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

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

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Sourcing and impurity profile

99% PURITY

Hydrolysis products

Additives

Impurities

Secondary reaction products

Persistent contaminants

Residual solvent

Decomposition Degradation

Precursors and unreacted raw materials

Byproducts

Distinctive signatures

Impurity profiles

Isotope ratios

Chemical

Attribution

Signature

Synthesis route

Reagents & conditions

Batch matching

Processing

Geographic information

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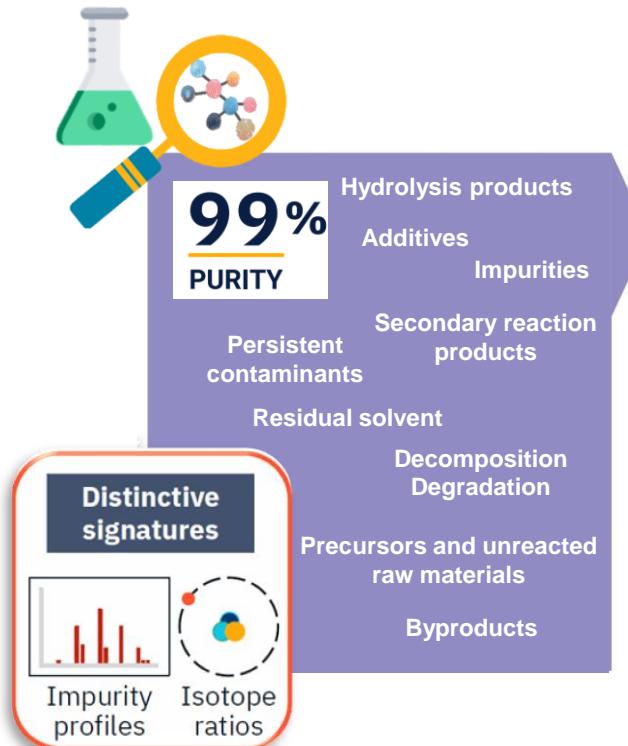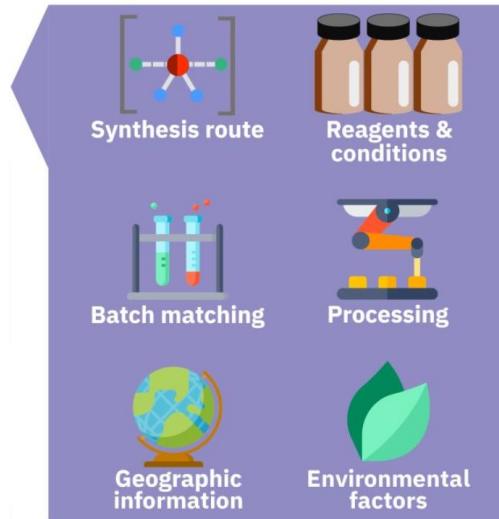
The role of chemical analysis is to provide well-founded findings about the source of the sample, to strengthen forensic investigations

# Metabolomics-based CAS discovery strategies

Approaches | Goals

Known

Known unknowns — Suspect Screening

unknowns unknowns — Non-targeted

Targeted — Unambiguous identification and quantification

Qualitative detection rate and half-quantitative values

New markers detection

Sensitivity

Information

Chlorpyrifos (CPF)

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

Sample Preparation | UHPLC-HRMS | Data processing | Curation | Chemometrics *(multivariate)* | Features detection *(RT, accurate mass, isotopic pattern, ion mobility)* | MS/MS ID | **Chemical Attribution Signature**

Wm^4

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Synthesis of chlorpyrifos samples

