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Differentiation of synthetic sources of an organophosphorus chemical by LC-HRMS based metabolomics

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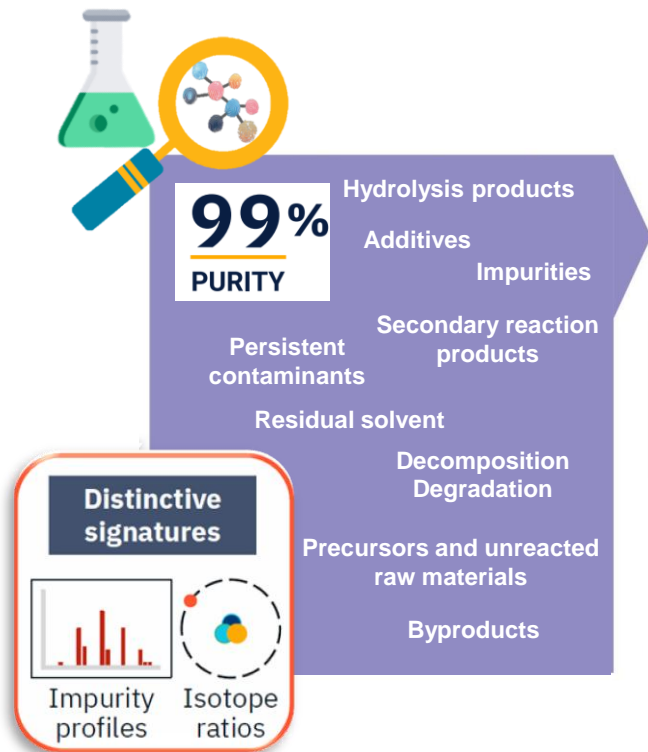
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➤ Differentiation of synthetic sources of an organophosphorous chemical by LC-HRMS based metabolomics

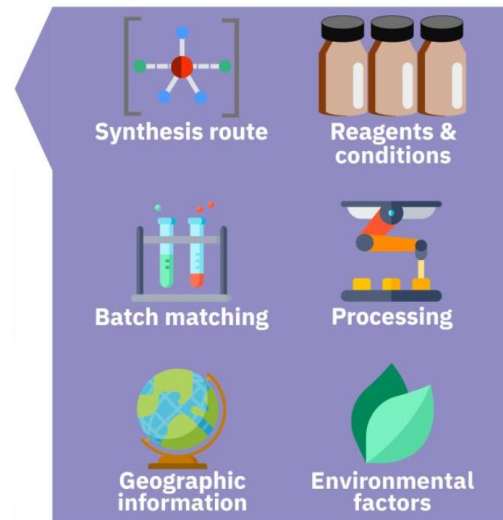
Carla ORLANDI
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> Sourcing and impurity profile

