APPNOTE

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GERSTEL AppNote 260

Determination of PFAS in Water according to EN 17892 using online-SPE-LC-MS/MS

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Keywords

EU Drinking Water Directive, LC/MS, online-SPE, SPE^{xos}, PFAS

Abstract

In the work presented here, the PFAS listed in EN 17892, including the substance list in EU Drinking Water Directive (EU 2020/2184), were determined by an automated method based on solid phase extraction with weak anion exchange sorbent combined with LC-MS/MS. Recovery rates, blank values, and limits of quantification (LOQ) were determined following the requirements of the EN 17892 method. The method accuracy was demonstrated based on analysis of spiked water samples from different sources. LOQs in drinking water were below 0.5 ng/L for the vast majority of compounds.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a family of highly fluorinated anthropogenic chemicals with special physicochemical properties. The lack of environmental degradation in combination with good solubility in water leads to a wide global distribution. PFAS are toxic and acute exposure could have detrimental health effects. Authorities worldwide are regulating the use of PFAS in general and in products, as well as their discharge to the environment.

The European Food Safety Authority (EFSA) has derived a tolerable weekly intake (TWI) for the sum of the four substances PFOA, PFNA, PFHxS and PFOS of 4.4 ng/kg body weight ([1]). The EU Drinking Water Directive (EU 2020/2184) [2] includes maximum limits for the sum of 20 PFAS of 0.1 µg/L. Monitoring such low levels requires a limit of detection (LOD) of 30 ng/L for the sum and 1.5 ng/L for individual compounds. Due to the low TWI, the EFSA recommends limits of detection for the analysis of PFOA, PFNA, PFHxS and PFOS far below the maximum parametric limit value of 0.1 µg/L.

Given the polar nature of most PFAS, especially carbonic and sulfonic acids, the analysis is mainly performed by LC/MS. The less polar carbonic chain allows reversed phase (RP) chromatography on C18-based columns. To reach low detection limits, water samples are usually extracted by means of SPE, using anion exchange sorbents (e. g. US EPA Method 533 [3] or DIN 38407-42 [4]). The direct injection of water samples is a competitive alternative for low level analysis of PFAS. Long chain acids tend to stick on all surfaces, leading to low (and irreproducible) recoveries. To overcome this drawback, the water samples need to be diluted with methanol and filtered prior to LC/MS analysis (e. g. US EPA Method 8327 [5]).

All these aspects were taken into consideration in the development of the EN 17892 standard [6], which includes a method using direct injection (Part A) and a method using SPE (Part B). As an alternative, online-SPE can be used, which relies on smaller cartridges that can be eluted directly onto the HPLC column, enabling quantitative transfer of analytes to the analysis system, and resulting in improved limits of detection and quantitation even when sample volumes are significantly reduced. Combining the efficiency of SPE with the simplicity of direct injection is a highly attractive proposition.

In a previous AppNote (GERSTEL AppNote 237) we presented a method for the analysis of PFAS on weak anion exchange (WAX) cartridges using an online-SPE system (GERSTEL SPE^{xos}), automating all steps of a typical SPE workflow including condi-

tioning, loading, rinsing, eluting, and changing the cartridge. The presented method enables fully automated determination of the PFAS compounds listed in the EU Drinking Water Directive in the low ng/L range. Rinsing the vial with methanol after the sample has been injected, and subsequently injecting the rinse solution to the analysis system, results in the transfer of adsorbed PFAS to the SPE cartridge, after which they are included in the analysis for improved recovery. Since online-SPE relies on small sample amounts, the main challenge was taking a representative small aliquot of the water sample for analysis. In the work presented here, besides aligning the method to EN 17892, we present a new approach for aliquotation, which simplifies sample preparation prior to analysis while also improving the accuracy and the robustness of the overall method.

Experimental

Materials and Solvents

Water samples were mixed 1:1 with 0.2% formic acid in methanol, preferably in the sampling vessel. This enables aliquotation without the loss of long chain PFAS, as described in EN 17892 for direct injection. Exactly 1.5 mL of this mixture were filled into 1.5 mL polypropylene vials (GERSTEL 093640-084-00) using a pipette. An internal standard solution was added, and the vials were sealed with screw caps with thin polypropylene/silicone septa (GERSTEL A00010-183-00). For the extraction, online-SPE cartridges for GERSTEL SPE^{xos} (Polymer WAX, GERSTEL 018804-023-00) were used.

For chromatography, methanol (hypergrade for LC-MS) and water (LC-MS grade) were used, fortified with ammonia solution 25% (for LC-MS) and/or formic acid 98-100% (for analysis, ACS, Reag. Ph Eur) all from Merck (Darmstadt, Germany).

Preparation of Samples and Calibration Standards

All standards were purchased as solutions from Wellington Laboratories (distributed by Campro Scientific, Berlin, Germany): Native PFC Stock Solution (PFAC-MXC) and Native Replacement PFAS Solution (PFAC-MXF) at 2000 ng/mL for each compound; PFUnS, PFTrS, PFMPA (PF4OPeA), Na4:2FTS, Na6:2FTS, Na8:2FTS, FOSA and N-EtFOSAA as individual solutions with 50 µg/mL; a mixture of isotopically labelled PFAS (MPFAC-24ES) and isotopically labelled HFPO (M3HFPO-DA) were used as internal standards. All substances with abbreviations are listed in table 1.

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Table 1: List of compounds.

