Detecting PFAS beyond the Current Regulative Request:
a Comprehensive Overview of the Contamination in Water by UPHLC-Ion mobility-HRMS

BRUKER

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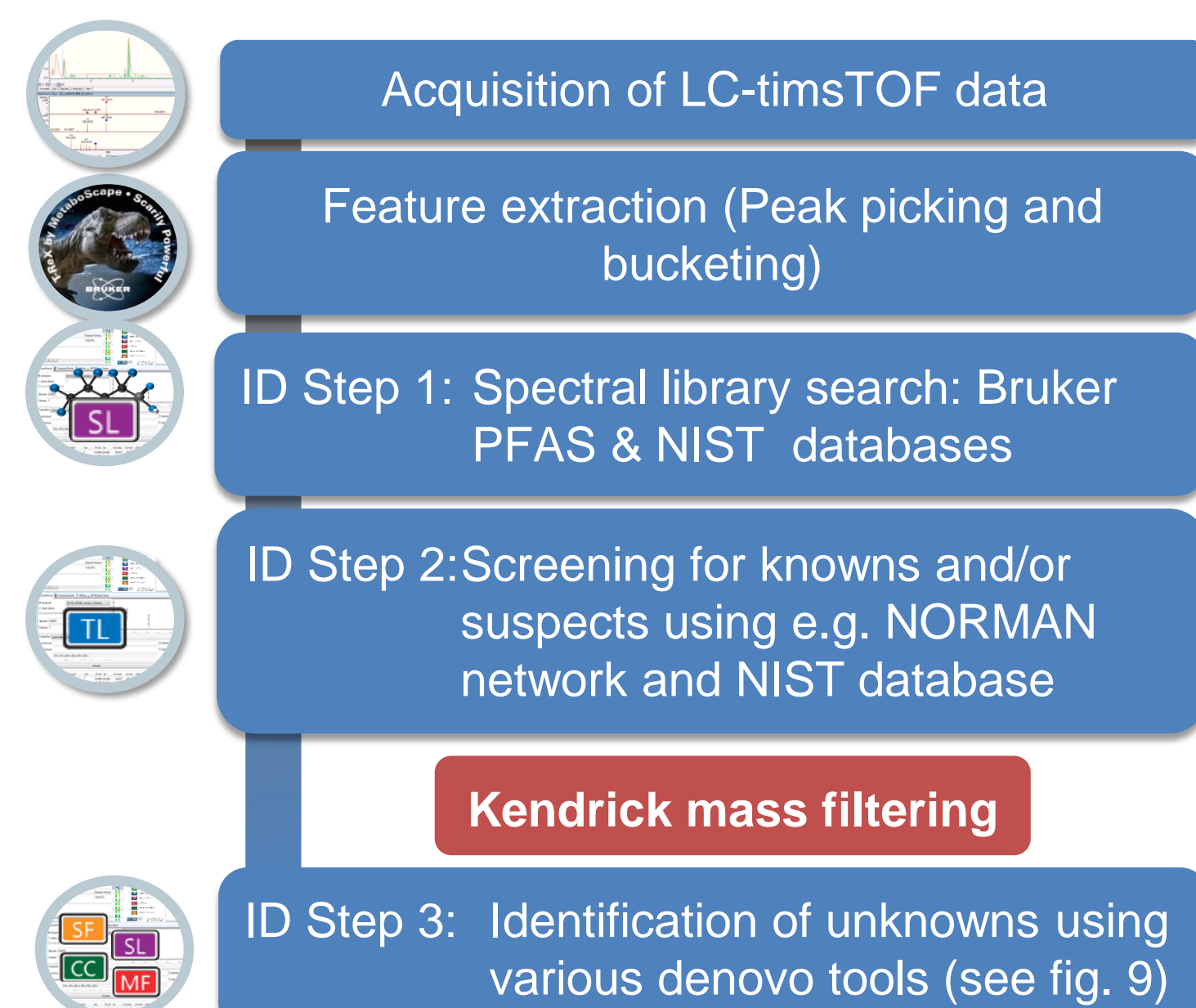
Introduction

- PFAS: Huge group of > 4,700 registered compounds plus degradation products and precursors
- Lack of reference standards or spectral libraries, plenty of isomers. Targeted analyses of the complete group is impossible.
- Persistent, bio-accumulative and toxic (PBT). Ubiquitous in environment and organisms.

