HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES INSTITUTE FOR FOOD SCIENCE AND TECHNOLOGY

Development and validation of SPE sample preparation method for the determination of 18 PFAS in drinking and surface water

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Development and validation of SPE sample preparation method for the determination of 18 PFAS in drinking and surface water

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5 INTRODUCTION

Addressing per-and polyfluoroalkyl substances (PFAS) contamination is an urgent environmental concern **(Zhou et al., 2024),** per- and polyfuoroalkyl substances (PFAS) are environmental contaminants with various adverse health effects in humans **(Sadrabadi et al., 2024).** The toxic heritage of PFOS begins with an accident, after acquiring a key patent in 1948, 3M started working on commercial applications for the first PFAS, known as fluorocarbon chemicals or fluorochemicals. “Almost every day we turned out a new molecule which had never been on the face of the earth before,” recalls J. Donald La Zerte, a 3M chemist who started in 1949 **(Pearson & Renfrew, 2024).** The European Commission’s current efforts to launch the largest proposal to restrict per- and polyfluoroalkyl substances (PFAS) in history reflect the dire global plight of PFAS accumulation in the environment and their health impacts. While there are existing studies on PFAS research, there is a lack of comprehensive analysis that both covers the entire research period and provides deep insights into global research patterns, incentives, and barriers based on various parameters. There is a demonstration of the increasing interest in PFAS research, although citation numbers are declining prematurely. Policy regulations based on proving and establishing the toxicity of PFASs have stimulated research in developed countries and vice versa, with increasing emphasis on ecological aspects. China, in particular, is investing increasingly in PFAS research, but without defining or implementing regulations **(Klingelhöfer et al., 2024)** These organic compounds are characterized by a carbon backbone surrounded by fluorine atoms in their structure which makes them chemically inert. PFASs have low molecular polarization, short C-F bond length and large C-F binding energy (110 kcal mol−1) and have different functional group heads including alcohols, carboxylic acids, sulphonates, and amides **(Podder et al., 2021)**. Owing to these characteristics PFASs become recalcitrant, hydrophobic and oleophobic, exhibiting thermochemical stability and lower surface tension properties **(Miralles-Marco & Harrad, 2015)**. Based on these unique characteristics the PFASs found a diverse range of use in various domestic, industrial and commercial applications such as surfactants in fluoropolymer production, metal plating, aqueous film-forming foams, paper, textile, landfills, and household products **(Riaz et al., 2023).** Widespread industrial use of per- and polyfluoroalkyl substances (PFAS) as surfactants has led to global contamination of water sources with these persistent, highly stable chemicals. As a result, humans and wildlife are regularly exposed to PFASs, which have been shown to bioaccumulate and cause adverse health

effects. Methods for detecting PFAS in water are currently limited and primarily utilize mass spectrometry (MS), which is time-consuming and requires expensive instrumentation. Thus, new methods are needed to assess the pollution level of water sources rapidly and reliably. While some fluorescent PFAS sensors exist, they typically function in high nanomolar or micromolar concentration ranges and focus on sensing only 1–2 individual PFAS. Developing monitoring system for both individual PFAS, as well as complex PFAS mixtures is crucial, and demonstrate its functionality in tap water samples**.** Evaluating the exposure hazard associated with potable water consumption across nations, Hungary inclusive, emerges as a pivotal endeavor. Presently, Hungary lacks a comprehensive survey dedicated to this pursuit. Given the stringent threshold values, it becomes imperative to devise an analytical approach characterized by the utmost sensitivity. The protocol outlined in the US EPA Method 537.1, titled "Determination Of Selected Per- And Polyfluorinated Alkyl Substances In Drinking Water By Solid Phase Extraction And Liquid Chromatography/Tandem Mass Spectrometry (LC MS/MS)," stands as a noteworthy guideline in this domain.

6 Objective

The objective of this research endeavor, aimed at fulfilling the requirements for a thesis diploma, involves the development and refinement of Solid Phase Extraction methods tailored for the analytical preparation of PFAS samples. These methods are intended for application in the quality assurance protocols governing drinking water, specifically targeting the detectability of PFAS compounds through analytical techniques such as UHPLC MS/MS. The ultimate goal is to contribute to the establishment of regulatory frameworks for the quality control of PFAS contaminants.

7 Literature review

7.1 Drinking water

Drinking water is our most important food, without which there is no life. The human right to safe drinking water was defined by Assembly in 2010, as follows.

The human right to safe drinking water and sanitation is derived from the right to an adequate standard of living and inextricably related to the right to the highest attainable standard of physical and mental health, as well as the right to life and human dignity" **(Assembly, 2010).** Therefore, drinking water was defined as follows.

7.1.1 Definition of drinking water

Drinking water is defined as potable water that is safe for household use, including food preparation and drinking. Most drinking water comes from an "improved source." An improved source is defined as rainwater, water from a well that is protected, or any water that is treated and piped into a home or public standpipe. According to the United Nations (UN), 91 % of the world's population has access to an "improved source" of drinking water **(Birkenholtz, 2016)**. Drinking water is very rich in mineral components, so it is an important source of nutrients.

7.1.2 Nutrients in drinkig water

Calcium (Ca), sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), iodine (I), cobalt (Co), molybdenum (Mo), and selenium (Se) are indispensable for human health. While it is not widely recognized, drinking water serves as a source of some of these essential elements. Another set of elements with certain health benefits includes fluorine (F) in preventing dental caries, as well as boron (B), manganese (Mn), nickel (Ni), silicon (Si), and vanadium (Va), which may attain essential status for humans based on emerging scientific evidence. The contribution of water to the overall dietary intake of select trace elements and electrolytes typically ranges between 1 and 20%. Among the micronutrients, calcium and magnesium exhibit the highest proportion of intake derived from drinking water relative to food, potentially supplying up to 20% of the recommended total daily intake for these elements. In contrast, for most other elements, drinking water accounts for less than 5% of the total intake **(Manuel, 2005).** Regarding the various sources of drinking water in the environment, contamination is a critical issue.

7.1.3 The critical issues of water contamination

The Environmental Protection Agency (EPA) establishes regulatory limits for over 90 contaminants in drinking water. These limits are set at levels designed to safeguard human health and are achievable by water systems utilizing the best available technology. Additionally, EPA regulations dictate the testing schedules and methodologies that water systems must adhere to. For instance the inorganic chemical contaminants are listed in Table 1 as follows:

Table 1. List of inorganic chemical contaminants regulated by the US Environmental Protection Agency in drinking water (MCL= Maximum Contaminant Level) **(EPA, 2024b)** and **(Tsaridou & Karabelas, 2021).**

Contaminant	MCL (mg/L)
Antimony	0.006
Arsenic	0.010
Asbestos (fiber > 10 micrometers)	7 MFL
Barium	2
Beryllium	0.004
Cadmium	0.005
Carbofuran	0.040
Carbon tetrachloride	0.050
Chlordane	0.002
Chlorite	1
Chromium (total)	0.1
Copper	Action Level=1.3
Cyanide (as free cyanide)	0.2
2,4-Dichloro-phenoxy-acetic acid	0.07
Endrin	0.002
Ethylbenzene	0.7
Fluoride	4.0
Lead	Action Level=0.015
Mercury (inorganic)	0.002
Methoxychlor	0.04

Nitrate (measured as Nitrogen)	10
Nitrite (measured as Nitrogen)	1
Selenium	0.05
Simazine	0.004
Styrene	0.1
Thallium	0.002
Vinyl chloride	0.002
Xylenes	10

Under the Safe Drinking Water Act (SDWA), individual states are granted authority to establish and enforce their own drinking water standards, provided that these standards are at least as stringent as EPA's national standards **(Tsaridou & Karabelas, 2021).** Beside the chemical contaminants the US EPA classified another group of contaminants, knowns as PFAS.

7.2 Per- and polyfluoroalkyl chemical contaminants

Per- and polyfluoroalkyl chemicals (PFAS) represent a class of synthetic compounds renowned for their distinctive properties, encompassing oil and water repellency, resilience to high temperatures, and friction reduction. Introduced in the 1940s, PFAS have found extensive application across commercial, military, and industrial sectors, including non-stick coatings, textile treatments, food packaging, insulating materials, and fire-fighting foams. However, despite their utility, PFAS are characterized by their enduring presence in the environment and in biological systems, contributing to global contamination and human exposure **(Gaber et al., 2023)**. The main concern with PFAS is their chemistry.

7.2.1 Chemistry of PFAS

As a main concern with PFAS is their resistance to degradation due to stability of the carbon–fluorine bond. Thus, the more highly fluorinated the organic molecule is, the more resistant to degradation the molecule is. However, what degree of fluorination means how many fluorine atoms are associated with a chemical structure. The various structures of PFAS fit different structural definition **(Su & Rajan, 2021).**The chemical structure of PFAS is shown in Figure 1.

