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This study is carried out within the "Bureau Environnement et Analyses Gembloux" (BEAGx). BEAGx is an environmental analysis laboratory accredited by "Service Public de Wallonie" (SPW) and GLP certified. BEAGx is accredited for the analysis of waste and toxic waste since 1991.

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Résumé

Les molécules poly- et perfluoroalkylées (PFAS) sont des molécules persistantes dans l'environnement. Selon un recensement du National Institute of environmental Health sciences (NIH), il existe plus de 9000 PFAS identifiés. Il a été démontré que certains d'entre-eux (dont le PFOA et le PFOS) présentent des effets néfastes pour la santé humaine. La directive européenne UE 2020/2184 entrant en vigueur le 12 janvier 2026, imposera une limite de 100 ng/L pour un ensemble de 20 PFAs dans les eaux potables. En Wallonie, la limite de 100 ng/L sera appliquée pour un sous-ensemble de 10 PFAS. Le présent travail visait à développer et valider une méthode d'analytique en UPLC-MS/MS de ces PFAS dans des eaux de surface, des eaux souterraines et des lixiviats de déchet par injection directe après filtration sur filtre en verre 0.7 µm sans delay column et sans standard interne isotopique. Une colonne BEH C18 (1.7 µm, 2.1 x 50 mm) et d'une pré-colonne HSST3 (1.8 µm, 2.1 x 5mm) ont été utilisée avec un gradient de 12 minutes comportant une solution 10 mM de NH_4Ac dans l'eau milliQ (éluant A) une solution 10 mM de NH_4Ac dans MeOH:ACN (82:20, v:v) (éluant B). La justesse, la répétabilité, la reproductibilité ont été évaluées durant 5 jours non consécutifs dans des conditions de reproductibilité intra-laboratoire par dopage d'échantillons pseudo-blancs à 40, 100 et 200 ng/L. La calibration externe retenue s'étend de 10 à 500 ng/L et est linéaire pour la majorité des analytes et des jours. Les LOQ instrumentales étaient comprises entre 3 et 53 ng/L. Les rendements de récupération (%Rec) étaient de 74–114% pour les eaux de surface et souterraines et 26–153% pour le lixiviat de déchet et étaient conformes aux critères de validation. Les répétabilités pour les matrices testées étaient de 1 à 20 %RSD avec seulement deux modalités à 22 et 30 % RSD pour le PFBA dans le lixiviat de déchet à 40 et 100 ng/L. Bien que les performances étaient moins bonnes pour les lixiviats de déchets, la justesse et la répétabilité étaient conformes aux critères fixés par les méthodes EPA 533, EPA 8327 et EPA 3512 qui sont de maximum ±30 % et maximum 20 %RSD, respectivement, pour les trois matrices testées. La reproductibilité était généralement comprise entre 4 et 65% RSD pour les 3 matrices. La méthode validée permet d'obtenir des résultats satisfaisants sans recours à une delay-column ni à des standards internes isotopiques pour les eaux de surface et souterraines testées. Compte tenu de l'effet de matrice, un standard interne isotopique ou une quantification par ajouts dosés reste nécessaire pour le lixiviat de déchet testé.

Abstract

Poly- and perfluoroalkylated molecules (PFAS) are persistent molecules in the environment. According to a survey by the National Institute of Environmental Health Sciences (NIH), there are over 9,000 PFAS identified. Some of them (including PFOA and PFOS) have been shown to have harmful effects on human health. EU Directive 2020/2184, which comes into force on 12 January 2026, will impose a limit of 100 ng/L for a group of 20 PFASs in drinking water. In Wallonia, the 100 ng/L limit will apply to a subset of 10 PFASs. The aim of this work was to develop and validate a UPLC-MS/MS analytical method for these PFASs in surface water, groundwater and waste leachates by direct injection after filtration on a 0.7 µm glass filter without delay column and without internal isotopic standard. A BEH C18 column (1.7 µm, 2.1 x 50 mm) and an HSST3 pre-column (1.8 µm, 2.1 x 5mm) were used with a 12-minute gradient comprising a 10 mM solution of NH_4Ac in milliQ water (eluent A) and a 10 mM solution of NH_4Ac in MeOH:ACN (82:20, v:v) (eluent B). Accuracy, repeatability and reproducibility were assessed over 5 non-consecutive days under intra-laboratory reproducibility conditions by spiking pseudo-white samples at 40, 100 and 200 ng/L. The external calibration used concentrations from 10 to 500 ng/L and is linear for most analytes and days. Instrumental LOQs ranged from 3 to 53 ng/L. Recovery percentages (%Rec) were 74–114% for surface and groundwater and 26–153% for waste leachate and met validation criteria. Repeatbility for the matrices tested were 1 to 20% RSD with only two modalities at 22 and 30% RSD for PFBA in waste leachate at 40 and 100 ng/L. Although performance was worse for waste leachate, accuracy and repeatability met the criteria set by EPA Methods 533, EPA 8327 and EPA 3512 which are maximum ±30% and maximum 20% RSD, respectively, for the three matrices tested. Reproducibility was generally between 4 and 65% RSD for the 3 matrices. The validated method makes it possible to obtain satisfactory results without the use of a delay-column or internal isotopic standards for the surface and groundwater tested. Given the matrix effect, an internal isotopic standard or quantification by dosed additions remains necessary for the waste leachate tested.

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Glossary

ACN: Acetonitrile
AFFF: aqueous film-forming foam
dSPE: dispersive solid phase extraction
ECHA: European Chemical Agency
EF: enrichment factor
EPA: United States Environmental Agency
ESI -: negative electrospray ionisationmode
FFF: firefighting foam
HDPE: high density polyethylene
HLB: hydrophilic-lipohilic balance
IED: Industrial Emissions Directive
L-PFBS: Potassium perfluoro-1-butanesulfonate
L-PFHxS: Sodium perfluoro-1-hexansulfonate
L-PFOS: Sodium perfluoro-1-octanesulfonate
MeOH: methanol
MRM: multiple reaction monitoring
MS: mass spectrometer
MS/MS: tandem mass spectrometry
NH_4Ac: ammonium acetate
PAN: polycrylonitrilePFAAs: perfluoroalkyl acids
PEEK: PolyEtherEtherKetone
PFASs: Per- and polyfluoroalkyls substances
PFBA: Perfluoro-n-butanoic acid
PFBS: Perfluorobutanesulfonic acid
PFCAs: perfluorocarboxylic acids
PFDA: Perfluoro-n-decanoic acid
PFHpA: Perfluoro-n-heptanoic acid
PFHxA: Perfluoro-n-hexanoic acid
PFNA: Perfluoro-n-nonanoic acid
PFOA: Perfluoro-n-octanoic acid
PFPeA: Perfluoro-n-pentanoic acid
PFSAs: perfluoroalkane sulfonic acids
ppb: part per billion (µg/L)
ppt: part per trillion (ng/L)
Pre-PFAAS: precursors to perfluoroalkyl acids
PTFE: polytetrafluoroethylene
QUEChERS: Quick Easy Cheap Efficient Rugged Safe
R^2: coefficient of determination
Rt: retention time
SPE: solid phase extraction
SPM: suspended particulate matter
SPME: solid phase micro extraction
TOP assay: total oxidizable precursor assay
U(H)PLC: Ultra-high performance liquid chromatography
WAX: weak anion exchange

1 Introduction

1.1 Synthetic environmental contaminants

Every day, new synthetic chemicals are produced. Even if synthetic chemicals have provided benefits to world population (pests control, additives or surfactants) they are now outbalanced by negative impacts such as environmental contamination, exposure to human and toxicity[1].

Because of their wide range of applications and chemical structures, it is impossible to group them all into only one category. Different classifications can be used to distinguish one type of molecule from another. One of the classes of chemical contaminants of greatest concern are Persistent Organic Pollutants (POPs).

POPs are considered persistent in the environment because of their resistance to natural degradation processes. POPs may also have adverse effects on human health[2]. The most common POPs are organochlorines pesticides (DDE, DDD, DDT[I]) and polychlorinated biphenyls (PCBs) and some of them are part the "dirty dozen" from the Stockholm convention[II].

POPs are not the only chemical contaminants which are synthetically produced. Another class of synthetic chemicals of rising concerns are Per- and PolyFluoroAlkyls Substances (PFASs). PFAS are used in numerous industrial applications[III] and in daily products such as food packaging and cookware[IV] or as surfactant in aqueous film-forming foam (AFFF)[3]. PFAs are a class of manufactured chemicals containing per- or polyfluoroalkylated alkyl chains. In addition, perfluorocarbon fractions are neither hydrophilic nor lipophilic giving them good surfactants properties[4]. Hydrogen atoms of alkyl chains are artificially replaced

[I] https://www.sciencedirect.com/science/article/pii/S0147651320311568#:~:text=Organochlorine%20pesticides%20(OCPs)%20are%20persistent,a%20wide%20variety%20of%20crops.

[II] http://www.pops.int/

[III] t.ly/o4RnW

[IV] t.ly/fOq-

by fluorine atoms (details in section 1.2.2). The carbon-fluor bonds makes them environmentally persistent due to the chemical stability of the bond[5]. That is one of the reasons they are called “forever chemicals”.

1.2 PFAs chemistry

1.2.1 Definition

PFAS constitute a vast class of molecules, there were more than 9000 different identified PFAS[I]. Different definitions can be applied to determine whether a molecule is considered as PFAS or not.

PFAS can be distributed in two main categories: polymers (PTFE or Teflon, aerospatial lubricant, etc…) and non-polymers[3]. In this work, the focus was put on the category of non-polymer PFASs. For the non-polymeric PFASs (per- and polyfluoroalkyls), it is possible to differentiate them according to the fluorination degree and the moieties attached to the alkyl chain. This classification will be explained more in details in the next section.

In 2011, Buck et al. defined PFAS as following “*the highly fluorinated aliphatic substances that contain 1 or more C atoms on which all the H substituents (present in the nonfluorinated analogues from which they are notionally derived) have been replaced by F atoms, in such a manner that they contain the perfluoroalkyl moiety* C_nF_{2n+1}”[6]. Recently, efforts have been made to consider PFAS as a more comprehensive group, because the definition from Buck et al. (2011) contained some gaps. The OECD (2021) proposed a revised PFAS definition: “*PFASs are defined as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it), i.e. with a few noted exceptions, any chemical with at least a perfluorinated methyl group (–*CF_3*) or a perfluorinated methylene group (–*CF_2*–) is a PFAS”*[7]. This definition

[I]https://www.niehs.nih.gov/health/topics/agents/pfc/index.cfm#:~:text=PFAS%20are%20used%20in%20hundreds,9%2C000%20PFAS%20have%20been%20identified.

allows to include more molecules into the PFAS group. Since there are more than 9000 of known PFAS, it could be interesting to develop an analytical method for total PFAS.

The analysis of PFAS is complex and it is mainly due to:

- More than 9000 PFAS listed: targeted analysis can become complex with external and/or internal standards.
- There is still no scientific consensus on the PFAS definition since different authors propose similar but different definitions.

1.2.2 PFAS structure

1.2.2.1 Fluorination degree and functional group

As seen before, PFAS are molecules that contain at least one fully fluorinated methyl or methylene carbon. Perfluoroalkyls are PFAS that have entirely fluorinated alkyl chains, whereas polyfluoroalkyls are PFAS that do not have fully fluorinated alkyl chains. In addition, the functional group attached on the alkyl can be different from a PFAS to another. PFAS can also be differentiated by the length of their alkyl chain. PFAS can also have side chain, for example: scotchgard (marketed by 3M) or GenX (2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid) used to replace PFOA in AFFF[8]. Depending on the synthesis pathway, a majority of PFAS will be produced in their linear or branched form. Figure 1.1 gives a glimpse of the different criteria mentioned above and the resulting complexity of PFAS chemistry. In the polyfluoroalkyl group, there are perfluoroalkane, sulfonamido and fluorotelomer substances. Those last two categories can undergo a transformation in the environment and are consequently considered as perfluoroalkyl polymers. They may contribute to the total potential contamination of PFAS as precursors[6]. This point will be developed later (section 1.2.4).

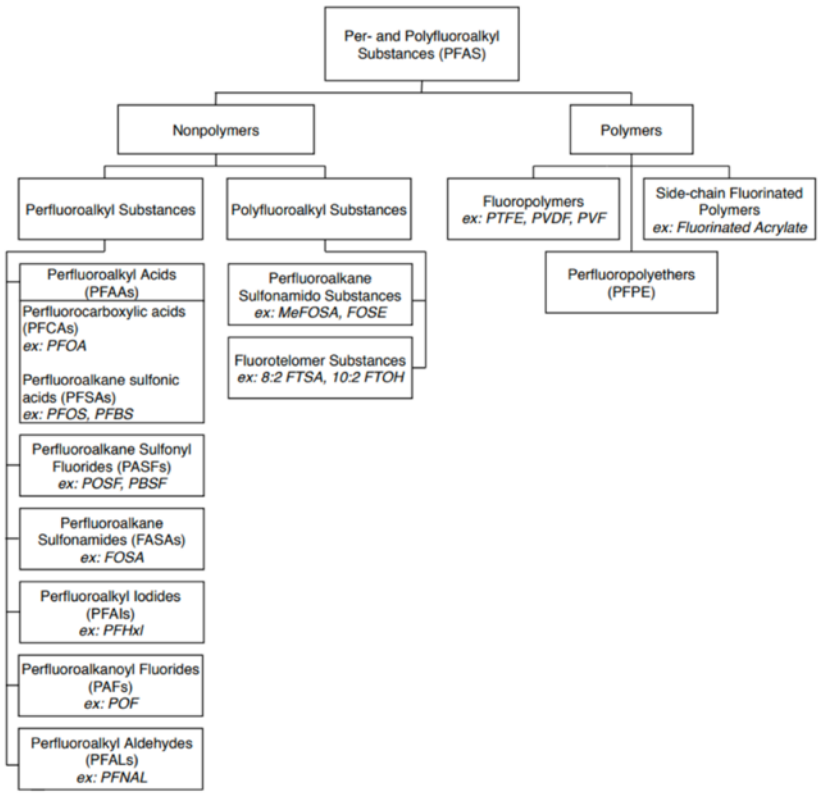


Figure 1. 1. PFAs categories using information from Buck et al. (2011)[6] and ITRC PFAs Team

A head functional group is attached to the alkyl chain, adding more PFAS subcategories. The functional group can include carboxylic acids, sulfonic acids, alcohols amongst other and their derivatives[9]. The most studied categories are perfluorocarboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs), which are characterized by a carboxylate or a sulfonate functional group, respectively. PFCAs and PFSAs are part of the perfluoroalkyl acids (PFAAs) class, are both acidic molecules. Their carboxyl or sulfonic acid groups have high polarity and their alkyl chain low polarity, resulting in a good solubility in organic solvents[10] .