This study:
- Water sampling at 10 sites in the Netherlands
- SPE pre-concentration
- Non-target, unbiased analysis using UHPLC-HRMS combined with trapped ion mobility (TIMS) with "4D" criteria: mass accuracy, isotope pattern fit, MS/MS, CCS
- Processing with TASQ and MetaboScape
- Subsequent identification by (1) library search, (2) screening data against NIST PFAS suspect list (4,700 entries) or (3) denovo unknown identification
- Kendrick mass defect (KMD) filtering for PFAS compounds from background, based on the fluorine content (repeating CF_2 units).

Workflow

- Acquisition of LC-timsTOF data
- Feature extraction (Peak picking and bucketing)
- ID Step 1: Spectral library search: Bruker PFAS & NIST databases
- ID Step 2: Screening for knowns and/or suspects using e.g. NORMAN network and NIST database
- Kendrick mass filtering
- ID Step 3: Identification of unknowns using various denovo tools (see fig. 9)
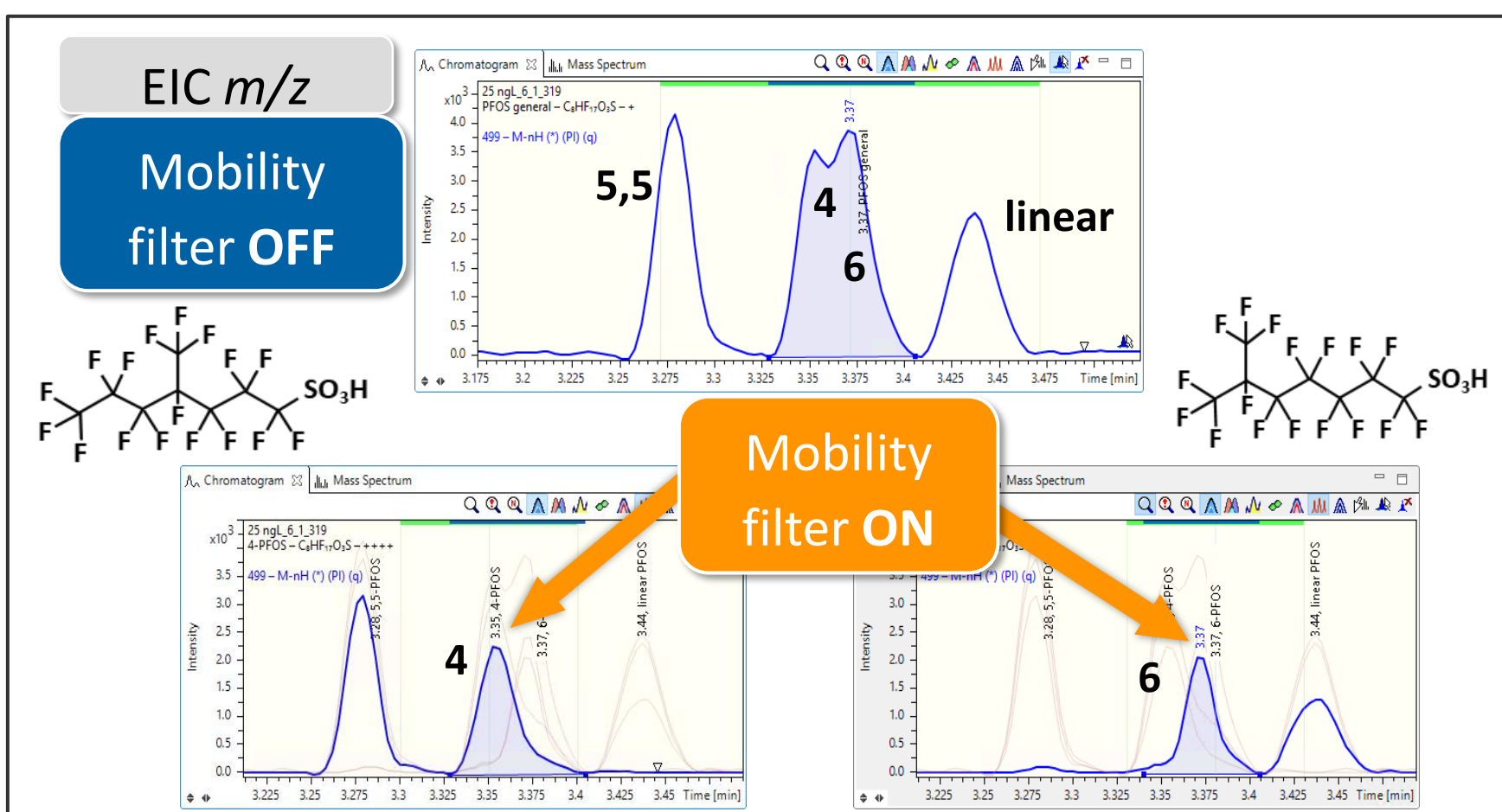
Benefits of TIMS

