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## Simplifying nontargeted PFAS analysis

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Official food control authority of the canton of Zürich,  
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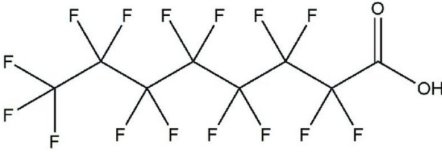


An illustration of a blindfolded archer in a white shirt and tie, holding a bow and arrow. Several arrows are scattered on the ground, missing a red target with a bullseye. The background is a light green field.

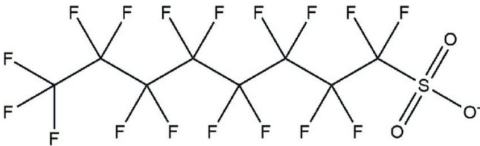


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## Polyfluoroalkyl substances (PFAS)



Perfluorooctanoic acid (PFOA)



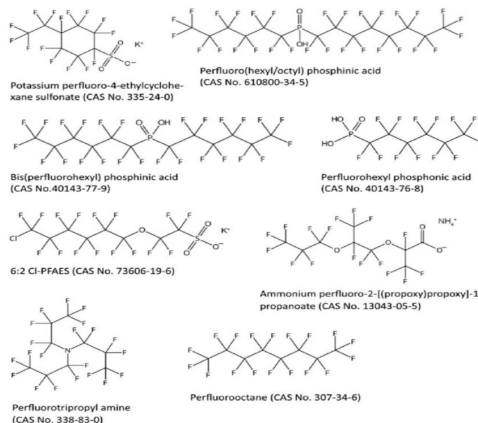
Perfluorooctane sulfonate (PFOS)

**C<sub>4</sub>-C<sub>16</sub>**

## PFAS

- Are everywhere (textile impregnation; firefighting foam etc.)
- Are chemically inert
- Can accumulate in animal and human tissue
- Are suspected to have various negative toxicological effects

## PFAS are chemically very diverse



**Some 6000 chemical structures have been reported !**

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**Targeted methods normally cover  
only the homologues of the two  
conventional PFAS families**

Perfluorooctanoic acid (PFOA)

Perfluorooctane sulfonate (PFOS)

**C<sub>4</sub>-C<sub>16</sub>**

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**Lack of commercial  
reference substances**

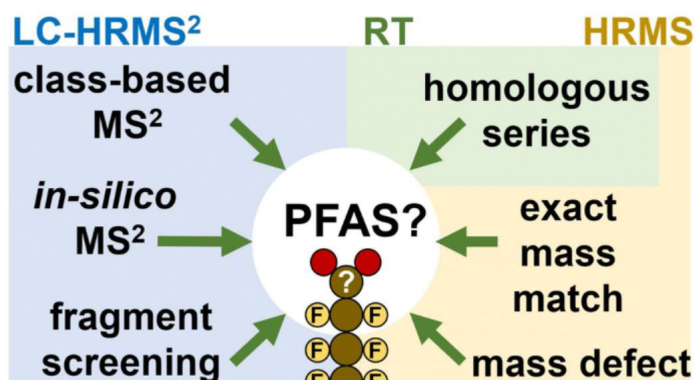
**Detection and quantification is only possible for  
a limited set of PFAS**