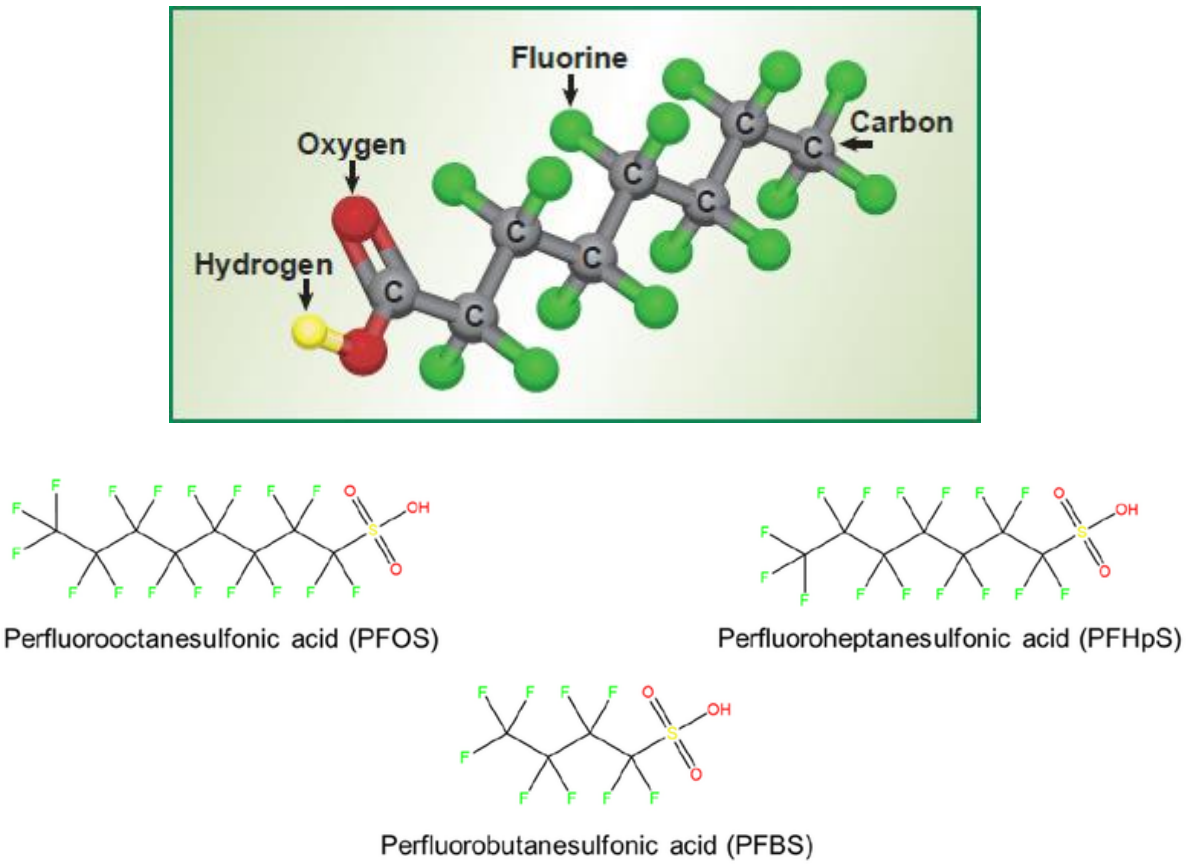


Figure 1.Chemical structure of some PFASs **(XDD, 2016).**

7.2.2 Health concern regarding PFAS

Notably, two prominent PFAS derivatives, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been associated with a spectrum of adverse health outcomes, encompassing pregnancy-induced hypertension, renal and testicular cancers, ulcerative colitis, restricted fetal growth, dyslipidemia, compromised immune function, reproductive and developmental impairments, as well as potential neurological and behavioral disorders. It is noteworthy that the detrimental effects of PFAS came to public attention only in the early 2000s, despite indications of industry awareness regarding these risks dating back to the 1970s **(Su & Rajan, 2021)**.

7.2.3 PFAS regulation

The absence of consistent global regulation and monitoring mechanisms for PFAS has facilitated their extensive contamination of drinking water systems, groundwater reservoirs, and rainwater,

not only in the United States but also worldwide. Regulatory initiatives aimed at controlling PFAS exposure have encountered obstacles stemming from industry influence on research, dissemination of scientific findings, and the establishment of safety thresholds. In response to mounting public apprehension and accumulating evidence of PFAS-related harm, regulatory bodies in the United States have initiated measures to address PFAS contamination. These measures include incorporating PFAS into the federal Toxics Release Inventory, issuing health advisories concerning PFAS in drinking water, and proposing enforceable limits on select PFAS compounds. Nonetheless, significant hurdles persist in monitoring and mitigating PFAS exposure, necessitating continued research efforts, enhanced regulatory oversight, and heightened public awareness campaigns to safeguard human health and the environment against the pervasive repercussions of PFAS contamination **(Nadia Gaber and all, 2023)**. Therefore several monitoring methods have been developed.

7.2.3.1 The US EPA and EFSA drinking water health advisories for PFAS limitation values

In June 2022, the US EPA unveiled health advisories concerning four PFAS, featuring interim updated non-regulatory lifetime drinking water health advisories for PFOA and PFOS at 4 pg/L and 20 pg/L, respectively. These advisories, deviating from prior ones, draw upon human epidemiological studies of populations exposed to these chemicals to establish concentrations in drinking water below which adverse health effects are not expected to occur. Previously, the US EPA's non-regulatory lifetime drinking water health advisories stood at 70 ng/L for the combined concentrations of PFOS and PFOA. In 2020, the European Food Safety Authority (EFSA) issued their Opinion on the risks to human health posed by PFAS in food, proposing a group tolerable weekly intake (TWI) of 4.4 ng/kg body weight for the combined sum of PFOA, PFNA, PFHxS, and PFOS. Immune system effects were deemed the most significant basis for risk assessment, relying on available studies in both animals and humans. Building on the TWI from the EFSA Opinion, the Danish Environmental Protection Agency, in June 2021, tightened their drinking water limit values, stipulating that drinking water should not exceed 2 ng/L of the sum of four PFAAs. Over the past 22 years, PFAS drinking water guidelines have consistently decreased. For instance, in the US, the PFOA drinking water guideline for West Virginia was previously 150,000 ng/L, which is 37.5 million times higher than the recently announced US EPA drinking water lifetime advisory for PFOA of 4 pg/L. Consequently, international drinking water

guidelines for PFAS now approximate or even fall below levels found in precipitation. While individuals residing in industrialized regions typically do not consume rainwater in modern life, it remains a reasonable expectation that the environment maintains a standard of cleanliness such that rainwater and precipitation-fed mountain stream water are safe for consumption. Moreover, in certain parts of the world, notably in some arid and tropical regions, rainwater remains a vital source of drinking water **(Cousins et al., 2022).** For the monitoring of PFAS contamination in drinking water different methods have been developed, the most important to consider are from US EPA.

7.2.3.2 The general analytical approach of PFAS analysis in drinking water

Based on our experimental work, the analytical approach for per- and polyfluoroalkyl substances (PFAS) in drinking water involves initial sample collection from various points within the distribution system, followed by sample preparation, wherein particulate matter is removed and the sample may be concentrated. Solid-phase extraction (SPE) is employed as the extraction method of choice to isolate PFAS from the water matrix. Following extraction, cleanup steps are performed to eliminate interfering compounds, enhancing analytical specificity. Subsequently, instrumental analysis utilizing high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) is conducted to detect and quantify PFAS compounds. Throughout the process, stringent quality control measures are implemented to ensure data reliability and accuracy. The obtained chromatographic data are then analyzed to assess PFAS levels against regulatory guidelines. The Figure 2 shown the process steps of the analytical approach of PFAS in drinking water.

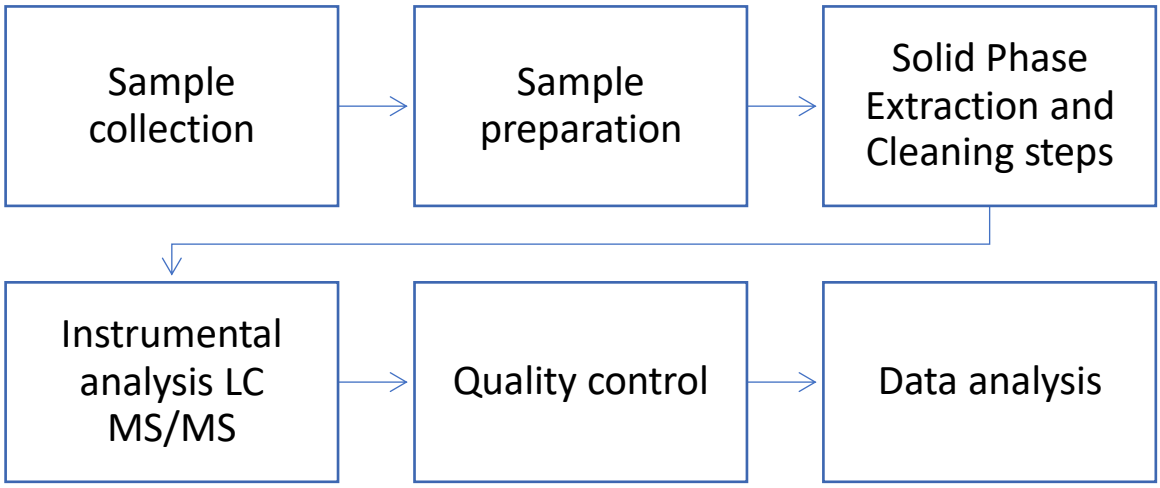


Figure 2. Analytical approach of PFAS analysis in drinking water.

7.2.3.3 US EPA Method 533 : DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER

This method involves fortified isotopically labeled analogues analytes that function as isotope dilution standards. The sample is passed through an SPE cartridge containing polystyrene divinylbenzene with a positively charged diamino ligand to extract the method analytes and isotope dilution analogues. The cartridge is rinsed with sequential washes of aqueous ammonium acetate followed by methanol, then the compounds are eluted from the solid phase sorbent with methanol containing ammonium hydroxide. The extract is concentrated to dryness with nitrogen in a heated water bath. The extract volume is adjusted water in methanol, and three isotopically labeled isotope performance standards are added. Extracts are analyzed by LC-MS/MS 533-3 in the MRM detection mode. The concentration of each analyte is calculated using the isotope dilution technique. For QC purposes, the percent recoveries of the isotope dilution analogues are calculated using the integrated peak areas of isotope performance standards, which are added to the final extract and function as traditional internal standards, exclusively applied to the isotope dilution analogues **(Rosenblum, 2019).**

Regarding the SPE methos of the US EPA 533 we can describe that the SPE cartridges contains weak anion exchange, mixed-mode polymeric sorbent (polymeric backbone and a diamino ligand). The SPE sorbent must have a pKa above 8 so that it remains positively charged during extraction. SPE cartridges containing sorbent (Phenomenex) were used during the method development 533 by US EPA **(Rosenblum, 2019).**

Regarding the LC system HPLC MS/MS was used in the US EPA 533 method, this system must provide consistent sample injection volumes and be capable of performing binary linear gradients at a constant flow rate. On some LC systems, PFAS may build up in PTFE transfer lines when the system is idle for more than one day. To prevent long delays in purging high levels of PFAS from the LC solvent lines, it may be useful to replace PTFE tubing with PEEKTM tubing and the PTFE solvent frits with stainless steel frits. These modifications are not used on the LC system used for method development. However, a delay column, HLB Direct Connect is placed in the mobile phase flow path immediately before the injection valve. This direct connect column may reduce the co-elution of PFAS originating from sources prior to the sample loop from the PFAS injected in the sample. It may not be possible to remove all PFAS background contamination **(Rosenblum, 2019).**

Regarding the calibration method the US EPA 533 method involves internal standard calibration technique. The internal standard technique calculates concentration based on the ratio of the peak area of the native analyte to that of the isotope dilution analogue.**(Rosenblum, 2019).**

US EPA METHOD 537.1: DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER.