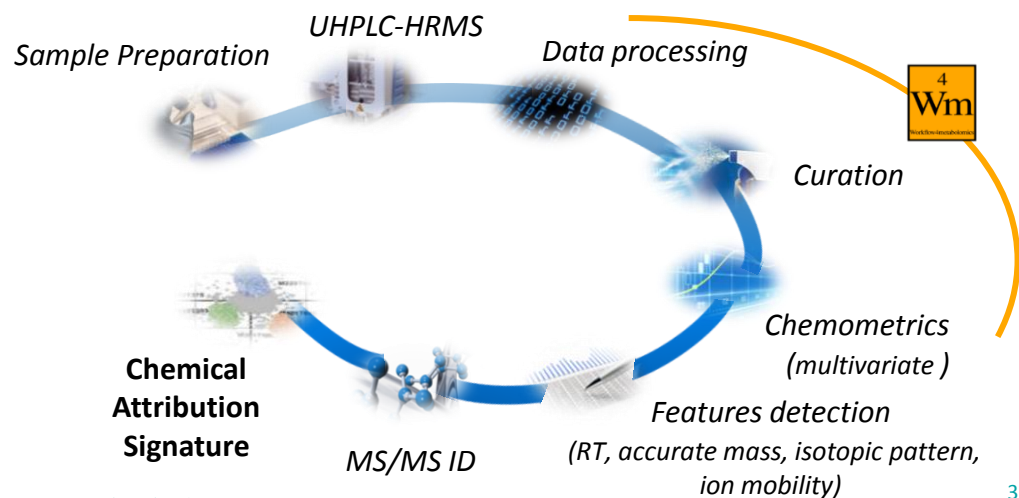
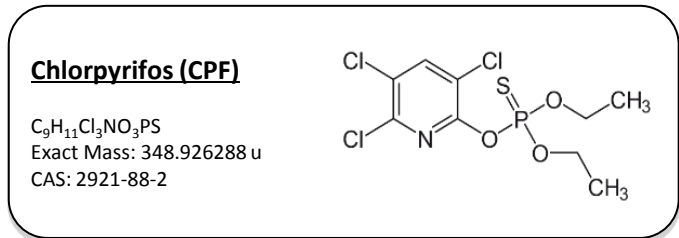
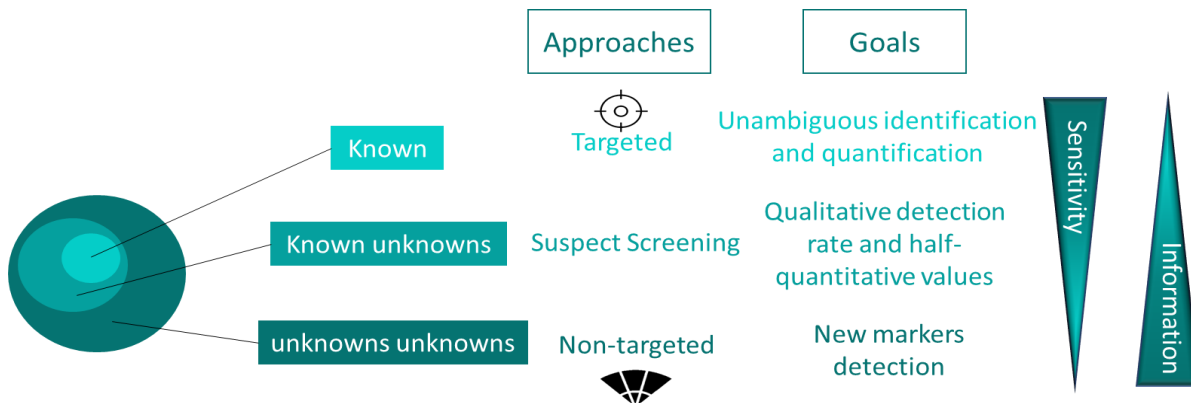


**Chemical
Attribution
Signature**



The role of chemical analysis is to provide well-founded findings about the source of the sample, to strengthen forensic investigations

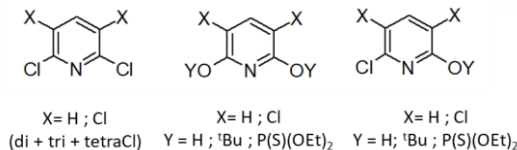
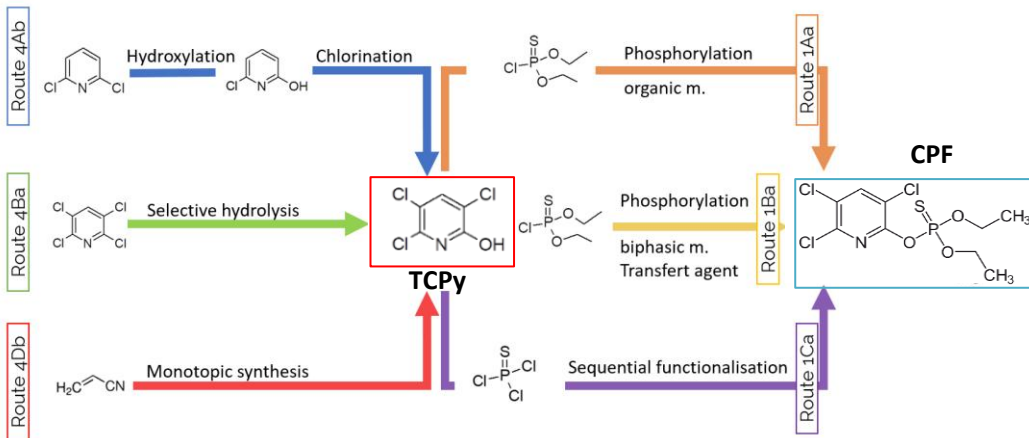
Metabolomics-based CAS discovery strategies