Perfluoro-n-octanoic acid (PFOA) and perfluoro-1-octanesulfonate (PFOS) are amidst the most studied PFAs[5] and belong to the PFAAs class. Their molecular structure is very similar, the main difference being the attached functional group. PFOS and PFOA have only their functional group differentiating from each other: a carboxylate group PFOA and a sulfonate group for PFOA (Fig. 1.2).

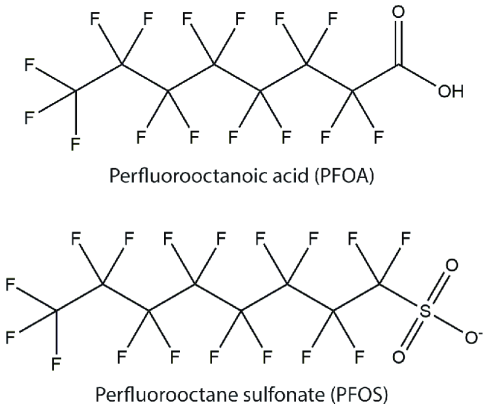


Figure 1. 2. Perfluorooctanoic acid (PFOA) and perfluoro-1-octanesulfonate (PFOS) 2D structure[11].

Depending on its pH level and its acid dissociation constant, a PFSA can be found in either their salt or acid form[6]. The functional group governs many properties of PFAS. The anionic or undissociated acid form can alter different PFAS properties[12]. Because of their low acid dissociation constants, PFAAS are found in their anionic state in the environment.

PFCAs and PFSAs differ by their global polarity. PFAAs can establish bond at both their polar and nonpolar regions. PFAAs are amphiphilic and can interact with organic molecules[13].

1.2.2.2 Alkyl chain length and ramification

PFAS can also be distinguished by the length and ramification of the alkyl chain. According to their chain length, they can be separated as follows[6]:

- Ultra-short chain (with 1 or 2 carbons, e.g., trifluoroacetic acid (TFA))
- Short-chain (with 3 to 6 carbons)
- Long-chain (with more than 6 carbons)

Short-chain PFASs have been manufactured to replace long-chain PFAS. The reason is that regulations first aimed long-chain PFAS. Ultra-short chain is a category apart from the other PFAS. Wallington et al. (2021) says that is not appropriate to include them in the PFAS chemical class because they are too small[14]. On the opposite, the OECD considers TFA, an ultra-short chain PFAS, as being part of PFAs. The fluorinated alkyl chain portion of PFAS molecules is nonpolar[5]. The chain length influences the polarity of the molecule: longer chain PFAS are more apolar compared to shorter chain PFAS[15].

1.2.3 PFAAS precursors

In the environment there can be co-occurring zwitterionic, cationic neutral and anionic PFAs that can be precursors to PFAAS (pre-PFAAS)[16]. Depending on ambient pH compared to their isoelectric point, pre-PFAAS can be found in their anionic, neutral or cationic form.

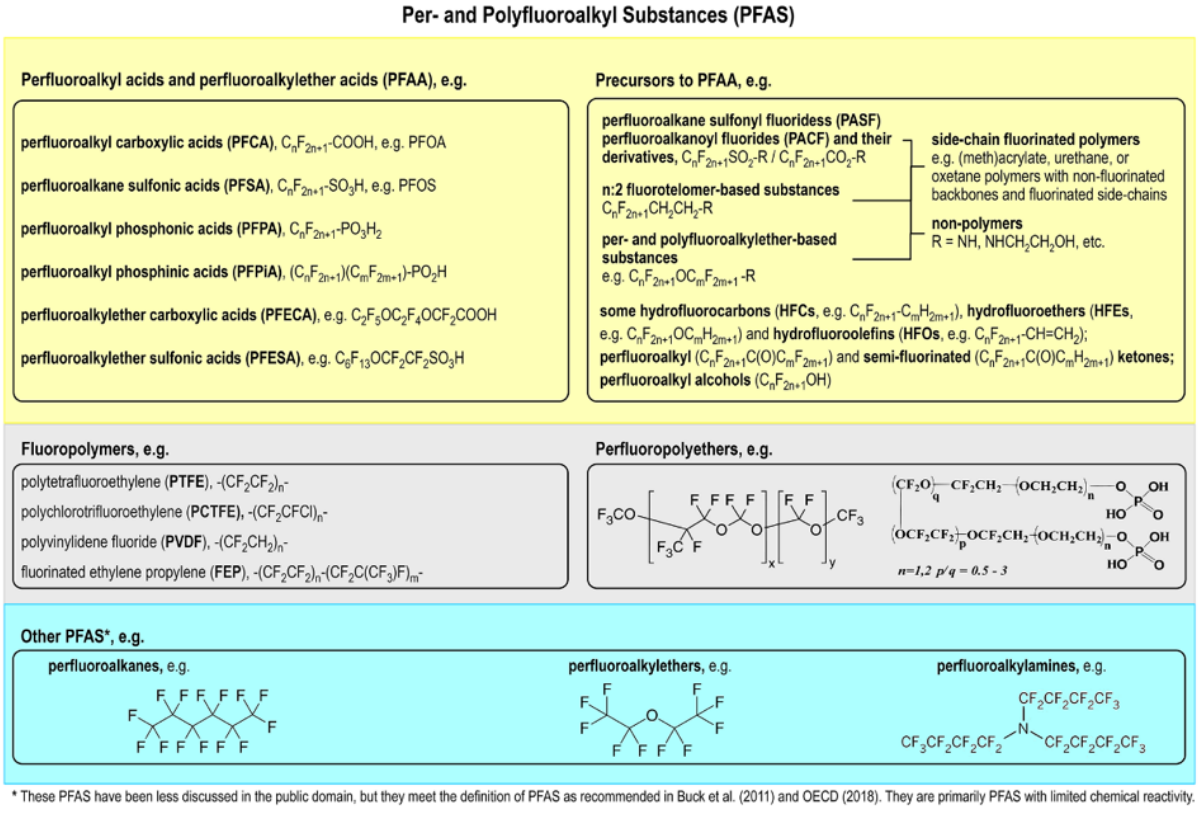


Figure 1. 3. Examples of PFAs chemistries. These PFAs have been less discussed in the public domain, nut they meet the definition of PFAs as recommended in refs 58 and 5. They are primarily PFAs with limited chemical reactivity.

Pre-PFAAS also include less studied PFAS containing amines, betaines, amine, oxides and quaternary ammonium moieties[16]. Different PFAS categories and their precursors are shown in Fig. 1.3.

1.3 Synthesis and Applications

1.3.1 Synthesis

Electrochemical fluorination (ECF) was the first industrial production process for PFAS. A more productive method, telomerization, has gained in popularity[5]. ECF is suitable to produce for carboxylic and sulfonic PFAS derivatives. Telomerization and ECF produce different proportions of linear PFAS, above 70 % for telomerization and below 70 % for ECF[17]. Some PFAS, like PFOA and L-PFOS, can be found in either their linear or branched forms. PFOS branched isomers are relatively more reactive and more polar than linear isomers[17,18]. It is why branched isomers are more likely to occur in aquatic environment than linear isomers[18]. It can be said that the PFASs industrial production process and their sources impact PFAS environmental fate.

1.3.2 Sources into the environment and Applications

There are two types of sources of PFAS into the environment: direct and indirect. Direct sources come from use, manufacture, and waste disposal. Direct sources account for most of the PFAS in the environment. The main contributors are fluorochemical production plants[19]. Indirect sources are PFAS produced from degradation or biotransformation of precursor compounds[6,19]. PFOA, for example, can be formed following the biotransformation of 8:2 fluorotelomer alcohol[6]. A representation of different emissions sources and exposure pathways are resumed in Fig. 1.4.

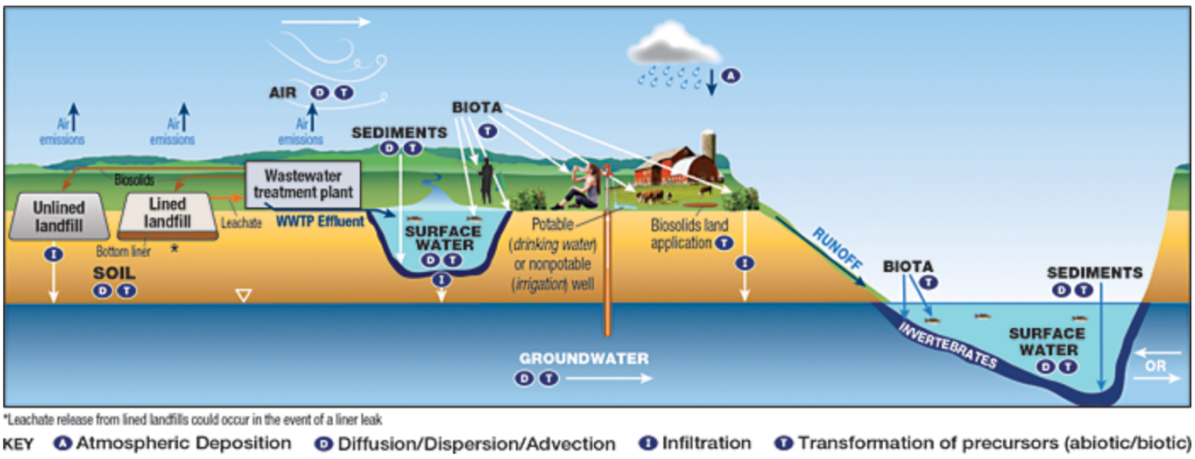


Figure 1. 4. common elements of conceptual site model associated with the potential release scenarios at waste management facilities.

The most prominent industrial and manufacturing sources are diverse. Building and construction have increasingly used composite wood and OSB. Bečanová et al. (2016) found concentrations of short chain PFCAs and PFOA ranging from 1.37 to 13.9 µg/kg in OSB samples[20]. The same study also showed that wood insulation fibre and other forms of building insulation may contain PFCAs. PTFE is another PFAS used in architectural fabrics. It also has been used in wire and cable industry. It is because PTFE confers chemical resistance, thermal resistance and water repellency[21]. PFAS are used in metal plating to reduce surface tension, allowing metal coating. They are then used as surfactants up to a concentration of 5 to 10% to reduce air bubbles production and aerosols emissions[22]. After this process, electrolyte solution can be treated to remove metals. But PFOA and PFOS can still be present in the effluent, leading to sewage sludge contamination[23].

PFAS are also used as grease proofing agents on food-contact materials. PFAS use for this purpose are restricted nowadays. In the USA, the most frequently PFAS found belong to fluorotelomer sulfonates (ex.: 6:2 FTS), PFCAs (ex.: PFOA and PFHxA) and PFSAs (ex.: PFBS) categories [24]. PFAS water and oil repellence properties are also used for textiles. This can result in exposure to long-chain PFAS[25].

The release of PFAS into the environment is principally related to solid waste management. The main reason is that landfills are the last repositories for PFAS contaminated waste[26]. PFAS can also be emitted into the environment from the use of wastewater treatment plants sludge[4]. Waste incineration ashes may also contain remaining PFAS[27]. Volatile PFAS can also be emitted from open air landfills and wastewater treatment plants[27].

In 2019, approximately 100000 sites were potentially emitting PFAS in Europe[28]. Fig. 1.5 clearly shows the ubiquity of PFAS contamination.

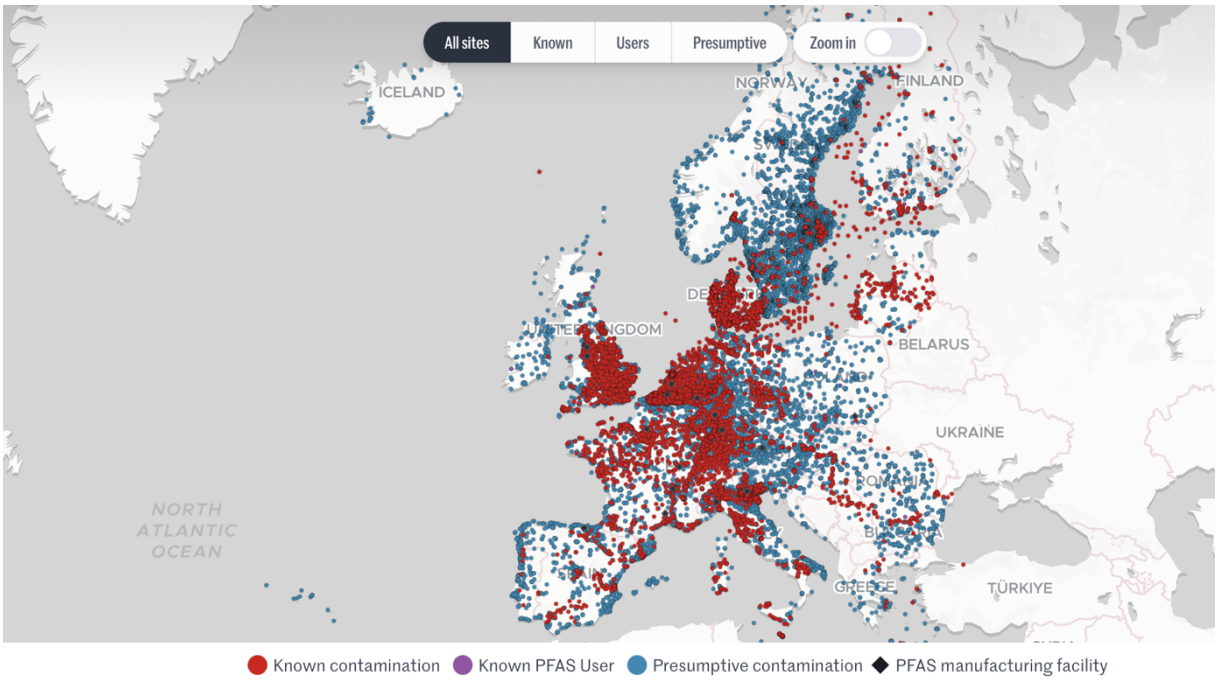


Figure 1. 5. This map shows PFAS production facilities, some sites where PFAS are used, as well as sites where contamination has been detected and sites where contamination is suspected but not yet confirmed (presumptive contamination). This map is originated from an investigation lead by "Le Monde" Journal [29].

