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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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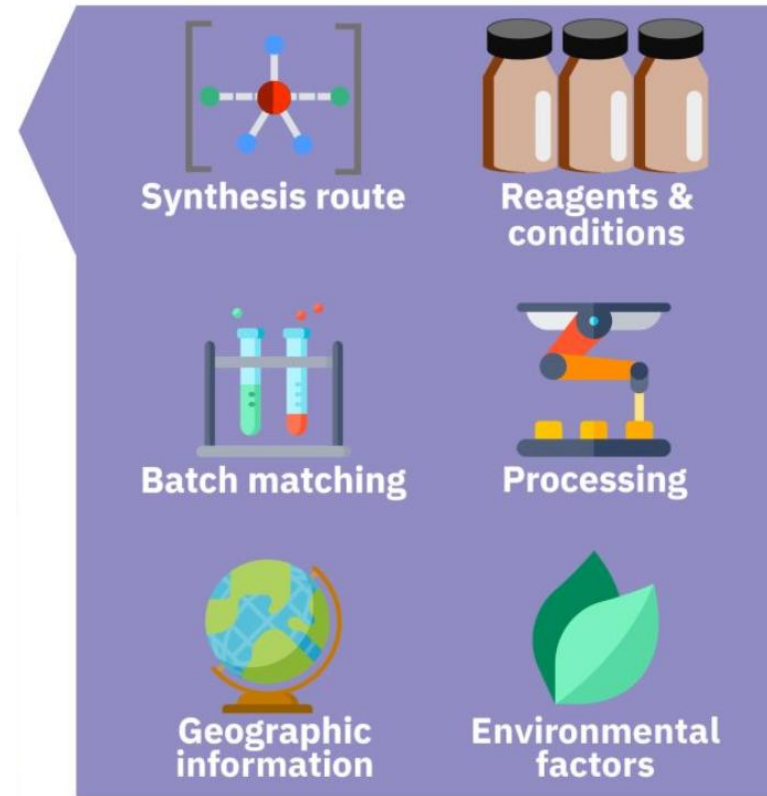
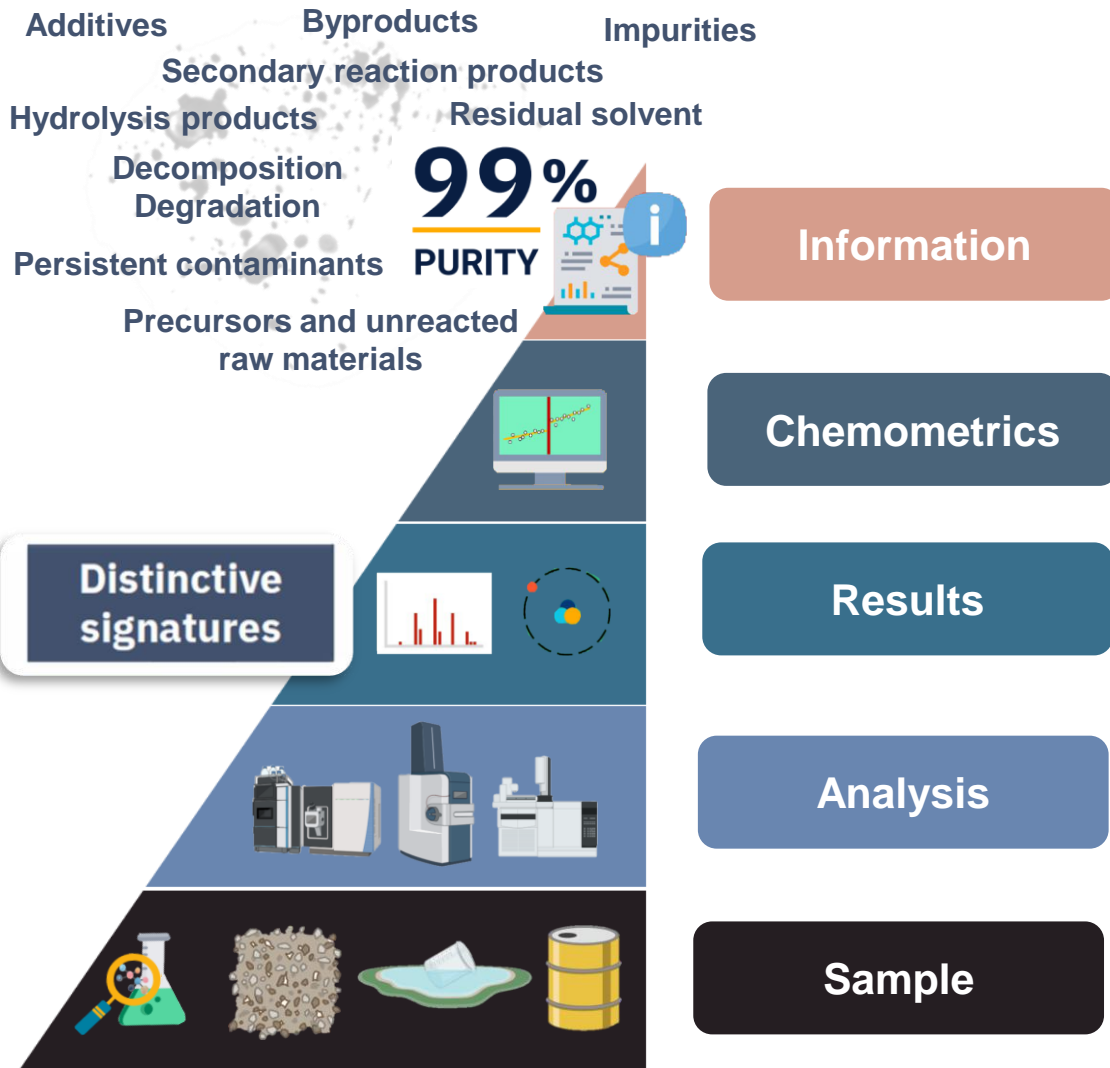