Fig. 1: The additional dimension of trapped ion mobility (TIMS) separates two co-eluting branched isomers, 4-PFOS and 6-PFOS.
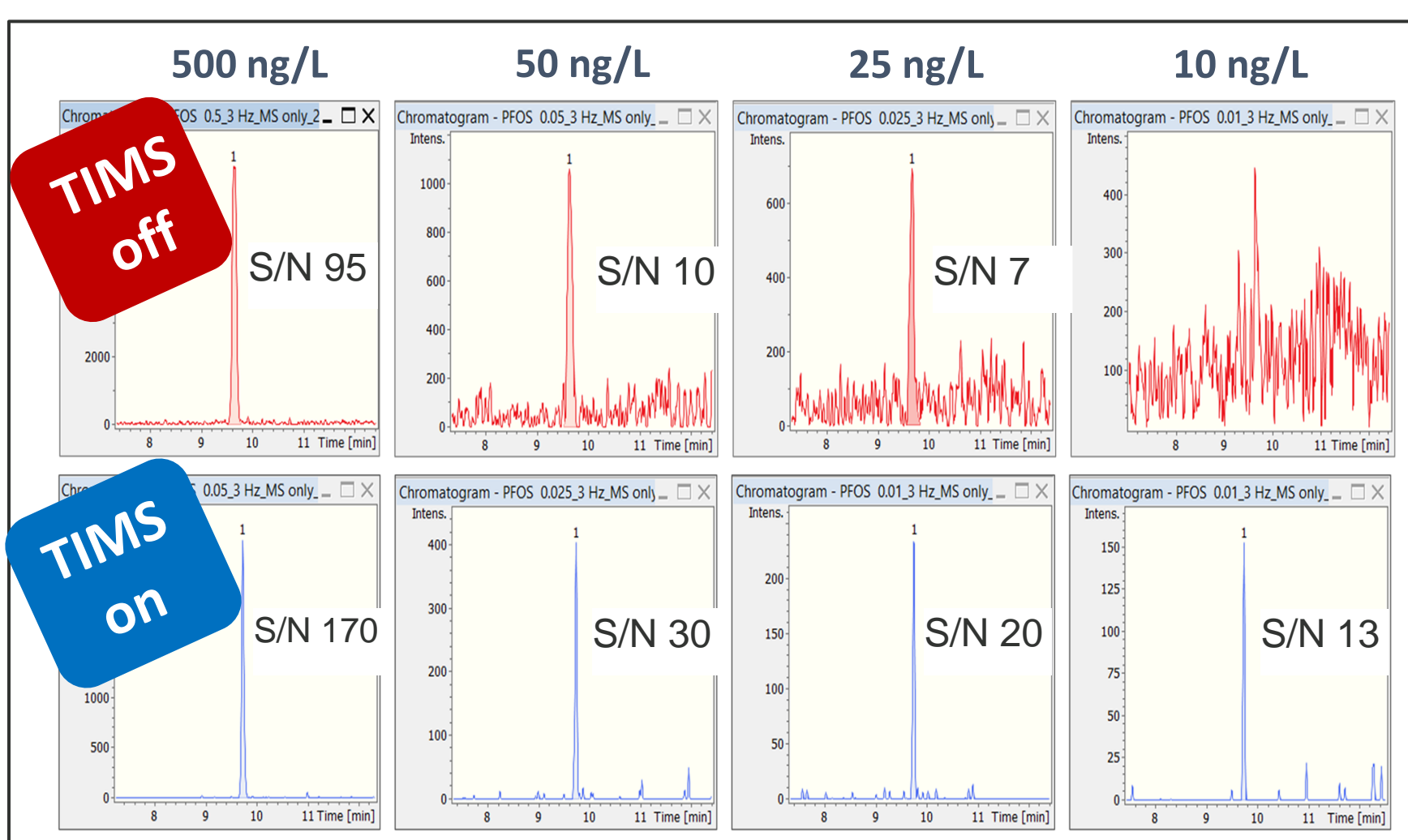

Fig. 2: Improvement of S/N values for chromatographic MS peaks by applying TIMS. The largest difference is visible for low concentrations of main interest.

