OPTIMIZATION AND COMPARISON OF SEVERAL EXTRACTION METHODS FOR DETERMINING PERFLUOROALKYL SUBSTANCES IN SOIL MATRICES USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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1 ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are highly persistent synthetic chemicals recently classified as persistent organic pollutants (POPs) under the Stockholm Convention. They include both legacy compounds such as PFOS, PFOA, and PFHxS, and new-generation alternatives like HFPO-DA, DONA, and PFECHS. Soil acts as a key reservoir and secondary source of PFAS, yet analytical protocols for this matrix remain less developed compared with water, biota, or biosolids. This study evaluated and compared three extraction and clean-up strategies for PFAS determination in soils using UHPLC–MS/MS. The ENVI-Carb™ method, routinely applied in our laboratory, was assessed alongside an alternative cleanup with Oasis WAX/GCB cartridges and a QuEChERS-based protocol adapted from biological and environmental matrices. A naturally contaminated soil sample was analyzed, targeting 38 PFAS. The results showed clear differences between methods. The ENVI-Carb method provided acceptable precision but recovered only a limited subset of analytes, failing in particular to quantify some short-chain PFCAs such as PFBA. Oasis WAX/GCB achieved broader coverage and lower RSD values, combining acceptable recoveries with improved reproducibility. QuEChERS enabled the detection of the widest range of analytes, including certain long-chain PFAS associated with humic fractions, but overall recoveries were lower and RSD higher. Rather than selecting a single method as definitive, the results suggest two directions for future work: optimization of the QuEChERS protocol to reduce matrix interferences and improve recoveries, and the evaluation of the full Waters Oasis WAX/GC protocol, including its extraction step, to determine whether it can further enhance performance in soil matrices.

2 RESUM

Les substàncies per- i polifluoroalquilades (PFAS) són contaminants sintètics altament persistents que recentment han estat inclosos dins la llista de contaminants orgànics persistents (POP) del Conveni d'Estocolm. Abarquen tant compostos de llegat, com el PFOS, el PFOA i el PFHxS, com alternatives de nova generació, com l'HFPO-DA, la DONA o el PFECHS. El sòl és un dels principals reservoris i alhora una font secundària de PFAS cap al medi, però els protocols analítics disponibles per a aquesta matriu són encara menys desenvolupats que en aigua, biota o biosòlids. En aquest estudi s'han avaluat i comparat tres estratègies d'extracció i neteja per a la determinació de PFAS en sòls mitjançant UHPLC–MS/MS. S'ha examinat el mètode ENVI-Carb™, utilitzat de manera rutinària al laboratori, juntament amb una neteja alternativa amb cartutxos Oasis WAX/GCB i un protocol QuEChERS adaptat a partir de mostres biològiques i ambientals. S'ha analitzat una mostra de sòl contaminada, incloent un total de 38 PFAS. Els resultats mostren diferències clares entre els mètodes. El mètode ENVI-Carb va oferir una bona precisió però només va recuperar un nombre limitat de compostos, i no va permetre quantificar alguns PFCAs de cadena curta com el PFBA. Oasis WAX/GCB va assolir una cobertura més àmplia i valors de RSD més baixos, combinant recuperacions acceptables amb bona reproductibilitat. El protocol QuEChERS va permetre detectar el major nombre de compostos, inclosos alguns PFAS de cadena llarga associats a fraccions húmiques, tot i que amb recuperacions més baixes i valors RSD més alts. Més que identificar un únic mètode com a definitiu, els resultats apunten cap a dues línies de treball futures: l'optimització del protocol QuEChERS per reduir interferències i millorar les recuperacions, i l'avaluació del protocol complet de Waters, incloent-hi l'etapa d'extracció, per comprovar si ofereix un rendiment superior en matrius de sòl.

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7 INTRODUCTION

PFAS are a large group of synthetic organic compounds that have been widely used since the mid-20th century due to their exceptional thermal stability, chemical resistance, and amphiphilic behavior. These properties come from their unique molecular structure, which consists of a fully or partially fluorinated carbon chain (the “tail”) attached to a polar functional group (the “head”) [1].

Figure 1 illustrates the basic structure of a PFAS molecule. The perfluorinated tail is highly hydrophobic and lipophobic, while the polar head, usually a carboxylic, sulfonic, or phosphonic acid group, Controls its solubility, interaction with environmental matrices, and toxicokinetic. This molecular duality plays a central role in the industrial versatility and long-term environmental persistence of PFAS [2].

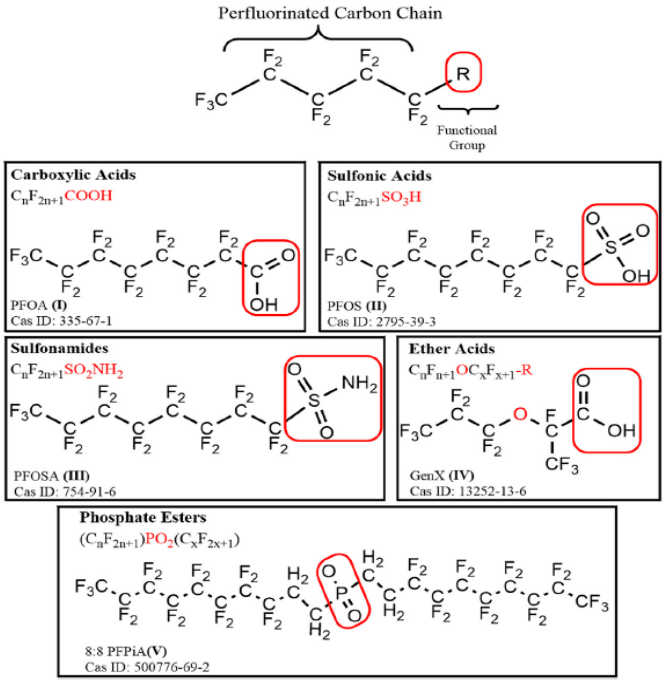

Figure 1. General structure of PFAS molecules, showing a hydrophobic perfluorinated carbon chain and a hydrophilic functional group (R), highlighted in red.

PFAS have been widely used in industrial and consumer products such as waterproof fabrics, food packaging, fire-fighting foams (AFFF), non-stick cookware. and personal care products. However, the strength of the carbon–fluorine bond also makes these compounds resistant to hydrolysis, photolysis, and microbial degradation. As a result, the very same chemical properties which give PFAS compounds their consumer-friendly resilience create nefarious drawbacks, namely extreme environmental persistence and biomagnification. To date, the U.S. Environmental Protection Agency (EPA) has identified over 14,700 different PFAS compounds in its PFASSTRUCT v5 list [3]. PFAS are highly persistent, capable of remaining in the environment for decades. They can accumulate in living organisms and travel long distances through air and water, reaching even remote ecosystems [1,4].

7.1 Classification of PFAS

The classification of per- and polyfluoroalkyl substances (PFAS) is still a topic of discussion within the scientific community, since there is no single system accepted by everyone. Different approaches have been proposed based on molecular structure, functional group, polymeric nature, or regulatory relevance. For instance, some classifications distinguish between polymeric and non-polymeric PFAS, or group them according to chain length [5] [6] .

In environmental and toxicological contexts, the most widely used classification separates PFAS based on their terminal functional group. This is the approach adopted by authoritative bodies such as the OECD and widely referenced in recent literature [1,2] According to this system, PFAS are divided into three main categories: Perfluorosulfonic acids (PFSAs) (e.g., PFOS, PFHxS, PFBS), Perfluorocarboxylic acids (PFCAs) (e.g., PFOA, PFNA), and emerging PFAS and precursors (including fluorotelomer-based compounds and sulfonamides such as 6:2 FTSA, DONA, FOSA, and GenX).

Figure 2 shows this classification as used in this study. We selected this system because it helps group PFAS that behave in similar ways in the environment. It also makes it easier to compare how these substances differ in terms of health effects, accumulation in living organisms, and how they are regulated.

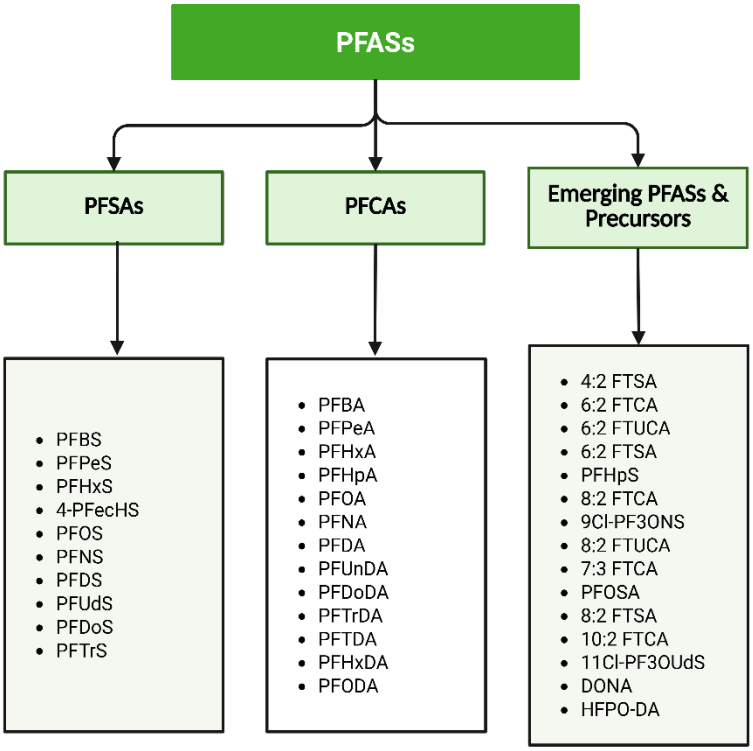

Figure 2. Classification of PFAS compounds into three main groups: Perfluorosulfonic acids (PFSAs), perfluoroalkyl carboxylic acids (PFCAs), and emerging PFAS and precursors. Each group includes representative compounds commonly found in the environment.

7.2. Environmental issues, ecological and health risk

PFAS are persistent and highly mobile pollutants with growing global relevance due to their widespread presence and documented health risks. Their resilience, especially the strength of the carbon–fluorine bond, allows PFAS to resist degradation by sunlight, microbes, or other natural processes [7]. Once released into the environment, they can persist for decades and spread through air, water, and soil systems.

A major environmental concern is the wide distribution of PFAS and their multiple pathways of release, especially in soil and water systems. As shown in **figure 3,** PFAS can enter the environment through fire-fighting foams (AFFF), landfill leachate, industrial wastewater, air emissions, and the use of biosolids or contaminated irrigation water. These pathways contribute to extensive contamination, including in agricultural regions [8,9].

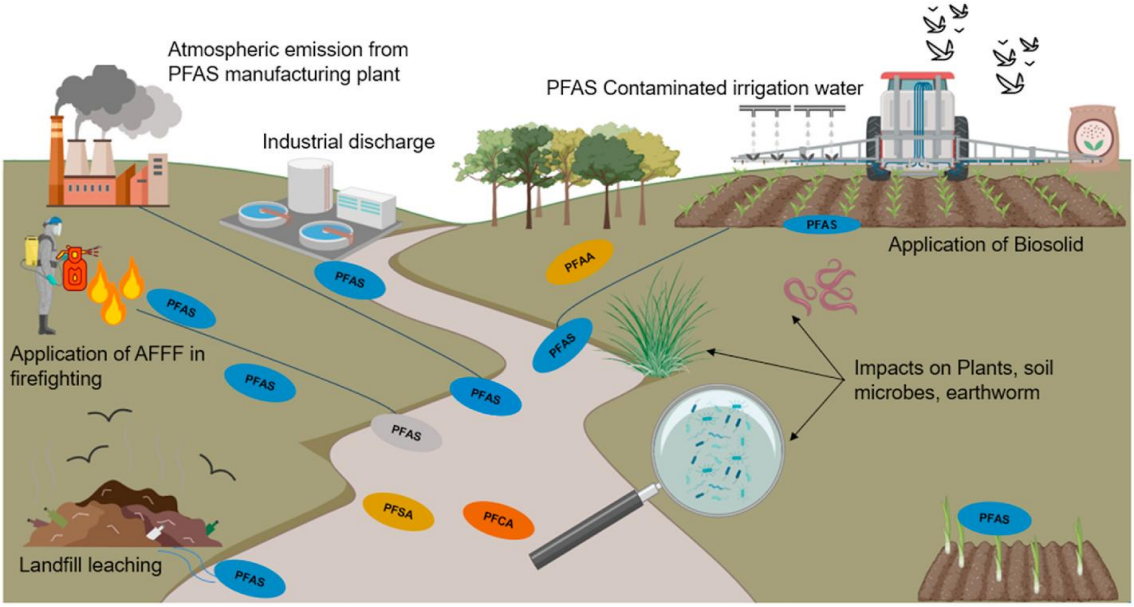

Figure 3. Schematic representation of PFAS origin, environmental mobility, exposure routes, and the resulting effects on ecosystems and human health.

Soil, in particular, serves as a long-term reservoir for PFAS. These compounds can attach to soil components such as organic matter and clay particles, especially under slightly acidic to neutral pH conditions [10]. However, depending on the soil's ionic characteristics, such as pH, ionic strength, and cation or anion exchange capacity, PFAS can desorb and leach through the soil, increasing the risk of groundwater contamination [11].

While the accumulation of PFAS in organisms and their potential for biomagnification are well established for widely studied compounds like PFOS and PFOA, these processes are not yet fully understood for the broader PFAS group. As result, their full ecological effects remain not completely understood. Recent studies have shown that PFAS can influence soil microbial activity, enzyme performance, and plant development, disrupting essential ecosystem functions such as nitrogen cycling and the breakdown of organic matter [2,5]. Earthworms, which play a vital role in maintaining soil structure and fertility, have also shown signs of PFAS bioaccumulation and physiological stress when exposed to contaminated soils [2,5].

On the human health side, epidemiological studies have linked PFAS exposure to an increasing number of adverse outcomes, including immunotoxicity, liver damage, reproductive and endocrine disruption, as well as metabolic disorders such as dyslipidemia and insulin resistance [1,2,6]. New research also raises concerns about neurological effects and links to neurodevelopmental disorders. The combination of environmental persistence, ecological impacts, and long-term health risks makes PFAS one of the most critical emerging contaminants. Addressing their presence requires both strong regulatory measures and the development of long-term monitoring strategies across different environmental systems [1,2,6].

7.3. PFAS in soil

Soil plays a central role in the environmental fate of PFAS. Although it can serve as a long-term sink for these substances, it may also act as a secondary source of contamination. The way PFAS are retained or move through soil depends on complex interactions between their chemical structure and the physical and chemical characteristics of the soil [10].

Key soil properties, such as organic carbon content, clay content, pH, moisture, and anion exchange capacity, can significantly affect how strongly PFAS are adsorbed or how easily they migrate. For example, long-chain PFAS like PFOS and PFOA generally bind more strongly to organic matter and show lower mobility, whereas short-chain PFAS and certain precursors can infiltrate deeper into the soil profile and reach groundwater [11].

Dissolved organic matter (DOM) also plays a dual function: it can promote PFAS retention by forming DOM-PFAS complexes or, alternatively, enhance their movement by generating mobile associations [10] This variability makes PFAS behavior highly site-specific, complicating predictive models and risk assessments.

Another major challenge is the complexity of cleaning up PFAS-contaminated soils. Conventional methods such as excavation or soil washing are expensive and often ineffective, particularly in extensive or hard-to-reach areas. Novel techniques like in situ stabilization, thermal desorption, or advanced oxidation are currently being explored, but remain mostly at the pilot stage [2].

As a result, soils not only retain PFAS over long periods but also act as a source of gradual and ongoing release into nearby ecosystems, highlighting the need for focused monitoring and long-term remediation strategies.

7.4. Regulation of PFAS

Regulatory action on PFAS in Europe has intensified in recent years. In January 2023, five Member States (Denmark, Germany, the Netherlands, Norway, and Sweden) submitted a joint proposal to ECHA aiming to restrict around 10,000 PFAS under the EU's Chemicals Strategy for Sustainability and the Zero Pollution Plan. The restriction proposal was officially published in February 2023, and a decision is expected by 2025, with implementation foreseen by 2026–2027 [12].

In terms of drinking water, the updated EU Drinking Water Directive (EU 2020/2184) introduced mandatory monitoring of PFAS. It establishes two parametric values: 100 ng/L

for the “Sum of 20 PFAS” and 500 ng/L for “Total PFAS”, giving Member States the choice of which parameter to adopt. Compliance becomes mandatory by January 2026 [12].

For surface water, groundwater, and biota, the Water Framework Directive and the Environmental Quality Standards Directive were amended in 2022, setting very stringent thresholds: 4.4 ng/L for a group of 24 PFAS in both surface and groundwater, and 77 ng/kg wet weight in biota. These limits are expressed as PFOA equivalents using a Relative Potency Factor approach to capture mixture toxicity [12].

With regard to food safety, the European Food Safety Authority (EFSA) revised its risk assessment in 2020, setting a Tolerable Weekly Intake (TWI) of 4.4 ng/kg body weight/week for the combined exposure to PFOA, PFOS, PFNA, and PFHxS. Additionally, the European Commission issued Recommendation (EU) 2023/915, which suggests indicative levels in foodstuffs. For example: 10 ng/kg for PFOS and PFOA, 5 ng/kg for PFNA, and 15 ng/kg for PFHxS in fruits, vegetables (except wild fungi), starchy roots, and tubers. Exceedance of these levels should trigger further investigation [12]

At the global level, the Stockholm Convention on Persistent Organic Pollutants has already banned PFOA, PFOS, PFHxS, and in 2022 extended the ban to long-chain perfluorocarboxylic acids (C9–C21), their salts, and related compounds, which are now listed in Annex A. This means their production and use are banned worldwide, with only a few specific exceptions. These rules apply across the environment, since PFAS have been found in surface water, groundwater, soil, and in food grown in contaminated areas [13,14].

Although soils act as the primary sink for PFAS, there are currently no specific regulatory established at the European level. However, in June 2024, the European Parliament approved the Soil Monitoring Law, which for the first time establishes a harmonized framework to monitor soil health across Member States. The law includes specific provisions to track pollutants such as PFAS and pesticides, particularly in sensitive or contaminated sites. Although concentration limits for soils are not yet defined, this initiative represents an important step toward systematic monitoring and the potential establishment of future threshold values [15].

7.5. Extraction Strategies for PFAS

Although different extraction strategies have been explored for PFAS determination in soils, including microwave-assisted extraction, accelerated solvent extraction (ASE), and modified Soxhlet procedures [2], most studies are based on adaptations of EPA Method 1633, which is based on solvent extraction followed by a SPE cleanup step [2,16]. Within this framework, ultrasound-assisted extraction (UAE) in combination with shaking and centrifugation has become the most frequently applied approach due to its simplicity, efficiency, and satisfactory recoveries in environmental matrices such as soils and biosolids [17,18] . By contrast, the QuEChERS method has been widely applied in food and biological samples but is rarely reported for PFAS extraction in soils. Nevertheless, its operational simplicity and low solvent consumption have motivated attempts to adapt it for this type of matrix [2,19,20]. For these reasons, we decided to describe both UAE and QuEChERS in more detail, as they represent two complementary approaches of particular relevance to the analysis of PFAS.

7.5.1. Ultrasound-Assisted Extraction

The use of ultrasound in combination with shaking and centrifugation enhances PFAS extraction by improving solvent–matrix interaction and fragmenting soil particles. UAE creates tiny gas bubbles that collapse and increase local temperature and pressure, which in turn helps release analytes trapped within the matrix. Shaking ensures full hydration of the sample, while centrifugation facilitates phase separation without filtration steps.

UAE using methanol as the extraction solvent has been widely applied for the recovery of PFAS from solid environmental matrices. This approach has been reported to provide satisfactory recoveries and reproducibility and has been successfully applied in studies investigating PFAS in biosolids and contaminated soils [2,16,16,17,17,18,18,21,21].

7.5.2. QuEChERS

The QuEChERS method, short for Quick, Easy, Cheap, Effective, Rugged, and Safe, was originally developed for pesticide extraction in food matrices but has since been adapted for environmental applications, including soil analysis [2]. Its effectiveness lies in the combination of acetonitrile-based extraction and salting-out partitioning using inorganic salts such as magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl), which facilitate efficient phase separation and promote the transfer of analytes into the organic phase [19] [20]

A fundamental part of QuEChERS is the clean-up step using dispersive solid-phase extraction (d-SPE), where specific sorbents like primary-secondary amine (PSA), C18, or graphitized carbon black (GCB) are used to selectively retain matrix interferences while allowing the analytes to remain in solution. This improves the quality and reproducibility of the final extract for chromatographic analysis. This method was included in the comparative design of the study due to its operational simplicity, low solvent use, and proven performance in multiresidue extractions.

7.6. Clean-up strategies

7.6.1. Solid-Phase Extraction (SPE)

SPE has become an essential technique in environmental analysis, especially for detecting trace levels of persistent pollutants like PFAS. Originally developed for water analysis, SPE is now widely used for cleaning and concentrating analytes from complex environmental matrices, including soil [22]. The method works by retaining either the analyte or the matrix interferences on a sorbent material packed into cartridges or columns. Its similarity with HPLC lies in the dependence on analyte–sorbent interaction and solvent polarity, making SPE a versatile option for selective sample treatment [22].

SPE process includes multiple sequential steps: conditioning, equilibration, sample loading, clean-up, elution, drying under nitrogen, reconstitution in solvent, and injection. These steps are visually summarized in **figure 4**, which illustrates the full workflow used for PFAS sample preparation prior to instrumental analysis.

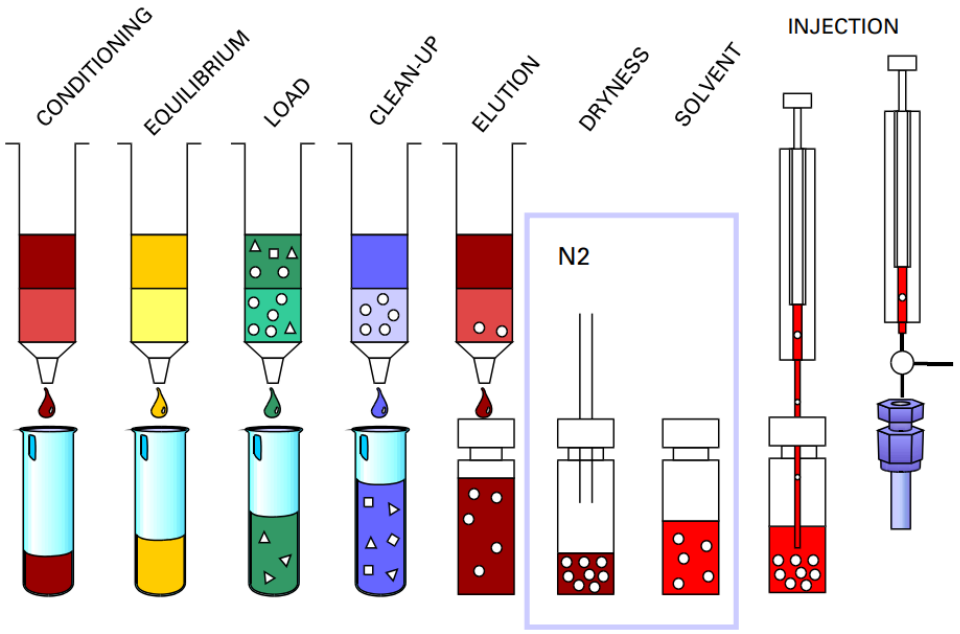

Figure 4. General schematic of the solid-phase extraction (SPE) procedure and sample preparation for instrumental analysis. The steps include conditioning, equilibration, sample loading, clean-up, elution, drying under nitrogen (N_2), reconstitution in solvent, and injection. Each step enables purification and concentration of the target analytes prior to analysis.

Numerous studies have demonstrated that incorporating SPE significantly reduces matrix interferences such as natural organic matter, salts, and co-extracted compounds, consequently improving chromatographic performance. For example, investigations on biosolids and sewage sludge have highlighted the importance of SPE in obtaining cleaner extracts and achieving more reproducible quantification across a broad range of PFAS [17]. Equally, research on landfill leachates and contaminated soils noted that SPE cartridges, especially those based on weak anion exchange (WAX) or hydrophilic–lipophilic balanced sorbents (HLB), play a crucial role in minimizing matrix effects and ensuring reliable detection of both short- and long-chain PFAS [8]. Overall, SPE has become a consolidated step in analytical workflows for PFAS in solid matrices, serving as an indispensable complement to initial solvent extraction strategies such as ultrasound-assisted or accelerated solvent extraction

Sorbents of solid-phase extraction

In PFAS analysis, sorbent-based clean-up steps are essential to minimize matrix effects and improve analytical reliability. Both SPE and d-SPE, as in QuEChERS methods, employ a variety of sorbents that selectively remove co-extracted interferences without compromising the recovery of target analytes. Graphitized carbon materials, anion-exchange phases, amine-functionalized sorbents, and reversed-phase C18 are among the most widely used.

a) ENVI-Carb™

ENVI-Carb™ (Supelclean) is a graphitized carbon black (GCB) sorbent used as a clean-up step in PFAS analysis, its flat carbon surface enables strong π–π and hydrophobic interactions with co-extracted organic substances like pigments, sterols, and aromatic compounds [20,23,24].

ENVI-Carb™ is employed as a clean-up rather than as a retention sorbent for PFAS. Soil extracts are passed through ENVI-Carb™ cartridges, and the loading fraction is collected, while matrix interferences are retained. This strategy allows PFAS compounds, which are mostly non-planar with SP^3 hybridized carbon atoms, to remain in solution and pass through the sorbent without significant loss.

b) Oasis® GCB/WAX

Oasis® WAX/GCB is a dual-layer cartridge that combines graphitized carbon black (GCB) with weak anion exchange (WAX) sorbent. The WAX sorbent is based on a polymeric reversed-phase backbone functionalized with weakly basic amine groups. These amines enable electrostatic interactions with anionic species, such as perfluorinated acids, which are negatively charged under near-neutral pH conditions. This makes WAX particularly suitable for retaining PFAS like PFOA, PFOS, and other carboxylic or sulfonic acid derivatives [2,25].

EPA Method 1633 specifies the use of WAX sorbents for the retention of PFAS, followed by a carbon-based cleanup step to remove co-extracted interferences, commonly performed with graphitized carbon materials such as ENVI-Carb [26]. Building on this framework, Waters has developed combined GCB/WAX cartridges that integrate both functions in a single step. In this configuration, PFAS are retained on the WAX phase through ionic interaction, while interfering organic compounds are captured by the GCB fraction. Elution with a basic solvent then disrupts the ionic binding, releasing the analytes into the final extract. This combined strategy has been reported to deliver reproducible cleanup and high recoveries for a broad spectrum of PFAS in complex solid matrices [25,26].

c) PSA (Primary Secondary Amine)

The PSA sorbent is a functionalized silica material containing both primary and secondary amine groups, commonly used in d-SPE during QuEChERS-based clean-up. PSA interacts with polar acidic interferences such as organic acids, sugars, and certain pigments, through hydrogen bonding and weak anion exchange, allowing more selective isolation of target compounds like PFAS from complex matrices [19,20].

PSA has been widely applied in PFAS extraction from biological and environmental samples. However, its use requires careful optimization, since it can interact with acidic PFAS under certain conditions, potentially reducing their recovery [19]. In fact, Liu et al. (2024) observed in fish samples that recoveries decreased when the PSA quantity exceeded 20 mg, suggesting that overloading the sorbent may increase PFAS retention [19].

PSA is employed during the d-SPE step following QuEChERS extraction, primarily to reduce matrix interferences without significantly affecting PFAS recovery. This aligns with method developments that balance cleanup efficiency with compound preservation [20]

d) C18

C18 is a reversed-phase sorbent composed of silica particles bonded to long-chain octadecyl (C18) groups. It is widely used in sample preparation to remove non-polar and hydrophobic compounds such as lipids and sugars from complex biological or environmental matrices [19,20].