# ➤ High-resolution mass spectrometry-based untargeted approach

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

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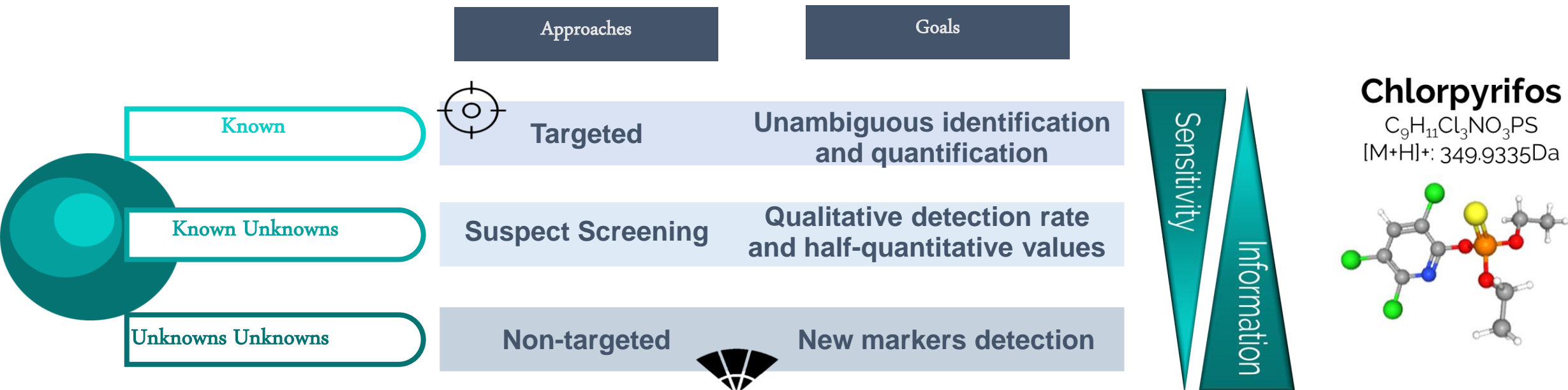
## ➤ Sourcing and impurity profile



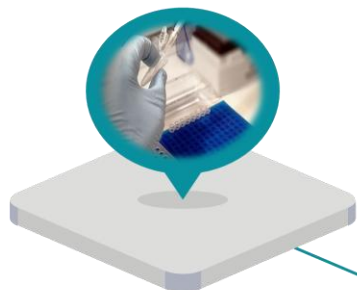
**Chemical  
Attribution  
Signature**

Distinctive signatures can reveal well-founded findings about a chemical's production history, to strengthen forensic investigations.

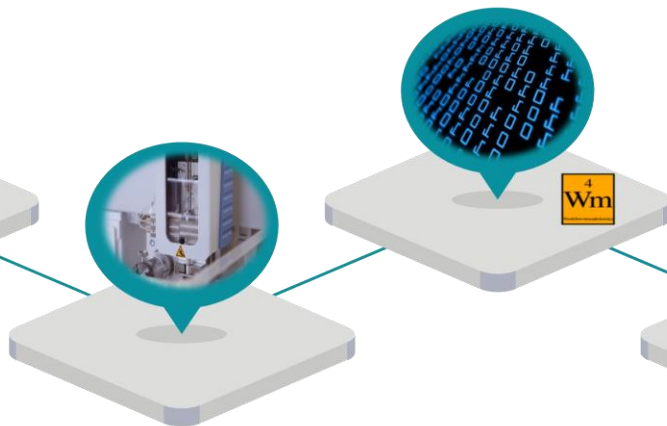
# Metabolomics-based CAS discovery strategies



