



High resolution mass spectrometry-based untargeted approach: a cutting-edge analytical technique for the detection and identification of organophosphorus synthesis sources

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➤ High resolution mass spectrometry-based untargeted approach

A cutting-edge analytical technique for the detection and identification of organophosphorus synthesis sources

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Research &
Innovation
6th International Conference

STRASBOURG
FRANCE
March 19th - 21st 2024

> Context



Events using toxic chemical threat agents → crucial to obtain as much historical information as possible

 Synthesis route and conditions

 Production batches and/or precursors

 Source or geographical origin



- aging
- low concentrations
- complex matrices

SOURCING

The process of **search** and **identification** of discriminating elements whose various properties allow us to attribute an origin to a product or sample

Additives

Persistent
contaminants

Decomposition
Degradation

Hydrolysis products

Impurities

Secondary reaction
products

Precursors and
unreacted raw
materials

Interaction by-
products



Chemical Attribution Signature

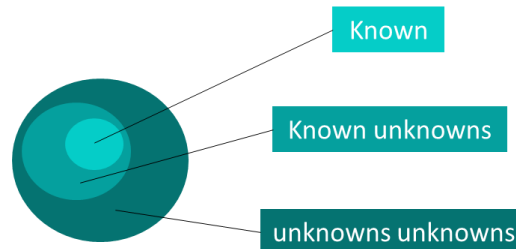
The role of chemical analysis is to provide well-founded information about the source of the sample, in support of forensic investigations

> Metabolomics-based trace discovery strategies

Methodology to support attribution of the origin of a toxic agent according to the chemical signature of substances resulting from its production pathway

Approaches

Goals



Targeted

Unambiguous identification and quantification

Suspect Screening

Qualitative detection rate and half-quantitative values

Non-targeted

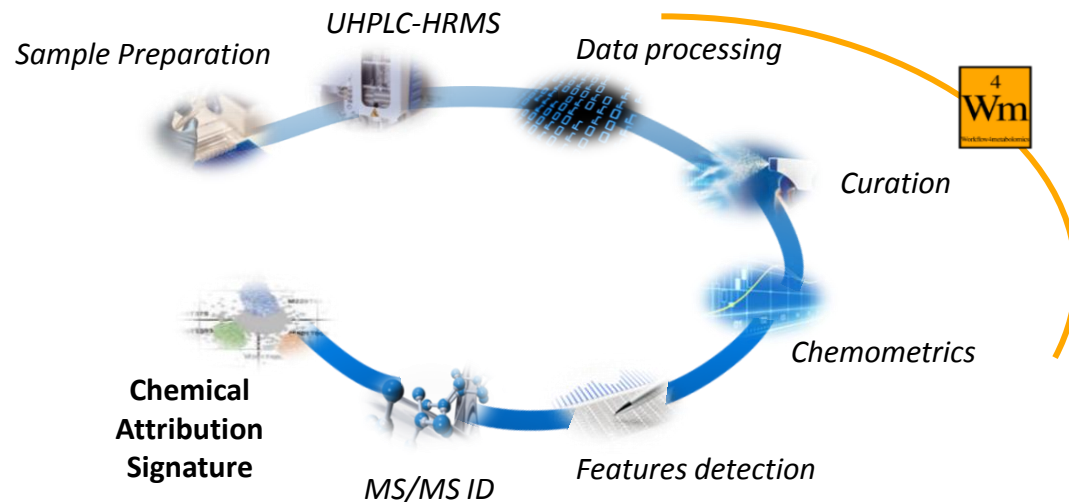
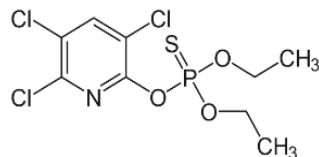
New markers detection

Sensitivity

Information

Chlorpyrifos (CPF)