Sampling sites and SPE pre-concentration

- Aarle-Rixtel
- Asten
- Bath
- Dordrecht
- Kralingseveer
- Leiden
- Nieuwerkerk aan den IJssel
- Oijen
- Tilburg
- Weert

Fig. 3: 10 water sampling sites in NL with different infrastructure (industrial, city, recreation).
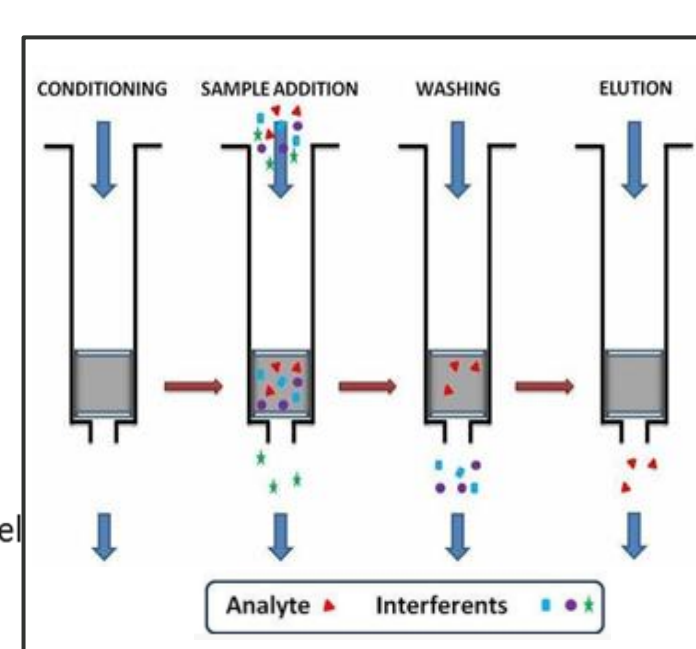
Sample pre-concentration by SPE. 25 mL surface water effluent + 50 uL IS. Blanks (50 uL IS). 4 mL 0.1% NH_4OH in MeOH for conditioning, 4 mL 0.1% NH_4OH in MeOH for elution + reconstitution.

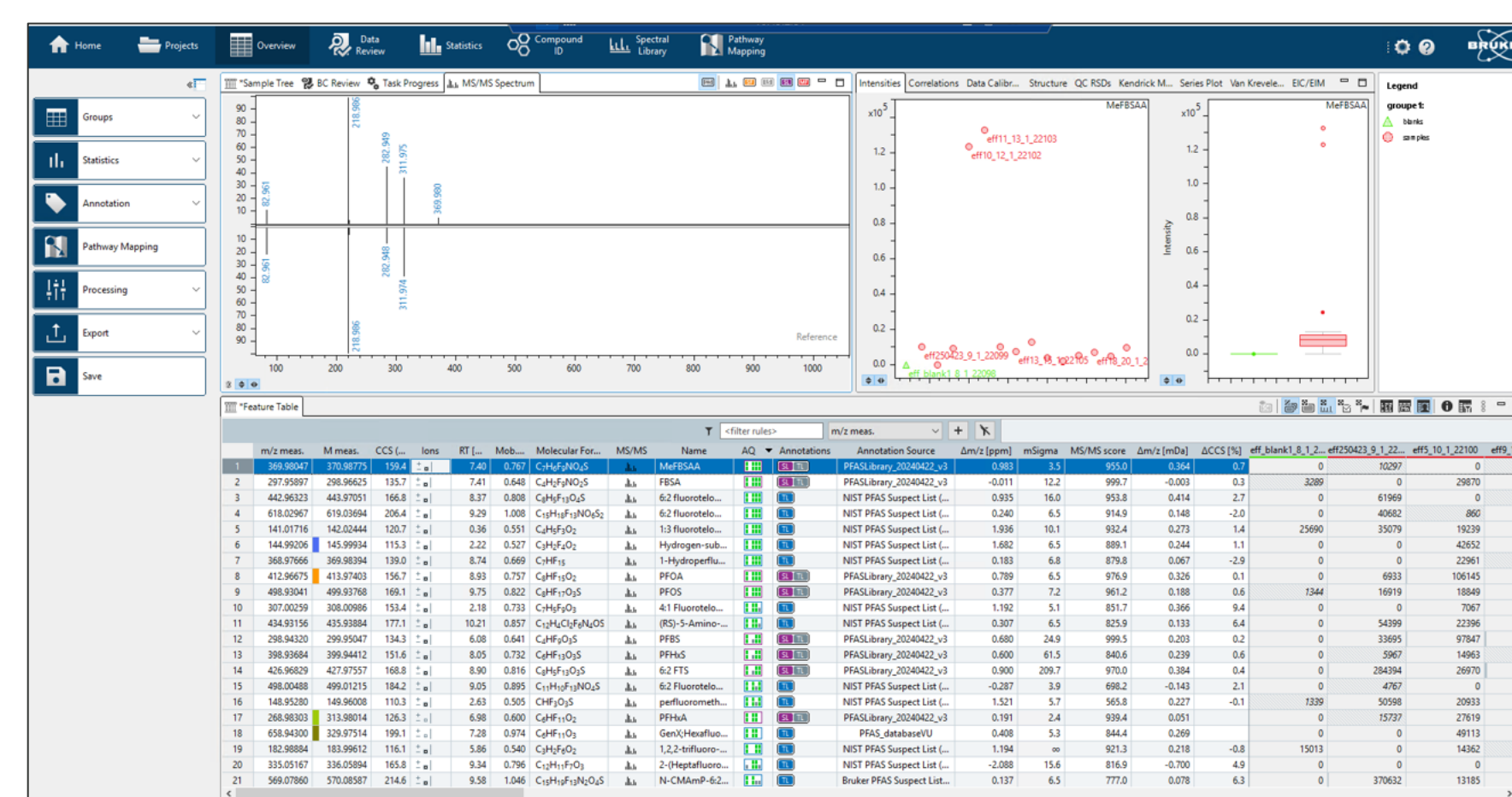## Non-targeted screening for PFAS by library search and suspect lists