Simplest and least specific → preserve as much information as possible
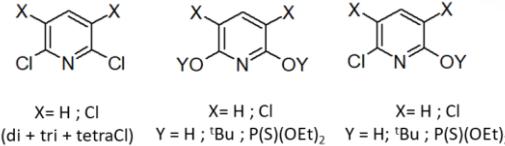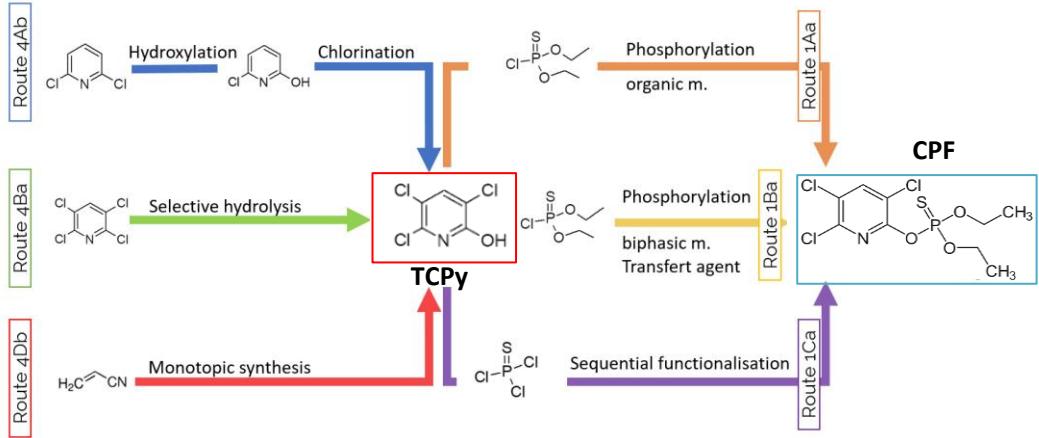
Wide range of compounds and possible impurities combinations

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)
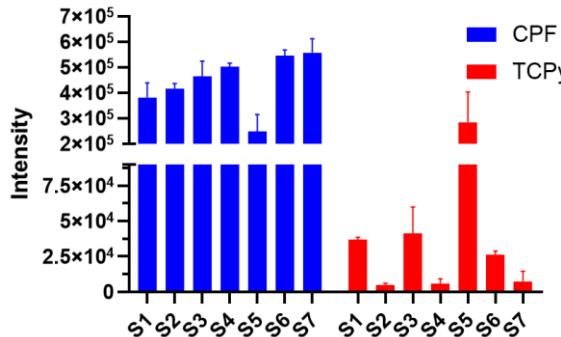
Mode and Ionization optimization
APCI & ESI

CPF | CPFoxon | DMTP | AcrN | mCPF | 4ClP

TCPy | ClOHP | 2ClBz | 2ClP | 2Cl2mP | 2OHP¤HCl
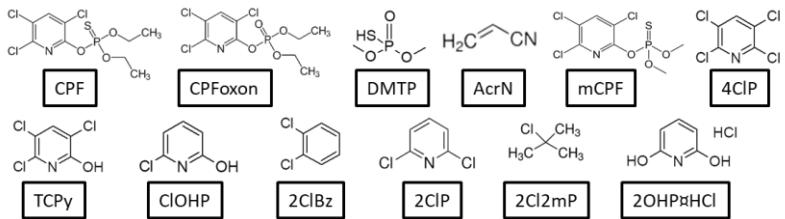
Positive mode ESI : more suitable for these compounds class and greater number of ions formed

ACQUITY UPLC I class Waters
- Hypersil Gold C18 (100x2.1mm, 1.9μm)
- Mobile phases:
  A) $H_2O/CH_3OH/AcOH$
  B) $CH_3OH/AcOH$
- 0.3mL/min at 40°C
- Injection volume: 10μL

