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D1.3 – Methods for global organic fluorinated content (TOP, TOF/AOF/EOF) for relevant matrices

Main authors: **Anne Togola (BRGM), Erni Saracevic (TUW), Tobias Hensel (BWB), Babatoundé Idjaton (IPGP/BRGM), Frederik Zietzschmann (BWB)**
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Executive Summary

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals known for their widespread use in various industrial and consumer applications. Due to their persistence, bioaccumulation, and potential health effects, there is a growing need for accurate and efficient analytical methods to detect and quantify PFAS in environmental samples. The aim of the current work within the PROMISCES project was to propose methods for assessment of global organic fluorinated content for relevant matrices. Two prominent analytical approaches for PFAS analysis include Total Oxidizable Precursor (TOP) assay and Combustion Ion Chromatographic (CIC) approaches have been developed to reach this objective, on different matrices of interest for the project.

Table 1: Comparative summary of the methods developed by PROMISCES partners (TUW, BRGM, BWB)

	TOP assay		CIC approaches	
Matrices	TUW	BRGM	BRGM	BWB
Waters (surface and groundwaters)	x	x	x	x
Waste water Effluent	x	x	x	
Waste water Influent	x			
Landfill leachates			x	
Sludge		x	x	

This deliverable presents the validation of the methods, but also their limitation, due to the high complexity of the not well defined "PFAS universe", this complexity is due to ignorance of the detailed composition of the samples, which does not allow the methods to be properly qualified.

While analytical methods such as TOP testing and CIC via adsorbable organic fluorine and extractable organic fluorine offer new types of information for a better characterization of PFAS contamination, continued research and development efforts are still necessary to remove the obstacles identified in this work. In particular, the development of interlaboratory tests is essential to supervise these methods, particularly in the case of complex matrices. First objective will be to identify the main factor affecting reproducibility of the method and second one will be to characterize the performance

At the same time, better characterize what the results obtained by these methods correspond to, is essential. Thus, the acquisition of data coupling these global approaches in a cross-referenced manner with targeted methods must be multiplied in different contamination contexts. This will make it possible to link global measurements of organic fluorine or oxidizable precursors to the presence of certain PFAS or certain types of contamination, and will promote the deployment of these tools.

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1   Introduction

Different methods for quantifying targeted PFAS have been developed and presented in the PROMISCES deliverable (D1.1). But the large universe of PFAS substances cannot be covered by targeted methods, since there the majority of substances are still completely unknown and there is a lack of reference standards for those that are known. Based on literature or standardized methods (US EPA, CEN, ISO...), the combination of all methods concerning targeted PFAS analysis covers less than 200 compounds, highlighting the need of global parameters. Different approaches are under development and two have been selected by the laboratories involved in the PROMISCES project: the Total Oxidizable Precursors (TOP) assay and the measurement of the organic fluorine content by Combustion Ion chromatography (CIC).

Development on these two methods are presented in this report, with some first applications on real samples. Limitations and gains of these methods are also discussed.

2   TOP assay principle

The Total Oxidizable Precursors (TOP) assay is a method used for measuring the total concentration of per- and polyfluoroalkyl substances (PFAS) able to form PFAA in various aqueous matrices such as drinking- surface- or groundwater, wastewater, and in eluates of soil, sediment, or biological tissues.

In the TOP assay, the quantity of perfluoroalkyl carboxylates (PFCAs) produced upon chemical oxidation by hydroxyl radical is used to quantify polyfluoroalkyl substances that contain linear or branched perfluoroalkyl groups. In theory, the distribution of PFCA chain-lengths, which depends on the precursor perfluorinated chain-length and synthesis method, provides insight into the chemical structure of precursors in the sample. The formation of PFCAs during oxidation can depend on various factors, including the nature of the precursor compounds, the oxidizing conditions employed, and the duration of the oxidation process. Additionally, some precursors may have higher propensity to form PFCAs compared to others.

After the oxidation step, conventional analysis of PFAS can be applied to estimate the production of PFCAs or the residual concentration of non-oxidized PFAS.

2.1   Analysis by TUW

2.1.1   General

After literature research and comparing the TOP assay methods implemented at different laboratories (eg Houtz & Sedlak (2012), Bannister (2020), Söderlund (2018) and Kaiser (2021)), it was decided to conduct research using the Houtz & Sedlak method as a base, which was adapted for PROMISCES work.

TOP assay, by Houtz and Sedlak established in 2012 involves analyzing PFAAs precursors before and after oxidizing using heat and alkaline activated persulfate.

PFAA precursors are then converted into PFCAs and afterwards, as a final product, are further quantified using LC-MS/MS.

2.1.2 Instruments, chemicals and reagents

Instruments

Instruments	Manufacturer	Description
Scale	Mettler Toledo XS205	Digital scale
Water bath	Julabo SW22	Shaker waterbath
Centrifuge	Sigma 3-16L	
LC system	PAL	PAL RTC
	Agilent	Agilent 1260 Infinity II
MS/MS system	sciex	Qtrap 6500+
Analytical Column	phenomenex	Phenomenex Luna Omega 3 µm PS C18; 150x5, 100 Å
Delay Column	phenomenex	phenomenex Luna C18 50x3 mm 110 Å

Material

Material	Manufacturer	Description
Polypropylen centrifuge tube	VWR	150 mL
Polypropylen Vials, 1.5 mL, screw cap	Macherey & Nagel	Short thread vial
Polypropylen Sample Flasks	Azlon	Rinsed with Acid, Base and DW
Pipettes	Eppendorf SE	10 µL- 5000 µL

Chemicals

Chemical	Manufacturer	Description
Methanol	Merck	CAS 67-56-1 (Merck, 20864.290)
Ultra-Pure Water	MilliQ TUW	MilliQ TUW
Ammonium hydroxyde	Merck	CAS 1335-21-6; (Merck1.05432.1000)
NaOH	Merck	Cas 1310-73-2
K2S2O8	Fluka	CAS 7727-21-1

Analytical Standards

Material	Manufacturer	Description
Native PFAS-Standards (Mix and Single Substances)	Wellington Laboratories	PFAC30PAR 1 mg/L; MXI 1mg/L,
Internal Standards EIS, NIS		MPFAC-HIF-IS 1 mg/L MPFAC-HIF-ES 1 mg/L

2.1.3 Sample collection, preservation and pre-treatment

Three types of samples were used for TOP assay analysis:

- native standard solution
- river water
- wastewater (influent and effluent).

The sample were prepared or collected without any contamination into a 1000 mL HDPE bottle, transported refrigerated and stored in the refrigerator at 5°C until measurement.

The necessary volume of samples for analysis and chemicals were dosed in 150 mL polypropylene bottle (PP) and then heated in a water bath at 85 degrees Celsius for 6 or 9 hours (Table 2).

Table 2 : Procedure at TOP analysis for water samples with different matrix

TOP Method TUW	
1	150mL PP bottle
2	100mL sample + EIS
3	1,6g $K_2S_2O_8$ (60mM end-concentration)
4	1,5mL 10M NaOH (150mM end-concentration)
5	85°C; 6/8 h
6	pH 5
7	SPE; phenomenex X-AW (33 µm, 200 mg/6 mL)
8	wash (after extraction); 4mL DW, 4mL 25mM ammonium acetate
9	centrifuge 4500 rmp; 10 min
10	Evaporation ⮕ 2 mL ⮕ 1mL + NIS
11	LC/MS/MS

After heating the sample, prior solid phase extraction (SPE), the calculated volume of internal standards (EIS) (to achieve the end concentration of 5 ng/L) were added , pH was corrected, and the samples were extracted. After SPE, non-extracted internal standards (NIS) were dosed to achieve the final concentration of 250 ng/L. For the exact assignment of internal standards to native substances see Table 3.