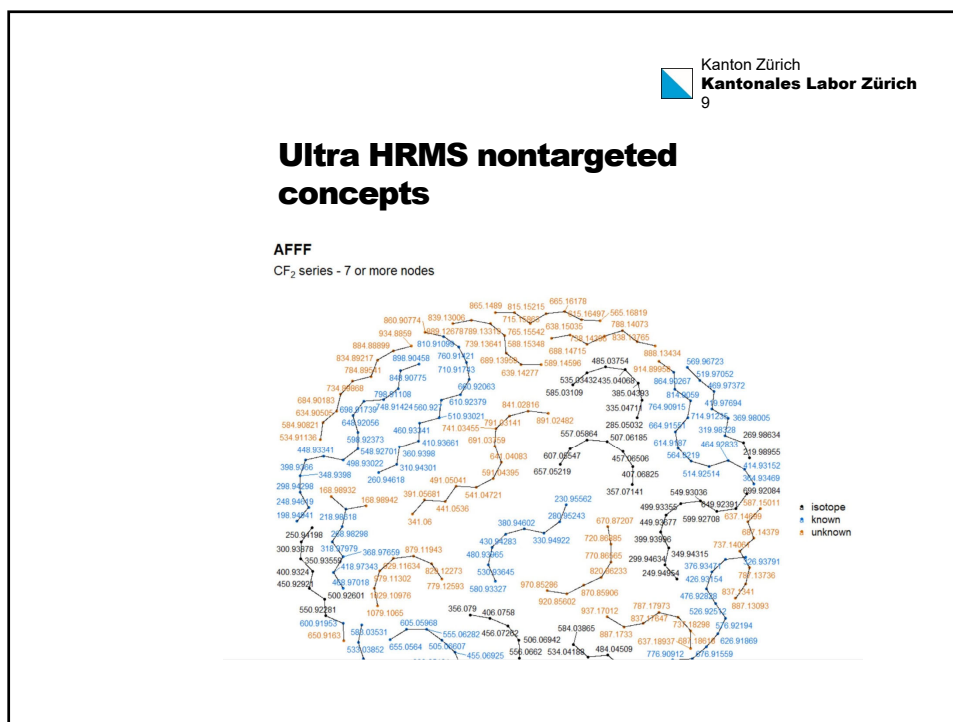
**Does monitoring a limited set of compounds answer  
the question, how humans are exposed to PFAS originating  
from the environment or food?**

## Nontargeted detection of PFAS



## Currently used HRMS nontargeted concepts





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## Fluoromatch 2.0

### the commercial solution

# FlouroMatch 2.0

Annotating thousands of PFAS with a few clicks

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<p><b>Emma Rennie</b> <a href="mailto:emma.rennie@agilent.com">emma.rennie@agilent.com</a> ACT-UR Grant Agilent Mentor</p>	<p><b>John Bowden</b> Professor <a href="mailto:john.bowden@ufl.edu">john.bowden@ufl.edu</a> PI</p>	

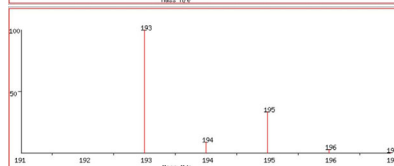
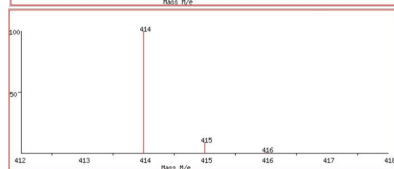
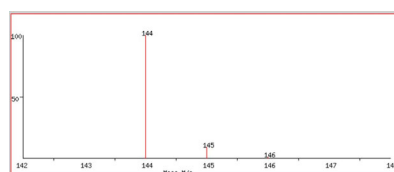
Yale SCHOOL OF PUBLIC HEALTH  
Agilent/Yale  
04/08/2021

## Nontargeted PFAS is hindered by the fact that fluorine has no natural isotopes

Atoms like S, Cl, Br, I show characteristic isotopes which facilitates MS based detection

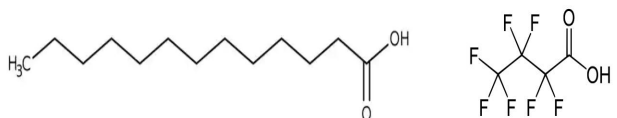
Fluorine has a slight mass defect, yet this is smaller than that of Cl, S or even O

## H or F: For the MS it is virtually the same !



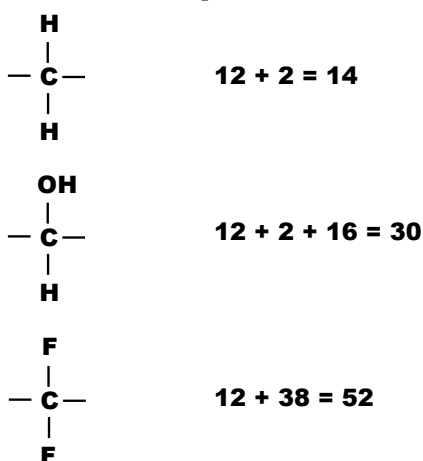
**How to distinguish  
a small, yet heavy PFAS ion  
from an equally heavy, but larger  
matrix compound?**