QToF Synapt G2-Si HRMS Waters
MS spectra m/z 50 - 800
MS resolution ~15000

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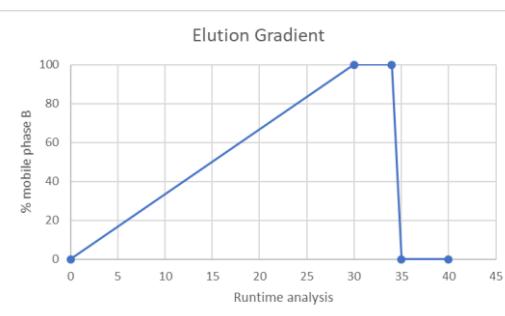
ESI parameters	POS
Capillary (kV)	0.7
Sampling Cone (V)	20
Source Offset	60
Source T (°C)	130
Desolvaton T (°C)	250
Cone Gas (L/Hr)	50
Desolvation Gas (L/Hr)	550
Nebuliser G (Bar)	6.5

Sequence acceptability criteria (QC)

Untargeted data processing

Workflow4metabolomics

Detection

XCMS

Data mzML →

Peak detection
↓
Peak grouping ⟲ Retention time alignment
↓
Integration of missing peaks

Filtration & correction

Data filtration within batch

Blank ion suppresion
blnk/smpl > 0.5
↓
Remove features:
RSD pool > 0.3
↓
Possibility to correct
batch LOESS
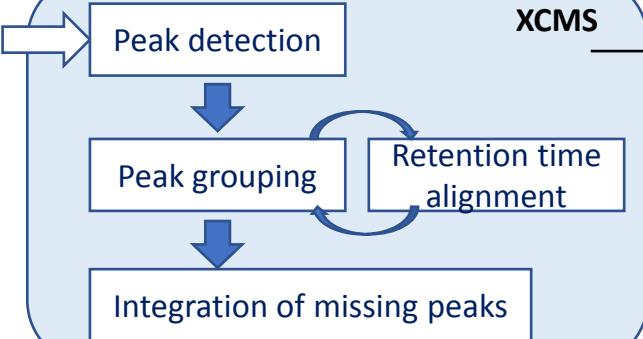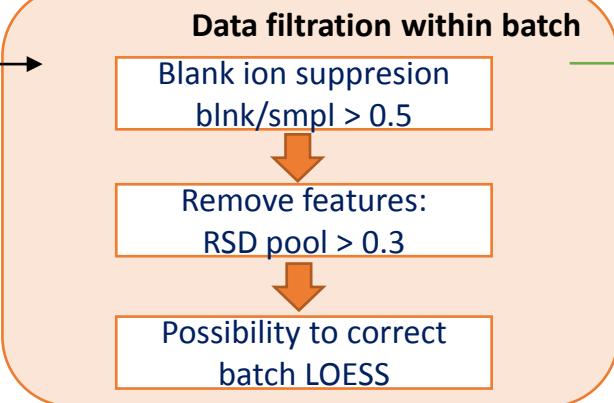
Chemometrics

Univariate & multivariate analysis

Data matrix

Samples

Features
(*m/z* + RT)

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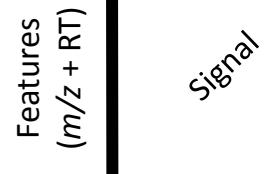
*Adapted from*
K. Dinis et al. Food Control 139 (2022) 109098