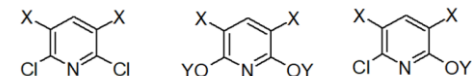
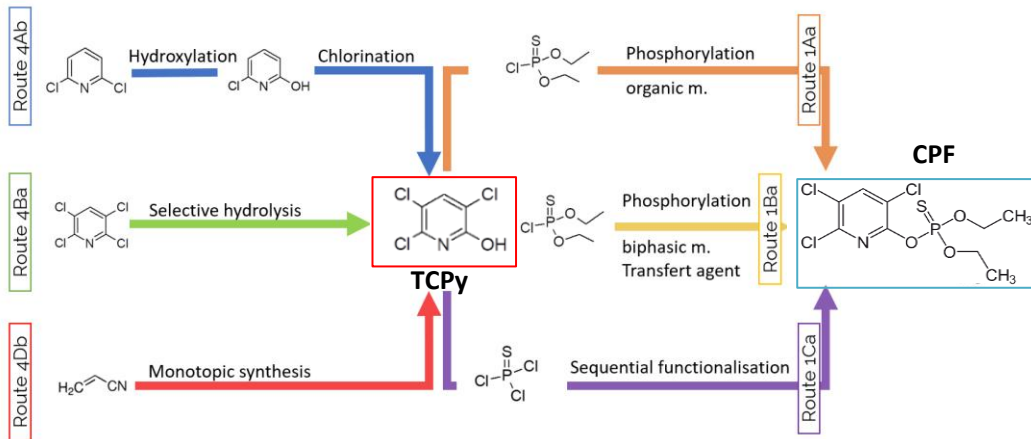
$C_9H_{11}Cl_3NO_3PS$
Exact Mass: 348.926288 u
CAS: 2921-88-2



> Syntheses of Chlorpyrifos



Simplest and least specific → preserve as much information as possible



X = H ; Cl
(di + tri + tetraCl)

X = H ; Cl
Y = H ; 'Bu ; P(S)(OEt)₂

X = H ; Cl
Y = H ; 'Bu ; P(S)(OEt)₂

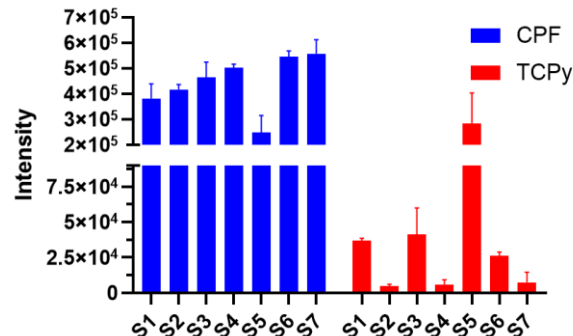
Wide range of compounds and possible impurities combinations

Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba

7 chlorpyrifos synthesis combinations (21 samples) – Exp1

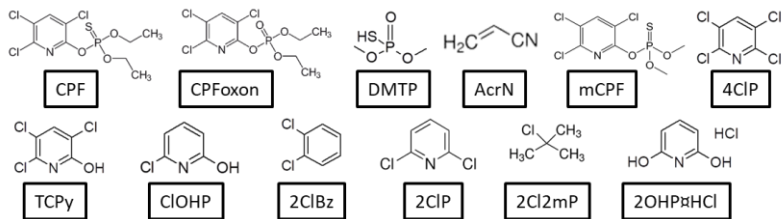
Wide variety of aspects → homogeneity of samples analyzed
(3 trials/triplicate/synthesis, i.e. 63 samples) – Exp2

Homogeneity of synthesis raw materials (Exp2)



> Analytical conditions LC-HRMS

Mode and Ionization optimization APCI & ESI



Positive mode ESI : more suitable for these compounds class and greater number of ions formed

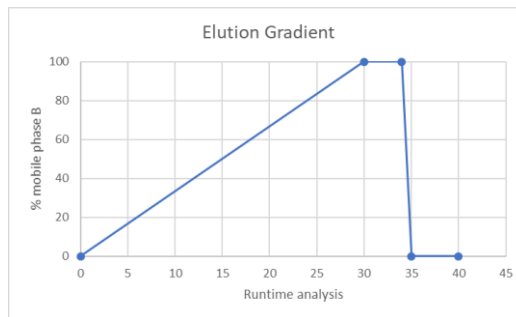


ACQUITY UPLC I class Waters

- Hypersil Gold C18 (100x2.1mm, 1.9 μ m)
- Mobile phases:
A) H₂O/CH₃OH/AcOH
B) CH₃OH/AcOH
- 0.3mL/min at 40°C
- Injection volume: 10 μ L