In this method the sample is fortified with surrogates and passed through an SPE cartridge containing SDVB (styrenedivinylbenzene) () to extract the method analytes and surrogates. The compounds are eluted from the solid phase sorbent with a small amount of methanol. The extract is concentrated to dryness with nitrogen in a heated water bath, and then adjusted methanol:water and addition of the internal standards. The injection is made into an LC equipped with a C18 column that is interfaced to an MS/MS. The analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC MS/MS conditions. The concentration of each analyte is determined by using the internal standard technique. Surrogate analytes are added to

all Field and QC Samples to monitor the extraction efficiency of the method analytes **(Tettenhorst, 2020)**.

Regarding the SPE method of US EPA 537.1, the SPE cartridges used contains SDVB polymeric sorbent phase. The sorbent may not be modified with monomers other than SDVB **(Tettenhorst, 2020).**

Regarding the analytical Instrument, the US EPA 537.1 used LC MS/MS capable of reproducibly injecting up to 10-µL aliquots and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method (0.3 mL/min). The usage of a column heater is optional. During the course of method development, it was discovered that while idle for more than one day, PFAS built up in the PTFE solvent transfer lines. To prevent long delays in purging high levels of PFAS from the LC solvent lines, they were replaced with PEEK tubing and the PTFE solvent frits were replaced with stainless steel frits. It is not possible to remove all PFAS background contamination, but these measures help to minimize their background levels. The LC MS/MS must be capable of negative ion electrospray ionization (ESI) near the suggested LC flow rate of 0.3 mL/min. The system must be capable of performing MS/MS to produce unique product ions for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision **(Tettenhorst, 2020).**

When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. PFAS analytes, internal standards (IS) and surrogate (SUR) standards commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers Primary Dilution Standard:(PDS) PDS and calibration standards were found to be stable for, at least, one month during method development. Laboratories should use standard QC practices to determine when standards need to be replaced. The target analyte manufacturer's guidelines may be helpful when making the determination **(Tettenhorst, 2020).**

7.2.4 Comparison between the US EPA methods 533 and 537.1

The key differences and similarities between Method 533 and Method 537.1 in terms of sample preparation, extraction, chromatography, and calibration techniques. Each method has its specific

requirements and procedures tailored to the determination of per- and polyfluoroalkyl substances (PFAS) in water samples.

Table 2. Comparison between the method US EPA 533 and 537.1 **(Rosenblum, 2019)** and **(Tettenhorst, 2020).**

Aspect	**Method 533**	**Method 537.1**
Sample Volume	100–250 mL	250 Ml
Sample Fortification	Isotopically labeled analogues as standards	Surrogates
Extraction Method	Solid-phase extraction (SPE)	Solid-phase extraction (SPE)
SPE Cartridges	Weak anion exchange, mixed-mode polymeric sorbent	Styrenedivinylbenzene (SDVB) polymeric sorbent
SPE Sorbent	Polystyrene divinylbenzene with positively charged diamino ligand	Polystyrenedivinylbenzene (SDVB)
SPE Cartridge Size	500 mg (development), 200 mg (acceptable)	500 mg
Elution Solvent	Methanol containing ammonium hydroxide	Methanol
Sample Volume Adjustment	Adjusted to 1.0 mL with 20% water in methanol	Adjusted to 1.0 mL with 96:4% methanol:water
LC System	Binary linear gradients, delay column (optional)	Binary linear gradients, PEEK tubing
Calibration	Internal standard calibration technique	Internal standard calibration technique
Calibration Curve	Linear or quadratic regression	Not specified
LC/MS/MS Calibration	Weighted (1/x) quadratic regression with forced zero	Not specified
Analyte Detection	LC-MS/MS in negative ion electrospray ionization MRM detection mode	LC MS/MS in negative ion electrospray ionization MRM detection mode

7.3 Sample preparations and the intrumental analyses

7.3.1 Solid phase extraction for sample preparation

The solid-phase extraction (SPE) method finds extensive application in the analysis of various compounds across a wide range of matrices. The availability of diverse sorbent types renders the SPE technique a highly suitable choice for fulfilling specific sample preparation requirements **(Ötles & Kartal, 2016)**. This method is a procedure for isolating target analytes from aqueous samples using solid-phase extraction (SPE) media. It describes conditions for extracting a variety of compounds from aqueous matrices that include groundwater and wastewater. This method describes the use cartridge extraction media. The extraction procedures are specific to the analytes of interest and vary by group of analytes and type of extraction media **(EPA, 2007).** SPE has different steps with specific parameters for the optimization of the extraction.

7.3.2 Solid phase extraction steps

The optimization process for solid-phase extraction (SPE) in the analysis of PFAS involves several critical stages. Initially, adjustments to loading sample conditions are made by modifying the sample pH to evaluate analyte retention and recovery, with pH 7 proving optimal for acidic analytes. Moreover, the necessity of an equilibration step is assessed, leading to its exclusion due to the sorbent's inherent activation at the sample pH. Following this, the washing and elution steps are refined to ensure efficient elimination of interferences while retaining acidic compounds on the sorbent. Through experimentation, it is determined that washing with 1 mL of methanol (MeOH) effectively removes compounds with higher pKa values while maintaining high recovery rates for acidic analytes. The implementation of an online SPE-LC system, particularly utilizing a mixed-mode weak anion exchange (WAX) sorbent coupled with liquid chromatography (LC), aids in selectively retaining analytes post-washing. Furthermore, optimization of the elution solvent is carried out, with an 80% NH_4Cl/NH_4OH buffer solution at pH 9.2 and 20% acetonitrile (ACN) identified as suitable for complete elution of analytes bound to the resin via ionic interactions.

Lastly, the impact of varying sample loading volumes on analyte recovery should be considered, aiming to ascertain the optimal volume for improving quantification limits. These iterative steps collectively contribute to the enhancement of SPE methodology for robust PFAS analysis in

environmental samples **(Fontanals et al., 2010).** The choice of SPE type is crucial for an efficient extraction .

7.3.3 Types of SPE cartridge

Depending on the functional group(s) appended to the mixed-mode sorbent, it can be categorized as either anionic or cationic, as well as either a strong or weak ion exchanger. For PFAS extraction the weak anion exchange cartridge is the most suitable.

7.3.4 Anion-exchange SPE cartridge

Anion-exchange sorbents typically incorporate quaternary ammonium groups or weakly basic functional groups like primary or secondary amines. Conversely, cation-exchange sorbents feature strongly acidic groups such as aromatic or aliphatic sulfonic acid groups, or weakly acidic functional groups like carboxylic acids. These functional groups enhance the selectivity of the solid-phase extraction (SPE) process when the sorbents are employed under suitable experimental conditions, thereby augmenting the sensitivity of detecting acidic or basic components within complex samples **(Fontanals et al., 2010).**

7.3.5 Weak anion-exchange SPE

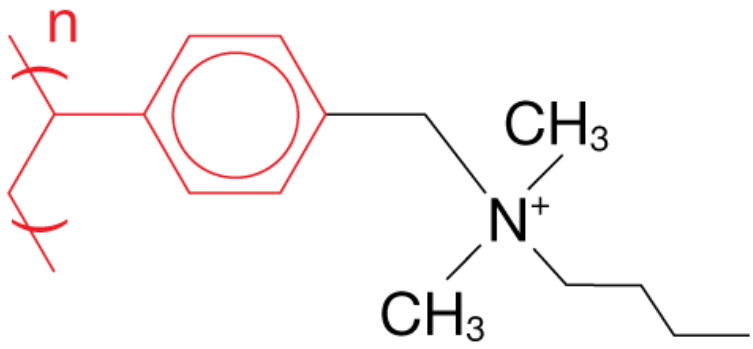


Figure 3. Weak anion exchange functional group **(Phenomenex, 2023).**

Phenomenex, 2023 recommends for acids with pKa values equal to or less than 5, the following chromatographic protocol. Initially, the column is equilibrated with 1 mL of methanol. Subsequently, equilibration is achieved by passing 1 mL of water adjusted to a pH range of 6 to 7. The diluted sample, also is adjusted to a pH range of 6 to 7, and then loaded onto the column. Following sample loading, the column is subjected to two successive washing steps: the first

wash involved 1 mL of 25 mM ammonium acetate buffered solution at pH 6 to 7, while the second wash utilized 1 mL of methanol. Elution of any acid species is performed using two consecutive 500 µL aliquots of 5% ammonium hydroxide in methanol. Finally, weak acids are specifically eluted using two successive 500 µL aliquots of 5% formic acid in methanol. This protocol ensures efficient separation and elution of acids with pKa values equal to or less than 5, facilitating downstream analysis or purification processes.

7.4 Analytical method validation strategy

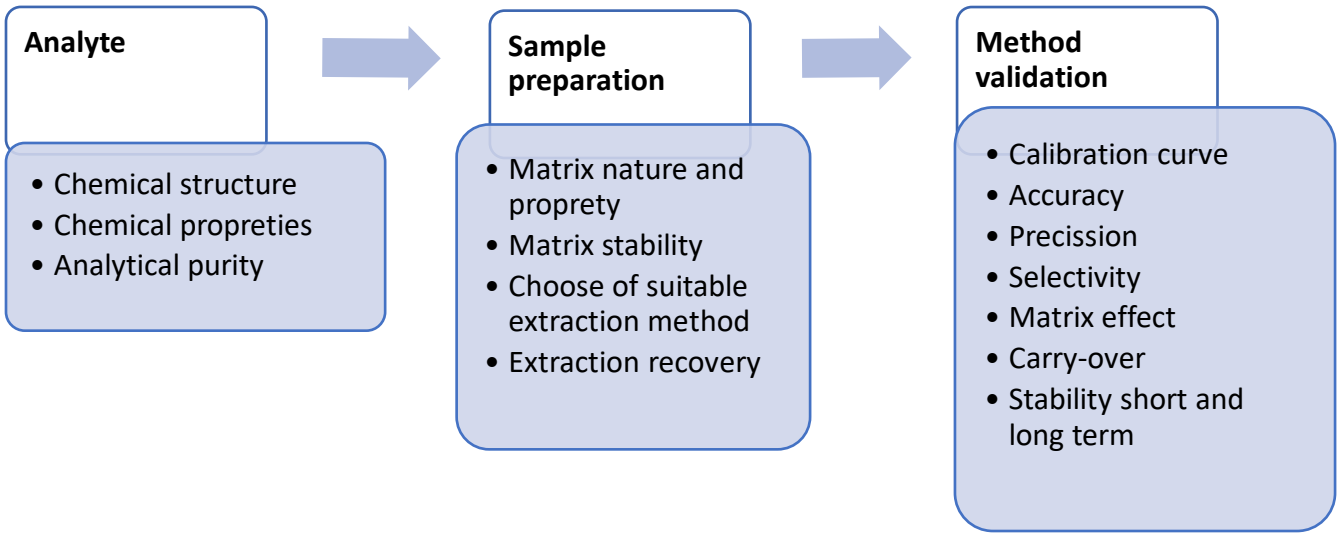


Figure 4. Analytical method development and validation strategies.