1.4 Physicochemical properties and environmental fate

When the non-polar chain is linked to a more polar structure, PFAS become good surfactants. PFAS are also environmentally persistent because of their C-F bonds. It makes them recalcitrant in the environment: non-biodegradable, non-reactive, non-photolytic, and hydrolysis-resistant[5]. Some fluorotelomer substances precursors can also be *in fine* degraded into PFAAs (a sub class of PFAS).

1.4.1 Physicochemical properties

The success of PFAS can be attributed to their physical and chemical properties resulting in surface tension reduction[3]. PFAS are amphiphile molecules because of their structure: a non-polar alkyl chain and a more polar head functional group (carboxylic or sulphonic acid). PFAS are considered as good surfactants.

According to various studies, some PFAS present in samples from aqueous environment can adsorb on plastic surfaces like high density polyethylene (HDPE)[30,31]. Long-chain PFAS

tend to adsorb on polypropylene containers walls, if preservation solution contains less than 40% of MeOH. As a function of the length of the alkyl chain, PFAS show different solubilities in water:

- 2-6 carbons chains are generally water soluble.
- > 9 carbons chains are generally not water soluble.

1.4.2 Environmental fate

As said previously, PFAS are particularly persistent in the environment. Due to their high persistence, PFAS accumulate in the environment and may accumulate in water bodies and in the biota[9].

PFAAs are mobile in the environment. To discuss about their mobility, they can be subdivided in two categories: short and long chains. Short-chain PFAAs are very mobile in the environment[32]. Combined with their persistency, this may cause them to accumulate in certain environmental compartments (water bodies, soils, etc.), depending on their intrinsic properties. Some PFAS can be very mobile in the air leading to their transport over long distances. Zhao et al. (2016) observed that short chain PFAS prevailed in surface water[33]. In contrast, long-chain PFAS are prevailing in suspended particulate matter (SPM) and sediments. In the same study, it was shown that both SPM-water and sediment-water distribution coefficients increased with the alkyl chain length. This may be partially explained by their molecular weight. Indeed, long-chain PFAS are heavier and sink faster than shorter chain PFAS. The environmental partitioning of PFAS can also be affected by the different properties of branched and linear forms[34].

1.5 Regulations

PFAS are gaining increasing public attention. Despite this, knowledge gaps regarding PFAS persist. A European commission staff working document underlined the need to develop targeted and non-targeted analytical methods[35]. These methods could help to develop and enforce future legislations.

1.5.1 European regulations and legislations

The European Chemicals Agency (ECHA) works with its partners to promote the safe use of chemicals[I]. The goal is to develop enough knowledge to serve EU policies. PFAS undergo different regulations. For example, PFOA and PFOS have been defined as POPs by the Stockholm convention and are therefore regulated by the Regulation (EU) 2019/1021[36].

In 2007 the Registration, Evaluation, Authorisation, and Restriction of CHemicals (REACH[II]) entered into force. This is a regulation adopted by the EU to improve general protection against the risks posed by chemicals. REACH stipulates that it is the responsibility of companies to prove that the chemicals they sell comply with European standards. Currently, the PFAS subject to restrictions under REACH are linear and/or branched C9 to C14 perfluorocarboxylic acids and their salts[37]. REACH also promotes alternatives to hazardous chemicals whenever possible.

Water legislations do not restrict all known PFAS. The Directive 98/83/EC set a concentration limit of 0.1µg/L for a sum of 20 PFAS. For PFAS total, the limit has been set at 0.5µg/L. Limit on PFAS total will be enforced only when an analytical technique able to measure PFAS total will be available[38]. It underlines the need to fill knowledge gap about PFAS. For groundwater, a review of the directive 2006/118/EEC lead to an initiative to obtain more data on PFAS posing potential risks. The result is a monitoring list of 10 PFAS, but no limit values have been set. In the Water Framework Directive (Directive 2000/60/EC), the only PFAS classified as a priority hazardous substance is PFOS. When new analytical methods become available, other PFASs will most likely be added to these lists.

Waste legislations differ depending on the source of emissions. Industrial waste is covered by Directive 2010/75/EU. Installations producing PFAS or using PFAS in their processes are covered by the Industrial Emissions Directive (IED) permits. IED permits set a production limit and are based on best available techniques. Where competent authorities set emission limit values in IED permits, these must not be exceeded. PFAS may be present in sludge from wastewater treatment plants[38] and are covered by IED. It can be a source of

[I] https://echa.europa.eu/about-us

[II] https://echa.europa.eu/regulations/reach/understanding-reach

PFAS into the environment. The directive 86/278/EEC on the protection of the environment and in the soil did not set limits for PFAS.

There are many examples of PFAS-contaminated soil in the EU (Fig. 1.5). Member States can set their own limit values for PFAS in soil. However, very few have done so. Belgium, Germany and the Netherlands have started to measure background PFAS contamination in soil. This will help identify the main sources of PFAS contamination and contribute to the development of guidelines and standards[30].

1.5.2 Consequences of legislations on PFAS diversity

Historically, the PFASs subject to restrictions have been those with long chains. This is mainly due to their widespread use in industry in the past, their potential toxicity and their tendency to bioaccumulate. As a result, long-chain PFASs have been replaced by shorter-chain PFASs. The shorter-chain PFASs are less bioaccumulative, but they are more mobile (including in the air) and have a similar environmental persistence[38]. It can be said that manufacturing industries are legally forced to reduce PFAS chain length. ECHA published a proposal for the restriction of shorter chain PFAS that has been driven by Germany, Denmark, Netherlands, Norway, and Sweden.

Replacing long-chain PFASs with short-chain PFASs poses certain problems. They are less efficient and will therefore potentially be used in greater quantities, leading to an increase in emissions[39]. Another problem with PFAS in general is their environmental persistence. Even if a regulation were applied soon, PFAS already present in the environment (including precursors) would still constitute a reserve of emissions into the environment in the future.

One possible way of addressing concerns about PFAS and avoiding unfortunate substitutions would be to treat them legally as a group and not regulate them individually. If legislation begins to treat PFASs as a group, it will be necessary to measure all PFASs. Analytical techniques for doing this could be Total Organic Fluorine (TOF) or Total Oxidisable Precursors (TOP), which are discussed below (section 1.6).

1.6 Analytical methods

Different analytical methods can be used to quantify PFAS levels in different environmental matrices. Most of the analytical methods are targeting specific PFAS, whereas non-targeted analyses are less regularly met. They each present different benefit and they can be complementary to one another.

1.6.1 Reference methods

There are different reference methods that use analytical methods targeting specific PFAS. Those methods can be reference methods issued by governmental institutions like the United States Environmental Protection Agency (EPA). Each method has been validated for specific PFAS, specific instrumentation, specific environmental matrix, extraction and purification protocol.

Because PFAS are omnipresent in the environment, they are also found in laboratory hardware such as filters and instrumental tubing. Therefore, different application notes[40,41] and reference methods as EPA 533[42] and EPA 8327[43] recommend the use of a delay column after solvent mixer and before analytical column (cf. Fig. 1.6). This point will be explained in more details later.

1.6.2 Non targeted analysis

Because of the vast diversity of structure within PFAS, classical analytical methods cannot focus on all known PFAS. Two alternative methods could be the total oxidizable precursor assay (TOP assay) and total organic fluorine (TOF) assay.

Non targeted analytical methods present different advantages over targeted analytical methods. The Staff Working Document from the European commission of 2020 on PFAS underlined the need to develop a technique that could be able to quantify PFAS in their totality[38]. It should allow a better understanding of the PFAS contamination in the environment by doing a more comprehensive approach of the problematic.

1.6.2.1 Total Organic Fluorine (TOF) assay

Total Organic Fluorine (TOF) assay constitutes a potential rapid screening tool to identify the total PFAS presence or absence[44]. It allows to quantify the total amount of organic fluorine which is also found on PFAS alkyl chain. This technique could be used as a complement or prior to targeted PFAS analysis. If the TOF content of a sample exceeds limits values of PFAS, it could be due to the presence of PFAS. TOF assay can be used for the analysis of a broad spectrum of PFAS. TOF assay presents some drawbacks: there are few comparable data, and it is not yet commercially available as itself, but different analytical methods can be used to perform it:

- Vacuum UltraViolet (VUV) photolysis coupled with ion chromatography: Li et al. (2022)[45] proposed first to convert TOF in fluoride by VUV photolysis. The fluoride analysis was realized with Ion Chromatography. This method showed satisfactory fluoride recoveries in real water samples[45].
- TOF assay by combustion ion chromatography: samples are prepared to eliminate inorganic fluorine. Extracts will then be combusted at temperatures > 1000 °C. Subsequently fluorine is quantified by ion chromatography[46].
- Oxygen combustion coupled with fluoride ion-selective electrode (F-ISE): this method presents a good accuracy and is relatively inexpensive compared to other TOF assay methods[47].
- Flow injection method: it can be coupled with two detection methods, in both case the first step is an offline sorption. Sorbent used is activated carbon, the reaction of defluorination is carried out with sodium biphenyl (SBP). Fluoride is analysed by ISE or potentiometric detection[48].

1.6.2.2 Total Oxidizable Precursor (TOP) assay

The PFAS TOP assay was first developed in 2012 as a non-targeted method for PFAS. It thus provides a better understanding of the extent of global PFAS contamination within a sample[49]. This method enables a more comprehensive understanding of PFAS contamination, consequently giving the potential to establish more relevant regulations. Briefly, it consists in the oxidation of oxidizable PFAS precursors, in strong basic conditions (pH 13 to 14),

while heating the samples up to 85°C[50]. An inter-laboratory study concluded, by comparing three different methods, that TOP assay was a semi quantitative method. It also concluded that it was not robust enough to help in future regulations. Unfortunately, there is not yet inter-laboratory validated standard methods[44].

1.6.3 Extraction

Different types of solid phase extrication (SPE) techniques exist. The main used technique to extract PFAS in environmental samples is the SPE Weak Anion eXchange (SPE-WAX) on cartridges . Other techniques exist such as Solid phase micro extraction (SPME) and dispersive solid phase extraction (dSPE).

1.6.3.1 Solid phase extraction (SPE)

WAX sorbent is commonly used for extraction of PFAS in complex matrixes. WAX moiety at a certain pH range is cationic and allows anion exchange with the negatively charged acid moieties of PFAS. SPE-WAX can be used in cartridges[40,51]. One advantage of the SPME technique compared to SPE-WAX is the use of less solvent for a similar sensitivity. The SPME extraction phase can be hydrophilic-lipophilic balance (HLB) based. HLB based extraction phases allow a balanced coverage of PFAS[52]. It interacts with the hydrophobic tail and the hydrophilic head of PFAS. Unfortunately, using HLB-based phase can lead to lower recoveries for PFOS and PFOA[52]. Olomukoro et al. (2021) underlined that HLB-WAX particles imbedded within polycrylonitrile (PAN) allowed a balanced coverage while increasing PFOA, PFBS and PFOS recoveries substantially. As we can see in SPME, an adequate fibre coating is crucial and so are extraction conditions. Method EPA 533[42] used a MeOH:H_2O (80:20, v:v) mixture to desorb PFAS from WAX sorbent and to avoid carryover contamination. This mobile phase is the one used in the presented work.

1.6.3.2 Dispersive SPE (dSPE)

Despite their performances to extract PFAS selectively, SPE-WAX methods do have drawbacks in comparison to dSPE:

- Time consuming

- Need of trained technician
- Cost of cartridges
- More steps leading to more variability and potential errors

1.6.4 Analytical columns

The large number of potential PFAS analytes that could potentially be present in a sample will inevitably challenge simple chromatographic separation approaches[53]. It appears that most of the used chromatographic columns for PFAS are using the reversed phase mode[54]. C18 phase chromatographic columns also meet the requirements of EPA method 537.1[55]. This stationary phase can bond to Ethylene Bridged Hybrid (BEH) particle[41]. A BEH C18 phase was used in the reference application note from which inspiration was taken for the method development of this work.

An additional column often used in PFAS analysis is a delay column. The aim is to delay PFAS contamination coming from the apparatus, tubings and mobile phases placed before the analytical column. It traps PFAS by holding them on a stationary phase similar to that of the analytical column[56]. The delay column is set after the solvent mixer and before the analytical column (Fig. 1.6).

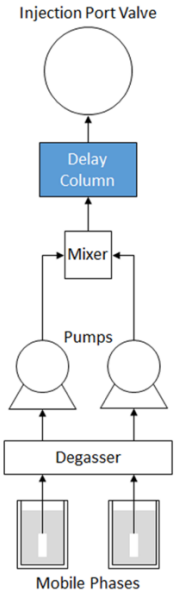


Figure 1. 6. This figure presents the position of a delay column when it is installed In a UHPLC configuration[56].