➤ Synthesis of chlorpyrifos samples



Simplest and least specific → preserve as much information as possible



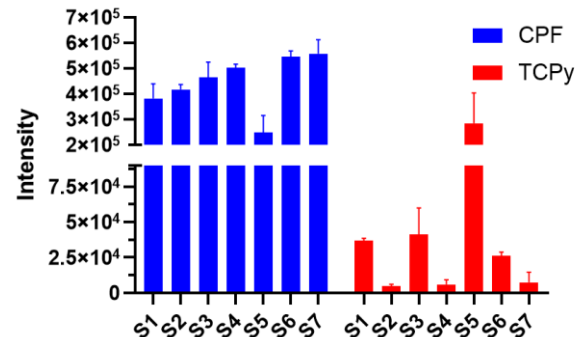
Wide range of compounds and possible impurities combinations

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

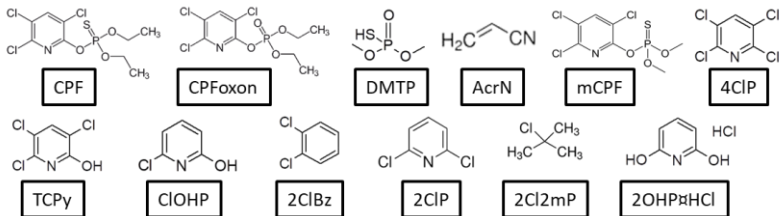
Homogeneity of synthesis raw materials (Exp2)



