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Improved and harmonized procedures and methods for the chemical analysis of new priority HS with very low limits of detection and quantification

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Abstract

The present document describes the findings and recommendations gathered during the implementation of the Interreg Danube Region Programme project Tethys within the activity focused on improving and harmonizing methods for the chemical analysis of hazardous substances, specifically per- and polyfluoroalkyl substances (PFAS) — in surface waters of the Danube River Basin. Effective management of hazardous substances requires high-quality, comparable monitoring data; however, laboratories across the region face challenges arising from diverse instrumentation, differing levels of expertise, and the lack of fully standardized analytical methods that meet the stringent requirements of the Water Framework Directive (WFD). This report therefore combines a review of international PFAS standard methods with a detailed comparison of analytical practices applied by project partner laboratories, including WRI Bratislava, TU Wien, and CETI Montenegro. Particular attention is paid to ultra-trace analysis using LC-MS/MS, solid-phase extraction protocols, sample handling, and difficulties associated with volatile PFAS and complex matrices.

A major outcome of the interlaboratory comparison exercises conducted within the project is the recognition of internal standards as the single most influential factor in achieving accurate and reliable PFAS quantification. The document provides extensive guidance on the correct assignment and use of isotope-labelled internal standards, demonstrating how insufficient or mismatched internal standards lead to systematic errors and poor recoveries. Workshops organized during the pilot action further highlighted key needs within the laboratory community, including greater opportunities for knowledge exchange, hands-on training, improved communication between authorities and laboratories, and coordinated development of analytical methods.

The document concludes with strategic recommendations for strengthening hazardous substances monitoring capacity in the Danube Region. These include proposals for establishing expert platforms for method development, enhancing participation in proficiency testing schemes and exploring collaboration possibilities through networks such as ICPDR's Monitoring and Assessment Expert Group and the NORMAN network. Overall, the report provides practical, experience-based guidance to support laboratories in implementing robust, harmonized PFAS analytical methods and in meeting the emerging monitoring requirements for new priority substances.

Introduction

The Interreg Danube Region project Tethys addresses the pressing challenge of water pollution in the Danube River Basin caused by hazardous substances (HS). This work takes place in a complex environment shaped by evolving legislation, high monitoring costs, and the technical difficulties of managing a large number of priority pollutants.

A key component of the project is the development and application of modeling tools to support the management of hazardous substances at both the international basin level and within participating countries. Reliable modeling depends on high-quality input data—particularly accurate concentration measurements across different environmental compartments.

Because water monitoring is carried out by multiple institutions within each country, harmonization of analytical result quality is essential. For many hazardous substances, standardized methods that meet the requirements of the Water Framework Directive (WFD) are not available; they often fail to include all relevant analytes or achieve the required limits of quantification (LOQs). As a result, laboratories adapt and modify various methods based on their resources and needs.

In the field of ultra-trace organic analysis, particularly for compounds such as poly and perfluoralkyl substances (PFAS), access to advanced instrumentation—liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)—is critical. Depending on instrument sensitivity, laboratories may need to apply sample pre-concentration or direct injection, which significantly affects detection limits and measurement uncertainty. Complete harmonization of analytical methods is therefore not feasible; instead, the focus must be on harmonizing the quality of analytical results and enhancing their reliability and comparability.

To address these challenges, the project implemented a pilot action aimed at knowledge transfer through workshops and harmonization of analytical quality using interlaboratory comparison studies. Based on the findings of the pilot action described in detail in the Output 1.2 (https://interreg-danube.eu/projects/tethys/library), this document provides guidance to laboratories on implementing or adapting critical steps in PFAS analysis.

The document includes:

- An overview of published standardized methods for PFAS analysis.
- A review of methods applied by laboratories of the Tethys project partners, adapted for surface water.
- Practical insights and tips from workshops for successful method implementation.
- Observations and recommendations derived from two interlaboratory comparison studies.

Review of standardized methods for analysis of PFAS

Several standards have been published for PFAS analysis in water; however, none of them cover all PFAS compounds included in the proposed new EU list of priority substances (https://environment.ec.europa.eu/publications/proposal-amending-water-directives_en). These standards can serve as a basis for method modification or development. Short review providing details that we consider to be important is presented in this chapter. Some of these standards also include matrices beyond surface water, such as drinking water, wastewater, solid matrices, and biota tissues. List of standard methods for determination of PFAS in water matrices:

ISO 21675:2019 Water quality — Determination of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in water — Method using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS)

ISO 25101:2009 Water quality — Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) — Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry

EN 17892:2024 - Water quality - Determination of selected per- and polyfluoroalkyl substances in drinking water - Method using liquid chromatography/tandem-mass spectrometry (LC-MS/MS)

Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry

Method 537: Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

EPA 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

Method 8327: Per-and Polyfluoroalkyl Substances (PFAS) by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

ASTM D7979 Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances in Water, Sludge, Influent, Effluent, and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

DIN 38407-42:2011-03 German standard methods for the examination of water, waste water and sludge - Jointly determinable substances (group F) - Part 42: Determination of selected polyfluorinated compounds (PFC) in water - Method using high performance liquid chromatography and mass spectrometric detection (HPLC/MS-MS) after solid-liquid extraction (F 42).

Proposed Standard Method for the Quantitative Analysis of Fluorotelomer Alcohols and Monomers in Water - QUANTITATIVE ANALYSIS OF FLUOROTELOMER ALCOHOLS_ASTM_DEC2021

Sampling and sample storage conditions are given in EN ISO 5667-3:2024 Water quality – Sampling - Part 3: Preservation and handling of water samples and ISO 21675:2019 Water quality — Determination of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in water — Method using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples should be taken in narrow-neck, flat-bottomed PP or PE bottles with conical shoulders and screw caps. Prior to use bottles should be washed with high purity water and methanol. Transport temperature is (5 ± 3)°C. Samples can be stored at (4 ± 3)°C for 60 days or at ≤-15°C for 180 days and defrizzed at ambient temperature. Contact of the sample with fluoropolymer plastics must be avoided. Options for sample storage are very important factors influencing organization of laboratory work and laboratory throughput, therefore, in Table 1 storage conditions as defined various standards can be found for comparison.

For sample preparation and pre-concentration all methods recommend weak anion exchanger on copolymer base as the SPE sorbent, one standard method also recommends hydrophilic-lipophilic balanced polymer material, and one standard method recommends styrene-divinylbenzene. Exact Solid Phase Extraction (SPE) protocols are listed in EPA 1633, EPA 533, EPA 537, ISO 21675 and ISO 21675. Other standard methods recommended following the manufacturer's SPE protocols. Most of the standard methods do not specify sample volumes and sample preconcentration factors. The maximum recommended flow rate for the sample loading is 6 ml/min. Recommend extract concentration are according to standards is in the range 250 ul, 500 ul or up to 1 ml or to dryness under a gentle stream of nitrogen followed by reconstitution in suitable solvent, maximum recommend temperature of heating is 65°C. Important note is that for analysis of volatile PFAS from the group of telomer alcohols, drying step should be carefully considered.

For LC-MS/MS separation and detection all standards recommend use of reverse phase system with C18 column. One standard method recommends the use of phenyl-hexyl analytical column. In general, LC parts of methods are quite common. The only critical point is the use of trap/delay/isolatory column placed in the mobile phase flow path immediately before the injection valve of the LC autosampler. This column is used to reduce the contamination by PFAS originating from sources prior to the sample loop from and to remove PFAS background contamination.

Table 1 provides overview of published standards with analytes, sample preparation details, parts of analytical methods and sample storage conditions.

Table 1: Overview of the substances included in the analysis, the application of SPE or direct injection techniques, the corresponding limits of quantification (LOQs), and the prescribed sample storage conditions prior to analysis.