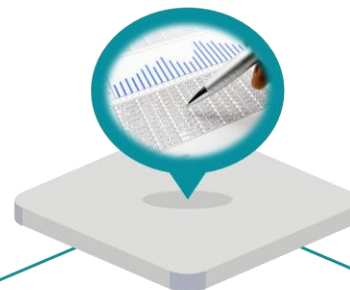
## Sample Preparation



## Data Processing and Curation



## Features detection



## Chemical Attribution Signature



*RT, accurate mass, isotopic pattern, ion mobility*

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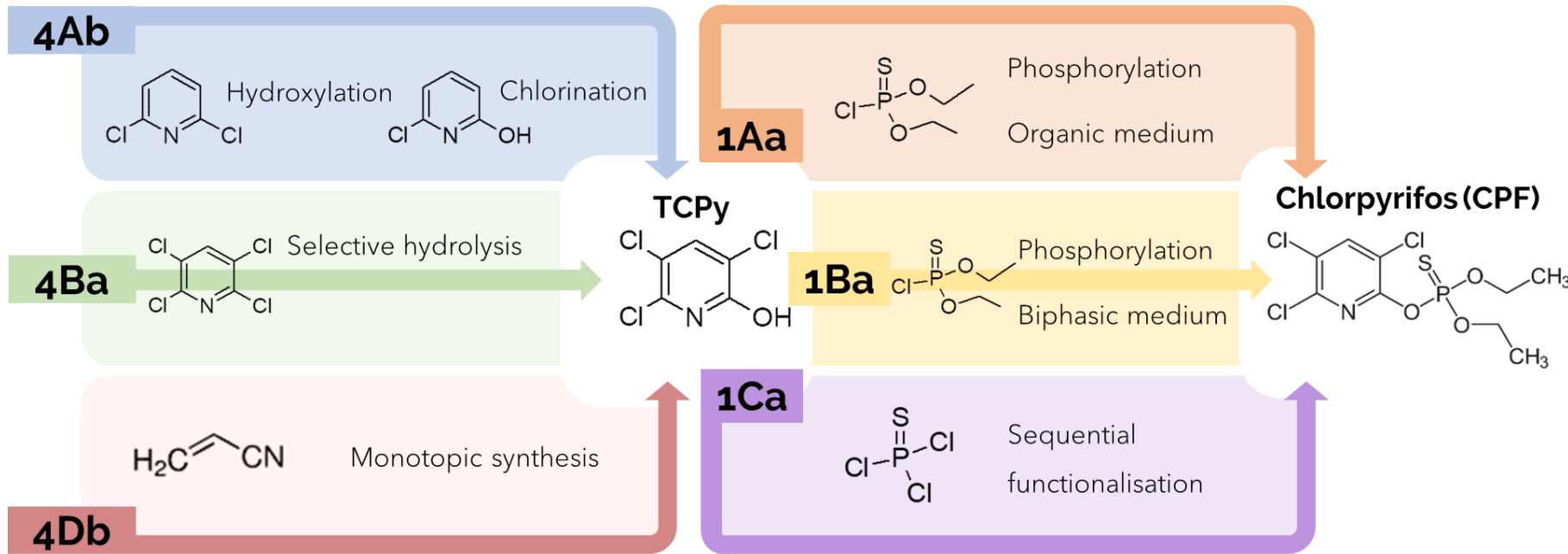
UHPLC-HRMS

Chemometrics

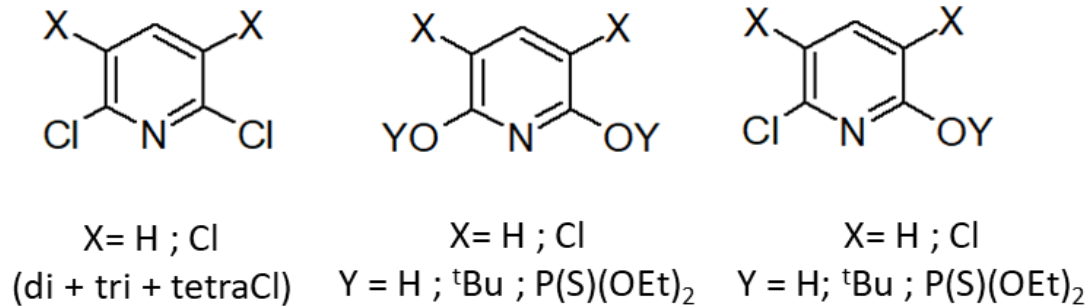
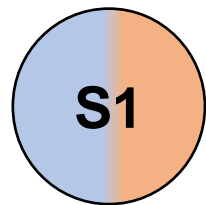
Multivariate analysis

MS/MS identification

➤ Our use-case: chlorpyrifos (various synthetic routes)