> 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

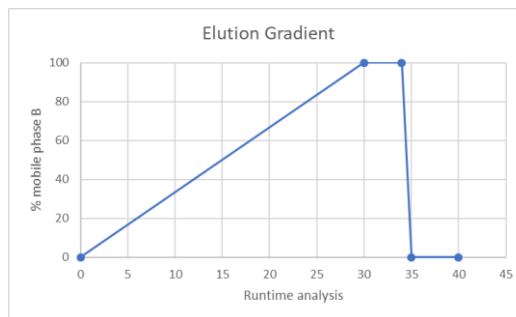


ACQUITY UPLC I class Waters

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

QToF Synapt G2-Si HRMS Waters

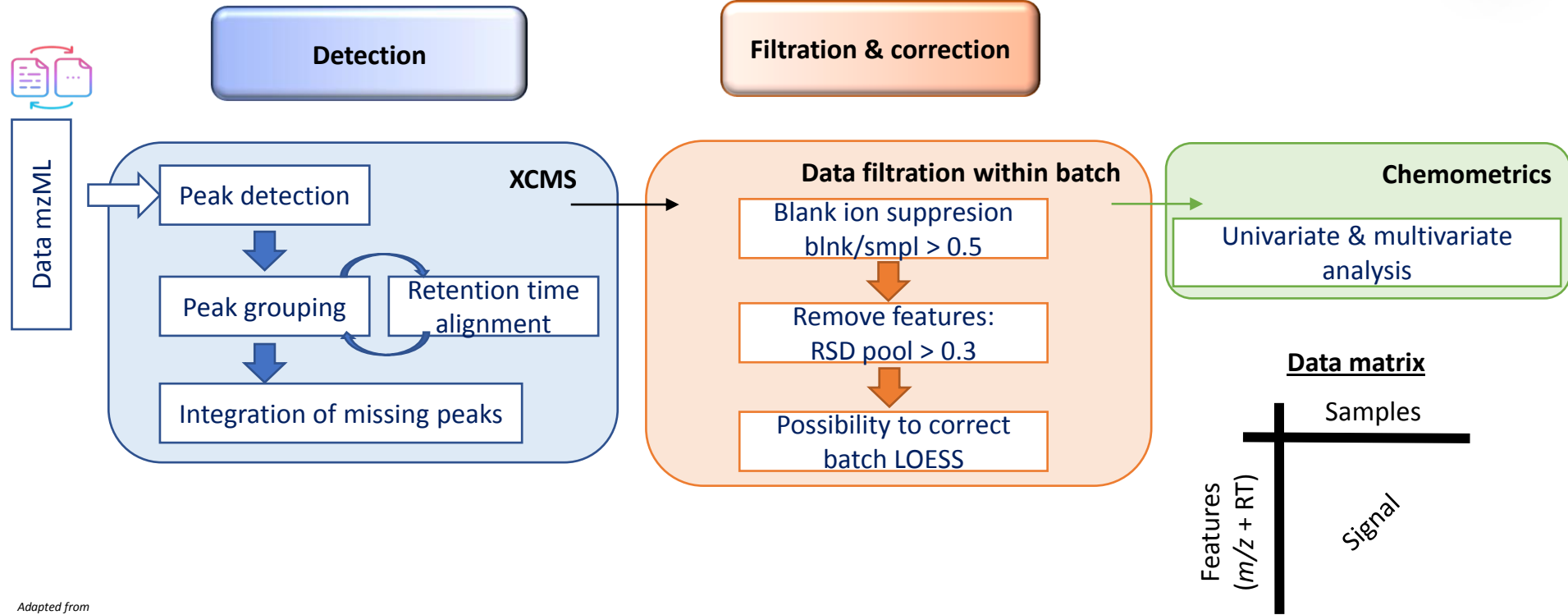
MS spectra m/z 50 - 800
MS resolution ~15000



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



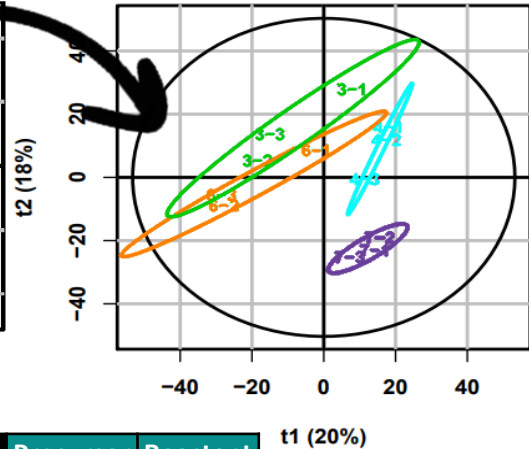
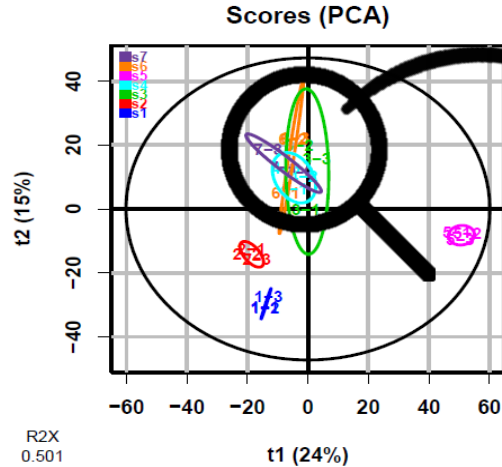
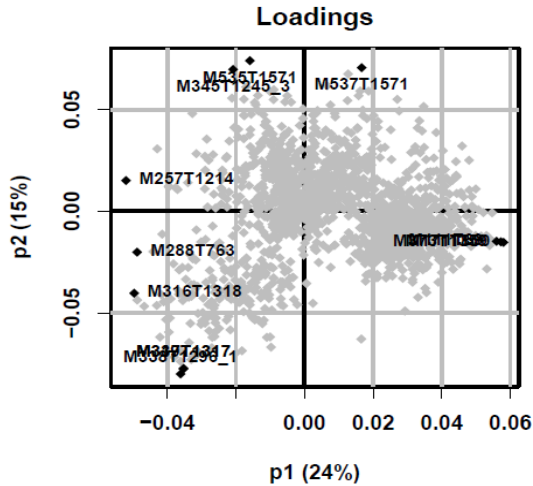
Adapted from
K. Dinis et al. Food Control 139 (2022) 109098