C18 was used as part of the d-SPE step following the initial extraction. Its role was to reduce matrix interference previous LC-MS/MS analysis. This is especially important in soil samples, where co-extracted organic matter can affect analytical performance. C18 was selected based on its proven efficiency in similar clean-up protocols.

7.7. High-Performance Liquid Chromatography Tandem Mass Spectrometry

7.7.1. High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is one of the most commonly used techniques for analyzing PFAS in environmental samples. In this method, the sample is injected into a liquid stream (mobile phase) that flows under high pressure through a column packed with solid material (stationary phase). In most applications, a C18-bonded silica column is employed. This stationary phase is non-polar and interacts strongly with the hydrophobic perfluorinated chains of PFAS, while the polar functional groups of these molecules determine their elution depending on the solvent composition and pH. As a result, longer-chain sulfonates and carboxylates tend to be retained longer, whereas short-chain acids elute earlier. This separation principle corresponds to reversed-phase liquid chromatography (RPLC), which has become the dominant mode in PFAS analysis due to its robustness, selectivity, and compatibility with electrospray ionization [27].

Several studies illustrate the widespread adoption of RPLC with C18 columns for PFAS determination. Lorenzo et al. (2015) optimized methanolic extraction of soils and sediments and coupled the extracts to LC-MS/MS on C18, achieving good recoveries across diverse PFAS [2]. Bugsel and Zwiener (2020) applied a Poroshell EC-C18 column with high-resolution QTOF-MS to identify more than 60 PFAS in contaminated soils [28]. Similarly, C18 reversed-phase chromatography has been combined with QuEChERS extraction for PFAS analysis in biotic matrices such as fish and rice [19,29], confirming the versatility of this chromatographic approach across different sample types. Taken together, these works highlight that RPLC with C18 remains the most reliable and widely used separation strategy for PFAS, balancing effective retention, reproducibility, and broad applicability across environmental and biological samples.

In recent years, ultra-high-performance liquid chromatography (UHPLC) has progressively replaced conventional HPLC in environmental analysis. By using sub-2 µm particle columns, UHPLC provides sharper peak shapes, higher resolution, and shorter analysis times while reducing solvent consumption compared to traditional HPLC. These advantages are particularly relevant for PFAS analysis, where compounds with very different chain lengths and polarities must be separated within the same run. For this reason, UHPLC coupled with tandem mass spectrometry has become the standard approach in most laboratories, as also reflected in EPA Method 1633 for aqueous and solid samples, Water is often used as the base of the mobile phase, and organic solvents like

methanol (MeOH) or acetonitrile (ACN) are added to improve the separation. Sometimes additives such as ammonium acetate or formic acid are included to improve peak shape and reproducibility. The mixture and proportion of these solvents can vary depending on the compounds being analyzed [2,26].

One of the main issues when working with PFAS is background contamination from the chromatographic system itself. Several authors suggest adding a trap column to the HPLC system to help distinguish between real signals from the sample and residual contamination coming from the instrument. Additionally, blank injections and thorough cleaning with solvents are recommended to reduce carryover and improve reliability [2].

7.7.2. Mass Spectrometry

MS is an analytical technique that allows for the quantitative and qualitative determination of a large number of compounds, including PFAS. All mass spectrometers consist of three main components: the ionization source, the mass analyzer, and the detector, which are described below.

a) Ionization source.

This step is where the analytes from the chromatographic system are ionized. In LC, the two main ionization sources that operate under atmospheric pressure are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). In ESI configuration the sample is introduced through a capillary that is coated with a positively charged metal surface. and is surrounded by a stream of nebulizing gas, such as helium or nitrogen. This creates an aerosol of charged microdroplets at the end of the capillary. This aerosol is then carried by the gas flow toward the mass analyzer. As it moves forward, the droplets get smaller due to solvent evaporation, eventually leaving only the ions of the sample. These negatively charged ions are accelerated by high-voltage positive plates and directed toward the detector, as represented in **figure 5.**

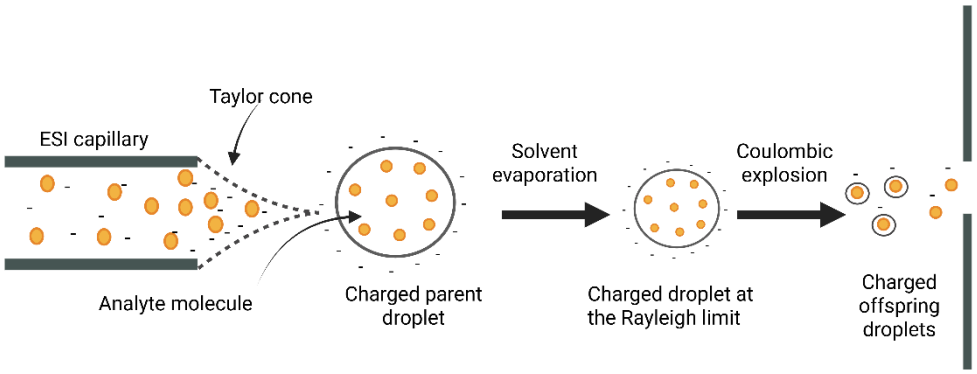

Figure 5. Schematic diagram of Electro spray ionization process

b) Mass analyzer.

There are different types of mass analyzers, they are responsible for separating and analyzing the ions generated in the ionization stage based on their mass-to-charge ratio (m/z). Low-resolution analyzers provide approximate values by rounding the m/z ratio to the nearest whole number. High-resolution analyzers, on the other hand, can determine this

ratio up to the fourth or fifth decimal place. Triple quadrupole (QqQ) instruments are by far the most frequently used for quantitative PFAS determination, as demonstrated in studies applying methanolic extraction followed by LC-MS/MS in soils and sediments [2] and in landfill leachate investigations [8]. In contrast, high-resolution instruments such as quadrupole time-of-flight (QTOF) mass spectrometers have been successfully applied for non-target and suspect screening, enabling the detection of dozens of PFAS in contaminated soils [28].

- **Triple quadrupole**

Are among the most commonly used instruments for contaminant analysis, mainly due to their high sensitivity, simplicity, and relatively low cost. One of the most widely used configurations is the triple quadrupole system, or QqQ shown in **figure 6.** This setup consists of three quadrupoles arranged in sequence. The first quadrupole (Q1) isolates the precursor ions of interest. These ions then enter the second quadrupole (q), also known as the collision cell, where they collide with an inert gas such as nitrogen, causing fragmentation. The resulting product ions are directed into the third quadrupole (Q3), which

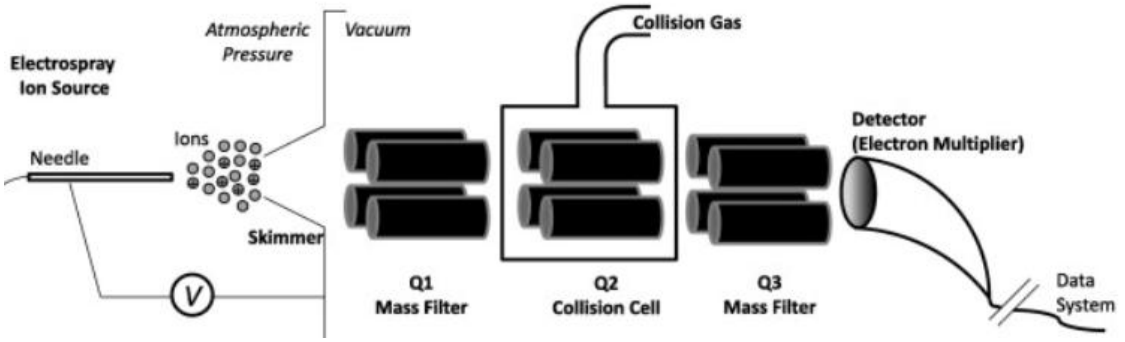

Figure 6. Schematic representation of a triple quadrupole (QqQ) mass spectrometer coupled with an electrospray ionization (ESI) source.

selects the specific fragments to be sent to the detector. The final output is the corresponding mass spectrum. Although QqQ systems are not high-resolution, their performance and reliability make them the preferred choice for routine contaminant analysis in most laboratories [30].

<u>Dynamic multiple reaction monitoring (dMRM)</u>

One of the most widely applied data acquisition strategies in triple quadrupole mass spectrometry is dynamic multiple reaction monitoring (dMRM) is applied to improve the selectivity and sensitivity of the analysis. This technique allows the instrument to focus on specific ion transitions only when the target compounds are expected to elute, based on their retention times. By limiting the monitoring to these time windows, the method reduces background noise and increases the measurement accuracy. Unlike conventional MRM, which monitors all transitions continuously, dMRM optimizes the use of instrument time and enhances performance, especially when working with complex mixtures or large compound lists.

Figure 7 illustrates the principle of MRM in a triple quadrupole system. The first quadrupole (Q1) selects the precursor ion, which then undergoes fragmentation in the collision cell (Q2). The resulting product ions are filtered by the third quadrupole (Q3), allowing only a specific transition to reach the detector. This targeted approach increases the signal-to-noise ratio and provides high specificity for quantifying trace-level compounds in complex matrices [31].

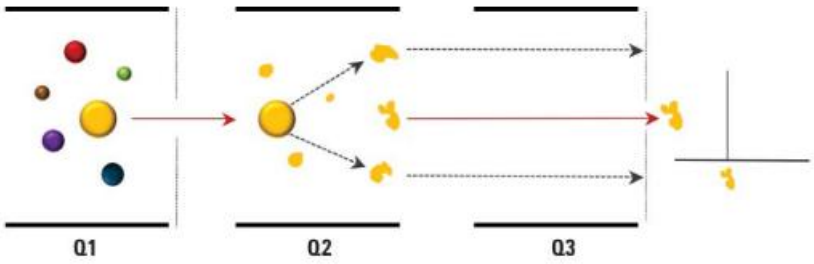

Figure 7. Schematic representation of dynamic Multiple Reaction Monitoring (dMRM) using a triple quadrupole mass spectrometer.

c) Detector

Once the ions are separated by their m/z ratio, they reach the detector. Its role is to measure and amplify the ion current. The most commonly used detector is the electron multiplier. The total ion signals recorded forms what is known as the total ion chromatogram (TIC), which represents all the ions detected during the analysis.

8 OBJECTIVES

General Objective

To evaluate and compare different extraction and clean-up strategies for the determination of PFAS in contaminated soils, with the aim of maximizing analyte coverage, increasing recoveries, and reducing RSD values.

Specific Objectives

1. To test the Oasis WAX/GCB cleanup step adapted from the Waters protocol [25], in order to evaluate whether this approach improves recovery and precision.
2. To design and evaluate a QuEChERS-based protocol for soil, adapted from methods originally applied to other solid and biological matrices (sediments, biosolids, fish, oysters), as a potential faster and simpler alternative.
3. To compare the three methods in terms of recovery and precision, and to identify the advantages and limitations of each approach, with particular focus on the optimization needs of the QuEChERS protocol.
4. To determine the most suitable method for routine application in environmental laboratories, balancing analytical robustness, reproducibility, and operational simplicity.

9 EXPERIMENTAL PART

9.1. Chemicals, reagents, and standard solutions

Isotopically Labeled Internal Standards

A total of 14 isotopically labeled per- and polyfluoroalkyl substances (PFAS) were used in this study as internal standards. These compounds were labeled with carbon-13 (13C) and were obtained from Wellington Laboratories Inc. (Ontario, Canada). Each compound was provided at high chemical purity (>98%) and was used to ensure robust quantification through internal standard calibration, compensating for matrix effects and instrumental variability during LC-MS/MS analysis.

The mixture of isotopically labeled internal standards used in this study included the following compounds: $13C_4$-perfluorobutanoic acid (PFBA), $13C_2$-6:2 fluorotelomer sulfonic acid (6:2 FTSA), $13C_2$-perfluorododecanoic acid (PFDoDA), $13C_2$-perfluorotetradecanoic acid (PFTDA), $13C_3$-perfluorobutanesulfonic acid (PFBS), $13C_3$-perfluorohexanesulfonic acid (PFHxS), $13C_4$-perfluoroheptanoic acid (PFHpA), $13C_5$-perfluoropentanoic acid (PFPeA), $13C_6$-perfluorodecanoic acid (PFDA), $13C_7$-perfluoroundecanoic acid (PFUnDA), $13C_8$-perfluorooctanoic acid (PFOA), $13C_8$-perfluorooctanesulfonic acid (PFOS), $13C_8$-perfluorooctanesulfonamide (PFOSA), and $13C_9$-perfluorononanoic acid (PFNA).

Regents and materials

All solvents and reagents used in this study were of analytical or LC-MS grade to ensure high sensitivity and minimal background contamination during PFAS extraction and quantification. Acetonitrile (LiChrosolv®, LC-MS grade, Merck, Germany) and methanol (Optima®, LC/MS, Fisher Chemical, USA) were used for sample extraction and preparation of standard solutions. Ultrapure water (18.2 MΩ·cm) was produced using a Milli-Q system (Merck Millipore, Germany) and used throughout the analytical method. Glacial acetic acid (EMSURE®, Merck), formic acid (≥99%, LC-MS grade, VWR Chemicals, USA), and ammonium hydroxide solution (25%) (Alfa Aesar, Germany) were used to adjust the pH during extraction and elution steps. Ammonium acetate (LiChropur® and EMSURE®, Merck) and magnesium sulfate heptahydrate (Merck) served as buffering agents and drying salts, respectively, and were used in specific clean-up protocols.

To reduce the risk of PFAS contamination, all plasticware and consumables used during sample processing were certified PFAS-free. SPE and sorbent materials were selected for their suitability in PFAS analysis and included Oasis® GCB/WAX cartridges (Waters, USA), ENVI-Carb™ cartridges (Supelco, USA), Supelclean™ PSA sorbent (Sigma-Aldrich, USA), and Kieselgel 60 C18 (Carl Roth, Germany).

In addition, further plastics, consumables, and auxiliary instruments employed throughout the study are presented below:

- **Plastics and Consumables:**
  - 50 mL polypropylene conical tubes – Falcon® (REF 352070)
  - Eppendorf Safe-Lock Tubes 2.0 mL – Eppendorf (Order no.: 0030 120.094)
  - Screw cap tubes 92 × 15.3 mm – Sarstedt (REF 60.610)
  - Pasteur pipettes 7 mL – VWR (REF 612-1681)
  - Syringe filters, 0.2 µm, 17 mm – Thermo Scientific
  - LC vials and caps, 1.5 mL PP 9 mm screw, SureSTART™ – Thermo Scientific
- **Auxiliary Equipment**
  - Analytical balance: Sartorius Cubis, max. 120 g, readability 0.01 mg
  - Top-loading balance: Sartorius, readability 0.01 g
  - Centrifuge (microtubes): Eppendorf 5430 R, compatible with 1.5–2.0 mL tubes
  - Centrifuge (conical tubes): Hermle Z 206 A, compatible with 50 mL tubes
  - Orbital shaker: IKA Vibrax VXR basic
  - Vortex mixer: IKA MS2 Minishaker
  - Pipettes: Rainin Pipet-Lite XLS, variable volume (including 500 µL–5 mL)
  - Nitrogen evaporator: Manifold with 12 positions
  - Ultrasonic cleaner: VWR USC - TH
  - Vacuum manifold: Manual system for SPE cartridge filtration.

9.2. Aqueous Film-Forming Foam Soil Sample

The analysis targeted 38 PFAS compounds. Detailed information on these analytes, including chemical structures, abbreviations, molecular formulas, CAS numbers, and molecular weights, is provided in **appendix 1.**

A soil sample was obtained from the grounds of a fire station in Korsør Nor, Denmark, by standard sampling protocols. The site was selected due to its known elevated PFAS concentrations, which allowed the analysis to be conducted without fortification with native compounds. This approach reduced sample preparation steps and ensured that the PFAS had been naturally adsorbed onto the soil matrix over an extended period, in contrast to spike samples where native compounds have limited interaction time with the matrix.

Prior to analysis, the sample was first subjected to partial drying. The main portion was placed on aluminum foil and left to dry under ambient conditions for approximately three days. After drying, the sample was homogenized and subsequently stored in a dehumidifier to prevent moisture reabsorption, allowing maximum water removal while minimizing analyte loss. An additional aliquot was oven-dried at 300 °C to achieve complete water removal. This high-temperature drying was performed exclusively for dry weight calculations, as it may cause losses of certain analytes, particularly short-chain PFAS. The dry weight factor obtained from this aliquot was applied to correct the final sample mass.

9.3. Experimental Procedure

To optimize PFAS determination in soils, three methods were evaluated. The first relied on ENVI-Carb™, a graphitized carbon sorbent currently used as the reference protocol at Aarhus University for soil and sediment samples. The second is an adaptation of this protocol, in which the cleanup step was replaced by the procedure proposed by Waters, using Oasis® WAX/GCB cartridges that combine weak anion exchange with graphitic carbon

and are designed to enhance recoveries while reducing matrix interferences. Since both of these approaches are highly labor-intensive and time-consuming, we also tested a third strategy based on the QuEChERS method. Originally developed for pesticides, QuEChERS has been successfully adapted for PFAS extraction in biological and food matrices, offering a faster and simpler workflow. This comparative evaluation aimed to determine which strategy provides the best balance between recovery efficiency, reduction of matrix effects, and overall practicality for routine PFAS determination in soils.

9.3.1. Method 1: Ultrasound Extraction + ENVI-Carb Cleanup

Sample Pre-treatment

In this method, 1 gram of previously homogenized soil was weighed into a 10 mL polypropylene tube. The sample was spiked with a mixture of ^13C-labeled PFAS internal standards. These standards were added to methanol and brought to room temperature before use to ensure proper mixing and analyte recovery.

Extraction Procedure

The extraction was performed in two consecutive steps using methanol as solvent. First, 5 mL of methanol was added to the soil, and then the sample was shaken vigorously for 5 minutes. The sample was then placed in an ultrasonic bath for 15 minutes. After sonication, the sample was centrifuged at 1000 rpm for 5 minutes, and the resulting supernatant was transferred to a clean polypropylene tube. The extraction was repeated using a second 5 mL of methanol, following the same procedure: shaking, ultrasonic treatment, and centrifugation. The supernatant from the second extraction was combined with the first.

The combined extract, approximately 10 mL, evaporated under a gentle nitrogen flow until the volume was reduced to approximately 2 mL. Prior to solid-phase extraction (SPE), 50 µL of glacial acetic acid were added to acidify the concentrated extract.

Solid-Phase Extraction (SPE) Clean-up

The clean-up was carried out using a Supelclean™ ENVI-Carb™ SPE cartridge (100 mg, 1 mL, 100–400 mesh; Supelco). Before sample loading, the cartridge was conditioned with 2 mL of acetonitrile and then 1 mL of 20% glacial acetic acid in acetonitrile. The acidified 2 mL extract was then loaded directly onto the SPE column. To ensure complete analyte recovery, the original sample tube was rinsed three times with 1 mL of methanol per rinse. Each rinse was passed through the SPE cartridge, and the loadings were combined.

The collected eluate evaporated to dryness under nitrogen. The dry residue was reconstituted in 500 µL of a methanol/5 mM ammonium acetate buffer solution. Finally, the sample was filtered using a nylon syringe filter directly into a 1.5 mL polypropylene LC vial with screw cap for a following instrumental analysis. The entire workflow of this method is summarized in **figure 8.**

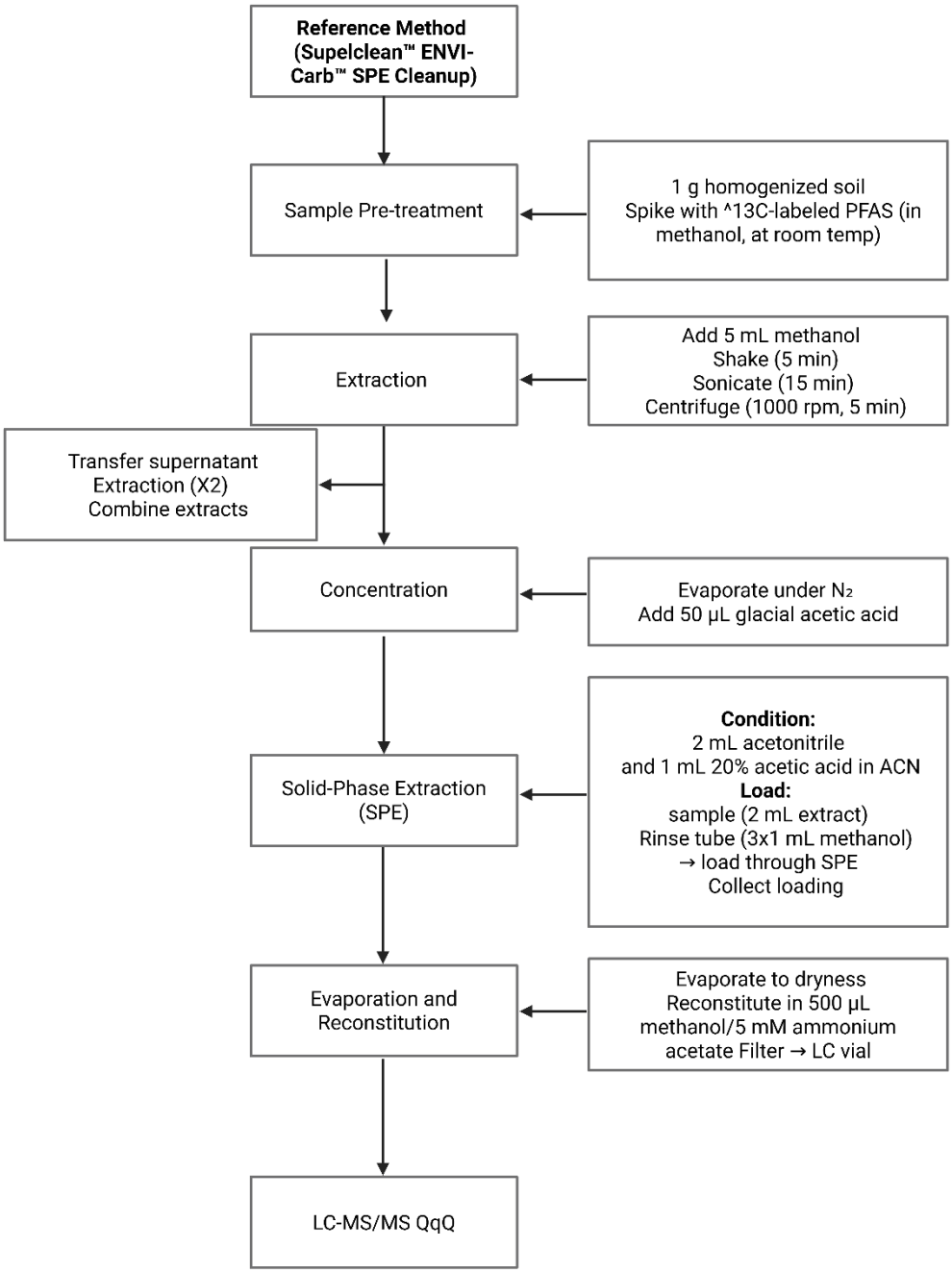

Figure 8. Workflow diagram of the reference method using Supelclean™ ENVI-Carb™ SPE cartridges for the extraction and clean-up of PFAS in soil samples.

9.3.2. Method 2: Ultrasound Extraction + Oasis WAX/GCB Cleanup

Sample Pre-treatment

The soil used was previously homogenized and stored under appropriate conditions until extraction. For each sample, 1 gram of soil was weighed into a 10 mL polypropylene tube. Before the extraction, the sample was spiked with 50 µL of a 100 µg/L solution of 13C-labeled PFAS internal standards prepared in methanol. The internal standard mixture was brought to room temperature before use.

Extraction Procedure

For the extraction step, methanol was used as solvent. First, 5 mL of methanol were added to the spiked soil. The tube was shaken at 1000 rpm for 5 minutes in the shaker and then placed in an ultrasonic bath for 15 minutes. After sonication, the sample was centrifuged for 5 minutes at 1000 rpm, and the resulting supernatant was transferred to a clean polypropylene tube. Following the first extraction, the remaining soil often formed a compact solid. A second extraction was carried out by adding another 5 mL of methanol and breaking up the solid with a spatula. The same extraction steps were repeated (shaking, sonication, and centrifugation), and the new supernatant was combined with the first extract. The total extract (≈10 mL) was then evaporated under a nitrogen flow until reaching an approximate volume of 7 mL Once concentrated, ultrapure water was added to bring the total volume to 47 mL Finally the solution was vortexed, and the pH was adjusted to 6.5 ± 0.5 using 50% formic acid or ammonium hydroxide.

Solid Phase Extraction (SPE)

For the clean-up, Oasis GCB/WAX cartridges (6 cc, 200/50 mg; Waters) were used. The cartridges were first conditioned with 15 mL of 1% ammonium hydroxide in methanol, followed by 5 mL of 3 M formic acid. Then the entire 47 mL sample was loaded onto the cartridge at a flow rate of ≈ 5 mL/min. After loading, the cartridge was washed with 10 mL of reagent water, followed by 5 mL of a 1:1 solution of 0.1 M formic acid and methanol. The sample tube was rinsed in both steps to ensure complete transfer and clean elution. The cartridge was then dried for 15 seconds using vacuum. For elution, 5 mL of 1% ammonium hydroxide in methanol were used, followed by a second elution with 2 mL of the same solution used to rinse the sample container. Both fractions were collected together [25]. A schematic of the SPE setup used during sample clean-up is presented in **figure 9.**

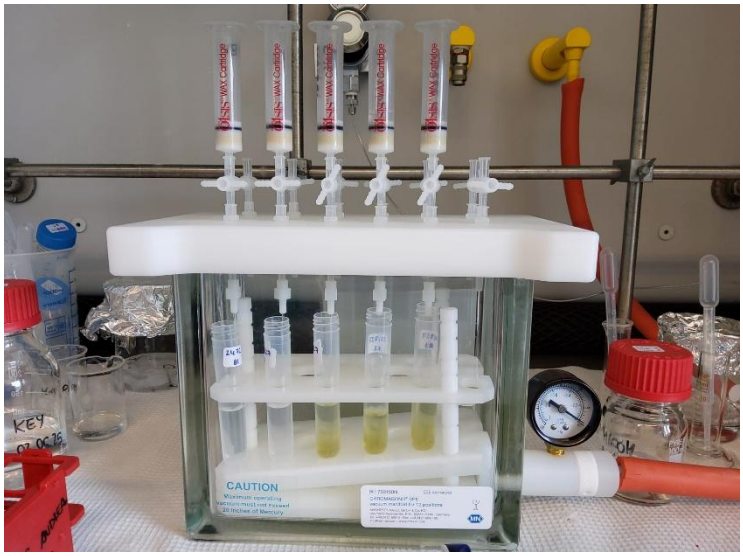

Figure 9. Solid-phase extraction (SPE) setup using Oasis® WAX/GCB cartridges. The image shows multiple cartridges connected to a vacuum manifold during the sample clean-up process. This configuration enables simultaneous processing of multiple samples, improving throughput and consistency.

The eluate was evaporated to dryness under nitrogen flow at 35ºC and redissolved in 500 µL of a methanol/5 mM ammonium acetate buffer solution. Finally, the extract was filtered using a nylon syringe filter directly into a 1.5 mL polypropylene LC vial with screw cap, ready for instrumental analysis. The entire workflow of this method is summarized in **figure 10**.