Table 3: Assignment of internal standards (EIS and NIS) to native substance

Chemical group	PFAS	CAS	EIS	NIS
Perfluoroalkyl carboxylic acids (PFCAs)	PFBA	375-22-4	MPFBA_13C4	M3PFBA_13C3
	PFPeA	2706-90-3	M5PFPeA_13C5	MPFHxA_13C2
	PFHxA	307-24-4	M5PFHxA_13C5	
	PFHpA	375-85-9	M4PFHpA_13C4	
	PFOA	335-67-1	M8PFOA_13C8	MPFOA_13C4
	PFNA	375-95-1	M9PFNA_13C9	MPFNA_13C5
	PFDA	335-76-2	M6PFDA_13C6	MPFDA_13C2
	PFUdA	2058-94-8	M7PFUdA_13C7	
	PFDoA	307-55-1	MPFDoA_13C2	
	PFTrDA	72629-94-8	M2PFTeDA_13C2	
	PFTeDA	376-06-7		
Perfluoroalkyl sulfonic acid (PFSA)	PFBS	375-73-5	M3PFBS_13C3	MPFHxS_18O2
	PFPeS	2706-91-4		
	PFHxS	355-46-4	M3PFHxS_13C3	
	PFHpS	375-92-8		
	PFOS	1763-23-1	M8PFOS_13C8	MPFOS_13C4
	PFNS	98789-57-2		
	PFDS	335-77-3		
Fluorotelomer sulfonic acid (n:2 FTCA)	4:2 FTS	27619-93-8	M2-4:2 FTS_13C2	
	6:2 FTS	27619-94-9	M2-6:2 FTS_13C2	
	8:2 FTS	27619-96-1	M2-8:2 FTS_13C2	
Perfluoroalkane sulfonamide (FASA)	PFOSA	754-91-6	M8FOSA_13C8	
	N-MeFOSA	31506-32-8	d-N-MeFOSA_d3	
	N-EtFOSA	4151-50-2	d-N-EtFOSA_d5	
Perfluoroalkane sulfonamido acetic acid (FASAA)	N-MeFOSAA	2355-31-9	d3-N-MeFOSAA	
	N-EtFOSAA	2991-50-6	d5-N-EtFOSAA	
Perfluoroether carboxylic acid (PFECA)	HFPO-DA	2062-98-8	M3HPFO-DA_13C3	MPFHxA_13C2
	ADONA	2250081-67-3	MPFDoA_13C2	MPFOA_13C4
	9Cl-PF3ONS	73606-19-6	M3PFHxS_13C3	MPFOS_13C4
	11Cl-PF3OUdS	83329-89-9	M3PFHxS_13C3	
	PFHxDA	67905-19-5	M4PFHpA_13C4	MPFDA_13C2
	PFODA	16517-11-6	M6PFDA_13C6	
	N-MeFOSE	24448-09-7	d7-N-MeFOSE	MPFOS_13C4
	N-EtFOSE	1691992	d9-N-EtFOSE	

2.1.4 Analysis

After sample preparation (heating and SPE) LCMS was used for direct injection and an external calibration for quantification was used. Therefore at least five different concentrations are diluted with methanol from the standard solution. The concentrations range from 50 ng/L to 1000 ng/L in the vial. In addition, 50 µL of the non-extracted internal standards (NIS) and extracted internal standards (EIS) is also added to each calibration solution. EIS was for establish of recovery and NIS to establish of initial calibration used.

The analytical procedures proposed by TUW in the deliverable (D1.1) have been applied for targeted analysis.

2.1.5 Performance

TOP Assay provides information into the actual total PFAS load on the system as well as whether a significant molar mass of precursors is present that can be transformed into simpler PFAA. That can help to assess the risk or potential risk related to PFAS and to design treatments plants in different contexts (for example WWTP, Drinking water, etc.).

In order to develop and optimize this method for TOP analysis the standard solutions with different concentration varying degrees have been analyzed. Figure 1 shows the results of analyses of standard solutions (mix of 32 PFAS, 50 ng/L and 100 ng/L).
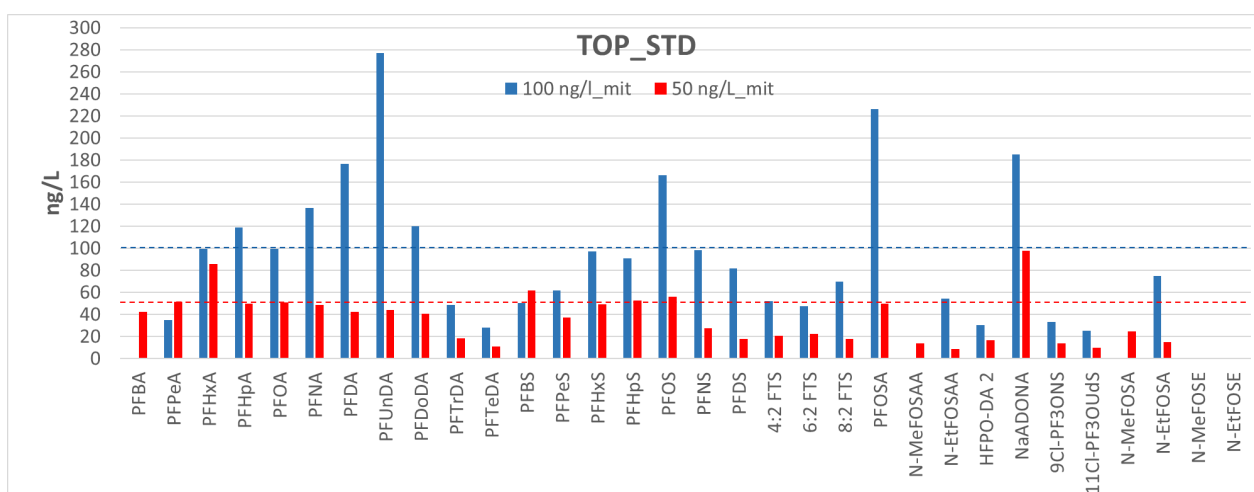
Figure 1 : Results TOP analysis for PFAS Standards 50 and 100 ng/L (mix solution 32 PFAS)

The figure 1 shows that generally the concentration of long chain PFAS decreases after oxidation, and a certain trend is to recognize that after TOP oxidation the PFAS are formed with shorter chain. Unfortunately, it was difficult to achieve sufficient reproducibility even for standard solutions for different concentrations. Very interesting, and we haven't clarified why, the concentration of long chain PFAS, ADONA, reproducible increase after oxidation.

2.1.6 Application to real samples

TOP assay method have been applied to monitor the presence of PFAA precursors into real water samples with different matrix (influent and effluent water, groundwater).
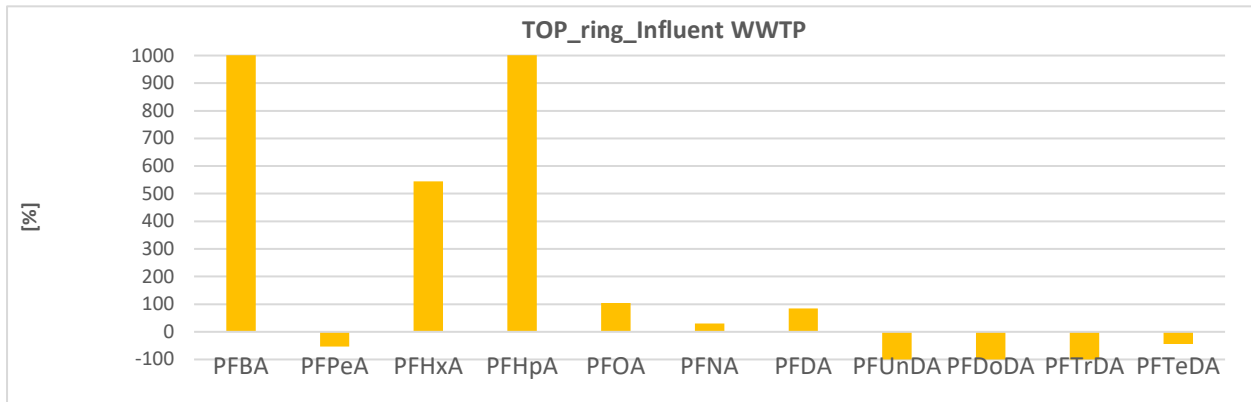
Figure 2 : Change of the concentration of individual substances after TOP analysis into Influent from WWTP

The figure 2 shows the percentage of change of 11 PFAS into influent from wastewater treatment plant after oxidation. The change of the concentration of individual substances after TOP analyses is expressed as a percentage of the difference between concentrations after and before oxidation. The concentration of short chain PFAS increase very strong, for example concentration of PFBA and PFHpA has increased approximately ten time. At the same time the long chain PFAS (for example concentration of PFBA and PFHpA) concentration decrease.
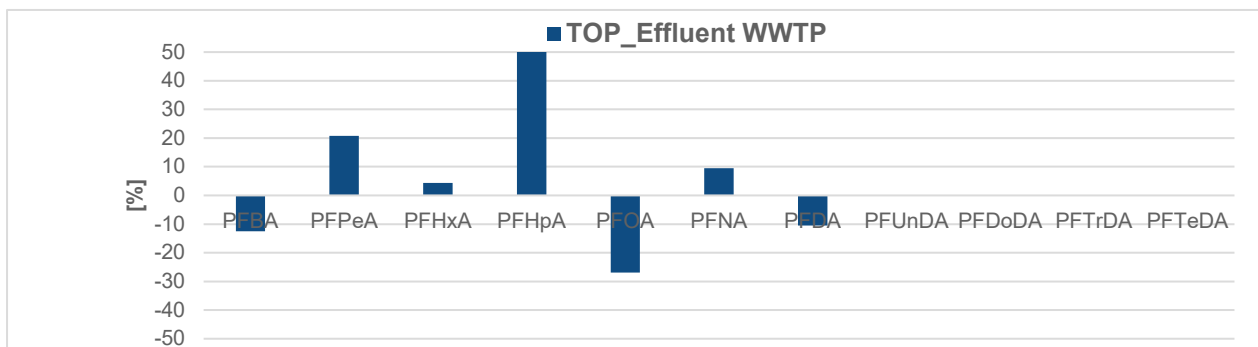
Figure 3 : Results of TOP analysis into effluent from WWTP

