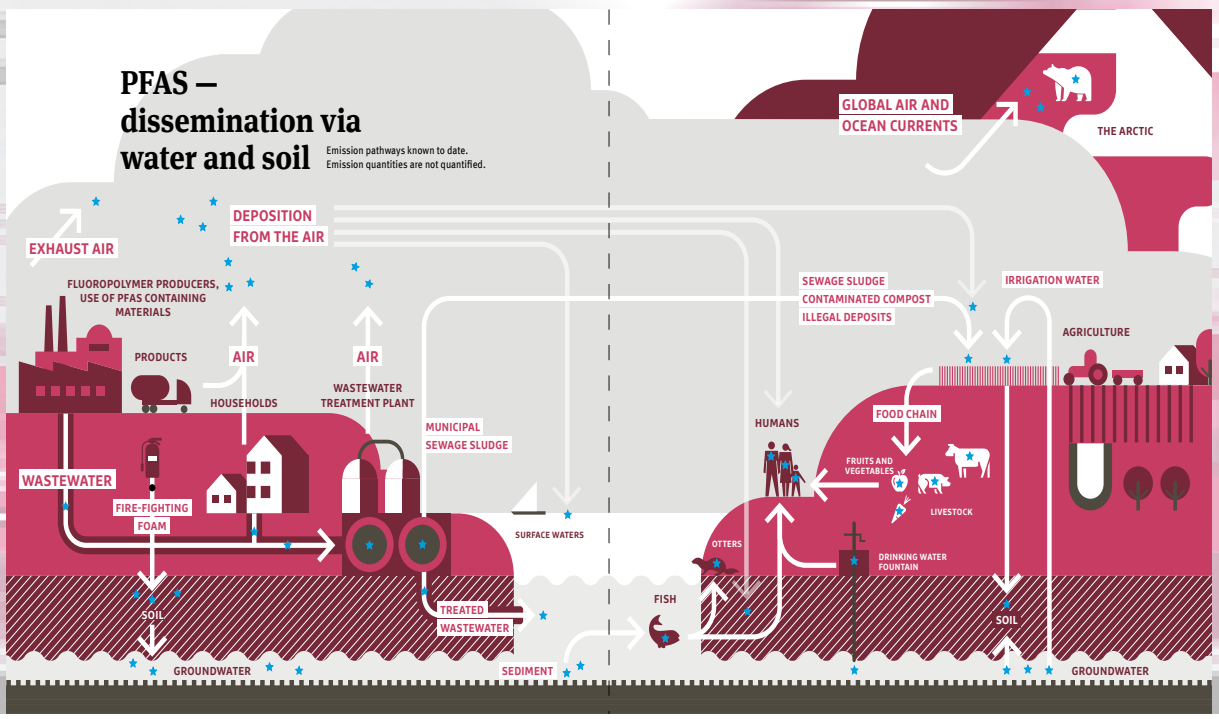


PFAS analysis miniaturized and automated

The EU Drinking Water Directive (EU 2020/2184) includes limits for total PFAS of 0.5 µg/L and sets a limit of 0.1 µg/L for the sum of 20 per- and polyfluoroalkyl substances (PFAS) of most concern. The Directive entered into force in January 2021. EU Member States have a 2-year transitional period to develop national laws. The listed perfluorinated carbonic and sulfonic acids can be determined by an automated method based on miniaturized solid phase extraction with weak anion exchange sorbent combined with LC-MS/MS. Surface adsorbed analytes are included using rinse cycles and effluent recovery, at the same time minimizing sample-to-sample carry-over. Based on just 1 mL sample volume, as opposed to the normally used 250 mL, the required quantification limit of 1.5 ng/L is reached for all listed analytes with good method accuracy.

By Thomas Brandsch, Ph.D.