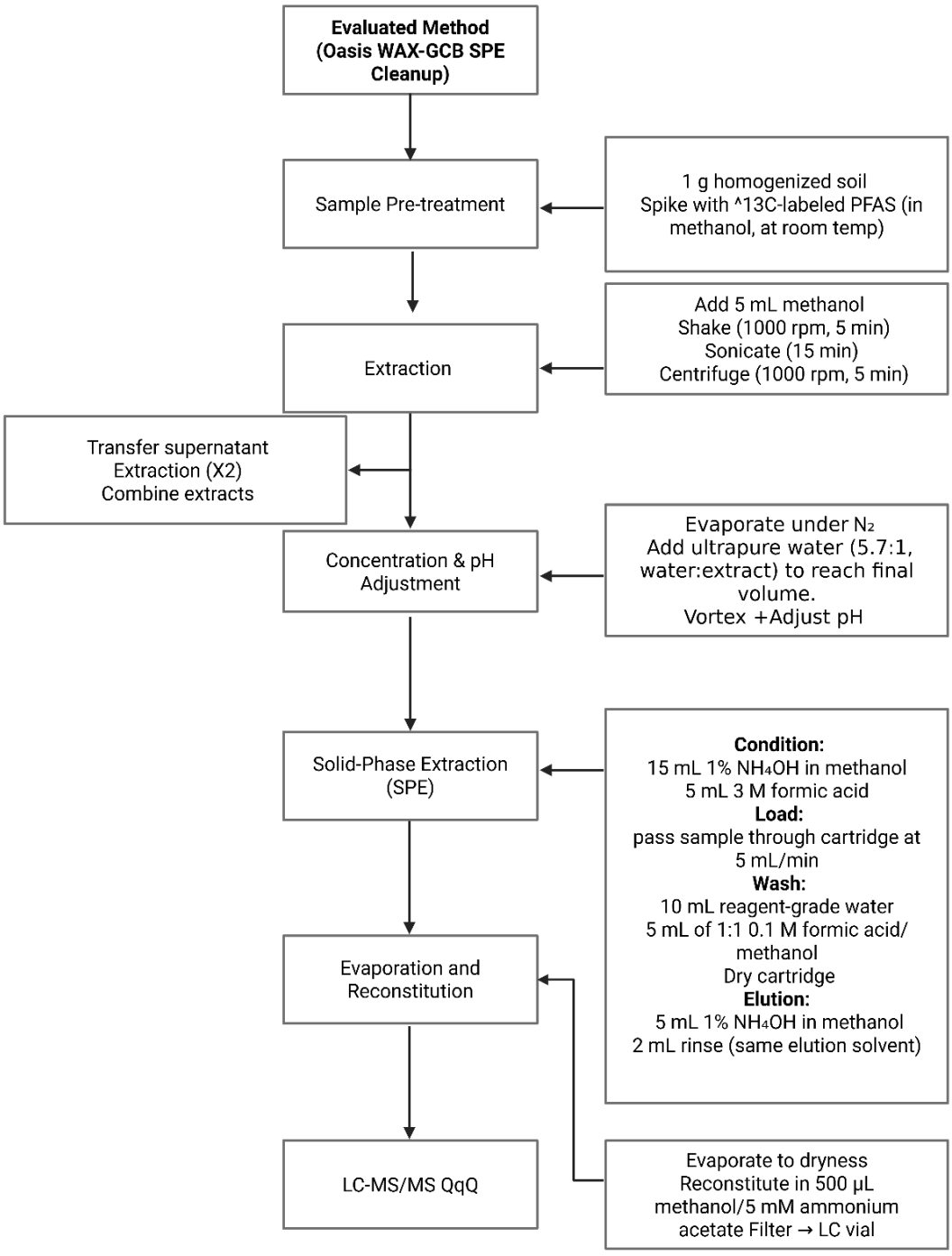

Figure 10. Workflow diagram of the evaluated method using Oasis® WAX-GCB SPE cartridges for the extraction and clean-up of PFAS in soil samples.

9.3.3. Method 3: QuEChERS Extraction Method

For the QuEChERS method, two different sorbent mixtures were tested during the clean-up step. The first mixture combined anhydrous $MgSO_4$ with PSA, a sorbent containing basic amine groups. Since these groups can also interact with PFAS acids, there is a risk of losing part of the analytes during extraction. For this reason, a second protocol was tested in which C18 was added to the PSA mixture, following a procedure already described in previous QuEChERS applications. The addition of C18 improves the removal of hydrophobic interferences from the soil matrix while helping to maintain PFAS recoveries.

QuEChERS Extraction Protocol I

In this method, 1 gram of previously homogenized soil was weighed into a 50 mL polypropylene centrifuge tube. The internal standard mixture, consisting of 50 µL of a 100 µg/L solution of 13C-labeled PFAS in methanol, was added to each sample. Prior to use**,** the internal standard solution was allowed to reach room temperature before use.

For the extraction step, 10 mL of deionized water were added to the sample and vortexed briefly. Following this, 10 mL of acidified acetonitrile (1% acetic acid) were added, and the sample was vortexed again to form a uniform soil suspension. Next, the extraction salts (1.5 g of sodium chloride and 2 g of anhydrous magnesium sulfate) were added to the tube. The mixture was vortexed. Once mixed, the samples were centrifuged for 5 minutes at 4000 rpm.

After centrifugation, the samples were placed in a freezer at –20 °C for approximately 60 minutes. This freezing step helped facilitate the separation of the supernatant by solidifying the water phase, as shown in **figure 11.** Once frozen, the upper organic phase (supernatant) was carefully transferred to a clean 50 mL polypropylene centrifuge tube.

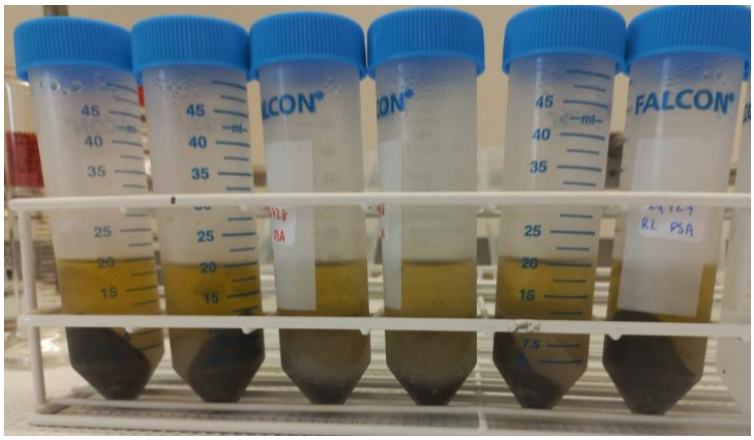

Figure 11. Soil sample extracts in 50 mL polypropylene centrifuge tubes after centrifugation and freezing at –20 °C. The image shows phase separation, with the water-rich lower phase solidified, allowing for easy collection of the upper organic phase.

For the clean-up step, 1200 mg of anhydrous $MgSO_4$ and 400 mg of PSA sorbent were added to the supernatant. The mixture was vortexed thoroughly to ensure adequate dispersive solid-phase interaction. The tube was then centrifuged at 1000 rpm for 10 minutes. After this, the clarified supernatant was transferred to a 15 mL polypropylene centrifuge tube.

The final extract was evaporated to dryness under a nitrogen flow. To reconstitute the dry residue, 500 µL of a methanol solution containing 5 mM ammonium acetate were added. Instead of filtration, the sample was placed on a shaker to assist with dissolution. The mixture was then transferred to smaller centrifuge tubes and centrifuged at 10,000 rpm for 15 minutes at 20 °C. Finally, the supernatant was carefully transferred into LC vials for instrumental analysis.

QuEChERS Extraction Protocol II

To evaluate the effect of an additional clean-up sorbent, QuEChERS Extraction Protocol II followed the same procedure described above, with the only modification being the addition of 400 mg of C18 along with PSA and $MgSO_4$ during the purification step. See **figure 12** for an overview of the modified protocol.

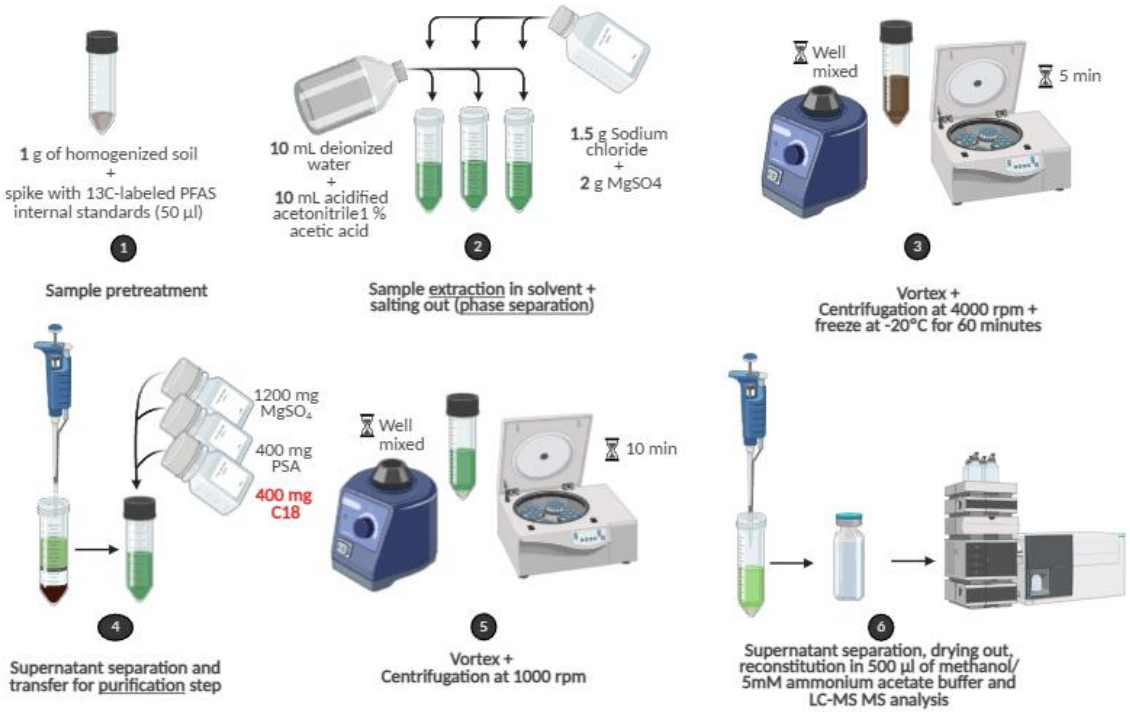

Figure 12. Workflow diagram of QuEChERS Extraction Protocol II for PFAS in soil

9.4. UHPLC-MS/MS analysis

The separation and quantification of target PFAS were performed using an ultra-high-performance liquid chromatography system (UHPLC) coupled to a triple quadrupole tandem mass spectrometer (UHPLC–MS/MS). The chromatographic system consisted of an Agilent 1260 Infinity II and 1290 Infinity UHPLC, coupled to an Agilent 6495 LC/TQ Triple Quadrupole Mass Spectrometer, operating in negative electrospray ionization mode (AJS ESI). Data acquisition and processing were carried out using Agilent MassHunter Workstation software.

Chromatographic separation was achieved on a ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm), maintained at 40 °C. The mobile phase A consisted of 5 mM ammonium

acetate in water, and mobile phase B consisted of 5 mM ammonium acetate in 90 % methanol. The gradient program is shown in **table 1**, at a constant flow rate of 0.4 mL/min. An injection volume of 10 µL was used, with automated direct injection (full loop) via the autosampler.

Table 1. Chromatographic gradient program used for PFAS analysis Chromatographic gradient program used for PFAS analysis. The mobile phase A consisted of 5 mM ammonium acetate in water, and mobile phase B consisted of 5 mM ammonium acetate in 90 % methanol.

Time (min)	%A	%B	FLOW (ml/min)
1,5	95	5	0,1
4	50	50	0,1
8	5	95	0,1
9,6	5	95	0,1
9,61	0	100	0,1

The ion source was operated with a capillary voltage of 2500–3000 V, gas temperature of 130 °C, nebulizer gas pressure of 25 psi, sheath gas temperature of 375 °C, and sheath gas flow rate of 13 L/min.

Detection was performed in dynamic multiple reaction monitoring (dMRM) mode, monitoring two transitions per analyte (precursor/product ion): one for quantification and one for confirmation. The corresponding parameters for 13C-labelled internal standards are provided in **table 2**, and optimized precursor ions, product ions, collision energies, and fragmentor voltages for all native PFAS are listed in **table 3.**

Table 2. Optimized parameters of the dMRM acquisition method for 13C-labelled internal standards.

ISTD	TR (min)	PRECURSOR ION (m/z) (Q)	FRAGMENTOR (V)	DAUGHTER ION 1 (m/z) (Q1)	COLLISION ENERGY 1 (EV)
13C4-PFBA	5,004	217	166	172	7
13C2-6-2 FTSA	8,513	429	166	409	27
13C2-PFDODA	9,838	615	166	570	11
13C2-PFTDA	10,561	715	166	670	12
13C3-PFBS	6,766	302	166	80	43
13C3-PFHXS	8,068	402	166	80	48
13C4-PFHPA	8,037	367	166	322	7
13C5-PFPEA	6,544	268	166	223	7
13C6-PFDA	9,281	519	166	474	8
13C7-PFUNDA	9,571	570	166	525	11
13C8-PFOA	8,538	421	166	376	7
13C8-PFOS	8,926	507	166	80	52
13C8-PFOSA	9,589	506	166	78	36
13C9-PFNA	8,944	472	166	427	8

Table 3. Optimized parameters of the dMRM acquisition method for each native compound

	COMPOUND	TR (min)	PRECURSOR ION (m/z) (Q)	FRAGMENTOR (V)	DAUGHTER ION 1 (m/z) (Q1)	COLLISION ENERGY 1 (EV)	DAUGHTER ION 2 (m/z) (Q2)	COLLISION ENERGY 2 (EV)
PFSAS	PFBS	6,768	299	166	90	36	80	43
	PFPeS	7,507	349	166	99	36	80	40
	PFHxS	8,068	399	166	99	43	80	48
	4-PFecHS	8,478	461	166	381	28	99	32
	PFOS	8,927	499	166	99	48	80	52
	PFNS	9,256	549	166	99	51	80	59
	PFDS	9,538	599	166	99	55	80	63
	PFUdS	9,796	649	166	99	63	80	50
	PFDoS	10,092	699	166	99	63	80	67
	PFTrS	10,477	749	166	99	63	80	50
PFCAS	PFBA	5,006	213	166	169	7	–	–
	PFPeA	6,545	263	166	219	7	–	–
	PFHxA	7,427	313	166	270	7	119	23
	PFHpA	8,045	363	166	319	7	169	19
	PFOA	8,538	413	166	219	15	169	19
	PFNA	8,945	463	166	419	8	219	16
	PFDA	9,281	513	166	469	8	219	16
	PFUnDA	9,571	563	166	519	11	319	16
	PFDoDA	9,838	613	166	569	11	169	28
	PFTrDA	10,158	663	166	619	11	169	32
	PFTDA	10,561	713	166	669	12	169	36
	PFHxDA	11,518	813	166	769	12	269	28
	PFODA	12,272	913	166	869	16	369	28
EMERGING PFASS & PRECURSORS	4:2 FTSA	7,359	327	166	307	19	80	36
	6:2 FTCA	8,206	377	166	313	1	293	16
	6:2 FTUCA	8,184	357	166	293	12	243	36
	6:2 FTSA	8,513	427	166	407	27	80	48
	PFHpS	8,536	449	166	99	44	80	48
	8:2 FTCA	9,090	477	166	413	1	393	12
	9Cl-PF3ONS	9,119	531	166	351	27	83	27
	8:2 FTUCA	9,074	457	166	393	12	343	44
	7:3 FTCA	9,067	441	166	337	11	317	23
	PFOSA	9,590	498	166	169	34	78	36
	8:2 FTSA	9,298	527	166	507	31	80	51
	10:2 FTCA	9,709	577	166	513	4	493	16
	11Cl-PF3OUdS	9,682	630.9	166	451	35	83	35
	HFPO-DA	7,625	285	166	185	19	169	3

Analyte identification was based on matching retention times with those of the corresponding isotopically labelled standards. All chromatograms corresponding to a fortified sample at 40 ng/mL are included in **appendix 2.**

Quantification was based on internal calibration using 13C-labelled PFAS as surrogate standards. Each target analyte was assigned to the most structurally similar isotopically labelled compound. The recoveries were calculated by dividing the sample area for the isotopic compound by the average of the calibration curve areas for the isotopic compound. Because recoveries were calculated from the peak areas of the internal standards, all analytes associated with the same labelled compound share the same recovery. The detailed assignment of analytes to their internal standards is provided in **table 4.**

For each soil sample, three independent subsamples were prepared and extracted in parallel. Each extract was then analyzed in duplicate by UHPLC–MS/MS, resulting in six replicate measurements per sample. The MassHunter Workstation software, Agilent, was configured to automatically correct analyte concentrations based on the assigned internal standards and the corresponding recoveries. The only additional correction performed manually was the adjustment for the dry weight of the sample, applied after the software-reported concentrations. Final results are expressed as ng/g on a dry weight basis, and method variability was evaluated from the six replicate determinations. It is reported as relative standard deviation (RSD), calculated as 100 × (standard deviation / mean concentration).

To evaluate the sensitivity of the analytical method, LOD and quantification LOQ were established for all target compounds. Depending on the availability of reference values, two different approaches were applied. For compounds with certified or assigned concentrations, LODs were derived from the variability of replicate analyses of an intercalibration material. For the remaining compounds, LODs were determined instrumentally based on signal-to-noise ratios. In both cases, LOQs were defined as three times the corresponding LODs. The resulting values are summarized in **table 4.**

Table 4. Assignment of native PFAS analytes to their corresponding 13C-labelled internal standards (ISTDs) and Limits of detection (LOD) and quantification (LOQ) expressed in ng/g wet weight for all target PFAS

ANALYTE	ISTD	LOD (ng/g)	LOQ (ng/g)
4:2 FTSA	13C2-6-2 FTSA	0,15	0,45
6:2 FTSA	13C2-6-2 FTSA	0,15	0,45
8:2 FTSA	13C2-6-2 FTSA	0,01	0,03
PFDODA	13C2-PFDoDA	0,07	0,21
PFDOS	13C2-PFDoDA	0,03	0,09
PFTRDA	13C2-PFDoDA	0,19	0,57
PFTRS	13C2-PFTDA	0,03	0,09
PFTDA	13C2-PFTDA	0,08	0,24
PFHXDA	13C2-PFTDA	0,1	0,3
PFODA	13C2-PFTDA	0,1	0,3
PFBS	13C3-PFBS	0,1	0,3
HFPO-DA	13C3-PFHxS	0,1	0,3
DONA	13C3-PFHxS	0,1	0,3
PFHXS	13C3-PFHxS	0,16	0,48
4-PFECHS	13C3-PFHxS	0,02	0,06
9CL-PF3ONS	13C3-PFHxS	0,02	0,06
PFBA	13C4-PFBA	0,1	0,3
PFHPA	13C4-PFHpA	0,07	0,21
PFHXA	13C5-PFHxA	0,1	0,3
6:2 FTCA	13C5-PFHxA	0,05	0,15
6:2 FTUCA	13C5-PFHxA	0,1	0,3
PFPEA	13C5-PFPeA	0,1	0,3
PFPES	13C5-PFPeA	0,1	0,3
7:3 FTCA	13C6-PFDA	0,05	0,15
PFDA	13C6-PFDA	0,13	0,39
10:2 FTCA	13C6-PFDA	0,05	0,15
PFUNDA	13C7-PFUnDA	0,1	0,3
11CL-PF3OUDS	13C7-PFUnDA	0,02	0,06
PFUDS	13C7-PFUnDA	0,03	0,09
PFOA	13C8-PFOA	0,09	0,27
8:2 FTCA	13C8-PFOA	0,05	0,15
8:2 FTUCA	13C8-PFOA	0,1	0,3
PFHPS	13C8-PFOS	0,01	0,03
PFOS	13C8-PFOS	0,05	0,15
PFDS	13C8-PFOS	0,08	0,24
PFOSA	13C8-PFOSA	0,09	0,27
PFNA	13C9-PFNA	0,18	0,54
PFNS	13C9-PFNA	0,04	0,12

9.5. Quality control and quality assurance

For each batch of samples, two procedural blanks were extracted and analyzed to control background contamination introduced during extraction from various laboratory sources. One spiked standard was analyzed to simultaneously verify the repeatability of the analytical method and detect any systematic errors. A delay column, identical in stationary phase to the main analytical column but with a slightly larger particle size, was installed between the pump and the injector to separate any contamination originating from the pump, which is constructed of PTFE.

For instrumental quality control, a methanol wash was performed at regular intervals between sample runs to control contamination in the LC system. Quantification of analytes was based on internal calibration using the corresponding 13C-labelled standard together with the native compound. The calibration curve consisted of a concentration series of 0.1, 1, 5, 10, 20, and 40 ng/mL, with a fixed amount of internal standard added to each level and was used for the quantification of target analyte concentrations.

10 RESULTS AND DISCUSSION

Three analytical protocols for PFAS extraction from soil were compared. The first corresponds to the method currently used for PFAS determination in this matrix, which includes an EnviCarb-based clean-up step. Initially, the objective was to evaluate only a part of the Waters procedure by modifying this clean-up step; however, since the two SPE-based methods are time-consuming and highly complex, they are less suitable for routine analysis. Therefore, two additional QuEChERS-based protocols were also evaluated, as QuEChERS offers a simpler workflow and considerable time savings compared to traditional SPE. First, the two QuEChERS protocols were compared against each other, and the best-performing one was subsequently evaluated against Methods 1 and 2.

10.1. Comparative Evaluation of QuEChERS Protocols for PFAS Extraction in Soil

Two QuEChERS-based protocols were evaluated. QuEChERS offers a simpler workflow and considerable time savings compared to the other methods, which could be advantageous for routine applications.

10.1.1. Recovery performance

For PFSAs, short-chain members (PFBS, PFPeS, and PFHxS) presented the highest recoveries, as shown in **figure 13**, reaching the upper part of the observed range. Mid-chain compounds, particularly PFNS, showed the lowest recoveries within the group. Long-chain PFSAs such as PFUdS, PFDoS, and PFTrS showed noticeably lower recoveries.

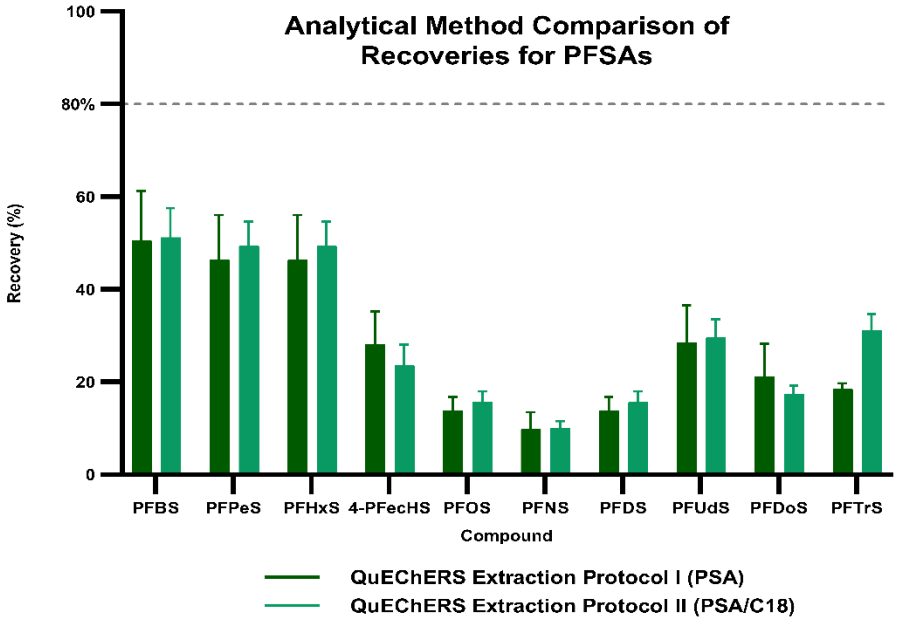

Figure 13. Recovery values (%) for perfluoroalkyl sulfonic acids (PFSAs) obtained with QuEChERS Extraction Protocol I (PSA) and Protocol II (PSA/C18).

A different pattern was observed for PFCAs, as shown in **figure 14**. Overall recoveries were similar across most compounds, with values generally between 20 and 40%, and lower than those obtained for PFSAs. An exception was PFNA, which showed the lowest recoveries among this group, although we found no evidence to explain this behavior. This result suggests that PFCAs remain challenging to extract from soil.

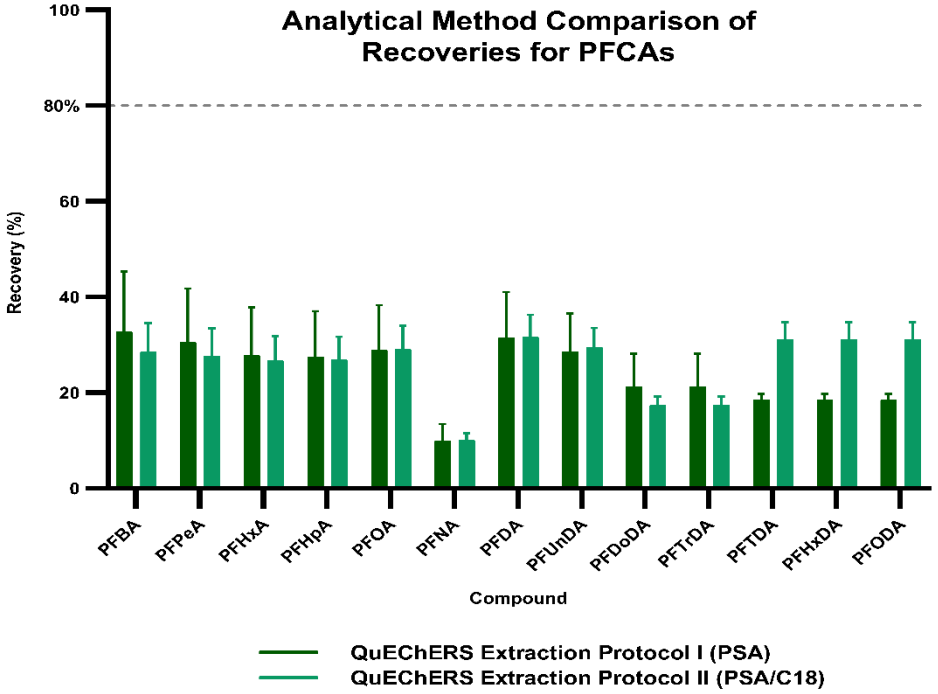

Figure 14. Recovery values (%) for perfluoroalkyl carboxylic acids (PFCAs) obtained with QuEChERS Extraction Protocol I (PSA) and Protocol II (PSA/C18).

For emerging PFAS and precursors, the pattern was more variable. Certain compounds, particularly the fluorotelomer sulfonates, achieved the highest recoveries among all analytes, as illustrated in **figure 15.** Others showed poor extraction performance, which can be linked not only to differences in chain length but also to their functional group chemistry. Unlike PFCAs and PFSAs, this group includes both fully anionic compounds and partially neutral species such as PFOSA and chlorinated sulfonamide derivatives. The presence of such precursors makes their extraction and detection more dependent on polarity, molecular structure, and ionization efficiency in ESI, as also noted in previous studies [32]. In addition, most emerging PFAS and their precursors do not have matching isotopically labeled standards, and their higher structural diversity compared to PFCAs and PFSAs makes recovery measurements less accurate. Therefore, recoveries for this group should be viewed with caution.