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



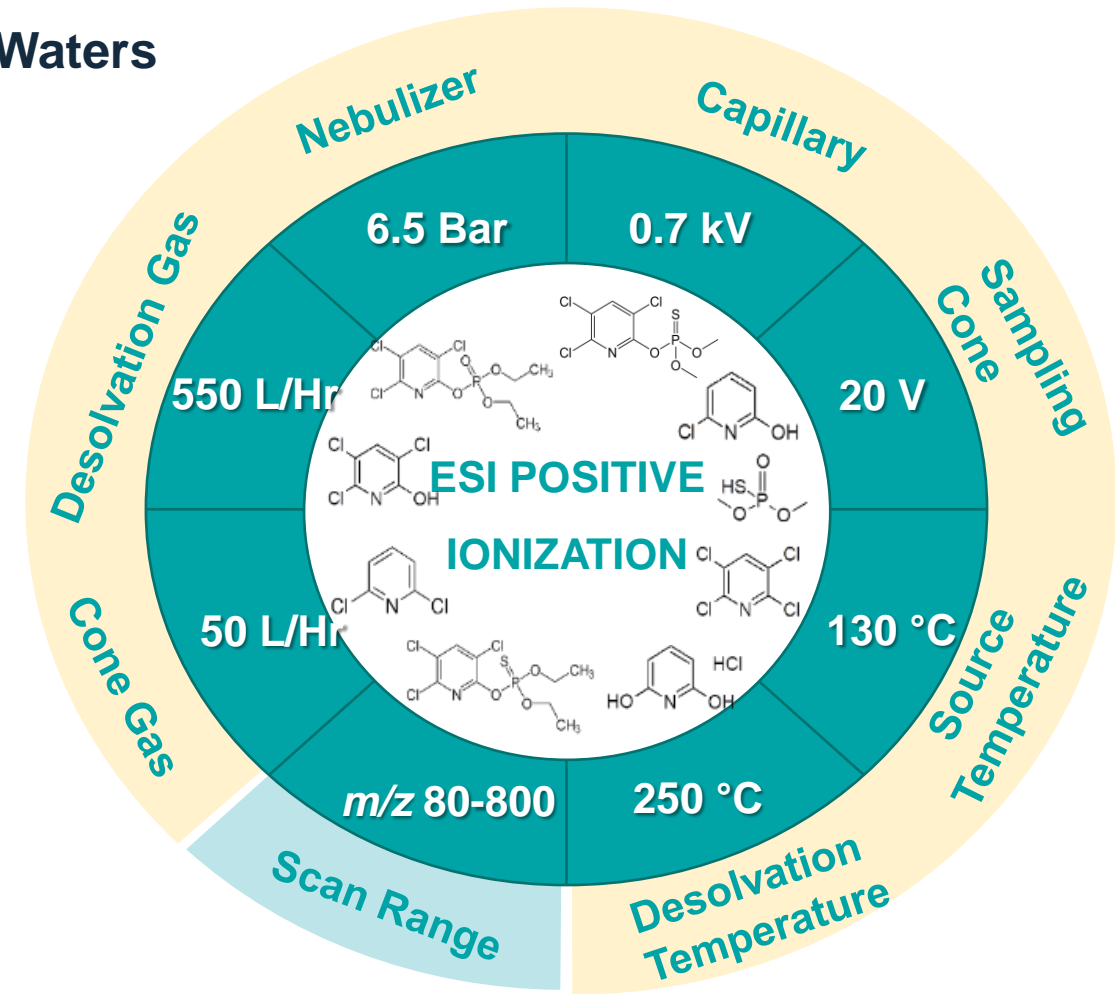
Wide range of compounds and possible impurities combinations

➤ Analytical LC-HRMS conditions



**ACQUITY UPLC I Class**  
**QToF Synapt G2-Si HRMS Waters**

<b>Column</b>	Hypersil Gold C18 (100 x 2.1mm, 1.9µm)
<b>Mobile phase</b>	A) H <sub>2</sub> O/CH <sub>3</sub> OH/AcOH (95/5/0.1) B) CH <sub>3</sub> OH/AcOH (100/0.1)
<b>Flow rate and Oven temperature</b>	0.3mL/min at 40°C
<b>Injection volume</b>	10µL



• MS Resolution : ~ 15000

# ➤ Untargeted data processing



## Workflow4metabolomics



Analytical and blank noise  
RSD pool features

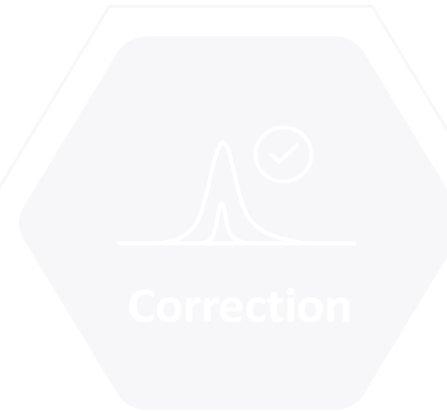
Univariate and multivariate



Universal data  
format  
(mzML)



Peak detection and grouping  
Retention time alignment  
Integrating missing data