Multivariate methods suitable to provide a more complete description of studied phenomena
- Major directions of variability → PCA (unsupervised) → exploratory
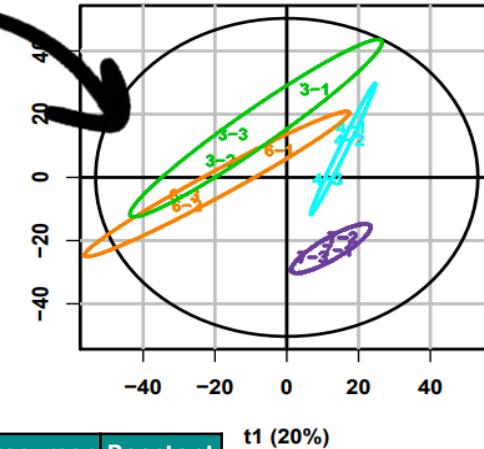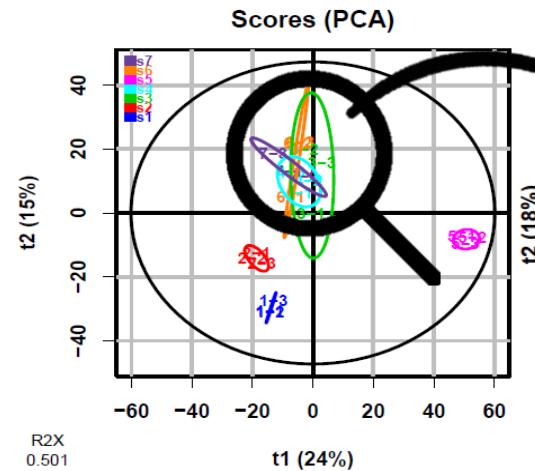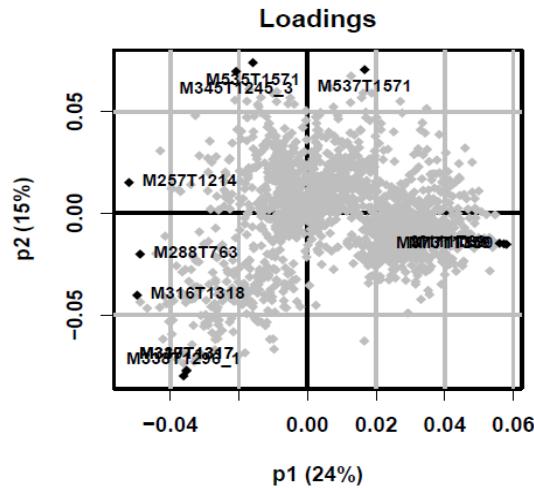
PCA: First overview of variability between samples and correlation between features

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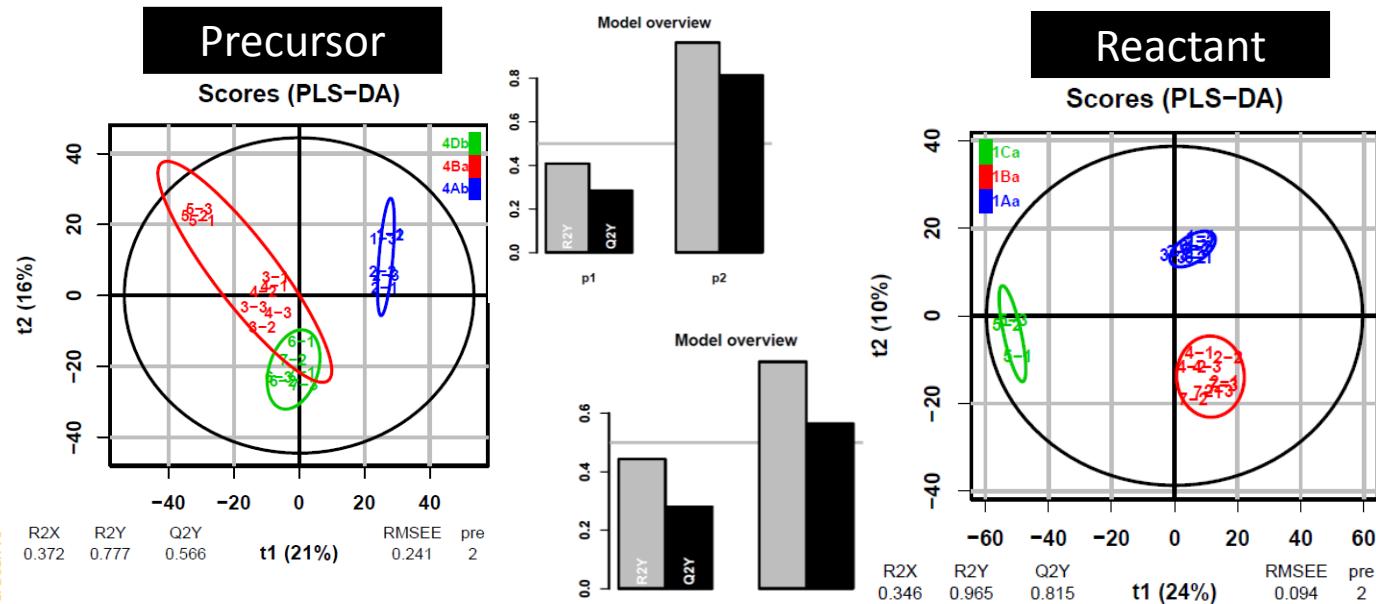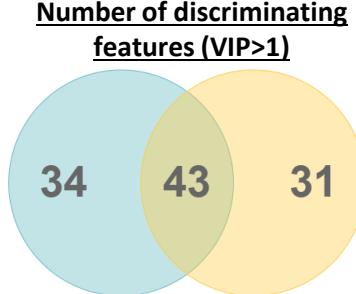
PLS-DA: discrimination of synthetic sources achieved
Quality of the model was assessed with $Q^2Y > 0.4$ and permutation test