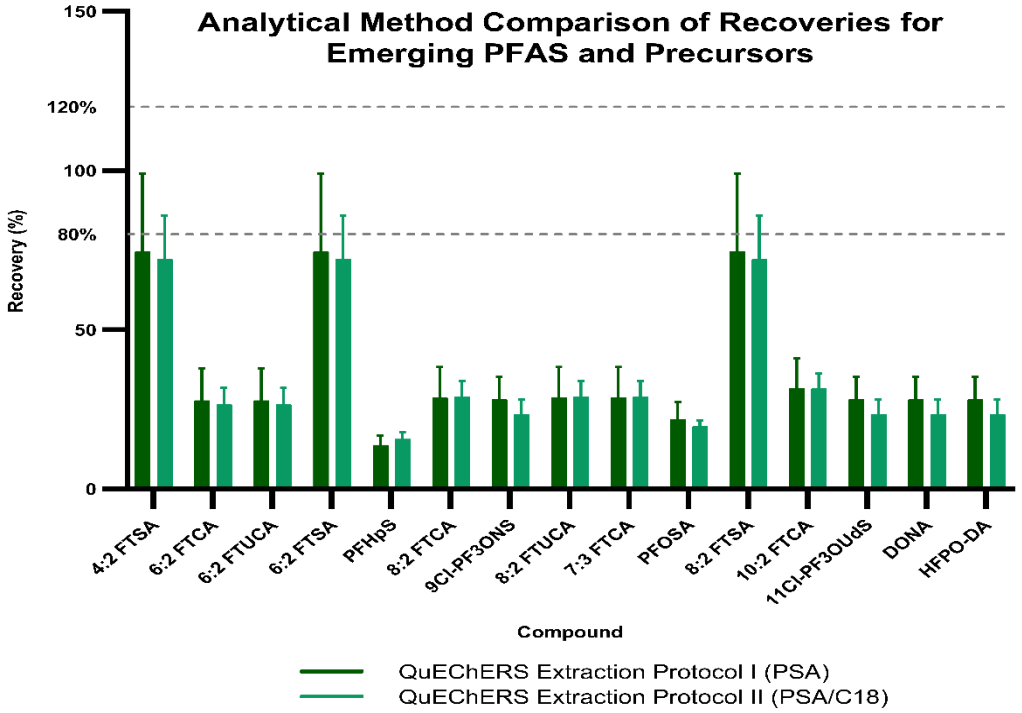

Figure 15. Recovery values (%) for emerging PFAS and precursors obtained with QuEChERS Extraction Protocol I (PSA) and Protocol II (PSA/C18).

The use of C18 in Protocol II led to slight improvements for certain compounds, particularly some mid- and long-chain species such as PFUdS and PFTrS in PFSAs, PFTDA, PFODA, and PFHxDA in PFCAs. However, when looking at the overall results, recoveries obtained with Protocol I (PSA) were slightly higher. The improvements observed with C18 are likely due to the removal of additional hydrophobic co-extractives, which can reduce matrix interferences during LC–MS/MS analysis. In fact, the study on biological matrices reported that adding C18 to the QuEChERS clean-up reduced matrix effect by more than 50% and led to more stable recoveries across replicate injections [20].

In general, recoveries for most compounds were moderate to low, with values ranging mainly between 10 and 60%, and none of the analytes reached the accepted optimal range of 80–120%. This indicates that, under the tested conditions, extraction efficiency from soil

is limited for all groups. Similar findings have been reported in other studies. QuEChERS applied to biosolids showed recoveries frequently outside the optimal range, with around 20% of analytes falling below 40% or above 120% [33], while EPA 1633 consistently achieved values between 70 and 120% [17]. In another study, methanol-based extractions provided considerably higher efficiencies than those using acetonitrile, with recoveries up to 70–130% for several PFAAs, whereas ACN-based workflows showed clearly lower values [8]. Likewise, work on mammalian tissue confirmed that methanol alone provided the highest recoveries (86–114%), while acetonitrile extracts performed substantially worse [34]. Taken together, these studies confirm that QuEChERS tends to underperform in complex solid matrices, and that the use of acetonitrile as the extraction solvent is a likely factor limiting recoveries, particularly for long-chain PFAS.

10.1.2. Precision evaluation

The precision, determined by the RSD values, was evaluated using QuEChERS Protocols I and II. For each AFFF-contaminated soil sample, three independent subsamples were extracted in parallel, and each extract was analyzed in duplicate by UHPLC–MS/MS, giving six replicate measurements per sample. Only isotopically labelled standards were spiked, while native PFAS were quantified directly from the contaminated matrix.

For PFSAs, the results showed a clear difference between the two QuEChERS protocols, as shown in **figure 16**. In Protocol I (PSA), most compounds had RSD values above 20%, and for several analytes such as PFDS, PFDoS, and PFNS, variability even exceeded 40%. By contrast, in Protocol II (PSA/C18), all compounds were below the 20% limit, showing better reproducibility across the group. In Protocol I, long-chain PFSAs such as PFUdS, PFDoS, and PFTrS tended to show higher variability than the short-chain members, whereas in Protocol II no clear difference was observed between short- and long-chain compounds.

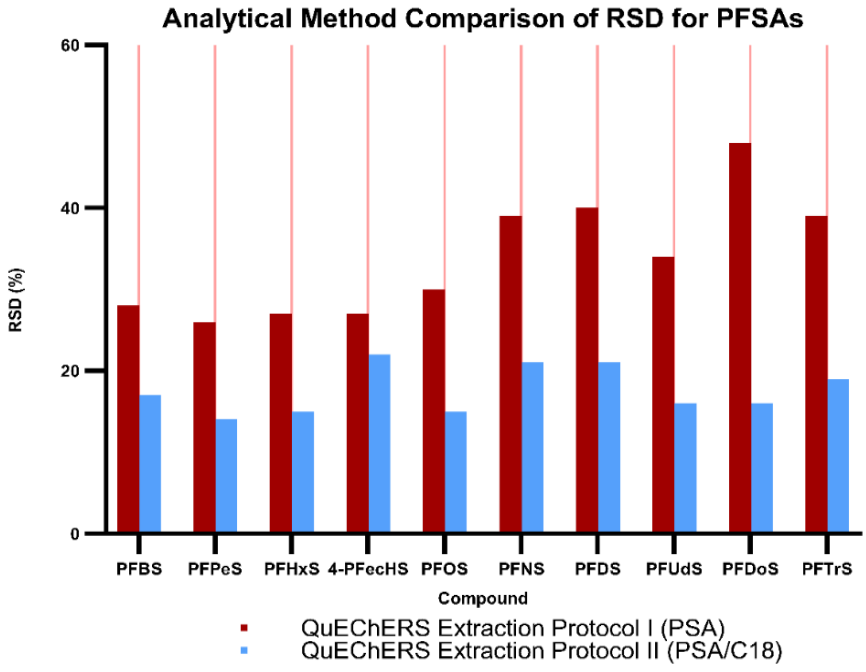

Figure 16. Relative standard deviation (RSD, %) for perfluoroalkyl sulfonic acids (PFSAs) obtained with QuEChERS Extraction Protocol I (PSA) and Protocol II (PSA/C18).

For PFCAs, the results again highlighted the differences between the two QuEChERS protocols, as shown in **figure 17.** In Protocol I (PSA), RSD values were generally high, with most compounds above 20% and some, such as PFBA and PFTDA, approaching or exceeding 40%. In contrast, Protocol II (PSA/C18) consistently produced RSD values below 20% for all analytes. An interesting observation in Protocol II is that variability tended to decrease as the chain length increased, with the longest-chain PFCAs (PFDoDA, PFTrDA, PFHxDA) showing the lowest RSD values in the group.

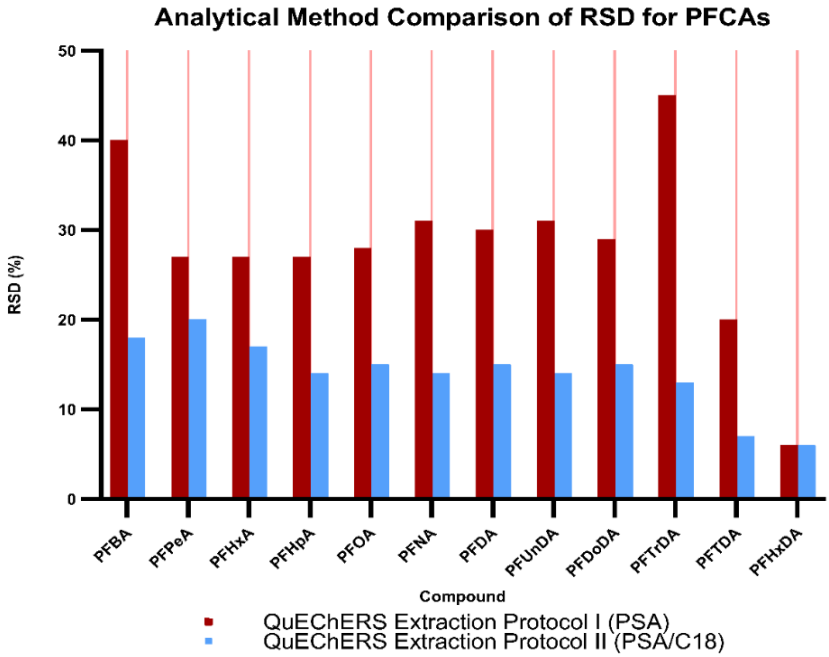

Figure 17. Relative standard deviation (RSD, %) for perfluoroalkyl carboxylic acids (PFCAs) obtained with QuEChERS Extraction Protocol I (PSA) and Protocol II (PSA/C18).

For emerging PFAS and precursors, variability was more compound-dependent, as shown in **figure 18.** No clear trend was observed across the group, indicating that precision for emerging PFAS depends strongly on individual compound properties rather than chain length alone.

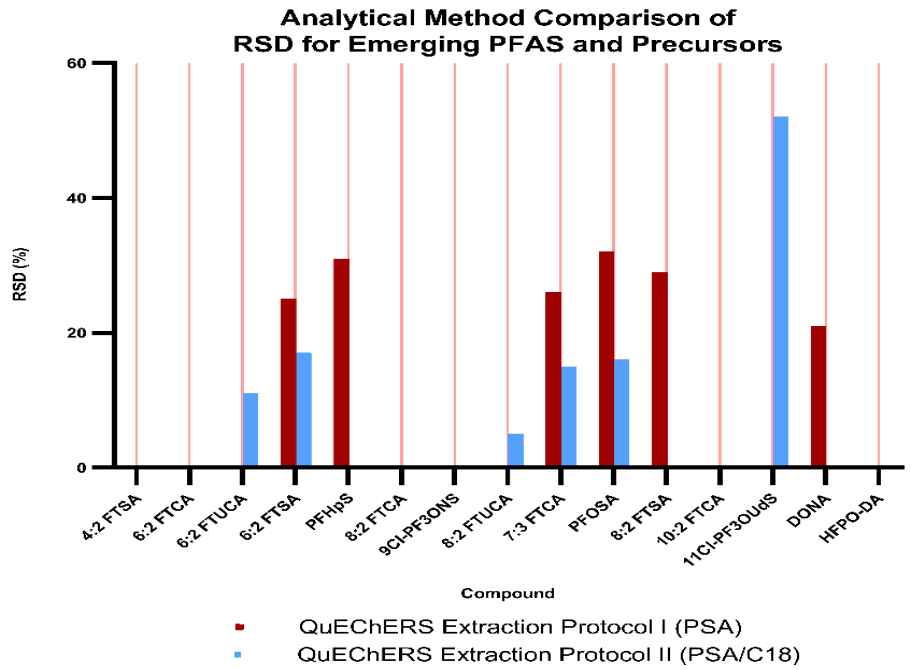

Figure 18. Relative standard deviation (RSD, %) for emerging PFAS and precursors with QuEChERS Extraction Protocol I (PSA) and Protocol II (PSA/C18).

In general, RSD values obtained with QuEChERS were lower in Protocol II (PSA/C18), where most compounds remained below the commonly accepted 20% limit, while Protocol I (PSA) showed higher variability, with several analytes exceeding 30–40%. This indicates that the addition of C18 improves reproducibility by reducing co-extracted interferences. Similar observations have been reported in other studies. A modified QuEChERS protocol applied to biological matrices also demonstrated that incorporating C18 reduced matrix effects and lowered RSD values across replicates [20]. Likewise, in contaminated soil samples, variability was shown to increase with chain length, particularly for PFCAs, due to stronger hydrophobic interactions with organic matter [16,32]. Taking together, these findings confirm that QuEChERS Protocol II provides more consistent precision, especially compared to Protocol I, and that the use of C18 is effective in reducing variability in complex solid matrices.

10.1.3. Method selection for further comparison

As a result of these observations, Protocol II (PSA/C18) was selected as the QuEChERS method to be compared with the other two methods. Considering all results, recovery values obtained with Protocol II were comparable to those of Protocol I, but the use of C18 provided a clear advantage in terms of precision, with consistently lower RSD values across most analytes. This improvement in reproducibility makes Protocol II more robust for further method comparison.

10.2. Comparison of QuEChERS, Ultrasound Extraction with ENVI-Carb Cleanup, and Ultrasound Extraction with Oasis WAX/GCB Cleanup

The three methods were evaluated, and their recovery performance was compared across three PFAS groups: PFSAs, PFCAs, and emerging PFAS/precursors. Each group was considered separately, with particular attention to compounds that showed better performance with one protocol than with the others. The discussion addresses possible reasons for these differences, relating them to the specific chemical properties of the compounds and their interactions with the soil matrix. Finally, a comparison across all PFAS is presented to summarize overall recovery performance.

10.2.1. Recovery performance

Figure19 shows the recovery results for PFSAs obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS. Short-chain PFSAs (PFBS, PFPeS, PFHxS) displayed the highest recoveries across all three methods. WAX/GCB and ENVI-Carb reached values close to or above 70%, while QuEChERS showed slightly lower values, generally between 50% and 60%. For mid-chain PFSAs (PFOS, PFNS), all three methods achieved moderate recoveries, with WAX/GCB and ENVI-Carb generally showing similar performance.

In the case of long-chain PFSAs (PFDS, PFUdS, PFDoS, PFTrS) results varied considerably among the methods, but a clear difference was observed for PFUdS and PFTrS. Both WAX/GCB and QuEChERS achieved measurable recoveries for these two compounds, while ENVI-Carb did not detect these compounds at all.

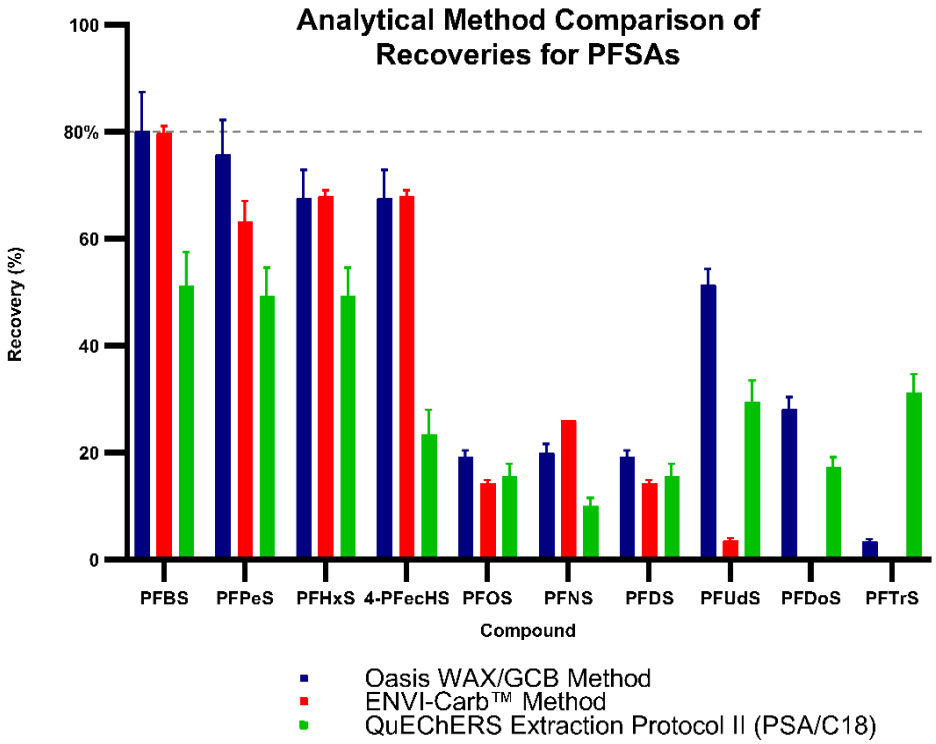

Figure 19. Recovery values (%) for perfluoroalkyl sulfonic acids (PFSAs) obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS.

Figure 20 shows the recovery results for PFCAs obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS. The general pattern was similar to that observed for PFSAs, Short-chain PFCAs (PFBA, PFPeA, PFHxA) displayed the highest recoveries overall, with WAX/GCB reaching values close to or above 80%, ENVI-Carb slightly lower, and QuEChERS showing the lowest values, generally between 20% and 40%. For mid-chain PFCAs (PFHpA, PFOA, PFNA, PFDA), WAX/GCB continued to show the highest recoveries, followed by ENVI-Carb, with QuEChERS remaining lower for most compounds. From PFDA to PFTDA, WAX/GCB generally predominated over the other two methods. In the case of the last three long-chain PFCAs (PFHxDA, PFODA, PFTrDA), recoveries were low, but QuEChERS achieved the highest measurable recoveries, while ENVI-Carb did not detect these compounds and WAX/GCB showed only minimal recovery.

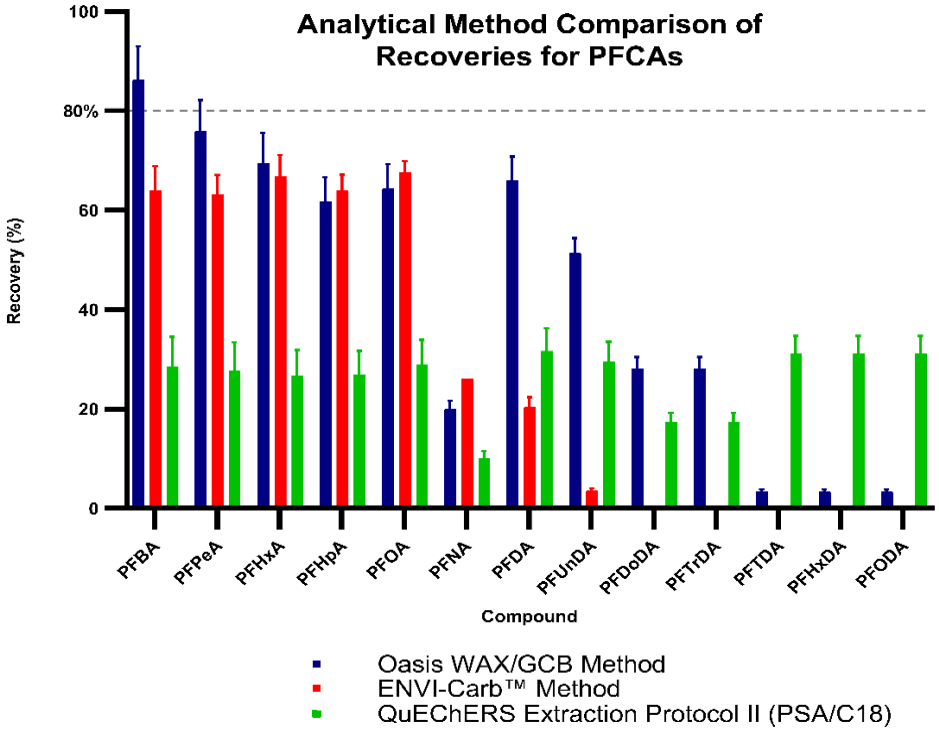

Figure 20. Recovery values (%) for perfluoroalkyl carboxylic acids (PFCAs) obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS PSA/C18.

The recovery results for emerging PFAS and precursors obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS. In contrast to PFSAs and PFCAs, the pattern observed here does not appear to be related to chain length but rather to other chemical properties such as polarity, functional group or molecular structure, as shown in **figure 21.** Fluorotelomer sulfonates (FTSA) showed the highest recoveries with WAX/GCB and ENVI-Carb, exceeding 150%. Although these values are high, they fall outside the generally accepted optimal range of 80–120% and are not necessarily indicative of good analytical performance. Such over-recoveries may result from matrix effects, ionization enhancement, or other analytical interferences. QuEChERS also achieved high recoveries for these compounds but remained within or closer to the optimal range. For fluorotelomer carboxylic acids and unsaturated carboxylic acids (FTCA, FTUCA), recoveries were moderate across all three methods, with WAX/GCB and ENVI-Carb generally achieving higher values than QuEChERS. Neutral or

partially fluorinated precursors (PFOSA, 9Cl-PF3ONS, 11Cl-PF3OUDS, DONA) displayed more

Across all groups, the recoveries obtained showed clear trends that can be linked to the

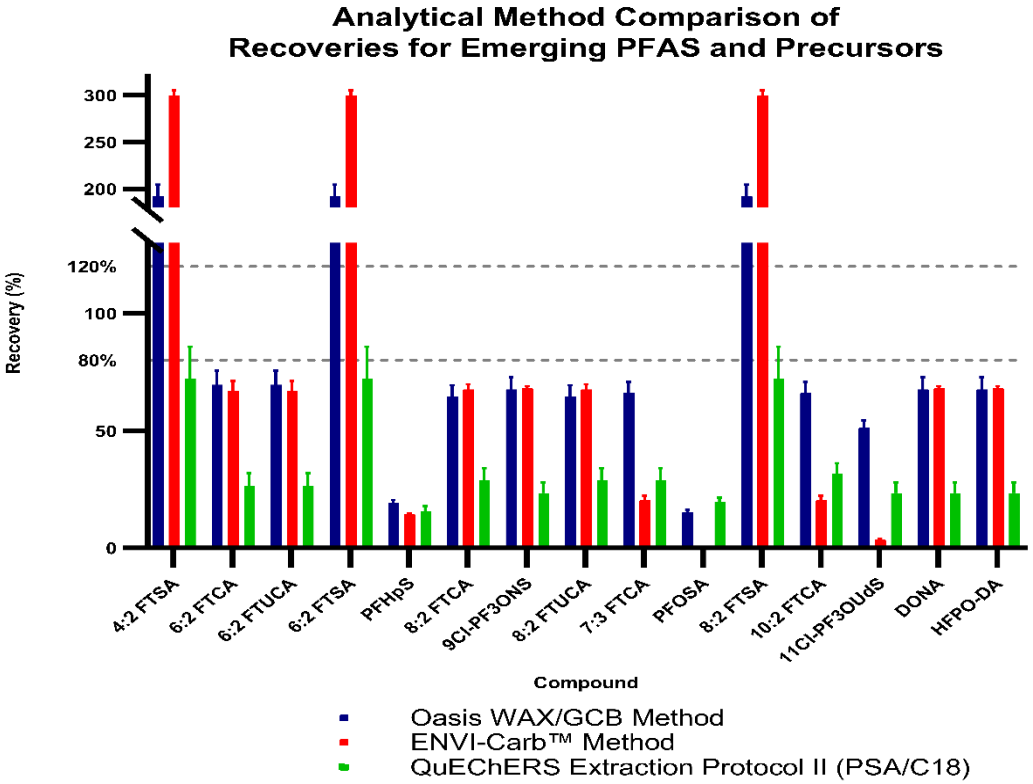

Figure 21. Recovery values (%) for emerging PFAS and precursors obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS PSA/C18

chemical properties of PFAS and their interactions with soil. Compounds with shorter carbon chains consistently presented higher recoveries, while those with longer chains were more difficult to extract. Similar behavior was reported by Nguyen et al. (2020), who observed that short-chain PFAS display lower distribution coefficients (K_d) and therefore higher mobility in soils, favoring their desorption during extraction. At the molecular level, Luft et al. (2022) showed that interactions between PFAS and soil components such as clay and humic substances become stronger as chain length increases, with PFOS binding more strongly than PFOA and both more than PFBA [10]. These findings are consistent with our results, where short-chain compounds reached the upper recovery range, while the long-chain homologues showed considerably lower values.

Another factor that may contribute to the poor performance of long-chain PFAS is their low solubility in aqueous media. After extraction and clean-up, the extracts were evaporated and reconstituted in 500 µL of a methanol/5 mM ammonium acetate buffer solution (50:50, v/v). Studies on mammalian tissues demonstrated that this solvent mixture supports the recovery of short- and medium-chain PFAS, but is less suitable for long-chain homologues, which are poorly soluble in water-rich conditions [34]. In that study, recoveries were within 86–114% for most compounds, but the longest-chain PFAS (M2PFTeDA) showed very low values. Increasing the methanol fraction improves solubility of long-chain compounds but compromises the separation of short-chain ones, while reducing methanol has the

opposite effect [34]. This balance explains why short-chain PFAS generally performed better in our study.

An exception to these patterns was observed for long-chain PFAS in the QuEChERS protocol, which showed comparatively higher recoveries than with ENVI-Carb or WAX/GCB. This can be attributed to the presence of fulvic and humic acids in the QuEChERS extracts. In the SPE-based methods, an additional filtration step was applied to remove humic fractions, while QuEChERS involved only centrifugation, and the extract retained a yellowish colour, suggesting that dissolved organic matter was still present. Long-chain PFAS are known to associate strongly with humic and fulvic acids, with interaction energies between 2 and 8 kcal mol^{-1}, and larger molecules showing a stronger tendency to aggregate within humic structure [10] s. As a result, when humic fractions are removed by filtration, a portion of long-chain PFAS is eliminated together with them, leading to lower apparent recoveries. In QuEChERS, their presence in solutions likely allowed more long-chain PFAS to be detected.

When looking at the emerging PFAS and precursors, the recoveries were more variable across compounds. Fluorotelomer sulfonates (FTSAs) showed the highest values, exceeding the upper acceptable range, while other species such as PFOSA or DONA had clearly lower recoveries. This variability reflects the mixed chemistry of this group, which includes both ionic and neutral species, making their extraction performance less uniform compared to PFCAs and PFSAs. This behavior is consistent with previous findings. Nguyen et al. (2020) reported that functional group chemistry plays a critical role in PFAS partitioning, with fluorotelomer species showing weaker sorption than fully fluorinated carboxylates or sulfonates, which may explain the high recoveries of FTSAs observed here. By contrast, neutral or partially ionizable compounds such as PFOSA or DONA are more strongly affected by matrix interactions, leading to lower and more variable recoveries. Similar trends have been described for PFAS interactions with dissolved organic matter, where neutral species display greater variability compared with fully anionic compounds [10,11,32].

The Oasis WAX/GCB method achieved recoveries comparable to those obtained with ENVI-Carb and, in some cases, was able to extract compounds that were not detected with the latter. An explanation for this behavior is related to the chemistry of the sorbent. WAX contains amine functional groups that can interact with the PFAS molecules, creating a competition between the sorbent and the soil matrix for binding these analytes. This competitive interaction may facilitate the release of PFAS from the soil into the extract. In contrast, ENVI-Carb primarily acts as a physical filter and adsorbent with high affinity for aromatic and planar compounds, but without functional groups capable of competing for PFAS binding. As a result, when ENVI-Carb is used, PFAS are more likely to remain bound to the soil matrix, leading to lower recoveries for certain analytes.

Finally, differences between sulfonic and carboxylic acids were also evident. In our results, PFSAs achieved higher recoveries than PFCAs. Nguyen et al. (2020) reported that PFCAs exhibit lower Kd values than PFSAs, reflecting greater mobility and weaker retention in soils [32]. By contrast, Luft et al. (2022) showed that PFSAs, due to their bulky sulfonate head group and the ability to form ordered layers on soil surfaces, display stronger sorption than

comparable PFCAs [10]. This mechanistic explanation supports our observations, where the stronger sorption of PFCAs likely contributed to their lower recoveries compared with PFSAs.

10.2.2. Precision evaluation

For each AFFF-contaminated soil sample, three independent subsamples were extracted in parallel, and each extract was analyzed in duplicate by UHPLC–MS/MS, giving six replicate measurements per sample. Only isotopically labelled standards were spiked, while native PFAS were quantified directly from the contaminated matrix.

Figure 22 shows the relative standard deviation (RSD, %) for PFSAs obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS. For ENVI-Carb, direct comparison is limited because several compounds were not detected. However, for those recovered, ENVI-Carb generally showed the lowest RSD values. Between WAX/GCB and QuEChERS, WAX/GCB method consistently had lower RSDs for all compounds in the group except PFDoS, where QuEChERS achieved slightly lower value.