QToF Synapt G2-Si HRMS Waters

MS spectra m/z 50 - 800
MS resolution ~15000



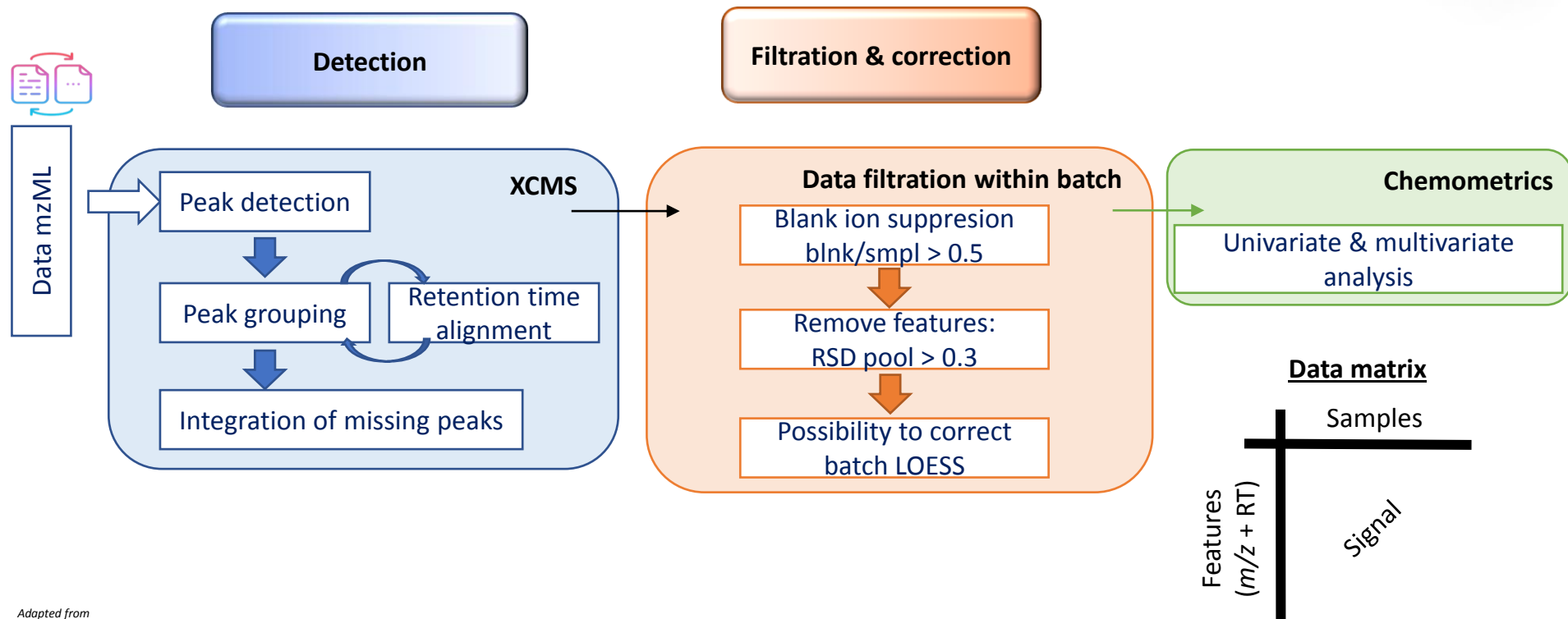
ESI parameters	POS
Capillary (kV)	0.7
Sampling Cone (V)	20
Source Offset	60
Source T (°C)	130
Desolvator T (°C)	250
Cone Gas (L/Hr)	50
Desolvation Gas (L/Hr)	550
Nebuliser G (Bar)	6.5

Sequence acceptability criteria (QC)