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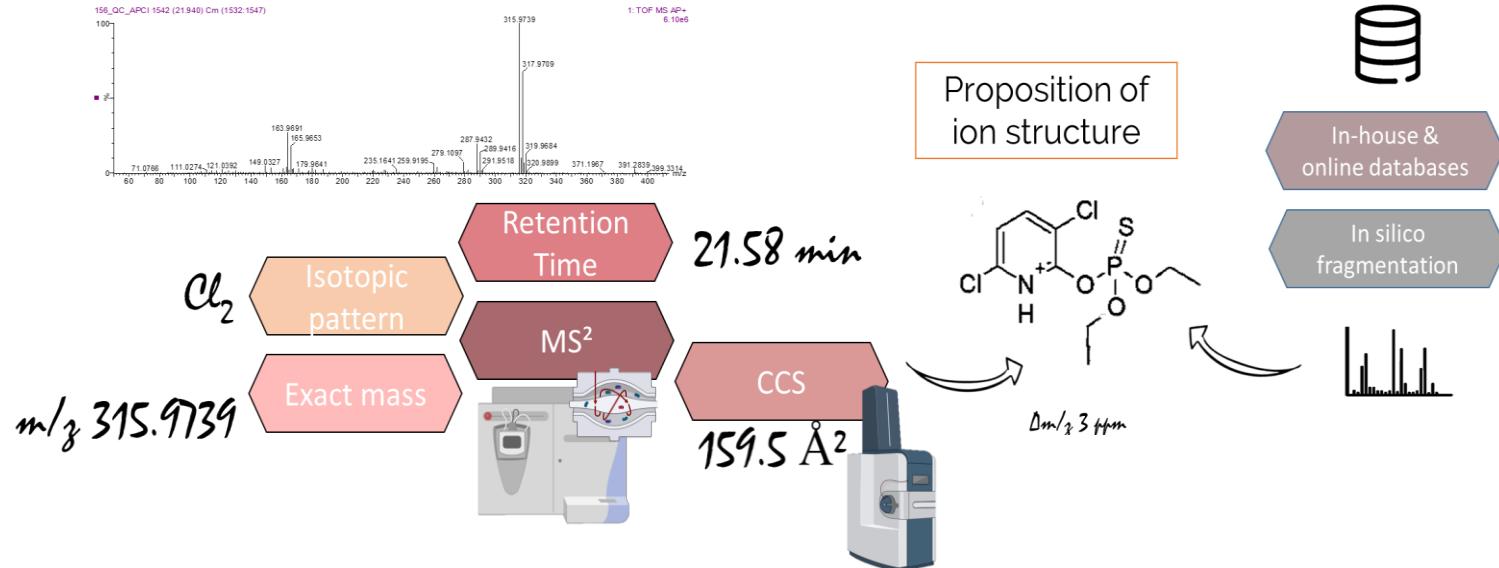
Sumner et al. Metabolomics (2007) 3, 211-221
*Schymanski et al. Environ Sci Technol. (2014) 48:2097-8*