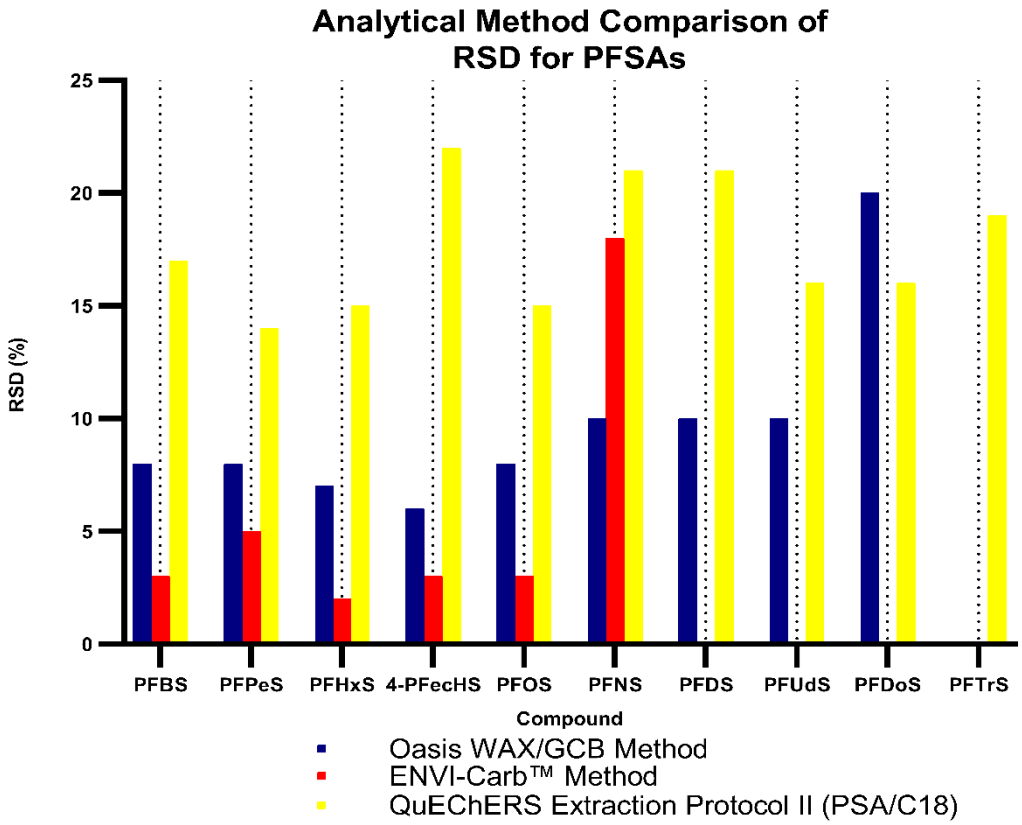

Figure 22. Relative standard deviation (RSD, %) for perfluoroalkyl sulfonic acids (PFSAs) obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS PSA/C18.

Figure 23 shows the RSD for PFCAs obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS. As with the PFSAs, direct comparison with ENVI-Carb is limited because several compounds were not detected. For the compounds with data, ENVI-Carb generally showed the lowest RSD values. Between WAX/GCB and QuEChERS, WAX/GCB had lower RSDs for almost all compounds in the group, with the exception of PFHxDA, where

QuEChERS showed better precision. This compound was present at concentrations close to the detection limit, which likely explains the higher variability observed.

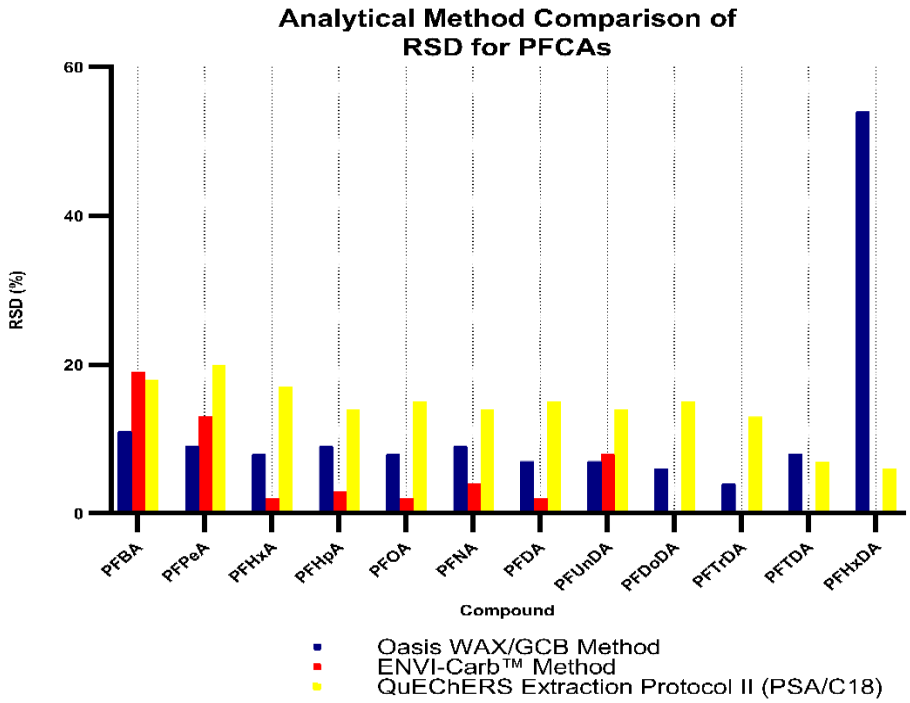

Figure 23. Relative standard deviation (RSD, %) for perfluoroalkyl carboxylic acids (PFCAs) obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS PSA/C18.

Figure 24 presents the RSD for emerging PFAS and precursors obtained with Oasis WAX/GCB, ENVI-Carb and QuEChERS. A considerable number of compounds in this group were not detected, which is consistent with their classification as emerging PFAS or precursors that that can be transformed into PFSAs or PFCAs during environmental processes. Among the compounds that were detected, variability between methods was

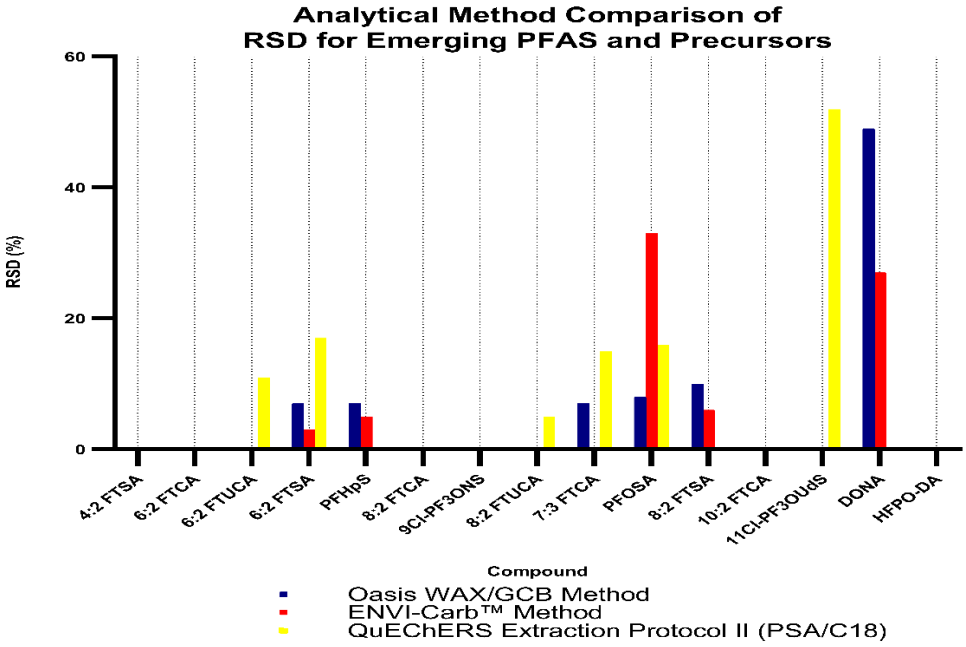

Figure 24. *Relative standard deviation (RSD, %) for emerging PFAS and precursors obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS PSA/C18.*

generally higher than that observed for the other two PFAS groups. Overall, the variability observed for these compounds did not seem to follow a consistent pattern across methods, making it difficult to draw clear conclusions from the results. Considering all three PFAS groups, most compounds showed RSD values below the commonly accepted limit of 20%, indicating acceptable precision for the majority of the dataset.

10.2.3. General Evaluation of Method Performance

The large number and variety of compounds make it difficult to select a single method that performs equally well for all PFAS. Therefore, it is necessary to find a balance between recovery, precision, and the ability to cover as many analytes as possible. Across the results, no compound reached the optimal recovery range of 80-120%, with the exception of PFBA using the WAX/GCB method, which was within range, and PFBS, which was close to it. In general, recoveries for PFAS in soil tend to be lower than in other matrices, mainly due to their strong sorption to soil organic matter and mineral surfaces [7,32].

ENVI-Carb achieved good recoveries for the compounds it extracted, but it was unable to extract a large portion of the target analytes, which restricts its applicability in cases where comprehensive PFAS coverage is needed. QuEChERS showed potential, as it was able to extract compounds that ENVI-Carb failed to recover, in some cases with relatively higher recoveries, although still below generally acceptable levels. However, it requires considerable optimization. This could include testing different sorbent ratios or adding an extra sorbent such as GCB. Oasis WAX/GCB offered the most balanced performance, extracting nearly all compounds with minimal chromatographic interferences. Although there is still room for improvement, this method currently provides the best compromise between recovery, precision, and analyte coverage. For this reason, the next logical step would be to evaluate the full Waters protocol, rather than only the clean-up stage, to determine whether optimizing the complete workflow can further enhance overall performance.

10.3. Concentration analysis across the three methods

The AFFF-impacted soil sample from Korsør Nor was analyzed using the three extraction–cleanup methods under evaluation: QuEChERS, ultrasound extraction with ENVI-Carb cleanup, and ultrasound extraction with Oasis® WAX/GCB cleanup. The concentration results highlight clear differences between the three approaches.The concentration results for the PFAS highlight clear differences between the three extraction–clean-up methods. For PFSAs, as shown in **figure 25,** PFBS and PFPeS were consistently quantified across the three methods, showing comparable concentrations. For PFNS and PFDS, Waters WAX/GCB and QuEChERS produced similar values, while ENVI-Carb showed a poor performance. PFHxS and PFOS were present at relatively high concentrations in the soil sample, but due to the chromatographic method limitations they could only be quantified

reliably up to about 20 ng/g. The remaining PFSAs mostly, long-chain, were either detected below this range or close to the limit of quantification (LOQ), depending on the method.

As shown in **figure 26,** The PFCAs compounds that could be quantified, The method showed a consistent order; QuEChERS reported the highest concentrations, Waters WAX/GCB gave intermediate values, and ENVI-Carb the lowest. Among the short and mid-chain PFCAs, at least two methods provided measurable results, with QuEChERS generally producing the highest concentrations and Waters WAX/GCB often close behind. ENVI-Carb usually produced lower concentrations, with several compounds not detected. The only

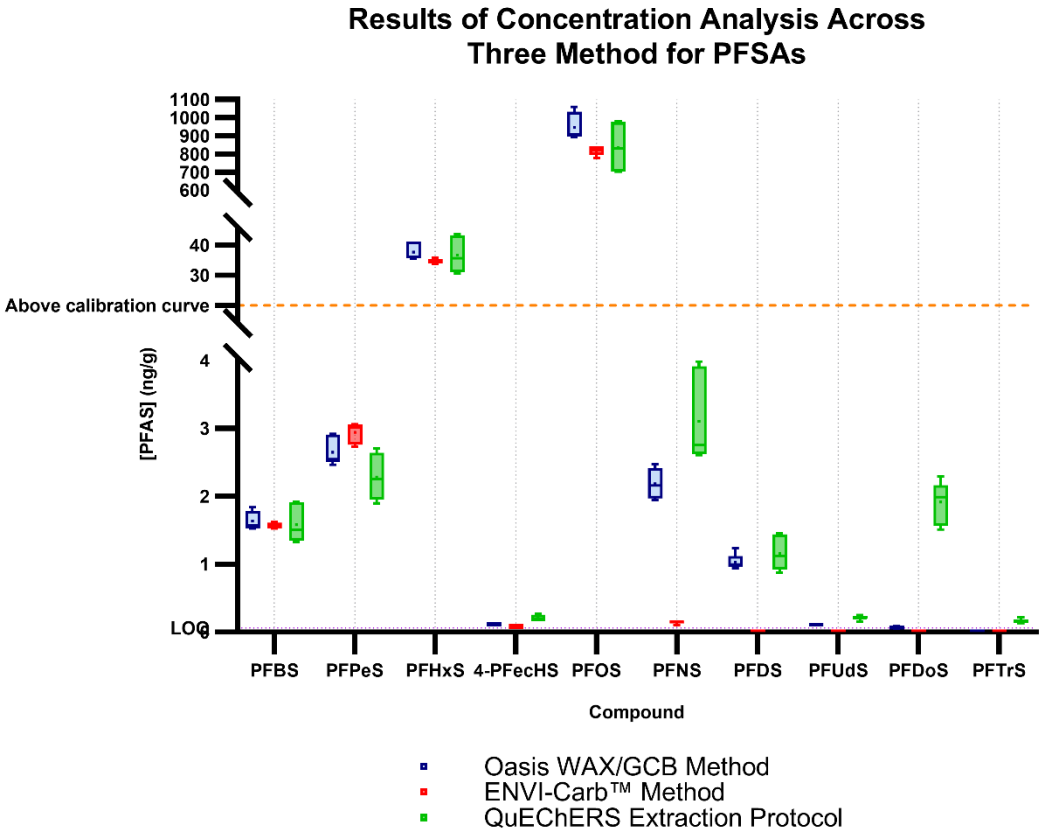

Figure 25. Recoveries (%) of perfluoroalkane sulfonic acids (PFSAs) obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS PSA/C18 protocols.

exception to this trend was PFPeA. In contrast, most long-chain PFCAs were below the LOQ and are therefore considered only qualitatively rather than quantitatively.

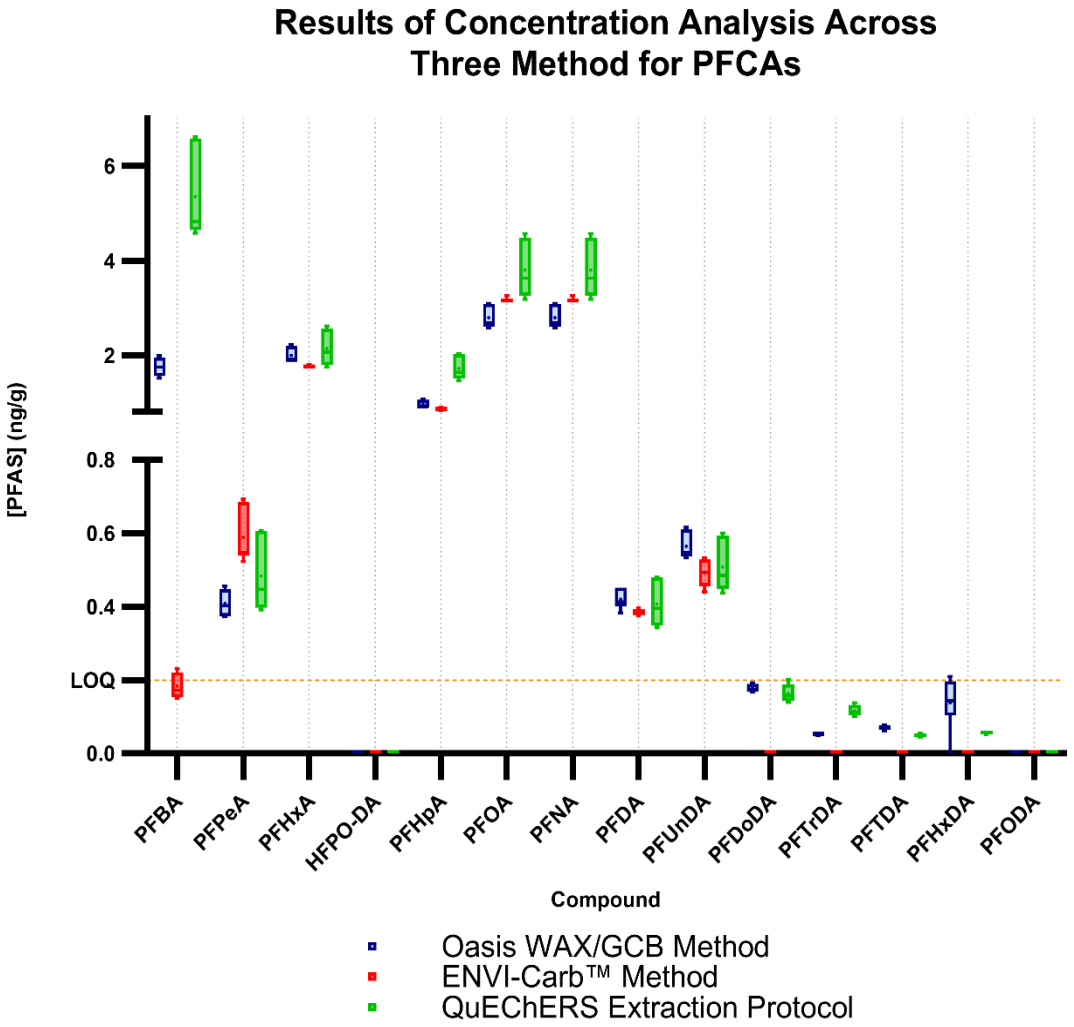

Figure 26. Recoveries (%) of perfluoroalkyl carboxylic acids (PFCAs) obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS PSA/C18 protocols.

As shown in **figure 27,** among the emerging PFAS and precursors only a few compounds were quantified within the calibration range. For the compounds that could be quantified, the methods did not show consistent order, as the results varied between analytes. For the rest of the compounds, results were either below the LOQ or above the calibration curve, and therefore not considered reliable for quantification.

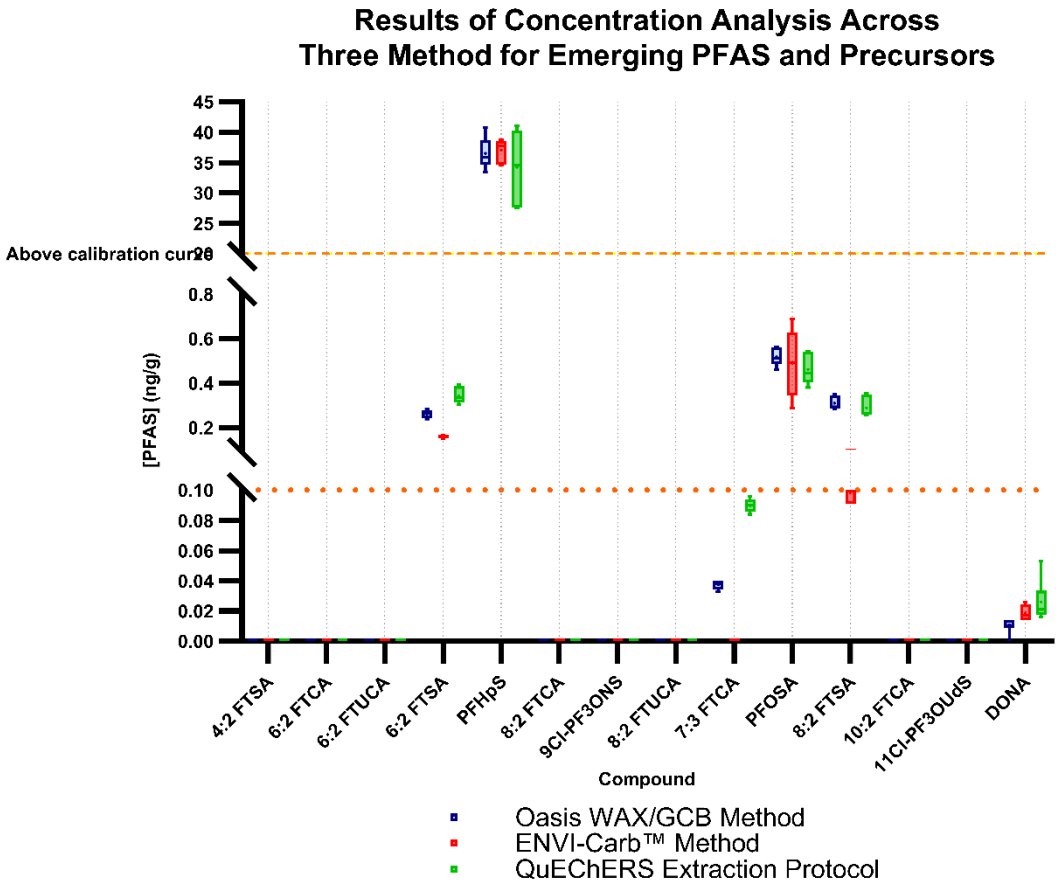

Figure 27. Recoveries (%) of emerging PFAS and precursors obtained with Oasis WAX/GCB, ENVI-Carb, and QuEChERS PSA/C18 protocols.

In all cases, short- and mid-chain PFAS were quantified more reliably than long-chain analogues, which often remained below the LOQ due to their stronger sorption to soils and lower extractability [16,33,35]. This trend has also been observed in other studies: Lorenzo et al. (2015) reported good recoveries for C4–C9 acids, but recoveries dropped markedly for long-chain sulfonates. Similarly, analyses of biosolids and agricultural soils showed that compounds such as PFOS and PFHxS could be quantified consistently, while PFDS or PFUdS were often close to or below detection limits [33,35]. High-resolution screening approaches (e.g., LC-QTOF) have detected the presence of long-chain PFAS in contaminated soils, but in most cases only at a qualitative level rather than with reliable quantification [28]. These results emphasize that while short- and mid-chain PFAS can be quantified with greater confidence, long-chain analogues remain particularly challenging due to both their stronger sorption to soil organic matter and the limited extraction efficiency of current methods.

11. CONCLUSIONS

This study evaluated and compared different extraction and clean-up strategies for the determination of PFAS in contaminated soils, with the aim of maximizing analyte coverage, increasing recoveries, and reducing RSD values. The results showed that the use of Oasis WAX/GCB cartridges provided a clear improvement over ENVI-Carb method, achieving comparable recoveries and, in addition, allowing the detection of compounds that were not quantified with ENVI-Carb. Precision was also enhanced, with RSD values consistently below the 20% acceptance limit, confirming that this approach improves the reliability of the analysis.

The QuEChERS-based protocol adapted for soils showed that it can be applied beyond biological and sludge samples, allowing the extraction of a broad range of PFAS, including some long-chain homologues not detected with SPE-based approaches. Nevertheless, the method produced lower overall recoveries and stronger matrix interferences, which indicates that further optimization is necessary before it can be considered suitable for routine use. Its main limitation was the high variability between replicates, as reflected by RSD values much higher than those of the other methods. Although the mean concentrations were corrected with isotopically labelled internal standards and were therefore comparable across protocols, the large dispersion of values in QuEChERS remains its most critical drawback.

When comparing the three methods, the same general trend was observed: short-chain PFAS were extracted more efficiently, while long-chain compounds showed lower recoveries. None of the methods reached the optimal recovery range for all compounds, but Oasis® WAX/GCB method provided the most balanced performance, combining broad analyte coverage with acceptable recoveries and good reproducibility. QuEChERS showed potential due to its simplicity and reduced solvent use, though the presence of interferences highlights the need to refine sorbent composition.

Despite its stronger performance, Oasis® WAX/GCB method is laborious and time-consuming, which limits its suitability for routine implementation in environmental laboratories. In contrast, QuEChERS, although less robust in its current form, offers a simpler and faster alternative, and with further optimization could become the most practical option for routine analysis.

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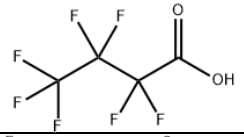
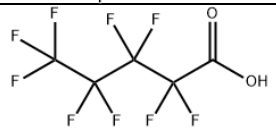
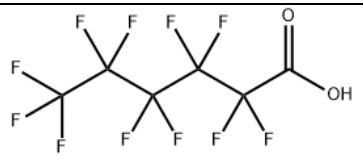
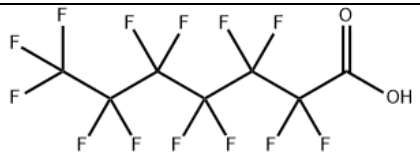
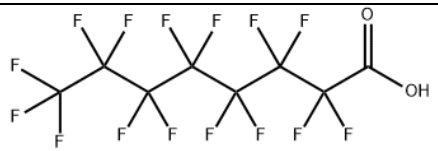
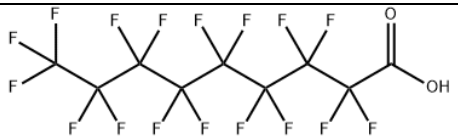
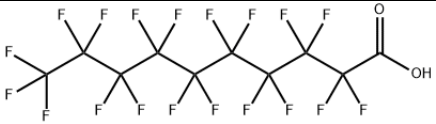
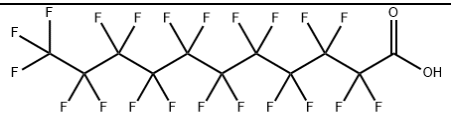
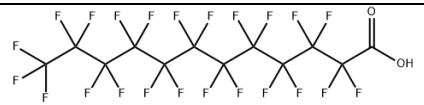
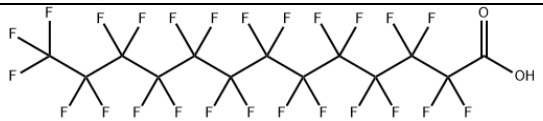
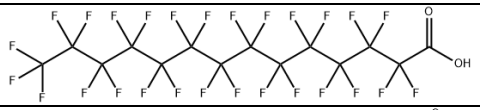
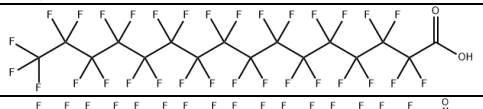
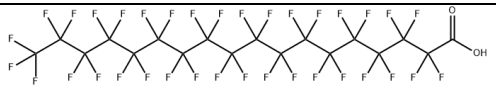
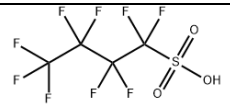
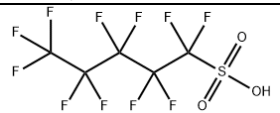
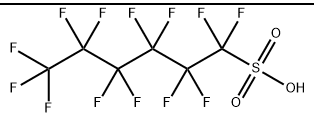
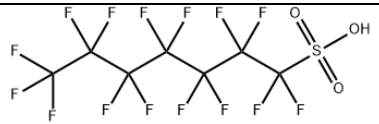
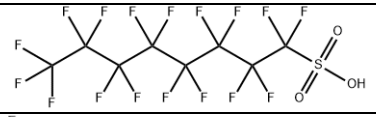
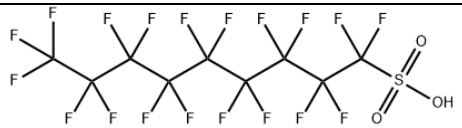
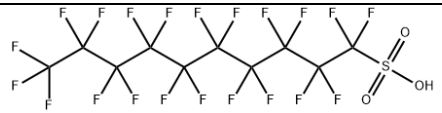
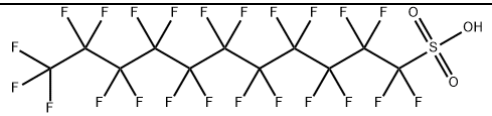
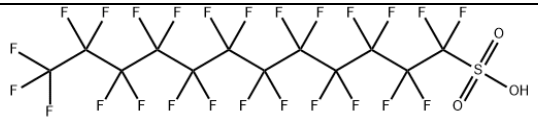
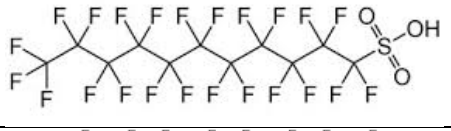
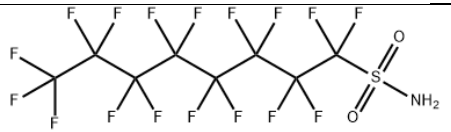
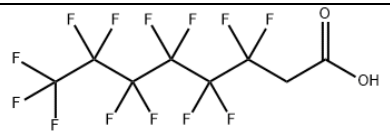
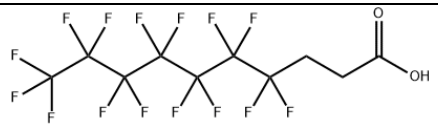
11 APPENDICES

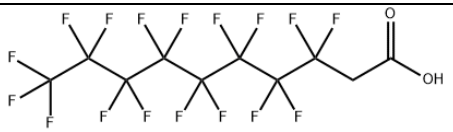
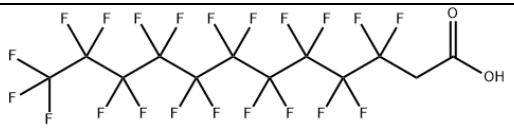
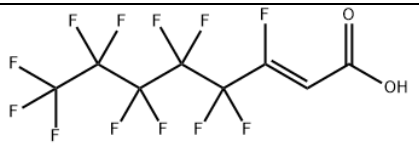
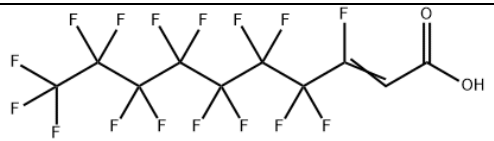
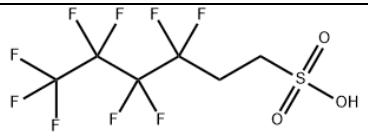
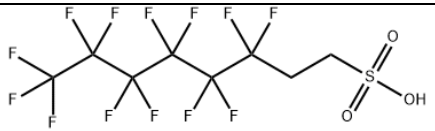
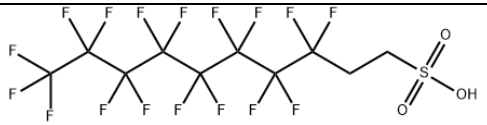
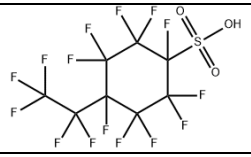
Appendix 1. List of Selected PFAS Analytes

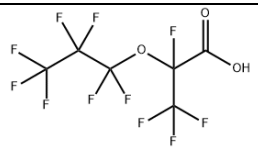
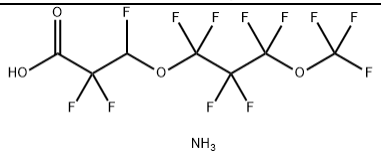
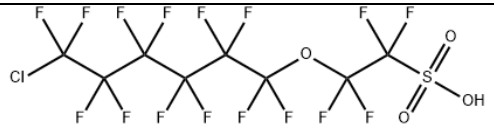
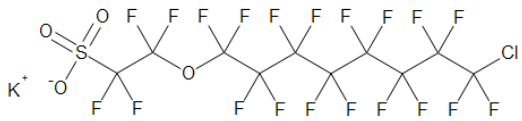
This appendix contains a comprehensive list of the PFAS considered in this study. The table compiles the essential information required for the analysis, including abbreviations, molecular formulas, CAS numbers, and molecular weights, providing a practical reference throughout the work.