Fig. 4 MetaboScape workflow and overview for the PFAS identification in the effluents using the library search and suspect lists.

Fig. 5: Detail of the NIST PFAS suspect list containing just very simple information about the name, elemental composition and InChI structure of 4700 PFAS. https://comptox.epa.gov/dashboard/

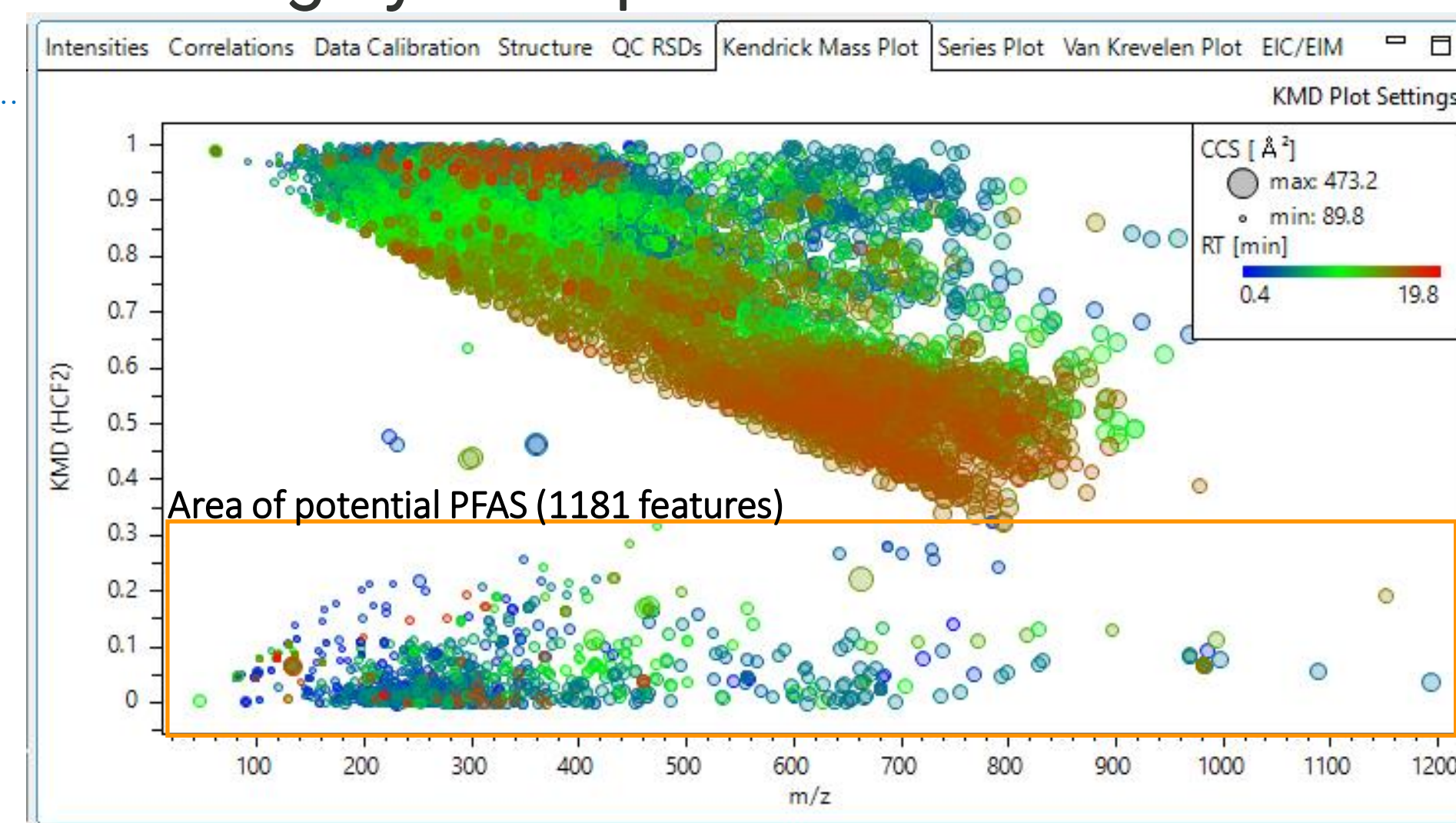## Filtering by KMD plots

Area of potential PFAS (1181 features)

