Revolutionizing PFAS Detection: Single HR-MS Run for Comprehensive Environmental Screening

Abstract

Per- and polyfluoroalkyl substances (PFAS) are a huge chemical group with more than 4,700 registered compounds plus associated degradation products. There is a lack of reference standards, molecular databases or spectral libraries. The low concentrations of PFAS in complex matrices and the presence of multiple isomers further complicate the PFAS analysis.

Here, Bruker presents a novel workflow based on the timsTOF Pro 2, a high-resolution QTOF instrument enabled with Trapped Ion Mobility Spectrometry (TIMS). This workflow aims to automate the screening and identification of PFAS against extensive suspect lists provided by the NORMAN network and NIST, encompassing nearly 5,000 entries.

Keywords:
PFAS, water, timsTOF Pro 2, CCS prediction, NORMAN network, NIST, VIP-HESI, MetaboScape

Introduction

Per- and polyfluorinated alkyl substances (PFAS) encompass a wide array of industrial compounds that have been manufactured and widely employed since the 1940s. They find application as heat, water, and grease repellents in various consumer products. They are classified as persistent pollutants, with known environmental accumulation and global circulation, found everywhere like in water, soil, food and living organisms (fish and other animals, humans). Due to their suspected toxicity, they pose a growing threat to both humans and wildlife. There is evidence that exposure to PFAS may cause adverse health effects, e.g., on reproduction and development, liver and kidney, the immune system, and it may cause tumors. Therefore, their analysis is an increasingly important task for analytical chemists.

Global regulations for PFAS in food and drinking water differ widely and are constantly evolving. The EU Directive 2020/2184 for drinking water, e.g., requires a limit of 500 ng/L for the total amount of all PFAS in a sample and 100 ng/L for the sum of 20 specific PFAS of highest health risk.

Currently proposed US EPA regulations would limit PFOA and PFOS to a Maximum Contaminant Level (MCL) of 4 ng/L.

In many laboratories, PFAS are quantified via liquid chromatography (HPLC) coupled to triple quadrupole (TQ) mass spectrometers. Although highly sensitive, TQ methods are dependent on standards for method development and limited in the number of compounds analyzed. For PFAS, current TQ methods cover a range of 50-100 compounds only, versus the vast amount of more than 5,000 known PFAS. A comprehensive screening and quantitation of such a high number of PFAS species is truly difficult to impossible for a targeted analysis by triple quads.

Here we propose a novel workflow which is based on the combination of UHPLC, trapped ion mobility spectrometry (TIMS) and a high-resolution (HR) mass spectrometer, the timsTOF Pro 2, for the detection and quantitation of a virtually unlimited number of PFAS in a comprehensive non-target sample screening approach.

Authors:
Arnd Ingendoh
Andrea Kiehne
Birgit Schneider

Methods

The timsTOF Pro 2 HR-QTOF is equipped with a highly sensitive vacuum insulated probe heated electrospray ionization (VIP-HESI) source for non-target screening and quantitation. On top of the UHPLC separation and the MS and MS/MS data acquisition, trapped ion mobility adds an additional dimension of separation for the huge compound group including its isomers in complex matrices, and at the same time provides high speed PASEF (described in detail in [1]) for a maximum MS/MS coverage. In PASEF acquisition (Parallel Accumulation – Serial Fragmentation), ion focusing by TIMS results in unmatched MS/MS speed without compromising sensitivity.

PFAS usually have to be detected in complex matrices with a high number of other naturally occurring compounds or contaminants. Kendrick mass analysis is included in Bruker's software MetaboScape®. This feature enables filtering for PFAS and thereby reducing data complexitiy. For the Kendrick mass scale, the mass of the recurring CF_2 repeating unit in PFAS is defined as nominal 50 Da. The Kendrick Mass Defect (KMD) is then calculated as KMD = $m_{NominalKendrick} - m_{Kendrick}$. Homologous PFAS species have the same KMD, align horizontally in the KMD plot and can therefore be differentiated from any other species. The PFAS list of the OECD (PFASOECDNA, at the EPA dashboard) contains 3,213 PFAS of which 2,982 (92.8%) have a KMD between - 0.25 and + 0.1, and to which KDM filtering can be applied.

Several parameters of the KMD filtered signals are used to screen against the NORMAN network [2] or NIST PFAS [3] suspect list with nearly 5,000 entries containing names, elemental compositions and InChI structures for PFAS (Fig. 1):

- The exact mass and isotope patterns of the KMD filtered signals are compared with the elemental compositions from the suspect list, and an initial list of candidates is generated.
- The MS/MS data is matched with *in-silico* fragments generated from the InChI structures of the retrieved suspect list entries using MetFrag [4].
- The experimentally determined CCS values are matched with those predicted by CCS-Predict Pro [5], which are also based on the InChI structures for the respective suspect list entries.
- If available, also retention time values could be applied for the search.