Compound	Structure	Abbreviation	Formula	CAS Number	Molecular Weight
Heptafluorobutyric acid		PFBA	C4HF7O2	375-22-4	214.04
Perfluoropentanoic acid		PFPeA	$C_5HF_9O_2$	2706-90-3	264.05
Perfluorohexanoic acid		PFHxA	$C_6HF_{11}O_2$	307-24-4	314.05
Perfluoroheptanoic acid		PFHpA	$C_7HF_{13}O_2$	375-85-9	364.06
Pentadecafluorooctanoic acid		PFOA	$C_8HF_{15}O_2$	335-67-1	414.07
Heptadecafluorononanoic acid		PFNA	$C_9HF_{17}O_2$	375-95-1	464.08
Nonadecafluorodecanoic acid		PFDA	$C_{10}HF_{19}O_2$	335-76-2	514.08

Perfluoroundecanoic acid		PFUnDA	$C_{11}HF_{21}O_2$	2058-94-8	564.09
Perfluorododecanoic acid		PFDoDA	$C_{12}HF_{23}O_2$	307-55-1	614.1
Perfluorotridecanoic acid		PFTrDA	$C_{13}HF_{25}O_2$	72629-94-8	664.1
Perfluorotetradecanoic acid		PFTDA	$C_{14}HF_{27}O_2$	376-06-7	714.11
Perfluorohexadecanoic acid		PFHxDA	$C_{16}HF_{31}O_2$	67905-19-5	814.13
Perfluorooctadecanoic acid		PFODA	$C_{18}HF_{35}O_2$	16517-11-6	914.1
Perfluorobutanesulfonic acid		PFBS	$C_4HF_9O_3S$	375-73-5	300.1
Perfluoropentanesulfonic acid		PFPeS	$C_5HF_{11}O_3S$	2706-91-4	350.11
Perfluorohexanesulfonic acid		PFHxS	$C_6HF_{13}O_3S$	355-46-4	400.12
Perfluoroheptanesulfonic acid		PFHpS	$C_7HF_{15}O_3S$	375-92-8	450.12

Perfluorooctanesulfonic acid		PFOS	$C_8HF_{17}O_3S$	1763-23-1	500.13
Perfluorononanesulfonic acid		PFNS	$C_9HF_{19}O_3S$	68259-12-1	550.14
Perfluorodecane sulfonic acid		PFDS	$C_{10}HF_{21}O_3S$	335-77-3	600.15
Perfluoroundecanesulfonic acid		PFUdS	$C_{11}HF_{23}O_3S$	749786-16-1	650.15
Perfluorododecanesulfonic acid		PFDoS	$C_{12}HF_{25}O_3S$	79780-39-5	700.16
Perfluoroundecanesulfonic acid		PFTrS	$C_{11}HF_{23}O_3S$	749786-16-1	650.15
Perfluorooctanesulfonamide		PFOSA	$C_8H_2F_{17}NO_2S$	754-91-6	499.15
2H,2H-Perfluorooctanoic acid		6-2 FTCA	$C_8H_3F_{13}O_2$	53826-12-3	378.09
Pentadecafluorodecanoic acid		7-3 FTCA	$C_{10}H_5F_{15}O_2$	812-70-4	442.12

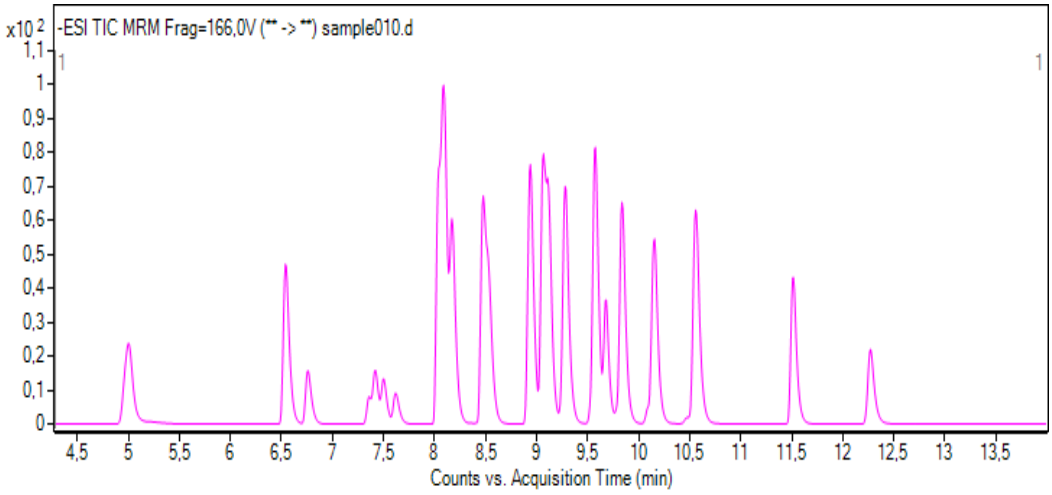
2-Perfluorooctyl ethanoic acid		8-2 FTCA	$C_{10}H_3F_{17}O_2$	27854-31-5	478.1
2H,2H-Perfluorododecanoic acid		10-2 FTCA	$C_{12}H_3F_{21}O_2$	53826-13-4	578.12
(Z)-6:2 Fluorotelomer unsaturated carboxylic acid		6-2 FTUCA	$C_8H_2F_{12}O_2$	161094-75-3	358.08
2H-Perfluoro-2-decenoic acid		8-2 FTUCA	$C_{10}H_2F_{16}O_2$	70887-84-2	458.1
4:2 Fluorotelomer sulfonic acid		4:2 FTSA	$C_6H_5F_9O_3S$	757124-72-4	328.15
1-Octanesulfonic acid		6-2 FTSA	$C_8H_5F_{13}O_3S$	27619-97-2	428.17
8:2 Fluorotelomer sulfonic acid		8-2 FTSA	$C_{10}H_5F_{17}O_3S$	39108-34-4	528.18
Perfluoro-4-ethylcyclohexane		PFECHS	$C_8HF_{15}O_3S$	646-83-3	462.13

2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic acid		HFPO-DA	$C_6HF_{11}O_3$	13252-13-6	330.05
Ammonium 4,8-dioxa-3H perfluorononanoate		ADONA (or acid form)	C7H5F12NO4	958445-44-8	395.1
Perfluoro(2-((6-chlorohexyl) oxy) ethanesulfonic acid)		9Cl-PF3ONS	$C_8HClF_{16}O_4S$	756426-58-1	532.58
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid		11Cl-PF3OUdS	$C_{10}HClF_{20}O_4S$	763051-92-9	632.60

Appendix 2. Representative chromatograms of a fortified sample at 40 ng/mL

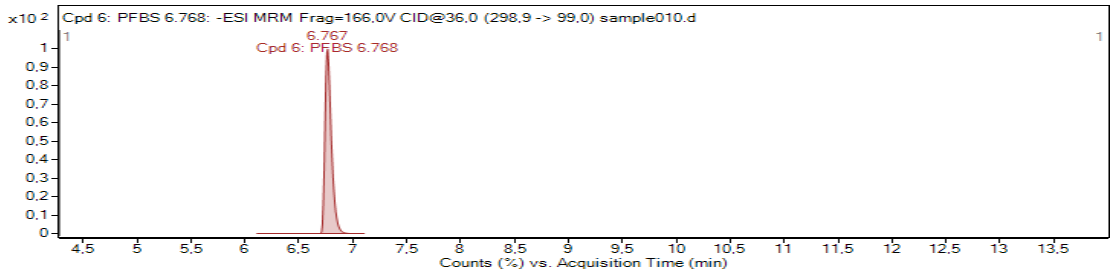
This appendix contains the chromatograms for a fortified sample with 40 ng/mL of each target PFAS, analyzed using the optimized UHPLC–MS/MS conditions described in Section 9.4. For each compound, two MRM transitions are shown: one used for quantification and the other for confirmation. The retention times and ion transition parameters match those reported in Tables 2 and 3. In addition, the total ion chromatogram (TIC) for the acquisition run is included to provide an overview of the complete signal profile obtained under these conditions.

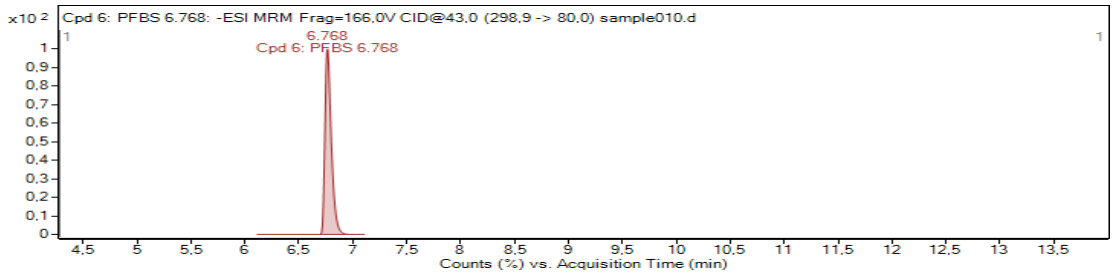
A few compounds (HFPO-DA, 6:2 FTCA, 8:2 FTCA, 10:2 FTCA) have a third possible MRM transition, but this was not included in the tables. In contrast, some short-chain PFAS are shown with only one transition because they fragment extensively in the first transition, making it difficult to detect a reliable secondary product ion.



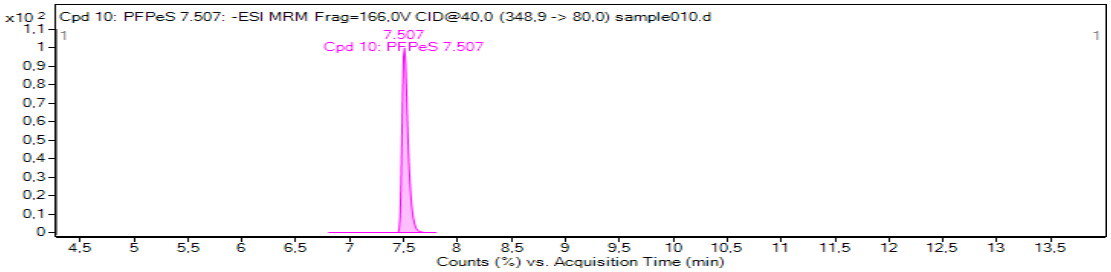
PFSAs

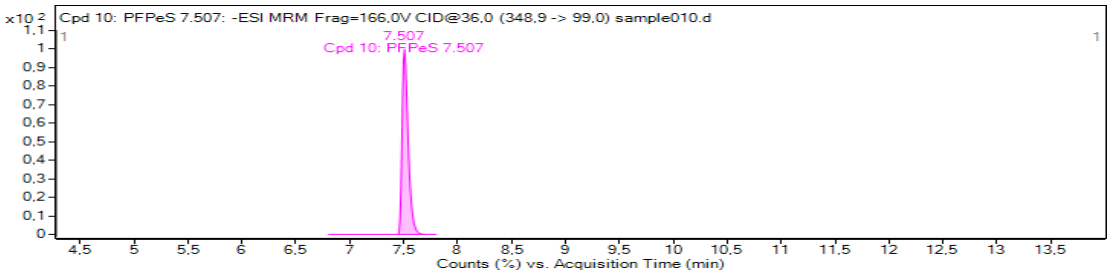
PFBS



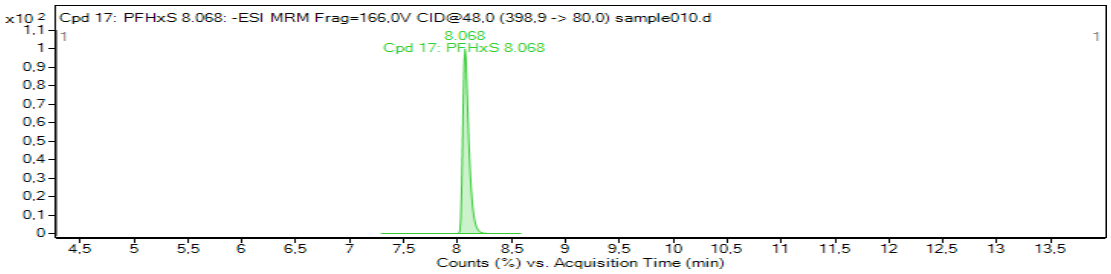


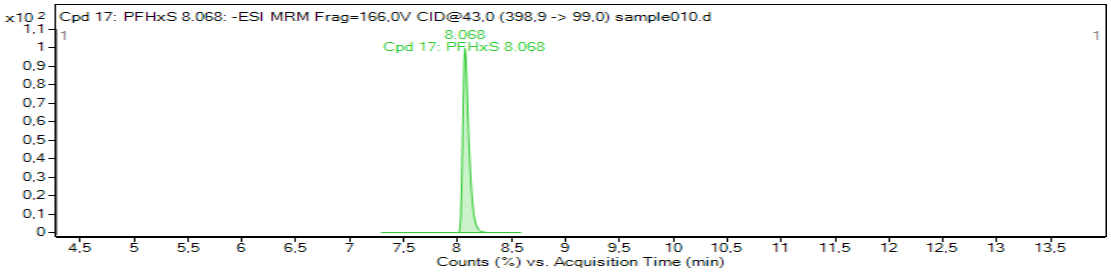
PFPeS



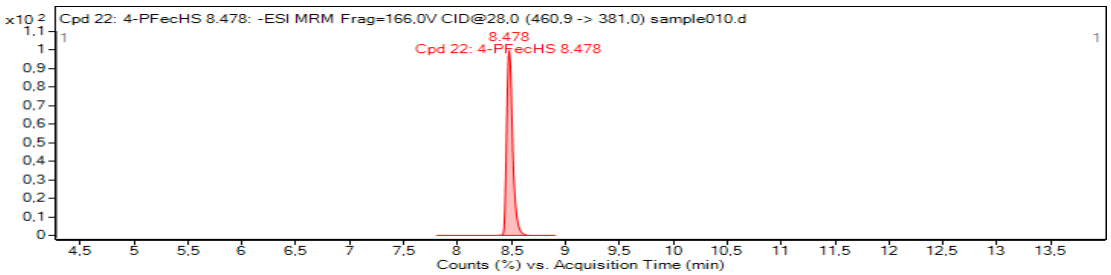


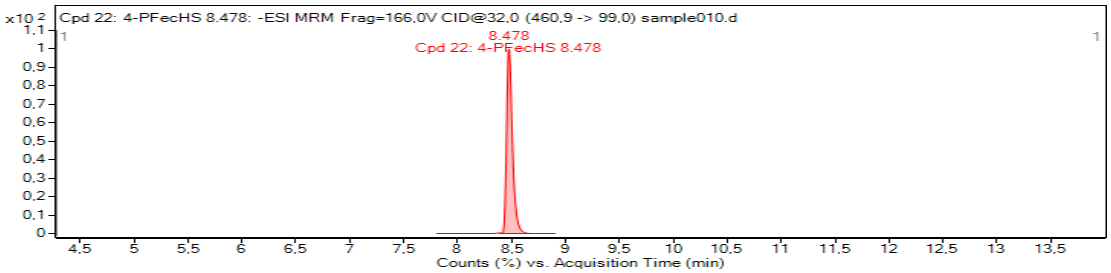
PFHxS



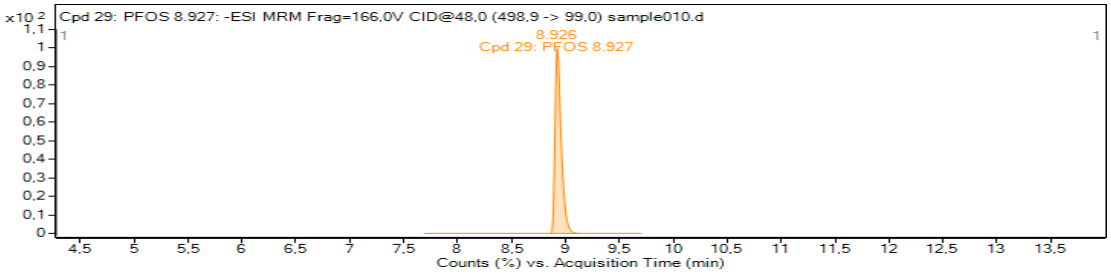


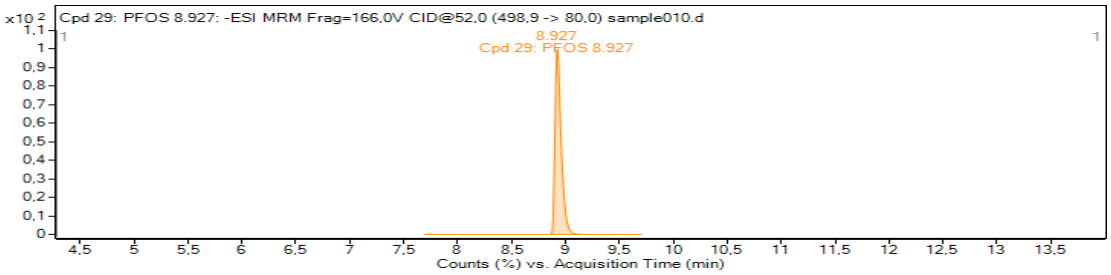
4-PFecHS



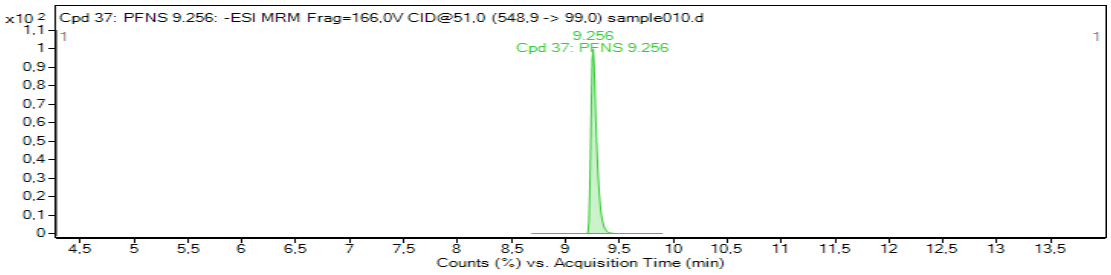


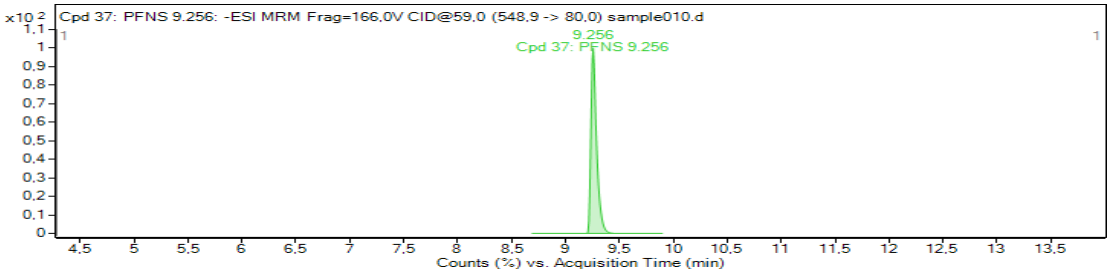
PFOS



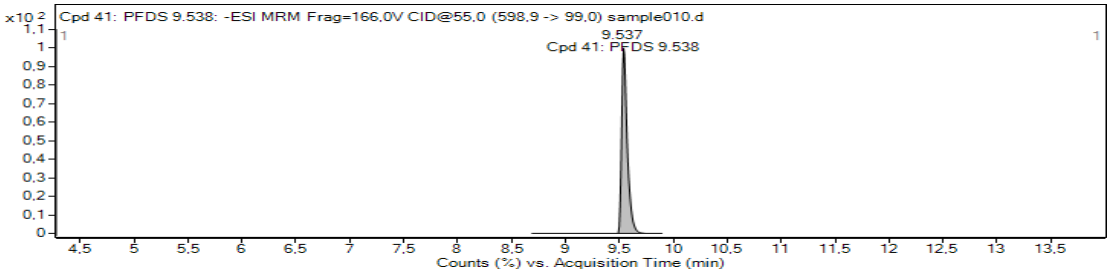


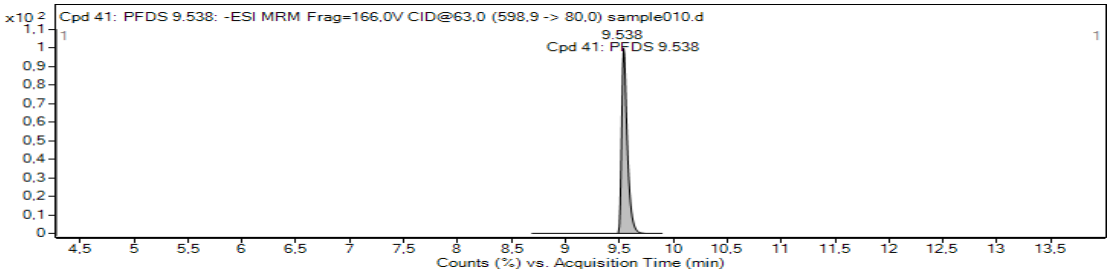
PFNS



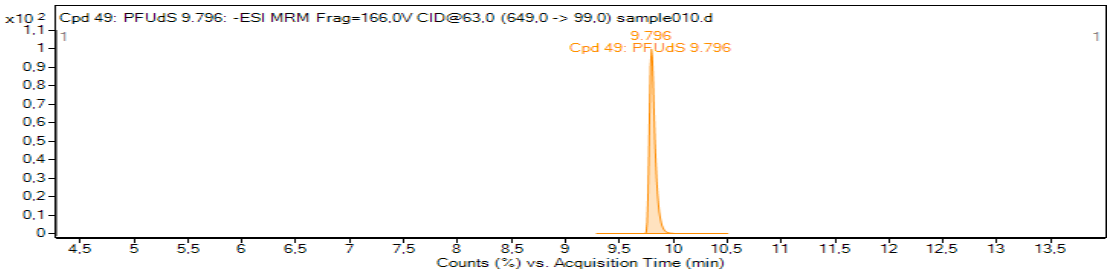


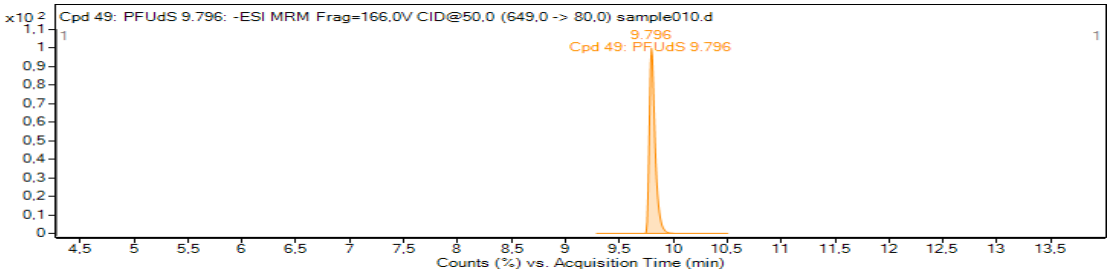
PFDS



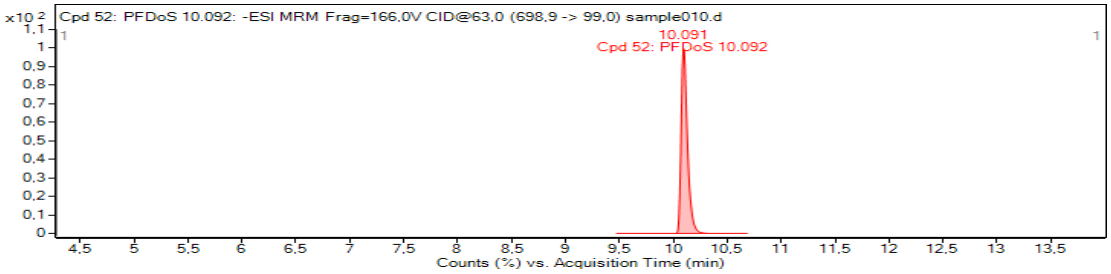


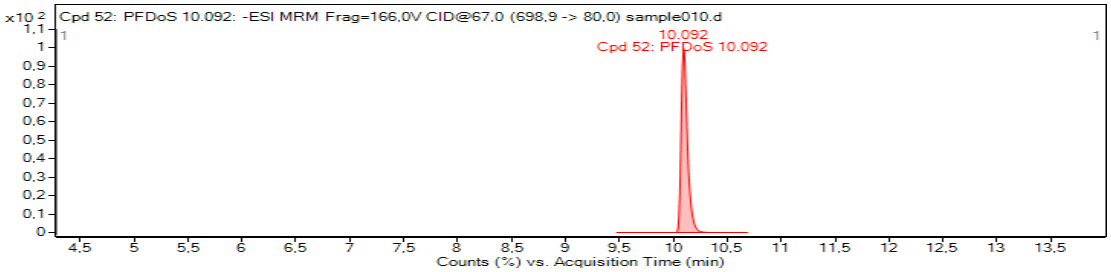
PFUdS


Cpd 49: PFUdS 9.796: -ESI MRM Frag=166,0V CID@63,0 (649,0 -> 99,0) sample010.d
9.796
Cpd 49: PFUdS 9.796
Counts (%) vs. Acquisition Time (min)