A delay column allows to mitigate interferences from background PFAS[57]. The polytetrafluoroethylene (PTFE) is often found throughout a typical lab and can contribute to trace PFAS contamination. Most LC-MS/MS instruments have PTFE components that can slowly leach out, interfering with PFAS analysis. It is continuously feeding interfering compounds at low levels in the analysis. One solution is to use a delay column[58]. The delay column will help to separate analytes and contamination coming from instrument parts before the analytical column. PolyEtherEtherKetone (PEEK) tubing can also replace traditional PTFE coated solvent tubing[41].

1.6.5 Solvents

As mentioned in Preston et al. (2021), current PFAS methods mainly rely upon C18 solid phase chemistry and simple methanol-ammonium acetate mobile phase gradients[53]. This was confirmed when comparing different methods in the literature. In different reference methods[42,55] and application notes[40,41], methanol (MeOH) is often used as the main solvent in the organic mobile phase. Acetonitrile (ACN) is also used alone in the organic mobile phase or in combination (certificate of analysis from *Wellington)* with MeOH[40,51]. Ammonium acetate or ammonium formate are sometimes added to mobile phases to increase the ionisation of the analytes in the mass spectrometer (MS) system. NH_4Ac can be used as pH buffer to avoid extreme acidification[59]. Most PFAS methods use NH_4Ac at concentration between 2 and 20 mM and this also helps ionization ESI mode[60].

1.6.6 Targeted Analysis and instrumentation

LC-MS/MS:

- UHPLC :
    - + : faster analysis, instrumental set up used in numerous application notes[41,61] and reference methods[43,55]
    - - : expensive, limited number of PFAS (available standards), does not account for the potential PFAAS environmental contamination on the opposite of TOP assay

- Mass spectrometer:

    - Triple quadrupole mass spectrometer (MS/MS or TQ MS):

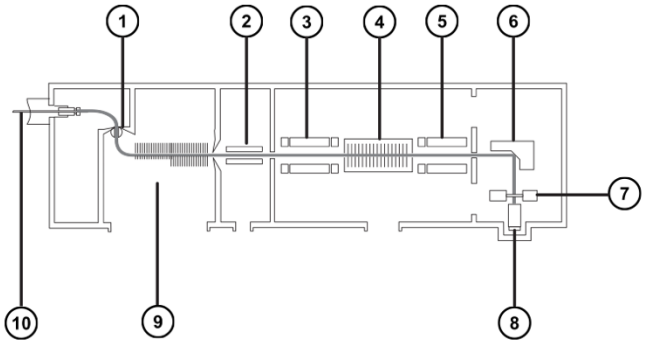


Figure 1. 7. Schematic diagram of tandem quadrupole mass spectrometer Xevo TQ-S micro from waters™. N° 1 : sampling cone, n° 2 : ion guide 2, n°3 : MS1, n° 4 : T-Wave collision cell, n°5 : MS2, n°6 : conversion dynode, n°7 : phosphorus disc, n°8 : photomultiplier, n°9 : ion guide 1, n°10 : probe.

A triple quadrupole mass spectrometer, or tandem quadrupole mass spectrometer (TQ MS or MS/MS), consists of three quadrupoles connected in series. The first and the third spectrometer are respectively called MS1 and MS2. Between MS1 and MS2 is located the collision cell. Precursor ions separated by the MS1 undergo collision induced dissociation in the collision cell (number 4 in Fig. 1.7). A MS/MS system allows to operate in different scanning mode such as multiple reaction monitoring (MRM). MRM is a scanning mode allowing a high specificity and selectivity and that can selectively quantify compounds[62]. The selection of a specific mass transition permits to avoid endogenous interferences[63]. MS/MS is not commonly used for untargeted analysis since previous knowledge of compounds is required to use MRM high sensitivity.

    - Quadrupole Time-of-Flight MS (Q-ToF-MS)

In Q-ToF-MS, MS2 from TQ MS is replaced by a time-of-flight analyser (Fig. 1.8). Q-ToF-MS provides a larger dynamic range than TQ MS, high mass resolution (if it is in HRMS) and resolution (Table 1.1). It is often used to identify unknown compounds such as in proteomics studies[64] and comprehensive drug screening [65]. Q-ToF-MS and its high mass resolution allows a precise acquisition of non-targeted mass. With the same injection volume, Q-ToF-MS is less sensitive than LC-MS/MS in MRM mode [66].

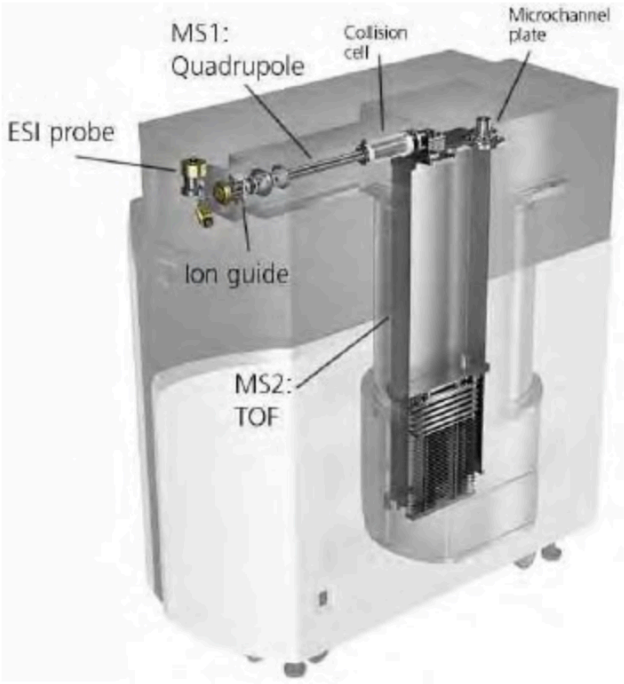


Figure 1. 8. Example of the instrumentation of a quadrupole Time-of-Flight (Q-ToF) MS[62].

Table 1. 1. Comparison between Triple Quadrupole Mass Spectrometer (TQ MS) and Quadrupole Time of Flight Mass Spectrometer.

MS/MS systems	Strengths	Limitations	Applications
TQ MS	Higher sensitivity (MRM mode) Wide dynamic range of detection Lower cost	Low mass resolution	Quantitative analysis in mrm mode Targeted analysis
Q-ToF-MS	High mass resolution Wide mass range Medium dynamic range of detection High sensitivity	Lower sensitivity than TQ MS in MRM mode	Qualitive analysis Structural elucidation sequencing

- High resolution mass spectrometry (HRMS)

HRMS thanks to high mass resolution can be used for molecule detection without hypotheses prior analysis[67]. HRMS can be used for the discovery of new PFAS or as a screening tool to discover new PFAS. Liu et al. (2019) inspected literature using HRMS for PFAS analysis. It resulted that more than 750 PFAS have been discovered while using HRMS[68]. HRMS is useful for the screening of untargeted PFAS. If no laboratory standards exist, it can achieve semi-quantification based on structurally similar analytes, retention times and masses[69]. HRMS system are expensive.

The different instrumental systems presented above have each their advantages and disadvantages. If the instrumental system available is suitable for the objective in question, it is preferable to use it, while being aware of its limitations.

1.7 Context and objectives

This study is carried out within the "Bureau Environnement et Analyses Gembloux" (BEAGx). BEAGx is an environmental analysis laboratory accredited by "Service Public de Wallonie" (SPW) and GLP certified since 1996. BEAGx is accredited for the analysis of waste and toxic waste since 1991.

As seen previously, PFAS will be subject to regulations in different environmental matrixes. The European directive UE 2020/2184 requires all European Union member states to comply with limit values for PFAS in groundwater. Directive UE 2020/2184 set two limits: at 0.1µg/L the sum of 20 PFAS (directive UE 2020/2184, annex I, part B) and 0.5 µg/L for "all PFAS" parameter. These limits are not yet in force in Wallonia and will have to be by 12th January 2026 at the latest. After consultation with the Walloon Groundwater directorate, the following interpretation can already be made: a limit of 0.1 µg/L for the sum of PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS and L-PFOS[70]. These acronyms are defined in table 2.1 (section 2.1).

Many reference methods and application notes exist already (section 1.6). These methods use different instrumental systems such as Q-ToF-MS or UPLC-MS/MS. If the instrumental set up is fit to purpose it can be used. BEAGx is equipped with an UPLC-MSMS from Waters™. Waters™ itself has published application notes for PFAS analysis using UPLC-MS/MS[41], as have ThermoFisher™[71] and Agilent™[40,51]. It is therefore necessary to develop an analytical method for the precited PFAS.

The objectives of this work are therefore to develop an analytical method for the list of 10 PFAS mentioned in this section for the following environmental matrices:

- Surface water (Orneau river)
- Groundwater (lysimeter)
- Waste leachate water

The second step of this work will be to verify the validity of this method.

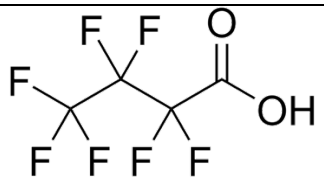
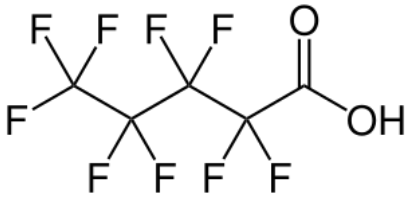
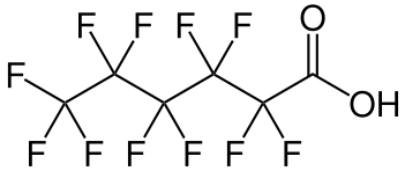
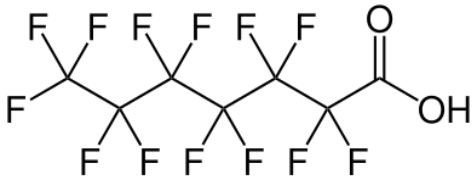
2 Materials and method

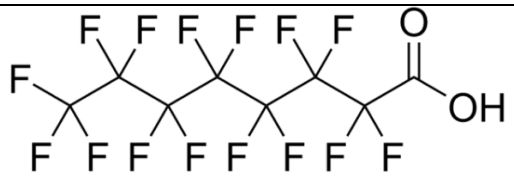
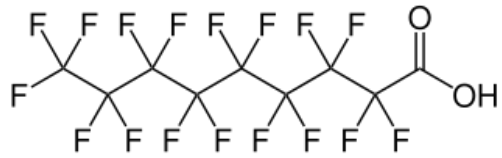
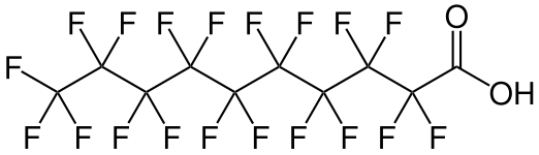
2.1 Analytical standard (PFAs Mix)

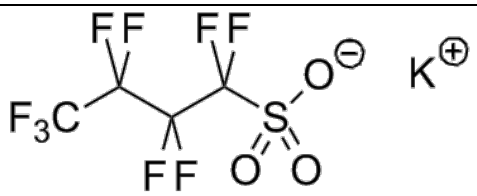
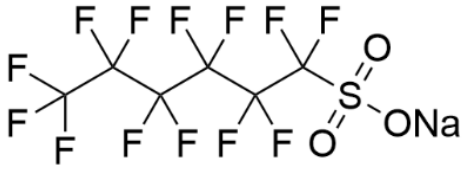
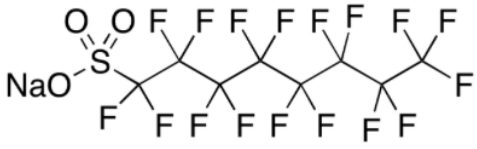
The mix of compounds analysed was supplied in an ampoule containing a standard solution from Wellington Laboratories (Canada). The solvent used in the ampoule containing the PFAs mix was MeOH (water < 1 %). The standard mixture was 4 molar of NaOH to prevent conversion of the carboxylic acids to their corresponding methyl esters.

The PFAs mix is a mixture seven perfluoroalkyl-carboxylic acids and three perfluoroalkyl-sulfonates whose full names, concentration and concentration are detailed in the Table 2.1.

Table 2. 1. PFAs mix compounds and concentrations (µg/L ± 5 % used in this study. In MeOH/H_2O (< 1 %)).

Compound		Concentration [µg/mL]
Perfluoro-n-butanoic acid (PFBA) (C4)		5.00
Perfluoro-n-pentanoic acid (PFPeA) (C5)		5.00
Perfluoro-n-hexanoic acid (PFHxA) (C6)		5.00
Perfluoro-n-heptanoic acid (PFHpA) (C7)		5.00

Perfluoro-n-octanoic acid (PFOA) (C8)		5.00
Perfluoro-n-nonanoic acid (PFNA) (C9)		5.00
Perfluoro-n-decanoic acid (PFDA) (C10)		5.00

Compound		Concentration [µg/mL]	
		As the salt	As the acid
Potassium perfluoro-1-butanesulfonate (L-PFBS) (C4)		5.00	4.44
Sodium perfluoro-1-hexansulfonate (L-PFHxS) (C6)		5.00	4.74
Sodium perfluoro-1-octanesulfonate (L-PFOS) (C8)		5.00	4.79

On reception, the standard mix solution was diluted 5 times in UPLC grade MeOH to reach a concentration of 1 µg/mL. It was stored in fridge at -20 °C.

2.2 Glassware cleaning

All the glassware was cleaned before and after use to not introduce RBS contamination in UPLC-MS/MS. It was first rinsed with technical acetone. The glassware was then immersed in a hot tap water bath for 30 minutes. It was then rinsed with tap water and then

rinsed with distilled water. The final rinse was with UPLC grade organic solvent (ACN or MeOH). The glassware was then left to dry overnight. This protocol complies with the recommendations issued from EPA method 8327 for reusable glassware cleaning in pfas analysis[72].