The calculated and predicted values allow the compound identification without the need of any standards or experimental libraries. All detected features can be searched against the PFAS list of nearly 5,000 entries, and thus the approach provides a very comprehensive coverage.

	A	B	C
1	Name	FORMULA	INCHI
2	1H-Perfluoro-3,3-bis(trifluoromethyl)hexyne	C8HF13	InChI=1S/C8HF13/c1-2-3(6(13,14)15,7(16,17)18)4(9,10)5(11,12)8(19,20)21/h1H
3	Bis(henelcosafluorodecyl)phosphinic acid	C20H9F34O2P	InChI=1S/C20H9F34O2P/c21-5(22,7(25,26)9(29,30)11(33,34)13(37,38)15(41,42)17(45,46)19(49,50)51)1-3-57(55,56)4-2-6(23,24)8(27,28)10(31,32)12(35,36)14(39,40)16(43,44)18(47,48)20(52,53)54/h1-4H2,(H,55,56)
4	Tris(2-(perfluorododecyl)ethyl)phosphate	C42H12F75O4P	InChI=1S/C42H12F75O4P/c43-7(44,10(49,50)13(55,56)16(61,62)19(67,68)22(73,74)25(75,80)28(85,86)31(91,92)34(97,98)37(103,104)40(109,110)111)1-4-119-122(118,120-5-2-8(45,46)11(51,52)14(57,58)17(63,64)20(69
5	2-Chloro-2-propenoic acid 3,3,4,4,5,5,6,6,6-nonafluorohexyl ester	C9H6ClF9O2	InChI=1S/C9H6ClF9O2/c1-4(10)5(20)21-3-2-6(11,12)7(13,14)8(15,16)9(17,18)19/h1-3H2
6	Bicyclo[2.2.1]hept-2-ene, 5,5,6-trifluoro-6-(heptafluoropropoxy)-	C10H6F10O	InChI=1S/C10H6F10O/c11-6(12)4-1-2-5(3-4)7(6,13)21-10(19,20)8(14,15)9(16,17)18/h1-2,4-5H,3H2
7	3,3,4-Trifluoro-4-(heptafluoropropoxy)tricyclo[4.2.1.0^2,5^]non-7-ene	C12H6F10O	InChI=1S/C12H6F10O/c13-8(14)6-4-1-2-5(3-4)7(6)9(8,15)23-12(21,22)10(16,17)11(18,19)20/h1-2,4-7H,3H2
8	3-(Nonafluorobutyl)bicyclo[2.2.1]hept-2-ene	C11H9F9	InChI=1S/C11H9F9/c12-8(13,7-4-5-1-2-6(7)3-5)9(14,15)10(16,17)11(18,19)20/h1-2,5-7H,3-4H2
9	3-(Heptafluorobutyryl)camphor	C14H15F7O2	InChI=1S/C14H15F7O2/c1-10(2)6-4-5-11(10,3)8(22)7(6)9(23)12(15,16)13(17,18)14(19,20)21/h6-7H,4-5H2,1-3H3
10	Perfluorobutylsulfonamide	C4H2F9NO2S	InChI=1S/C4H2F9NO2S/c5-1(6,3(9,10)11)2(7,8)4(12,13)17(14,15)16/h(H2,14,15,16)
11	N-(3,4-Dichlorophenyl)-2,2,3,3,4,4,4-heptafluorobutanamide	C10H4Cl2F7NO	InChI=1S/C10H4Cl2F7NO/c11-5-2-1-4(3-6(5)12)20-7(21)8(13,14)9(15,16)10(17,18)19/h1-3H,(H,20,21)
12	N-(3,5-Dichlorophenyl)-2,2,3,3,4,4,4-heptafluorobutanamide	C10H4Cl2F7NO	InChI=1S/C10H4Cl2F7NO/c11-4-1-5(12)3-6(2-4)20-7(21)8(13,14)9(15,16)10(17,18)19/h1-3H,(H,20,21)
13	2-Propenoic acid, 2-methyl-, 2-[[[[3-[[[2-[ethyl[(heptadecafluorooctyl)su	C28H28F17N3O8S	InChI=1S/C28H28F17N3O8S/c1-6-48(9-10-54-19(50)46-16-8-7-14(4)17(11-16)47-20(51)56-15(5)12-55-18(49)13(2)3)57(52,53)28(44,45)26(39,40)24(35,36)22(31,32)21(29,30)23(33,34)25(37,38)27(41,42)43/h7-8,11,15H
14	2-Propenoic acid, 2-methyl-, 2-[[[[5-[[[2-[ethyl[(pentadecafluoroheptyl)s	C27H28F15N3O8S	InChI=1S/C27H28F15N3O8S/c1-6-45(54(49,50)27(41,42)25(36,37)23(32,33)21(28,29)22(30,31)24(34,35)26(38,39)40)9-10-51-19(47)43-16-8-7-14(4)17(11-16)44-20(48)53-15(5)12-52-18(46)13(2)3/h7-8,11,15H,2,6,9-10,

Figure 1. Part of the NIST PFAS suspect list with in total 4,967 compounds, listing names, elemental compositions and InChI structures.