It occasionally takes a while, before we as consumers realize that synthetic chemicals, which make our lives easier and more convenient, are harmful in addition to being useful. The category of synthetic chemical troublemakers includes Persistent Organic Pollutants (POPs). Once released, the members of the so-called dirty dozen can hardly be recovered and neutralized since mother nature has no effective mechanism to do so. Per- and polyfluoroalkyl substances (PFAS) should now be added to that list. Their resistance to photolytic, hydrolytic, oxidative, and reductive breakdown mechanisms are the exact properties designed into these compounds to make them fulfill their purpose. PFAS belongs to a family of highly fluorinated anthropogenic organic chemicals with special physical chemical properties. Adding PFAS to the surface makes a material oil and water repellant in addition to heat resistant. These are useful properties in many household and industry applications. They are used in commercial products like cooking utensils, food packaging, clothing, carpets, cleaning products, and in firefighting foams. And there is more to PFAS than just thermal and chemical stability.

Peeking into the molecular cosmos

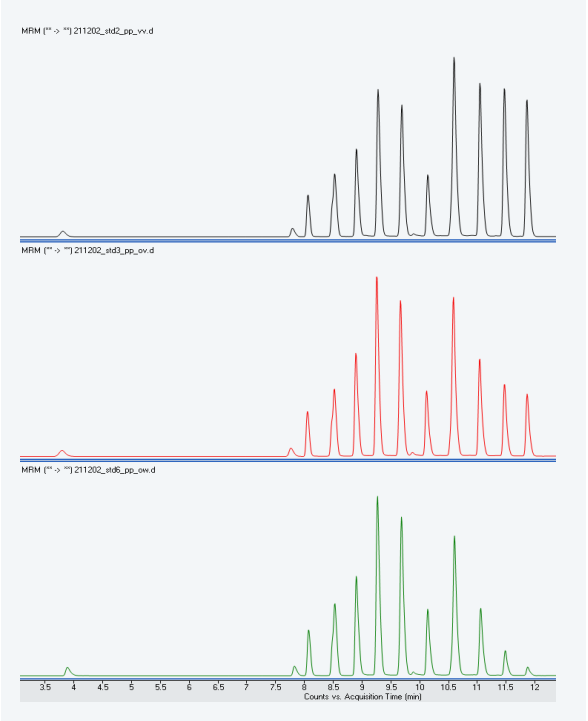
The PFAS carbon atom chain is hydrophobic, whereas the head of many PFAS molecules is hydrophilic. The resulting amphiphilic character is what makes a compound useful as surfactant. As opposed to classical surfactants, the hydrocarbon chain of the PFAS is also oil repellent, which is why PFAS is used as water, oil, fat, and dirt repellant. At the same time, PFAS are sufficiently water soluble to access and accumulate in the food chain, ground water, rivers, and surface waters, which means in our main drinking water reservoirs.

Clear and present danger

An estimated 4700 chemical compounds are classified as PFAS. 20 of these have been targeted in the EU Drinking Water Directive 2020/2184 due to their toxicity [1]. They are strongly suspected of causing liver damage, thyroid disease, adiposity, fertility disorders, and cancer [2] leading to their inclusion in the EU Directive:

- To minimize the risk of adverse effects through potentially contaminated drinking water, the EU Drinking Water Directive 2020/2184 establishes a limit of 0.5 µg/L for the sum of all PFAS.
- The Directive also sets a limit of 0.1 µg/L for the sum of the 20 per- and polyfluoroalkyl substances (PFAS) of most concern.
- The lower limits of quantitation (LOQ) subsequently required are: 30 ng/L for the sum of the 20 PFAS and 1.5 ng/L for the compounds individually.

To minimize the overall environmental impact and to limit adverse effects on humans, livestock, and wildlife, not only drinking water will need to be monitored for contamination with the relevant PFAS compounds, but also ground-, surface-, and wastewater. Unlike drinking water, the latter types can be expected to contain up to significant amounts of particulate matter and solid matrix, which will impact the analysis. Extracting adsorbed PFAS from solid matrix material and subsequently eliminating the solids from the extract will become key objectives of the sample preparation.