> Untargeted data processing



Workflow4metabolomics

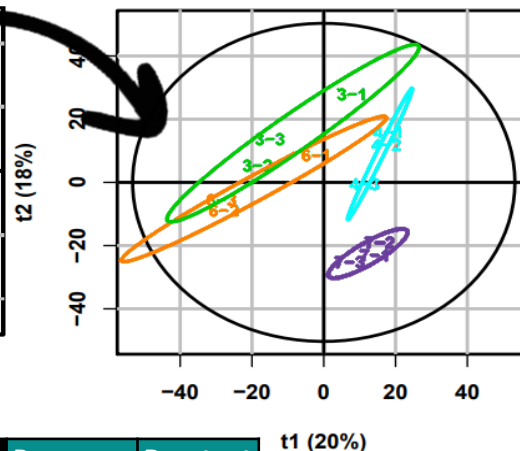
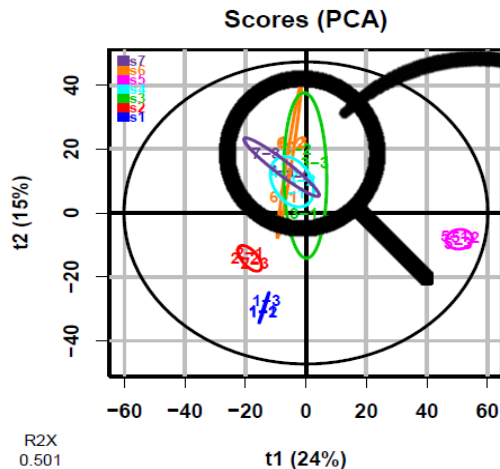
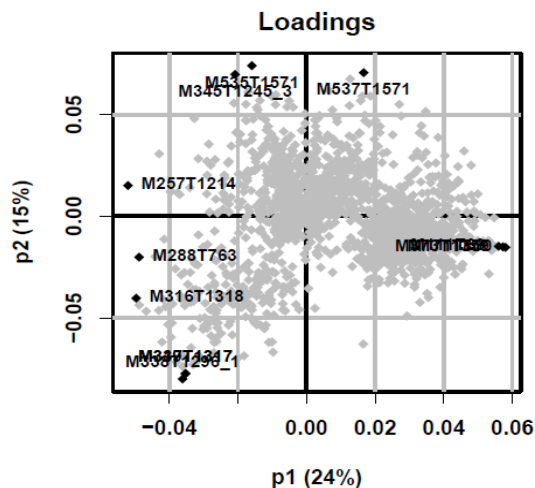


Adapted from
K. Dinis et al. Food Control 139 (2022) 109098

➤ Chemometrics

Multivariate methods suitable to provide a more complete description of studied phenomena

- Major directions of variability → PCA (unsupervised) → exploratory



PCA: First overview of variability between samples and correlation between features

Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba

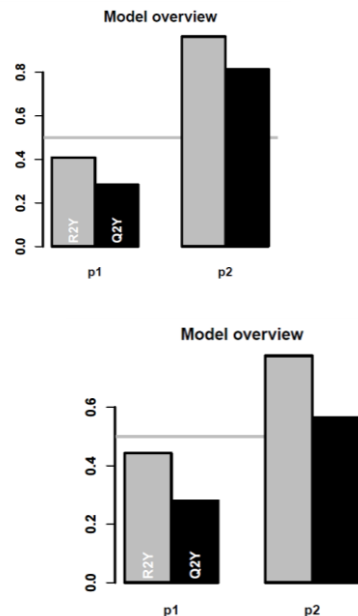
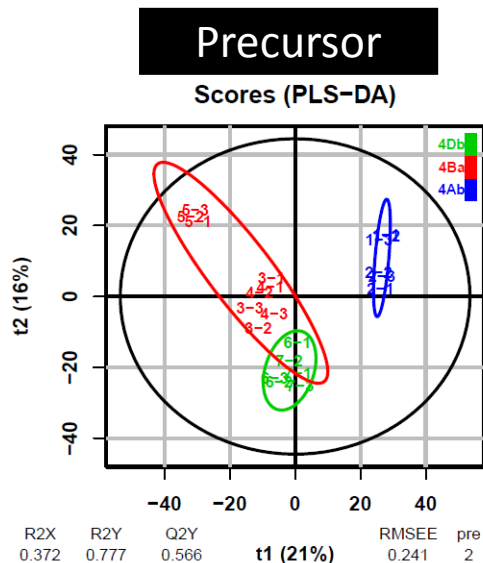
➤ Chemometrics

Multivariate methods suitable to provide a more complete description of studied phenomena

- Specific directions of variability → PLS (supervised) → predictive of regression or discrimination

Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba

Number of discriminating features (VIP>1)