Standard (year of pubication)	**Analytes**	**Sample preparation (SPE material)**	**Analytical method (LOQ) (sample volume)**	**Sample storage and holding times**
EN 17892 (2024)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, FOSA, EtFOSAA, HFPO-DA, ADONA, PFMPA (PF4OPeA), 9Cl-PF3ONS	Part A: Method using direct injection Part B: Method using SPE (weak anion exchanger on a polymer base with reversed-phase moiety or hydrophilic-lipophilic balanced polymer material)	LC-MS/MS (1 ng/l) (-)	storage (4±3)°C 60 days storage ≤-15°C 180 days
ISO 21675 (2019)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFOcDA, PFBS, PFHxS, PFHpS, PFOS, PFDS, 6:2 FTSA, 8:2 FTSA, FOSA, NMeFOSA, NEtFOSA, NMeFOSAA, EtFOSAA, HFPO-DA, ADONA, 9Cl-PF3ONS, 8:2 FTUCA, 8:2 diPAP	SPE (weak anion exchanger on copolymer base)	LC-MS/MS (≥ 2 ng/l) (-)	storage (5±3)°C 4 weeks
ISO 25101 (2010)	PFOS, PFOA	SPE (copolymer)	LC-MS/MS (PFOS 2 ng/l PFOA 10 ng/l) (-)	storage (4±2)°C 2 weeks or freezed
DIN 38407-42 (2011)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS	SPE (weak anion exchanger on polymer base)	LC-MS/MS (0,01 ug/l) (-)	cool place no longer than 14 days
ASTM D7979-17 (2019)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFOS, 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, PFecHS, 8:2 FTUCA, 6:2 FTUCA, 7:3 FTUCA	direct injection (-)	LC-MS/MS (0,7-106,8 ng/l) (5 ml)	storage 0-6°C 28 days

Standard (year of pubication)	Analytes	Sample preparation (SPE material)	Analytical method (LOQ) (sample volume)	Sample storage and holding times
EPA 1633 (2024)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFOS, 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, FTOH, NMeFOSAA, EtFOSAA, NMeFOSE, NEtFOSE, HFPO-DA, ADONA, PFMPA (PF4OPeA), PFMBA, NFDHA, 9Cl-PF3ONS, 11Cl-PF3OUdS, PFEESA, 3:3FTCA, 5:3FTCA, 7:3FTCA	SPE (Waters Oasis WAX or equivalent)	LC-MS/MS (1,6-40 ng/l) (500 ml, extract volume 5 ml)	transport 0-6°C storage 0-6°C 28 days storage ≤-20°C 90 days sample extracts 0-4°C 90 days
EPA 8327 (2021)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, NMeFOSAA, EtFOSAA, FOSA	-	LC-MS/MS (-) (-)	storage ≤6°C 14 days sample extracts ≤6°C 30 days
EPA 533 (2019)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, HFPO-DA, DONA, PFMPA (PF4OPeA), PFMBA, NFDHA, 9Cl-PF3ONS, 11Cl-PF3OUdS, PFEESA	SPE (weak anion exchange, mixed-mode polymeric sorbent (polymeric backbone and a diamino ligand), particle size approximately 33 µm)	LC-MS/MS (1,6-13 ng/l) (100 – 250 ml, extract volume 1 ml)	transport ≤10°C, storage ≤6°C 28 days, sample extracts at room temperature 28 days
EPA 537.1 (2018)	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFOS, PFNS, NMeFOSAA, EtFOSAA, HFPO-DA, 9Cl-PF3ONS, 11Cl-PF3OUdS, ADONA	SPE (styrenedivinyl-benzene (SDVB) polymeric sorbent phase)	LC-MS/MS (0,53-6,3 ng/l) (250 ml, extract volume 1 ml, injection volume 10 µl	transport ≤10°C storage ≤6°C 14 days, sample extracts at room temperature 28 days

Standard (year of pubication)	Analytes	Sample preparation (SPE material)	Analytical method (LOQ) (sample volume)	Sample storage and holding times
EPA Proposed Standard Method for the Quantitative Analysis of Fluorotelomer Alcohols and Monomers in Water (2021)	4:2FTOH, 6: 2FTOH, 8:2FTOH, 10:2FTOH, 7:2sFTOH, 4:3FTOH, 5:1FTOH, 6:1FTOH, 7:1FTOH, 8:1FTOH, 9:1FTOH, 10:1FTOH, 11:1FTOH, 6:2FTAc, 6:2FTMAc,8:2FTAc, 8:2FTMac,10:2FTAc,10:2FTMac,FOSE, NMeFOSE, NEtFOSE	liquid/liquid microextraction	GC-MS/CI (7-101 ng/l) (10 ml)	storage ≤6°C 7 days

Based on the results of interlaboratory comparison studies conducted during the Tethys pilot action, we identified that the most significant factor influencing the quality of analytical results is the correct use of appropriate internal standards. These standards should closely match the physicochemical properties of analytes, elute as close as possible to them, and avoid any interference. For this reason, current practice relies on isotope-labeled compounds with identical structures to the analytes, most commonly carbon-13 labeled substances, and occasionally those labeled with other isotopes.

Although the use of isotope-labeled internal standards may appear costly at first glance, accurate quantification is virtually impossible without them. Laboratories that did not apply internal standards in quantitative analysis failed for most parameters in the interlaboratory comparison. The use of an insufficient number of internal standards or their incorrect assignment to analytes was likely the most critical reason for failure in some cases, while good results were achieved for other compounds.

To support laboratories in improving analytical reliability, we also provide an overview showing recommended combinations of internal standards and analytes according to various standards in Table 2.

Table 2: Overview internal standards as applied to quantitate analytes by various standard methods.

Standard (year of publication)	Internal Standard	Analyte
EPA 533 (2019)	13C4-PFBA	PFBA, PFMPA
	13C5-PFPeA	PFPeA, PFMBA
	13C3-PFBS	PFBS, PFEESA
	13C5-PFHxA	PFHxA,NFDHA
	13C2-4:2FTS	4:2FTS
	13C3-PFHxS	PFPeS, PFHxS
	13C3-HFPO-DA	HFPO-DA

Standard (year of publication)	Internal Standard	Analyte
	13C4-PFHpA	PFHpA, ADONA
	13C2-6:2FTS	6:2FTS
	13C8-PFOA	PFOA
	13C8-PFOS	PFHpS, PFOS, 9Cl-PF3ONS, 11Cl-PF3OUdS
	13C9-PFNA	PFNA
	13C2-8:2FTS	8:2 FTS
	13C6-PFDA	PFDA
	13C7-PFUnA	PFUnA
	13C2-PFDoA	PFDoA
EPA 1633 (2024)	13C4-PFBA	PFBA
	13C5-PFPeA	PFPeA, PFMPA, PFMBA
	13C5-PFHxA	PFHxA, NFDHA, PFEESA, 5:3FTCA, 7:3FTCA
	13C4-PFHpA	PFHpA
	13C8-PFOA	PFOA
	13C9-PFNA	PFNA
	13C6-PFDA	PFDA
	13C7-PFUnA	PFUnA
	13C2-PFDoA	PFDoA
	avg.13C2-PFTeDA and13C2-PFDoA	PFTrDA3
	13C2-PFTeDA	PFTeDA
	13C3-PFBS	PFBS
	13C3-PFHxS	PFPeS, PFHxS
	13C8-PFOS	PFHpS, PFOS, PFNS, PFDS, PFDoS
	13C2-4:2FTS	4:2FTS
	13C2-6:2FTS	6:2FTS
	13C2-8:2FTS	8:2FTS
	13C8-PFOSA	PFOSA
	D3-NMeFOSA	NMeFOSA
	D5-NEtFOSA	NEtFOSA
	D3-NMeFOSAA	NMeFOSAA
	D5-N-EtFOSAA	NEtFOSAA
	D7-NMeFOSE	NMeFOSE
	D9-NEtFOSE	NEtFOSE
	13C3-HFPO-DA	HFPO-DA, ADONA, 9Cl-PF3ONS, 11Cl-PF3OUdS
	13C5-PFPeA	3:3FTCA
EN 17892 (2024)	13C3-PFBS	PFBS/PFPeS
	13C3-PFHxS	PFHxS/PFHpS
	13C4-PFOS	PFOS/PFDS/9Cl-PF3ONS

Standard (year of publication)	Internal Standard	Analyte
	13C8-PFOS	PFOS/PFDS/9Cl-PF3ONS/PFNS
	13C8-FOSA	FOSA
	d5-EtFOSAA	EtFOSAA
	13C2-4:2 FTSA	4:2 FTSA
	13C2-6:2 FTSA	6:2 FTSA
	13C2-8:2 FTSA	8:2 FTSA
	13C4-PFBA	PFBA
	13C5-PFPeA	PFPeA
	13C2-PFHxA	PFHxA
	13C5-PFHxA	PFHxA
	13C4-PFHpA	PFHpA/DONA
	13C4-PFOA	PFOA
	13C8-PFOA	PFOA
	13C5-PFNA	PFNA
	13C9-PFNA	PFNA
	13C2-PFDA	PFDA
	13C6-PFDA	PFDA
	13C2-PFUnDA	PFUnDA
	13C7-PFUnDA	PFUnDA
	13C2-PFDoDA	PFDoDA/PFTrDA
	13C3- HFPO-DA	HFPO-DA
ISO 21675 (2019)	13C3-PFBS	PFBS
	13C3-PFHxS/18O2-PFHxS	PFHxS
	13C4-PFOS	PFOS/PFDS/9Cl-PF3ONS
	13C8-PFOS	PFOS/PFDS/9Cl-PF3ONS/PFNS
	13C8-FOSA	FOSA
	D3-NMeFOSA	NMeFOSA
	D5-NEtFOSA	NEtFOSA
	D3-NMeFOSAA	NMeFOSAA
	D5-N-EtFOSAA	NEtFOSAA
	13C2-6:2FTSA	6:2 FTSA
	13C2-8:2FTSA	8:2 FTSA
	13C4-PFBA	PFBA
	13C5-PFPeA	PFPeA
	13C2-PFHxA	PFHxA
	13C5-PFHxA	PFHxA
	13C4-PFHpA	PFHpA/DONA
	13C4-PFOA	PFOA