Batch and analytical drift  
Standardization



Data matrix

	Samples
Features (m/z + RT)	Intensity



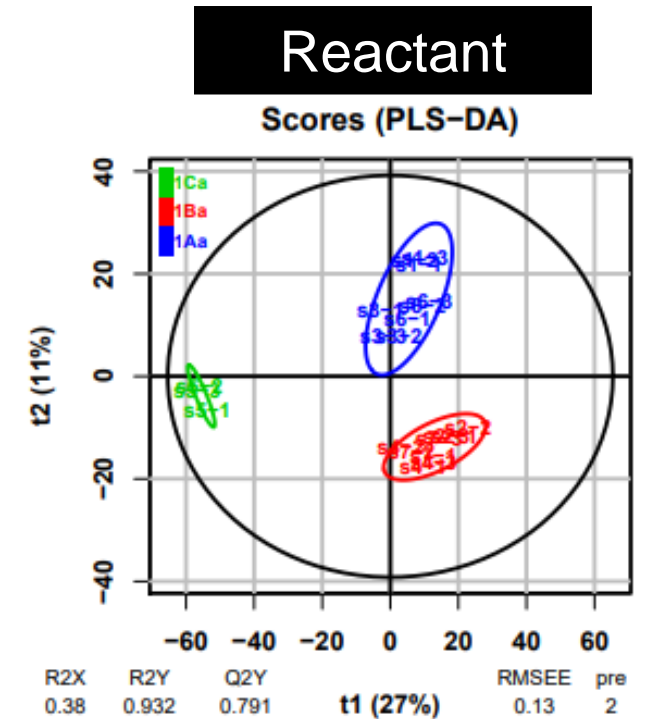
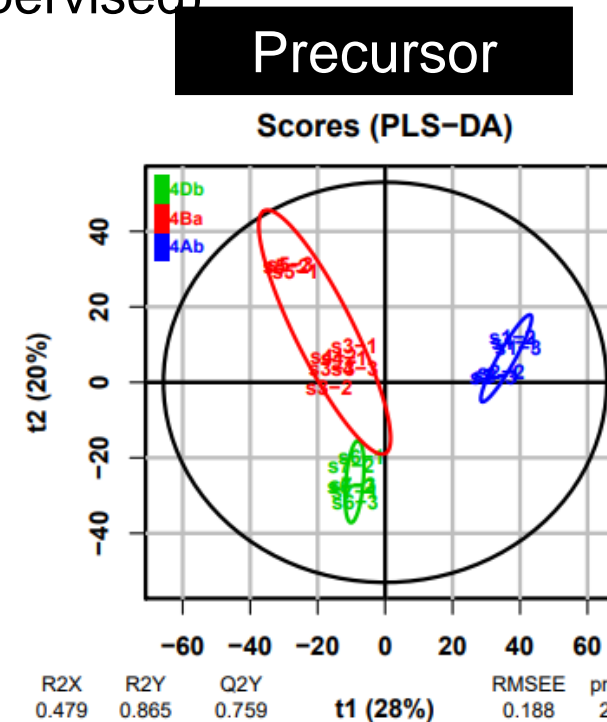
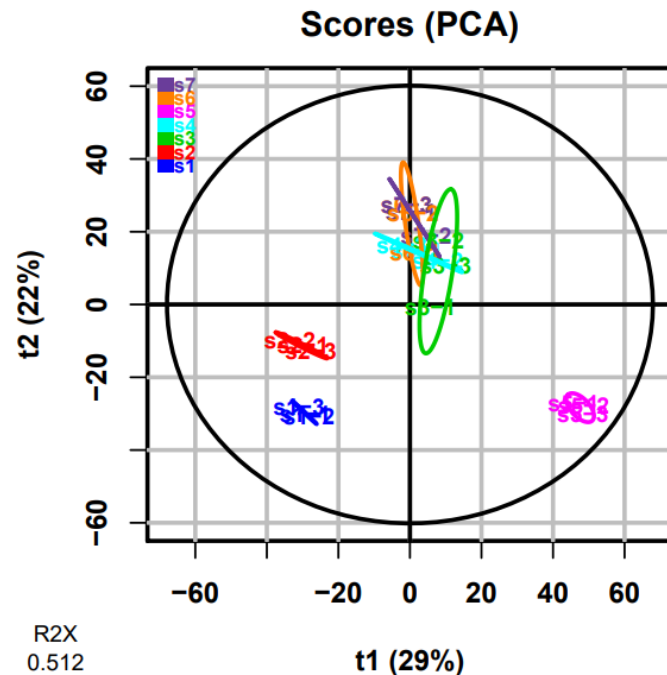


## ➤ Chemometrics

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

- Major sources of variability → PCA (unsupervised)
- Specific sources of variability → PLS (supervised)

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



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

**PLS-DA:** Discrimination of various used synthetic sources

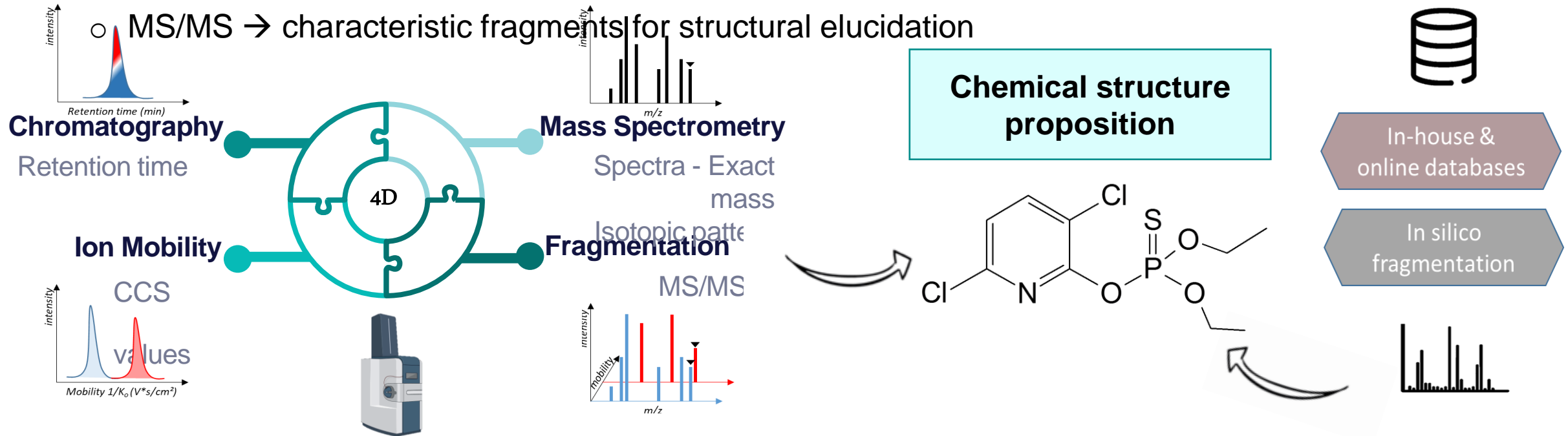
Quality of the model assessed with  $Q^2Y > 0.4$  and permutation test