Fig. 6 Plotting the Kendrick Mass Defect (KMD) with CF_2 as the base unit versus m/z. Starting from a total of 15,700 detected features, 1181 potential PFAS have been filtered (92% data reduction).
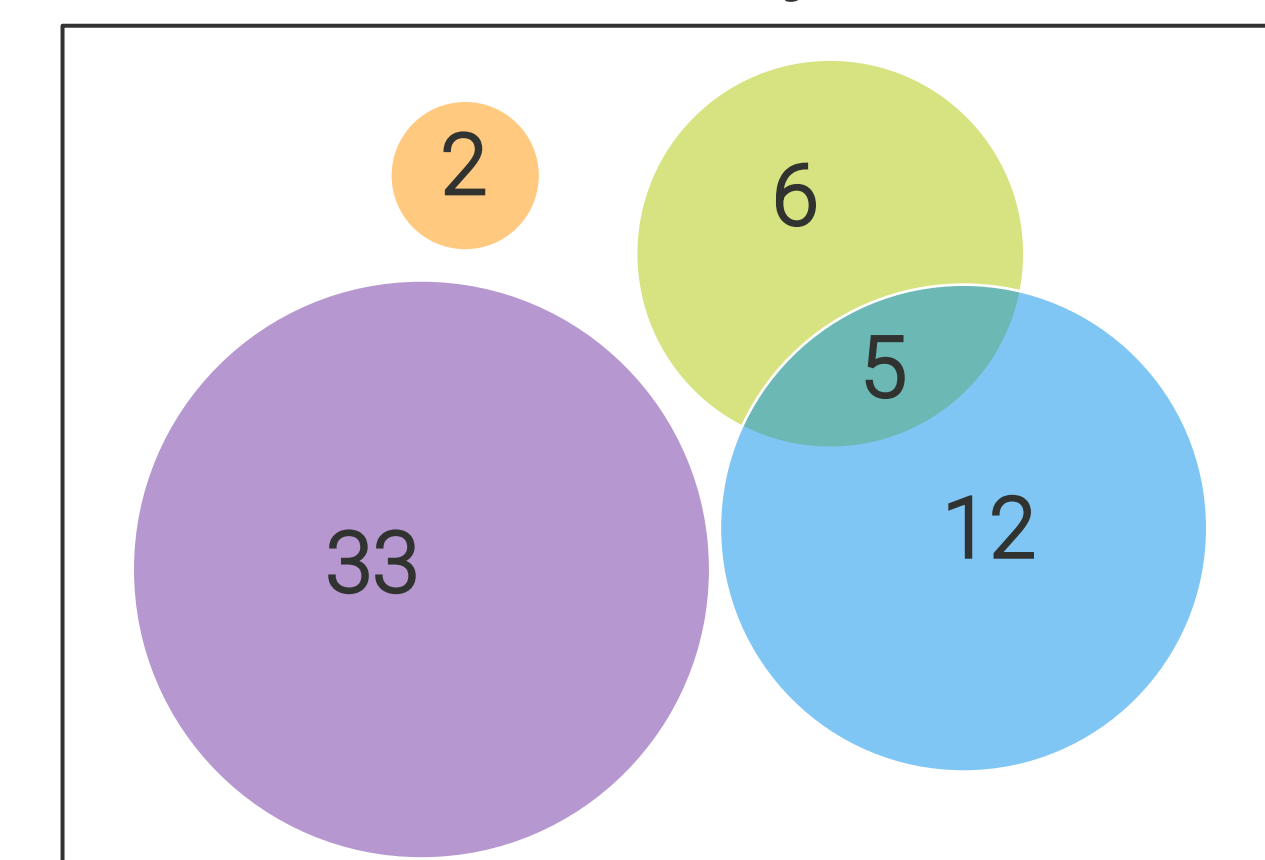
Identified PFAS by various methods

Fig. 7: Venn diagram as a summary of the identified PFAS by the various identification steps. From 15,700 extracted features in the LC/MS/MS run, the different ID steps lead to:
- ID step 1: 23 PFAS could be annotated by library searches in the Bruker PFAS library (green) and NIST PFAS library (blue) with an overlap of 6 compounds.
- ID step 2: Additional 33 PFAS were identified by the comparison with the NIST PFAS suspect list (purple).
- ID step 3: KMD was used to filter the remaining features systematically for potential PFAS. 2 more PFAS were found via the by denovo identification workflow (orange).