Standard (year of publication)	Internal Standard	Analyte
	13C8-PFOA	PFOA
	13C5-PFNA	PFNA
	13C9-PFNA	PFNA
	13C2-PFDA	PFDA
	13C6-PFDA	PFDA
	13C2-PFUnDA	PFUnDA
	13C7-PFUnDA	PFUnDA
	13C2-PFDoDA	PFDoDA/PFTrDA
	13C2-PFTeDA	PFTeDA
	13C2-PFHxDA	PFHxDA/PFOcDA
	13C4-8:2 FTUCA	8:2 FTUCA
	13C4-8:2 diPAP	8:2 diPAP
	13C3- HFPO-DA	HFPO-DA

As a supplement to the overview of standards for PFAS analysis in water and water-related matrices, we also include standards for the analysis of:

- Waste: EPA 8327: Per-and Polyfluoroalkyl Substances (PFAS) by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)
- Solids: ASTM D7968-23 Standard Test Method for Determination of Polyfluorinated Compounds in Soil by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS); DIN 38414-14:2011-08 German standard methods for the examination of water, waste water and sludge - Sludge and sediments (group S) - Part 14: Determination of selected polyfluorinated compounds (PFC) in sludge, compost and soil - Method using high performance liquid chromatography and mass spectrometric detection (HPLC-MS/MS) (S 14)
- Biota: EURL POPs Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed.

Review of methods applied for the analysis of PFAS within the Tethys project

During the Tethys project, PFAS compounds in surface water samples were analyzed across three laboratories of the project consortium. In this chapter, we provide concise summaries of the methods employed.

WRI Bratislava

The method applied at WRI was developed for the analysis of PFAS compounds in accordance with the requirements of the Directive on Priority Substances (2008/105/EC, 2013/39/EC), the proposed amendment to this directive, and the Drinking Water Directive (EU 2020/2184). This method includes the analysis of 29 PFAS compounds, including telomeic alcohols, with the exception of C6O4 due to the commercial unavailability of a reference material.

For pre-concentration step manual solid phase extraction is applied using 6 ml SPE columns containing 150 mg PFAS WAX (Oasis, Waters) or WAX (Oasis, Waters) sorbent. First step is conditioning of SPE column (4 ml methanol, followed by 4 ml 0,1% NH3 in MeOH) and its equilibration (4 ml pure water). Sample is fortified with solution of internal standards prepared in methanol (concentration 100 µg/l, volume added 50 ul) to obtain the final concentration 20 ng/ml. Both, native and isotopically labeled standards were obtained from Wellington Laboratories. Sample (250 ml) is then loaded at a flow rate of max 5 mL/min. Next step is washing with acetate buffer solution at pH=4 (4 ml), followed by drying the cartridge under vacuum. Elution is carried out in two steps. First, elution with methanol (2 ml) provides fraction containing telomeric alcohol PFAS compounds. In second step using methanolic ammonium hydroxide (4 ml, 0,1% NH3 in MeOH) all acidic PFAS compounds are eluted. Both fractions are concentrated using gentle nitrogen stream, for telomeric alcohols to volume of 0,5 ml and for acidic PFAS to dryness, followed by reconstitution with 250 µl of metanol.

Basic methanol fraction is analysed using LC-MS/MS method:

HPLC Agilent 1260 and triple quadrupole MS/MS Thermo Scientific Endura in ESI- mode. Column: Poroshell 120 EC-C18 2.7 µm, 2.1x100 mm (Agilent Technologies), delay column: Poroshell 120 EC-C18 4.0 µm, 3.0x50 mm (Agilent Technologies), column temperature 45°C, injection volume 5 ul. For elution gradient mobile phase is applied: Solvent A 5 mM ammonium acetate in water and Solvent B: Methanol. Gradient program is presented in Table 3 and Table 4 describes parameters of MS. In Table 5 MS/MS transitions, precursors, qualifier ions and internal standards are presented. The method is not yet fully validated, therefore we present LOQs only in approximate range of 1 – 2 ng/l.

Table 3: Gradient and flow program of analytical method.

Time [min]	B [%]	Flow rate [ml/min]
0	10	0,3
0,5	50	0,3
7,5	100	0,3
11	100	0,4
11,1	10	0,4

13	10	0,3

Table 4: Paramters of MS.

Parameter	**Value**
Ion source type	H-ESI
Polarity	Negative, 3500 V
Sheath gas	40 arbitrary units
Aux gas	7 arbitrary units
Sweep gas	0 arbitrary units
Ion transfer tube temp.	333 °C
Vaporizer temp.	317 °C

Table 5. List of analytes, quantifier and qualifiers transition and internal standards applied for quantification.

Compound	Precursor (m/z)	Quantifier (m/z)	Qualifier 1 (m/z)	Qualifier 2 (m/z)	ISTD
PFBA	213	169	-	-	M3PFBA
PFPA	262,97	218,946	-	-	M3PFPeA
PFBS	298,97	80,125	99,04	-	M3PFBS
PFPS	349	80,097	99,058	-	M3PFBS
PFHxA	312,97	268,946	119,071	-	PFHxA-13C2
HFPO-DA	285,183	169,18	185	-	M3HFPO-DA
PFHxS	398,878	80,125	99	-	PFHxS-18O2
PFHpA	362,909	318,85	169	-	M4PFHpA
ADONA	377	250,88	85,111	-	M4PFHpA
PFHpS	448,878	80,125	99,071	-	PFHxS-18O2
PFOA	412,787	368,746	168,858	218,892	PFOA-13C4
PFNA	463	418,946	218,933	-	PFNA-13C5
PFOS	498,909	99,143	80,15	129,964	MPFOS
PFNS	548,939	80,125	99	-	MPFOS
PFDA	512,97	468,875	218,929	-	PFDA-13C2
PFDS	598,878	99,071	80,125	-	MPFOS
PFUnDA	562,97	518,946	268,804	-	PFUdA-13C2
PFUnDS	648,909	98,99	80,04	-	MPFOS
PFDoDA	612,97	568,946	169,25	-	PFDoA-13C2
PFDoDS	698,939	98,992	80,125	-	MPFOS
PFTrDS	748,878	80,097	229,866	-	MPFOS
PFTrDA	662,97	618,875	169	-	M2PFTeDA
PFTeDA	712,939	669,875	168,91	-	M2PFTeDA
PFHxDA	812,909	768,818	268,861	218,946	M2PFHxDA
PFODA	912,878	868,818	468,889	569,296	M2PFHxDA

For analysis methanolic fraction containing fluorinated telomer alcohols GC-MS/MS method with positive chemical ionization is used (GC Agilent 7890B with Multi-Mode Inlet and MS/MS Agilent 7010B) equipped with analytical column DB-Wax UI (30 m x 250 µm x 0.25 µm, Agilent Technologies). Analytical column is connected to backflush device which is via restrictor (1m x 0,1 mm deactivated) connected to the MS. Carrier gas is He and the flow for analytical column is 1,3 ml/min and flow for restrictor is 1,5 ml/min. For injection pulsed splitless mode is applied (45 psi, 0,3 min), Restek Double Goosneck liner is used, and inlet temperature is 240°C. Injection volume is 5 µl. Oven temperature program is listed in Table 6.

Table 6. Oven temperature program.

	Temp. gradient [°C/min]	Temperature [°C]	Time [min]
Initial temperature		45	0
	20	120	1
	20	135	0
Final temperature	15	175	0

Transferline temperature is 240°C, MS ion source is held at 250°C and quadrupoles are held at 106°C. For positive chemical ionization 20% flow of methane is used. MS/MS transitions, retention times and collision energies applied are listed for analyzed compounds in Table 7. The method is not yet fully validated, therefore we present only approximate values of LOQs for all 4 compounds on the level of 1 ng/l.

Table 7. List of fluorinated telomer alcohols, retention times, MS/MS transitions and collision energies applied.