## Annotation Challenges

### HRMS: sensitive and selective detection of discriminating impurities

- Features (PLS-DA; VIP>1) annotated using LC (RT), HRMS, MS/MS and IMS data to propose a putative structure



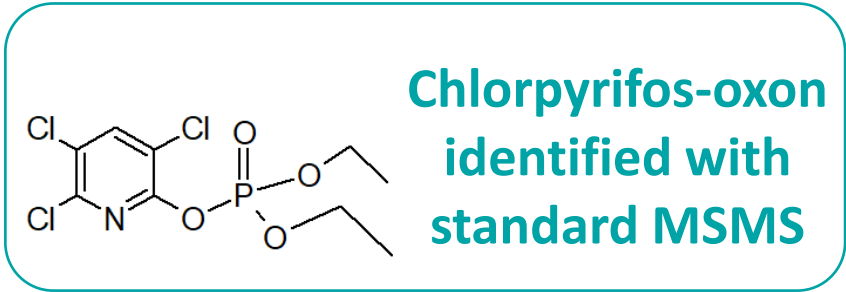
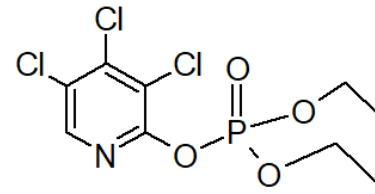
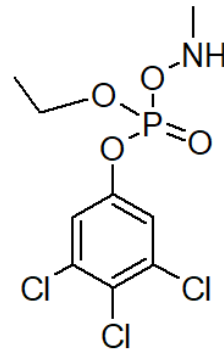
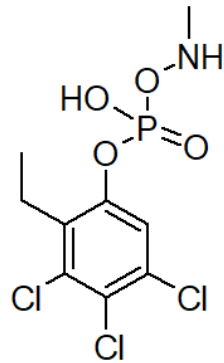
[1] Confirmed Structure	[2] Probable Structure	[3] Tentative Candidate	[4] Unknown
5	72	36	9

➤ Ion mobility spectrometry: a valuable aid for annotation



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

**CCS measurement: 162.7 Å<sup>2</sup>**



<b>Predicted CCS</b>	166.1 Å <sup>2</sup>	165.5 Å <sup>2</sup>	165.1 Å <sup>2</sup>	165.5 Å <sup>2</sup>
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5% accuracy acceptance

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



## ➤ Analysis of unknown samples

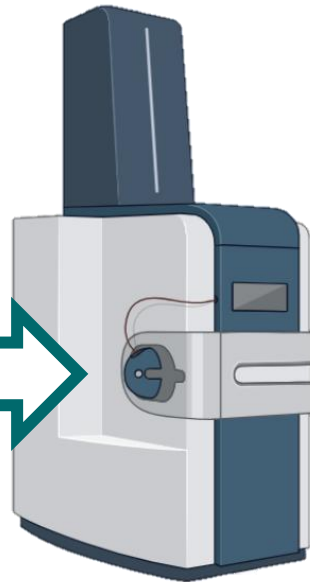
**4D**

RT, accurate mass, MS/MS, ion mobility



Coupled separation technique

(LC)  
Annotation with database  
(RT)



Methodology validation



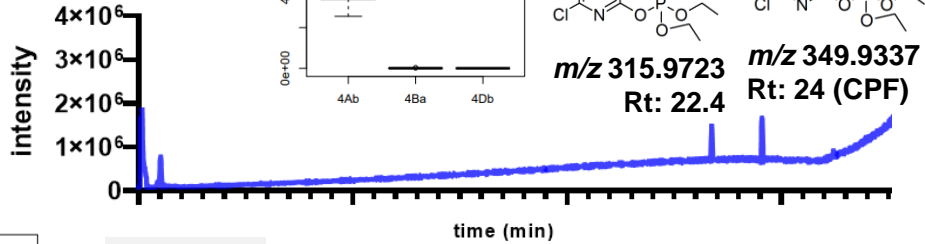
**Blind analysis** of new unknown samples to **classify** their **synthesis route** according to their **discriminating impurities** with the **constructed model**