2.3 Ultra-performance liquid chromatography (UPLC)

The UPLC system was an Acquity UPLC H Class PLUS®. An Acquity UPLC® HSST3 VanGuard™ (1.8 µm, 2.1 x 5 mm) pre-column was installed before an Acquity UPLC® BEH C18 (1.7 µm, 2.1 x 50 mm) column. The column temperature was constant at 35 °C. The samples were kept at 10 °C in the autosampler manager. Installed injection loop had a maximum capacity of 250 µL. The injection volume was set to 200 µL during method development. Needle was washed with MeOH UPLC grade between each injection.

Other columns tested in this work for method development were:

- HSST3, 1.8 µm, 2.1 x 150 mm (HSST3 15)
- BEH C18, 1.7 µm, 2.1 x 50 mm (BEH C18)
- HSST3, 1.8µm, 2.1 x 5 mm (as a precolumn) (HSST3 5)

2.3.1 Elution gradient

Various solvent gradients were tested during the development of the analysis method. The elution gradient selected consisted of two phases. Only ultrapure water (18.2 MΩ at 25 °C, filtrated at 0.22 µm) was used for the mobile phases. From now on, ultrapure water will be referred to as milliQ water. Phase A consisted of 10 mM of ammonium acetate (NH_4Ac) in milliQ water. Phase B consisted of a mixture of MeOH:ACN (80:20, v:v) + 10 mM of NH_4Ac. The following table describes selected elution gradient at the end of method development (Table 2.2).

Table 2. 2. Elution gradient of the mobile phases A and B during the analytical gradient. Phase A consisted in milliQ water + 10mM of ammonium acetate. Phase B consisted in a mix of MeOH:ACN (80:20, v:v) + 10mM of ammonium acetate.

Time [min]	Flow rate [mL/min]	Mobile Phase A [%]	Mobile Phase B [%]	Gradient Curve
Initial	0.3	99	1	Initial
0.5	0.3	99	1	6
2	0.3	30	70	6
8	0.3	5	95	6
9	0.3	5	95	6
9.5	0.3	99	1	6
12	0.3	99	1	1

2.4 Mass spectrometer system

The mass spectrometer was a tandem quadrupole mass spectrometer Xevo TQ-S micro from Waters™. The ionisation mode used was in negative electrospray mode (ESI-). MS conditions were the same as those used in Rosnack et al. (2019)[41] (Table 2.3):

Table 2. 3. MS conditions used during the analysis of PFAs. The desolvation gas was nitrogen and the cone gas was argon The MS conditions detailed in this table are the same as those used in the application note from Waters™ (Rosnack et al., 2019)[41].

Ionization mode	ESI-
Capillary voltage	0.5 kV
Desolvation temperature	350 °C
Desolvation gas flow	900 L/hr
Cone gas flow	100 L/hr
Source temperature	100 °C

The mass spectrometer used in this work and the mass spectrometer used and in the Rosnack et al. (2019) were the same. The next table shows the MS tuning conditions used in this study (Table 2.4).

Table 2. 4. MS tuning conditions used in this study. CV and CE respectively stand for cone voltage and collision energy.

Analyte	Precursor [m/z]	Product [m/z]	CV [V]	CE [V]
PFBA	212.9	169	10	10
PFPeA	262.9	219	10	5 – 0
L-PFBS	298.9	80.1 – 99.1	15	30
PFHxA	312.9	119 – 269	5 – 5	20 – 10
PFHpA	362.9	169 - 319	15 – 15	15 – 10
L-PFHxS	398.9	81.1 – 99.1	10 – 10	35 – 30
PFOA	412.9	169 – 369	10 - 10	15 – 10
PFNA	462.9	219 - 418	10 – 10	15 – 10
L-PFOS	498.9	80.2 – 99.1	15 – 15	40 – 40
PFDA	512.9	219.9 – 468.9	15 – 15	15 - 10

The analytes were grouped in four separate functions (i.e., retention time windows) named there after function one, two, three and four. The four functions are presented in Table 2.5.

Table 2. 5. MRM functions distributed in retention time windows.

Function	Analytes	Retention windows [min]	Dwell time [s]
1	PFBA	3.5 to 4.2	0.165
2	PFPeA and L-PFBS	4.2 to 4.55	0.032
3	PFHxA, PFHpA and L-PFHxS	4.45 to 5.05	0.032
4	PFOA, PFNA, L-PFOS and PFDA	5.05 to 6.5	0.040

At the start of every validation batch, one system suitability test at 500 ng/L of PFAS mix in H_2O + 10 mM NH_4Ac was injected. This made it possible to correct the retention windows if the analytes retention times (Rt) had shifted outside that initial retention window.

2.5 Sample preparation

2.5.1 Environmental water sample

Surface water was sampled from the river Orneau upstream (Escaille nature reserve - 50°34'13.90"N, 4°42'18.25"E) and downstream (INASEP - 50°32'10.27"N, 4°41'14.13"E) of the town of Gembloux (Belgium). They were sampled in 3.3 borosilicate glass bottles hermetically sealed with polypropylene cap on 23 May 2023. Afterward they were stored in a fridge at 6 ± 5°C.

Underground water was sampled from four lysimeters and pooled together. Lysimeters were in agricultural fields in Wallonia. Samples were stored in 3.3 borosilicate glass bottles hermetically sealed with polypropylene cap in a fridge at 6 ± 5 °C.

Underground and surface water samples were filtered through glass microfibre filter with a porosity of 0.7µm (glass microfibre filter 0.7µm, Art. Nr. 7699901, LABSOLUTE®). It was filtered and stored in a fridge at 6 ± 5°C until further analysis.

2.5.2 Waste leachate

Leachates of waste were prepared at the laboratory. Their internal identification number were 82281 and 82282. First waste leachate of both samples has been analysed.

Firstly, a fresh weight corresponding to 100 g of dried sample is weighed and placed in a 2 L polyethylene bottle. One litre of milliQ water is then added. The bottle is then shaken on a planetary shaker at 150 rpm for 40 minutes. After shaking, the waste leachate is filtered using a steel filtration apparatus. A filter (Pall™, Supor® 0.45 µm, diameter of 142 mm PES membrane) with a porosity of 0.45 µm is placed in the filtration apparatus. The waste leachate is introduced in the apparatus. An overpressure is applied by injecting pressurized air in the system allowing the waste leachate to be filtered. The liquid obtained is transferred directly into a borosilicate 3.3 glass bottle. The waste leachate is stored in a fridge at 6 ± 5°C until further analysis.

2.5.3 Acidification

PFAS can be found in their salt/ion or acid form in the environment. It is one of the reasons why water the pH of the samples is adjusted prior to filtration and SPE. Before injection PFAS in UPLC-MS/MS samples were acidified to pH values < 3 with glacial acetic acid (GAA) (Fisher chemical™, purity ≥ 99.8 %). In method development and validation, samples were acidified after being spiked with PFAS mix to test if %Rec changed.

2.5.4 Solid Phase Extraction (SPE)

During the method development, SPE has been tested. The sample preparation protocol used was the similar as for Rosnack et al. (2019)[41]. Briefly in this study, milliQ water samples were spiked before having their pH adjusted to < 3 with GAA. The model of SPE cartridge used was a Chromabond® HR-XAW (85 µm, 6 mL, 500 mg) from Macherey-Nagel™.

SPE cartridges were conditioned first with 0.5 % ammonia/MeOH solution, then with pure methanol and lastly with milliQ water. After that 250 mL of samples were loaded onto the cartridge. When samples entirely passed through the cartridge, they were evaporated to dryness. When the cartridges were dried, they were loaded with 4 mL of 25 mM acetate buffer at pH 4 and once again dried. Afterwards cartridges were loaded with a first fraction of methanol sent to waste and finally analytes were eluted with a solution of 0.5 % ammonia/MeOH and collected. The eluate was completely dried under N_2 flow at temperature < 40 °C. After drying, a volume of milliQ water +10 mM NH_4Ac (recovery volume or RV) was added to match the desired enrichment factor (EF) (Table 2.6). Injection volume was also adapted. The objective was to have an injected quantity of PFAS in ng for a sample that was covered by the calibration curve (Table 2.6).

Table 2. 6. Details of samples spiking. Recovery volume (RV) corresponds to the added volume of milliQ water + 10 mM NH_4Ac after the SPE final eluate phase has been dried out. EF stands for enrichment factor.

Spiking [ng/L]	Spiking solution [ng/mL]	Spiking volume [mL]	Spiked volume [mL]	RV [mL]	EF	Injection volume [mL]	PFAS content [ng]
100	125	1	250	5	50	0.05	0.25
500	25	1	250	5	50	0.025	0.625

Enrichment factor (EF) is calculated as follows:

$$Enrichment\ Factor = \frac{Spiked\ volume\ (mL)}{Recovery\ volume\ (mL)}$$

Recovery percentage (%Rec) is calculated as follows:

$$\%Rec = \frac{Calculated\ concentration\ from\ calibration\ curve\ in\ ng/L}{Expected\ concentration\ in\ ng/L}$$

Sample list corresponded to the order of injections. While testing SPE and filtration sample concentrations were measured using a bracket calibration. Table A.5 represents an example of sample list. Calibration curves concentrations ranged from 25 to 2500 ng/L. For the test where spiking before or after filtration have been compared, bracket calibration concentrations ranged from 10 to 2500 ng/L. An injection of milliQ water was interposed between each sample injection. Two injections of milliQ water were interposed between each sample injection for the spiking before or after filtration test. Interposing milliQ water between the samples was used to ensure that there was no analytes carry-over.

2.5.5 Standard solution preparation

Working concentrations were obtained from the standard mix at 10^6 ng/L. The following tables show how dilution plans were implemented:

Table 2. 7. Analytical standard dilution steps used for column, solvent gradient and diluent solvent selection. Pure UPLC grade MeOH has been used to dilute standard mix.

Target concentration [ng/L]	Standard concentration [ng/L]	Dilution factor	Final volume [mL]	Standard solution [mL]	MeOH [mL]
100000	1000000	10	1	0.1	0.9
10000	100000	10	1	0.1	0.9
5000	10000	2	1	0.5	0.5
2500	5000	2	1	0.5	0.5
1000	5000	5	1	0.2	0.8
500	5000	10	1	0.1	0.9
250	5000	20	1	0.05	0.95
100	1000	10	1	0.1	0.9
50	500	10	1	0.1	0.9

Table 2. 8. Analytical standard dilution steps used for diluent solvent choice. Pure UPLC grade and H_2O + 10 mM NH_4Ac have been used to dilute standard mix.

Target concentration [ng/L]	Standard concentration [ng/L]	Dilution factor	Final volume [mL]	Standard solution [mL]	MeOH or H_2O + 10 mM NH_4Ac [mL]
10000	100000	10	1	0.1	0.9
5000	100000	20	1	0.05	0.95
2500	100000	40	1	0.025	0.975
1000	10000	10	1	0.1	0.9
500	5000	10	1	0.1	0.9
250	5000	20	1	0.05	0.95
100	1000	10	1	0.1	0.9

Standard preparation for Injection volume selection, test on SPE and filtration, and method validation, were prepared from standard solution in MeOH at 100000 ng/L. All subsequent dilutions were made with H_2O + 10 mM NH_4Ac as the diluent.

2.6 Method development strategy

The method developed in this work was initially based on the method for PFAS analysis found in Rosnack et al. (2019). Method development can be divided in different steps:

1. Column selection

Different columns with diverse stationary phases are proposed in application notes and scientific paper. Columns tested here met EPA methods recommendations, were manufactured by waters™ and were already available at the laboratory.

2. Solvent gradient

After column selection, solvent gradient with different durations and time steps have been tested. Solvents used to select gradient duration and time steps were the same than mobile phases in Rosnack at al. (2019). When the solvent gradient is selected mobile phases from the certificate of analysis from wellington will be compared and see which ones give the best peak separation in time.

3. Diluent solvent

UPLC grade pure MeOH and milliQ water + 10 mM NH_4Ac have been compared, to see which one provided the best peak shape.

4. Injection volume

Proposed injection volume in Rosnack et al. (2019) is 10 µL because of their SPE protocol. The injection loop on the UPLC had a maximum capacity of 250 µL. Five injection volumes will be tested: 10 – 25 – 50 – 100 – 200 µL. The objective is also to decrease calibration curves highest concentrations by increasing PFAS quantity injected.

5. Direct injection or SPE

SPE and direct injection after filtration were two possibilities to prepare samples before UPLC-MS/MS analysis. Both will be tested for Orneau river and waste leachates samples. Based on PFAS %Rec and required time, one or the other will be retained for method validation. Filtration can occur before or after spiking sample, both procedures will be compared by PFAS %Rec.

2.7 Validation

2.7.1 Experimental design

Method validation will be realized as follows:

- 6 days:
    - MS cone has been washed before validation day 1 and between validation day 3 and 4.
- Three environmental water matrices: surface water (Orneau river), underground water (lysimeter) and water from wastes (waste leachates)
- Three spiking concentrations: 40 – 100 – 200 ng/L (Table 2.10). The aim is to validate the method at concentrations framing those that will apply in future legislation. That is to say 100 ng/L for the sum of the 10 PFAS analysed in this work.
- Two replicates for each concentration and each matrix. It will account up to 18 samples per validation day. Two blank replicates will also be analysed for each matrix.
- Each method step has been repeated for each separate validation day: standard dilution (Table 2.9), spiking, acidification, calibrations curves, and injection. The three matrices were filtered before the method validation began.

Quantification will be completed using a bracket calibration. The calibration concentrations used will be: 0 – 10 – 25 – 50 – 100 – 250 – 500 – 1000 ng/L. Dilution plan used to establish calibration curves and spiking solutions was repeated each validation day (Table 2.9).