Results

Ion mobility using TIMS has several unique benefits for the analysis of such complex samples and groups of species:

1) TIMS allows the separation of isomers which are co-eluting. Fig. 2 shows an example of four Perfluorooctanesulfonic acid (PFOS) with identical elemental composition and an exact mass of 498.9302 Da but different molecular structures. While two of them, 5,5-PFOS and L-PFOS, can be separated already by chromatography, the other two, 4-PFOS and 6-PFOS, co-elute. Trapped ion mobility forms two distinguished peaks which can be used for the identification and quantitation of each of these isomers.

2) TIMS increases the S/N in both MS and MS/MS by filtering the respective compounds from the background. Fig. 3 illustrates the different data quality with TIMS OFF and ON for MS and MS/MS spectra. In particular at low concentrations like 10 ng/L, the S/N ratios and sensitivity for the extracted ion

chromatograms are highly improved in MS mode. In broad-band MS/MS (bbCID) mode TIMS greatly enhances the visualization of diagnostic fragments by efficiently minimizing background interferences, consequently enhancing the reliability of database searches.

These benefits lead to sensitivity levels for PFAS well below the current legal regulations. Table 1 represents LOD values for common PFAS standards. These ranges between 0.25 ng/L and 5 ng/L with an average value of 1.3 ng/mL and match those typically achieved by high-performance TQ systems.

To validate the efficiency and benefits of the workflow reliant on the timsTOF Pro 2 instrument, an analysis was performed using a sample of drinking water*. This sample was analyzed in native state as well as spiked with several PFAS compounds at concentration levels relevant for legal limits.
The workflow depicted in Fig. 4 was employed for the examination of samples.