Substance *	Abbreviation	Molecular Formula	CAS No	Internal Standard used
Perfluorobutanoic acid	PFBA	$C_4HO_2F_7$	375-22-4	$^{13}C_4$-PFBA
Perfluoropentanoic acid	PFPeA	$C_5HO_2F_9$	2706-90-3	$^{13}C_5$-PFPeA
Perfluorohexanoic acid	PFHxA	$C_6HO_2F_{11}$	307-24-4	$^{13}C_5$-PFHxA
Perfluoroheptanoic acid	PFHpA	$C_7HO_2F_{13}$	375-85-9	$^{13}C_4$-PFHpA
Perfluorooctanoic acid	PFOA	$C_8HO_2F_{15}$	335-67-1	$^{13}C_8$-PFOA
Perfluorononanoic acid	PFNA	$C_9HO_2F_{17}$	375-95-1	$^{13}C_9$-PFNA
Perfluorodecanoic acid	PFDA	$C_{10}HO_2F_{19}$	335-76-2	$^{13}C_6$-PFDA
Perfluoroundecanoic acid	PFUnDA	$C_{11}HO_2F_{21}$	2058-94-8	$^{13}C_7$-PFUnDA
Perfluorododecanoic acid	PFDoDA	$C_{12}HO_2F_{23}$	206-203-2	$^{13}C_2$-PFDoDA
Perfluorotridecanoic acid	PFTrDA	$C_{13}HO_2F_{25}$	72629-94-8	$^{13}C_2$-PFTDA
Perfluorotetradecanoic acid	PFTDA	$C_{14}HO_2F_{27}$	376-06-7	$^{13}C_2$-PFTDA
Perfluorohexadecanoic acid	PFTDA	$C_{16}HO_2F_{31}$	67905-19-5	$^{13}C_2$-PFTDA
Perfluorooctadecanoic acid	PFTDA	$C_{18}HO_2F_{35}$	16517-11-6	$^{13}C_2$-PFTDA
Perfluorobutanesulfonic acid	PFBS	$C_4HO_3F_9S$	375-73-5	$^{13}C_3$-PFBS
Perfluoropentanesulfonic acid	PFPeS	$C_5HO_3F_{11}S$	630402-22-1	$^{13}C_3$-PFBS
Perfluorohexanesulfonic acid	PFHxS	$C_6HO_3F_{13}S$	355-46-4	$^{13}C_3$-PFHxS
Perfluoroheptanesulfonic acid	PFHpS	$C_7HO_3F_{15}S$	357-92-8	$^{13}C_3$-PFHxS
Perfluorooctanesulfonic acid	PFOS	$C_8HO_3F_{17}S$	1763-23-1	$^{13}C_8$-PFOS
Perfluorononanesulfonic acid	PFNS	$C_9HO_3F_{19}S$	98789-57-2	$^{13}C_8$-PFOS
Perfluorodecanesulfonic acid	PFDS	$C_{10}HO_3F_{21}S$	335-77-3	$^{13}C_2$-PFDoDA
Perfluoroundecanesulfonic acid	PFUnS	$C_{11}HO_3F_{23}S$	749786-16-1	$^{13}C_2$-PFDoDA
Perfluorododecanesulfonic acid	PFDoS	$C_{12}HO_3F_{25}S$	79780-39-5	$^{13}C_2$-PFDoDA
Perfluorotridecanesulfonic acid	PFTrS	$C_{13}HO_3F_{27}S$	791563-89-8	$^{13}C_2$-PFTDA
4:2 Fluorotelomer sulfonic acid	4-2 FTSA	$C_6H_5O_3F_9S$	757124-72-4	$^{13}C_2$-4-2 FTSA
6:2 Fluorotelomer sulfonic acid	6-2 FTSA	$C_8H_5O_3F_{13}S$	27619–97–2	$^{13}C_2$-6-2 FTSA
8:2 Fluorotelomer sulfonic acid	8-2 FTSA	$C_{10}H_5O_3F_{17}S$	39108–34–4	$^{13}C_2$-8-2 FTSA
Perfluorooctanesulfonamide	PFOSA	$C_8H_2O_2NF_{17}S$	754-91-6	$^{13}C_8$-PFOSA
N-ethyl perfluorooctanesulfonamidoacetic acid	N-EtFOSAA	$C_{12}H_8O_4NF_{17}S$	2991-50-6	$^2H_5$-N-EtFOSAA
Hexafluoropropylene oxide dimer acid	HFPO-DA	$C_6HO_3F_{11}$	13252-13-6	$^{13}C_3$-HFPO-DA
4.8-Dioxa-3H-perfluorononanoic acid	DONA	$C_7H_2O_4F_{12}$	919005-14-4	$^{13}C_4$-PFHpA
Perfluoro-3-methoxypropanoic acid	PFMPA	$C_4HO_3F_7$	377-73-1	$^{13}C_5$-PFPeA
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	$C_8HO_4ClF_{16}S$	73606-19-6	$^{13}C_8$-PFOS
11-Chlorooctadecafluoro-3-oxaundecane-1- sulfonic acid	11Cl-PF3OUdS	$C_{10}HO_4ClF_{20}S$	763051-92-9	$^{13}C_2$-PFDoDA

* For the sulfonic acids and telomer sulfonic acids the corresponding potassium (for PFBS) and sodium salts were used for calibration and concentrations are given as such.

The native compounds were mixed to result in a stock solution of 200 ng/mL, which was diluted consecutively with methanol resulting in the working solutions (0.0015 to 15 ng/mL) to be used for spiking calibration samples. The mixture of labeled compounds was diluted to 0.3 ng/mL.

Calibration samples were prepared in 1.5 mL vials by adding 20-100 µL of stock solution to 0.75 mL LC-MS grade water, filled up to 1.5 mL with methanol, followed by adding 50 µL of a solution of internal standards.

Different water samples were analyzed: Tap water from our laboratory, water from the river Ruhr in Mülheim an der Ruhr, and mineral water with high salt content from a local supermarket. After dilution 1:1 with 0.2% formic acid in methanol, 50 µL of the solution of internal standards were added to 1.5 mL sample in the vial.

Instrumentation

The automated system consists of a MultiPurpose Sampler (MPS robotic, GERSTEL) and an online-SPE System (SPE^{xos}, GERSTEL) coupled to an LC-MS/MS system (figure 1). Every LC-MS/MS can be linked to the GERSTEL equipment to perform online-SPE. We used Infinity II 1290 High Speed Pump and 6495 LC/TQ, both from Agilent Technologies, Waldbronn, Germany. SPE elution is performed using 0.25% ammonia in methanol delivered from an additional isocratic HPLC pump (Infinity II 1260 Iso Pump, Agilent Technologies). The eluate is merged with the starting level buffer of the binary analytical pump in a valve fitted with a special T-rotor used in the SPE^{xos} system. As analytical column a Poroshell 120 EC-C18, 4.6x100 mm, 2.7 µm (Agilent Technologies) was used. Between the binary pump and MPS, a delay column (Poroshell 120 EC-C18, 4.6x50 mm, 2.7 µm, Agilent Technologies) was installed.

Figure 1: Online-SPE system, consisting of GERSTEL MPS and SPE^{xos}, coupled to an LC-MS/MS system from Agilent Technologies.

Injection was performed with a 2.5 mL syringe into the injection valve on the MPS, fitted with a 1 mL stainless steel sample loop.

Analysis conditions

The automated workflow started out with conditioning of the cartridge, using 0.25% ammonia in methanol at first, followed by methanol and water. After injection, the sample was loaded onto the SPE cartridge by pumping water through the injection loop. These steps were performed by the High-Pressure Dispenser (HPD) unit of the SPE^{xos}. The injection is split into three steps, each consisting of aspiring 500 µL water and 500 µL sample into the syringe, injecting into the loop and loading onto the cartridge with water. Using *SimultaneousMode* in MAESTRO, the HPD and MPS can perform parallel processing of these steps, reducing the overall sample preparation time.

0.25% ammonia in methanol from a solvent reservoir on the MPS is added to the vial and the vial contents then aspirated and injected into the sample loop of the injection valve, before starting the pumps and switching the valves to elution position. The isocratic pump elutes the cartridge with 0.25% ammonia in methanol and the binary pump delivers 0.05% formic acid in water, merged in the T-rotor valve of the SPE^{xos} (see figure 2). After 6 minutes, the elution phase is finished and chromatography starts. During the following 10 minutes the binary pump delivers a gradient flow of 0.6 mL/min employing water with 0.05% formic acid and methanol with 0.25% ammonia and 0.05% formic acid. During this time the sample introduction and sample preparation system can be cleaned, and preparation of the next sample can be started (prep-ahead).