Ion mobility: an annotation aid


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

CCS measurement: 162.7 Å²
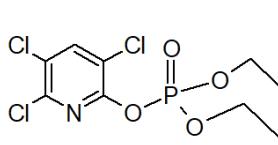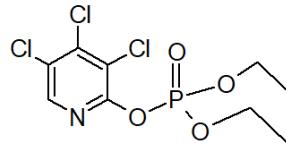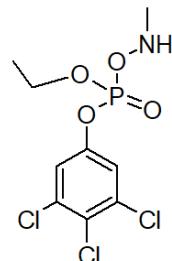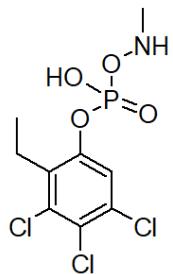
Chlorpyrifos-oxon identified with standard MSMS

CCS predicted	166.1 Å²	165.5 Å²	165.1 Å²	165.5 Å²

5% accuracy acceptance

CCS: theorical 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

ROC curve: Data/Synthesis 4
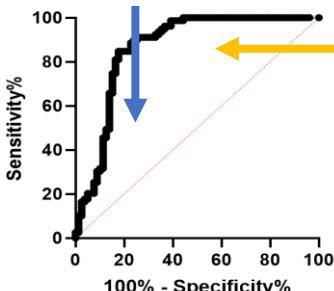
All the AUC values > 0.5 → results are not random
Model still valid to variations modeling real case samples

Heatmap

Boxplot

Hierarchical Clustering Analysis

Volcano Plot (S1 vs. S2)
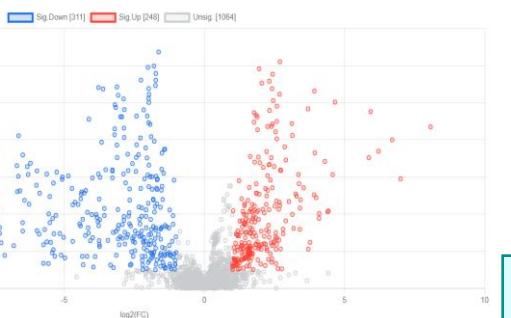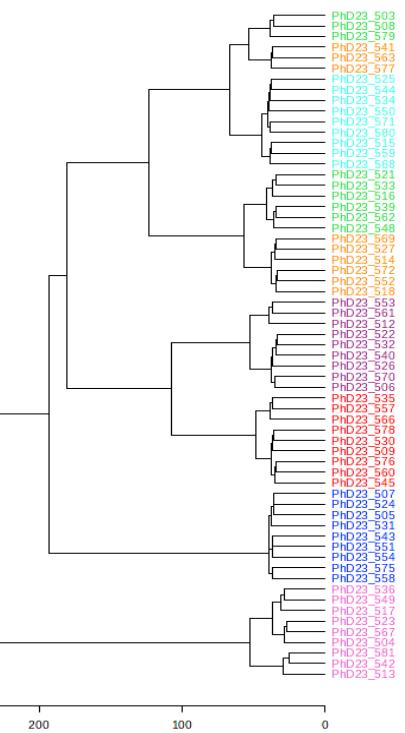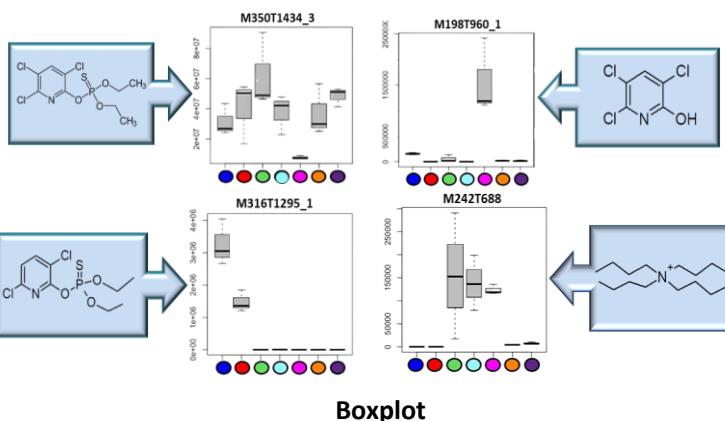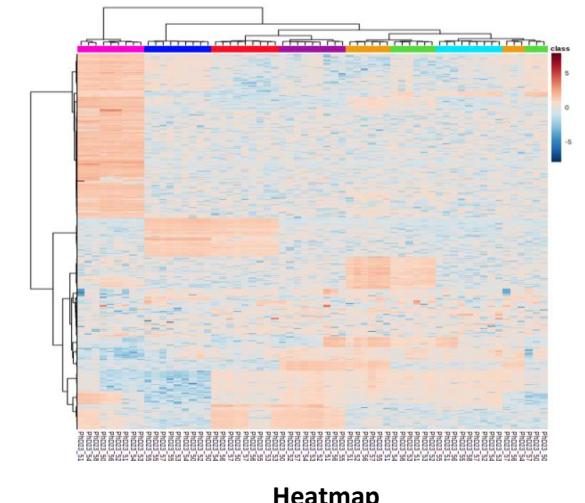
Different graphical representations can be used to illustrate synthesis impurities giving information about sourcing