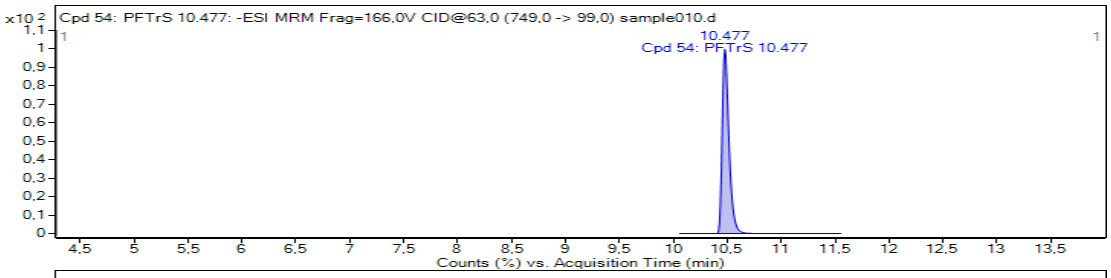

Cpd 49: PFUdS 9.796: -ESI MRM Frag=166,0V CID@50,0 (649,0 -> 80,0) sample010.d
9.796
Cpd 49: PFUdS 9.796
Counts (%) vs. Acquisition Time (min)

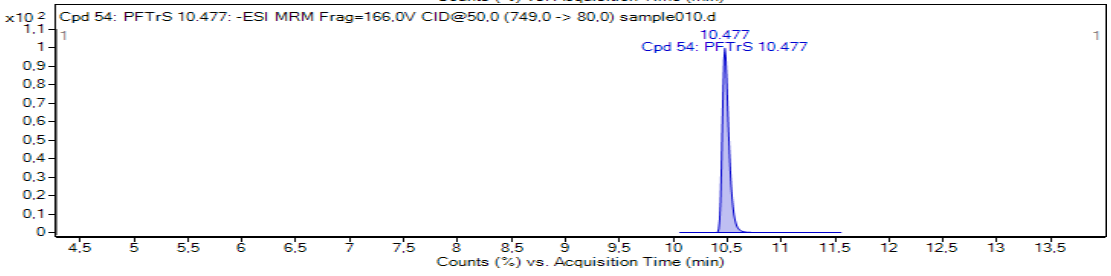
PFDoS


Cpd 52: PFDoS 10.092: -ESI MRM Frag=166,0V CID@63,0 (698,9 -> 99,0) sample010.d
10.091
Cpd 52: PFDoS 10.092
Counts (%) vs. Acquisition Time (min)


Cpd 52: PFDoS 10.092: -ESI MRM Frag=166,0V CID@67,0 (698,9 -> 80,0) sample010.d
10.092
Cpd 52: PFDoS 10.092
Counts (%) vs. Acquisition Time (min)

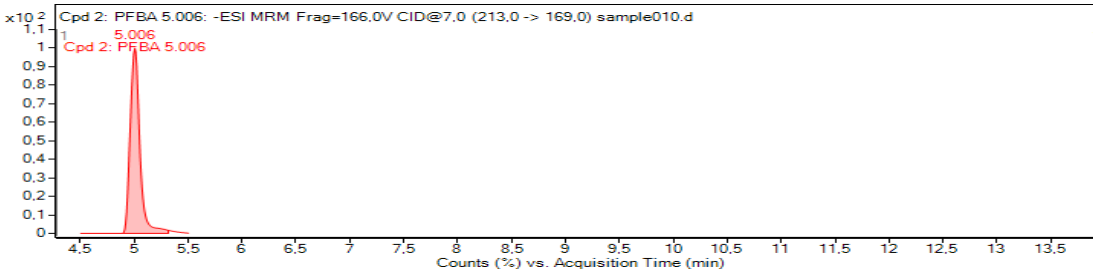
PFTrS


Cpd 54: PFTrS 10.477: -ESI MRM Frag=166,0V CID@63,0 (749,0 -> 99,0) sample010.d
10.477
Cpd 54: PFTrS 10.477
Counts (%) vs. Acquisition Time (min)

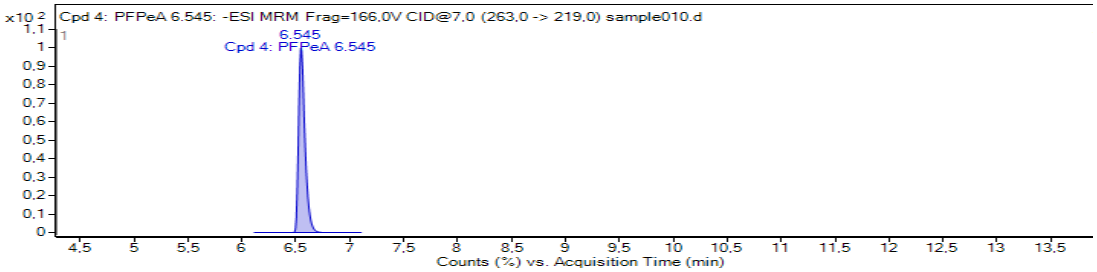

Cpd 54: PFTrS 10.477: -ESI MRM Frag=166,0V CID@50,0 (749,0 -> 80,0) sample010.d
10.477
Cpd 54: PFTrS 10.477
Counts (%) vs. Acquisition Time (min)

PFCAs

PFBA



PFPeA



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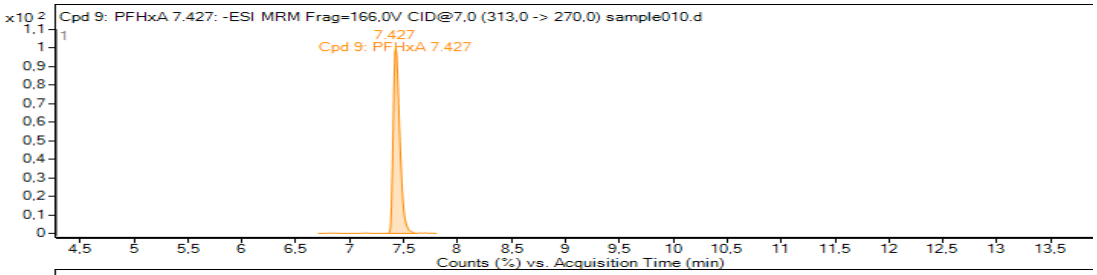

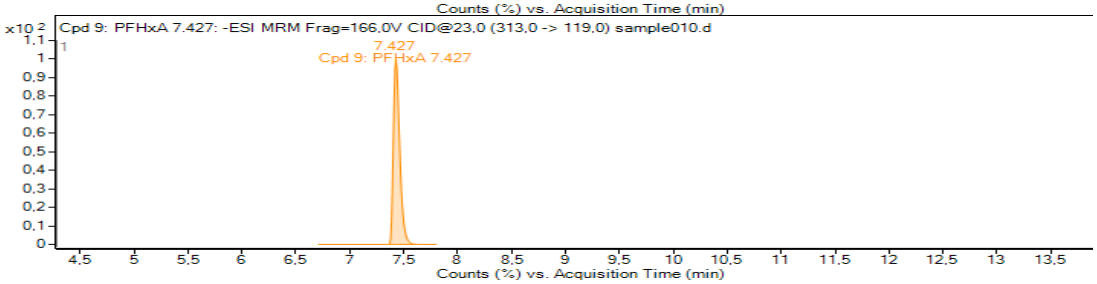

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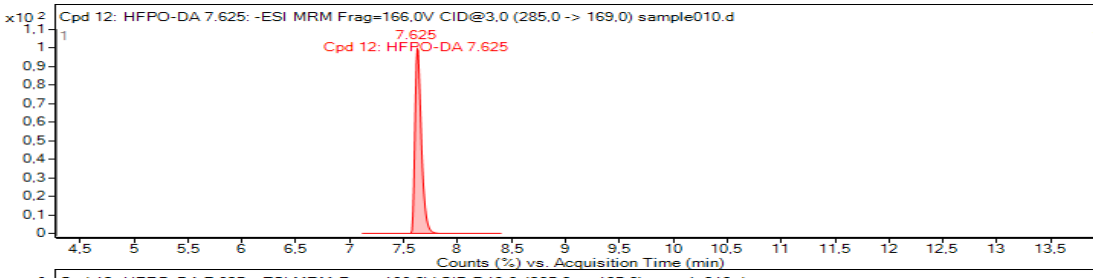

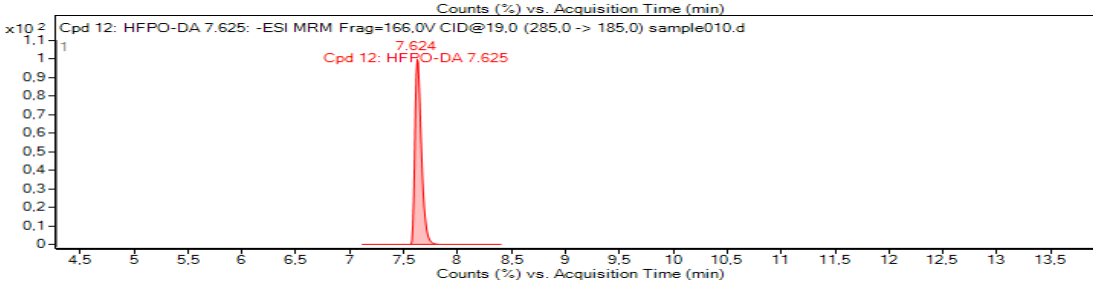

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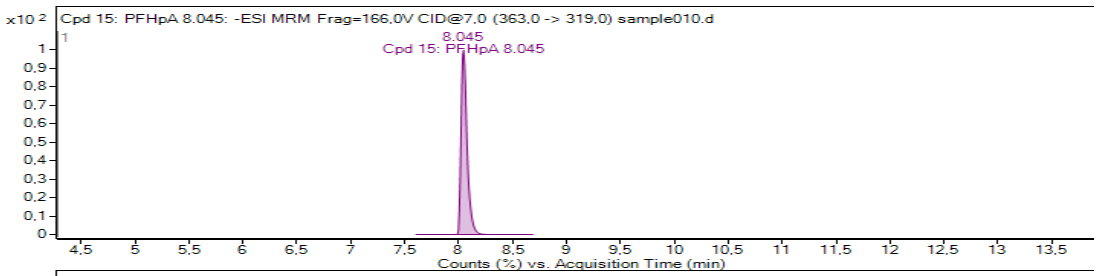

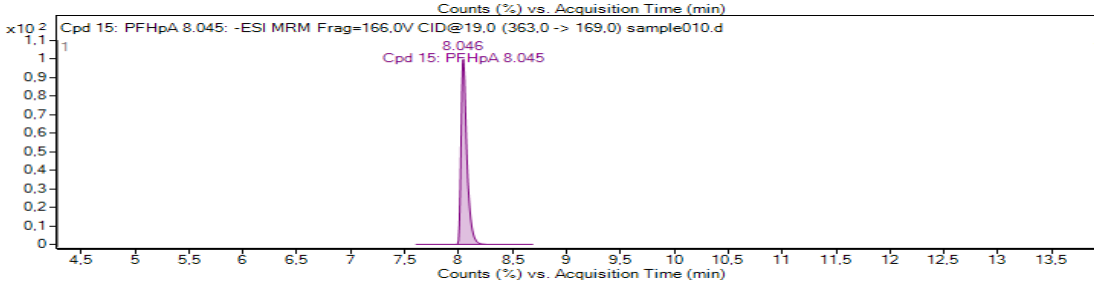

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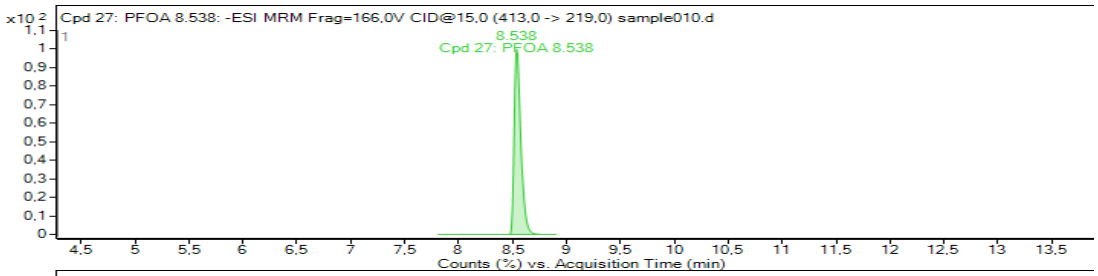

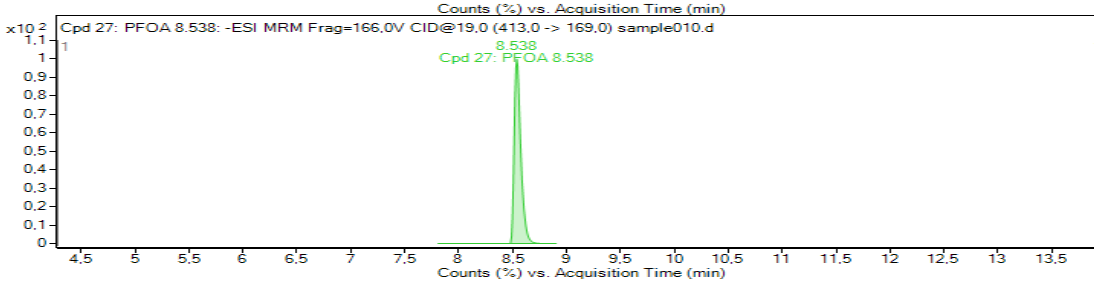

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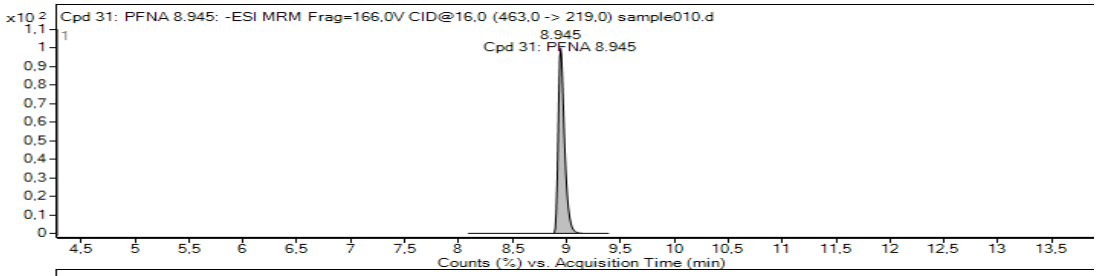

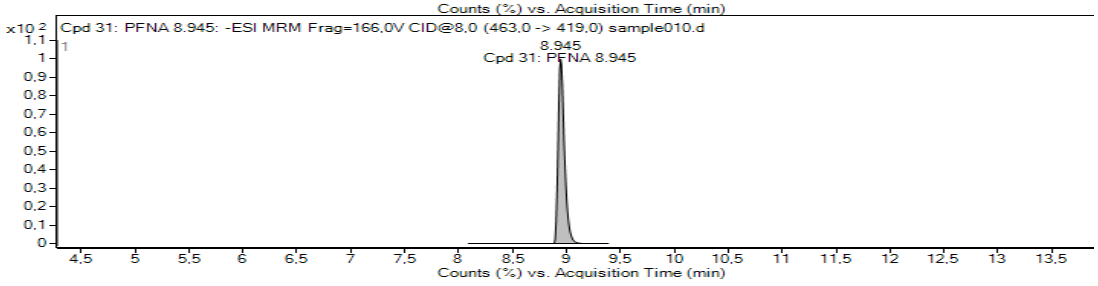

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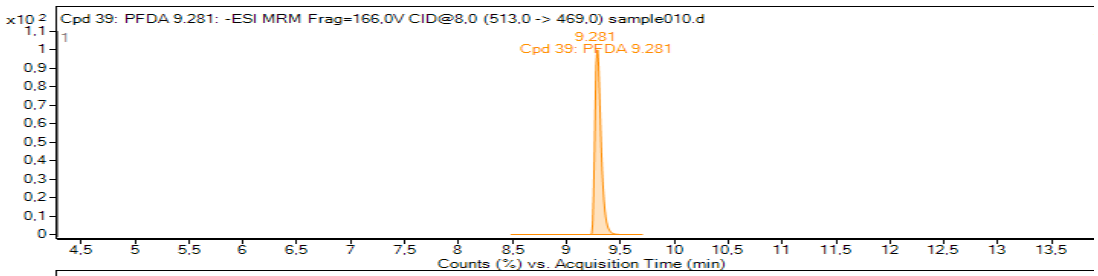

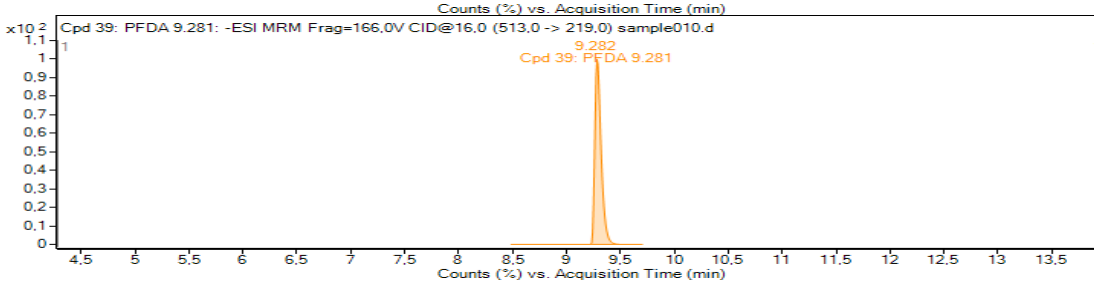

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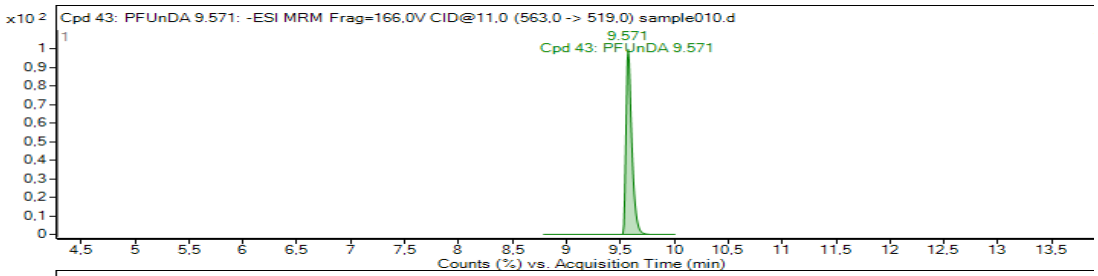

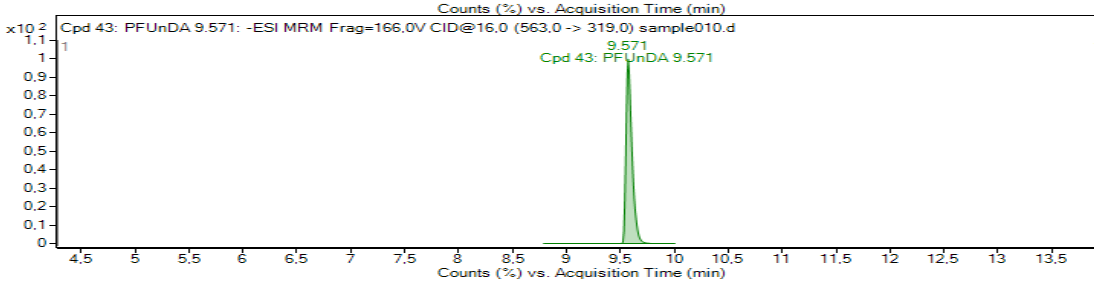

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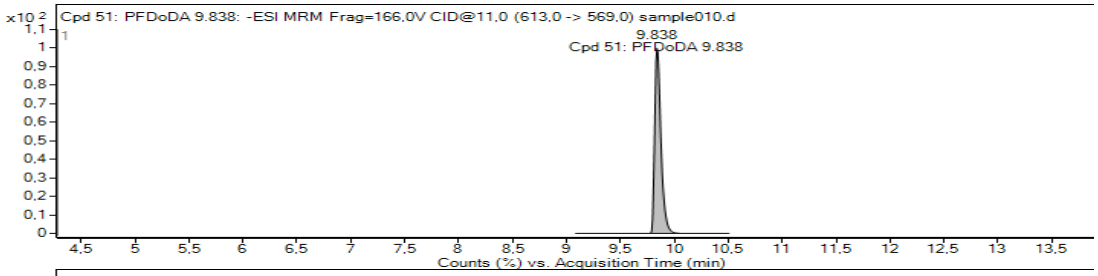

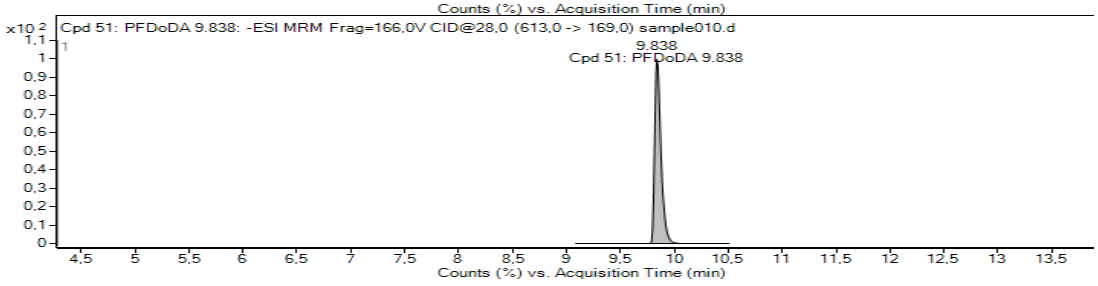

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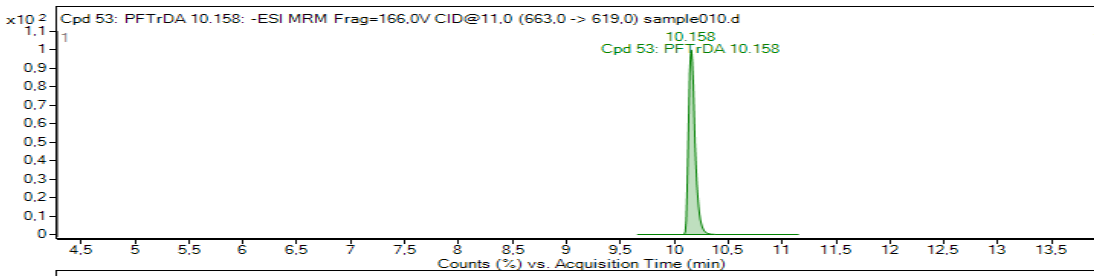

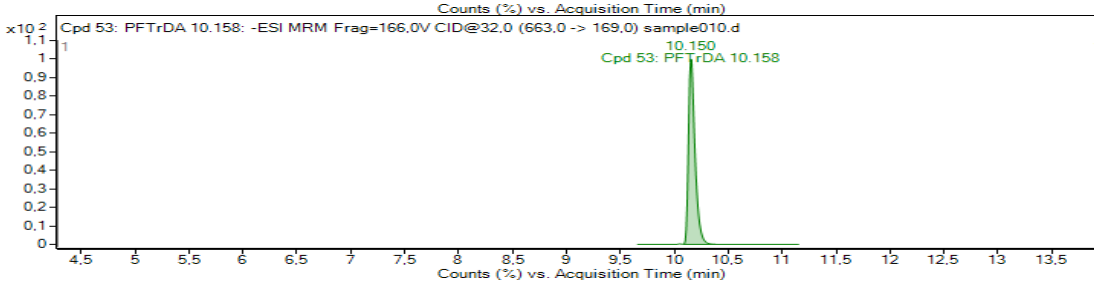

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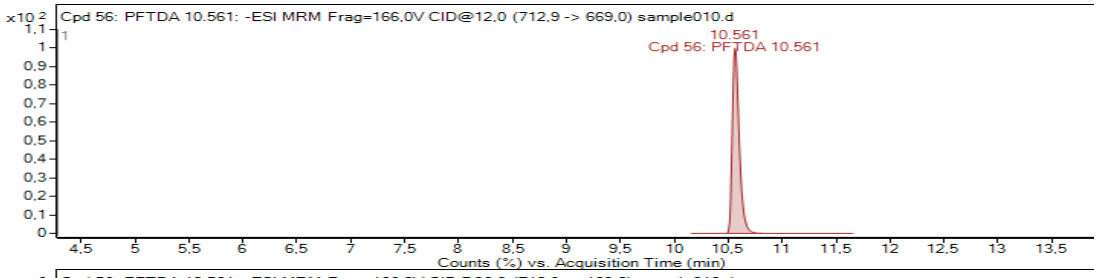

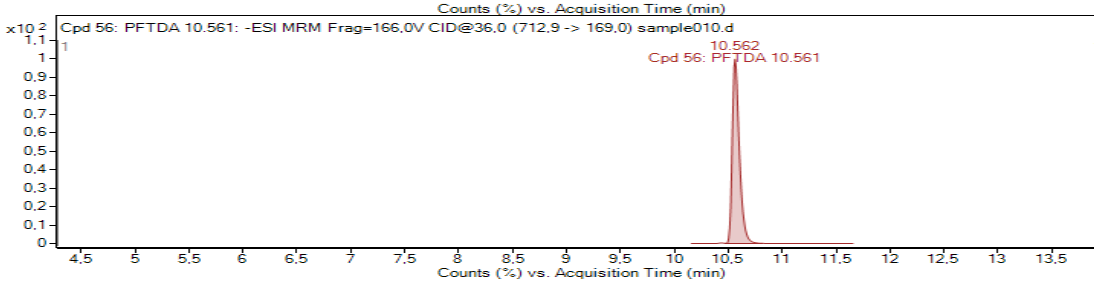

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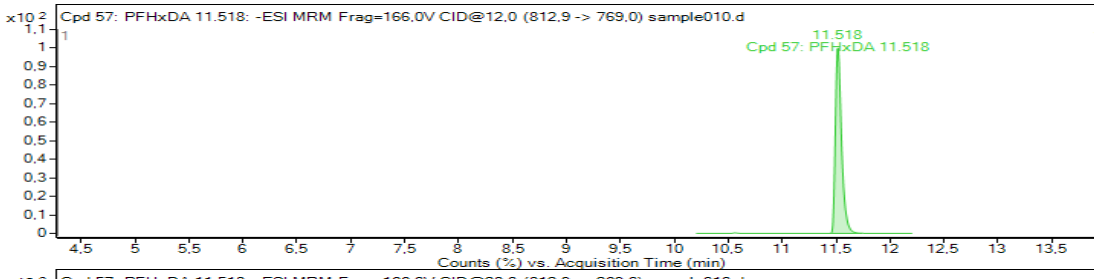