The figure 3 shows the change of 11 PFAS into effluent from waste water treatment plant after oxidation. The long chain PFAS were not detected into effluent. For some short chain PFAS the trend of concentration changes after TOP analyses is similar to the influent (PFHxA, PFHpA) but the concentration of PFBA has decreased.
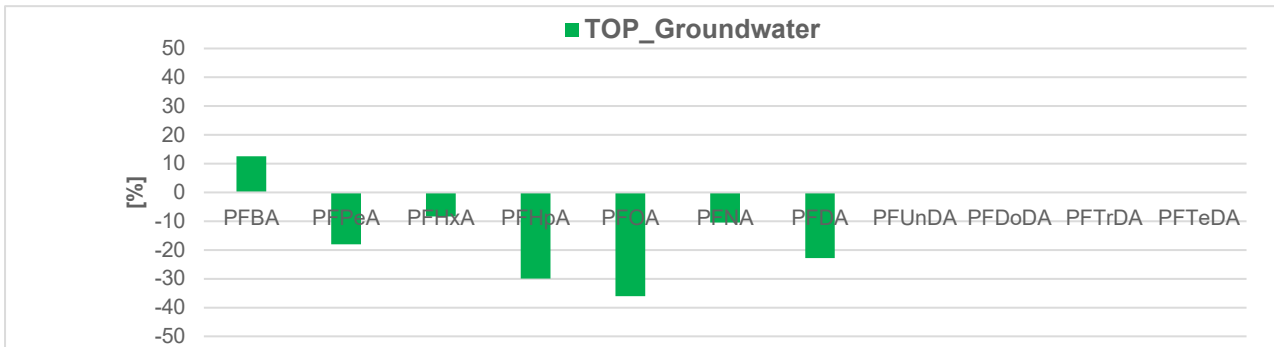
Figure 4: Results of TOP analysis for groundwater

The concentration of PFAS in the groundwater was low and figure 4 shows that only PFBA concentration increased by approximately 10% and at the same time the concentration of all other PFAS decreased to − 35%.

Generally for real samples a certain trend is recognizes as after TOP oxidation the PFAS with shorter chain are formed (Tsou et al., 2023). Calculating the TOP assay is difficult if all fluorinated substances are not analyzed (such in this case TFA were not determined).

That would be a possible explanation why the laboratory was not able to get a satisfying reproducibility into standard solutions, or when analyzing samples with different matrix despite multiple repetitions (figure 5 and figure 6).

The next step is to try to optimize the TOP assay method, for example individual steps in analytics (the chemicals concentration, etc.), to better understand the factors affecting efficiency of the oxidation.
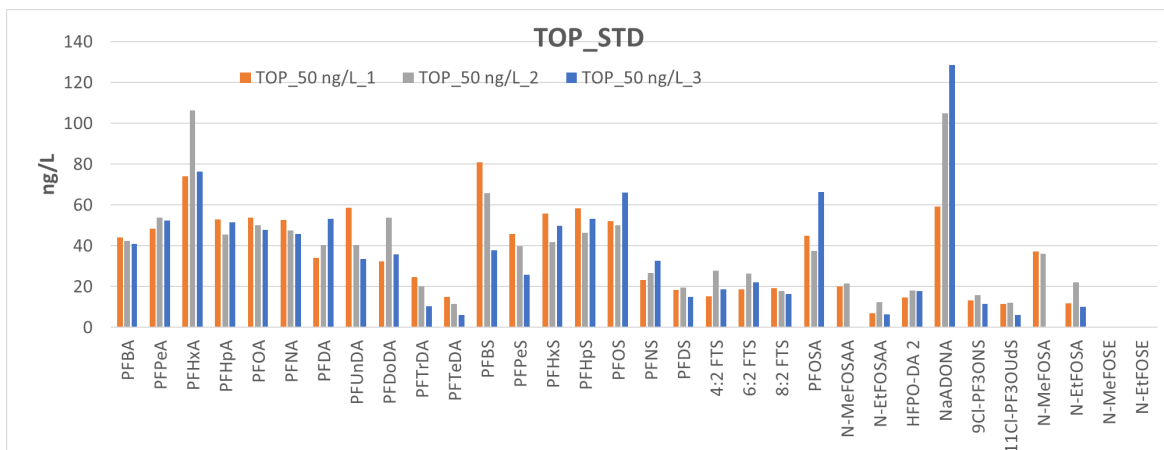
Figure 5: Multiple repetitions of TOP analysis for standard solutions (50 ng/L)
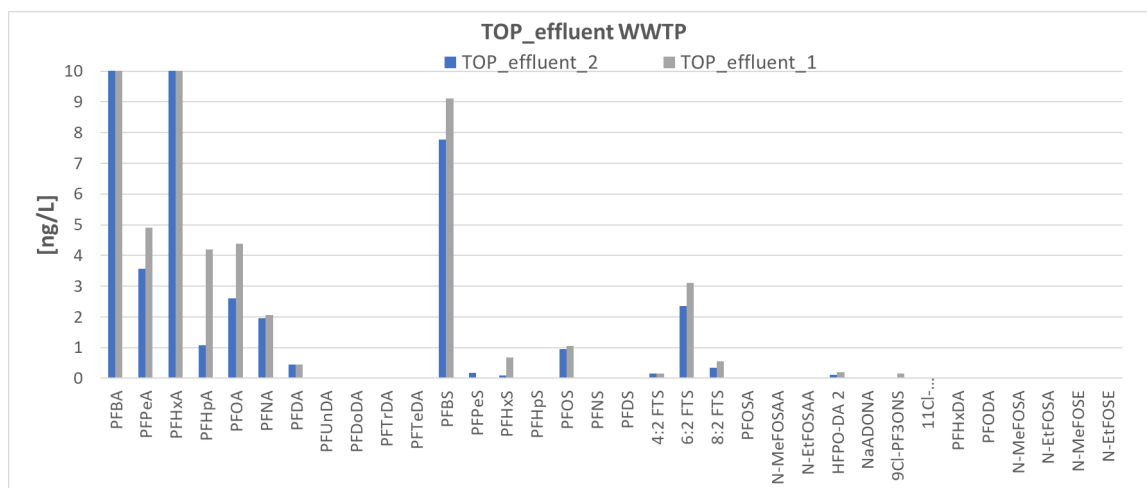
Figure 6: Multiple repetitions of TOP analysis of effluent WWTP

2.2 Analysis by BRGM

2.2.1 General

As TUW, BRGM has compared two different methods for TOP assays, based on Houtz et al. (2012) and Hutchinson et al. (2020). Differences in protocols are related to the amount of oxidizing agents, as presented in Table 6.

2.2.2 Instrument, chemicals and reagents

Table 4 : List of Instruments, material and chemicals for the BRGM method.

Instruments	Manufacturer	Description
LC system	Waters®	ACQUITY H-CLASS
MS system	Waters®	XEVO-TQXS
Analytical Column	Waters®	ACQUITY UPLC BEH C18 1.7µm 2.1x100mm

Material	Manufacturer	Description
Injection vials	Waters	Polypropylen ,0.7 mL, screw cap
20 mL Tubes	Falcon	
Centrifuge tube.		15 mL polypropylen

Chemical	Manufacturer	Description
Methanol	BIOSOLVE CHIMIE	Abs. ULC-MS
Ammonium hydroxide	Fischer Scientific	
Ammonium acetate	Fischer Scientific	
Potassium persulfate	SUPELCO	EMSURE®
NaOH	Merck	

Standards are the same than those used for targeted analysis. They were purchased from Wellington, Neochema, Cambridge Isotope Laboratory (CIL), Ehrenstorfer GmbH, Chiron and HPC standards. For

radiolabeled compounds, standards were purchased from Wellington (both mix and individual compounds).

2.2.3 Sample collection, preservation and pre-treatment

The water samples are collected in 10 mL PP tubes, stored in the refrigerator at 5°C until measurement (within 2 weeks) or keep frozen at -20 °C.

For sludges, 10 mL sample is previously diluted tenfold. 10mL is collected and the same protocol is applied than for water sample.

Method developments have been done on spiked waters with a mix of 56 PFAS (concentration of 250 ng/L for each compound) and on a diluted sample of the aqueous film foaming foam (AFFF) used in PROMISCES CS6 experiment (same concentration range). The characterization of this product is described in Table 5.

For the method development, two methods have been compared, from Houtz et al. (2012) and from Hutchinson et al. (2020).

Table 5 : PFAs content of the AFFF product used for spike.

Compound	LQ (mg/L)	AFFF (pur product) (mg/L)	spiked water with AFFF (ng/L)
PFBA	0,01	0,48	<LQ
PFPeA	0,005	0,34	<LQ
PFHxA	0,005	8,9	<LQ
PFHpA	0,002	0,1	<LQ
PFOA	0,002	0,11	<LQ
PFDA	0,002	0,028	<LQ
PFDoDA	0,002	0,016	<LQ
5:3 FTCA	0,002	0,005	<LQ
4:2 FTSA	0,002	3,4	<LQ
6:2 FTSA	0,02	2 251	142
8:2 FTSA	0,005	3,8	<LQ
10:2 FTSA	0,01	1,7	<LQ
6:2 FTAB	0,01	3 409	215
6:2 FTSAB ? No standard but area equiv to 6:2 FTAB			

6:2 FTSAB is an identified candidate, not confirmed by analytical standard, no quantification is also provided for this compounds.