❯ 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?

Matrices

✓ Model Validation
✓ Crude yields

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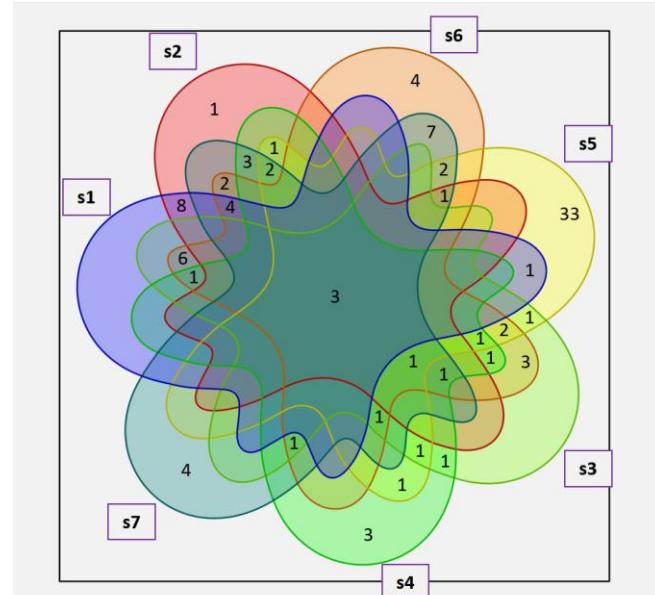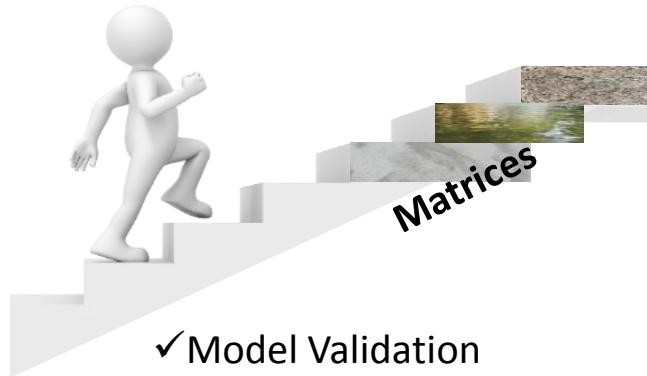
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Thank you for your attention