The method validation process for the determination of per- and polyfluoroalkyl substances (PFAS) utilizing solid-phase extraction (SPE) coupled with ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) involves rigorous testing to ensure the accuracy, precision, sensitivity, selectivity, and robustness of the analytical method. Initially, calibration curves are constructed using appropriate standards to quantify PFAS concentrations accurately. The extraction efficiency and matrix effects are assessed by analyzing spiked samples at different concentration levels, and recovery rates are calculated to evaluate the method's accuracy. Precision is evaluated by analyzing replicate samples, and both intra-day and inter-day variations are assessed. Selectivity is determined by examining potential interferences

from matrix components, and chromatographic resolution is optimized to separate PFAS analytes from co-eluting compounds effectively. Additionally, method robustness is evaluated by testing the method's performance under different experimental conditions, such as variations in extraction and chromatographic parameters. Overall, method validation ensures that the SPE-UHPLC-MS/MS method for PFAS analysis meets regulatory requirements and can reliably quantify PFAS concentrations in complex environmental or biological matrices with high accuracy and precision.

In the realm of analytical method validation for quantitative analyses, it is imperative to conduct within-laboratory validation procedures to ascertain the suitability of a method for its intended application. This validation process is mandated by accreditation bodies and should be reinforced and augmented through ongoing method performance verification during routine analytical procedures. Ideally, all procedural steps within a method should undergo validation. Representative matrices are utilized to validate both multi-residue and single-residue methods. When the method is applied to a broader range of matrices, supplementary validation data should be obtained, such as through ongoing quality control during routine analyses. The validation process must encompass sensitivity/linearity assessment, mean recovery to gauge trueness or bias, precision evaluated through repeatability (RSDr), and determination of the limit of quantification (LOQ). Additionally, identification parameters, such as ion ratio and retention time, must be evaluated. A minimum of 5 replicates at the targeted LOQ or reporting limit (RL) of the method and at least one higher concentration level (e.g., 2-10 times the targeted LOQ or the maximum residue limit [MRL]) are required to assess recovery and precision. When the residue definition encompasses multiple analytes, the method should ideally be validated for all analytes **(Pihlström et al., 2021).**

7.4.1 Calibration methods

7.4.1.1 Matrix matched calibration

Matrix effects, which can cause inaccuracies in quantifying compound concentrations, are a concern in analytical chemistry. To mitigate these effects, various clean-up methods have been devised, the impact of solid-phase extraction (SPE) as a sample clean-up technique is investigated and integrated into the validation process **(Gerssen et al., 2010)**. Evaluation of the method performances by preparing spiked samples, mix standards in neat solvent and matrix-matched calibration standards at different calibration levels. For the spiked sample preparation, a

model matrix is needed. Each of them followed the sample preparation procedure that consisted in a dilution 1:1 with a specific solvent **(Cortese et al., 2020).**

In the MSc of Food Safety and Quality Engineering we learnt the importance and use of the Matrix-matched calibration, that is considered as a vital component of the validation and application of ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) methods in analytical chemistry. This calibration approach involves preparing calibration standards in a matrix that closely mimics the composition of the sample matrix to be analyzed. The use of matrix-matched calibration standards helps to mitigate matrix effects, which can lead to ion suppression or enhancement and impact the accuracy and precision of quantitative results in complex sample matrices.

In matrix-matched calibration, calibration standards are prepared by spiking known concentrations of analytes of interest into a blank matrix extract obtained from the same or similar sample matrix as the samples to be analyzed. This ensures that the calibration standards closely resemble the sample matrix in terms of composition and matrix effects. The calibration standards are then subjected to the same sample preparation and analysis procedures as the actual samples.

Matrix-matched calibration standards are used to generate calibration curves relating the response of the UHPLC-MS/MS instrument to the concentration of the analytes in the sample matrix. These calibration curves are used to quantitate the concentrations of analytes in the samples by comparing their instrument response to that of the calibration standards.

7.4.1.2 Internal calibration

In my MSc Food Safety and Quality Engineering, Internal standard calibration is a pivotal technique utilized in ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analytical methods to enhance the accuracy and precision of quantitative analyses. This calibration approach involves the addition of stable isotopically labeled internal standards (IS) to the sample matrix prior to analysis. The internal standards closely resemble the analytes of interest but contain isotopic substitutions, ensuring their distinguishability from the native analytes during mass spectrometric detection. The purpose of the internal standard is to behave similarly to the analyte but to provide a signal that can be distinguished from that of the analyte. Ideally, any factor that affects the analyte signal will also affect the signal of the internal

standard to the same degree. Thus, the ratio of the two signals will exhibit less variability than the analyte signal.

Internal standards are often used in chromatography, mass spectroscopy and atomic emission spectroscopy. They can also be used to correct for variability due to analyte loss in sample storage and treatment. During the chromatographic separation, both the native analytes and their corresponding internal standards elute from the column and enter the mass spectrometer for detection. The internal standards serve as reference compounds, allowing for correction of variations in sample preparation, instrument response, and chromatographic performance. By quantifying the ratio of the peak areas of the native analytes to their respective internal standards, the method can compensate for fluctuations in analyte recovery, matrix effects, and instrument response, thus improving accuracy and precision.

8 Materials and methods

8.1 Materials

8.1.1 Chemicals

In this research endeavor, our focus centers on the utilization of multi-perfluoroalkyl substances (PFAS) solutions, pivotal for various analytical processes. These solutions encompass three distinct formulations, each serving a crucial role in the experimental setup. Firstly, the Analyte Primary Dilution Standard (analyte PDS) comprises 18 analytes, each meticulously calibrated to a concentration of 2mg/L. Secondly, the Surrogate Primary Dilution Standard (SUR) features four isotopically labeled standards, strategically employed to aid in surrogate operations throughout the experimental procedures, meticulously calibrated to a concentration of 4mg/L for N-EtFOSAA (D5), 1mg/l for each of PFDA (^{13}C9), PFHxA (^{13}C6) and HFPO-DA (^{13}C13). Lastly, the Internal Primary Dilution Standard (IS) consists of three isotopically labeled standards meticulously calibrated to a concentration of 4mg/L for N-MeFOSAA (D3), 3mg/L for PFOS (^{13}C8) and 1(mg/l) for PFOA (13C8), essential for precise calibration and internal referencing within the analytical framework. Together, these solutions form the foundational elements underpinning our investigation into PFAS compounds. (All of Primary Dilution Standards are shown in **Table 6**). All of Primary Dilution Standards are supplied by Agilent Technologies.

Table 3. List of Analyte Primary Dilution Standard measured in the experiment.

PFAS Name	Concentration mg/L
Analytes PDS	
11Cl-PF3OUDS	2
9Cl-PF3ONS	2
ADONA	2
N-EtFOSAA	2
N-MeFOSAA	2
PFBS	2
PFDA	2
PFDoA	2
PFHpA	2
PFHxS	2
PFHxA	2
PFNA	2
PFOS	2
PFOA	2
PFTeDA	2
PFTrDA	2
PFUnDA	2
HFPO-DA (GenX)	2
Surrogate PDS	
N-EtFODAA (D5)	4
PFDA (C13C9)	1
PFHxA (13C6)	1
HFPO-DA (13C13)	1
Internal Standard PDS	
N-MeFOSAA (D3)	4
PFOS (13C8)	3
PFOA (13C8)	1

8.1.2 SPE solvents

In preparation for the solid-phase extraction (SPE) procedure, we meticulously assemble a set of solvents, each tailored to fulfill specific requirements. These solvents are dispensed into polypropylene (PP) Falcon tubes or glass vials, with a volume of 45 mL allocated for each. Firstly, a solution of 0.1% NH_4OH in MeOH is meticulously concocted by combining 161 µl of 28% ammonium hydroxide with 45 ml of methanol (MeOH). Secondly, pure methanol (MeOH) is precisely measured and dispensed into the respective container, constituting an essential

solvent for the extraction process. Thirdly, deionized water, totaling 45 ml, is introduced into its designated Falcon tube or vial, serving as a vital component for the extraction procedure. Additionally, a solution of 1% acetic acid with a pH of 4 is meticulously prepared by initially adding approximately 30 ml of water to the Falcon, followed by the addition of 450 µl of acetic acid, and finally adjusting the volume to 45 ml with water. Lastly, a 25 mM acetate buffer with a pH of 4 is meticulously formulated by sequentially placing 86.7 mg of ammonium acetate into the Falcon tube, adding around 30 ml of distilled water, adjusting the pH to 4 with approximately 150 µl of acetic acid, and filling the solution to a total volume of 45 ml with water. These meticulously prepared solvents ensure optimal conditions for the subsequent SPE procedure, facilitating efficient extraction and analysis of target compounds.

8.1.3 Instruments and Apparatus

8.1.3.1 SPE apparatus

The SPE syringe used contains WAX cartridge Strata-X-AW (Di-amino) weak anion exchange, supplied by Phenomenex Figure 5. The SPE apparatus has a vacuum capacity of 20 inches of Mercury.