Table 2. 9. Analytical standard dilution plan used during method validation. Two different diluents were used: UHPLC grade pure MeOH (diluent 1) and H_2O +10 mM NH_4Ac (diluent 2).

Target concentration [ng/L]	Standard concentration [ng/L]	Dilution factor	Final volume [mL]	Standard solution [mL]	Diluent 1 [mL]	Diluent 2 [mL]
100000	1000000	10	5	0.5	4.5	-
20000	100000	5	10	2	-	8
5000	20000	4	1	0.25	-	0.75
2500	20000	8	1	0.125	-	0.875
1000	20000	20	1.5	0.075	-	1.425
500	5000	10	1	0.1	-	0.9
250	2500	10	1	0.1	-	0.9
100	1000	10	1	0.1	-	0.9
50	500	10	1	0.1	-	0.9
25	250	10	1	0.1	-	0.9
10	100	10	1	0.1	-	0.9
0	-	-	1	-	-	1

The samples spiked were realised as follows (Table 2.10):

Table 2. 10. This table describes how the different samples have been spiked. The 20000 ng/L has been obtained by diluting 5 times, with H_2O + 10 mM NH_4Ac, a standard solution of the PFAS mix in MeOH at 100000 ng/L.

Target concentration [ng/L]	Standard concentration [ng/L]	Dilution factor	Sample Volume spiked [mL]	Standard solution [mL]
40	20000	10	50	0.10
100	20000	5	50	0.25
200	20000	4	50	0.50

2.7.2 Performance characteristics

2.7.2.1 Linearity test

Linearity has been tested by a partial F-test. The p-value has been set at 0.05. The H_0 is : regression is linear. If $F_{obs} < F_{crit}$, H_0 is accepted and the regression is linear, otherwise it is a quadratic regression. F_{obs} is calculated as follows:

$$F_{obs} = \frac{(\frac{RSS_{reduced} - RSS_{full}}{p})}{(\frac{RSS_{full}}{n-k})}$$

Where:

- $RSS_{reduced}$: residual sum of squares of the reduced model, model containing a subset of the predictor variable
- RSS_{full}: residual sum of squares of the full model
- p: number of predictors removed from the full model
- n: total observations in the dataset
- k number of coefficients (including the intercept) in the full model

F_{crit} was determined with Df1 = 1, DF2 = 9 and p-value = 0.05. F_{crit} consequently equals 5.1.

2.7.2.2 Instrumental Limit of detection (LOD) and limit of quantification (LOQ)

Instrumental LOD and LOQ have been defined as the concentrations equivalent to a signal over noise ratio (S/N) of 3.3 and 10 respectively. Fig 2.1. shows how signal and noise were measured. The black line length corresponds to the noise intensity (Fig. 2.1). The dark blue line corresponds to the signal and is measured from middle of noise to the peak summit (Fig. 2.1). S/N is calculated as follows:

$$S/N = \frac{Dark\ blue\ line\ length\ in\ cm}{Black\ line\ length\ in\ cm}$$

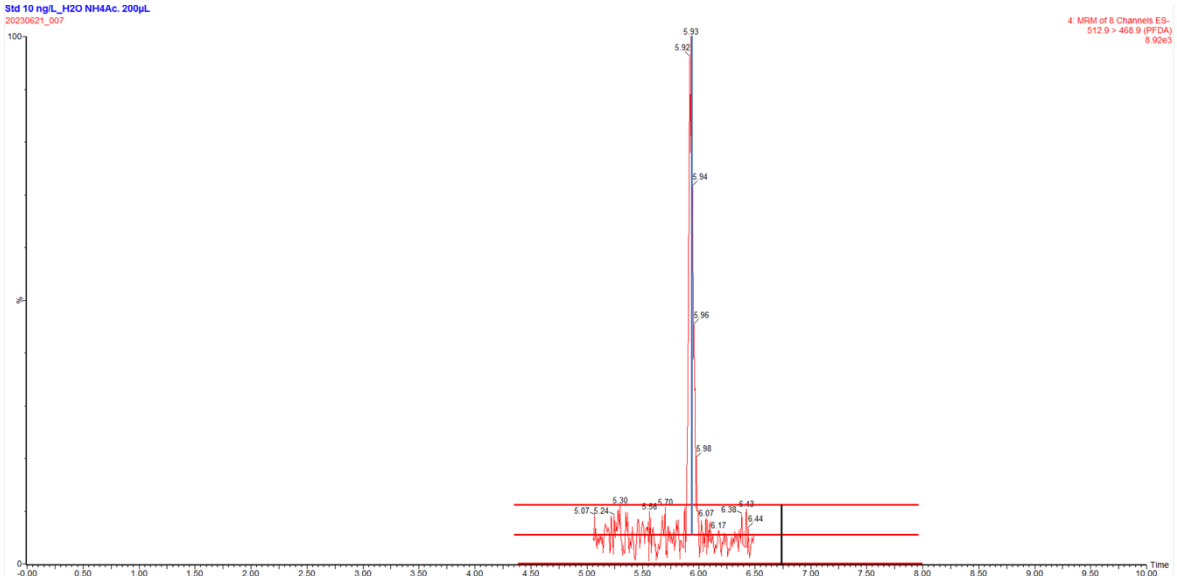


Figure 2. 1. Illustration of how Signal over Noise ratio (S/N) has been measured. Black line length corresponds to the noise and blue line corresponds to the signal.

S/N was calculated at 10 and 25 ng/L from the first curve of the bracket calibration for validation day 1 and 4. A rule of 3 was applied to determine which concentrations corresponded to the instrumental LOD and LOQ.

$$LOD\ or\ LOQ = \frac{25\ ng.L^{-1} - 10\ ng.L^{-1}}{S/N\ molecule\ i_{\text{à }25\ ng.L^{-1}} - S/N\ molecule\ i_{\text{à }15\ ng.L^{-1}}} \times y$$

Where "y" equals 3.3 or 10 for LOD and LOQ respectively.

2.7.2.3 Precision

The repeatability relative standard deviation (RSD_r), is obtained by taking the square root of the within group mean square term which represents the within-group variance (s_r) divided by the mean then multiplied by 100:

$$s_r = \sqrt{MS_w}$$

The contribution to the total variation from the grouping factor ($s_{between}$) is also obtained from the ANOVA table:

$$s_{between} = \sqrt{\frac{MS_b - MS_w}{n}}$$

The intermediate precision (S_I) can now be calculated by combining the within- and between group variance components above:

$$s_I = \sqrt{s^2{}_r + s^2{}_{between}}$$

Reproducibility is calculated by dividing "s_I" by the mean then multiplied by 100.

2.7.2.4 Validation criteria

The results obtained during method validation must be compared with validation criteria. If these criteria are met, the method used is statistically valid. Various criteria can be used, a non-exhaustive list of which is presented in the appendix. Validation criteria in standards methods EPA 533, 8327 and 3512 were chosen to assess the acceptability of this method (Table 2.11).

Table 2. 11. Validation criteria used for the method validation in this work. Validation criteria were taken from EPA methods 533, 8327 and 3512.

Criteria	Limits
Coefficient of determination (R^2)	≥ 0.99
Precision (repeatability)	%RSD ≤ 20 %
Retention time	± 0.2 min
Recovery for calibration point	LLOQ: 50 to 150 % Other concentrations: 70 to 130 %
%Rec	70 to 130 % for target analyte

The coefficient of determination (R^2) is the square of the linear correlation coefficient R between the predicted values

$$R^2 = corr(\hat{y}, y)^2$$

2.8 Standard additions

Standard additions matching calibration points were realised in the 82588 sample. Sample 82588 was discharge water from a waste treatment centre. Calibration points were the same used in bracket calibration for method validation (section 2.7.1). Table 2.12 shows how dosed additions were prepared.

Table 2. 12. Preparation of standard additions in sample n° 82588. The diluent used was H_2O + 10 mM NH_4Ac. The same dilution plan than the one presented in Table 2.9 was used to get standard solution of 10000, 5000, 2500 and 1000 ng/L.

Calibration point [ng/L]	Sample volume [mL]	Standard solution		Diluent [mL]
		Volume [mL]	Concentration [ng/L]	
0	1	0	0	0.5
10	1	0.15	100	0.35
25	1	0.15	250	0.35
50	1	0.15	500	0.35
100	1	0.15	1000	0.35
250	1	0.15	2500	0.35
500	1	0.15	5000	0.35
1000	1	0.15	10000	0.35

Sample concentration equals to the abscissa at origin and was calculated as follows:

$$Abscissa\ at\ origin = \left|\frac{-\ intercept}{curve\ slope}\right|$$

One mL of sample will be diluted in 1.5 mL (FD = 1.5). To get the real sample concentration, abscissa at origin will have to be multiplied by 1.5.

2.9 Data acquisition

The raw data was acquired using MassLynx software v4.2 SCN977(MassLynx). Peak integrations were also carried out using the same software. MassLynx was the interface for giving instructions and changing the experimental analysis parameters.

3 Results and discussion

3.1 Method development

The method developed in this work is based on the application from Waters[41]. It has been chosen for three main reasons:

- Water type matrix
- Same MS/MS system
- UPLC column used available at the lab

3.1.1 Analytical column selection

For the following tests, injected concentration was 10 µg/L. The first column tested was an HSST3 (2.1 x 150 mm, 1.8 µm). No delay nor precolumn were added in this first test. Mobile phases and gradient elution were the same than the ones used in Rosnack et al. (2019). This configuration allowed a satisfactory peak separation as it can be seen on Fig.3.1. Column used in Rosnack et al (2019) was BEH C18 (2.1 x 100 mm, 1.7 µm). Having a such good peak separation was to be expected. HSST3 column was longer than the BEH C18 used in Rosnack et al. (2019) and theoretical number of separation plates was consequently higher. HSST3 15 was adequate for the analysis of the 10 PFAS. The gradient used for PFAS in Fig. 3.1 lasted for 22 minutes. It was taken from the reference application note in which 40 different PFAS were analysed. Only 10 PFAS were analysed in this work. So, a shorter elution gradient was tested. Time steps duration of the elution gradient, from reference application note, were divided by two (table 3.1).

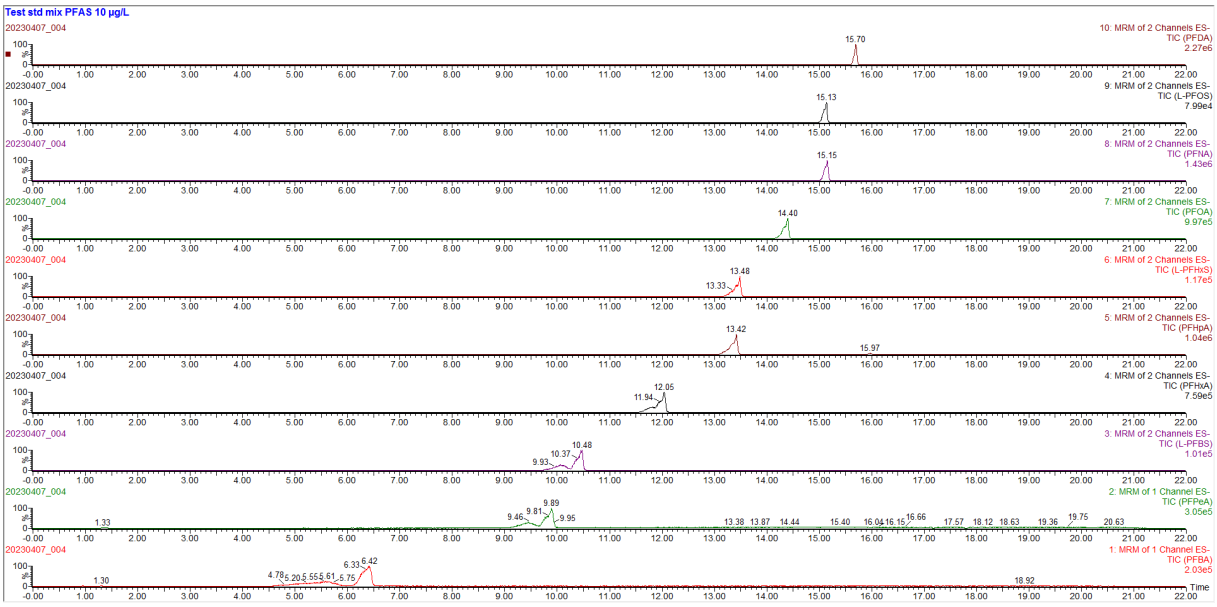


Figure 3. 1. TIC chromatograms of the 10 PFAS analyzed in this work. The column used was an HSST3 HSST3 (2.1 x 150 mm, 1.8 µm). PFAS mix was diluted to 10 µg/L in MeOH and injection volume was 10 µL. Mobile phase A was a mix of H_2O:MeOH (95:5, v:v) + 2 mM NH_4Ac. The mobile phase B was MeOH + 2 mM NH_4Ac. The solvent gradient lasted for 22 min and was as follows: started at 0 %B at 0.3 mL/min to reach 20 %B after 1 min. After 6 minutes %B reached 45 % with a flow of 0.3 mL/min. At 13 min %B was 80 % with a flow of 0.3 mL/min to ramp up at 95 % after 14 min with a flow of 0.4 mL/min. It stayed at 95 %B with a flow of 0.4 mL/min until 17 min. In one minute, %B decreased from 95 to 0 % with a flow of 0.3 mL/min to remain constant until 22 min.

PFAS were eluted faster when time steps were divided by two. Analytes all eluted in a shorter time window: 3.68 min for the 10 minutes gradient (Fig. 3.2) and 9.28 min for 22 minutes gradient (Fig. 3.1). When comparing the two gradients, PFAS that eluted at similar Rt in the 22 minutes gradient still eluted at similar Rt in the 10 minutes gradient. For example, PFHpA and L-PFHxS followed that behaviour such as PFNA and L-PFOS. Dividing time steps (gradient 2) by two provided a similar peak separation than the initial elution gradient.