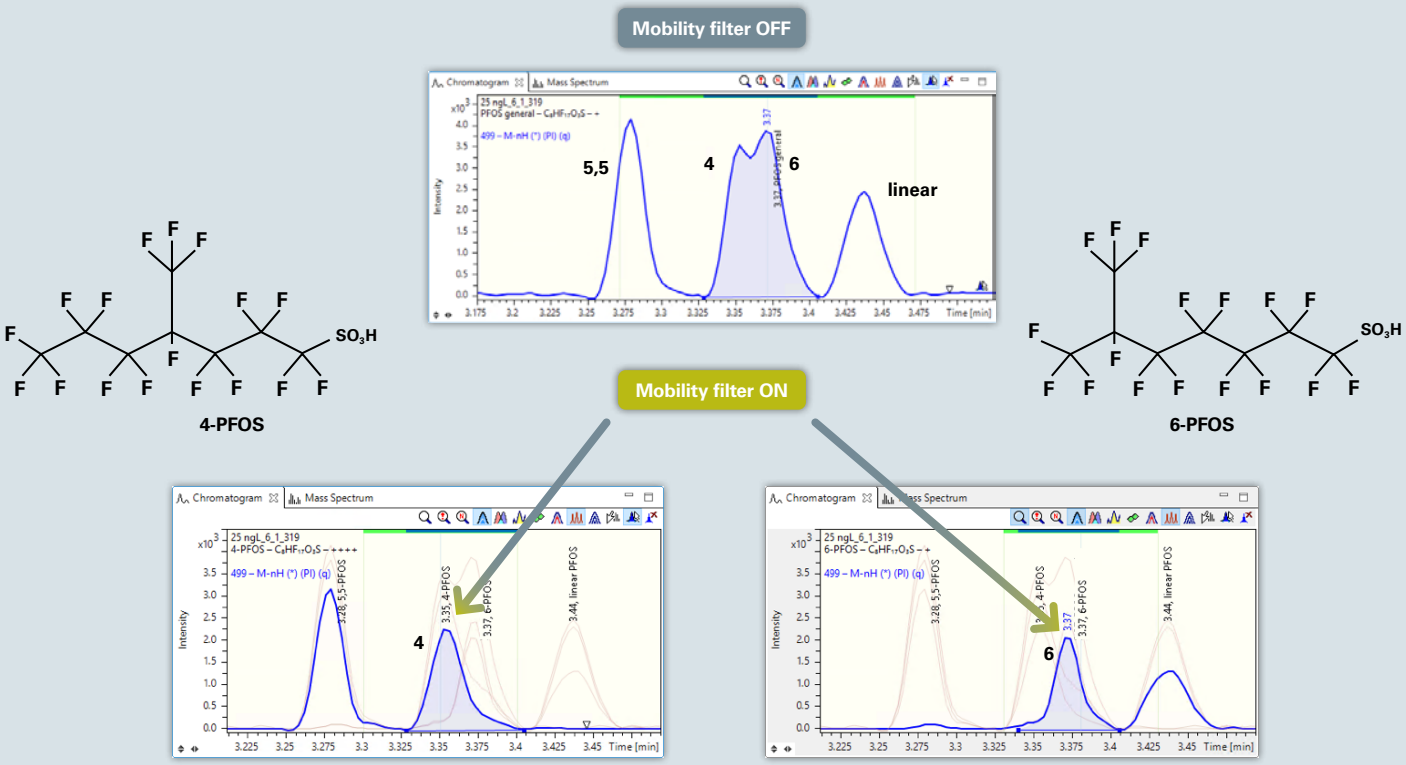


Figure 2. The additional dimension of trapped ion mobility spectrometry (TIMS) separates two co-eluting PFOS isomers, 4-PFOS and 6-PFOS.

Table 1: LOD of a typical set of PFAS standards. Conditions for the analysis: S/N >3, at least one additional qualifier ion, identified in three replicates and significantly higher intensity than in the blank. Calibration lines were measured in spiked bottled water.

PFAS name	LOD [ng/L]
11Cl-PF3OUdS	5.0
3,6-OPFHpA	2.5
4:2 FTS	0.5
6:2 FTS	1.0
9Cl-PF3ONS	1.0
ADONA	0.5
L-PFBS	0.5
L-PFHpS	0.25
L-PFOA	0.25

PFAS name	LOD [ng/L]
L-PFOS	1.0
L-PFPeS	0.25
PF5OHxA	2.5
PFDA	2.5
PFEESA	0.5
PFHpA	0.5
PFHxA	2.5
PFHxS	0.5
PFNA	1.0

*Sample kindly provided by M. Sadia, S. Absalah, Freshwater and Marine Ecology IBED-FAME, University of Amsterdam, Netherlands

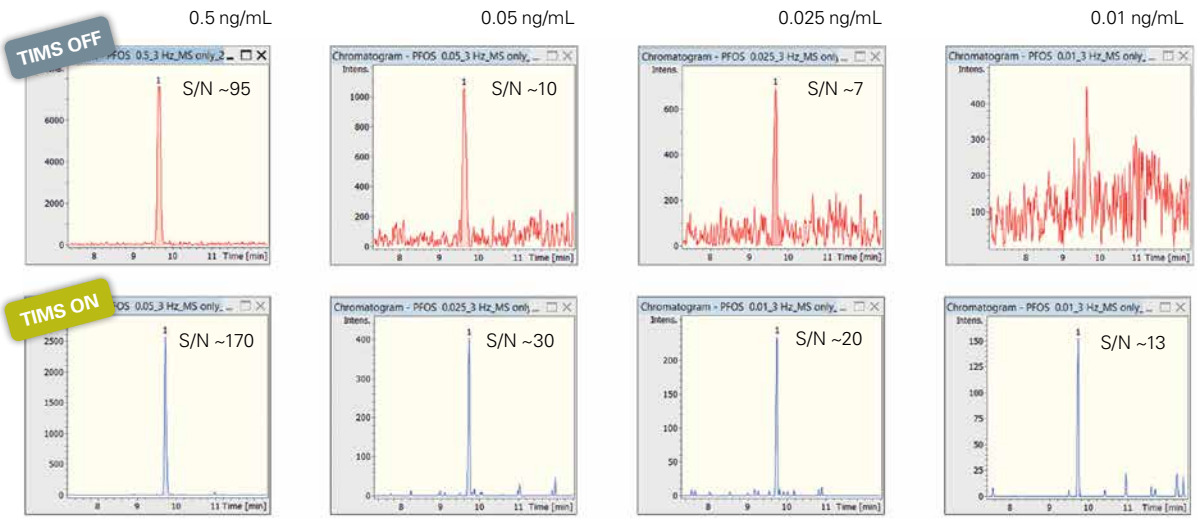


Figure 3. A) Improvement of S/N values for chromatographic MS peaks by applying TIMS. The main difference is visible for low concentrations which are of main interest.