Table 6 : Procedure for TOP analysis in water samples

TOP Method Houtz et al. (2012)	TOP Method Hutchinson et al. 2020
10 mL spiked water	10 mL spiked water
162 mg $K_2S_2O_8$ (60mM end-concentration)	540 mg $K_2S_2O_8$ (200mM end-concentration)
150 µL 10M NaOH (150mM end-concentration)	250 µL 10M NaOH (250mM end-concentration)
85°C; 6 h	
Stop reaction at 4°C, 45 min	
Neutralisation (pH7) with HCl	
Collection of 25 µL, add of internal standard, methanol and acetic acid to obtain 500 µL of extract with the ratio (80/20; MEOH/water 3%Acetic Acid) for LC/MS analysis	
LC/MS/MS	LC/MS/MS

As described in the Table 6, the main steps for TOP assay are related to the sample preparation. The analysis is the same as the one developed for water analysis in D1.1. A dilution step (factor 20) is added at the end to avoid matrix effect due to the addition of chemicals for oxidation step.

For the validation, internal standards have been added after oxidation step to have a better understanding of the behaviour of compounds. As expected, there is the formation of PFCA compounds, especially PFOA, but also shorter chain PFCA such as PFBA, PFPeA, PFHxA and PFHpA.

2.2.4 Analysis

The decrease in the concentration of longer chain PFCA can be related to the sorption of PFAS on the polypropylene vial during the oxidation step (hypothesis confirmed by the use of control solution that have followed the entire process without add of $K_2S_2O_8$ and NaOH, and a part can be hydrolyzed during the process. Results with ("after TOP") and without ("without TOP") the oxidation's step are compared.
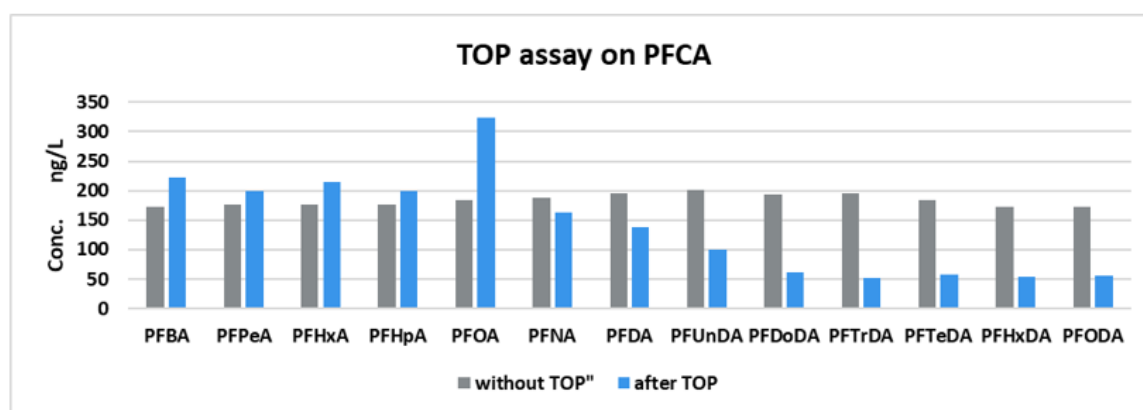
Figure 7 : Results of TOP analysis (Houtz protocol) on spiked water with PFAS mix, focus on PFCA.

Regarding impact of TOP assay on other chemical groups of PFAS, different results can be highlighted, such as the predictable inefficiency on PFSA compounds (impacted on the longer chain by sorption)
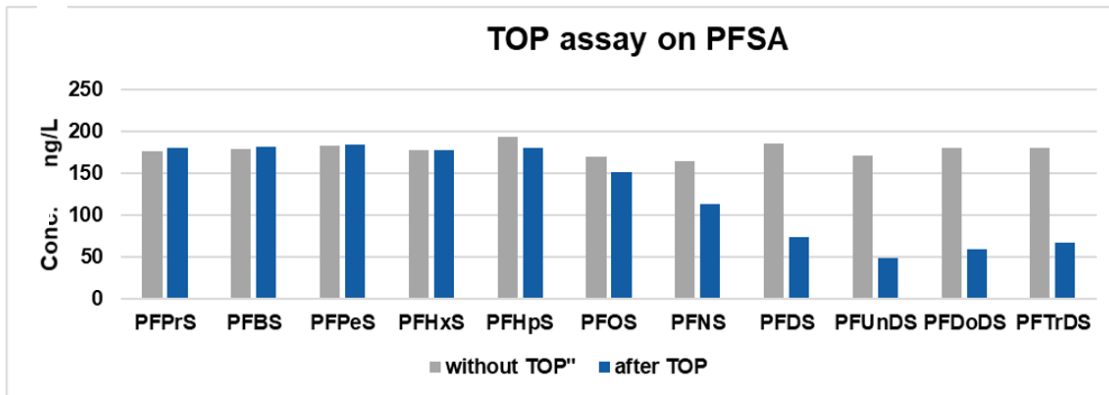
Figure 8 : Results of TOP analysis (Houtz protocol) on spiked water with PFAS mix, focus on PFSA.

Results on different chemical groups are less easy to interpret, since compounds from the same chemical groups react very differently, such as presented in Figure 9 and Figure 10. Results on FTSA compounds are surprising (Figure 9), since only the longer compounds seem to be affected by oxidation process.
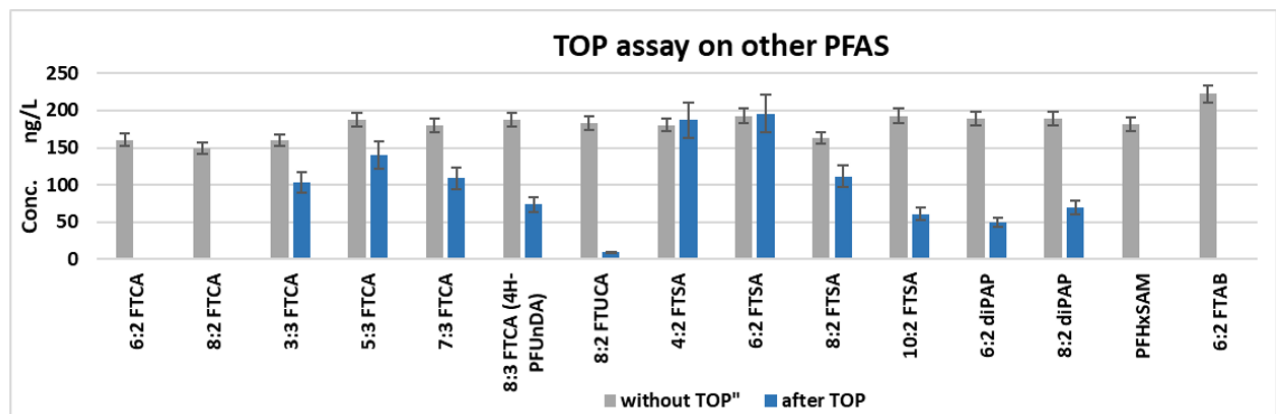
Figure 9 : Results of TOP analysis (Sedlak protocol) on spiked water with PFAS mix, focus on other PFAS.
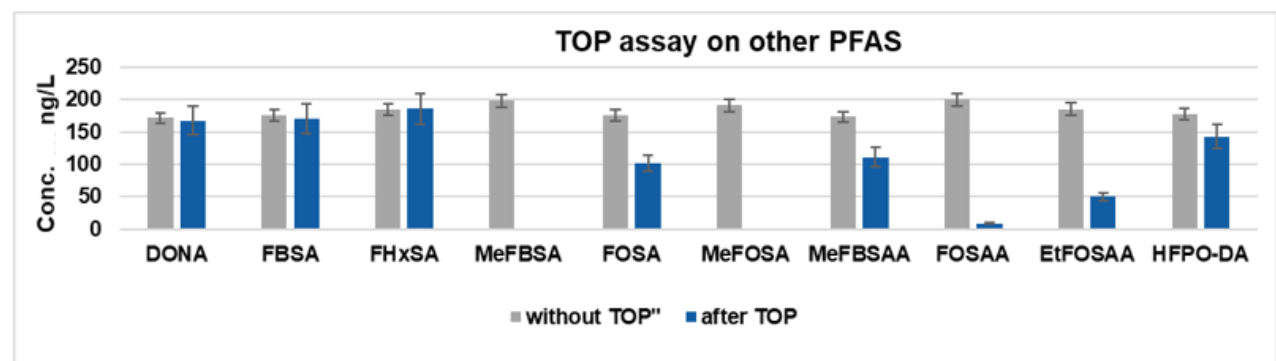
Figure 10 : Results of TOP analysis (Sedlak protocol) on spiked water with PFAS mix, focus on other PFAS (b)