> 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

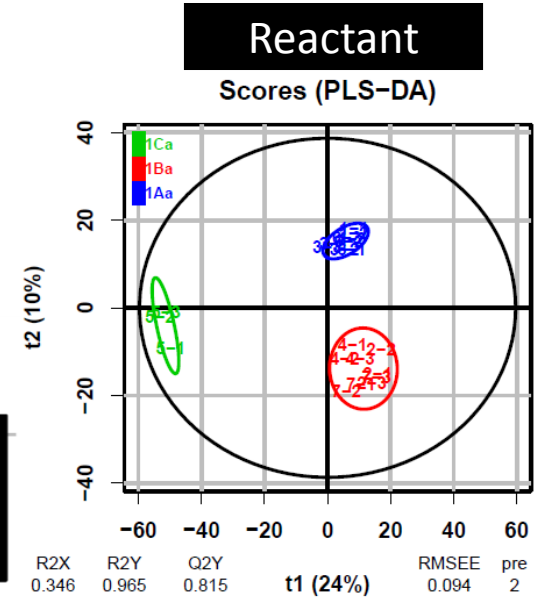
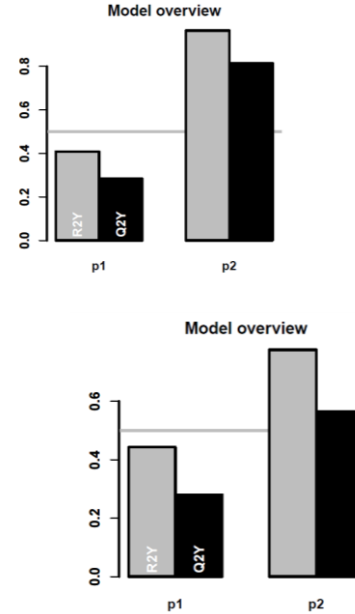
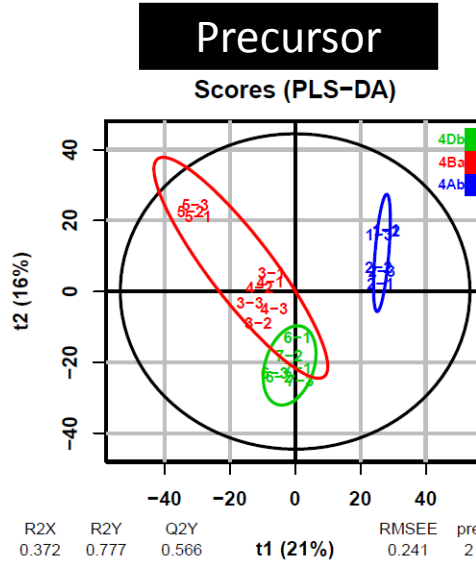
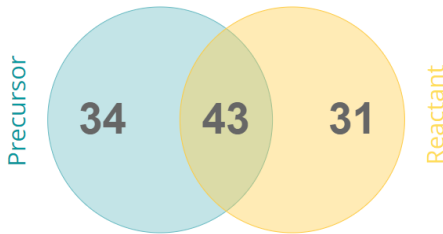


Multivariate methods suitable to provide a more complete description of studied phenomena

- 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)