The importance of rinsing and recovering the rinse effluent can be seen in the above comparison of chromatograms obtained through direct injection of 1 mL of PFAS standard solutions into the online-SPE-LC-MS/MS system. The uppermost chromatogram resulted from subsequently rinsing the vial, syringe and injection loop and adding the rinse effluent to the SPE cartridge before the analysis. For the middle chromatogram, only the syringe and injection loop were rinsed, and the effluent recovered. Finally for the bottom chromatogram, no rinsing was performed resulting in significantly decreased recovery of relevant analytes.

Solid Phase Extraction

The separation technique of choice specified in German standard procedures for the analysis of waste water and sludge (DIN 38407-42) [2] is solid phase extraction (SPE). Due to the anionic properties of the analytes the extraction is performed using weak anion exchange (WAX) resins. The matrix is purged from the SPE cartridge, and retained analytes are subsequently eluted using a methanolic ammonia solution. How efficiently the SPE and thereby the analysis is performed depends to a large extent on the SPE technique used.

Chemistry of PFAS

Per- and Polyfluoroalkyl compounds are purely synthetical man-made chemicals. They are formed by chemical reactions substituting hydrogen atoms with fluorine atoms in carbonic and sulfonic acids with a chain length from C4 to C18. Two categories of PFAS are especially relevant for environmental and food analysis: Perfluorinated alkyl sulfonates (PFAS) with perfluorooctanoic sulfonate (PFOS) as the most widely known representative, and perfluorinated carbonic acids (PFCA), for which perfluorooctanoic acid (PFOA) is the most widely known. Among the estimated 4700 PFAS compounds, are alkaline, neutral, and poly- rather than perfluorinated compounds.

As opposed to standard dimension SPE cartridges, as described in the DIN 38407-42 method, the Online-SPE (GERSTEL SPE^{XOS}) relies on smaller cartridges from which the eluate is transferred directly and quantitatively to the HPLC mobile phase for 100 % analyte recovery and significantly lower limits of detection and of quantification. In other words, the required limits can be reached even with substantially smaller sample amounts.

Online SPE the method of choice

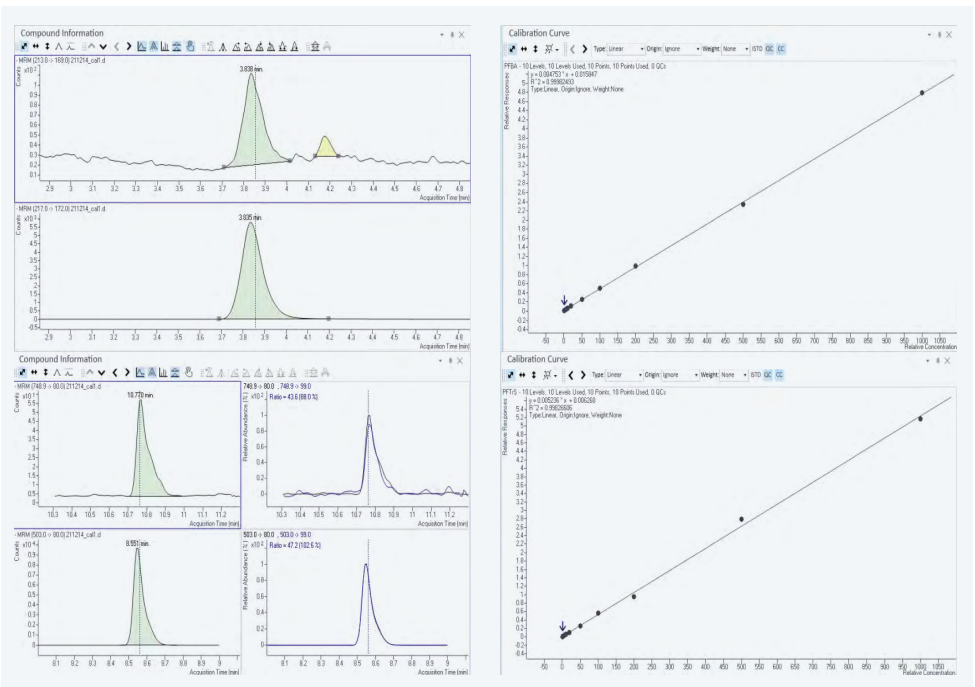
The combination of the GERSTEL SPE^{XOS} and the MultiPurpose Sampler (MPS) robotic makes PFAS analysis both efficient and simple to perform. SPE^{XOS} automates all steps normally associated with standard SPE. These include conditioning, loading, rinsing, eluting into the HPLC mobile phase, and finally exchanging the cartridge. The MPS, on the other hand, injects the sample into the SPE system and then rinses the vial, syringe, and injection volume to recover surface adsorbed PFAS. The rinse effluent is transferred to the SPE cartridge for inclusion in the analysis leading to improved analyte recovery and reduced sample to sample carry over. Following analyte elution, SPE^{XOS} removes the cartridge from the HPLC mobile phase flow path and prepares the system for the next analysis in parallel to the ongoing HPLC-MS/MS analysis.