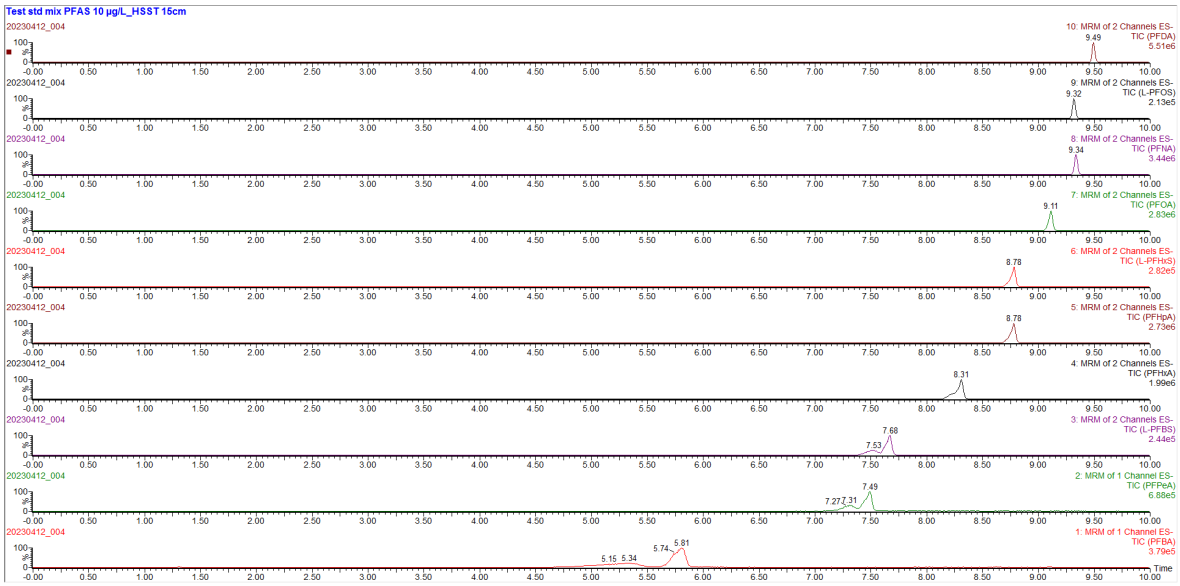


Figure 3. 2. TIC chromatograms of the 10 PFAS analysed in this work. The column used was an HSST3 (2.1 x 150 mm, 1.8 µm). PFAS mix was diluted to 10 µg/L in MeOH and the injection volume was 10 µL. Mobile phase A was a mix of H_2O:MeOH (95:5, v:v) + 2 mM NH_4Ac. The mobile phase B was MeOH + 2 mM NH_4Ac. The solvent gradient lasted for 10 min and was as follows: started at 0 %B at 0.3 mL/min to reach 20 %B after 0.5 min. After 3 minutes %B reached 45 % with a flow of 0.3 mL/min. At 6.5 min %B was 80 % with a flow of 0.3 mL/min to ramp up at 95 % after 7 min with a flow of 0.4 mL/min. It stayed at 95 %B with a flow of 0.4 mL/min until 8.5 min. In 0.5 minute, %B decreased from 95 to 0 % with a flow of 0.3 mL/min to remain constant until 10 min.

The 10 min gradient is detailed in the legend of Fig. 3.2. It is important to stress that mobile phase B ramped up from 0 % to 20% in 0.5 min. When using the 10 minutes gradient with BEH 5 cm, all ten PFAS eluted around 0.5 minute (Fig. 3.3). BEH 5 cm is 3 times shorter than the HSST3 15 cm. Thus, Rt will be shorter. There was no hold time where gradient stayed at the initial conditions, with mobile phase A at 100 %. It appears that when BEH 5 cm column is used, having a hold time before the start of the gradient is necessary. In an application note from Macherey-Nagel studying PFAS, a hold time was also used[73]. That specific gradient allowed to completely separate analysed PFAS[73] (Fig. A.1). The column used in that application note was an EC NUCLEOSHELL® RP 18plus column (100 x 2.0 mm, 2.7 µm).

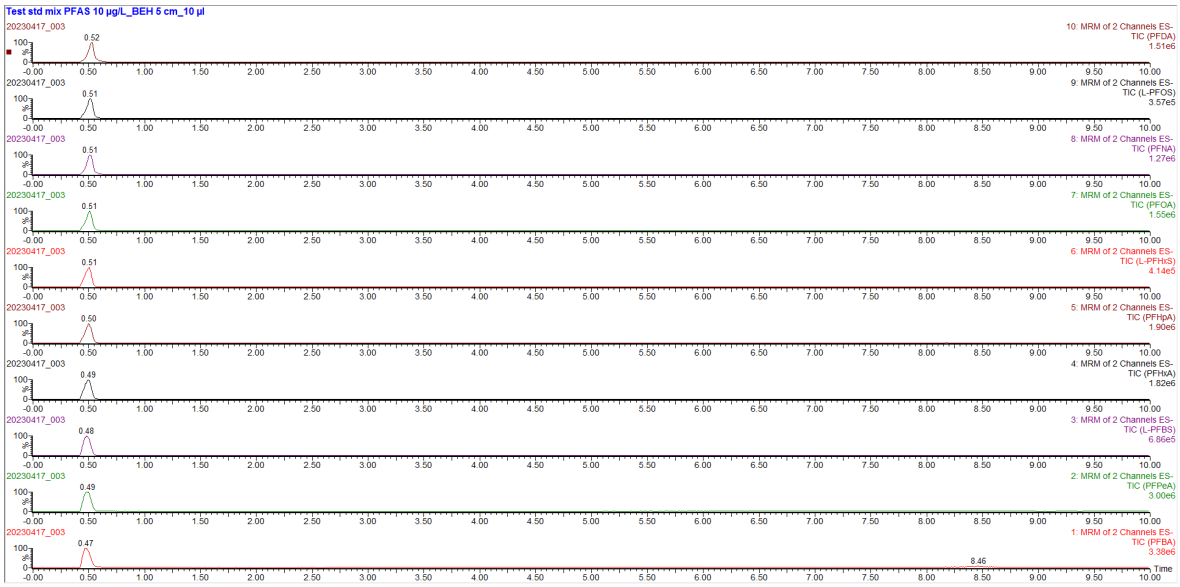


Figure 3. 3. TIC chromatograms of the 10 PFAS analysed in this work. The column used was a BEH C18 (2.1 x 100 mm, 1.7 µm). PFAS mix was diluted to 10 µg/L in MeOH, and injection volume was 10 µL. Mobile phase A was a mix of H_2O:MeOH (95:5, v:v) + 2 mM NH_4Ac. The mobile phase B was MeOH + 2 mM NH_4Ac. The solvent gradient lasted for 10 min and was as follows: started at 0 %B at 0.3 mL/min to reach 20 %B after 0.5 min. After 3 minutes %B reached 45 % with a flow of 0.3 mL/min. At 6.5 min %B was 80 % with a flow of 0.3 mL/min to ramp up at 95 % after 7 min with a flow of 0.4 mL/min. It stayed at 95 %B with a flow of 0.4 mL/min until 8.5 min. In 0.5 minute, %B decreased from 95 to 0 % with a flow of 0.3 mL/min to remain constant until 10 min.

Total duration of the gradient was extended by 2 minutes to include an initial hold time. Then, initial mobile phases mix changed only after the hold time of 1 minute. Only PFBA (Rt = 3.64 min) and PFPeA (Rt = 6.36 min) eluted between 1 and 6.5 minutes (Fig. 3.4). The other PFAS eluted after the solvent gradient between 6.67 min (L-PFBS) and 8.27 min (PFDA). Time window from 7 to 8.5 minutes is used to "wash out" all analyte injected. Mobile phases proportions vary less than between 1 to 6.5 minutes ("elution window"). The "elution window" can be defined as: the period between the end of the hold time and the start of the period when the gradient reaches its highest proportion of strong eluent. The goal is to elute analytes during the elution window. Even in such conditions, the BEH 5 cm column allowed good separation of analyte peaks. BEH 5 cm and 12 minutes duration for elution gradient were then chosen for the rest of method development.

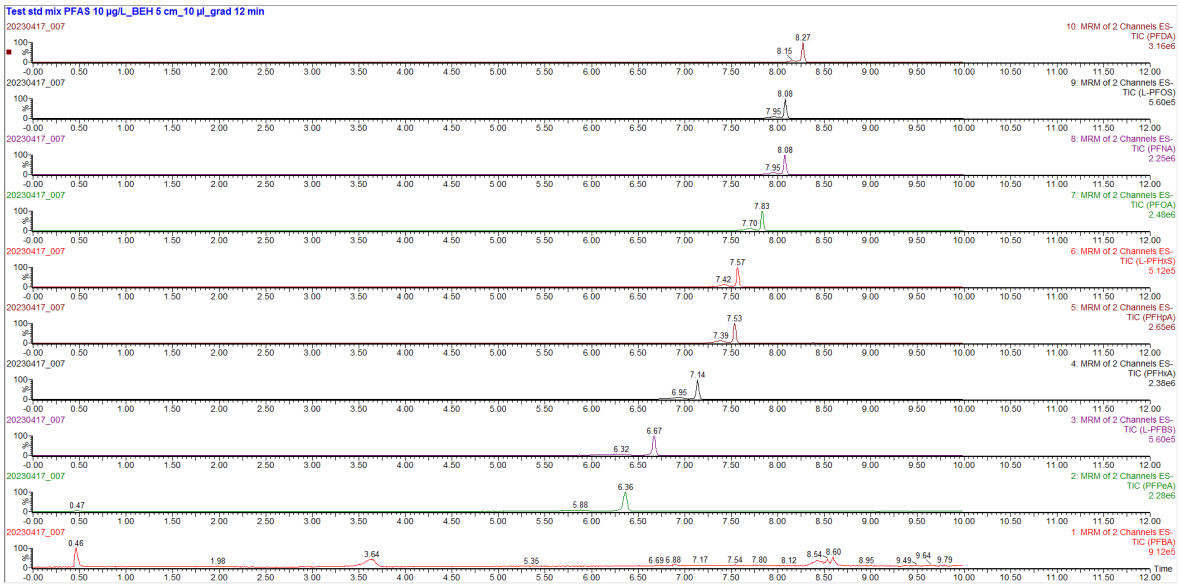


Figure 3. 4. TIC chromatograms of the 10 PFAS analysed in this work. The column used was a BEH C18 (2.1 x 100 mm, 1.7 µm) with a HSST3 (2.1 x 5 mm, 1.8µm) as the precolumn. PFAS mix was diluted to 10 µg/L in MeOH and injection volume was 10 µL. Mobile phase A was a mix of H_2O:MeOH (95:5, v:v) + 2 mM NH_4Ac. The mobile phase B was MeOH + 2 mM NH_4Ac. The solvent gradient lasted for 12 min and was as follows: remained constant at 0 %B at 0.3 mL/min for 1 min. Then, %B ramped up from 0 to 80 % in 5.5 min with a flow of 0.3 min/mL. %B reached 95 % after 7 min with a flow of 0.4 mL/min. It stayed at 95 %B with a flow of 0.4 mL/min until 8.5 min. In 0.5 minute, %B decreased from 95 to 0 % with a flow of 0.3 mL/min to remain constant until 12 min.

In the literature, most of the application notes used C18 stationary phases. PFAS affinity to C18 ligand increases with their chain length. A relatively short and narrow column can be used to rapidly and successfully resolve target analytes, since retention is strong enough[54]. A raptor C18 (50 mm x 21 mm, 2.7 µm) easily separated 25 PFAS compounds including those analysed in this study (Fig. A.1). A Gemini C18 (50 mm x 2 mm, 3 µm) also offered for PFAS a solid retention and predictable resolution[42]. In comparison, the Gemini 100 mm (100 mm x , 3 µm) provided stronger retention leading to a longer runtime and less shift in retention times[15]. Compromises must be found between runtime and Rt shift. In this study a shorter column has been chosen. Since this column provide sufficient peak separation it therefore respects EPA method 537.1 requirements[55]. A HSST3 (1.8µm, 2.1 x 5 mm) has also been installed as precolumn. No delay column was installed. This is why it is very important to perform blank injections before any sample or calibration solution.

3.1.2 Elution gradient

Gradient-12 min 2 and 3 only differed by the time needed to reach 80% of mobile phase B. Gradient 2 reached 80% of mobile phase B after gradient 3 (Fig. 3.5). For gradient 2, only PFBA and PFPeA eluted before 5.5 min. For gradient 3, the 10 PFAS eluted between 3.38 min (PFBA) and 5.34 (PFDA). With gradient 3, all analytes were eluted before the "wash out phase". It is to be noted that only PFBA eluted before gradient 3 reached 80 % of mobile phase B. So, for the PFAS analysed in this work, except for PFBA, it is necessary to get a higher ratio of organic phase to elute out the column. BEH C18 is a reversed stationary phase column. The longer the PFAS chain, the more apolar the molecule will be overall. To elute those compounds, it is then essential to have a strong eluent. Extending the "apolar" period of the elution gradient allows to elute more apolar PFAS before the gradient reaches the "wash out" phase.