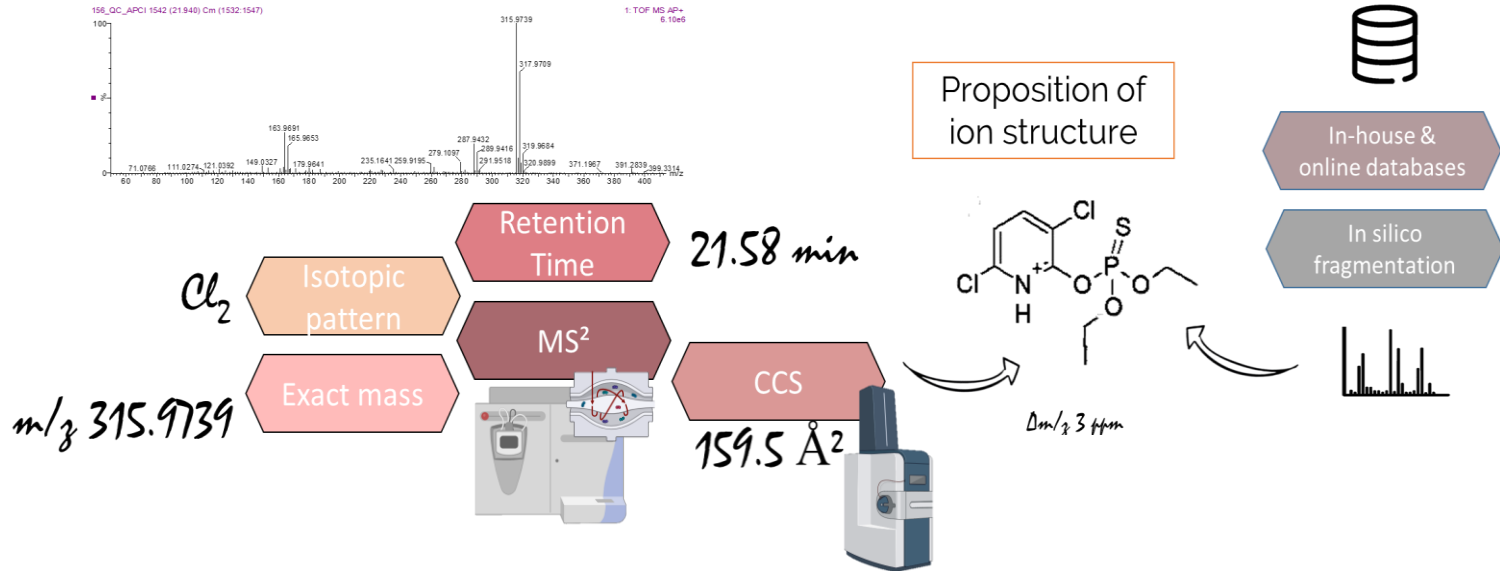
PLS-DA: discrimination of synthetic sources achieved
 Quality of the model was assessed with $Q^2Y > 0.4$ and permutation test

Annotation Challenges



HRMS : sensitive and selective detection of discriminating impurities

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



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

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

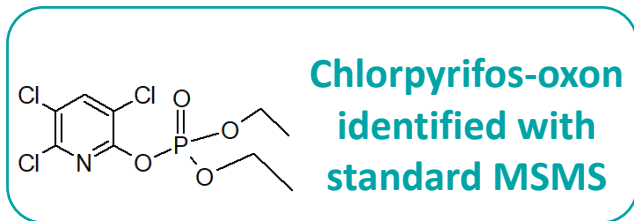
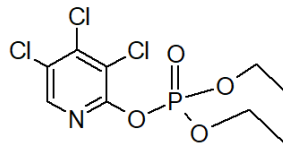
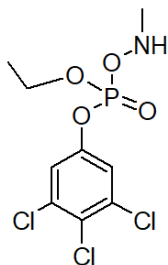
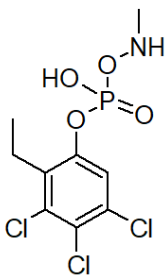
> Ion mobility: an annotation aid



m/z 333.9468
Rt: 20 min
 $C_9H_{11}Cl_3NO_4P$



CCS measurement: 162.7 Å²



CCS predicted	166.1 Å ²	165.5 Å ²	165.1 Å ²	165.5 Å ²
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5% accuracy acceptance

CCS: theoretical values similar between structure proposals
Support to annotation → remove hypothesis, putative information of structure

➤ How sensitive is the model to variation?