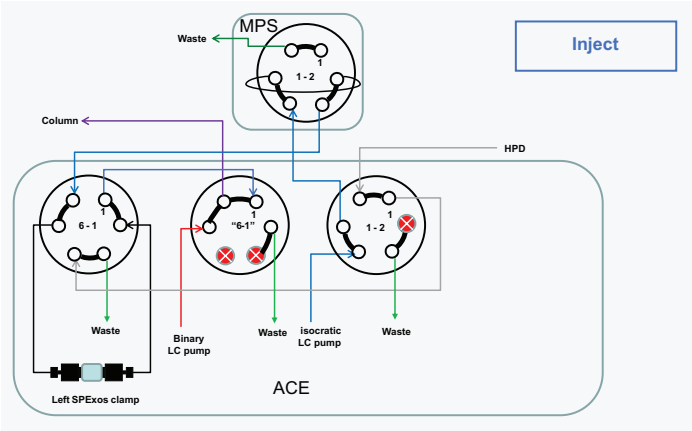

Figure 2: Flow diagram showing valve settings during injection: Elution of the cartridge with ammonia in methanol going through the injection loop, merging with formic acid in water in the T-rotor valve (middle).

Isocratic Pump (0.25% NH_3 in methanol)

0.0 min	0.1 mL/min
0.5 min	0.3 mL/min
5.5 min	0.3 mL/min
6.0 min	0.0 mL/min
6.5 min	0.0 mL/min
7.0 min	1.0 mL/min
8.0 min	1.0 mL/min
8.5 min	0.0 mL/min

Binary Pump (eluent A: 0.05% formic acid in water, eluent B: 0.25% NH_3 and 0.05% formic acid in methanol)

0.0 min	0.1 mL/min	5% B
0.5 min	0.9 mL/min	0% B
3.5 min	0.5 mL/min	0% B
5.5 min	0.3 mL/min	40% B
6.0 min	0.6 mL/min	70% B
16.0 min	0.6 mL/min	95% B
16.5 min	0.6 mL/min	5% B
19.5 min	1.0 mL/min	5% B
20 min	0.1 mL/min	5% B

Detection was performed in dynamic multiple reaction monitoring mode (dMRM) using the following source parameters (negative ion mode):

Gas Temperature	150°C
Gas Flow	18 L/min
Nebulizer Pressure	25 psi
Sheat Gas Temp.	390°C
Sheat Gas Flow	11 L/min
Capillary Voltage	2500 V
Nozzle Voltage	0 V
High Pressure RF	90 V
Low Pressure RF	60 V

For each target compound and isotope labeled internal standard (ISTD) two MRM transitions were chosen, one quantifier and one qualifier (except PFBA, for which only one transition has sufficient intensity).

Results and Discussion

Splitting the injection into three steps, allows us to introduce a larger sample volume than the capacity of the sample loop. By diluting the water/methanol mixture with pure water in the syringe, the methanol proportion is reduced and PFAS are better retained on the cartridge. If the water samples contain high concentrations of inorganic salts, these will form a cloudy precipitate when methanol is added. Diluting with water redissolves the precipitate, enabling injection to the system without prior filtration. These additional steps are time consuming, but using SimultaneousMode in MAESTRO, where HPD and MPS work in parallel, the overall analysis time for one sample can be kept at 20 minutes.

Usually, in online-SPE, elution is achieved with a gradient delivered by the analytical pump. However, WAX cartridges are eluted with ammonia in methanol and this eluate cannot be transferred directly to the HPLC column. For this reason, an extra (isocratic) HPLC pump elutes the cartridge and subsequently, the eluate is merged with the starting level buffer of the binary analytical mobile phase. This takes place in the SPE^{xos} system, using a valve fitted with a special rotor. During this stage the analytes reach the analytical column starting with 25% methanol (with 0.25% NH_3) and 75% water (with 0.05% formic acid). As the methanol proportion increases, the short chain PFAS begin to migrate on the column, while the longer chain PFAS are trapped at the head of the column. After switching the valve, which ends the SPE elution step and starts the gradient chromatography, the methanol content is increased rapidly up to 70%, leading to a focusing of the early eluting peaks, while the later eluting peaks are separated in the second gradient stage. The result is a chromatogram with nearly equidistant peaks for the carbonic acids from C5 to C14. PFBA and PFMPA are eluting during the SPE elution phase, and the sulfonic acids are near the carbonic acids with one C atom more (see figure 3).

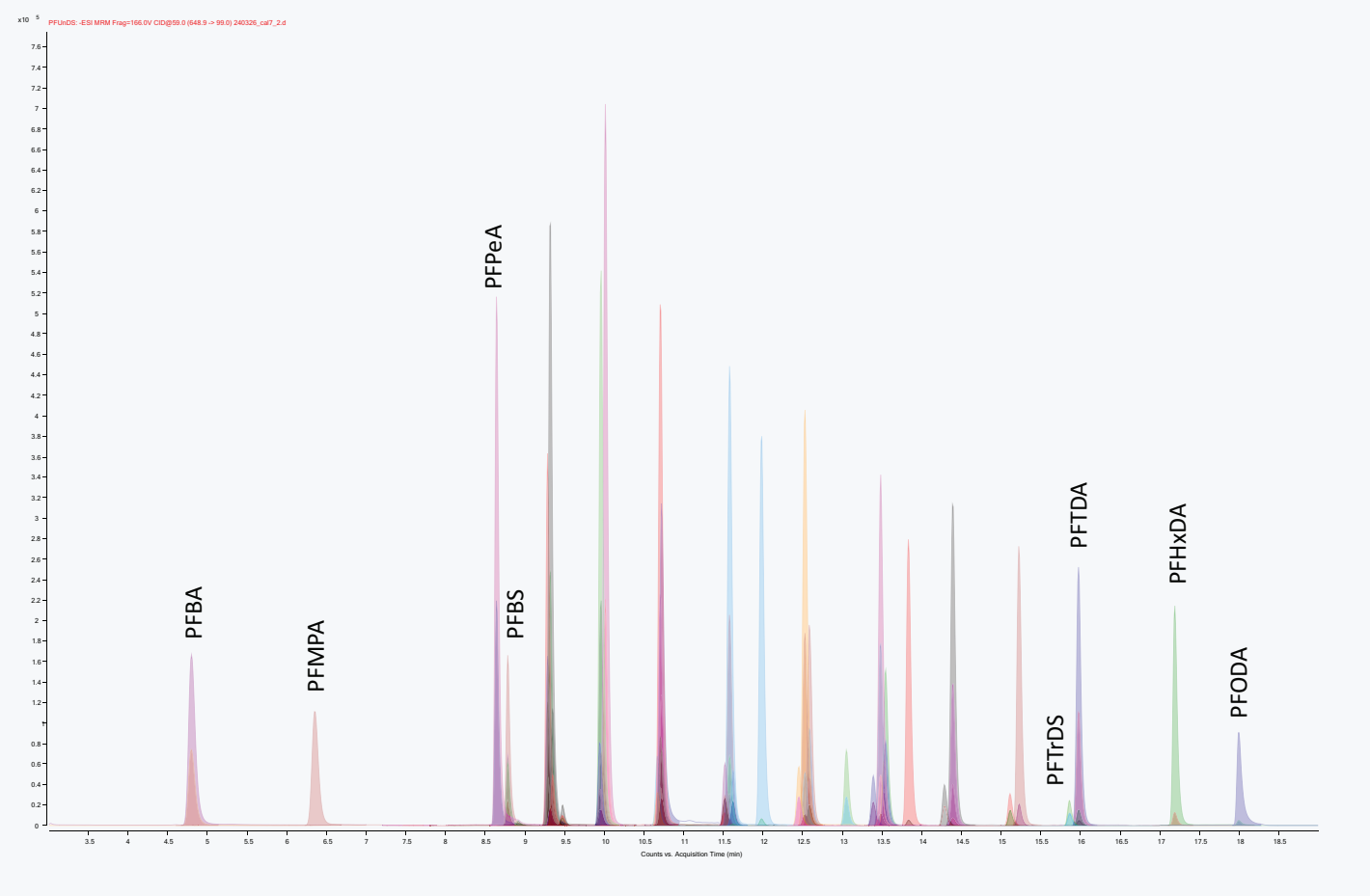

Figure 3: Example chromatogram for a standard solution (50 ng/L) in water with all recorded MRMs, first and last eluting peaks are annotated.