# Analysis of unknown samples

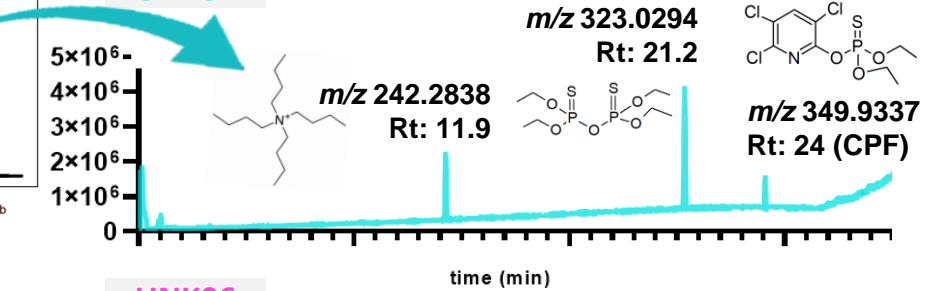


Unknown Samples

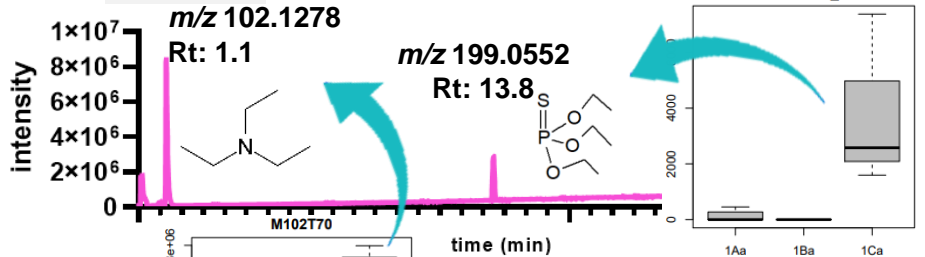
UNK04



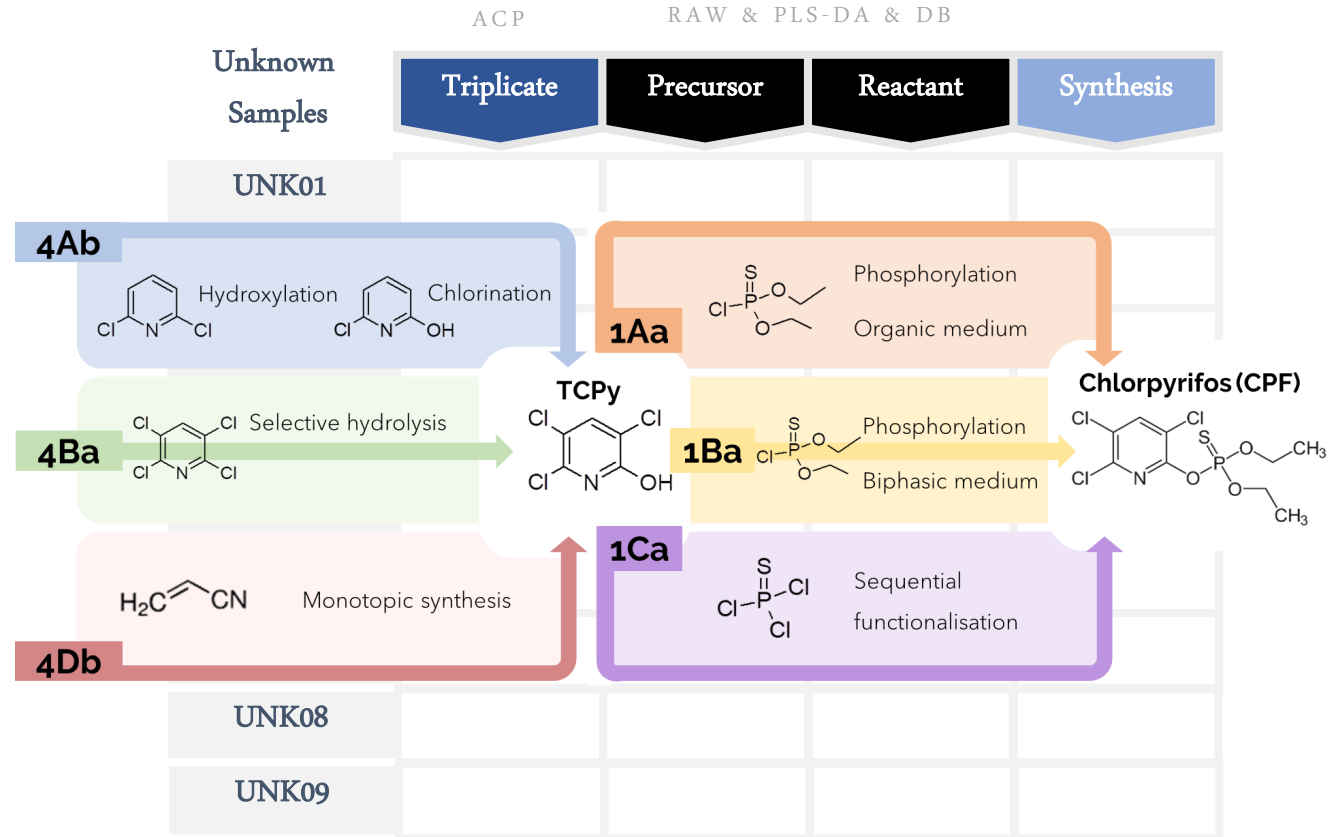
UNK02



UNK06



# Methodology validation



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

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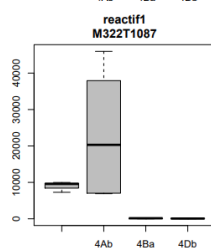
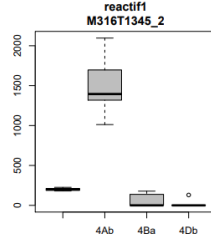
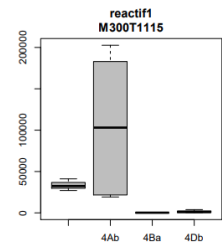


# ➤ What about UNK01,03,06 « Precursor »?



## Targeted Approach

Discriminant features	Presence?
<b>Precursor 4Ab</b>	<i>m/z</i> 299.9963 <i>rt</i> 18.5min <b>YES</b>
	<i>m/z</i> 315.9736 <i>rt</i> 22.4min <b>YES</b>
	<i>m/z</i> 321.9771 <i>rt</i> 17.6min <b>YES</b>
	<i>m/z</i> 287.9421 <i>rt</i> 12.7min <b>YES</b>
<b>Precursor 4Ba</b>	<i>m/z</i> 242.2849 <i>rt</i> 12min <b>NO</b>
<b>Precursor 4Db</b>	<i>m/z</i> 554.7456 <i>rt</i> 27.5min <b>NO</b>
	<i>m/z</i> 504.8106 <i>rt</i> 26.6min <b>NO</b>
	<i>m/z</i> 532.8596 <i>rt</i> 26.2min <b>NO</b>
	<i>m/z</i> 424.9442 <i>rt</i> 21.9min <b>NO</b>



## Methodology validation

Unknown Samples	ACP	Raw & DB	PLS-DA & DB	Synthesis
	Triplicate	Precursor	Reactant	
UNK01	♥	4Ab	1Ca	S8