Remove 20% of the weakest features

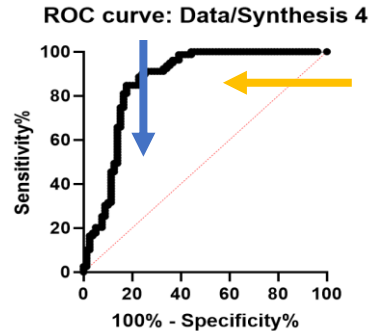
ROC CURVES	CTRL: Exp1 (21 samples)	PAT: Exp2 (63 samples)
Common discriminating features	intensity	intensity



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

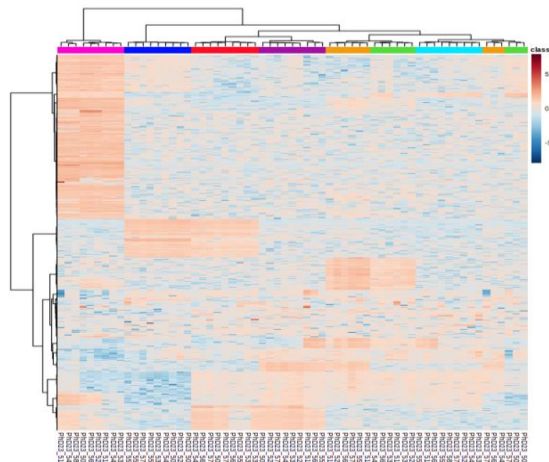
Specificity (Rmv 20% Exp1)	S1	S2	S3	S4	S5	S6	S7
Area	0.86	0.85	0.83	0.86	0.76	0.78	0.82
95% confidence interval	0.79 to 0.92	0.78 to 0.91	0.76 to 0.90	0.80 to 0.92	0.68 to 0.83	0.71 to 0.85	0.75 to 0.89
P value <0.0001 ?	yes	yes	yes	yes	yes	yes	yes

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

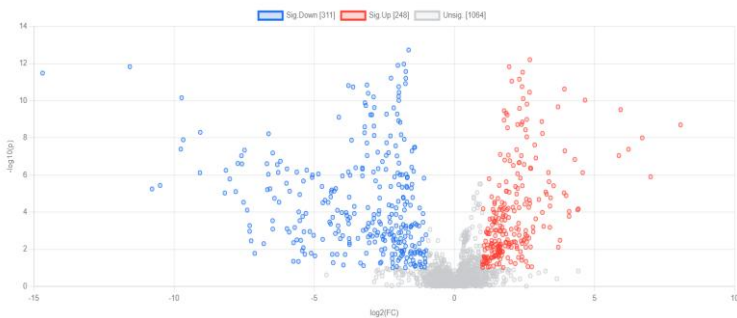


All the AUC values > 0.5 → results are not random
 Model still valid to variations modeling real case samples

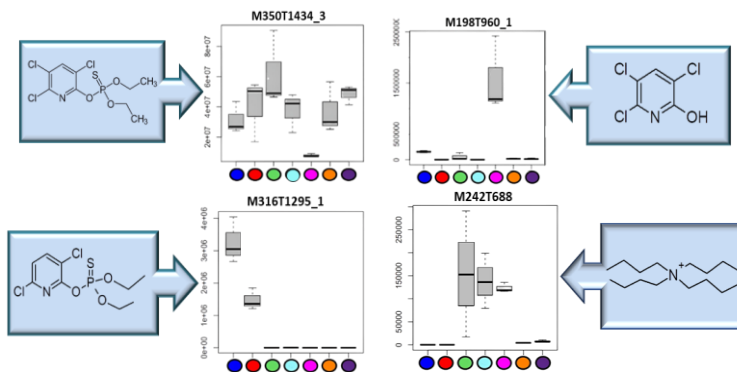
➤ Chemical Attribution Signature (W4M, MetaboAnalyst)