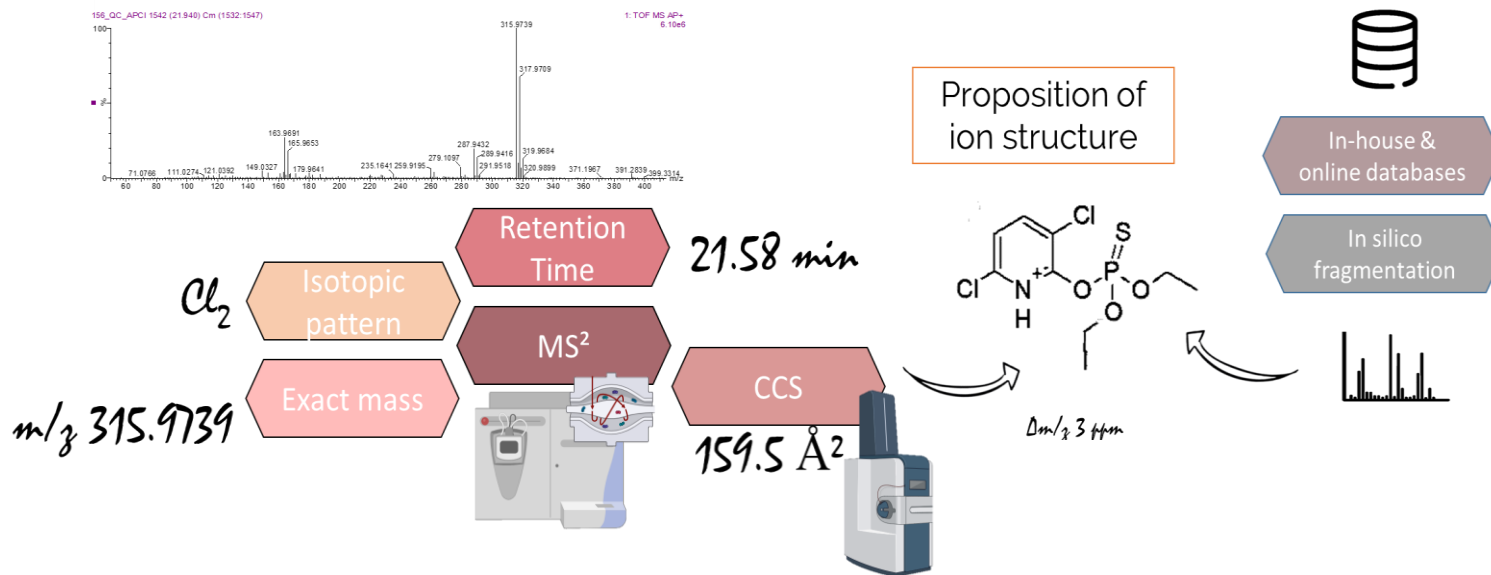


PLSDA: discrimination of synthetic sources achieved
Quality of the model was assessed with $Q^2Y > 0.4$ and permutation test

> Annotation Challenges

HRMS : sensitive and selective detection of discriminating impurities

- Features (PLSDA; VIP>1) annotated using LC, MS, MSMS and IMS to propose a putative structure
- MS² → characteristic fragments for structural elucidation



Sumner et al. *Metabolomics* (2007) 3, 211-221
Schymanski et al. *Environ Sci Technol.* (2014) 48:2097-8

[1] Confirmation Structure	[2] Probable Structure	[3] Tentative Candidate	[4] Unknown
4	14	28	55

> How sensitive is the model to variation?

Remove 20% of the weakest features

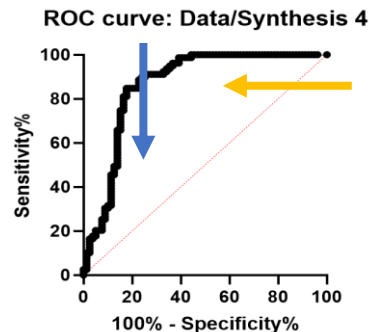
ROC CURVES	CTRL: Exp1 (21 samples)	PAT: Exp2 (63 samples)
Common discriminating features	intensity	intensity



Area under the ROC curve	S1	S2	S3	S4	S5	S6	S7
Area	0.85	0.85	0.83	0.86	0.76	0.78	0.82
95% confidence interval	0.78 to 0.92	0.78 to 0.91	0.76 to 0.90	0.80 to 0.93	0.68 to 0.83	0.71 to 0.85	0.75 to 0.89
P value <0.0001 ?	yes	yes	yes	yes	yes	yes	yes

