jms.4962

**RESEARCH ARTICLE**


**Characteristic response of formulation ingredients revealed by ultra high performance liquid chromatography-electrospray ionization-high resolution mass spectrometry-based untargeted screening of pesticides in soil**

Hikmat Ghosson<sup>1,2</sup> | Delphine Raviglione<sup>1,2</sup> | Marie-Virginie Sallia<sup>1,2</sup> | Cédric Bertrand<sup>1,2,3</sup>

Ghosson et al. *J Mass Spectrom* (2023), 58(7):e4962



Ghosson et al. *Anal Chem* (2024), 96(7):2810-2821

### Performance assessment

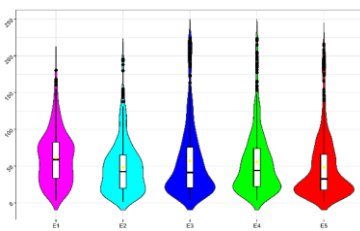
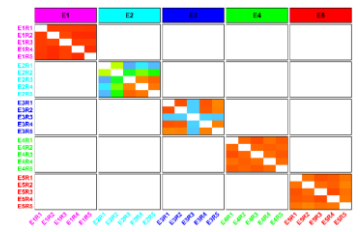
- Metabolome coverage
- Quantitative capacity

314V



### Repeatability assessment

- Intra-group

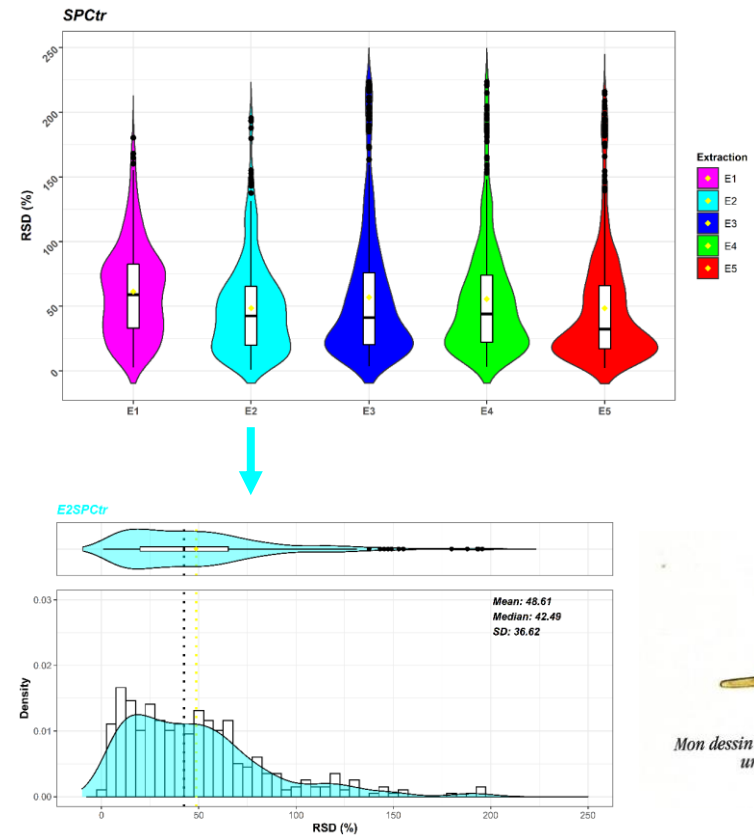


861V

# Repeatability: Le Petit Prince vs. Euclid

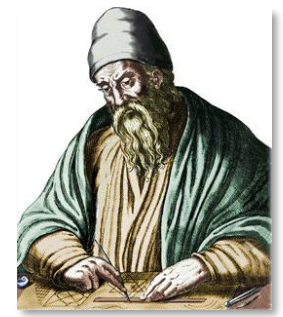
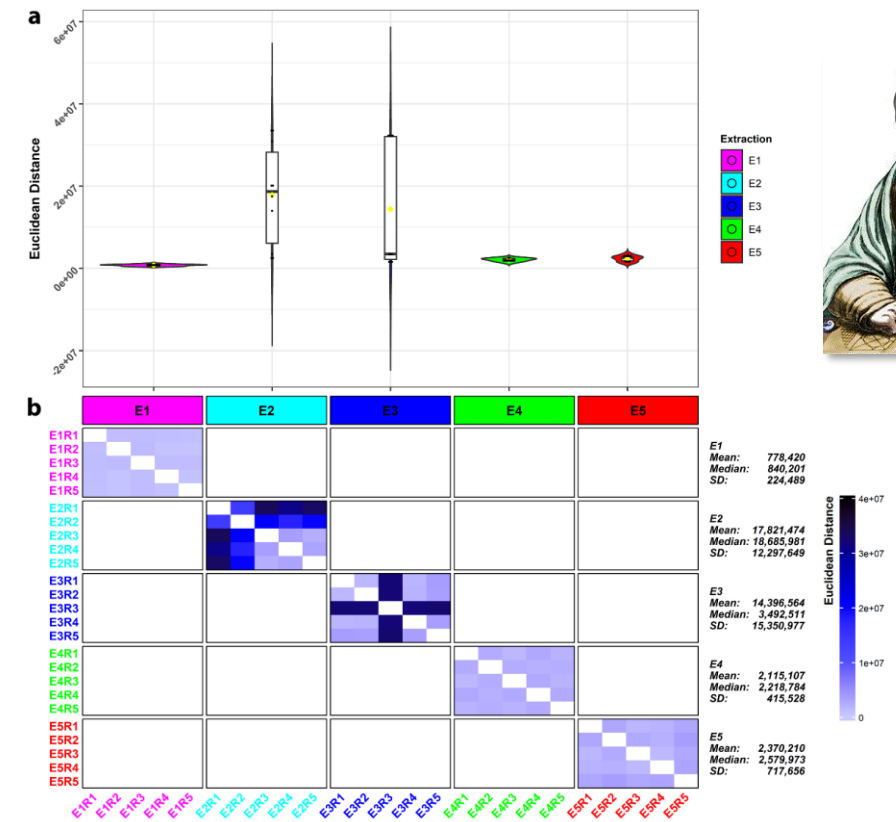


## Dispersion of intensities' RSD of variables



*Mon dessin ne représentait pas un chapeau. Il représentait un serpent boa qui digérait un éléphant*

## Euclidean Distances between replicates



### Performance assessment

- Metabolome coverage
- Quantitative capacity

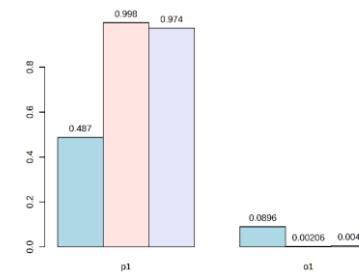
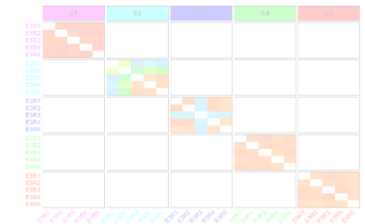


314V

Sample	Group	D	E	F	G	H	I	J	K	L	M	N	O	P
E1SPB0R1	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPB0R2	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPB0R3	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPB0R4	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPB0R5	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R1	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R2	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R3	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R4	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R5	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R1	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R2	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R3	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R4	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R5	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R1	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R2	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R3	