In total, 58 PFAS were found and identified by this holistic approach, providing an overview of the total content of PFAS in each sample. All steps represent tentative results, the post-processing is still work-in-progress, and more PFAS are expective to be found after a complete evaluation of the data from all sampling sites.
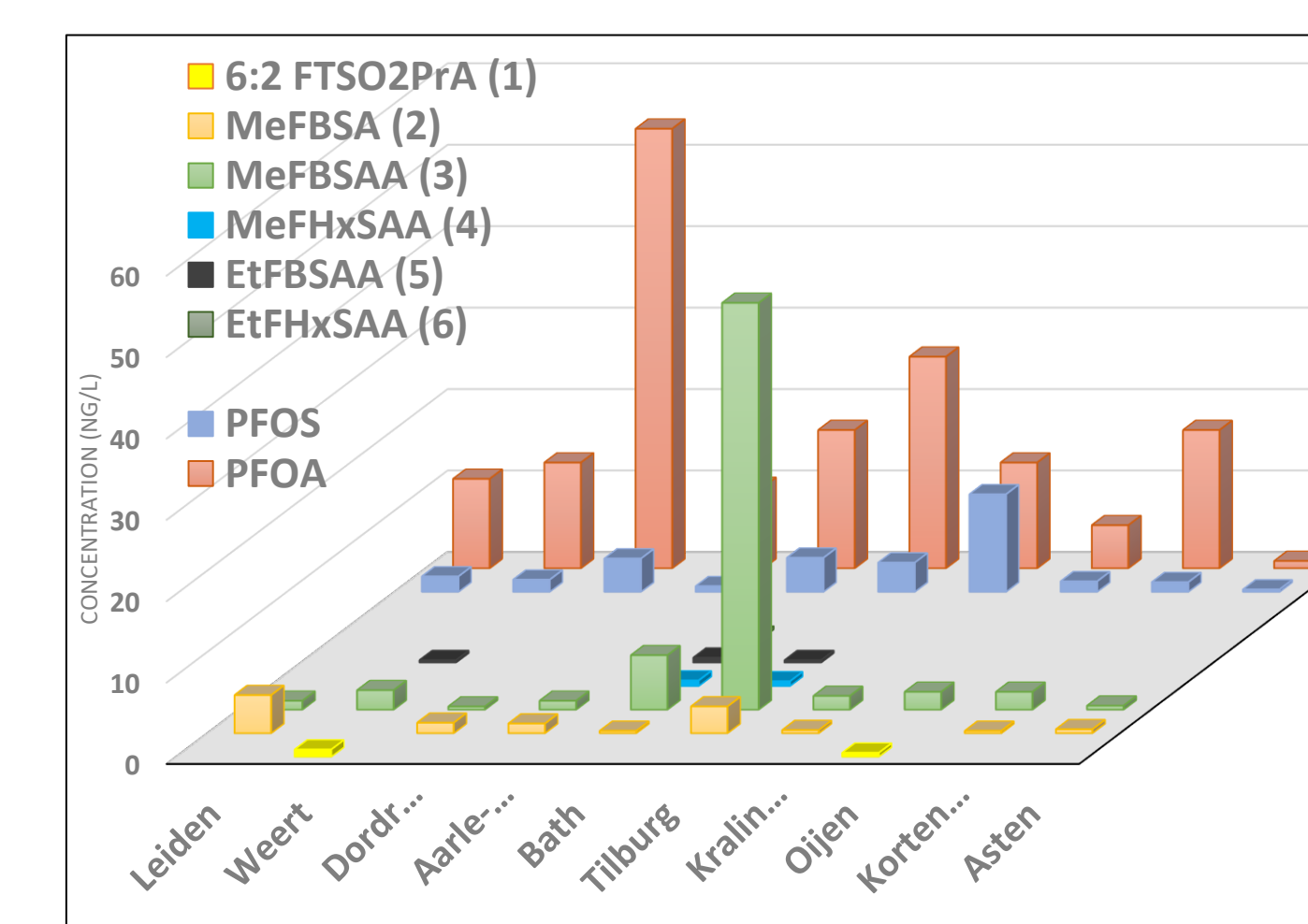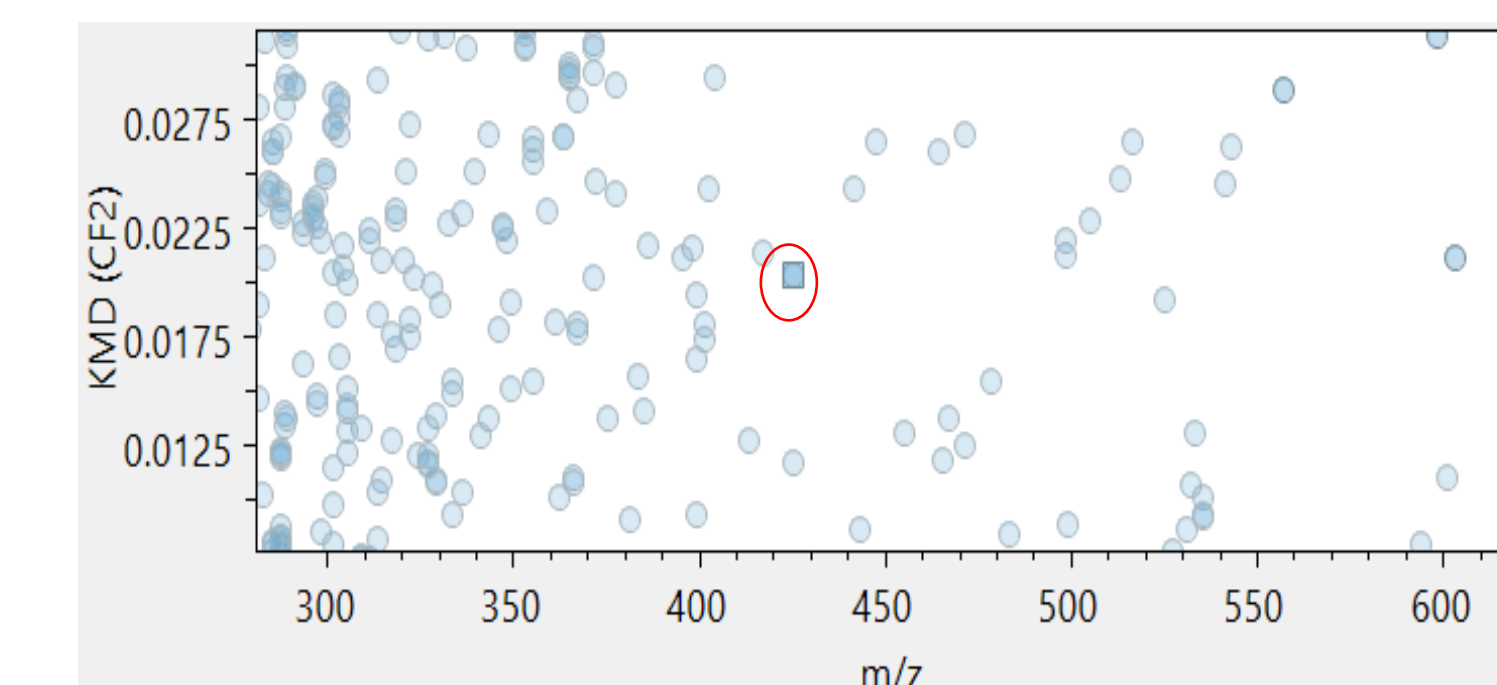