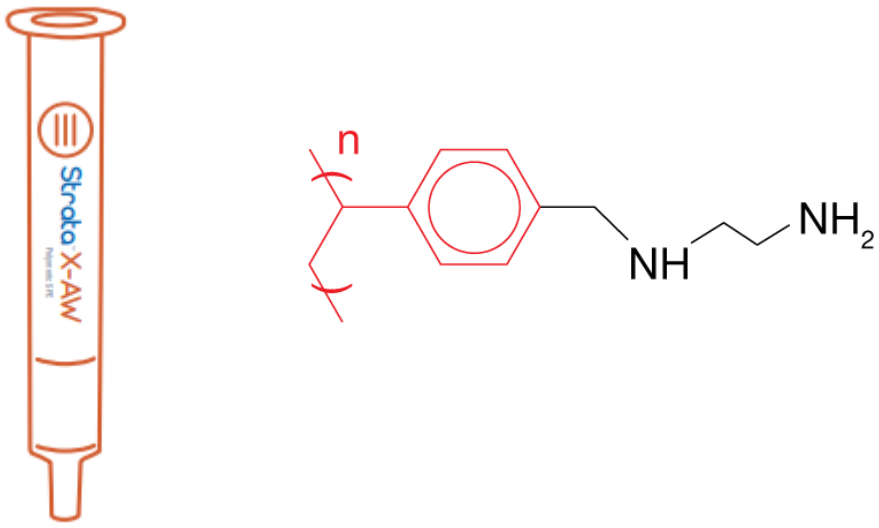
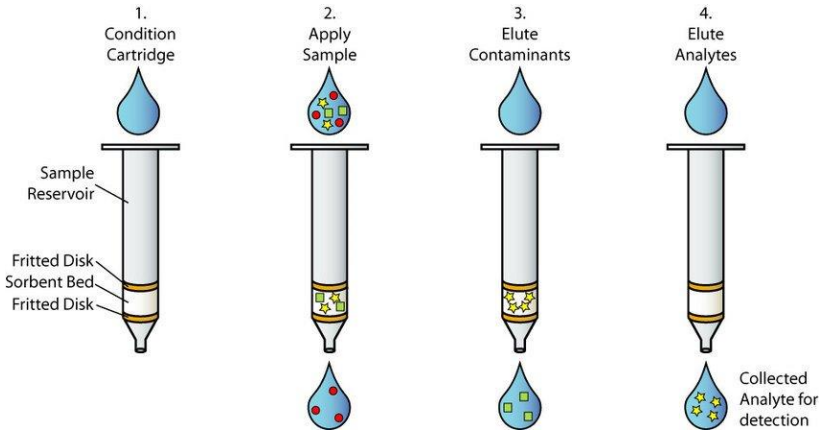


Figure 5. Strata-X-AW SPE and the chemical structure of its cartridge surface **(Phenomenex, 2023).**



.Figure 6. The Solid Phase Extraction Syringe **(Hawach, 2024)**.

8.1.3.2 UHPLC MS/MS

UHPLC-MS/MS instrument, Zorbax Eclipse Plus C18 RRHD chromatographic column with the particle size of 1.8µm and with a dimension of 2.1 x 50mm. UHPLC instrument composed of a pump, autosampler complete with a temperature control module. Eluent A and eluent B were used as the mobile phase (Mobile phase: A: 4mM ammonium-HCO3, 0.01% AA, B: MeOH). Glass equipment and pipettes were purchased from the same company.

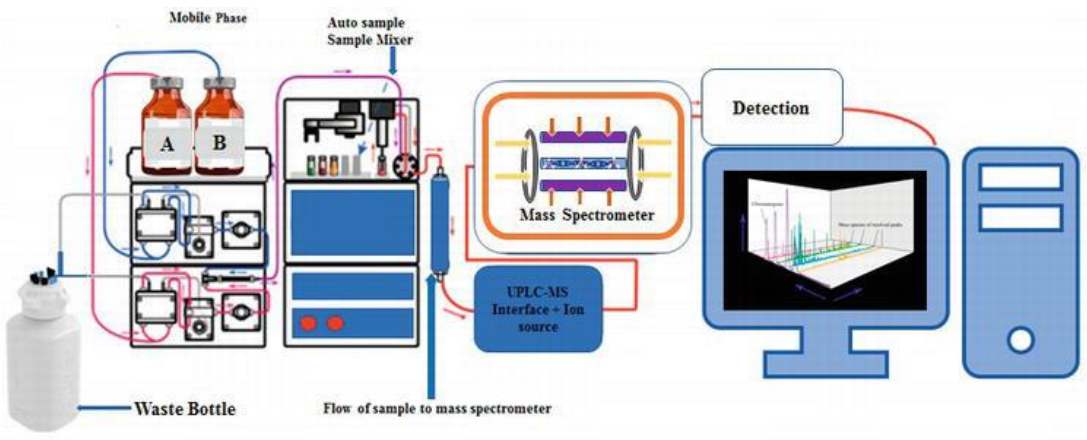


Figure 7. Flow diagram of ultra performance liquid chromatography-mass spectrometry **(Ashraf et al., 2020).**

8.2 Method

8.2.1 Experiment 1. Initial accuracy and precision determination of multi-PFAS method

8.2.1.1 Sample spiking

The solid-phase extraction step was optimized by studying several concentrations. In each test water was spiked with a mixture of standards in methanol. Each condition was tested in triplicate, enabling calculation of RSDs as a measure of repeatability**.**

The objective of this experimental work endeavor is to execute the solid-phase extraction (SPE) elution procedure and subsequently determine the final concentration of the pre-concentrated solution, aiming to achieve recoveries around 100% with an acceptable accuracy range of 70-130%. The experimental workflow entails four main steps: (a) sample preparation by spiking, involving the introduction of known quantities of analytes into the solution to create spiked samples for controlled analysis; (b) preparation of the SPE Solvent, where a specialized solvent mixture is meticulously prepared to facilitate efficient extraction of target compounds from the sample matrix; (c) SPE procedure, wherein the prepared samples undergo solid-phase extraction to selectively retain target analytes on the solid phase while removing interfering compounds, leading to sample pre-concentration; and (d) measurement, wherein the final concentration of the pre-concentrated solution is determined through analytical techniques, enabling the calculation of recovery rates and assessment of accuracy against predefined criteria.

To achieve precise results, we need a method with an accurately recover the spiked value within an acceptable range of 70-130%. Our plan involves conducting spiking experiments at four different levels, including a blank sample. We anticipate that at least two of these levels will yield satisfactory outcomes.

8.2.1.2 SPE procedures

8.2.1.2.1 Conditioning

Conditioning involves several steps to prepare the sorbent for optimal performance. Firstly, 4ml of 0.1% NH_4OH in MeOH is used to wash away any anion-related impurities from the sorbent. This is followed by 4ml of MeOH to remove any non-polar impurities. Subsequently, 4ml of water is introduced to saturate the sorbent with the aqueous phase. Finally, 4ml of 1% acetic acid

in water with a pH of 4 is applied to adapt the sorbent to acidic conditions and prevent the presence of anions on the column.

8.2.1.2.2 Load

The procedure involves loading the water samples into the system at a flow rate of 2-3ml/min, while also rinsing the sample bottle with 10ml of water beforehand. Subsequently, the washing phase consists of two steps: Firstly, using 4ml of 25mM acetate buffer with a pH of 4 to remove polar matrix interferences, ensuring the elimination of salts without introducing anions. Then, 4ml of water is applied to further eliminate any remaining salts from the sorbent. Following this, a drying period of 10 minutes is implemented to complete the process.

8.2.1.2.3 Elution

In the elution step, 4ml of MeOH is used to remove the apolar and nonionic matrix compounds from the sorbent. Following this, the loading process entails applying 2ml of 0.1% NH_4OH in MeOH twice.

8.2.1.3 Preconcentration

For preconcentration, we begun by evaporating the solution under a stream of nitrogen (N_2). Next, we added 960µl of MeOH, vortex to mix thoroughly, then add 40µl of water and vortex again. The mixture solutions were transferred the into an Eppendorf tube and store it at -20°C.

The calibration solutions are prepared in polypropylene (PP) Eppendorf tubes. After thorough vortexing, 200µl of each solution is pipetted into polypropylene-inserted HPLC vials for subsequent measurement. Similarly, the samples are transferred into PP-inserted vials for transportation. From the Eppendorf tubes, 200µl of each sample is pipetted into the inserted HPLC vials to facilitate analysis.

The analytical method employed for these measurements is UHPLC MS/MS utilizing a PFAS column and a mobile phase comprising 4mM ammonium-HCO_3 (A) and MeOH (B). The eluent preparation protocol involves diluting 4ml of the stock solution, stored at 100mM in the refrigerator, to approximately 50ml with water. Subsequently, 10ul of acetic acid is added, and the volume is adjusted to 100.0ml with a water-MeOH mixture.

8.2.1.4 Experiment steps

To achieve this experiment, deionized water samples are spiked with multi-analytes at a singular level, conducted across seven replicates. The Surrogate Primary Dilution Standard (SUR) is introduced into each sample, serving to mimic the presence of the analytes. Consequently, the SUR is administered alongside the spiked analytes in all samples. Additionally, the Internal Primary Dilution Standard (IS) is employed for calibration purposes, ensuring accuracy in measurements. The concentration levels employed are as follows: the spiked level of water samples across seven parallels is set at 80 parts per trillion or 80 ng/L spike. Subsequently, 10 µl of the SUR Primary Dilution Standard (PDS) is added to each sample. Following evaporation, 10 µl of the IS PDS is introduced to the dry extracts, further enhancing the calibration process.

In the conditioning phase, the sorbent is prepared through a series of steps: initially, 4 mL of 0.1% NH4OH in MeOH is employed to eliminate anionic contaminants, followed by the addition of 50 µL of ammonia in 50 mL of MeOH. Subsequently, 4 mL of MeOH is used to remove nonpolar impurities, while 4 mL of water is introduced to saturate the sorbent with the aqueous phase. Additionally, 4 mL of 1% acetic acid in water with a pH of 4 is applied to precondition the sorbent under acidic conditions, mitigating anionic interferences. The loading process involves the introduction of water samples at a flow rate of 2-3 mL/min, followed by rinsing the sample bottle with approximately 10 mL of water. Washing is performed using 4 mL of 25 mM acetate buffer at pH 4 to eliminate polar matrix interferences, with particular attention to avoiding anionic species. Elution is achieved through the sequential use of 4 mL of MeOH and 2 mL of 0.1% NH4OH in MeOH, conducted twice. Finally, preconcentration involves evaporation under N2 stream, addition of 10 µL of Internal Standard Primary Dilution Standard (IS PDS) to 990 µL of MeOH (containing 4% water), vortexing, and storage at -20°C, followed by filtration with a NYLON filter prior to measurement.