UNK02	♣	4Ba <sup>4Ba</sup>	1Ba	S4
UNK03	♥	4Ab	1Ca	S8
UNK04	♦	4Ab <sup>4Ab</sup>	1Aa	S1
UNK05	♦	4Ab	1Aa	S1
UNK06	♥	4Ab	1Ca <sup>1Ca</sup>	S8
UNK07	♣	4Ba	1Ba	S4
UNK08	♦	4Ab	1Aa	S1
UNK09	♣	4Ba	1Ba	S4

Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba
S8	4Ab	1Ca

New combination of synthesis pathways discovered

## > Conclusions

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

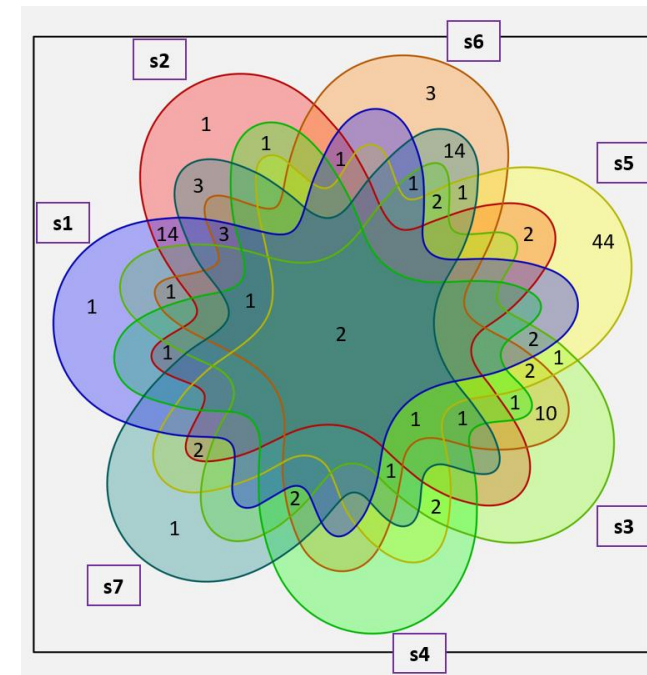
- ⊕ Chemical footprints were linked to a manufacturing process from synthetic raw materials
- ⊕ Methodology validated under blinded conditions with unknown new samples

### Untargeted Approach

Discriminant impurities

### Targeted Approach

Shotgun and specific analysis



Applicable to others scientific fields: biomarkers of chemical exposure on health and sourcing of toxic treat agents

« *Better understand how it is produced, better understand how it works!* »

## > Perspectives

⊕ **Ruggedness:** Apply the methodology to complex samples



⊕ **Structural Elucidation:** Trap and isolate impurities (2D-LC-HRMS) to complete elucidation by NM



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Thank you for your attention

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