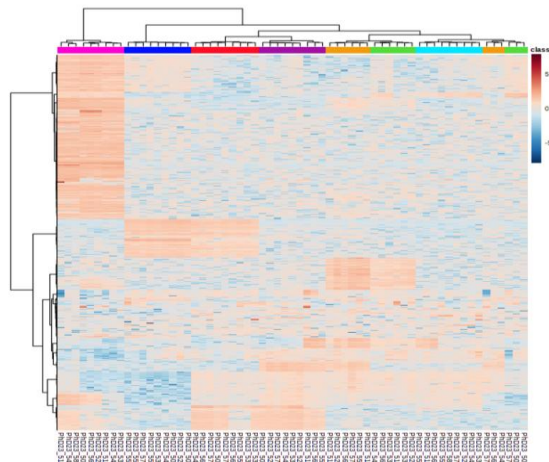
Specificity (Rmv 20% Exp1)	S1	S2	S3	S4	S5	S6	S7
Area	0.86	0.85	0.83	0.86	0.76	0.78	0.82
95% confidence interval	0.79 to 0.92	0.78 to 0.91	0.76 to 0.90	0.80 to 0.92	0.68 to 0.83	0.71 to 0.85	0.75 to 0.89
P value <0.0001 ?	yes	yes	yes	yes	yes	yes	yes

Sensitivity (Rmv 20% Exp2)	S1	S2	S3	S4	S5	S6	S7
Area	0.71	0.71	0.69	0.71	0.67	0.69	0.69
95% confidence interval	0.62 to 0.80	0.62 to 0.80	0.61 to 0.78	0.62 to 0.80	0.58 to 0.76	0.60 to 0.77	0.60 to 0.78
P value <0.0001 ?	yes	yes	yes	yes	no	yes	yes

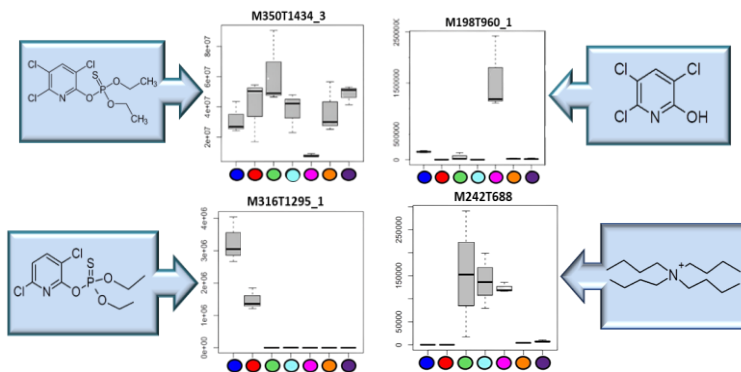


All the AUC values > 0.5 → results are not random
Model still valid to variations modeling real case samples

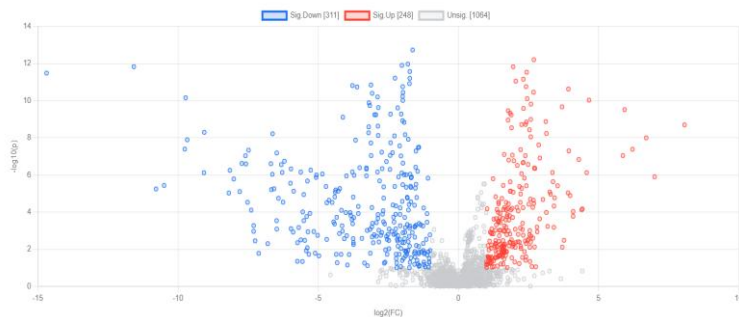
➤ Chemical Attribution Signature (W4M, MetaboAnalyst)



Heatmap

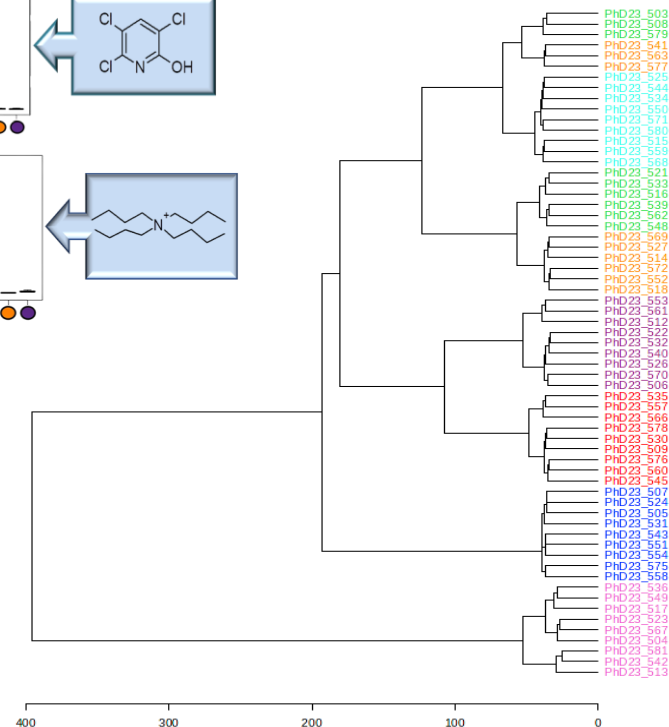


Boxplot



Volcano Plot (S1 vs. S2)