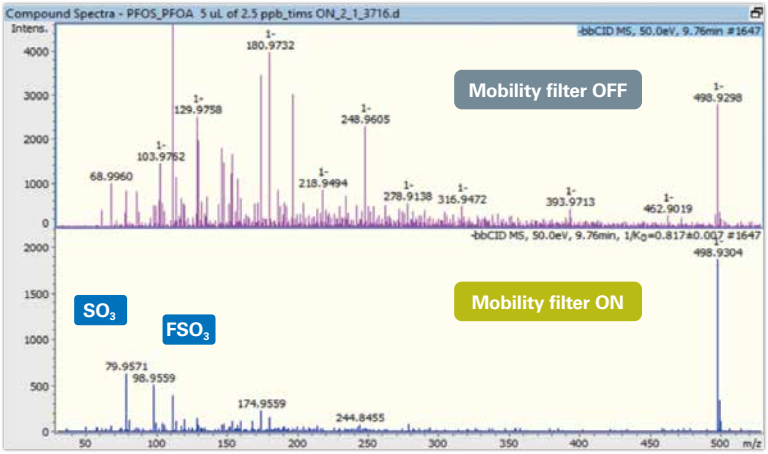


Figure 3. B) TIMS cleans up MS/MS spectra (here bbCID) and increases the S/N of diagnostic ions which are of major importance for an ambiguous compound identification.

Workflow

1. Acquisition of MS and MS/MS data by UHPLC-PASEF. PASEF is based on trapped ion mobility and enables a very fast duty cycle for MS/MS fragmentation on the majority of the detected features.

2. Feature extraction (combining isotopes, adducts etc. into a single feature) including mass and mobility calibration, retention time alignment and MS/MS spectrum import in the software MetaboScape.

3. Kendrick mass filtering and/or statistics (PCA, t-test, PLS-DA).

4. Screening for knowns and/or suspects using a compound target list such as the NORMAN network PFAS list or the NIST PFAS suspect list.

5. Identification of unknowns using various tools in MetaboScape. MetaboScape is a unique "all in one" software package that performs all key tasks of an untargeted screening experiment without having to export and import data from one application to the other. Following the compound identification, alignment across samples and statistical evaluation are done to identify differentially regulated features between groups or timepoints.

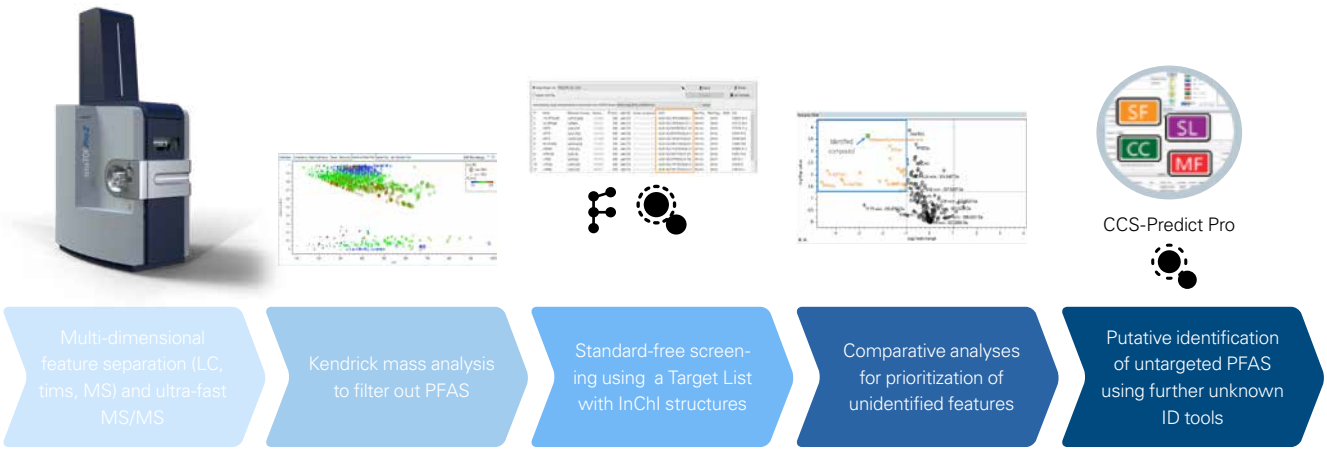


Figure 4. Workflow for the unambiguous identification of compounds.

Fig. 5 shows the heatmap of the complete UHPLC-TIMS-HRMS run. Data processing in MetaboScape revealed more than 1,300 features. Since PASEF provides an extremely high MS/MS speed with > 100 spectra/sec, a very high ratio of 95% of all features is covered by MS/MS data. This uniquely high MS/MS coverage is key for the subsequent comparison with *in-silico* fragments and the final unambiguous identification against 5,000 reference compounds. The features were then filtered by KMD analysis for the CF_2 repeating unit, characteristic for PFAS. As seen in Fig. 6, potential compounds which may be related to PFAS show up in vertical lines at KMD values between -0,1 and 0.3. This is a very effective data reduction by 90% that allows to limit the following post-processing to analytically relevant species only. An important condition for this efficient filtering is the high mass accuracy provided by the timsTOF Pro 2. Accurate mass and the isotope patterns are compared to the elemental compositions of the suspect list for the generation of an initial list of elemental compositions which needs further reduction and refinement. As a next step, the InChI structures present in the NORMAN network and NIST suspect lists are used to predict *in-silico* fragments (MetFrag) and collision cross section (CCS) values (CCS-Predict Pro) for the candidates on the initial list from the fly. The total match of these various and orthogonal criteria, namely exact mass, isotope pattern, MS/MS fragments and CCS values generate a highly confident and correct assignment of the PFAS compounds in MetaboScape. Below, the automated workflow for standard-free PFAS screening is illustrated and shows as an example the identification of the signal at *m/z* 789.9816 as 6:2 diPAP. Accurate mass and isotope pattern data generated a list of potential elemental compositions. These were checked against respective *in-silico* fragments and predicted CCS values, resulting in a unique match with 6:2 diPAP.