Despite the degradation of PFAS in the mix, the objective of obtaining a complete mass balance by measuring the increase in PFCA concentration was not achieved. In order to better estimate the transformation of the precursor into PFCAs, the same experiment has been done on water spiked with AFFF containing both 6:2 FTSA and 6:2 FTAB, and no PFCA. Results presented in Figure 11, demonstrate the formation of PFCA but not equivalently to disappearance of precursors.
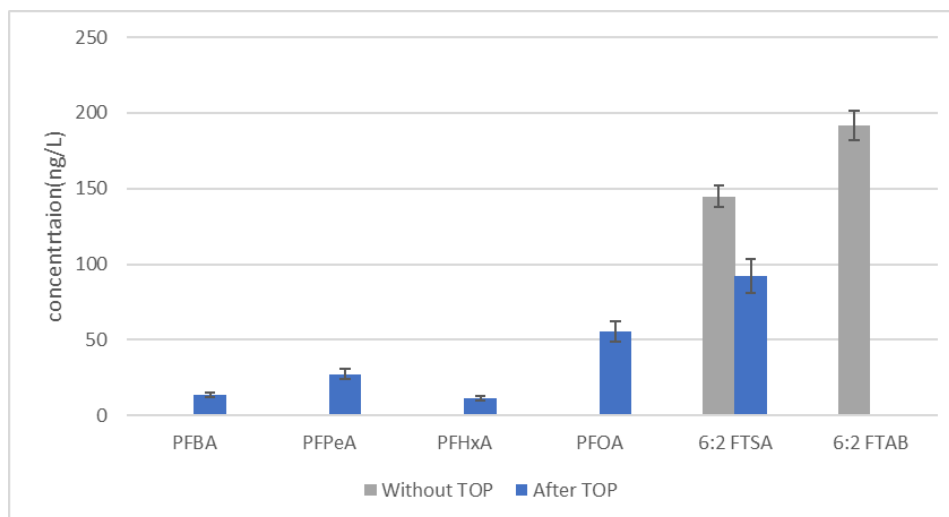
Figure 11 : Results of TOP analysis on spiked water with AFFF.

Comparison with the both protocols (Houtz and Hutchinson) has been done on water spiked by AFFF and on the complex mix on 56 PFAS. The chemical agents for oxidation are the same but with different concentrations, as presented in Table 6. A better oxidation is demonstrated when more reactants are used, as shown on Figure 12 , but in the PFCAs formed there is more short chain PFAS than expected, as highlighted by disappearance of PFOA. Two hypotheses can reach to this result: either the PFAS chain length is reduced upon oxidation, or unknown precursors are oxidized in shorter PFAS. As the analytical method developed is not adapted to PFCA shorter than C4 (such a TFA or PFPrA), this hypothesis cannot be confirmed. A specific method is needed for shorter PFAS, but it will make the TOP assay more expensive.

As a specific method on a dedicated equipment is required with associated method development, this data gap cannot be fulfill in the frame of the current project but it is an important need recently highlighted by Tsou et al. (2023).
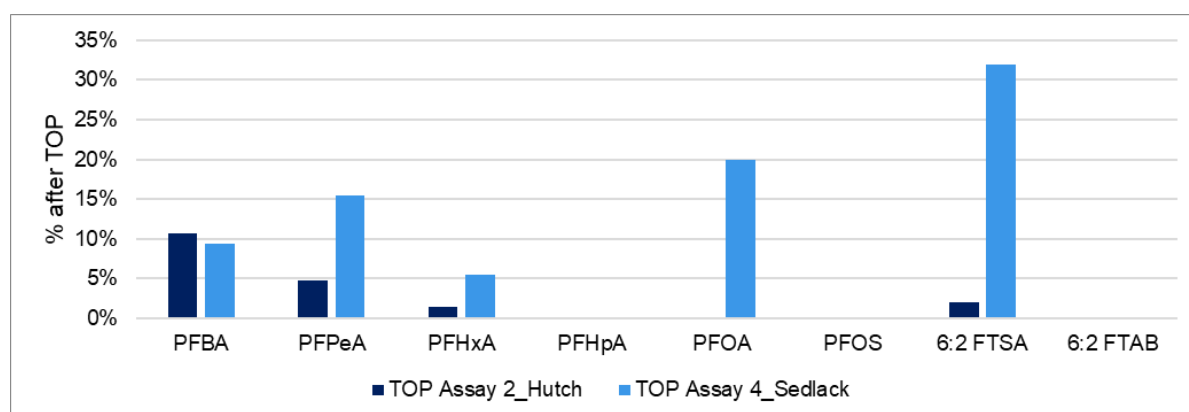
Figure 12 : comparison on the both protocol on water spiked with AFFF.

This large variability in the efficiency of the method is highlighted in several publications involved in the development of TOP assays, as the overview on several publications demonstrate in Table 7. Based on individual solutions, oxidation products obtained for different PFAS have been characterized and quantified by different authors. High variability within PFAS but also within authors on the same compounds can by highlighted.

Table 7 : Variability in the TOP assay results from literature.

| compounds	TFA	PFPrA	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	reference
5:3 FTCA	29	18	28	13	2					Tsou et al., 2023
7:3 FTCA	11	14	21	25	40	17	3			Tsou et al., 2023
4:2 FTSA	29	26	28	0,05						Tsou et al., 2023
6:2 FTSA	NA	NA	22	27	22	2				Houtz et al., 2012
	NA	NA	16	26	4					Macorps, 2023
	11	15	26	24	28	3				Tsou et al., 2023
8:2 FTSA	NA	NA	11	12	19	27	21	3		Houtz et al., 2012
	NA	NA	9	12	13	19	5			Macorps, 2023
	2	10	12	25	32	13	3	3		Tsou et al., 2023
10:2 FTSA	NA	NA	3	3	5	8	8	19	8	Macorps, 2023
6:2 diPAP	NA	NA	27	47	33	15				Houtz et al., 2012
	NA	NA	20	40	28	12				Rehnstam et al., 2023
8:2 diPAP	NA	NA	10	17	24	43	38	13		Houtz et al., 2012
	NA	NA	5	13	18	38	26			Rehnstam et al., 2023
FOSA	NA	NA					97			Houtz et al., 2012
	NA	NA	4	6	5	11	44			Macorps, 2023
EtFOSA	NA	NA			5		90			Rehnstam et al., 2023
FOSAA	NA	NA	1	3	3	11	29			Macorps, 2023
MeFOSAA	NA	NA					110			Houtz et al., 2012
	NA	NA	4	5	5	15	18			Macorps, 2023
EtFOSAA	NA	NA					92			Houtz et al., 2012
			4	4	5	18	21			Macorps, 2023
6:2 FTAB			7	9	2					Macorps, 2023
6:2 FTSaB	11	14	19	38	8	2				Tsou et al., 2023

So there is a need of an interlaboratory assay involving a sufficient number of laboratories to compare impact of the oxidation process (such as effect of reactants concentration), but above all, that consider different matrices well characterized, and taking into account not only the conventional PFCA but also the shortest ones (Below C4).

2.2.5 Performance

The notion of performance is difficult to characterize for this global method compared to classical target method. Concerning the limit of quantification, the dilution step (factor 20) applied for this method has suppressed the potential matrix effects due to chemicals used for the oxidation steps.