Compound	RT [min]	Precursor (m/z)	Product (m/z)	Collision energy (eV)	ISTD
FTOH 6:2	5,42	365	365*	0	2-perfluorohexyl (1,1–2 H2, 1,2–13C2)ethanol (6:2 FTOH-iso)
		365	326,8	5	
MFTOH 6:2	5,41	369	331,1	10	2-perfluorohexyl (1,1–2 H2, 1,2–13C2)ethanol (6:2 FTOH-iso)
		331,1	331,1*	0	
FTOH 8:2	5,87	465	465*	0	2-perfluorooctyl (1,1–2 H2, 1,2–13C2)ethanol (8:2 FTOH-iso)
		465	426,9	10	
MFTOH 8:2	5,86	469,1	469,1*	0	2-perfluorooctyl (1,1–2 H2, 1,2–13C2)ethanol (8:2 FTOH-iso)
		469,1	463,1	0	

Other method developed at WRI for analysis of volatile fluorotelomer alcohols using stir bar sorptive extraction is published in Journal of Chromatography A 1759 (2025) 466246 by Tölgyessy et al., (https://doi.org/10.1016/j.chroma.2025.466246).

TU Wien

The following methodology is used at TU Wien laboratory for quantitative analysis of PFAS in different matrices. Water matrix samples (river water, wastewater) are prepared using solid phase extraction (SPE) or using direct injection and analysed by LCMS. In the following tables configuration of LC-MS/MS instrument (Table 8), materials used (Table 9), chemicals (Table 10) and reference materials (Table 11) are described.

Table 8. Configuration of LC-MS/MS instrument.

Instruments	Manufacturer	Description
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

Table 9. Materials used.

Material	Manufacturer	Description
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

Table 10. Chemicals used.

Chemical	Manufacturer	Description
Methanol	Merck	CAS 67-56-1 (Merck, 20864.290)
Ultra Pure Water	MiliQ TUW	MiliQ TUW
Ammoniumhydroxyde	Merck	CAS 1335-21-6; (Merck1.05432.1000)

Table 11. Reference materials.

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

Sample should be collected without any contamination from any material (e.g. Tubing Sealing, O-rings). The sample can be taken with a PP-scoop depending on the sampling location. The sample is filled bubble-free into a 1000 mL HDPE plastic bottle, transported refrigerated and stored in the refrigerator at 5°C until measurement.

For direct injection an external calibration is used for quantification. For calibration at least five different concentrations are diluted with methanol from the standard solution. The concentrations of calibration solutions range from 50 ng/L to 1000 ng/L. In addition, 50 µL of the non-extracted internal standards (NIS) and extracted internal standards (EIS) is also added to each calibration solution. EIS is used to determine recovery and NIS to establish initial calibration. The exact assignment of internal standards to native substances is listed in Table 12.

Table 12. Assignment of internal standards (EIS and NIS) to native substances.

PFAS group	Analyte	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	
	HFPO-DA	2062-98-8	M3HPFO-DA_13C3	MPFHxA_13C2
Perfluoroether carboxylic acid (PFECA)	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	

PFAS group	Analyte	CAS	EIS	NIS
	N-MeFOSE	24448-09-7	d7-N-MeFOSE	MPFOS_13C4
	N-EtFOSE	1691992	d9-N-EtFOSE	

To reach lower LOQs or to protect instrumentation from contamination, extraction and pre-concentration SPE is performed. To enrich PFAS in samples of ground and surface water an aliquot of 250 mL and for wastewater (influent, effluent) 100 ml is extracted via manual SPE. Depending on the dilution an enrichment factor of 10 - 100 is achieved. Details of SPE are described in Table 13.

All reusable items (HDPE bottle, tubes, syringe etc.) are rinsed with ethanol and ultrapure Water prior to use. An Aliquot of the water sample is filled in the sample bottle and spiked with 50 µL of the internal standard solution (EIS). As a positive control sample, reference compound PFAS in ultra-pure water (1 ng/L) is used.

Table 13. Details of SPE.

Step	Details
1. Conditioning	1. 2 mL MeOH 2. 2 mL Ultra-Pure Water
2. Loading	1. Loading Volume: 250 mL, (groundwater surface water, effluent 100 mL, influent WWTP, Landfill Leachate 2. Loading Speed: 10 mL/min
3. Washing	1. 1 mL 25 mM Ammonium acetate in Ultra-Pure-Water
4. Drying	1. Put cartridges in falcon tubes and centrifuge at 4000 rpm for 6 min.
5. Elution	1. 2x1 mL MeOH (+1% NH4OH), each into appropriate glass vessel using pressurized air 2. Elution Speed: 0.8 mL/min
6. Transfer	1. 1 mL of the eluate into a HPLC vial

After application of SPE, 2 mL of the eluate with MeOH (+1% NH4OH) is collected. 1 mL of the eluate is transferred to polypropylene vails (1,5 mL) and spiked with 50 µL of the internal standard solution (NIS).

For calibration of LC-MS/MS instrument a direct injection of calibration standards is used. The stability of the system is checked always after analysis of 12 samples by injection of calibration standard series. A blank sample is injected before each calibration series and is used for the determination of the limit of quantification.

For injection a PAL RTC sampler, for separation an Agilent 1290 Infinity II HPLC pump and for measurement a sciex Qtrap 6500+ with electrospray ionization source (ESI) was used. As analytical column a Phenomenex Luna Omega 3 µm PS C18 100 x 3.0 mm 100 Å was used at a temperature of 40 °C and an injection volume of 40 µL. Furthermore, a delay column Phenomenex Luna C18 50 x 3 mm 110 Å was installed. The separation is achieved using a binary gradient mobile phase consisting of ultra-pure water with 20 mM Ammonium acetate buffer (A) and HPLC-MS grade methanol (B) at a constant flow of 0.6 mL/min. The conditions of gradient separation are presented in Table 14.

Table 14. LC elution gradient program.

Time	Mobile phase A	Mobile phase B	Flow
min	%		mL/min
0.00	90	10	0.600
1.50	35	65	0.600
8.00	5	95	0.600
8.10	1	99	0.600
12.00	1	99	0.600
12.10	90	10	0.600
16.00	90	10	0.600

For determination of low concentration levels of PFAS without sample preparation large volume injection (LVI) is used. LVI can be used only for clean matrices due to high risk of instrument contamination. The great advantage is labor and cost reduction due to SPE procedure omitting. Instrumental parameters are the same as in case of SPE enrichment except for injection volume. Samples are diluted (1:1) with 20 mM Ammonium acetate buffer prior injection: Methanol (90:10) and injection volume of 40 µL is applied. Disadvantages of direct LVI analysis are higher LOQs obtained and risk of HPLC system contamination.

The mass spectrometers ESI is operated in negative ion mode using multiple reaction monitoring (MRM) for the components as shown in Table 15. The capillary voltage was set to -3000 V. The sheath gas temperature was 350 °C and the drying gas temperature 80°C.

Table 15. List of PFAS, MRM transitions and MS parameters.

Name	Q1 mass	Q3 mass		dwell time	DP	EP	CP	CXP
		Fragment Mass 1	Fragment Mass 2					
	(m/z)			ms	V			
PFBA	213	169		5	-20	-10	-13	-19
PFPeA	263	219	197	5	-20	-10	-12	-20
PFHxA	313	269	119	5	-20	-10	-14	-26
PFHpA	363	319	169	5	-20	-10	-14	-26
PFOA	413	369	219	5	-30	-10	-15	-20
PFNA	463	419	218.9	5	-30	-10	-14	-4
PFDA	513	469	269	5	-30	-10	-16	-29
PFUdA	563	519	269	5	-30	-10	-17	-4
PFDoA	613	569	319	5	-30	-10	-18	-4
PFTrDA	663	619	169	5	-30	-10	-20	-4
PFTeDA	713	669	169	5	-40	-10	-20	-4
PFBS	299	80	99	5	-50	-10	-55	-4
PFPeS	349	99	119	5	-60	-10	-44	-12
PFHxS	399	80	99	5	-80	-10	-75	-4
PFHpS	449	80	99	5	-80	-10	-83	-4
PFOS	499	80	99	5	-130	-10	-120	-10
PFNS	549	80	99	5	-120	-10	-110	-4
PFDS	599	80	99	5	-130	-10	-112	-4
4:2 FTS	327	307	81	5	-30	-10	-28	-4
6:2 FTS	427	407	81	5	-30	-10	-32	-4
8:2 FTS	527	507	81	5	-30	-10	-40	-4
PFOSA	498	78	64	5	-30	-10	-85	-4
N-MeFOSAA	570	419		5	-30	-10	-36	-4
N-EtFOSAA	584	419	526	5	-30	-10	-36	-4
HFPO-DA	285,1	169,1	285	5	-30	-10	-10	-10
ADONA	377	251	85	5	-30	-10	-15	-20
9Cl-PF3ONS	531	351		5	-30	-10	-36	-28
11Cl-PF3OUdS	631	451	199	5	-30	-10	-40	-12

CETI, Montenegro

The methodology presented herein outlines the procedure for quantitative analysis of per- and polyfluoroalkyl substances (PFASs) in water samples. Water matrices of higher complexity, including river water and wastewater, must undergo preparatory treatment by solid-phase disk extraction (SPE). Following sample preparation, the determination and quantification of PFAS compounds are carried out using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

UHPLC-MS/MS system with following configuration is used:

- Agilent, UHPLC 1290 Infinity II
- Agilent, 6475 LC/TQ
- HPLC column – Zorbax, Eclipse Plus C18, 100 x 2.1 mm, 1.8 µm, Agilent
- Delay Column, InfinityLab PFC Delay Column, 4.6 x 30 mm, Agilent

Automatic water extractor, Biotage, Horizon 5000
Vacuum evaporator, Heidolph, Laborta 4002

Analytical Standards used are presented in Tables 16 and 17.