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R4	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R5	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R1	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R2	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R3	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R4	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R5	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STG0R1	E1STGv	0	0	0	0	0	0	0	0	0	0	0	0	0

### Repeatability assessment

- Intra-group



### Discrimination capacity comparisons

- Contaminated vs. Non-contaminated

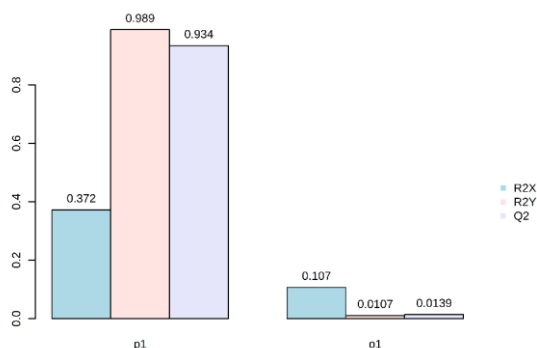
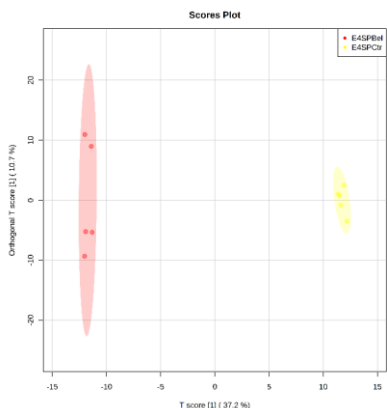
861V

Sample	Group	D	E	F	G	H	I	J	K	L	M	N	O	P
E1SPB0R1	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPB0R2	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPB0R3	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPB0R4	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPB0R5	E1SPBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R1	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R2	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R3	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R4	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPC0R5	E1SPCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R1	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R2	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R3	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R4	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1SPG0R5	E1SPGv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R1	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R2	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R3	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R4	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STB0R5	E1STBel	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R1	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R2	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R3	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R4	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STC0R5	E1STCv	0	0	0	0	0	0	0	0	0	0	0	0	0
E1STG0R1	E1STGv	0	0	0	0	0	0	0	0	0	0	0	0	0