Fig. 8: Various PFAS species with different intensities at the sampling sites. Dordrecht sticks out for PFOA, e.g., while Tilburg has a higher MeFHxSAA concentration. As well, new PFAS precursors have been found which are not screened yet in legal directives.

Criterion	Score
MS/MS	814
Δm/z	-0.37
ΔCCS	5.8
mSigma	6.5
rt	8.96
CCS	166.3

Fig. 9: Example for the denovo ID step 3. PFAS H2-U-PFOS was identified by the MetaboScape tools SmartFormula, Compound Crawler, in-silico fragmentation (MetFrag) and CCS prediction. This unsaturated PFOS has already been identified before in the environment, but a standard is not commercially available yet. known

Conclusion

- The 4D scoring of exact mass, isotope pattern match, MS/MS data and CCS values provides a reliable basis for confident identification.
- Different workflows including (1) library search, (2) comparison with suspect lists and (3) denovo identification have been successfully applied.
- In total, 58 PFAS were found in the water samples, with distinct differences for the various sampling sites which point to individual environmental circumstances and origins for PFAS.
- This wide-scope screening of real-life samples proved to be a comprehensive approach for a fast and efficient PFAS identification and will highly assist in the understanding of the chemical universe of PFAS in the environment and for protecting the environment, wildlife, and human health.

timsTOF Pro 2

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