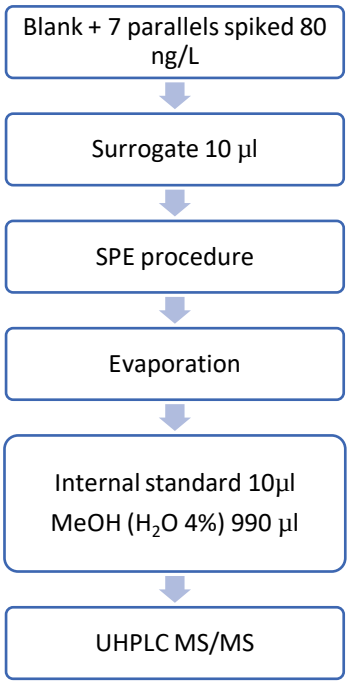


Figure 8**.** Experiment steps for the demonstration of SPE sample preparation accuracy and precision.

8.2.2 Investigating the potential loss of analytes The aim of this experiment is to address the potential loss of analytes, therefore we propose the following investigation: Firstly, we will augment the concentration of ammonia in the elution step. Additionally, we will adopt a comprehensive approach by collecting all phases during solid-phase extraction (SPE), subsequently evaporating each phase and conducting measurements collectively. This strategy will enable us to pinpoint where analytes are being lost. The experimental procedure will involve the analysis of 10 mL water samples, each acidified with 100 µl of acetic acid. Blank samples will also be included. Six fractions will be collected from each sample, resulting in a total of 18 samples for analysis. These samples will then undergo measurement. For calibration, a one-point calibration will be implemented to achieve the proposed level. For instance, if 10 µl of analyte (ANA) are introduced into the cartridge and subsequently dissolved in 500 µl of solvent, then 2 µl of ANA will be dissolved in 198 µl of MeOH (containing 4% water).

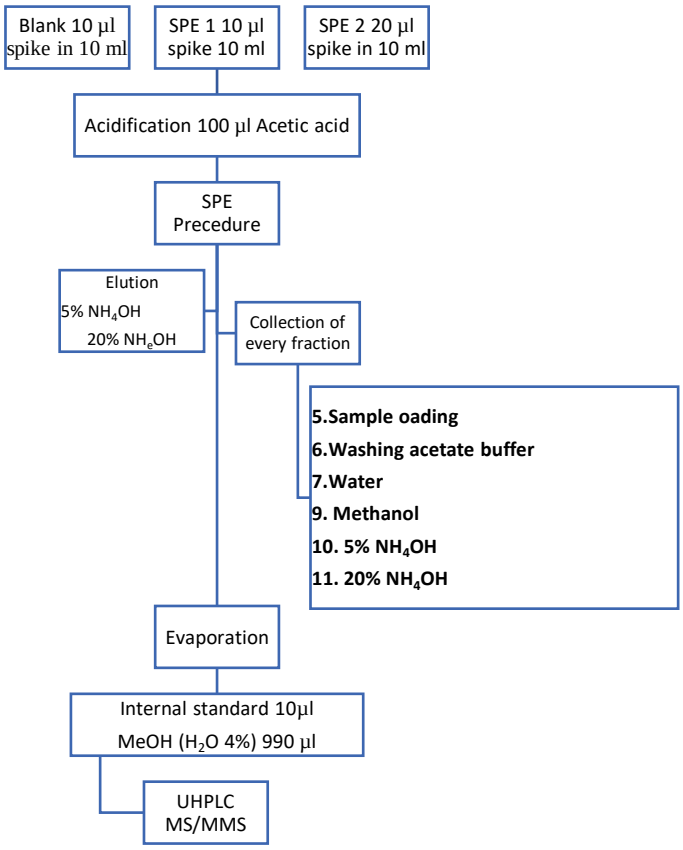


Figure 9. Investigation of Analyte Loss in Solid-Phase Extraction (SPE)

8.2.1 Experiment 3. Optimization of SPE procedure for high-recovery extraction

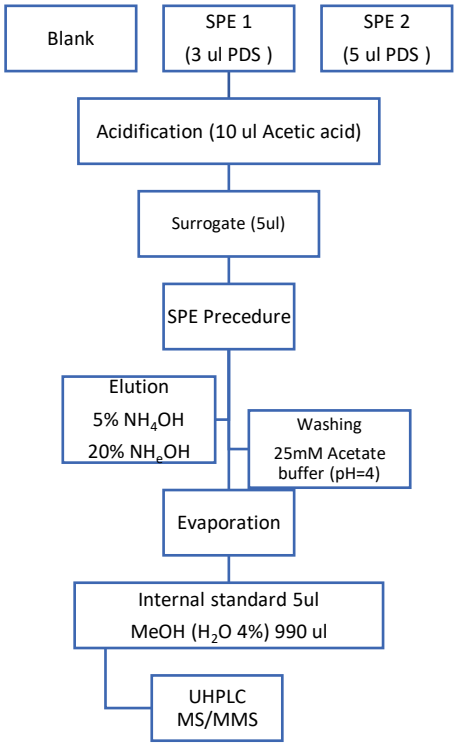


Figure 10.

optimization of the solid-phase extraction (SPE) procedure, that was conducted with a focus on achieving high-recovery extraction without any losses throughout the steps. Notable changes were implemented in the washing step, particularly by introducing only 10 µl of acid to the water samples, which differs from the previous approach.

For calibration purposes, a one-point calibration was established to attain the desired level. For instance, if 5 µl of analyte (ANA), 5 µl of surrogate standard (SUR), and 5 µl of internal standard (IS) were introduced into the cartridge and subsequently dissolved in 500 µl of solvent, then 2 µl of ANA, 2 µl of SUR, and 2 µl of IS would be dissolved in 194 µl of MeOH (containing 4% water). Calculations were performed to determine the concentrations of ANA, SUR, and IS in both the analytical sample and calibration solutions. Specifically, the ANA concentration in the analytical sample was computed to be 15 ppb initially and 20 ppb at the end, while the SUR concentration was determined to be 40 ppb initially and 10 ppb at the end. The IS concentration

remained consistent at 5 µl to 500 µl in the analytical sample and 2 µl to 200 µl in the calibration solution. The calibration standards were prepared using 5% v/v of 28% NH4OH in MeOH and 1% acetic acid in water. Loading of water samples was conducted at a flow rate of 2-3 ml/min, with the sample bottle rinsed using approximately 10 ml of water. Washing was performed using 25 mM acetate buffer at pH 4, and subsequent steps involved the preparation of various solutions, including 0.1% acetic acid in a 1:1 water:MeOH mixture and 20% NH4OH in MeOH.

8.2.2 Validation of Analytical Method for Tap Water Samples: Accuracy, Precision, and LOQ Determination using Surrogate-Matrix-Normalization Method

The aim of this experiment is the validation of analytical method for water samples are spiked at four concentration levels (4ng/L, 8ng/L, 16ng/L, and 80ng/L) in five replicates each, aiming for results within ±20% accuracy. Additionally, the recovery of surrogate standards (SUR) is assessed, with acceptable values ranging from 30% to 140%, aligning with Hungarian regulations and guidelines from US EPA and SANTE. Precision, characterized by RSD ≤ 20%, provides an initial estimation of measurement uncertainty, with Hungarian regulations allowing up to 50%. For LOQ determination, tap water is spiked at a single level, with expected accuracy within ±35% and precision ≤ 25%. The contribution of blank levels of analytes should not exceed 30% of the LOQ. The validation process starts with the spike concentration of 80ng/L in tap water. The surrogate method is employed for calculating accuracy, LOQ, and all measurements, involving the introduction of known amounts of surrogates into field samples to calculate sample analyte recoveries based on surrogate recovery. The selection of surrogates is based on chemical similarity, with logP values aiding in analyte grouping. While isotopically labeled surrogates for each unique analyte would be ideal, the limited availability of only four surrogates necessitates careful selection. Extrapolation based on percent recovery is conducted for target analytes, ensuring data usability while adhering to set limit values for surrogate recovery.

Figure 11Validation of Analytical Method for Tap Water Samples: Accuracy, Precision, and LOQ

9 Results and discussion

9.1 Experiment 1: accuracy and precision determination of multi-PFAS method

In this study, we conducted a series of experiments utilizing High Performance Liquid Chromatography (HPLC) to assess the initial accuracy and precision of determining multiple PFAS following SPE sample preparation. Seven deionized water samples were spiked at 80ng/L level with the multianalyte standard stock solution, which contains 18 PFAS compounds. Before the SPE sample preparation, the isotopically labelled surrogate analytes were also added to the samples in order to check the recovery of them, as well. For quantification, isotopically labelled internal standards were added immediately before the measuring.

As a result, we found that, apart from IS, no components were detected in the spiked water samples. These findings suggest a potential deficiency in the experimental protocol. Moreover, the presence of trace levels of PFOA and GenX points towards potential contamination originating from the analytical system rather than from the isotopically labeled compounds themselves.

Therefore the experiment will be repeated with the aim of investigating the deficiency and identify the level of the spiked analytes loss as following in Experiment 2.

9.2 Experiment 2: Investigating the potential loss of analytes

The aim of this experiment is to address the potential loss of analytes**.** In this experiment only 3 deionized water samples with the volume of 10 ml (in order to work faster) of each were used. The samples were spiked by 0, 10 and 20ul of multianalytes standard mix stock solution, and no surrogate and no IS were added in order to save. That is why we think, that the result of this experiment is only semiquantitative, however will inform us about in which step of SPE we loose the analytes.