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**It is the mass per carbon atom**

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**Fluorine is much heavier than hydrogen**

## Turning a weakness into a strength

The absence of F isotopes means that the isotopic profile remains unchanged when H is substituted by F

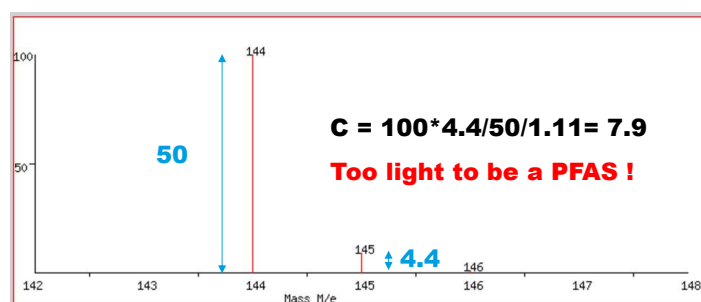
This means that the ion abundance of the first isotopic ion of a PFAS is primarily defined by the number of carbon atoms present within the chemical formula

This permit us to estimate the number of carbons and with this a PFAS marker parameter:

mass of unknown/ estimated number of carbon within the unknown:

$$(m/z) / C$$

## Estimating the number of carbons

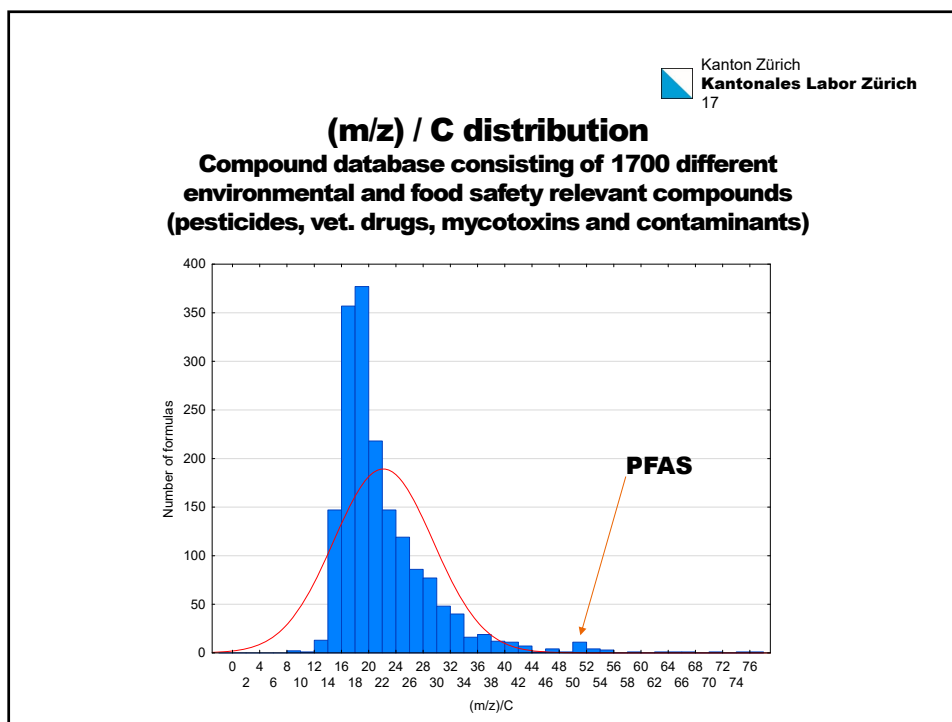


mass / number of carbon

$$(m/z) / C$$

$$144 / 7.9 = 18$$





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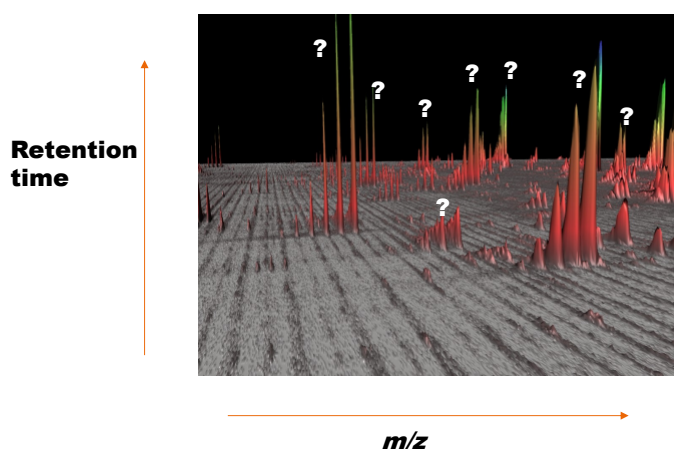
### Deconvolution and componentization