Long chain perfluorinated acids dissolved in water tend to be adsorbed on all surfaces. The EN method therefore recommends the dilution of the water sample 1:1 with methanol prior to direct injection. If samples with such high methanol content are injected on a SPE cartridge, short chain PFAS are not retained. The addition of formic acid and the dilution with water in the syringe allow a much higher injection volume due to the higher retention of the short chain perfluorinated acids. Using the MPS as injector, the sample vial can be rinsed with methanol after the injection of the sample. Subsequent injection of this solution not only recovers analytes adsorbed on the surface of the vial, but also rinses injection syringe, sample loop, and associated tubing with the result that all adsorbed analytes are released, recovered, and transferred to the SPE cartridge. In the method described here, the transfer of the rinse solution from the injection loop to the cartridge is done during the elution step, in which the isocratic pump is connected to the sample loop. This is possible because the rinse solution only contains long chain PFAS, which are trapped during the elution step on the front of the analytical column, and no peak broadening or splitting occurs.

Blank determination

Our online-SPE method is a combination of the direct injection method (Part A of EN 17892) and the SPE method described in Part B. Our method involves a large volume direct injection, and this has to be considered when evaluating the blanks. Injecting 1.5 mL of a water/methanol mixture results in higher blanks than a run without any injection. This in effect corresponds to a procedural blank of the SPE contribution. The high volumes of methanol injected lead to considerable blanks mainly for 6-2 FTSA, PFBA and PFPeA, depending on the purity of the solvent and on batch-to-batch variations. However, all peak areas are below the peak areas resulting from a standard solution with a concentration of 0.1 ng/L for each compound (see figure 4). Blank values have a great impact on quantification limits and will be considered accordingly.

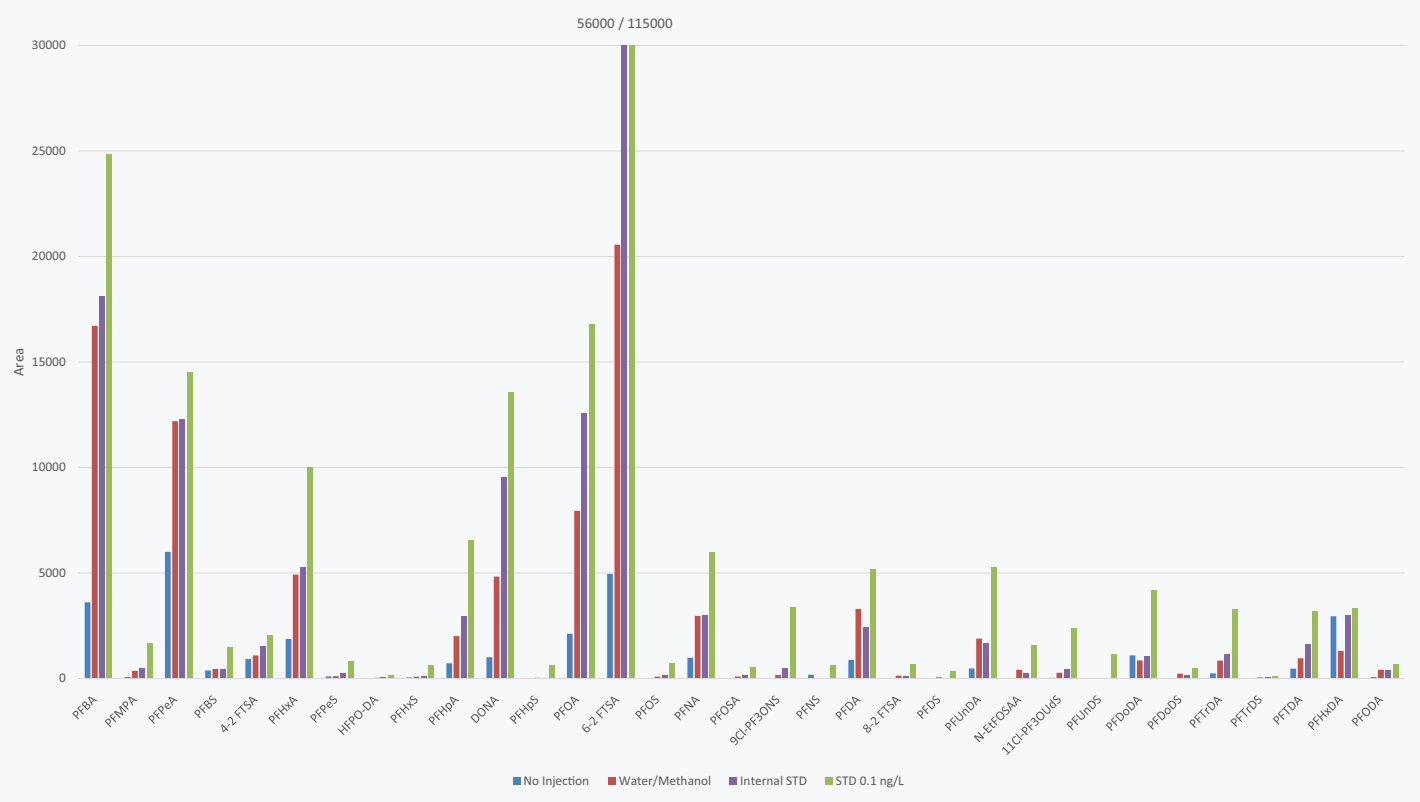

Figure 4: Blank determination and comparison with a low level calibration standard (0.1 ng/L).

Calibration

For all compounds the linear calibration range spans up to 2 µg/L (for an example see figure 5). To ensure linearity, accuracy, and the validity of the quantification limits, we recommend limiting the calibration range when analyzing low concentrations. For the water samples analyzed in this work, a 7-point calibration from 0.5 to 50 ng/L with a weighting of 1/x was used.

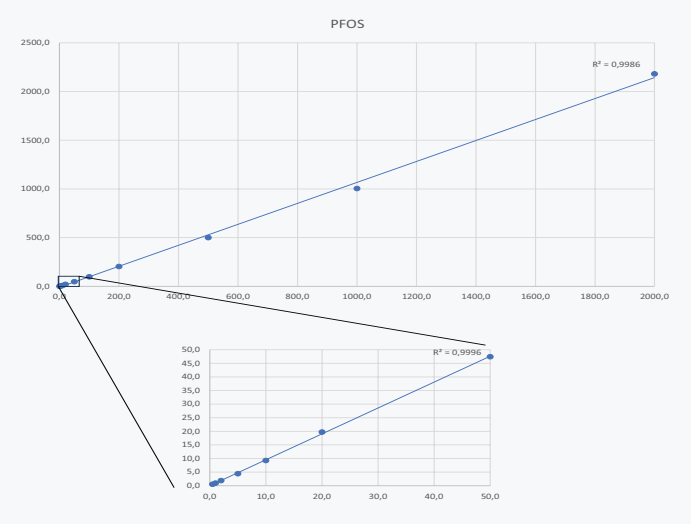

Figure 5: Example calibration curve in the range 0.5 – 2000 ng/L (enlarged area 0.5 – 50 ng/L).