Table 16. Native Perfluorinated Compound Solution Mixture, Wellington Laboratories.

No.	Substance	Acronym	Conc. (ng/ml)
1.	Perfluoro-n-butanoic acid	PFBA	2000
2.	Perfluoro-n-pentanoic acid	PFPeA	2000
3.	Perfluoro-n-hexanoic acid	PFHxA	2000
4.	Perfluoro-n-heptanoic acid	PFHpA	2000
5.	Perfluoro-n-octanoic acid	PFOA	2000
6.	Perfluoro-n-nonanoic acid	PFNA	2000
7.	Perfluoro-n-decanoic acid	PFDA	2000
8.	Perfluoro-n-undecanoic acid	PFUnA	2000
9.	Perfluoro-n-dodecanoic acid	PFDoA	2000
10.	Perfluoro-n-tridecanoic acid	PFTrDA	2000
11.	Perfluoro-n-tetradecanoic acid	PFTeDA	2000
12.	Perfluoro-n-hexadecanoic acid	PFHxDA	2000
13.	Perfluoro-n-octadecanoic acid	PFODA	2000
14.	Potassium perfluoro-1-butanesulfonate	PFBS	2000
15.	Sodium perfluoro-1-pentanesulfonate	PFPeS	2000
16.	Sodium perfluoro-1-hexanesulfonate	PFHxS	2000
17.	Sodium perfluoro-1-heptanesulfonate	PFHpS	2000
18.	Sodium perfluoro-1-octanesulfonate	PFOS	2000
19.	Sodium perfluoro-1-nonanesulfonate	PFNS	2000
20.	Sodium perfluoro-1-decanesulfonate	PFDS	2000
21.	Sodium perfluoro-1-dodecanesulfonate	PFDoS	2000

Table 17. Mass-labelled PFAS extraction standards solution, Wellington Laboratories.

No.	Substance	Acronym	Conc. (ng/ml)
1.	Perfluoro-n-(13C4)butanoic acid	MPFBA	2000
2.	Perfluoro-n-(13C5)pentanoic acid	M5PFPeA	2000
3.	Perfluoro-n-(1,2,3,4,6-13C5)hexanoic acid	M5PFHxA	2000
4.	Perfluoro-n-(1,2,3,4-13C4)heptanoic acid	M4PFHpA	2000
5.	Perfluoro-n-(13C8)octanoic acid	M8PFOA	2000
6.	Perfluoro-n-(13C9)nonanoic acid	M9PFNA	2000
7.	Perfluoro-n-(1,2,3,4,5,6-13C6)decanoic acid	M6PFDA	2000
8.	Perfluoro-n-(1,2,3,4,5,6,7-13C7)undecanoic acid	M7PFUdA	2000
9.	Perfluoro-n-(1,2-13C2)dodecanoic acid	MPFDoA	2000
10.	Perfluoro-n-(1,2-13C2)tetradecanoic acid	M2PFTeDA	2000
11.	Sodium perfluoro-1-(2,3,4-13C3)butanesulfonate	M3PFBS	2000
12.	Sodium perfluoro-1-(1,2,3-13C3)hexanesulfonate	M3PFHxS	2000
13.	Sodium perfluoro-1-(13C8)octanesulfonate	M8PFOS	2000

For automated SPE sample preparation Horizon Biotage 5000 is used. Detailed program of sample preparation is listed in Table 18. After elution of the analyte, the extract is evaporated to a volume of 1 ml.

Table 18. Procedure of sample preparation using Horizon Biotage 5000.

Phase	Solvent	Purge (sec)	Pump flow rate	Saturate (sec)	Soak (sec)	Drain (sec)
Disk conditioning	Metanol, 15ml	60	2	1	120	30
Disk conditioning	Metanol, 15ml	60	2	1	120	30
Disk conditioning	Water, 15 ml	60	2	1	120	30
Sample loading	Vacuum pump rate: 1		Done Loading Delay: 0			
Air dry disk	Dry: 500 sec		Pump rate: 6			
Elution of analytes	Metanol, 15ml	60	1	1	150	60
Elution of analytes	Metanol, 15ml	60	1	1	150	60
Elution of analytes	Metanol, 15ml	60	1	1	150	60

LC part of the analytical method is described in Table 19 and gradient elution program with mobile phase A: 2mM Ammonium Acetate in water and mobile phase B: Acetonitrile: Water=95:5 is described in Table 20.

Table 19. LC parameters of method.

Analytical column:	*Zorbax, Eclipse Plus C18, 100 x 2.1 mm, 1.8 µm, Agilent*
Column temperature:	*50°C*
Vial tray temperature:	15°C
Flow:	0.4 ml/min
Injection volume:	4 µl

Table 20. UHPLC gradient program.

Time (min)	% Mobile Phase A	% Mobile Phase B
0,0	85	15
0.5	85	15
1.5	75	25
7.0	40	60
10.0	0	100
12.0	0	100
12.1	85	15

The mass spectrometer's electrospray ionization (ESI) source was operated in negative ion mode, utilizing multiple reaction monitoring (MRM). The operational parameters of the mass spectrometer, including ion source settings, gas flows, voltages, and optimized MRM transitions, are detailed in Tables 21 and 22.

Table 21 Ion source parameters.

Ionisation mode	ESI -
Gas Flow (l/min)	8.0
Nebulizer (psi)	20.0
Sheath Gas Flow	10.0
Capilare Voltage:	
Positive setpoint	4000 V
Negative setpoint	2500 V
Gas temperature (^{0}C)	230
Sheat gas temperature (^{0}C)	355

Table 22. Optimized MRM parameters.

Compound name	Precursor m/z	Product m/z	Fragmentor (V)	CAV (V)	CE (V)	Polarity
13C2-PFDoA	615	570	90	2	12	Negative
13C2-PFDoA	615	269	90	2	12	Negative
13C2-PFTeDA	715	670	90	2	12	Negative
13C2-PFTeDA	715	369	90	2	12	Negative
13C3-PFBS	302	99	130	2	32	Negative
13C3-PFBS	302	80	130	2	44	Negative
13C3-PFHxS	402	99	156	2	44	Negative
13C3-PFHxS	402	80	156	2	48	Negative
13C4-PFBA	217	172	72	2	8	Negative
13C4-PFHpA	367	322	72	2	8	Negative
13C4-PFHpA	367	169	72	2	16	Negative
13C5-PFHxA	318	273	72	2	8	Negative
13C5-PFHxA	318	120	72	2	24	Negative
13C5-PFPeA	268	223	72	2	4	Negative
13C6-PFDA	519	474	72	2	8	Negative
13C6-PFDA	519	219	72	2	20	Negative
13C7-PFUdA	570	525	100	2	8	Negative
13C7-PFUdA	570	270	100	2	8	Negative
13C8-PFOA	421	376	72	2	8	Negative
13C8-PFOA	421	172	72	2	20	Negative
13C8-PFOS	507	99	148	2	52	Negative
13C8-PFOS	507	80	148	2	54	Negative
13C9-PFNA	472	427	72	2	8	Negative
13C9-PFNA	472	223	72	2	16	Negative
PFBA	213	169	72	2	8	Negative
PFBS	298.9	99	154	2	34	Negative
PFBS	298.9	80	154	2	36	Negative
PFDA	513	469	72	2	12	Negative
PFDA	513	269	72	2	16	Negative
PFDA	513	219	72	2	20	Negative
PFDoDA	613	569	100	2	8	Negative
PFDoDA	613	269	100	2	24	Negative
PFDoDA	613	169	100	2	32	Negative
PFDoS	698.9	99	156	2	62	Negative
PFDoS	698.9	80	156	2	67	Negative
PFDS	598.9	99	148	2	56	Negative
PFDS	598.9	80	148	2	60	Negative
PFHpA	363	319	72	2	8	Negative