Our assumption was that the mistake is in the elution step of the SPE procedure. We investigated the publications about this question and we saw, that in some cases more concentrated NH_4OH solution is used for elution. By Jurikova et *al* in "The occurrence of perfluoroalkyl substances (PFAS) in drinking water in the Czech Republic" the researchers investigated 100ml of water samples spiked by surrogate analytes. They used 1% NH_4OH in MeOH in order to elute the

target compounds from the weak anion-exchange SPE column. We also studied the suggestions of Phenomenex, which is accompanied to the weak anion-exchange SPE cartridges. They suggest even more concentrated alkaline medium for elution: 5% of NH_4OH. Based on the above mentioned data, we changed our SPE protocols in this experiment, and applied a two-step elution at the end of the SPE protocol: one with 4ml of 5% NH_4OH in MeOH, and the second one with 4ml of 20% NH_4OH in MeOH. In this experiment, each eluted liquid from the SPE steps were collected and were screened for PFAS residues, afterwards (see the protocol in the Material-method section, Figure (8).).

We can explain our results for experiment 2, referring to the published researches by (Olomukoro et al., 2021) and (Sanan & Magnuson, 2020).

The (Figure 11) shows the results of recovery in each fraction of each step of SPE. From the results we conclude that our PFAS analytes eluted in the tenth step with 5% ammonia. That means the analytes were not lost but remained in the cartridge because the concentration of ammonia was not sufficient.

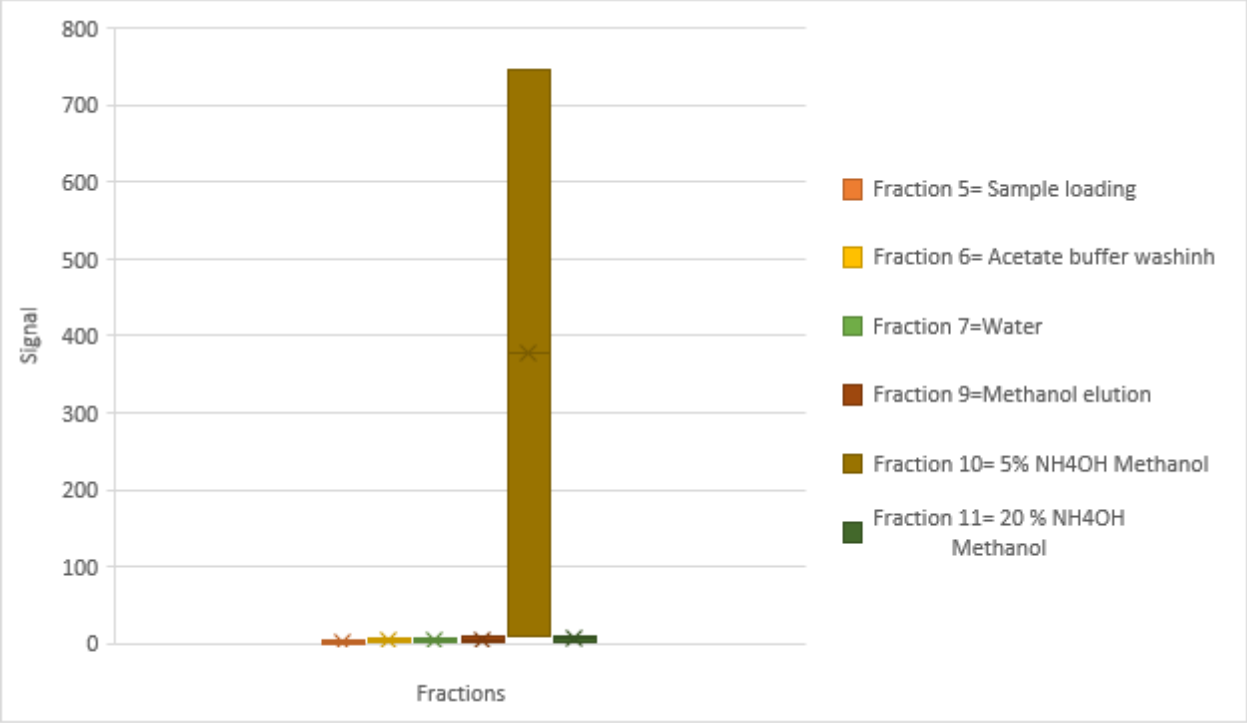


Figure 12. UHPLC MS/MS detection ratios of SPE fractions.

The detection results of PFAS using UHPLC MS/MS, shown an efficient elution of our target analyte in the 10th step, using 5% NH_4OH in MeOH.

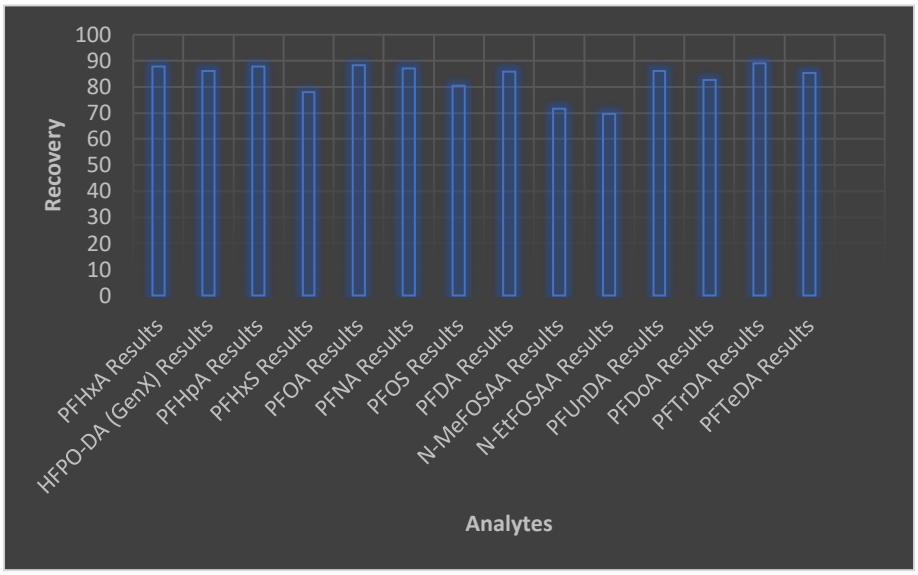


Figure 13. PFAS Recovery results by UHPLC MS/MS detection of the SPE fraction 10.

The recovery of extraction fits in the criteria for the different analytes, based on our experiment a specific cartridge needs specific conditions of desorption, that were optimized to ensure quantitative desorption of analytes from the extraction phase while maintaining the throughput of the method. Moreover, as the Strata-X-AW weak anion exchange SPE coating was found to be optimal for extraction, it was essential that the pH of the desorption solution was able to neutralize the WAX functional groups to facilitate the quantitative desorption of the model analytes. A desorption solution of methanol ammonium hydroxide to adjust the pH with a gradient desorption using two elution steps with 5% NH4OH, was chosen according to results from EPA method 533, which uses this solvent composition as the optimal solution for elution of PFAS from SPE cartridges.

9.3 Experiment 3: Optimization of SPE procedure for high-recovery extraction

The aim of this experiment is the optimization of PFAS recovery with a well-chosen SPE protocol, based on the last mesurements. We spiked three water samples again in 10 ml of each, by 0, 3 and 5ul of multianalite standard stock solution. In this experiment we applied acidification of the deionized water by 10ul of concentrated formic acid, and we used isotopically labelled surrogates and internal standards again, in order to check the recovery and

have a really quantitative data. The SPE procedure was the same as in the experiment 2. We used internal standard calibration for quantification of recovery of analytes and surrogates, as well. The (Figure 13). shows the recovery results.

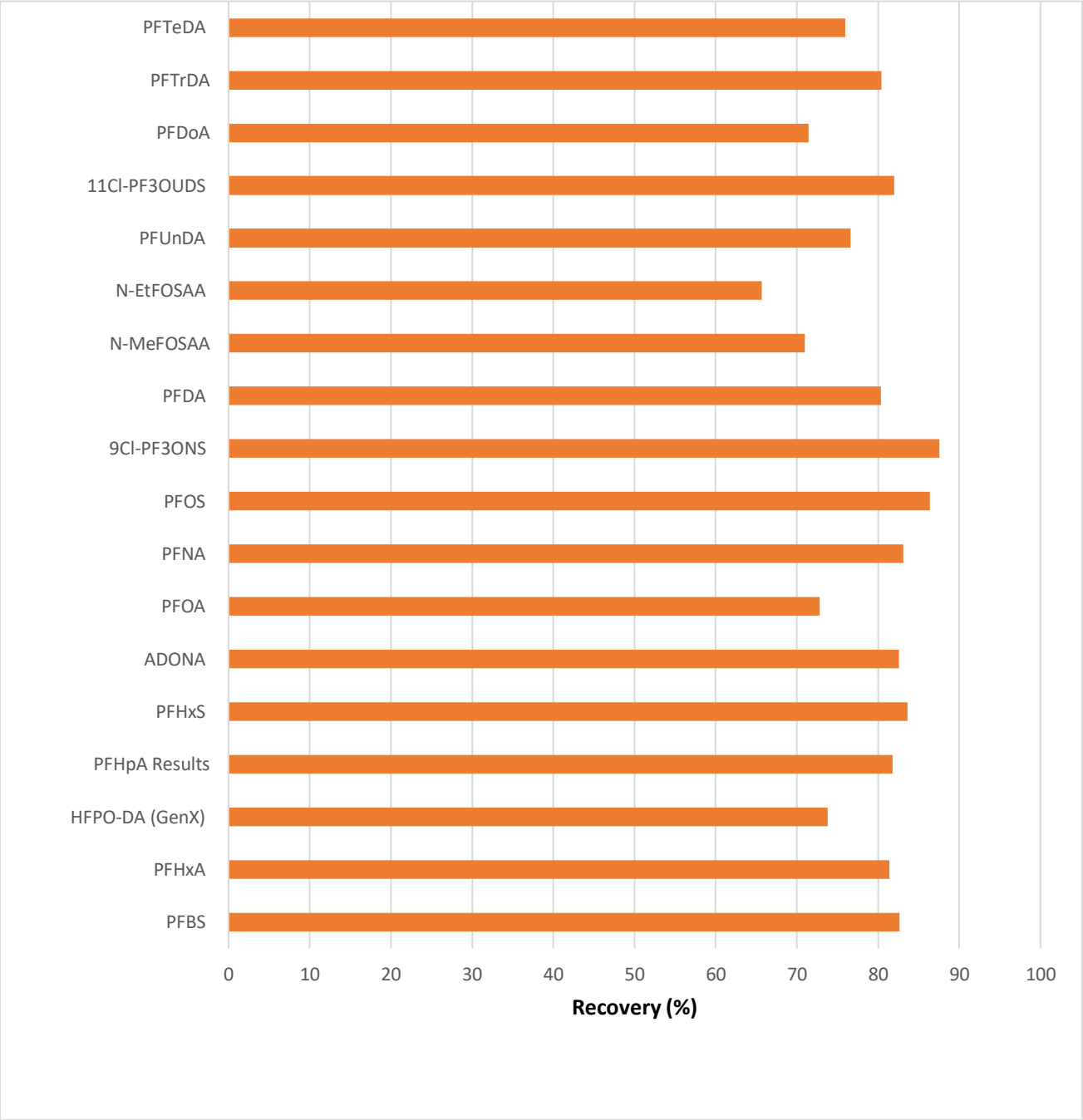


Figure 14. Optimized recovery results of SPE.