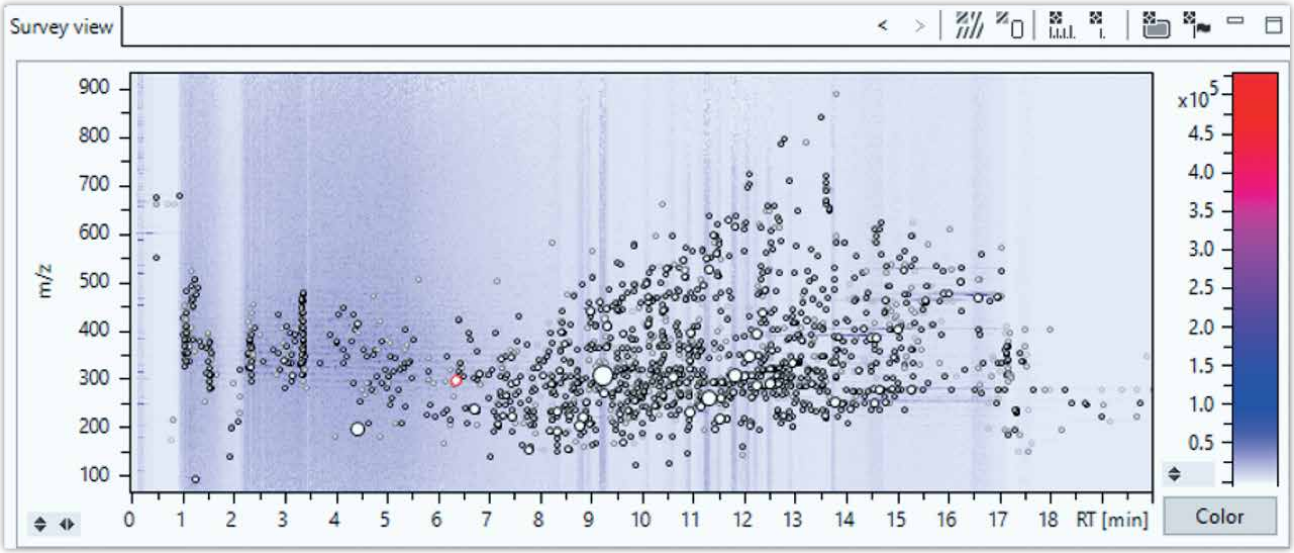


Figure 5. Analysis of the water samples spiked with PFAS. Data processing in MetaboScape revealed > 1300 features with > 95% TIMS PASEF MS/MS coverage.

A total of 67 PFAS was annotated using the NIST PFAS suspect list. Of these, 46 spiked compounds were clearly confirmed, 5 additional PFAS isomers were detected, and 8 more PFAS annotations which were not spiked were found from the NIST PFAS suspect list. They belong to low abundant signals which are present in both samples. The remaining 8 features were identified as isCID (in-source collision-induced dissociation) fragments of carboxylic acids (loss of CO_2) using the "Feature Correlation" plot in MetaboScape.

As seen in the volcano plot in Fig. 7, which reveals differences between the native and the spiked water sample, only 13 of the 46 spiked features show a significantly higher intensity in the spiked sample (area of the blue square). That means that most of these PFAS are already present in the non-spiked, native sample and proves the high sensitivity of the method. As an example, the intensity plot for MeFBSA shows its clear detection in the original water sample already, although at a lower concentration.