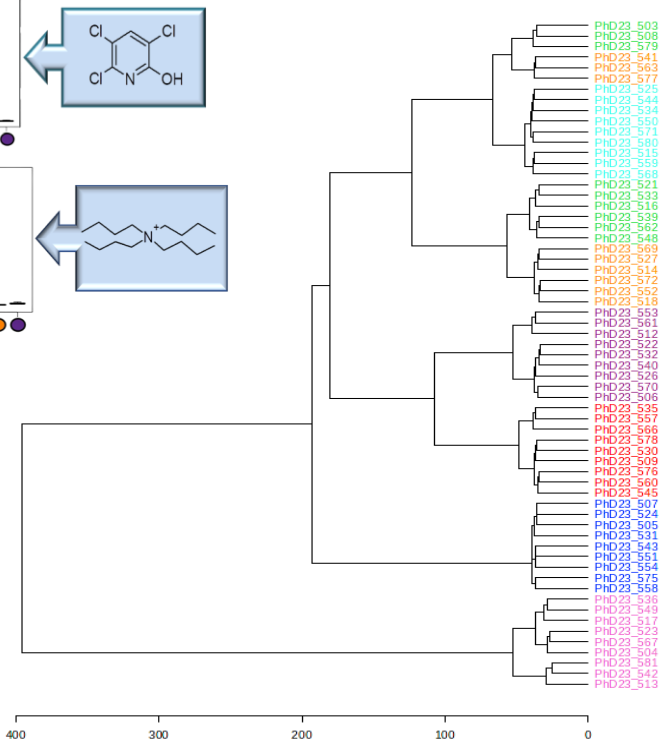
Heatmap



Volcano Plot (S1 vs. S2)



Boxplot



Hierarchical Clustering Analysis

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

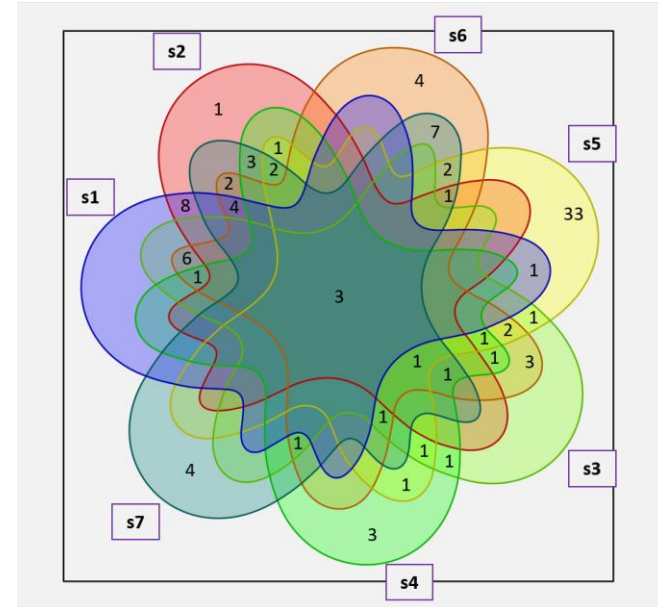
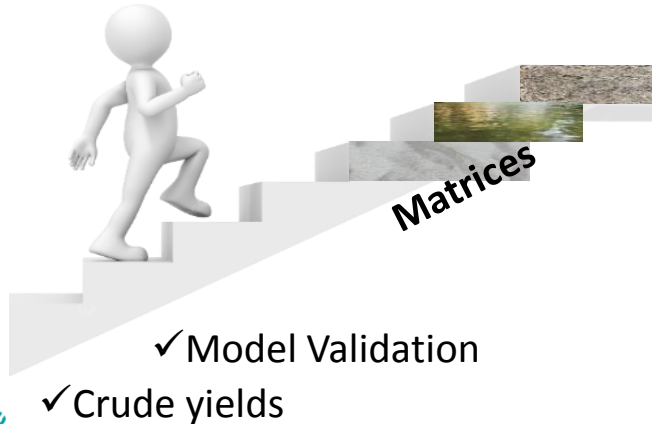
> Conclusions

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

- ⊕ **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?



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Thank you for your attention