Recovery rates

It is difficult to check the absolute recovery of PFAS from water samples with this method because the absolute intensities of detector signals are highly dependent on the pH value and on the methanol content of the buffer reaching the MS. Injecting a small volume of standard solution directly to the column would lead to a completely different chromatogram. Therefore, the system was modified, placing the injection valve after the valve with the T-rotor seal and substituting the sample loop with a smaller one (10 µL, see figure 6). This allows the injection of a concentrated standard solution to the column, using the same gradient as for the online-SPE elution. Retention times and therefore signal intensities are the same, allowing the determination of recovery rates for the SPE part of the method.

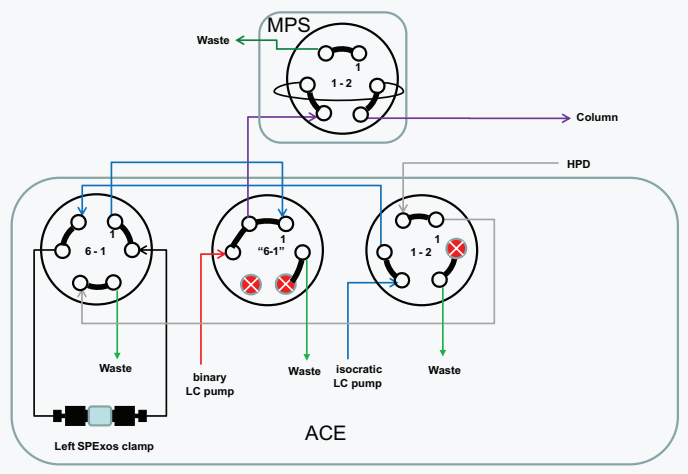

Figure 6: Modified system for the determination of recovery rates by direct injection onto the column under online-SPE conditions.

This was done for three different concentrations; the results are shown in figure 7. At 1 ng/L the recovery rates for a few compounds are higher, caused by blank values or low signal intensities, but the overall recovery rates of all compounds are between 70 and 115%.

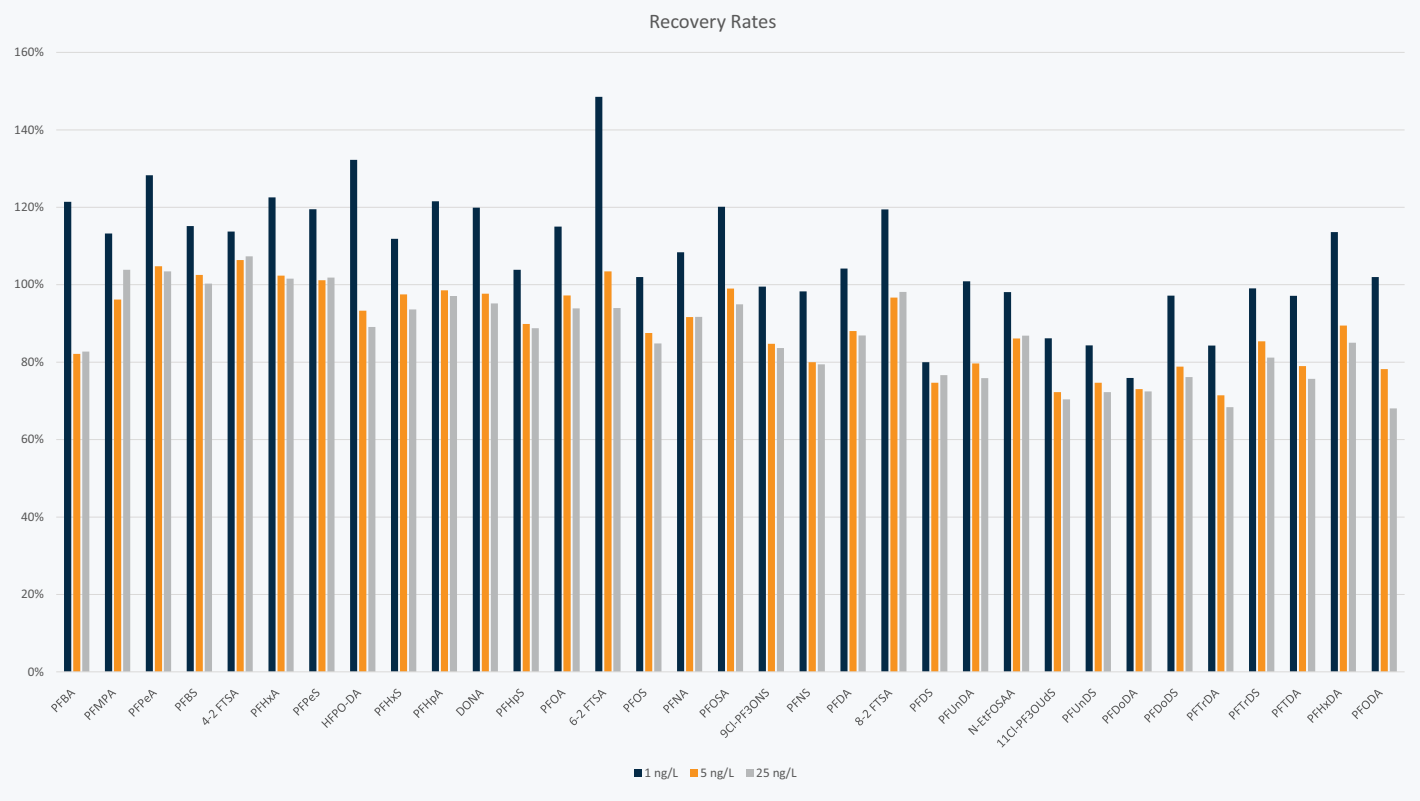

Figure 7: Recovery for standard solutions in water/methanol at different concentrations.

When analyzing real water samples, in some cases, the recovery of labeled PFBA was lower, but for all other internal standards the recovery rates were within the limits of 50 to 150% required by EN 17892. Even for wastewater they are mostly in the range of 75 to 125%.

Limits of Quantification

Method detection limits are not determined only by the sensitivity of the instrument, but also by the unavoidable blank values at sub ng/L level. The contribution from the buffers in the binary pump can be trapped on the delay column used, but for the isocratic column this is not possible. EN 17892 stipulates that in the case of considerable blanks, the quantification limit must be at least three times the blank value.

Limits of detection (LOD) and limits of quantification (LOQ) were calculated from calibration lines near the expected LOQ (0.05 – 0.5 ng/L) as per the requirements of DIN 32645 [7]. In addition, the blank values for each compound were calculated by extrapolating the linear regression curves to their intersection with the x-axis. As an example, this is shown for PFOA in figure 8, compared to PFNA without considerable blank. As a result, LOQs are considered the higher value of calculated LOQ and three times the calculated blank value, shown in figure 9. Excepting PFBA and PFPeA, LOQs are all below 1 ng/L, most of them are even below 0.3 ng/L.