The main difficulties, as mentioned by TUW before is the poor reproducibility of the method, without the reason being clearly stated (Figure 13 and Figure 14).
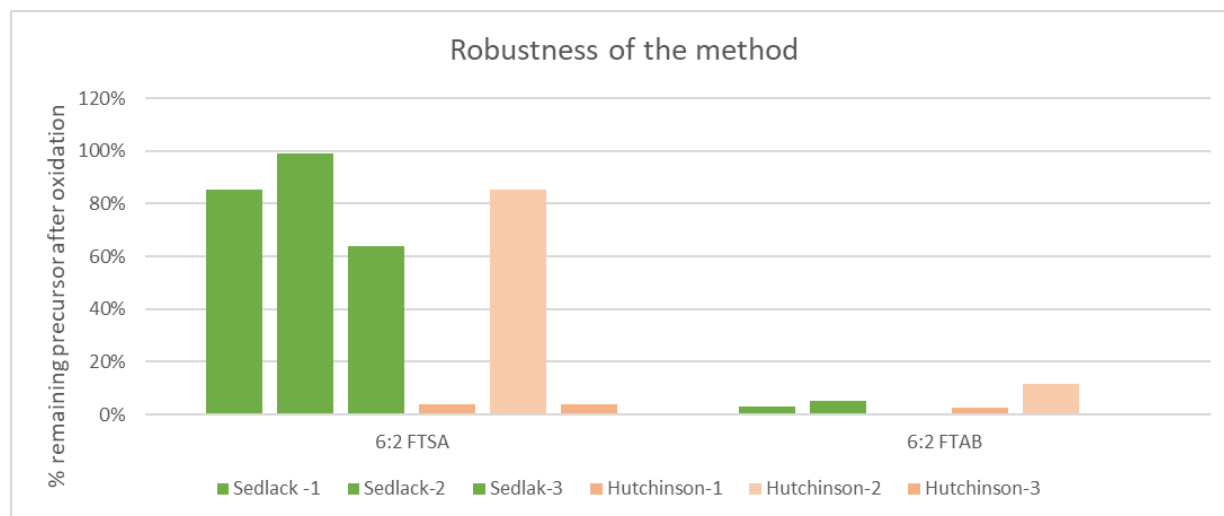
Figure 13 : Results of the different experimentations on degradation of precursors
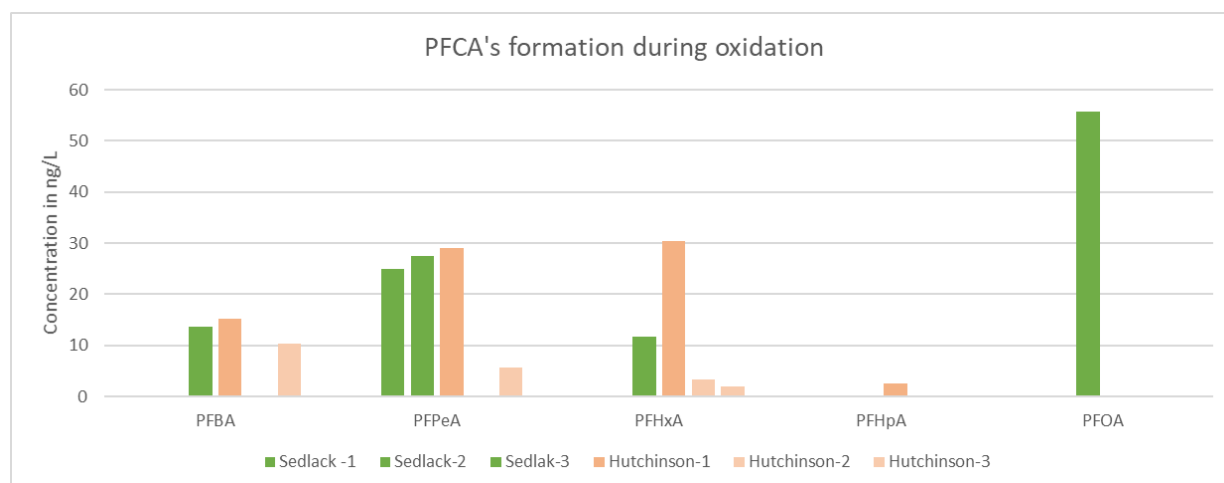
Figure 14 : Variability in the formation of PFCA's during oxidation 'step.

The main hypothesis explaining this variability is the high organic carbon content of sludge and effluent samples. To limit this impact, further work has been carried out to reduce the sample size, and thus increase the reagent ratio. This approach appears to reduce the variability of results.

Despite the fact that the robustness of the method needs to be improved (to this end, discussions for the organization of an inter-laboratory trial are underway within the framework of the European PARC project), the value of TOP-assay analysis is highlighted. By simplifying the PFAS signal through this oxidation step, the formation potential of terminal PFAS is highlighted. Moreover, the degradation pattern of the molecules will (when the analyses are stabilized) provide information on the structure (chain length, chemical groups…) of the precursor PFAS.

3 Estimation of the Organic fluorine content using the Combustion Ion chromatography

Having a global overview of the PFAS content seems currently an unattainable goal considering partial knowledge due to non-exhaustive list of compounds to consider or the potential transformation products formed in the environment… However, one of the most promising ways of getting around these difficulties seems to be the development of global methods measuring Organic fluorine content. For these global methods, several approaches have been proposed based on the different fluorine chemical species.

Different fractions of the sample can be identified. Figure 10 provides a comprehensive illustration of the different forms of fluorine compounds according to their inorganic or organic character as well as their adsorptibility or extractability features.
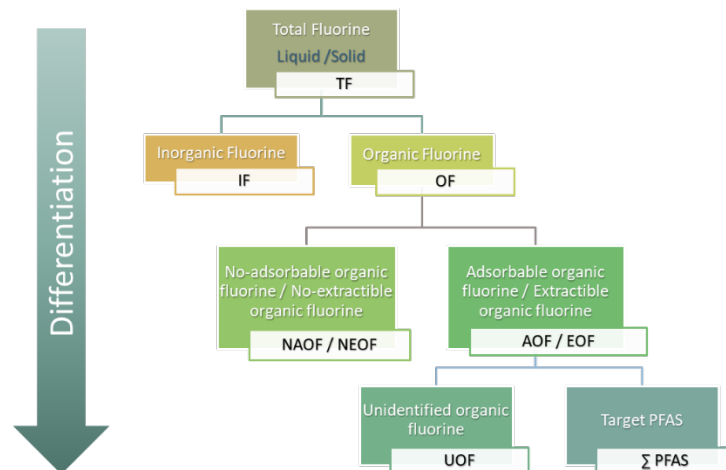
Figure 15: Overview of the different fluorine chemical species according to their inorganic or organic character as well as their adsorptibility or extractability features of different matrices (liquid or solid) (Modified from Aro et al. 2021).

Based on the experience with the determination of global adsorbable organic halogens (AOX) parameter, the Combustion Ion Chromatography (CIC) seemed to be the more reliable method for PFAS compounds, compared to other research area for a global characterization, such as TOP assay, HR–CS–GFMAS (Simon et al., 2022) or HRMS approaches (Liu et al, 2019). So different methods have been developed in the PROMISCES project using this technology in two laboratories (BWB for liquid samples and BRGM for solid and liquid samples).

3.1 Analysis by BRGM

3.1.1 General

As BRGM has the objective of developing methods for different typologies of samples, the generic scheme summarizing the different methods is presented in Figure 16.
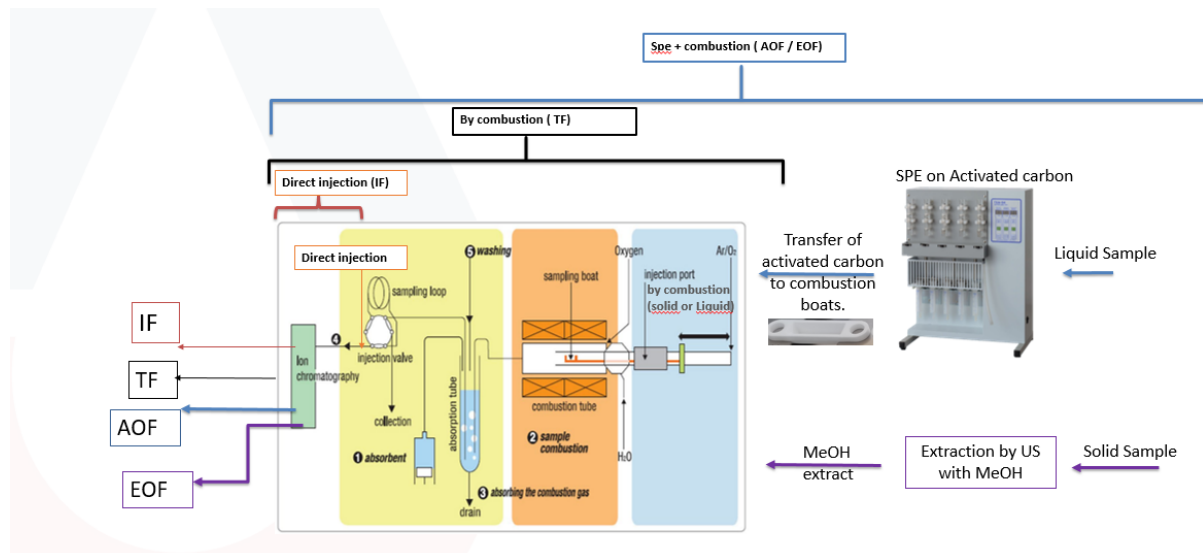
Figure 16: General scheme of methods developed by the BRGM using CIC equipment.

The ion chromatography (IC) allows fluoride analysis from the fluorine content of the sample. Direct combustion of samples (100 µg or 100 µL) before analysis provides total fluorine (TF) content, while direct injection in the IC provides inorganic fluorine (IF) measurement. Previous sample preparation such as solvent extraction or solid phase extraction allows the determination of EOF or AOF, respectively.

3.1.2 Chemicals and reagents

List of material

Instruments	Manufacturer	Description
Combustion boat	envirosciences	
Activated carbon	envirosciences	AOX Pack / Premium Pack
Centrifugation tube.		15 mL polypropylene , 50 mL polypropylen

List of chemical products

Chemical	Manufacturer	Description
Methanol	Fisher chemical	Optima LCMS Grade
Ammonium hydroxide	Fischer Scientific	
Ammonium acetate	Fischer Scientific	
Sodium Nitrate	Fischer Scientific	

List of instruments

Instruments	Manufacturer	Description
SPE enrichment module	envirosciences	TXA-04 : 5-channel
Sample changer	envirosciences	ASC-2700LS liquid auto sampler, an ABC-210 automatic boat controller
Combustion system	envirosciences	HF-210 ET AQF-2100H
Absorption system	envirosciences	GA-211
Ion chromatography	ThermoScientific	ICS 6000
Analytical pre-column	ThermoScientific	Dionex IonPac AG20 RFIC™ 2*50mm
Analytical column	ThermoScientific	Dionex IonPac AS20 RFIC™ 2*250mm
Laboratory centrifuge		
Ultrasonic bath		

3.1.3 Sample collection, preservation and pre-treatment for solid and liquids

Solid samples:

For TF, 100 µg of dried samples is directly introduced in the combustion system in combustion boat.