Compound name	Precursor m/z	Product m/z	Fragmentor (V)	CAV (V)	CE (V)	Polarity
PFHpA	363	169	72	2	16	Negative
PFHpS	448.9	99	148	2	42	Negative
PFHpS	448.9	80	148	2	50	Negative
PFHxA	313	269	72	2	8	Negative
PFHxA	313	119	72	2	24	Negative
PFHxDA	812.9	369	120	2	25	Negative
PFHxDA	812.9	269	120	2	28	Negative
PFHxDA	812.9	219	120	2	28	Negative
PFHxS	398.9	99	156	2	40	Negative
PFHxS	398.9	80	156	2	56	Negative
PFNA	463	419	72	2	8	Negative
PFNA	463	219	72	2	16	Negative
PFNA	463	169	72	2	20	Negative
PFNS	548.9	99	148	2	52	Negative
PFNS	548.9	80	148	2	56	Negative
PFOA	413	369	72	2	8	Negative
PFOA	413	219	72	2	16	Negative
PFOA	413	169	72	2	16	Negative
PFODA	912.9	868.9	110	2	16	Negative
PFODA	912.9	269	110	2	30	Negative
PFODA	912.9	169	110	2	39	Negative
PFOS	498.9	99	148	2	50	Negative
PFOS	498.9	80	148	2	54	Negative
PFPeA	263	219	72	2	4	Negative
PFPeS	348.9	99	144	2	40	Negative
PFPeS	348.9	80	144	2	44	Negative
PFTeDA	712.9	669	100	2	12	Negative
PFTeDA	712.9	219	100	2	28	Negative
PFTeDA	712.9	169	100	2	32	Negative
PFTrDA	663	619	100	2	12	Negative
PFTrDA	663	319	100	2	20	Negative
PFTrDA	663	269	100	2	20	Negative
PFUnDA	563	519	100	2	12	Negative
PFUnDA	563	319	100	2	20	Negative
PFUnDA	563	269	100	2	20	Negative

Experiences and recommendations for the analysis of PFAS gathered during project workshops

As a part of the pilot action aimed at the improvement and harmonization of procedures of methodologies in the field of hazardous substances analysis, two workshops were organized. In this chapter we gathered advice and recommendations from experts presenting at these workshops.

A. Choice of analytical method:

a) properties of organic compounds,

b) type of matrix in which the analytes are to be determined (water, sediment, biota),

c) review of published methods - for method development it is good to base it on standardized methods as ISO, ASTM, EPA, application notes – most of those methods require LC-MS/MS for the analysis of PFAS

d) required limits of determination, which are derived from environmental quality standards

B. Optimization of chromatography:

Column - selection depends on the physical and chemical properties of the compound of interest. Reversed phases with C18 column are used for PFAS separation.

For the mobile phase, methanol, acetonitrile and water or a mixture of the solvents are commonly used. Addition of acids or ammonium salts may be used to enhance peak resolution and shape.

After selecting a suitable column and mobile phase, one needs to optimize LC conditions including the flow rate, mobile phase gradient and column temperature.

C. Optimization of Transitions and Collision Energies in LC-MS/MS

LC-MS/MS uses multi-reaction monitoring (MRM) in a triple quadrupole system. In the first quadrupole, a charged precursor ion is selected and allowed to pass through to the second quadrupole, where it collides with gas to form product ions in a process called collision-induced dissociation (CID). Finally, a single product ion is selected in the third quadrupole and allowed to pass through to the detector. The instrument measures a combination of precursor and product ions

First it is recommended to use single substance reference material (standard) that is free from other compounds, but it is possible also use a mix of standards for the optimization

Depending on the instrumental sensitivity, the standard is diluted to a suitable concentration (usually at µg/l level) with an appropriate solvent.

PFAS generally ionize in a negative mode.

In negative mode the mass of the Precursor is usually its molecular weight minus a proton from the original molecule [M–H]–.

All Perfluoroalkyl acids lose their carboxylic acid function group (fragment with mass 44), and the perfluoroalkyl chain always fragment at the fourth, fifth, or sixth carbon when the chain is long enough.

The Perflouroalkyl sulfonic acids systematically provide the sulfite anions SO_3^- (fragment with mass 80) and sulfite anions plus fluorine FSO_3^- (fragment with mass 99). Perflouroalkyl sulfonic acids with longer chains can also fragment on the perfluoroalkyl chain.

Product ions with a very low mass (fragment with mass lower then 50) should be avoided because they are also produced by many interfering substances and lack selectivity resulting in poor LOQs and specificity/selectivity.

It is a common practice and identification criteria to have at least two MRMs for each compound. The first pair is usually for quantification (Quantifier) while the second pair is used for confirmation of identity (Qualifier).

The quantifier should be the most abundant ion.

D. Calibration

Calibration curve with a minimum of 5 calibration points are used.

The first calibration point is usually between the limit of detection LOD and limit of quantification LOQ.

Calibration curve with an internal standards should be used to determine the concentration of an analyte by comparing it to a series of calibration standards that contain a constant concentration of an internal standard. This method helps to correct for variations in sample preparation and instrumental response.

The ISO 8466 recommends for the organic trace analysis to use isotopically labeled compounds as internal standards.

In general, a coefficient of determination R^2 value with more than 0.990 is considered satisfactory, but for SPE methods the coefficient of determination is not so strict as can seen in the ASTM D7979.

E. Quality assurance

QC - quality control samples are typical samples which over a given period of time are sufficiently stable and homogeneous to give the same result, and available in sufficient quantities to allow repeat analysis over time. Over this period the precision of the method can be checked by monitoring values obtained from analysis of the QC sample, usually by plotting them on a control chart.

In control charts limits are set for the controlled values – usually ‘warning limits’ are set at 2sigma above and below the mean value, and ‘action limits’ are set at ±3sigma around the mean value.

Sigma – standard deviation of min. 20 points.

Other option is the use of a spiked sample:

A known quantity of the analyte (a "spike") is added to a representative portion of the sample matrix. The spiked sample is then analyzed along with the unspiked (or "blank") sample.

Matrix effects can arise from various factors, including interference with the analytical instrument, changes in the analyte's ionization or extraction behavior, or competition with other compounds in the sample.

A key aspect of spiking is assessing analyte recovery. The recovery is the percentage of the spiked analyte that is accurately measured in the spiked sample and is used to quantify the effects of the matrix.

In summary, spiked samples are a valuable tool in analytical chemistry to evaluate the performance of a method within a specific sample matrix and to identify potential interference or matrix effects that may affect the accuracy of the results.

Duplicate samples, also known as replicate samples can be used as well. They are used to assess the precision of a measurement method. They involve collecting two or more samples from the same source and analyzing them independently under identical conditions.

Last possibility is Blank –pure water should be analyzed as the sample to see the background contamination.

Blank sample, also known as a method blank, serves as a control to ensure the absence of contamination or interferences in the analytical system. It's a sample that is free of the analyte being measured and is processed through the entire analytical method, including any sample preparation or instrumental analysis steps.

The ISO 21675 recommends analysis of 1 blank per batch run.

In each sample preparation batch a blank sample, a quality control sample, and a spiked or duplicate sample should be prepared to assess the performance of method during each batch.

E. Practical advice for analysis of PFAS

LC system - replace PFC materials with PFC-free solvent lines and tubing.

Use of delay column for maximum PFAS background reduction.

Use PE or PP materials for sample preparation.

Avoid using products containing Teflon or other flouropolymer plastics.

WAX (weak anion exchange) is often used for specific applications involving acidic compounds, while HLB (hydrophilic-lipophilic balanced) is a more versatile sorbent for general-purpose extraction – PFAS are mostly acidic compounds and our tests of different SPE cartridges (different phases/sorbents and producers) showed lower recoveries for HLB sorbents.

Use PE not high-density PE bottles for sample freezing.

Freeze the samples and fill the bottles to the top because fluorotelomer alcohols are volatile.

Fluorotelomer alcohols ionize poorly in ESI-. There are two ways to increase the detectability: derivatization prior LC-MS/MS detection and the use of GC-MS/MS method with positive chemical ionization. As fluorotelomer alcohols are volatile GC method is preferred.