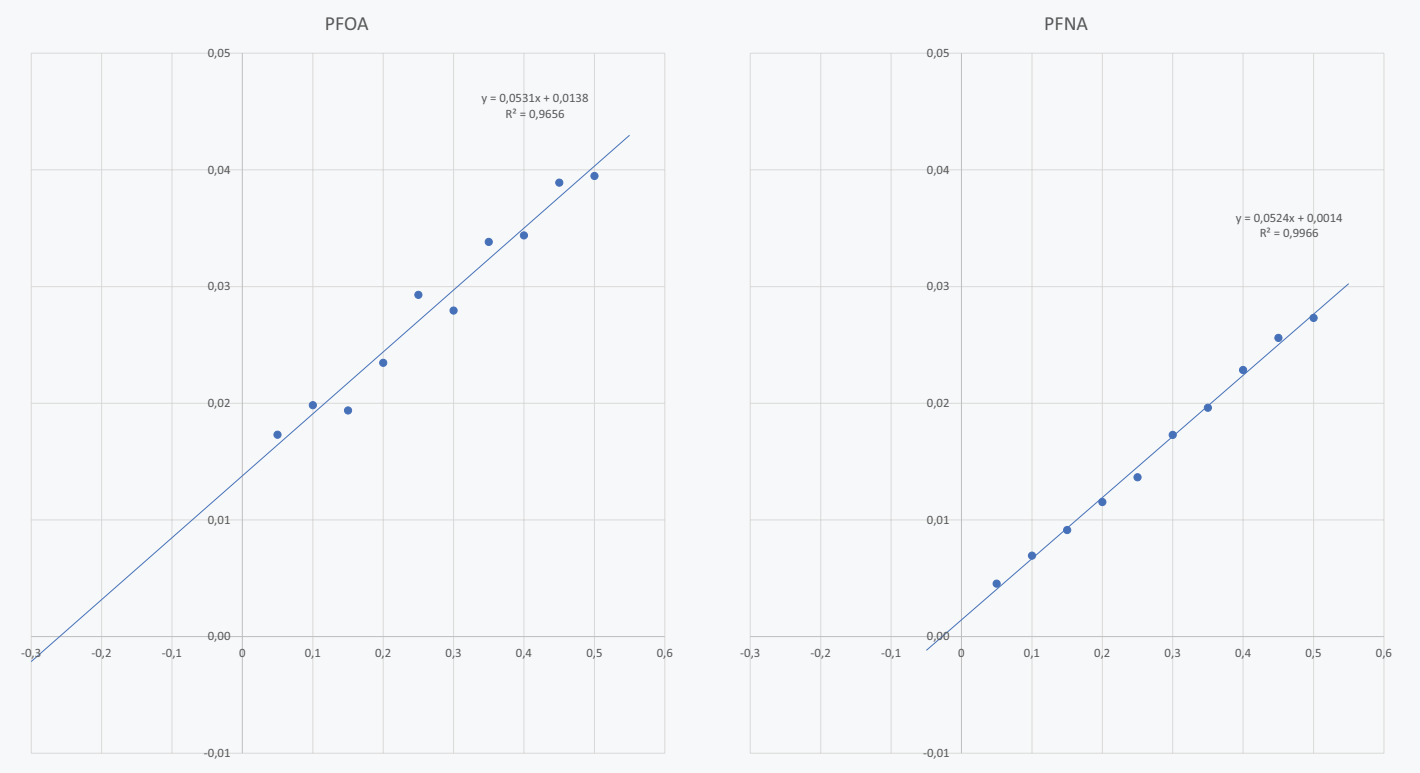

Figure 8: Example calibration curves in the range 0.05 – 0.5 ng/L with and without significant blank.

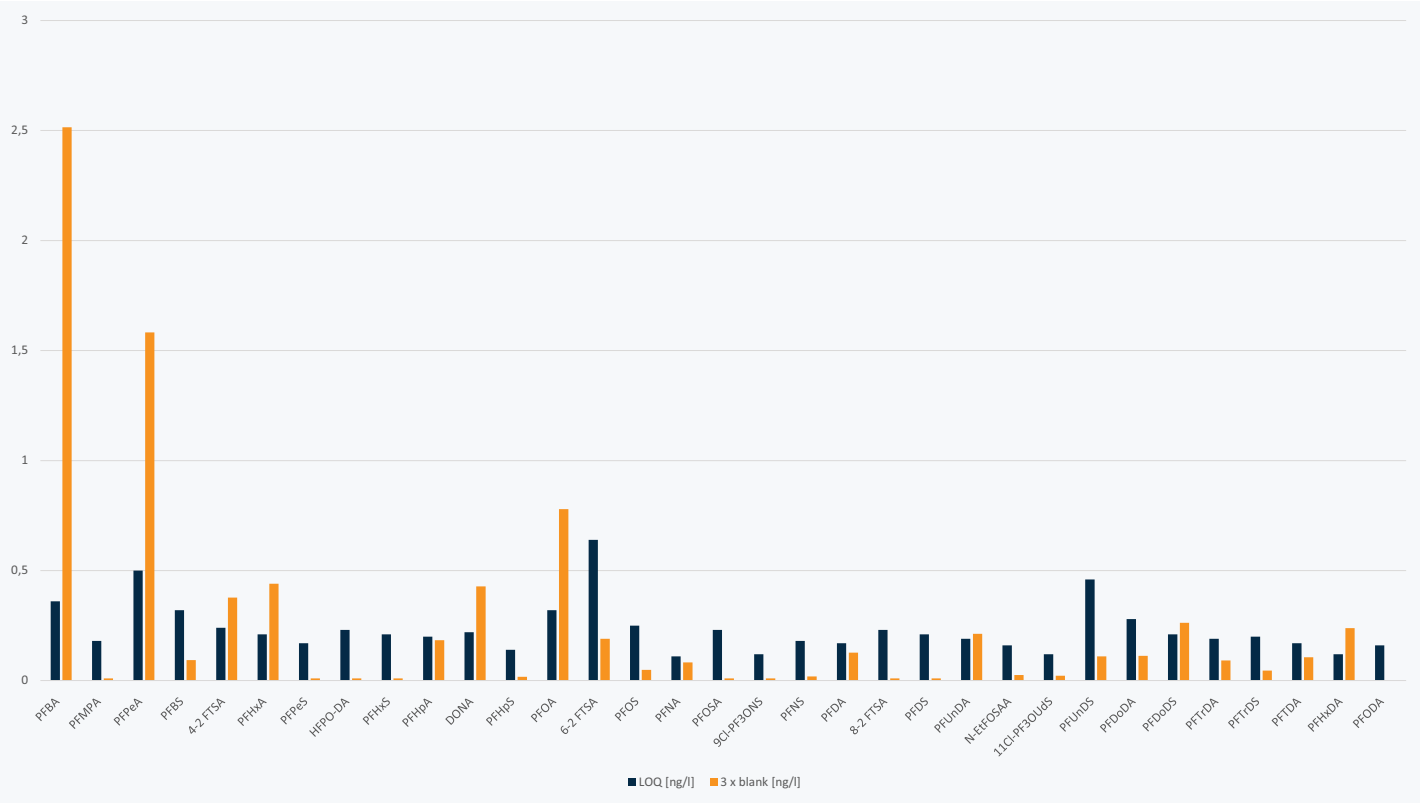

Figure 9: Limits of quantification compared to the blanks calculated from calibration curves in the range 0.05 – 0.5 ng/L.