We can see on the (Figure 13), that in the case of analytes, with the exception of N-EtFOSAA, all other PFASs were recovered properly (70-130%).

The surrogate matrix does not necessarily have to reproduce the functions of the authentic matrix, but it must at least in part replicate its composition to ensure comparable recovery and matrix effects for the analyte **(Agrawal et al., 2021).** In our study we used four different surrogate isotopically labeled standards,with the following recovery results: N-EtFOSAA,(130%), PFDA (^{13}C9) (68.8%), PFHxA (^{13}C6) (69.1%) and HFPO-DA (^{13}C13) (61%). We calculated the recovery of each analyte using the appropriate surrogate and the closer one in a chemical standpoint.

PFASs recovery corrected with surrogate recovery, from spiked water is between 65% and 87% one had recovery below 70% Fig.13. The accuracy of matrix recoveries in our method is an improvement over previously reported methods for a similar number of PFAS according to **(Coggan et al., 2019).**

9.4 Experiment 4: Validation of Analytical Method for Tap Water Samples: Accuracy, Precision, and LOQ Determination using Surrogate-Matrix-Normalization Method

The aim of this experiment is the validation of analytical method using for the three criteria that are the accuracy, the Precision and the LOQ. Using drinking water (tap water) as an analytical sample and Surrogate-Matrix-Normalization method as an approach for the calibration of the analytes recovery.

Commencing with an initial spike of 80ng/L, tap water serves as the medium for our experimental procedures. Our analytical approach employs the surrogate methodology. This technique involves the introduction of known quantities of surrogate compounds into field samples. Subsequently, the recovery of sample analytes can be determined by assessing the measured recovery of these surrogate compounds. While this calculation method may not be commonplace, it is employed in challenging analytical scenarios, such as those encountered in volatile organic compound (VOC) analysis, as elucidated in the US EPA's "User’s Guide for Surrogate Matrix Normalization".

The crux of this technique lies in the surrogate compounds, each of which represents a group of analytes. The task at hand involves establishing the relationship between surrogates (SUR) and target analytes (ANA). In essence, this entails determining which surrogate compound should be utilized for the correction of each target analyte. It is imperative to associate target analytes with surrogate compounds that exhibit chemical similarities, a principle guided by the logarithm of the compound's partition coefficient (logP). Ideally, each unique analyte would possess an isotopically labeled surrogate compound for precise correction. However, the reality is that only four surrogate compounds are available.

Following the judicious selection of surrogate compounds for each target analyte, extrapolation based on percentage recovery is undertaken to ascertain the concentration of target analytes. However, it is essential to note that excessively low recoveries of surrogate compounds can compromise the usability of the data. Therefore, a threshold value for surrogate compound recovery is established to ensure data integrity and reliability.

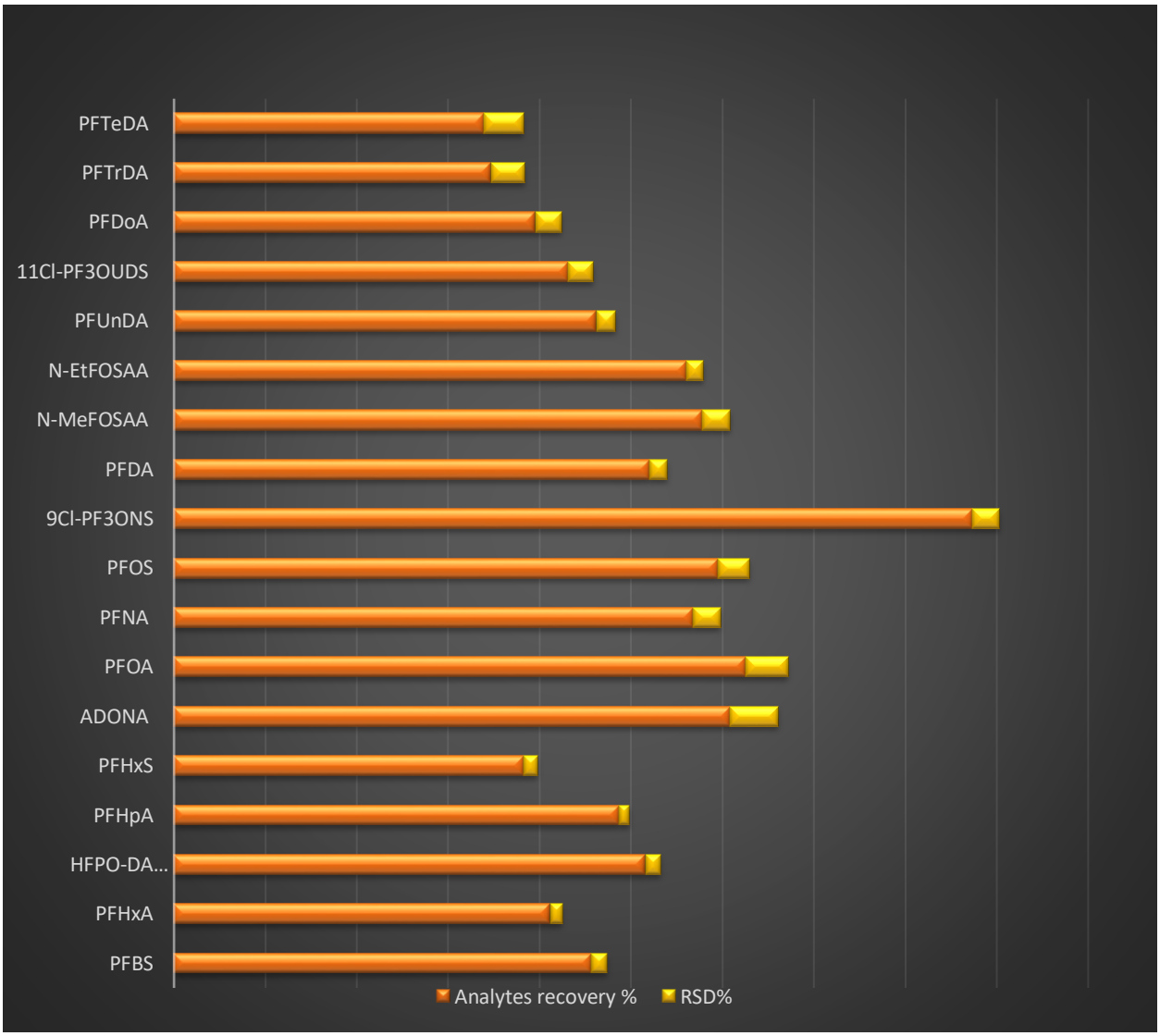


Figure 15. Analytes recovery (%) and RSD (%).

Table 4. Surrogte recovery(%).

Surrogate PDS	**Recovery %**
PFHxA (13C6)	105.5

HFPO-DA (13C3)	94.7
PFDA (13C9)	72.1
N-EtFOSAA (D5)	57.7

Table 5. Analytes recovery (%) and RSD (%).

Analytes+D2B6:D24	Recovery %	RSD (%)
PFBS	91.28	3.5
PFHxA	82.24	2.7
HFPO-DA (GenX)	103.09	3.3
PFHpA	97.26	2.3
PFHxS	76.42	3.1
ADONA	121.63	10.5
PFOA	124.98	9.3
PFNA	113.68	5.9
PFOS	119.10	6.7
9Cl-PF3ONS	174.60	6.0
PFDA	104.01	3.9
N-MeFOSAA	115.57	6.1
N-EtFOSAA	112.13	3.7
PFUnDA	92.36	4.3
11Cl-PF3OUDS	86.14	5.6
PFDoA	79.05	5.7
PFTrDA	69.32	7.5
PFTeDA	67.72	8.7

In this experiment we are considering the recovery of analytes PDS(%), the RSD(%) and the surrogate recovery (%) as well. Based on our results in the table (7) and the figure (14), The

requirements are not fulfilled for 5 analytes : PFHxS Results, PFOA Results, 9Cl-PF3ONS Results, PFTrDA Results and PFTeDA Results. The results fulfilled the requirements for the surrogate PDS. The requirement for precision is fulfilled with RSD% ≤ 20% for all the analytes .

The FDA method can quantify analytes with maximum levels identifed in Commission Regulation (EU) 2022/2388 in priority foods below the recommended LOQs. For monitoring purposes, the FDA reports values above the MDLs and these are comparable with LOQs required by the EURL POPs for surveillance samples. This indicates that values above similar thresholds are being reported in the US and Europe but an extra concentration step is needed in the FDA method to reach the required LOQs **(Genualdi et al., 2024)**

10 Conclusion

This study presents the development and validation of a solid-phase extraction (SPE) sample preparation method tailored for efficient extraction of per- and polyfluoroalkyl substances (PFAS) from drinking water samples, with subsequent analysis using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC MS/MS). With the need for establishing regulations for PFAS monitoring in Hungary, this research represents a significant milestone, demonstrating the efficacy of the developed SPE method utilizing a weak anion exchange cartridge. Results confirm the method's ability to selectively capture PFAS compounds while minimizing matrix interference. The subsequent UHPLC MS/MS analysis enables sensitive and selective detection of PFAS, supporting accurate quantification for regulatory purposes. This integrated approach offers a robust solution for routine PFAS monitoring in drinking water, contributing valuable data to ensure water quality and public health protection. Further research may explore expanding the method's applicability to other environmental matrices and addressing potential analytical challenges in PFAS analysis.

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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, (2019).

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METHOD 537.1 DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS), (2020).

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