Parallel sample preparation and analysis (PrepAhead) ensures maximum efficiency and throughput.

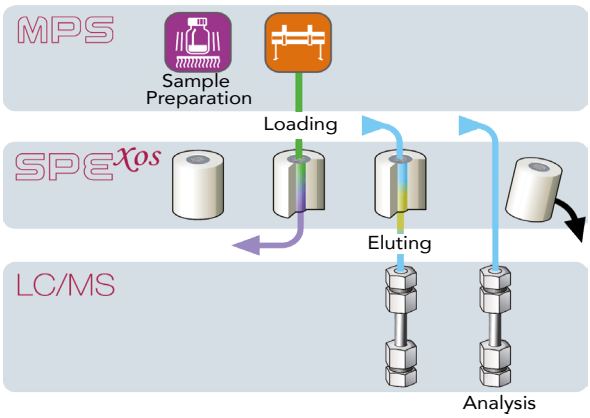
SPE^{XOS} method details

During the development project, the method was extensively tested and validated, resulting in a rugged and robust miniaturized method for determination of the PFAS listed in the EU Drinking Water Directive 2020/2184:

- An Agilent Technologies HPLC-MS/MS system was used (1260 Infinity II LC and ULTIVO LC/TQ MS) combined with a GERSTEL MPS robotic and SPE^{XOS}, which performed all sample preparation and introduction, including SPE cartridge exchange (SPE^{XOS} Polymer WAX, 25-35 µm) in parallel to the ongoing analysis.
- Analyte elution was performed using a 0.25 % solution of ammonia in Methanol. HPLC separation (duration 15 min) was performed using a Poroshell 120 EC-C18 column, 3.0 x 100 mm, 2.7 µm (Agilent Technologies) with a gradient of 0.1 % formic acid in water and 0.05 % formic acid in a 0.25 % solution of ammonia in methanol at a flow rate of 0.6 mL/min.
- Analyte detection was performed in dynamic Multiple Reaction Monitoring Mode (dMRM). For every target compound and every isotopically labelled internal standard (ISTD), two MRM transitions were selected, one Quantifier and one Qualifier, with the exception of PFBA and PFPeA, for which only one transition was available.



Calibration was performed using water spiked with a standard solution containing the 20 relevant PFAS (carbonic- and sulfonic acids ranging from C4 to C13) in the range from 1–1000 ng/L. To each reference solution and each sample, a standard mixture of isotopically labelled compounds was added. All calibration curves were linear in this range with correlation coefficients R2 > 0.998. Calibration curves for the first and last eluting compounds (PFBA and PFTrDS) are shown in the figure.