The critical role of internal standards for accurate PFAS quantification

The quantitative analysis of per- and polyfluoroalkyl substances (PFAS) in water, usually found at trace levels in the range of ng L^{-1}, is quite challenging and requires highly sensitive and robust analytical techniques, such as liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

Two main reasons can be accounted for the difficulties in PFAS analysis:

- Diversity of chemical nature of PFAS itself, their strong sorption to surfaces and losses during handling and extraction;
- Matrix complexity of water samples, which can vary significantly in composition, from relatively clean groundwater and treated drinking water to complex wastewater or surface water with high organic and particulate content, which can differently affect recovery and detection.

For these reasons, the accuracy and reliability of such analyses is critically dependent on effective quality control measures, especially on the use of internal standards.

Internal standards (IS) are compounds that are added in known concentration to all samples, calibration standards, and quality control blanks prior to analysis. Their primary function is to account for variability and potential biases introduced during various stages of the analytical workflow, including extraction, clean-up, concentration, and instrumental detection. For PFAS analysis, variability can arise from matrix effects (e.g., ion suppression or enhancement in electrospray ionization), incomplete recovery during solid-phase extraction (SPE), and differences in instrumental sensitivity across runs.

PFAS internal standards are isotopically labeled analogs—typically ^{13}C or ^{18}O labeled PFAS, and are chemically identical to their native counterparts, except for the isotopic substitution, which makes them distinguishable by mass spectrometry. According to EPA 1688 two classes of IS are used for PFAS analysis:

1) Extracted Internal Standards (EIS): added before sample extraction and carried through the whole sample preparation workflow. They track PFAS losses or biases introduced during extraction, clean-up and concentration of PFAS from water samples.

2) Non Extracted Internal Standards (NIS): added after extraction, but prior to instrumental injection. Their role is to account for instrument variability and it helps to detect anomalies in instrumental performance (e.g. ion- suppression, injection volume variance).

The structural similarity and chemical properties between the IS and the native target analyte is crucial for good analytical results. Ideally, each target PFAS should have a corresponding labeled internal standard. This one-to-one matching allows for the most accurate correction of recovery losses and matrix effects. However, due to the limited availability of labeled standards, surrogate internal standards are used, particularly for emerging or less commonly monitored PFAS. In such cases, the

selected IS should be as structurally and behaviorally similar as possible, preferentially sharing chain length, functional groups (e.g., carboxylates vs. sulfonates), and retention times. Using inappropriate or poorly matched internal standards introduces significant uncertainty and reduces method accuracy.

Figure 1 shows the availability of corresponding internal standard and recovery of native PFAS in ultra-pure water matrix.

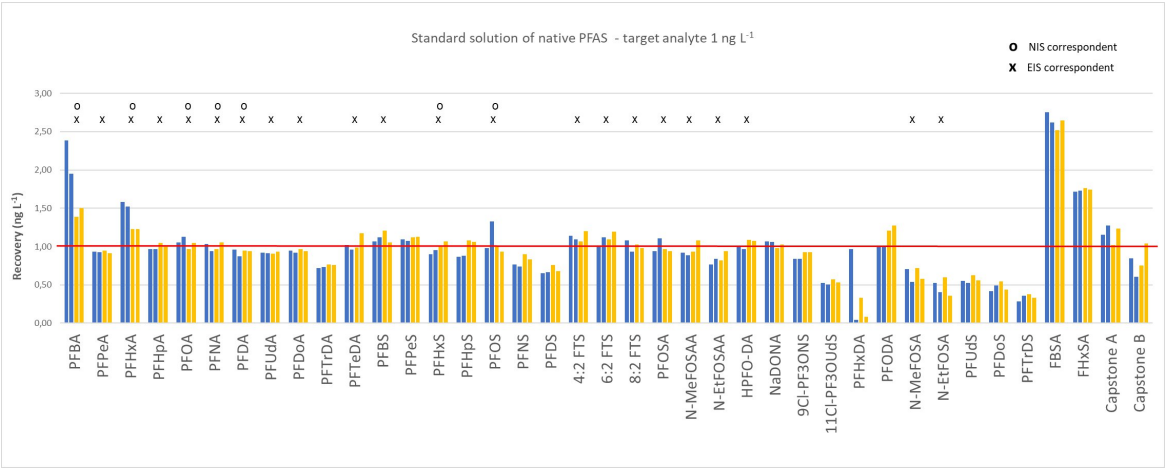

Figure 1: Availability of corresponding internal standard and recovery of native PFAS in ultra-pure water matrix. Ultra-pure water was spiked with 39 PFAS at a concentration of 1 ng/l each, followed by SPE extraction and LC-MS/MS analysis (Figure 1). Columns represent data of four independent analyses, carried out at two different time points (blue and yellow column, respectively). The red line represents the expected theoretical concentration of 1 ng/l. Above each PFAS is indicated the availability of corresponding internal standards (o for NIS; x for EIS). No symbol means that IS-surrogates are used.

The use of IS-surrogates that are chemically related, but with a different carbon chain size, for example, can already lead to a drop in accuracy. Examples from PFAS with poor IS-matching are the long chain sulfonic acid PFAS, 11Cl-PF3OUdS, FBSA, FHxSA and the Capstones A and B. They have either a much longer carbon chain as the available IS with the same functional group or they have unique chemical structures that do not match properly with the available IS. Figure 2 shows the results of recovery for long-chain Perfluorsulfonic acid. In this case, PFOS was the longest Perfluorsulfonic acid PFAS with an available internal standard (both EIS and NIS). All PFAS containing the same functional group but with longer carbon chains must therefore use these 8C internal standards as surrogates. A major short-coming of this approach is that a decrease in quantification accuracy could be observed for every addition of carbon in the chain length. In cases like these, it would be necessary to conduct investigations to select the best IS surrogates for a wide variety of matrices.

Figure 2: Decrease in recovery for perfluorsulfonic acid PFAS after using IS surrogates. The red line in the y axis represents the expected theoretical concentration of 1ng/l. Bars represent results of four independent analysis, carried out at two different time points.

In summary, the selection of appropriate internal standards is fundamental to any rigorous PFAS analytical method, particularly in matrices where trace-level detection is required. When matched correctly, internal standards ensure data accuracy, reproducibility, comparability and are crucial for environmental monitoring, risk assessment, and regulatory compliance. As analytical capabilities and interest in new PFAS continue to evolve, strategies for selecting internal standards and verifying accuracy must also evolve.

Lessons learned on good methodologies and approaches during interlaboratory comparison studies

During the implementation of the Tethys project, two rounds of interlaboratory comparison studies were organized on analytical methods for the determination of PFAS water samples. Based on results, which are available in Tethys Output Document 1.2, we have prepared following recommendations for testing laboratories to consider for improvement of testing methods applied.

- Correct use proper isotopically labeled internal standards. The results obtained from laboratories employing analytical methods without internal standards or inadequately matched internal standards to native analytes indicate significant problems in determination of correct values of PFAS. Significant deviations of the reported results from the reference value indicate the presence of systematic errors that may originate from various steps of the analytical process. These errors may be related to sample preparation, calibration as well as the analytical procedure itself.

- Correct calculation of uncertainty values. In several cases, the reported values may be underestimated by the uncertainty associated with analyses at the ultra-trace level. Especially in ultra-trace analysis using sample preparation procedures, LC-MS/MS detection with very strong effects of ions suppression or enhancement it is likely to expect values of relative uncertainty to be in ranges of single digit of %.
- Analytical work should be performed according to an appropriate quality system, including validated analytical methods, proficiency testing, internal laboratory quality control and external assessment where necessary.
- Validation procedures should include deviation checks using CRM. Laboratories performing chemical analysis should determine and report uncertainties with the results of analyses. Reporting information on values below the LOD will lead to an underestimation of uncertainty.
- Correct number of digits for reporting concentrations and uncertainties. Numerical values of the result and its uncertainty should not be reported with an excessive number of digits. Regardless of whether expanded uncertainty or a standard uncertainty is provided, in most cases it is sufficient to state no more than two significant digits for the uncertainty. The reported results should be rounded to ensure consistency with the stated uncertainty.
- Laboratories' measurements often underestimate uncertainty due to the failure to account for systematic errors (bias).
- An excessively low uncertainty may lead to an underestimation of the true value, primarily because it can indicate the presence of unaccounted systematic errors or an inaccurate representation of measurement variability.
- Suitable tools for calculating the uncertainty of analytical procedures can be found, for example in:
    - ISO 11352:2012 Water quality – Estimation of measurement uncertainty based on validation and quality control data
    - Setting and Using Target Uncertainty in Chemical Measurement Eurachem/Citac Guide, 2015
- We recommend that participants in the second round of PT reconsider their methods for determining expanded uncertainty based on the statistical outputs of the analytical data evaluation.
- Laboratories should consider participation in PTs as option to receive high quality feedback on applied methods and understand potential failure in PT as an opportunity for improvement rather than as proof of poor work quality. For laboratory should be important to be able to compare its performance with others. Therefore, we promote participation in PT also already at the stage of method development before method validation.