# Discrimination capacity: Who's the lynx?

OPLS-DA and its Cross-Validation test to discriminate Control from Contaminated



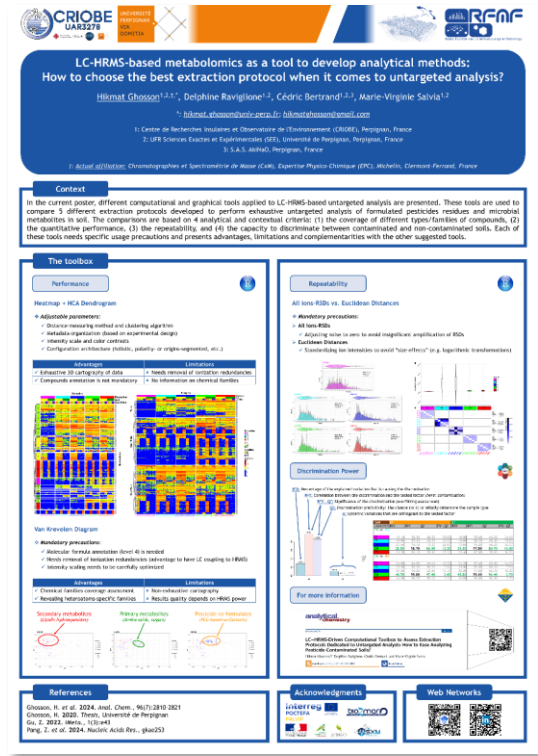
Contaminated vs. Non-contaminated  
OPLS-DA results summary


## Indicators

- ✓ R2X: Variation percentage explained
- ✓ Q2: Discrimination power
- ✓ R2Y - Q2: Discrimination significance

Soil	SP				ST			
	R2X	R2Y	Q2	R2Y - Q2	R2X	R2Y	Q2	R2Y - Q2
<i>Ctr vs. Gly</i>								
E1	19.10	98.30	68.50	29.80	18.00	98.60	64.00	34.60
E2	19.90	97.60	69.30	28.30	18.90	98.20	68.40	29.80
E3	20.50	96.70	71.20	25.50	19.60	96.20	66.20	30.00
E4	<b>28.50</b>	<b>98.70</b>	<b>86.50</b>	<b>12.20</b>	<b>24.50</b>	<b>97.50</b>	<b>80.70</b>	<b>16.80</b>
E5	24.40	96.10	78.90	17.20	23.60	97.30	78.80	18.50
<i>Ctr vs. Bel</i>								
E1	26.00	96.90	81.60	15.30	26.30	97.60	83.40	14.20
E2	33.30	98.20	90.30	7.90	34.00	95.50	88.00	7.50
E3	29.50	95.50	82.40	13.10	30.00	97.00	86.70	10.30
E4	<b>48.70</b>	<b>99.80</b>	<b>97.40</b>	<b>2.40</b>	<b>45.80</b>	<b>99.10</b>	<b>96.40</b>	<b>2.70</b>
E5	46.80	98.20	95.00	3.20	45.90	99.00	96.30	2.70

# For more information...







pubs.acs.org/ac Article

## LC–HRMS-Driven Computational Toolbox to Assess Extraction Protocols Dedicated to Untargeted Analysis: How to Ease Analyzing Pesticide-Contaminated Soils?

Hikmat Ghosson,<sup>\*</sup> Delphine Raviglione, Cédric Bertrand, and Marie-Virginie Salvia

 Cite This: *Anal. Chem.* 2024, 96, 2810–2821
  Read Online

Section 5 - Poster 22

Ghosson et al. *Anal Chem* (2024), 96(7):2810-2821

## Acknowledgments

Cédric Bertrand (CRIOBE, Perpignan)

Marie-Virginie Salvia (CRIOBE, Perpignan)

Delphine Raviglione (CRIOBE, Perpignan)

Marie-Louise Brassier (CRIOBE, Perpignan)

Yannick Brunato (CRIOBE, Perpignan)

Thierry Courp (CEFREM, Perpignan)

Bruno Charrière (CEFREM, Perpignan)

Ludivine Garcia (IUT, Perpignan)

Aude Lusetti (CA66, Torreilles)

Laurent Debrauwer (Toxalim, Toulouse)

Emmanuelle Vulliet (ISA, Lyon)

Yann Guitton (LABERCA, Nantes)

Julien Bocard (ISPSO, Genève)

Carole Calas-Blanchard (LBBM, Perpignan)

Nicolas Le Yondre (CRMPO, Rennes)

Philippe Jéhan (CRMPO, Rennes)

# #BBBS Thank You!

Interreg  
POCTEFA  
PALVIP



AKINAO



CHAMBRE  
D'AGRICULTURE  
PYRÉNÉES-ORIENTALES