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Repeatability and Trueness

To show the applicability of the method and the trueness of determination, water samples from different sources were analyzed in duplicate, as well as spiked with two concentration levels (1.5 and 30 ng/L). The results from the 6-fold determination of PFAS in these samples are summarized in table 2. Only low concentrations of some PFAS below 10 ng/L were detected in tap water and river water. In some cases, this leads to higher values for the calculated trueness or repeatability, when spiking with the low concentration of 1.5 ng/L. All other values are between 80 and 120% for trueness and below 10% for repeatability expressed as relative standard deviation. At high concentrations repeatability and trueness are even better.

Table 2a: Results from 6-fold determination of tap water analyzed directly and spiked with 1.5 ng/L, respectively 30 ng/L of each compound.

	Tap water		spiked with 1.5 ng/L			spiked with 30 ng/L		
	Average [ng/L]	RSD [%]	Average [ng/L]	RSD [%]	Trueness [%]	Average [ng/L]	RSD [%]	Trueness [%]
PFBA	2.2	5%	4.7	7%	164%	30.6	2%	95%
PFMPA	< 0.5	-	1.3	8%	89%	23.8	5%	79%
PFPeA	2.3	8%	4.2	2%	130%	31.6	2%	98%
PFBS	2.5	5%	4.4	6%	131%	30.8	2%	94%
4-2 FTSA	< 0.5	-	1.7	9%	111%	27.3	3%	91%
PFHxA	1.8	8%	3.9	11%	140%	29.8	3%	93%
PFPeS	< 0.5	-	1.8	6%	118%	28.2	1%	94%
HFPO-DA	< 0.5	-	1.8	16%	121%	29.9	5%	100%
PFHxS	< 0.5	-	1.8	7%	119%	27.2	2%	91%
PFHpA	1.1	2%	3.1	3%	130%	29.6	4%	95%
DONA	< 0.5	-	1.7	4%	112%	28.9	2%	96%
PFHpS	< 0.5	-	1.6	10%	108%	27.4	2%	91%
PFOA	0.5	12%	2.4	9%	129%	29.1	2%	95%
6-2 FTSA	< 0.5	-	1.6	20%	108%	30.1	4%	100%
PFOS	< 0.5	-	1.7	9%	114%	28.3	3%	94%
PFNA	< 0.5	-	1.7	9%	114%	28.6	3%	95%
PFOSA	< 0.5	-	1.6	10%	109%	28.7	3%	96%
9Cl-PF3ONS	< 0.5	-	1.7	8%	113%	29.4	2%	98%
PFNS	< 0.5	-	1.7	10%	115%	29.5	3%	98%
PFDA	< 0.5	-	1.7	9%	113%	28.1	4%	94%
8-2 FTSA	< 0.5	-	1.6	9%	109%	28.0	3%	93%
PFDS	< 0.5	-	1.7	10%	111%	30.0	3%	100%
PFUnDA	< 0.5	-	1.7	9%	114%	28.8	3%	96%
N-EtFOSAA	< 0.5	-	1.7	8%	112%	28.5	2%	95%
11Cl-PF3OUdS	< 0.5	-	1.7	7%	113%	30.6	3%	102%
PFUnDS	< 0.5	-	1.8	9%	120%	30.8	3%	103%
PFDoDA	< 0.5	-	1.7	8%	113%	28.5	3%	95%
PFDoDS	< 0.5	-	1.8	9%	121%	31.3	3%	104%
PFTrDA	< 0.5	-	1.6	9%	108%	27.7	3%	92%
PFTrDS	< 0.5	-	1.7	8%	110%	27.8	3%	93%
PFTDA	< 0.5	-	1.7	9%	112%	28.0	4%	93%
PFHxDA	< 0.5	-	1.6	10%	105%	27.5	4%	92%
PFODA	< 0.5	-	1.6	12%	104%	25.5	3%	85%

Table 2b: Results from 6-fold determination of river water analyzed directly and spiked with 1.5 ng/L, respectively 30 ng/L of each compound.

	River water		spiked with 1.5 ng/L			spiked with 30 ng/L		
	Average [ng/L]	RSD [%]	Average [ng/L]	RSD [%]	Trueness [%]	Average [ng/L]	RSD [%]	Trueness [%]
PFBA	2.3	9%	3.6	11%	88%	27.9	4%	85%
PFMPA	< 0.5	-	1.7	12%	117%	38.5	14%	128%
PFPeA	2.3	5%	4.2	6%	128%	29.1	3%	89%
PFBS	8.3	3%	9.7	3%	94%	34.7	3%	88%
4-2 FTSA	< 0.5	-	1.2	4%	79%	27.8	4%	93%
PFHxA	2.1	5%	3.7	8%	103%	29.1	3%	90%
PFPeS	< 0.5	-	1.4	14%	90%	25.9	4%	86%
HFPO-DA	< 0.5	-	1.3	11%	85%	25.4	4%	85%
PFHxS	< 0.5	-	1.6	8%	106%	27.0	4%	90%
PFHpA	1.0	7%	2.3	2%	87%	27.0	3%	87%
DONA	< 0.5	-	1.4	16%	96%	25.5	3%	85%
PFHpS	< 0.5	-	1.3	8%	86%	26.6	3%	89%
PFOA	2.6	4%	3.8	2%	84%	28.3	4%	86%
6-2 FTSA	1.4	8%	2.6	4%	84%	28.4	5%	90%
PFOS	5.5	8%	6.8	2%	83%	32.0	2%	88%
PFNA	< 0.5	-	1.5	7%	98%	25.8	4%	86%
PFOSA	< 0.5	-	1.3	4%	87%	27.0	4%	90%
9Cl-PF3ONS	< 0.5	-	1.2	3%	82%	25.6	2%	85%
PFNS	< 0.5	-	1.3	7%	84%	26.1	2%	87%
PFDA	0.5	5%	1.9	4%	90%	27.1	4%	89%
8-2 FTSA	< 0.5	-	1.3	7%	87%	26.7	4%	89%
PFDS	< 0.5	-	1.3	4%	84%	26.9	4%	90%
PFUnDA	< 0.5	-	1.4	6%	93%	26.2	4%	87%
N-EtFOSAA	< 0.5	-	1.6	5%	106%	27.9	3%	93%
11Cl-PF3OUdS	< 0.5	-	1.3	4%	87%	26.9	4%	90%
PFUnDS	< 0.5	-	1.3	6%	85%	27.8	4%	93%
PFDoDA	< 0.5	-	1.6	3%	109%	26.8	3%	89%
PFDoDS	< 0.5	-	1.3	8%	85%	25.9	5%	86%
PFTrDA	< 0.5	-	1.4	5%	93%	27.2	5%	91%
PFTrDS	< 0.5	-	1.4	6%	91%	28.2	4%	94%
PFTDA	< 0.5	-	1.5	7%	102%	27.1	5%	90%
PFHxDA	< 0.5	-	1.3	9%	84%	24.2	7%	81%
PFODA	< 0.5	-	1.4	9%	95%	31.3	6%	104%

Table 2c: Results from 6-fold determination of mineral water (2000 mg/L salts) analyzed directly and spiked with 1.5 ng/L, respectively 30 ng/L of each compound.