Lessons learned from workshops on analytical methods

As part of the pilot action, two workshops on analytical methods for monitoring hazardous substances were organized. As a part of the second workshop, the facilitated co-creation method "World Café" was applied to explore and better understand the needs of laboratories in implementing new analytical methods. Details on this method can be found in Output Document 1.2 of the Tethys Project. In Table 23 questions asked and highest rated responses gathered during the facilitated co-creative activity are presented.

Table 23. questions and highest rated responses gathered from the World Café facilitation co-creation activity.

1, What are the reasons behind delays in development and implementation of analytical methods for monitoring?	Points received
Workshops/trainings for "know-how"	7
Communication and lab-network	7
Laboratory network (shared experience)	5
Communication (laboratory – government)	5
Money	5
Limited budget (instruments, new skilled people, routine work management, maintenance)	4
2, What kind of support would help laboratories involved in monitoring to develop and implement analytical methods required by legislation?	
Sharing information between institutions	10
Exchanging experience	9
Training for staff for instruments, methods	9
Governmental support (money for instruments, chemicals…)	7
PT tests	5
Support for method developing	4
3, Regarding this workshop, what can we do better?	
Professional networking – contact list of participants, stay in touch	12
More frequent workshops	9
More examples in presentations – real problems	7
After official part, there could be an unofficial small group talk (walk around the city, dinner, drinks)	7
More lab work, practical presentations	6
Practical problems – problems solving	6
Hands-on experience – laboratory work	5
More opportunities for asking questions about other WFD analysis	4
Meeting colleagues in person	4

Based on the responses collected, it can be concluded that for better and earlier implementation of newly required analytical methods, it is essential to ensure adequate funding. This would allow laboratories to hire new experts, procure analytical instruments, secure necessary chemicals and reference materials, and provide training. This finding is not new, and apart from recommending this to managing authorities in individual countries, there is little more that can be done within the scope of the Tethys project.

Much more interesting findings indicate that laboratories need stronger communication as part of the support process. Improved communication between laboratories and national authorities could help clarify what the authorities expect from testing laboratories and, conversely, what laboratories need to meet these objectives. For better implementation of methods, the need for information sharing among laboratories also appears to be very important.

In the process of selection and definition of new lists of priority substances, so-called Environmental Quality Standards Dossiers are developed at expert level. These documents contain extensive information on proposed substances (use, properties, amounts use, toxicity, environmental fate, sometimes also possible methods for determination), derived from high-quality scientific sources, and are used by experts in the Working Group Chemicals (WG Chemicals) under the Common Implementation Strategy of the Water Framework Directive (WFD). Analytical chemistry experts should review these documents as a potential source of information. Within a EU Member State, the focal point - national expert from this working group should communicate these documents to laboratories. For non-EU countries, these documents are publicly available on the EU CIRCABC server: https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/73b2d635-4cb1-4d7d-975c-da1b5db594d8?p=1&n=10&sort=modified_DESC The location of these documents changes over time, so search engines should be used to find them.

Further, the responses clearly show the need for laboratories to participate in specialized workshops, which should include hands-on sessions and opportunities to share information about existing methods, recent developments and also troubleshooting. Experts responsible for development and implementation of analytical methods could be be organized within professional group, possibly using professional social networks such as LinkedIn. From the perspective of the Danube Region, we assume that it would be most appropriate for such working group and workshops to be organized under the auspices of the Monitoring and Assessment Expert Group (MA EG) of the ICPDR. Successful establishment of such sub-group with regular activities and active participation of experts could significantly improve and streamline communication and also support of organization and participation of laboratories in proficiency testing schemes, as a tool for harmonization of analytical results quality. At the EU level, the approved work program of WG Chemicals, which includes sharing best practices, could under some circumstances be used for this purpose as well, although the scope of WG Chemicals is more towards implementation of WFD regarding chemicals, then development of analytical methods. The European Commission's Joint Research Centre (JRC) already organizes similar workshops on analytical methods but for the Watch List compounds and is also active in developing Effect-Based Methods for estrogen analysis. In the past, JRC has developed standard methods for the analysis of several problematic analytes in line with WFD requirements, and it would be highly beneficial if JRC could also develop standard methods for new priority substances that pose challenges due to very low LOQ requirements. JRC is also part of the team revising the list of priority substances, has a department for preparing reference materials, and organizes proficiency testing schemes.

The newly proposed Directive amending WFD, GWD, and EQSD also includes the establishment of an entity called the **"Monitoring Facility."** Based on available information, it is unclear what exactly this entity should do. The name suggests it could be a physical institution performing sample analyses,

which would be difficult to implement in practice, or it could be a financial mechanism to support monitoring. Based on the findings from this pilot activity, it would be highly appropriate for the Monitoring Facility to support laboratories in developing monitoring methods and providing training activities for implementing new methods in practice.

Another organization that already brings together a large number of experts and leading scientists working primarily in the field of environment and water is the NORMAN network (norman-network.net). The NORMAN network enhances the exchange of information on emerging environmental substances and encourages the validation and harmonization of common measurement methods and monitoring tools so that the requirements of risk assessors and risk managers can be better met. It specifically seeks both to promote and to benefit from synergies between research teams from different countries in the field of emerging substances. Membership in NORMAN is recommended for competent authorities, reference laboratories, and research institutions working in the field of scientific and technical support for environmental protection.

Finally, we would like to emphasize that specialized workshops should be organized in a physical format. To balance knowledge among laboratories at different levels, they should include hands-on sessions demonstrating laboratory procedures. It is important to consider the social aspect of workshops, such as enabling discussions in smaller groups and including a social program. Workshops should also contribute to relationship-building and allow sharing of participants' contact information (GDPR requirements must be respected, and consent for sharing must be obtained). From the perspective of understanding the situation in the Danube Region, it would be most productive for workshops to be organized under the auspices of MA EG ICPDR.

Outlook and advice to improve implementation of analytical methods for new pollutants monitoring

The continuous emergence of new pollutants in water environment and continuous development of legislation presents a growing challenge for countries to effectively implement the Water Framework Directive. Monitoring these pollutants requires use of validated analytical methods employing highly sophisticated instrumentation, expert laboratory staff, long-term experience in method development and sufficient financial resources. The Danube River Basin consists of countries significantly varying in size, population and economic performance. For many smaller non-EU and EU Member States, the development and application of such advanced monitoring methods methodologies represent a considerable challenge, both in terms of technical infrastructure and human resource capacities available.

Based on the pilot action of the Tethys project, we have identified key elements that could help to overcome existing limitations and thus to improve the quality of monitoring and new methods implementation:

- Enhanced sharing of Information
    - Between decision making bodies at country level and monitoring laboratories – transparent clarification of what is needed by the authority, what is needed by the laboratory, realistic estimation of budget, human resources and time scales.

    - Between national experts, e.g. focal point at the Working Group Chemicals and laboratories – information on legislation under preparation, sources of information (Environmental Quality Standards Dossiers, workshop and interlaboratory comparisons organized by the Joint Research Center).
- Establishment of a platform – an expert group focused on the development of analytical methods for monitoring, which would serve to share information on new methods development, modifications, but also to organize workshops, trainings and, finally, it could also serve as a platform for sharing information/requirements for organizing PTs. From the point of view of the Danube region, such a platform could be covered by the Monitoring and Assessment Expert Group ICPDR, who has already carried out similar activities for biological quality elements and intercalibration.
- Creation of an expert network using professional social networks such as LinkedIn to support communication between experts.
- From the point of view of the EU as a whole, it would be appropriate if the Joint Research Centre could be more involved in the development and standardization of analytical methods, especially for substances at extremely low concentration levels, which poses a challenge to a significant proportion of European countries.
- The draft directive revising the WFD foresees the establishment of a Monitoring Facility, which could also serve as a hub or coordinator for the development of standard analytical methods for the WFD.
- Laboratory participation in proficiency tests (PTs). Laboratories should participate in PTs already during method development to help them identify gaps and opportunities for improvement before final validation. At the same time, participation in PT will also help them to estimate a realistic goal regarding possible uncertainties or achieving extremely low LOQs.
- Consideration on membership in NORMAN network – recommended for competent authorities, reference laboratories, and research institutions working in the field of scientific and technical support for environmental protection.