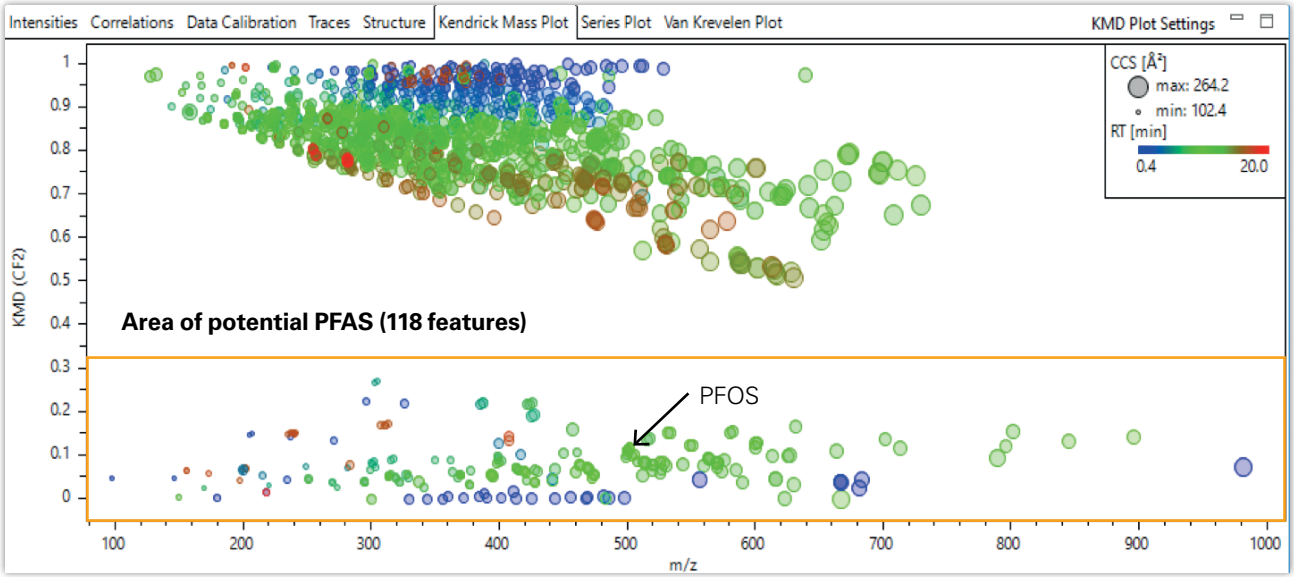


Figure 6. Plotting the Kendrick Mass Defect (KMD) of CF_2 versus *m/z*, separates the PFAS from other features and reduces the data amount by 90%. PFAS show up in vertical lines at KMD values between -0,1 and 0.3.

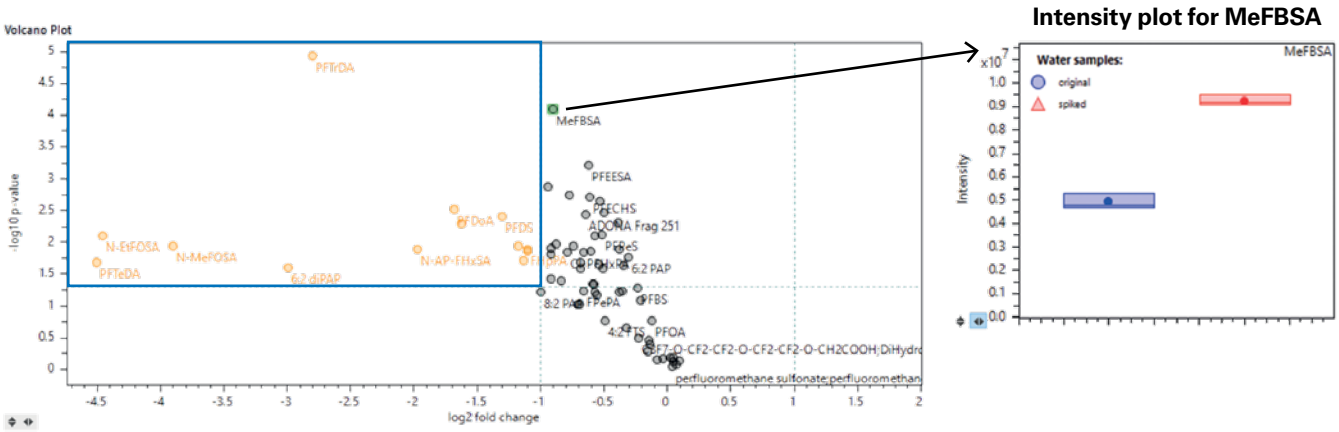


Figure 7. The volcano plot reveals differences between the native and the spiked water sample. Only 13 of the 46 spiked features show a significantly higher intensity in the spiked sample (area of the blue square).

Automatic workflow for standard-free PFAS screening

Exemplary identification of 6:2 diPAP

The elemental composition of $C_{16}H_9F_{26}O_4P$ matches best with the data according to the criteria accurate mass (deviation -0.955 ppm) and isotope pattern (mSigma value of 14.2).