For EOF, 1 g of dried sample is extracted with 3 consecutive extraction steps: 10 mL of solvent, ultrasonication for 20 min, centrifugation for 5 min (4612 g) and transfer into a polypropylene 50 mL centrifuge tube. The 3 solvents were MeOH with 10 mM NH_4OH, MeOH with 100 mM CH_3COONH_4 and 10 mL MeOH. The three extracts were combined. An aliquot volume of 10 mL is reduced under nitrogen to 1 mL. One hundred microliter is collected and analysed by CIC to determine EOF.

Liquid samples:

For TF, 100 µL of sample is directly introduced in the combustion system in combustion boat.

For AOF, samples are pre-concentrated on pre-packaged activated carbon columns (AOXPack / Premium Pack) using the 5-channel AD enrichment module.

150 ml of the homogenized sample are adjusted to pH7 (with $ACNH_4$ or HNO_3). SPE system is rinsed with 40mL of sample. 100 mL of water sample is loaded on two activated carbons (50 mg each) placed in the column with a constant flow of 3 mL per minute. The column is washed with 15 mL of $NaNO_3$ solution at 0.01 M and flushed with 5 ml of air using a syringe. The column is then dismantled and the two activated carbons are placed in the combustion boat and deposited in the CIC sampler.

3.1.4   Analysis

The instrument used for OF quantification comprises an ASC-2700LS automatic liquid sampler, an ABC-210 automatic combustion boat controller, an HF-210 horizontal furnace and a GA-211 sample absorption unit. All this is coupled to an ICS 6000 ion chromatography unit (Thermo Scientific). Chromatographic conditions are the same for all analysis. Changes occur in the combustion program, by changing the duration and positioning of the sample in the oven. The method varies according to the nature of the sample and the presence of solvent, leading to 3 programs: one for AOF, "volatile"one for EOF (occurrence of solvent) and "inorganic"one for TF.

Chromatographic conditions:

Parameter	Settings
Eluent*	KOH gradient 15 – 80 mM
Eluent generator	EGC 500 KOH
Flow rate	0.250 ml/min
Injection volume	1 µL
Pre-column	Dionex IonPac AG20 RFIC™ 2*50mm
Column	Dionex IonPac AS20 RFIC™ 2*250 mm
Column temperature	35°C
Suppressor temperature	35°C
Suppressor	ADRS_2mm
Detection	Conductivity (F−,Cl−,Br−)

Elution gradient:

Time (min)	Concentration mM
0.000	Run
0.000	15
12.00	15
12.010	80
16.000	80
16.010	15
20.00	Stop Run

Programs for combustions:

volatil			AOF			inorganic		
Position in the oven	Wait Time (s)	Speed	Position in the oven	Wait Time (s)	Speed	Position in the oven	Wait Time (s)	Speed
65	10	20	65	30	10	110	30	20
100	30	0,06	END	600	20	150	30	10
130	30	0,06	COOL	60	20	180	30	10
END	60	20	HOME	200	40	END	600	20
COOL	60	40				COOL	60	40
HOME	90	40				HOME	90	40

To ensure the quality of the analytical work several blanks were analyzed. A protocol for cleaning the combustion-absorption tube of the CIC was carried out before each series of analyses. Particular attention was paid to the memory effect after eluting a highly concentrated sample, which requires a blank analysis after each sample. The addition of a systematic blank afterwards eliminates the residual effect of high fluoride concentrations.

3.1.5 Performance per matrix

Calibration has been done by direct injections ranging 0.005 mgF.L^{-1} to 10 mgF.L^{-1} for IF as NaF. Calibration controls were performed for each analytical run. The 20 µgF.L^{-1} (as IF) calibration level is used in each sequence as a quality control (QC) of the reference calibration range used for several analytical runs. Water, activated carbon and combustion vessel blanks were routinely performed between each analytical run. The limit of quantification (LOQ) of each method was calculated as the average concentration measured in blank extracts plus 10 times its standard deviation.

Multiple assays on the analysis of individual consumables (reused boat, new boat, activated carbon) have shown that contamination level for the analytical parts is less than 0.21 ± 0.06 µgF.L^{-1} for combustion boat and 0.47 ± 0.12 µgF.L^{-1} for entire process (combustion boat + activated carbon).

Similarly, multiple tests of AOF blanks including entire protocol (extraction and analysis) before and after real samples were carried out with HPLC water. They demonstrated excellent repeatability (<12%, n=11) and good control of residual contamination (by cleaning enrichment module after each sample elution) leading to a background level of fluoride of 0.88 ± 0.11 µgF.L^{-1}, called "procedural blank" which constrains the limits of quantification (LOQ) for the different measurements.

Considering LOQ from apparatus sensitivity (0.005 µgF.L^{-1}) and analytical parameters of each method, theoretical LOQ for AOF will be 0.5 µgF.L^{-1} for 100 mL sample, 500 µgF.kg^{-1} for TF and 500 µgF.L^{-1} for EOF extract injected.

For AOF, calculation of LOQ based on residual contamination leads to 1.98 µgF.L^{-1}. Then the LOQ set as 2 µgF.L^{-1} for 100 mL sample is therefore driven by the system blank. For TF and EOF, the method blank value was 136 ± 29 µgF.kg^{-1}, leading to a LOQ of 500 µgF.kg^{-1} for TF (sample 100 mg) and 500 µgF.L^{-1} for EOF (sample 100 µL extract injected). The LOQ for IF in the leachate from solid is 0.025 mgF.L^{-1}.

This method is on line with the method under ISO normalization process (ISO/TC 147/SC 2 N 2147), based on the German standard DIN 38409-59:2021.

DOC impact on AOF measurements has been assessed on artificial samples spiked with humic acids (up to 150 mgC.L^{-1}) and on two real leachate samples (with DOC concentrations of 2135 and 3167 mgC.L^{-1}). Signal extinction has been highlighted in the two leachate samples. By the way, for complex water samples (effluent or leachates) two systematic preparation have been applied: a direct and a ten-fold dilution to characterize matrix effects. Depending the results, complementary dilutions are undertaken considering signal extinction and measured concentration.

Concerning suspended matter, the main impact is identified during the sample loading on the activated carbons that are protected by a piece of cotton that can create clogging. Assays have been undertaken until 250 mg/L without any important clogging, but a second sample, diluted, is analyzed systematically to confirm the measurement.

All the developments on CIC analysis have been submitted for publication in Science of Total Environment (accepted).

3.1.6   Application to real samples

The LOQ obtained for AOF (0.5 µgF.L^{-1} for 100 mL sample) should be confirmed on real samples to confirm its applicability. Even if this LOQ is very high compared with the performance achieved with targeted analysis, the inclusion of a wider range of fluorinated organic compounds is a major benefit. In the first instance, this approach can be used to identify sites with the greatest potential for contamination. In conjunction with targeted analysis (usually carried out on the most commonly found PFAS), it can reveal the presence of PFAS not previously expected.

These complementary benefits between approaches will be developed in the future works by comparing monitoring strategies.

3.2   Analysis by BWB

3.2.1   General

BWB method is used for the determination of absorbable organic fluorine (AOF) along with organically bound other halogens (AOCl, AOBr, and AOI) in aqueous solutions. The method is according to the industrial codes: DIN EN ISO 10304-1:2009-07, DIN EN ISO 9562:2005-02 and DIN EN ISO 38409-59:2020-11. Using combustion ion chromatography (CIC) as the detection method, it is possible to determine the organically bound halogens fluorine, chlorine, bromine, iodine as F$^-$, Cl$^-$, Br, I$^-$ by upstream high-temperature combustion.

The analytes in the aqueous samples are first absorbed in activated carbon, whereupon the activated carbon is then digested at 950 °C under an argon atmosphere and combusted with a constant flow of oxygen and water. The resulting gaseous compounds are fed into an absorption solution, which is then transferred inline to an ion chromatography system (Metrohm 920 Compact IC Flex) for analysis.

As in the AOX determination, the sample is enriched by adsorption onto activated carbon. In this way, non-polar and polar hydrogen halides are detected. During combustion in the furnace, CO_2, H_2O and hydrogen halide are formed and any free halogen X_2 that is still present is reduced to HX, since the reaction is endothermic.

3.2.2 Chemicals and reagents

List of chemicals and reagents used in the BWB AOF method.