The MPS-Online-SPE system automates liquid sample preparation as well as SPE with automated cartridge exchange

Successful implementation

The combined MPS-SPE^{XOS}-HPLC-MS/MS system was successfully validated for the target compounds listed in EU Drinking Water Directive 2020/2184. To prove its usefulness for standard laboratory analysis work and the accuracy and trueness of the results, drinking water (tap water) samples as well as surface water samples from the river Ruhr were spiked at two concentration levels (5 and 100 ng/L) and analyzed. The fivefold analyses showed only minimal concentrations of some short chain PFAS (below 10 ng/L). These results were confirmed by determining low concentrations spiked into the samples. Trueness was determined at between 90 and 110 %, except PFPeA for which trueness was at 70 %. The higher-level spiked samples were analyzed resulting in trueness for all compounds between 70 and 130 %, as well as relative standard deviations with a median of 2.6 % and an upper range of 8.6 %, demonstrating the good performance of the method.

Summary

The presented online SPE-LC-MS/MS-System enables the fully automated determination of the 20 target PFAS compounds listed in EU Drinking Water Directive 2020/2184 at low ng/L concentrations while meeting the requirements of DIN 38407-42. The added value of the dedicated analysis system includes vastly simplified handling, dramatically reduced solvent consumption for improved laboratory sustainability, as well as excellent accuracy and reproducibility. In addition, water samples need not be filtered. By rinsing the sample vials with methanol, PFAS compounds adsorbed on the glass surface as well as fine particulate matter are recovered and transferred to the SPE cartridge where particle adsorbed PFAS compounds can equally be desorbed and included in the analysis for a true picture of the PFAS load in the entire water sample.

For more details on the analysis, please consult GERSTEL AppNote 237: Determination of PFAS in Water according to EU 2020/2184 and DIN 38407-42 using online-SPE-LC-MS/MS. Download AppNote 237: https://gerstel.com/en/Determination-of-PFAS-in-Water

Limits of Determination (LODs) and Limits of Quantitation (LOQs) determined by sixfold injection of 1 mL zero blind water sample into the Online-SPE^{XOS}-LC-MS/MS-System according to DIN 32645.

Compound	Acronym	Formula	LOD [ng/L]	LOQ [ng/L]
Perfluorobutyric acid	PFBA	$C_4HO_2F_7$	0.4	1.2
Perfluoropentanoic acid	PFPeA	$C_5HO_2F_9$	0.1	0.3
Perfluorohexanoic acid	PFHxA	$C_6HO_2F_{11}$	0.3	0.8
Perfluoroheptanoic acid	PFHpA	$C_7HO_2F_{13}$	0.2	0.5
Perfluoroctanoic acid	PFOA	$C_8HO_2F_{15}$	0.4	1.2
Perfluorononanoic acid	PFNA	$C_9HO_2F_{17}$	0.2	0.5
Perfluorodecanoic acid	PFDA	$C_{10}HO_2F_{19}$	0.2	0.5
Perfluoroundecanoic acid	PFUnDA	$C_{11}HO_2F_{21}$	0.3	0.8
Perfluorododecanoic acid	PFDoDA	$C_{12}HO_2F_{23}$	0.3	0.9
Perfluorotridecanoic acid	PFTrDA	$C_{13}HO_2F_{25}$	0.4	1.1
Perfluorobutanesulfonic acid	PFBS	$C_4HO_3F_9S$	0.2	0.5
Perfluoropentanesulfonic acid	PFPeS	$C_5HO_3F_{11}S$	0.1	0.4
Perfluorohexanesulfonic acid	PFHxS	$C_6HO_3F_{13}S$	0.2	0.5
Perfluoroheptanesulfonic acid	PFHpS	$C_7HO_3F_{15}S$	0.1	0.3
Perfluorooctanesulfonic acid	PFOS	$C_8HO_3F_{17}S$	0.2	0.5
Perfluorononanesulfonic acid	PFNS	$C_9HO_3F_{19}S$	0.2	0.5
Perfluorodecanesulfonic acid	PFDS	$C_{10}HO_3F_{21}S$	0.4	1.3
Perfluoroundecanesulfonic acid	PFUnS	$C_{11}HO_3F_{23}S$	0.3	1.0
Perfluorododecanesulfonic acid	PFDoS	$C_{12}HO_3F_{25}S$	0.5	1.4
Perfluorotridecanesulfonic acid	PFTrS	$C_{13}HO_3F_{27}S$	0.3	0.9