	Mineral water		spiked with 1.5 ng/L			spiked with 30 ng/L		
	Average [ng/L]	RSD [%]	Average [ng/L]	RSD [%]	Trueness [%]	Average [ng/L]	RSD [%]	Trueness [%]
PFBA	< 0.5	-	1.6	35%	105%	27.6	2%	92%
PFMPA	< 0.5	-	1.0	13%	70%	24.8	3%	83%
PFPeA	(0.4)	29%	2.3	6%	124%	30.3	3%	99%
PFBS	< 0.5	-	1.5	4%	97%	28.5	2%	95%
4-2 FTSA	< 0.5	-	1.4	15%	94%	28.6	4%	95%
PFHxA	0.5	20%	1.8	7%	90%	28.7	3%	94%
PFPeS	< 0.5	-	1.4	6%	93%	28.4	2%	95%
HFPO-DA	< 0.5	-	1.3	15%	90%	28.7	4%	96%
PFHxS	< 0.5	-	1.4	5%	94%	28.3	2%	94%
PFHpA	< 0.5	-	1.3	8%	90%	27.8	2%	93%
DONA	< 0.5	-	1.4	7%	92%	27.9	1%	93%
PFHpS	< 0.5	-	1.4	7%	90%	28.2	2%	94%
PFOA	< 0.5	-	1.4	10%	96%	28.2	2%	94%
6-2 FTSA	< 0.5	-	1.3	1%	86%	28.3	2%	94%
PFOS	< 0.5	-	1.4	5%	90%	28.4	2%	95%
PFNA	< 0.5	-	1.4	6%	91%	28.5	2%	95%
PFOSA	< 0.5	-	1.4	5%	95%	28.0	1%	93%
9Cl-PF3ONS	< 0.5	-	1.4	5%	94%	29.0	2%	97%
PFNS	< 0.5	-	1.5	6%	97%	29.1	2%	97%
PFDA	< 0.5	-	1.4	5%	91%	28.3	2%	94%
8-2 FTSA	< 0.5	-	1.4	7%	95%	28.2	2%	94%
PFDS	< 0.5	-	1.4	8%	93%	28.3	2%	94%
PFUnDA	< 0.5	-	1.3	7%	89%	28.3	2%	94%
N-EtFOSAA	< 0.5	-	1.4	5%	94%	28.4	2%	95%
11Cl-PF3OUdS	< 0.5	-	1.4	6%	92%	29.4	2%	98%
PFUnDS	< 0.5	-	1.3	7%	89%	29.1	2%	97%
PFDoDA	< 0.5	-	1.4	6%	91%	28.2	2%	94%
PFDoDS	< 0.5	-	1.4	10%	96%	29.0	3%	97%
PFTrDA	< 0.5	-	1.4	5%	95%	27.9	3%	93%
PFTrDS	< 0.5	-	1.4	9%	92%	29.6	5%	99%
PFTDA	< 0.5	-	1.4	7%	95%	27.8	2%	93%
PFHxDA	< 0.5	-	1.3	8%	90%	24.6	4%	82%
PFODA	< 0.5	-	1.2	7%	83%	21.8	4%	73%

Wastewater

To check the applicability of the online-SPE method for wastewater, two samples from the outlet of a sewage treatment plant were analyzed in duplicate, as well as spiked at 30 ng/L. The samples contained sediment, which was re-suspended by agitation and allowed to settle for about one hour. Thus, fine particles were included in analysis. All samples were also analyzed after dilution 1:5, to check if matrix effects could be reduced in this way. This would increase the quantification limit, but also the quality of the results. However, with one exception (PFBA, where the recovery

of the internal standard increases significantly) this was not the case. Concentration findings, calculated trueness, and repeatability from recovery of spiked standards including diluted sample are shown in table 3. With exception of PFMPA and 4-2 FTSA, the repeatability of results and consistency of findings in undiluted and diluted samples are all very good.

Table 3: Results from double determination of wastewater, directly and spiked with 30 ng/L of each compound.

Wastewater	Sample 1			Sample 2		
	Average [ng/L]	Trueness [%]	RSD spike [%]	Average [ng/L]	Trueness [%]	RSD spike [%]
PFBA	21.0	112%	9%	2.2	107%	2%
PFMPA	< 0.5	166%	17%	< 0.5	150%	25%
PFPeA	25.2	114%	11%	7.0	103%	8%
PFBS	4.0	107%	1%	4.6	105%	5%
4-2 FTSA	< 0.5	67%	21%	< 0.5	107%	3%
PFHxA	67.5	107%	4%	9.8	106%	2%
PFPeS	< 0.5	128%	3%	0.6	112%	3%
HFPO-DA	< 0.5	107%	3%	< 0.5	99%	2%
PFHxS	< 0.5	107%	3%	0.9	102%	2%
PFHpA	2.2	107%	3%	2.1	106%	2%
DONA	< 0.5	106%	2%	< 0.5	103%	3%
PFHpS	< 0.5	108%	4%	< 0.5	103%	1%
PFOA	4.3	107%	1%	6.9	105%	2%
6-2 FTSA	21.0	114%	7%	4.9	110%	8%
PFOS	0.7	101%	5%	< 0.5	105%	2%
PFNA	0.7	106%	1%	0.6	103%	1%
PFOSA	< 0.5	106%	2%	< 0.5	103%	3%
9Cl-PF3ONS	< 0.5	93%	8%	< 0.5	101%	1%
PFNS	< 0.5	101%	6%	< 0.5	106%	2%
PFDA	< 0.5	110%	2%	< 0.5	105%	2%
8-2 FTSA	< 0.5	108%	2%	< 0.5	106%	3%
PFDS	< 0.5	103%	1%	< 0.5	102%	2%
PFUnDA	< 0.5	108%	2%	< 0.5	104%	3%
N-EtFOSAA	< 0.5	101%	2%	< 0.5	102%	6%
11Cl-PF3OUdS	< 0.5	107%	2%	< 0.5	104%	2%
PFUnDS	< 0.5	109%	3%	< 0.5	105%	2%
PFDoDA	< 0.5	106%	2%	< 0.5	102%	3%
PFDoDS	< 0.5	109%	4%	< 0.5	105%	5%
PFTrDA	< 0.5	112%	8%	< 0.5	103%	2%
PFTrDS	< 0.5	101%	3%	< 0.5	100%	5%
PFTDA	< 0.5	94%	12%	< 0.5	103%	6%
PFHxDA	< 0.5	76%	14%	< 0.5	93%	4%
PFODA	< 0.5	112%	9%	< 0.5	107%	2%

* Including undiluted and diluted samples.

Proficiency test

Using the described method, we took part in the interlaboratory trial for the validation of the CEN standard EN 17892 in summer 2023 [7]. All 20 PFAS of the EU Drinking Water Directive (PFAS-20), plus additional PFAS were determined in 5 different matrices. For all compounds our results were included for evaluation, none of them were considered outliers.

Conclusions

The online-SPE-LC-MS/MS system combined with the presented method enables fully automated determination of PFAS compounds in accordance with EN 17892. The sample handling required corresponds to the direct injection method, while achieving the full benefits of the SPE based method, lowering the limits of detection, and improving the accuracy of the results thanks to the sample clean-up. There is no need to filter water samples, including fine sediment particles. The method accuracy and trueness were demonstrated for water samples from different sources, resulting in relative standard deviations below 10% and trueness mainly between 80 and 120%.

References

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