Chemical	Manufacturer	Description
Sodium carbonate	Bernd Kraft	For analysis
Ortho-Phosphorous Acid	Merck	85 %, For analysis
Nitric Acid	Merck	For analysis
ethanol	Merck	Absolute, For analysis
Cleaning solution	Metrohm	
Sodium sulfite	Merck	For analysis
Silver nitrate	Merck	For analysis
Sodium nitrate	Merck	For analysis
material	**Manufacturer**	**Description**
Glass tubes filled with activated carbon	Analytik Jena	Particle size: 50-150 µm, Tested for blind values
Ceramic wool	Analytik Jena	
Absorbent Cotton		Tested for blind values
SPE Cartridges Bakerbond	JT Baker	SDB-1,3 mL
Standard solution	**Manufacturer**	**Description**
Calibration solutions F⁻ ; Cl⁻ ; Br⁻ ; I⁻	Merck	Each 1000 mg/L
Reference Material F⁻ ; Cl⁻ ; Br⁻ ; I⁻ (inorganic)	Bernd Kraft	1000 mg/L
Reference Material F⁻ ; Cl⁻ ; Br⁻ ; I⁻ (organic) – VOC Mix 4	Neochema	10 mg/L
Iodide	Merck	1000 mg/L

3.2.3 Sample collection, preservation and pre-treatment for solid and liquids

The sample should be collected with caution, to avoid contamination of any material (especially tubing, seals, O-rings). The sample is filled bubble-free into a 250 mL conical shoulder glass bottle and transported refrigerated. The sample is stored in the refrigerator at 5 °C until measurement (up to 5 days) or deep frozen at -25 °C (up to 4 weeks).

To prevent particle interferences, both ends of the activated carbon tubes are stuffed with ceramic wadding.

3.2.4 Analysis

100 ml of the homogenized sample are directed through two consecutive enrichment columns containing at least 50 mg of activated carbon at a constant flow of 3 mL per minute. The columns are then rinsed with 25 mL of nitrate wash solution (pH=7). Upon start-up, the device must equilibrate, including heating of the combustion oven up to 950 °C, starting of the IC flow and heating of the IC column oven to 60 °C, as well as stabilization of the signal base line. Then, measurements can start, with three options of combustion and analysis (combined combustion and analysis of both activated carbon tubes, separate combustion and combined analysis, separate combustion and separate analysis, respectively).

As blank, 0.5 mL nitrate solution is added to 100 mL of ultra-pure water and is enriched in the same way as the samples.

A dilution of the reference material (0.5 mg/L) serves as calibration solution. The calibration solution is injected automatically in different volumes for analysis by the device. The calibration curve should have a correlation factor $R^2 > 0.99$.

3.2.5 Performance

According to DIN 38409-59 for the evaluation of the method, 7 different concentrations spiked in ultra-pure water must be measured. Table 8 shows the recoveries of AOF in different concentration levels as well as the mean method recovery. For spiking 4-Fluorobenzoic acid solution is used. The blind value of each individual measurement is removed from the measured concentrations.

Table 8: Recovery of AOF in different concentration levels of the BWB AOF method.

Spiked Concentration in µg/L	Measured Concentration (blind value removed) in µg/L	Recovery in %
2	1,98	99
5	4,71	94
10	10,28	103
15	15,67	104
20	21,49	107
40	40,38	101
60	60,91	102
	Mean recovery:	101

The measured concentrations are plotted over their spiked concentrations in Figure 17. The correlation factor (R^2) of the linear plot must be grater or equal than 0.99 and the slope of the recovery equation needs to be between 0.9 and 1.1.
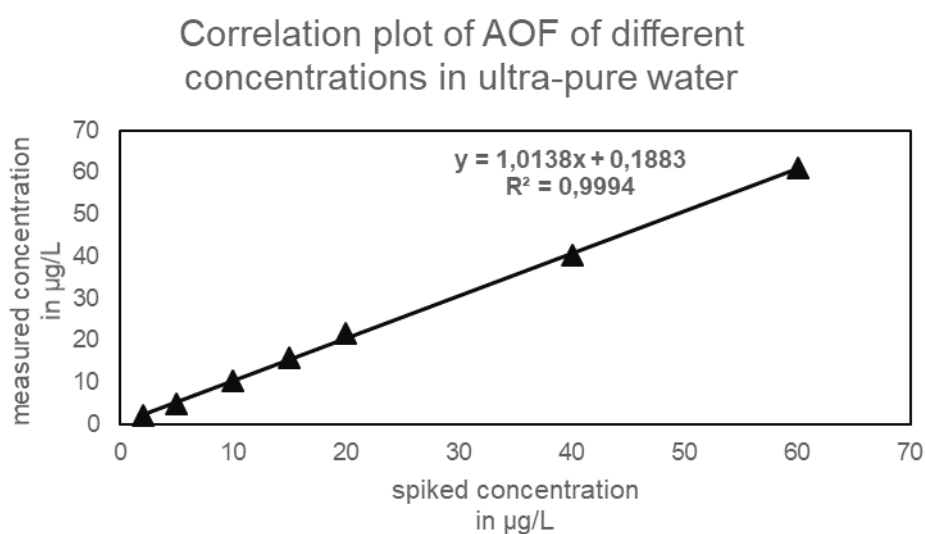

Figure 17 : Correlation between the spiked and measured concentrations of the BWB AOF method.

The limit of quantification is calculated according to DIN 8466-2 and is derived from 13 individual concentrations. After adsorption of 100 mL of sample the LOQ of AOF is 0.86 µg/L.

For daily routine measurements the individual calibration function is checked against an independent check standard once a day. The recovery must be between 95 % and 105 %. After every 10th sample a reference standard (4 µg/L) is analyzed.

3.2.6   Application to real samples

CIC-AOF analysis requires DOC/TOC (dissolved and total organic carbon, respectively) of 10 mg/L or below, to ensure unimpeded adsorption of organic halides. Therefore, raw wastewater and wastewater treatment plant effluent samples need dilution in most cases. In addition, checking for completeness of adsorption requires that at least two different dilutions of each sample undergo analysis, followed by an assessment of similarity of the results. According to the corresponding DIN 38409-59 annex H the concentration difference must be under 10%, otherwise additional measurements in other dilutions are necessary. For very clean samples with little to no known contamination (e.g., groundwaters, drinking waters), measurement at two dilutions is not required.

3.3   Interlaboratory comparison

The two AOF methods developed by BRGM and BWB were compared by analyzing the same samples in the two laboratories. 3 samples have been selected (wastewater influent, effluent and groundwater). Repeatability is good on triplicates (7 to 12% depending on the matrix).

Between the two laboratories, results are consistent on all 3 matrices. The greatest heterogeneity concerns influent waters, undoubtedly due to the impact of suspended solids.

4 Conclusions

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals known for their widespread use in various industrial and consumer applications. Due to their persistence, bioaccumulation, and potential adverse health effects, there is a growing need for accurate and efficient analytical methods to detect and quantify PFAS in environmental samples.

The aim of this work was to propose methods for assessment of global organic fluorinated content for relevant matrices. Two prominent analytical approaches for PFAS analysis including Total Oxidizable Precursor (TOP) assay and Combustion Ion Chromatographic (CIC) methods have been developed to reach these objectives, on different matrices of interest for the project.

Table 9: Comparative summary of all methods

	TOP assay		CIC approaches	
Matrices	TUW	BRGM	BRGM	BWB
Waters (surface and groundwaters)	x	x	x	x
Waste water Effluent	x	x	x	
Waste water Influent	x			
Landfill leachates	x		x	
Sludge		x	x	

The deliverable presents the validation of the methods, but also their limitation, due to the high complexity of the "PFAS universe", complexity due to ignorance of the composition of the samples, which does not allow the methods to be properly qualified.

While analytical methods such as TOP testing and CIC via adsorbable organic fluorine and extractable organic fluorine offer new types of information for better characterization of PFAS contamination, continued research and development efforts are still necessary to remove the obstacles identified in this work.

In particular, the development of interlaboratory tests is essential to supervise these methods, particularly in the case of complex matrices.

At the same time, better characterizing what the results obtained by these methods correspond to is essential. Thus, the acquisition of data coupling these global approaches in a cross-referenced manner with targeted methods must multiply in different contamination contexts. This will make it possible to link global measurements of organic fluorine or oxidizable precursors to the presence of certain PFAS or certain types of contamination and will promote the deployment of these tools.

Maturity of the methods can be described based on different criteria, as presented on Table 10, identifying the various obstacles that need to be overcome to make these methods more appropriate. Two main criteria are considered: robustness of the method (that can be enhanced by method normalization) and clarity/characterization of information provided by the method (that need more sample testing and exchange with the stakeholders using the results.

Table 10: Maturity of CIC and TOP assay approaches

Methods Matrices	TOP assay		CIC methods	
	Robustness	information	Robustness	information
Waters	++		++	
Waste water Effluent	++		++	
Waste water Influent	-	+	-	-
Landfill leachates	-		-	
Sludge	-		-	

Improving understanding, showing the advantages and weaknesses, but above all the complementarity of the various analytical approaches, based on feedback from the different case studies on which they have been implemented is one of the main objectives of the final stages of the project (Deliverable D1.7, February 2025).

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