- **Ten thousands of chromatographic peaks are extracted**
- **Based on retention time and peak shape extracted mass traces are grouped to produce a "feature"**
- **A feature represents a chromatographically resolved compound**

### Analyzing the spectrum of each feature

- The spectrum of a feature contains the monoisotopic ion, the isotopic peaks and possible adducts or fragments
- Ratios of abundances of isotopic peaks versus the monoisotopic peak and the accurate mass of the monoisotopic peak for each extracted feature are exported into Excel

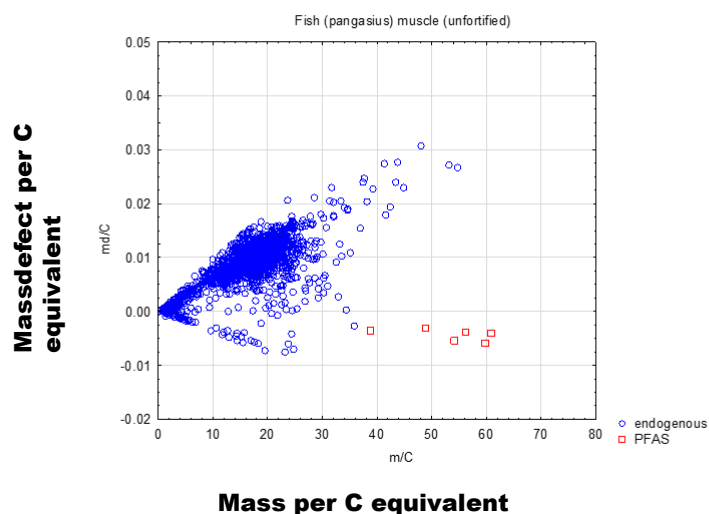
### Deconvolution and componentization

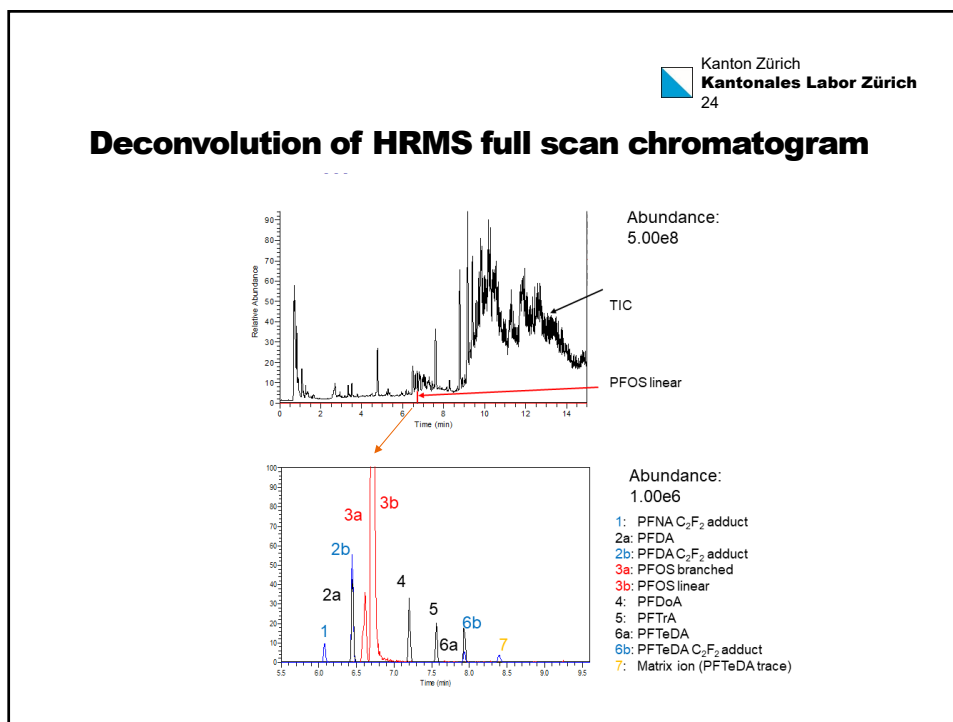
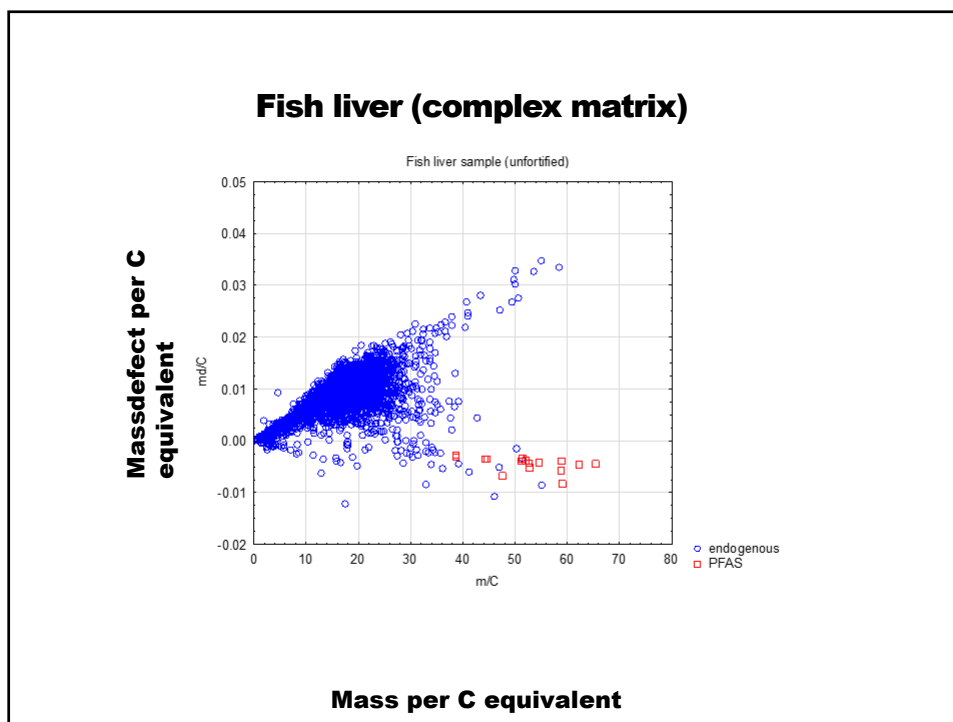


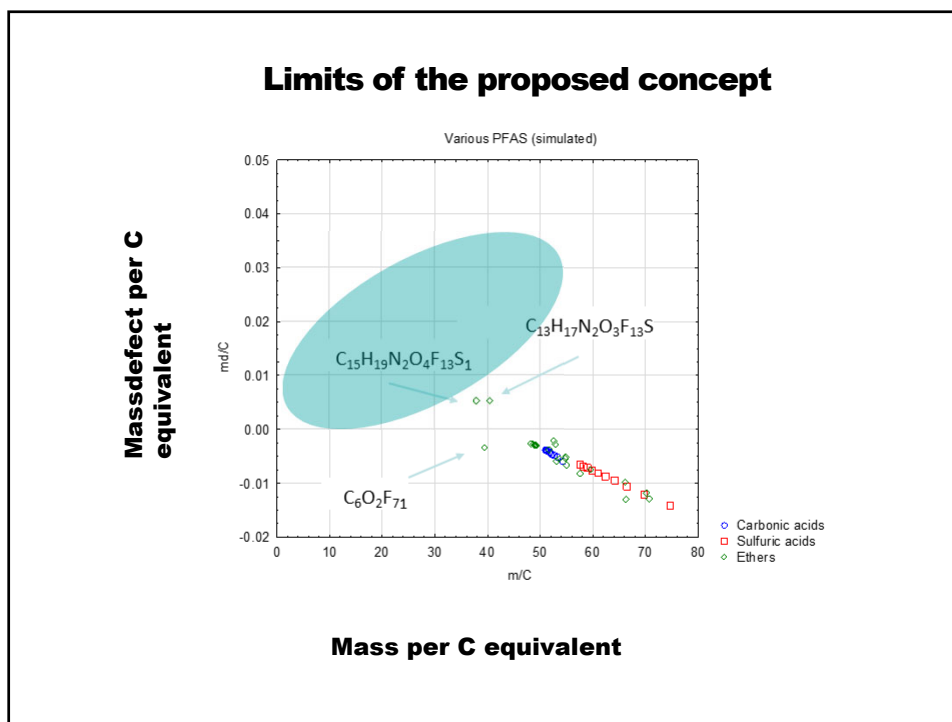
### How well does it work?