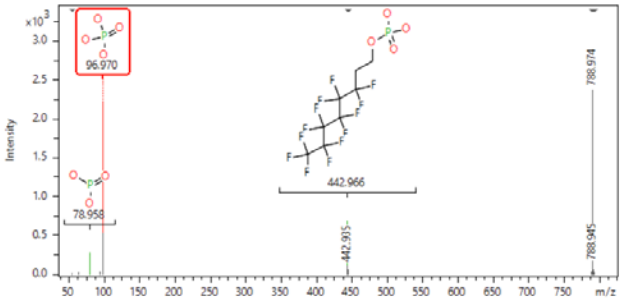
1

RT [min]	m/z meas.	M meas.	Ions	MS/MS	CCS (Å²)	Name	Molecular Formula	Δm/z [ppm]	Δm/z [mDa]	mSigma
12.68	788.97430	789.98157	± ▫ |		227.4	6:2 diPAP	$C_{16}H_9F_{26}O_4P$	-0.955	-0.754	14.2

In-silico fragments calculated from InChI structure match with the MS/MS spectrum with a coverage of 83.2%.

2

Name	Molecular Formula	Score	Raw Value	# Frag	IntCov. [%]
6:2 diPAP	$C_{16}H_9F_{26}O_4P$	1.0	10069325.866	3	83.186



CCS value predicted from InChI structure using CCS-Predict Pro matches the measured value with a deviation of -3.4% (confident values are typically < 5%).

3

RT [min]	m/z meas.	M meas.	Ions	CCS (Å²)	MS/MS	Name	Molecular Formula	ΔCCS [%]
12.68	788.9743	789.9816	± ▫ |	227.4		6:2 diPAP	$C_{16}H_9F_{26}O_4P$	-3.4

Overview for the scoring of final result, using accurate mass (deviation -0.955 ppm), isotope pattern matching (mSigma 14.2), MS/MS score (728) and CCS match (deviation -3.4%). The scoring is visualized by color coding on the right side. "Green" means a perfect match. The retention time (RT) was not considered as criteria due to the lack of database information. However, RT is not needed for a confident hit at all.

4

RT [min]	m/z meas.	M meas.	Ions	MS/MS	CCS (Å²)	Name	Molecular Formula
12.68	788.97430	789.98157	± ▫ |		227.4	6:2 diPAP	$C_{16}H_9F_{26}O_4P$

Annotations	Δm/z [ppm]	Δm/z [mDa]	mSigma	MS/MS score	ΔCCS [%]	AQ
TL SF	-0.955	-0.754	14.2	728.0	-3.4	

Summary

This study demonstrates a comprehensive and truly unique workflow for a complete, non-target screening of PFAS data for real-life samples versus thousands of PFAS compounds. Combining TIMS and HR MS and MS/MS data results in a unique, extremely high 4-dimensional data quality which can be used to search against large PFAS suspect lists for a confident non-target identification of PFAS.

- timsTOF Pro 2 provides superior MS/MS coverage in PASEF mode, high mass accuracy MS and MS/MS data, perfect isotope pattern matches and accurate CCS values.
- Kendrick mass defect analysis separates PFAS from other non-fluorinated compounds.
- The NORMAN network and the NIST PFAS suspect list with nearly 5,000 entries provide molecular formulas and InChI structures which are a sufficient input for the search of the timsTOF Pro 2 data.
- The utilization of the truly orthogonal criteria accurate mass, isotope fidelity, *in-silico* MS/MS fragment and CCS prediction enables the confident identification of PFAS in real-life samples without the need of standards or libraries.
- Statistical tools (PCA, t-test, …) facilitate the comparison of samples enabling the tracking of PFAS changes.

References

1. Parallel accumulation – serial fragmentation (PASEF): Multiplying sequencing speed and sensitivity by synchronized scans in a trapped ion mobility device; F. Meier, S. Beck, N. Grassl, M. Lubeck, M. A. Park, O. Raether, and M. Mann; J Proteome Res. 2015 Dec 4;14(12):5378-87.
2. K. Sims, A. James, A. Kärrman, A. Heggelund, B. González-Gaya, D. Gray, G. Jacobs, L. Vergeynst, N. Perkola, R. Carter, S. van Leeuwen, U. Borchers, 2021 NORMAN network PFAS Analytical Exchange, Provided by Environment Agency (UK) (2022)
3. https://data.nist.gov/od/id/mds2-2387
4. Wolf, S., Schmidt, S., Müller-Hannemann, M. et al., BMC Bioinformatics 11, 148 (2010). https://doi.org/10.1186/1471-2105-11-148
5. Zhou, Z., Luo, M., Chen, X., Yin, Y., Xiong, X., Wang, R. & Zhu, Z. J.; Nature communications, 11 (4334), (2020)

Bruker Applied Mass Spectrometry is continually improving its products and reserves the right to change specifications without notice. © 2023, Bruker Applied Mass Spectrometry. AMS-04 1904205

For Research Use Only. Not for use in clinical diagnostic procedures.

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

marketing.bams.us@bruker.com - www.bruker.com

Learn more at https://info.bruker.com/pfas-resource-lp-dec22