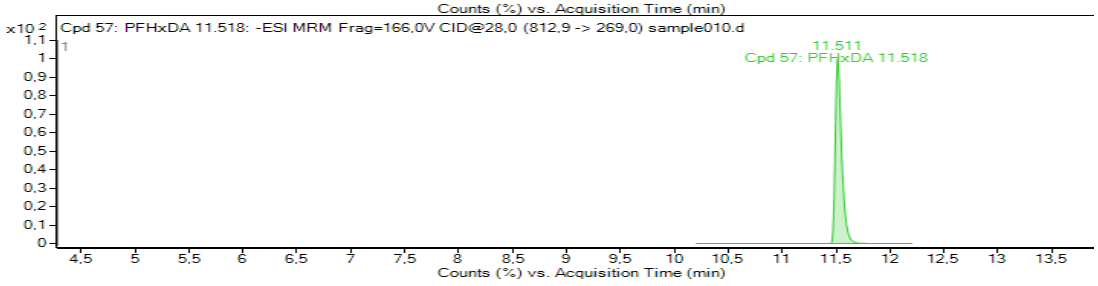

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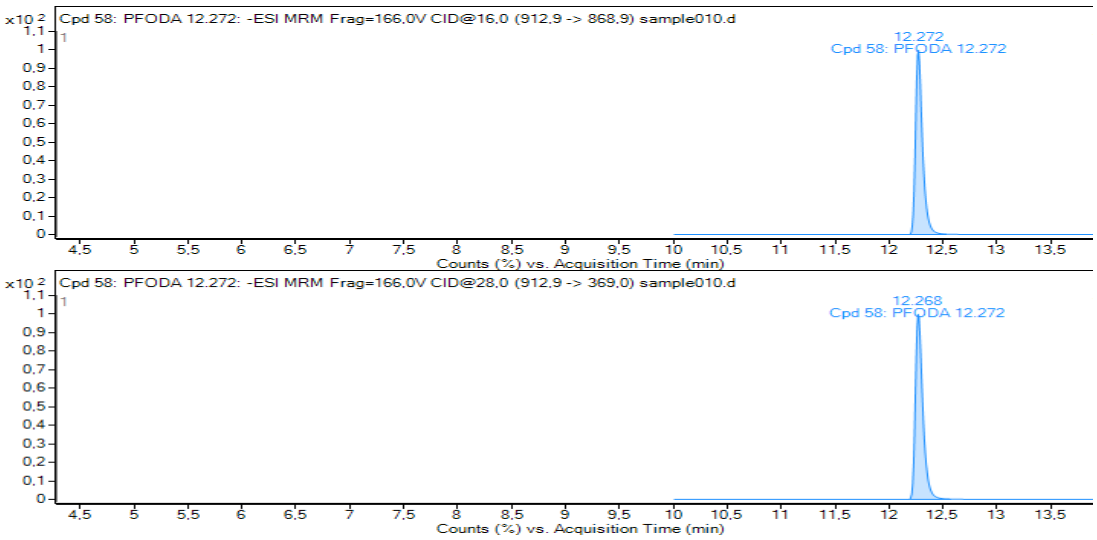
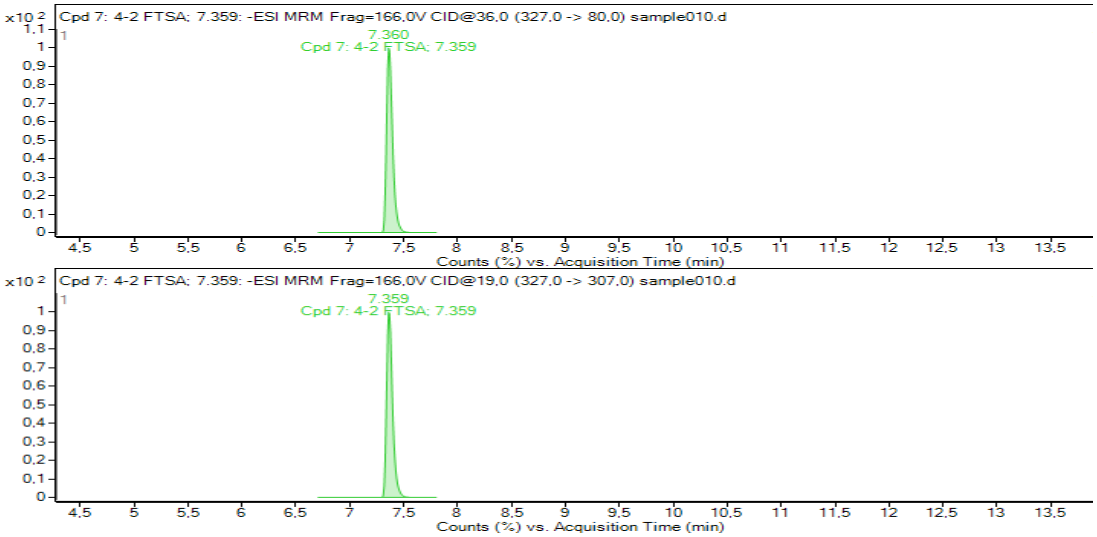
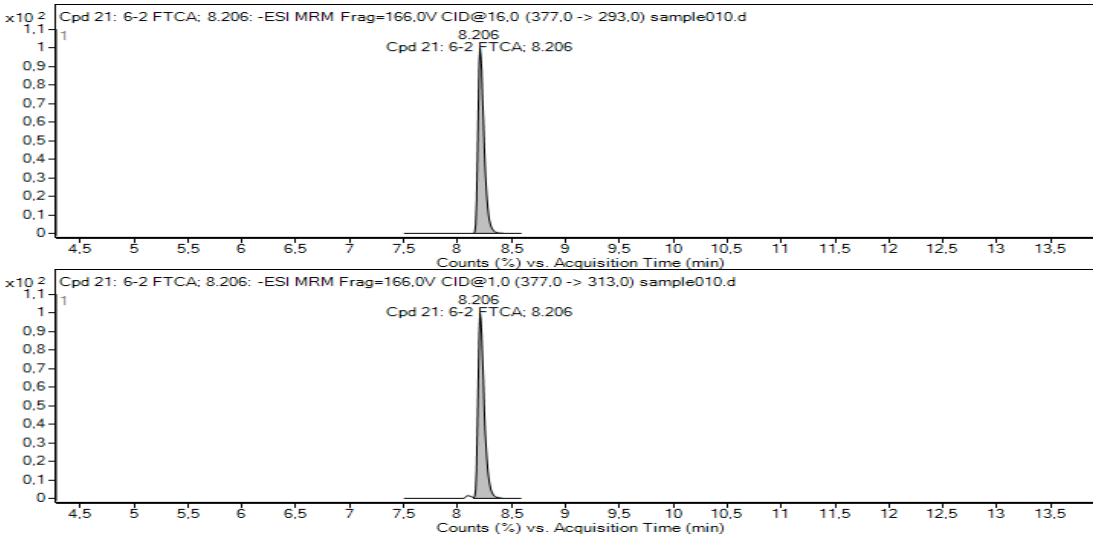


Emerging PFASs & Precursors

4:2 FTSA



6:2 FTCA



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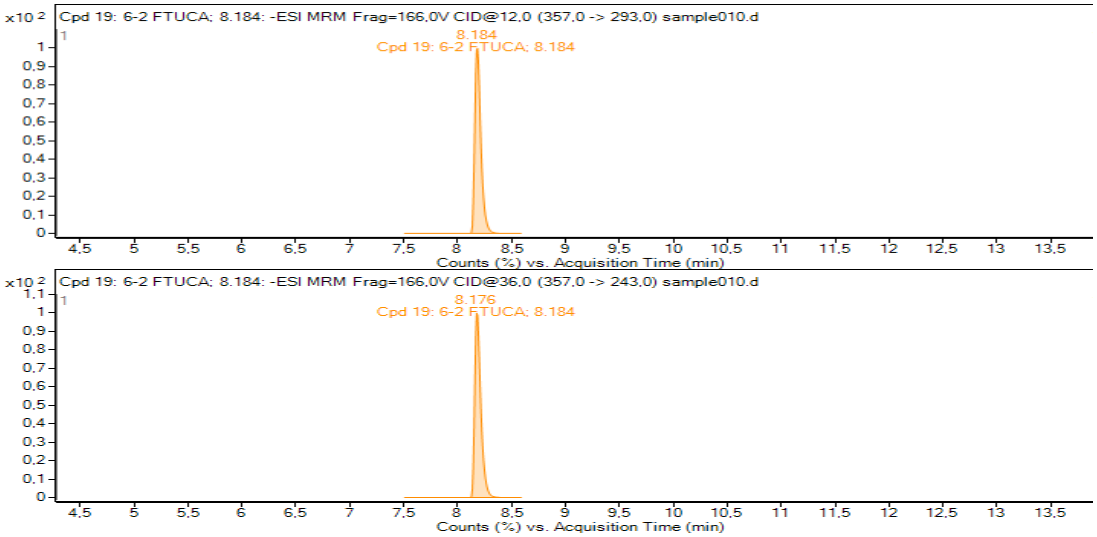

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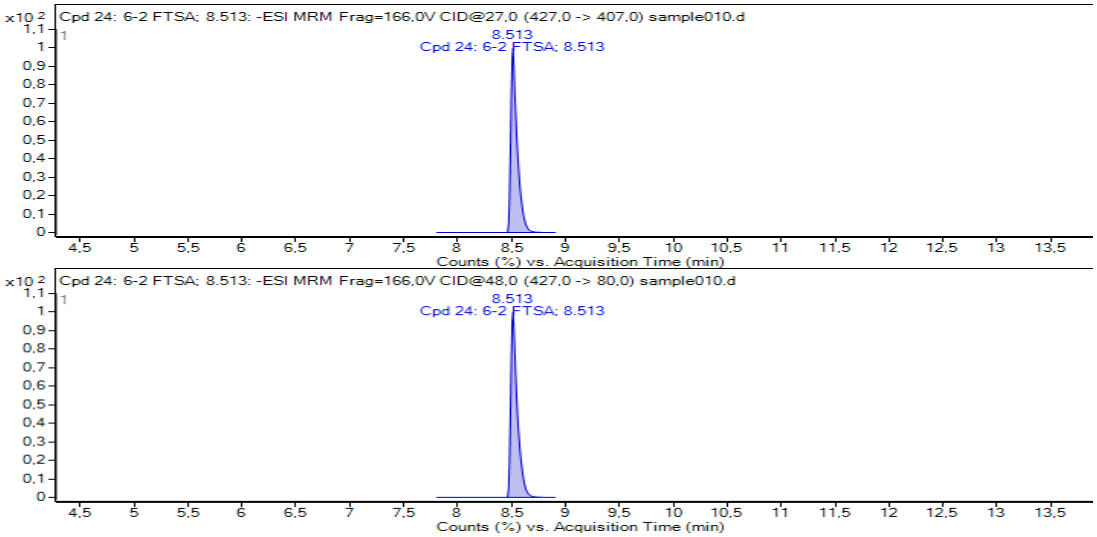

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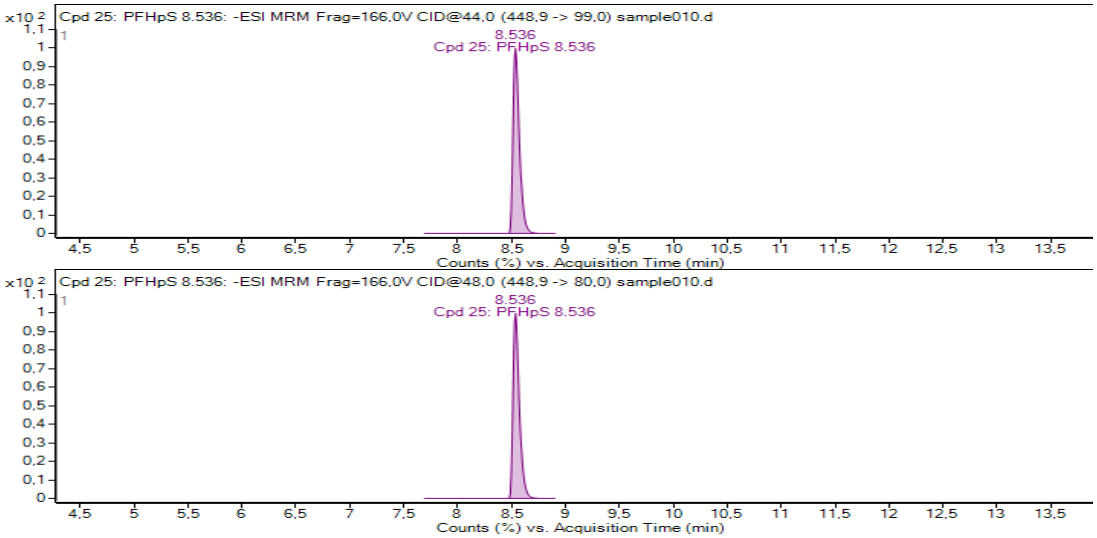
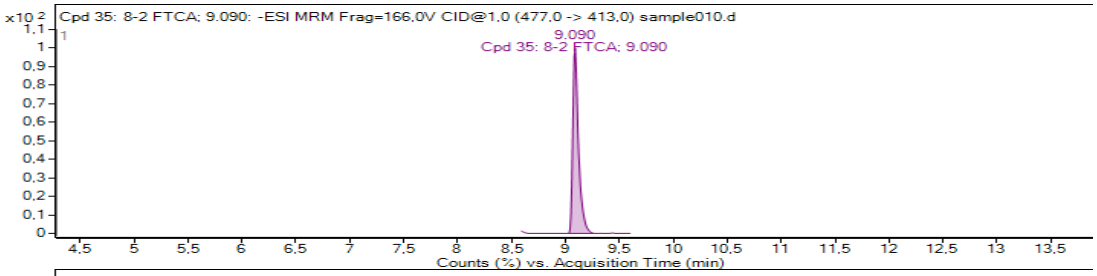
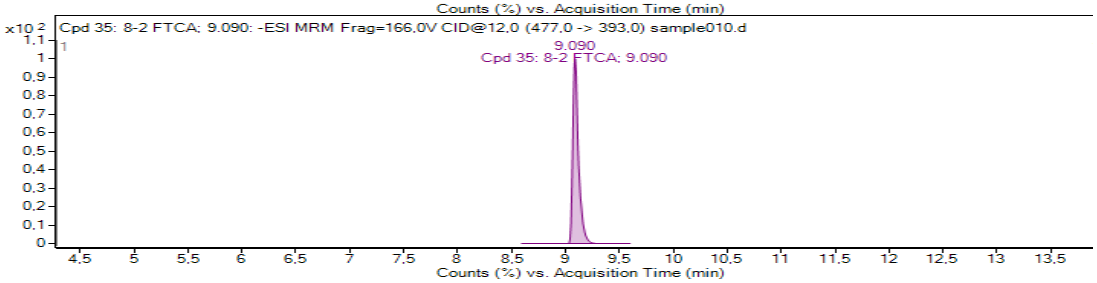
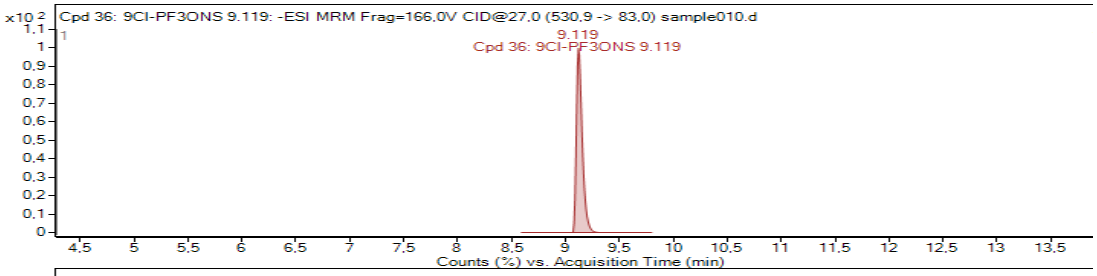


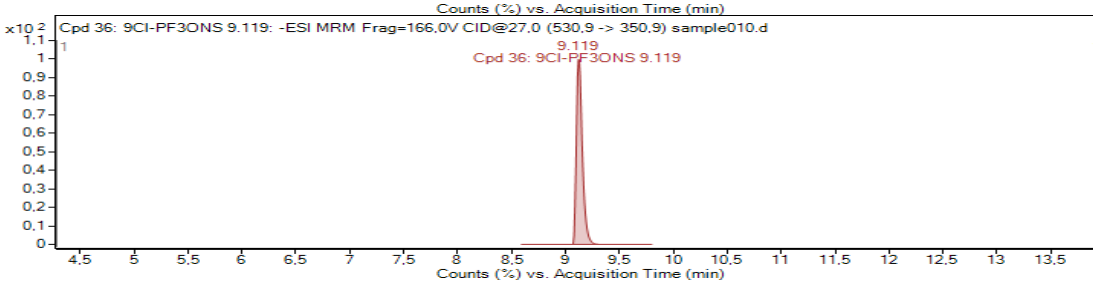
8:2 FTCA





9Cl-PF3ONS





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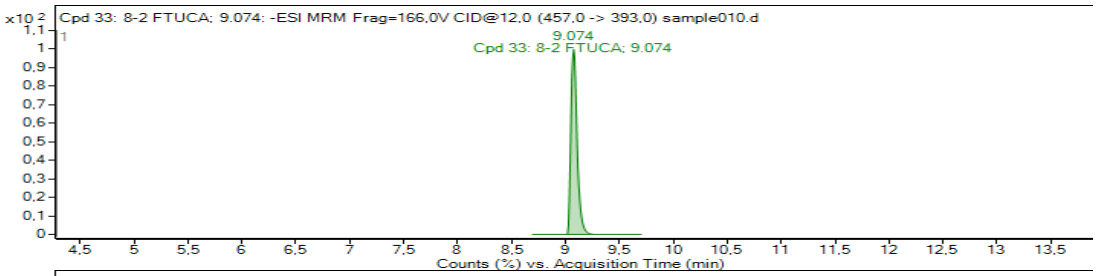
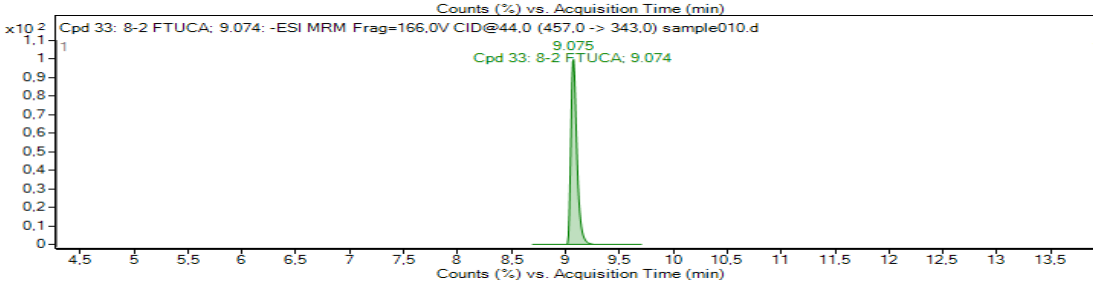
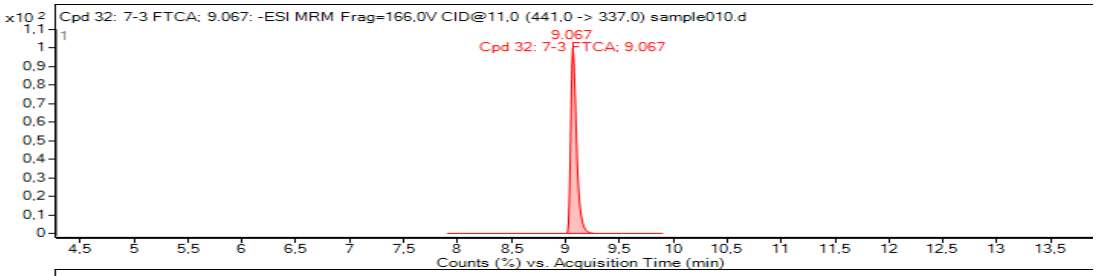
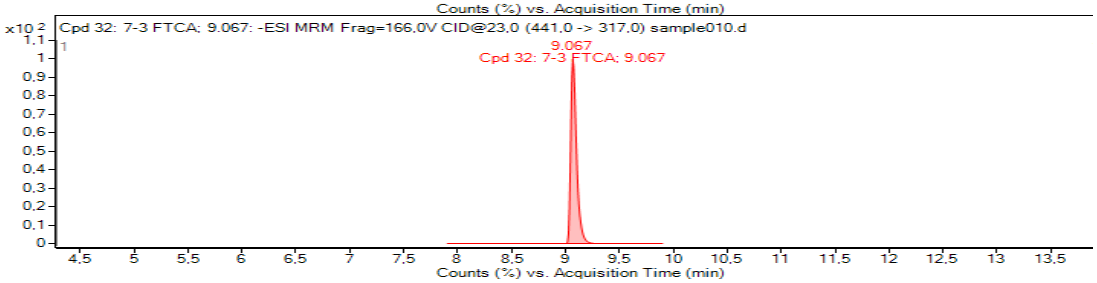




7:3 FTCA





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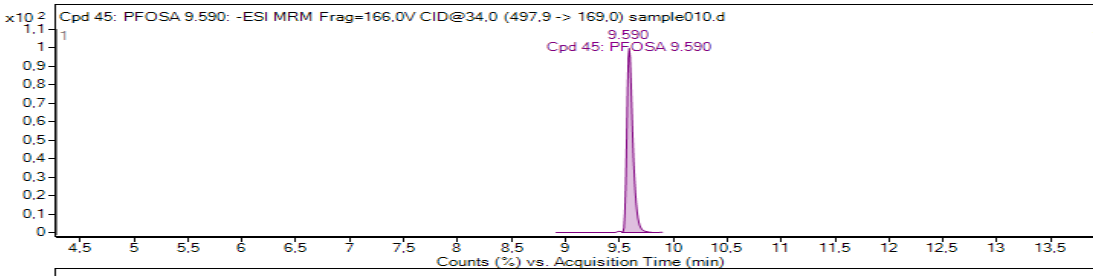
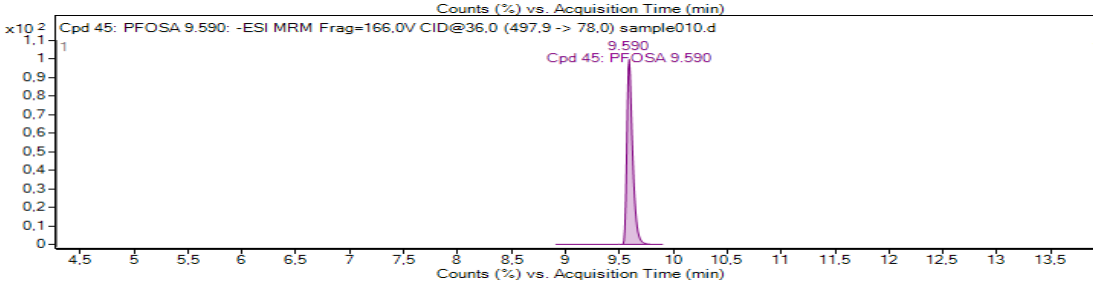
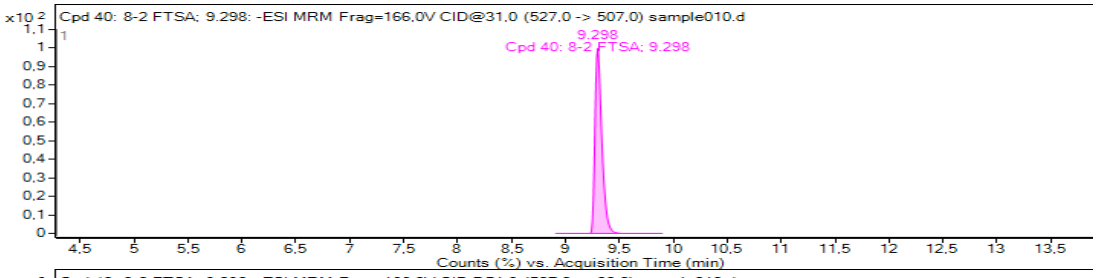
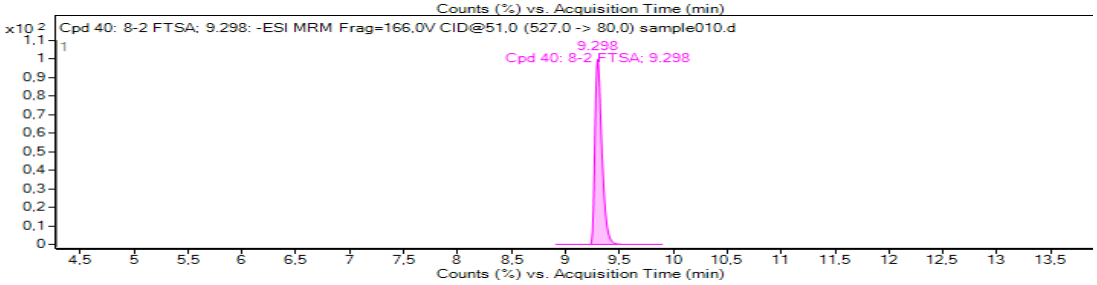




8:2 FTSA





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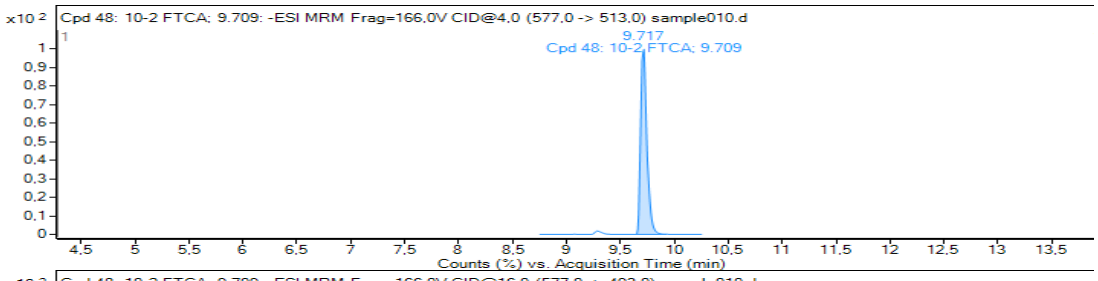
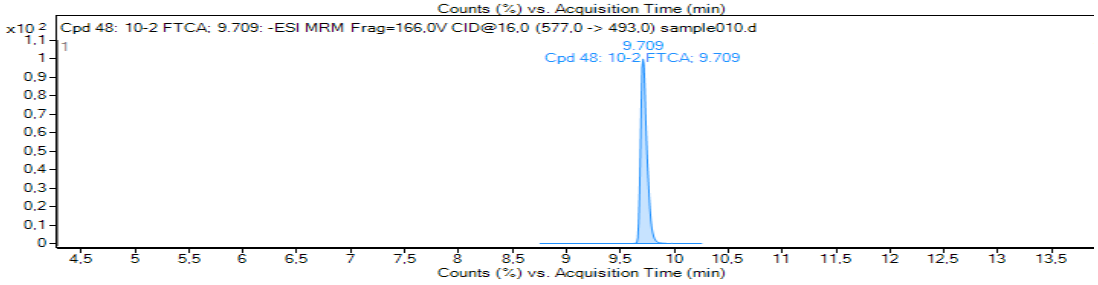
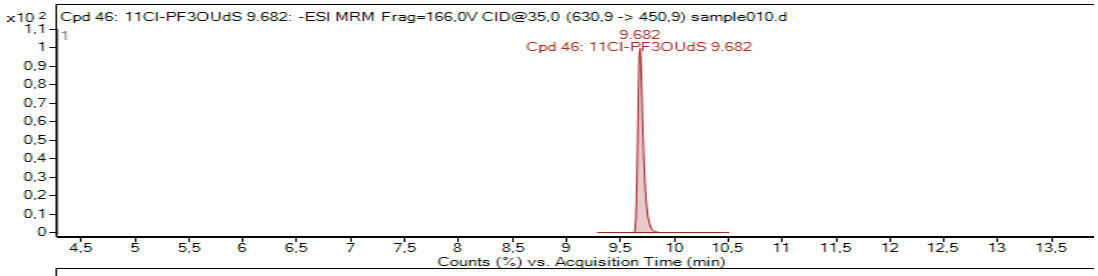
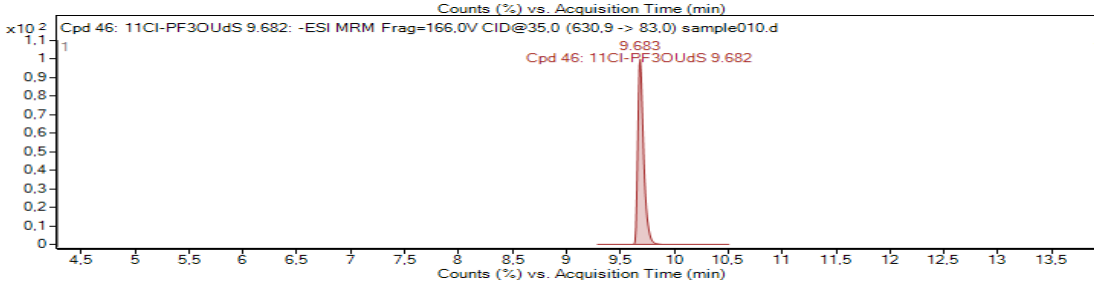




11Cl-PF3OUdS





DONA