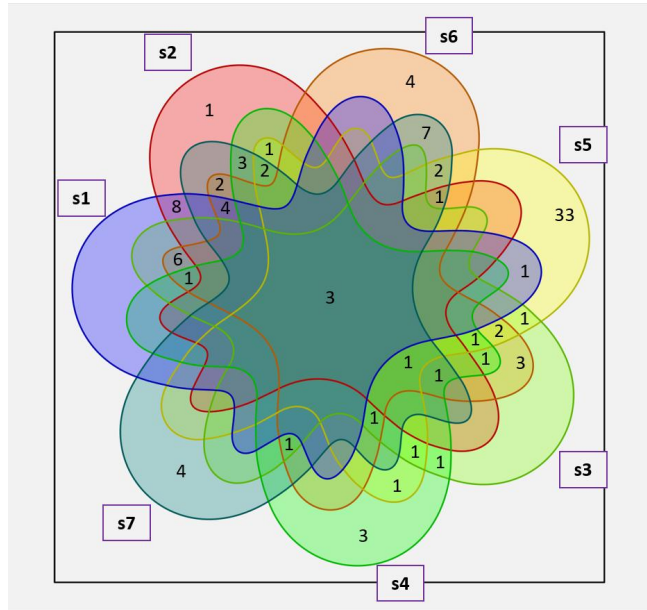
Hierarchical Clustering Analysis



Different graphical representations can be used to illustrate synthesis impurities giving information about sourcing

➤ Conclusions

- ⚙️ **Sensitivity** : Link a manufacturing origin/process to a chemical footprint from synthetic raw materials
- ⚙️ **Selectivity** : Footprint characteristic of a single route or set of pathways with common steps



> Perspectives

⊕ Ruggedness

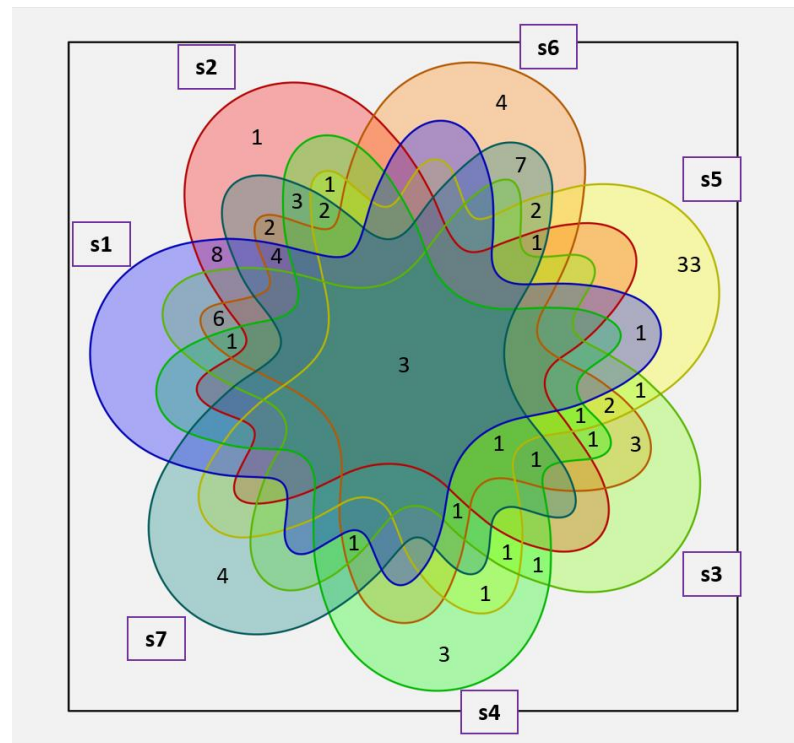
⊕ Is it applicable to complex samples?



Matrices
Sand, Water, Soils, Air

✓ Model Validation

✓ Crude yields



To be continued...

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Monitoring committee



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Thank you for your attention

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