- **Processing HRMS data from extracts which were produced by a validated, targeted PFAS method**
- **Anion exchange based clean-up of fish matrix samples**

### Fish muscle (easy matrix)







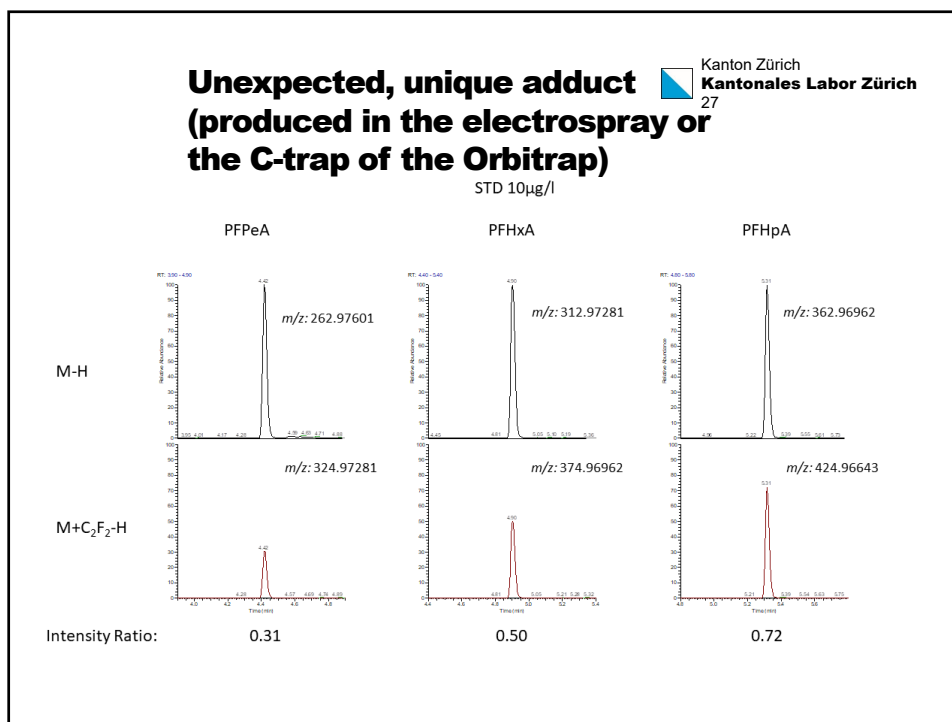
### How did we perform ?

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- **The nontargeted approach successfully detected the PFAS covered by the targeted method**
- **Perfluorotetradecanoic acid was only detected by the nontargeted methodology (the compound was not present in the standard mix)**
- **A set of previously unreported MS adducts was elucidated**

## 2. "NTMs – qualitative or quantitative?"

Application example: "PFAS analysis by use of NTMs"

2<sup>nd</sup> online webinar, June 6 2023

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### Recent developments

**A. Kaufmann: Simplifying Nontargeted Analysis of PFAS in complex Food matrices;  
J. AOAC int. 2022, Sep. 6 (105), 1280-1287**

- **The commercial software "Compound discoverer" integrated the proposed algorithm.**
- **J. Zweigle: Efficient PFAS prioritization in Non-target HRMS data: systematic evaluation of the novel MD/C-m/C approach;  
J. ABC, 2023, 415, 1791-1801**

## **Conclusion**

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**Nontargeted works if we can clearly describe a common property of our compounds of interest**

**This property has to be significantly different from the properties of our matrix compounds**

**If this is not the case, the exogenous analytes will be hidden within the dense cluster of endogenous compounds**