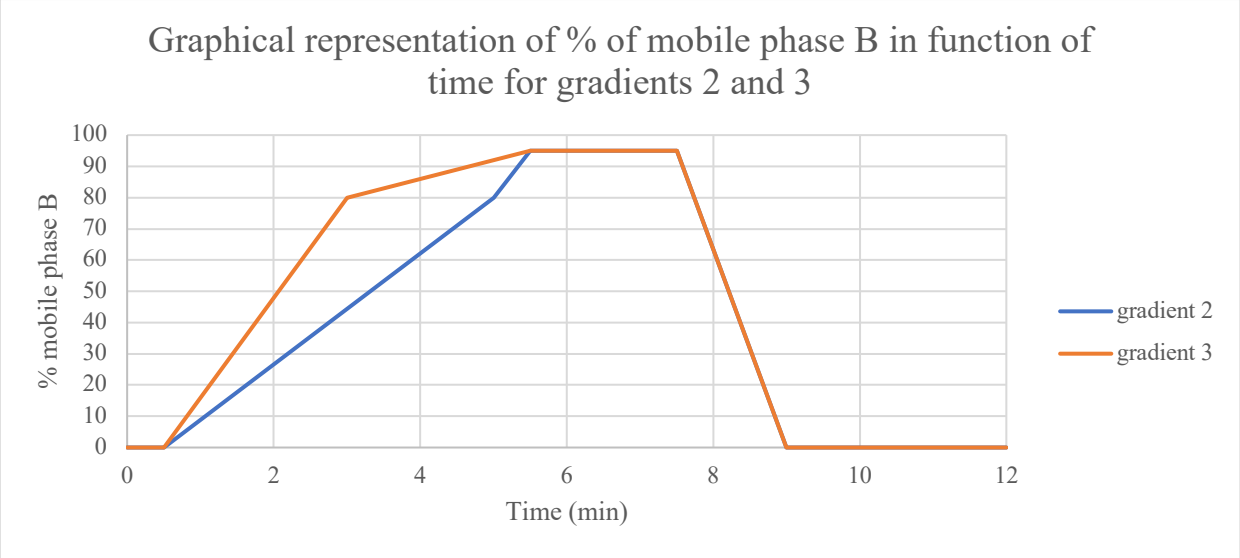


Figure 3. 5. Graphic representing solvent gradient 12 min 2 and 3. Both gradients lasted 12 minutes. Here is represented mobile phase B % in function of time. Mobile phase A was a mix of H_2O:MeOH (95:5, v:v) + 2 mM NH_4Ac. The mobile phase B was MeOH + 2 mM NH_4Ac.

Gradient 4 and 5 both lasted for 12 minutes. Both gradients had a constant flow of solvent set at 0.3 mL/min. Gradient 4 had two steps during elution phase. Gradient 4 reached 80 % of mobile phase B after 2.5 minutes, and it reached 95% of mobile phase after 5.5 min. Comparatively to gradient 3, gradient 4 had a longer "more polar phase". Gradient 5 went to 95 % of mobile phase B in one sitting in 7 minutes. For gradient 3 and 4, all PFAS were eluted before 5.5 min, before the wash out phase. Gradient 5 only had PFBA, PFPeA,

L-PFBS and PFHxA eluting before the wash out phase, before 5.5 min. Gradient 4 reached faster a higher percentage of the organic phase than gradient 5. It shows the importance to quickly increase organic phase proportion in the solvent mix to elute PFAS before wash out phase.

Different gradients were tested with an intermediate step during the elution phase. All had a constant flow set at 0.3 mL/min. Gradient 6 and 7 both reached 80 % of mobile phase B respectively after 1.5 minutes and 2 minutes. Gradient 9 reached 70 % of mobile phase B after two minutes. Gradient 6 reached 95 % of mobile phase B after 7 minutes. Gradients 7 and 9 reached 95 % of mobile phase B after 8 minutes (Fig. 3.6). PFAS eluted during 1.27 min, 1.58 min and 2.26 min respectively for gradient 6, 7 and 9 respectively. Gradient 6 had a longer period of its elution phase at higher organic phase proportion, but it reached the wash out phase faster. It appeared that having a more equilibrate elution gradient, between low and high organic phase proportion (i.e., gradients 7 and 9, Fig. 3.6), gives longer retention window (i.e., gradients 7 and 9).

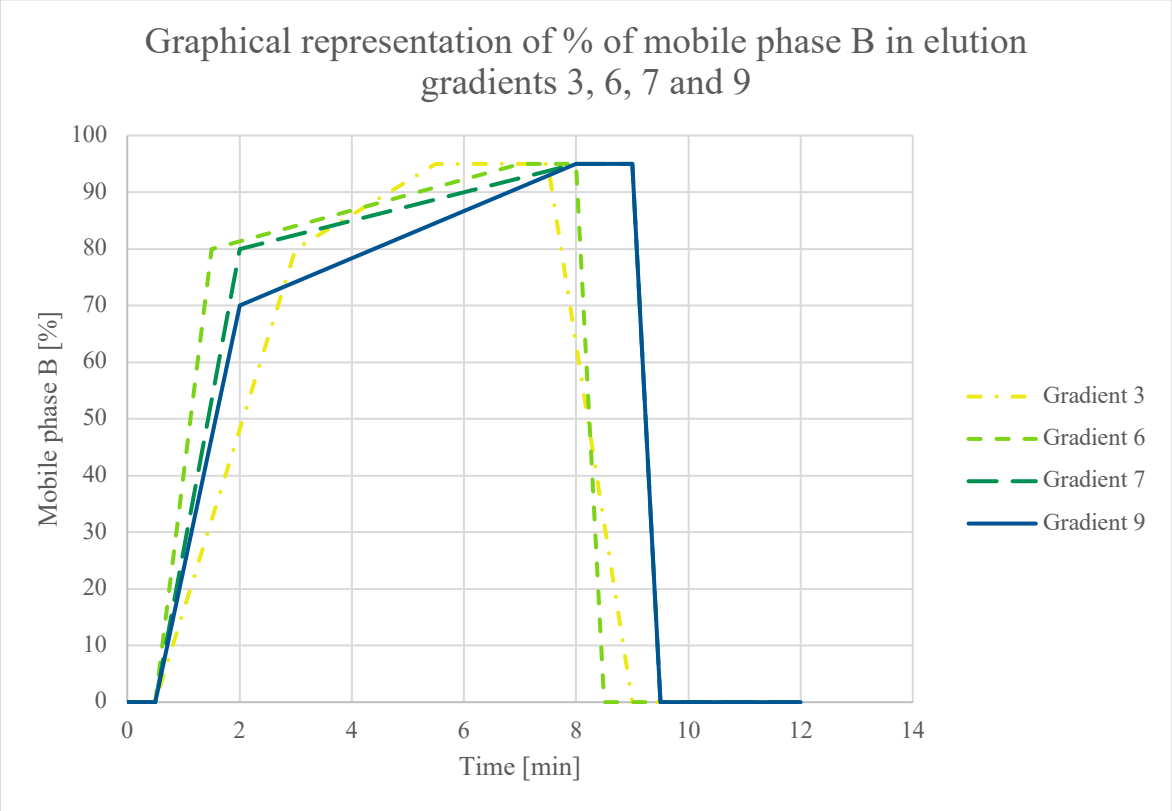


Figure 3. 6. Solvent gradient 3, 6,7 and 9. Four gradients were binary and lasted 12 minutes. Here is represented mobile phase B % in function of time. Mobile phase A was a mix of H_2O:MeOH (95:5, v:v) + 2 mM NH_4Ac. The mobile phase B was MeOH + 2 mM NH_4Ac. Gradients 6, 7 and 9 had a constant flow set at 0.3 mL/min.

Gradient 9 was preferred over gradient 7 because the 10 PFAS eluted further apart. Having more separated analytes in time allows to set functions regrouping specific analytes for specific retention times. It allows to set MRM detection mode target for few molecules on a specific time window instead scanning mass transitions of all molecules at every moment and therefore allows better sensitivity.

3.1.3 Eluent choice

PFAS mix was provided by Wellington laboratories. With it was provided a certificate of analysis in which a chromatogram of the analysis of the same mix was given. Mobile phase A was 10 mM of NH_4Ac in milliQ water and mobile phase B was 10 mM of NH_4Ac in MeOH:ACN (80:20, v:v). The column used was an Acquity UPLC BEH Shield RP_{18} (1.7 µm, 2.1 x 100 mm). The gradient used in the certificate of analysis is detailed in the Table 3.1.

Table 3. 1. Elution gradient used in the certificate of analysis of the analytical standard from Wellington laboratories. Mobile phase A was milliQ water + 10 mM NH_4Ac. Mobile phase B was a mix of MeOH:ACN (80:20, v:v) + 10 mM NH_4Ac.

Time [min]	Flow [mL/min]	Mobile phase A [%]	Mobile phase B [%]
0	0.3	60	40
8	0.3	10	90
10	0.3	10	90
11	0.3	60	40
12	0.3	60	40

These chromatographic conditions were tested with BEH C18 (50 mm) column and the HSST3 (5 mm) precolumn installed. All PFAS had Rt around 0.56 min and PFNA, L-PFOS and PFDA showed a second peak at later Rt (Fig. 3.7). The installed column is shorter than in the certificate of analysis. Consequently, the starting proportion of organic phase might be too high leading to an early leach out of PFAS compounds out the column.

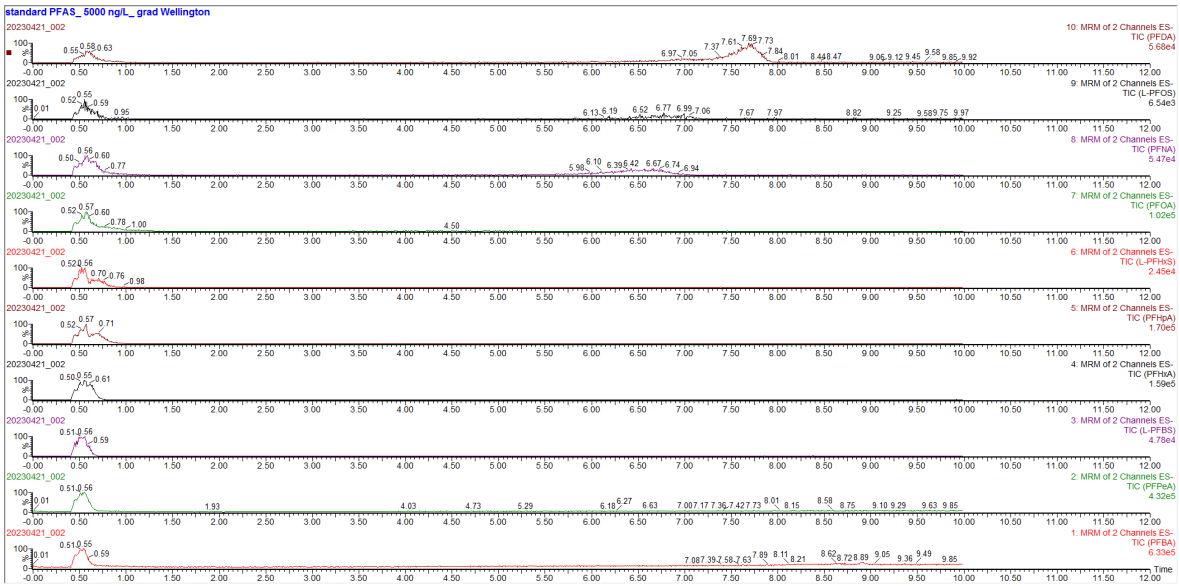


Figure 3. 7. TIC chromatograms of the 10 PFAS analysed in this work. The column used was a BEH C18 (2.1 x 100 mm, 1.7 µm) with a HSST3 (2.1 x 5 mm, 1.8µm) as the precolumn. PFAS mix was diluted to 10 µg/L in MeOH, and injection volume was 10 µL. Mobile phase A was milliQ water + 10 mM NH_4Ac. Mobile phase B was a mix of MeOH:ACN (80:20, v:v) + 10 mM NH_4Ac. The flow was constant at 0.3 mL/min. The gradient lasted for 12 min. The gradient started at 40 % of phase B and stayed constant until 0.5 min. Between 0.5 and 8 min, phase ramped up to 90 %. Phase B stayed at 90 % for two more minutes. Then, phase B decreased to 40% in one minute to remain constant until 12 min.

Mobile phases A and B from Wellington were tested using the same time steps and solvent proportions than gradient 9. Because Wellington mobile phase A is only constituted of H_2O + 10 mM NH_4Ac and the column used is a BEH C18, elution starting point was 99 % of mobile phase A and 1 % of mobile phase B. PFAS had Rt ranging from 3.85 min (PFBA) to 5.94 min (PFDA), when mobile phases from Wellington were used with gradient 9. PFNA and L-PFOS had close Rt, 5.5 and 5.55 min respectively. Then, it was decided to use the same mobile phases than in the certificate of analysis but with the gradient 9. This elution gradient is detailed in Table 2.2 (Section 2.3.1).

Here the column was flushed with 76 % of MeOH (80 % of 95 % of mobile phase B). It was the highest proportion of MeOH during sequence batch. Flushing column and priming it with at least 90 % MeOH before a batch sequence could lower background contamination[1]. Reducing interferences is particularly important as PFAS are ubiquitous in the environment. Even more that without a delay column, it is practically impossible to separate PFAS contamination originating from the instruments[52]. Even without delay column, the instrumental LOQ calculated in the present work were fit for purpose (Table 3.7, section 3.2.1), meaning background noise was sufficiently low. But between each injection, the needle was washed with pure MeOH which seems appropriate in this case. The highest

organic phase proportion was 95% of an 80:20 mix of MeOH:ACN, which is close to 100 % of organic solvent. In this study no carry over has been detected between samples. During validation, two blank milliQ water injections were performed between each calibration solution to further check zero presence of carry-over.

3.1.4 Functions

The analytes eluted separately enough to be grouped into different time functions. During each function, only the mass transitions of the compounds eluting during the said function were scanned by the MS/MS. PFAS were then regrouped in four functions (Fig. 3.8):

- Function 1 : PFBA
- Function 2: PFPeA and L-PFBS
- Function 3: PFHxA, PFHpA and L-L-PFHxS
- Function 4: PFOA, PFNA, L-PFOS and PFDA

The number of analytes eluting within a specific Rt window should be minimized to allow enough scans across each peak[74]. It is preferable to have at least 10 scans for each chromatographic peak to reach decent integration [75],[42]. Regrouping PFAS in four functions separated in time allows to increase sensitivity. Indeed, for a specific time window scans are focused on less MRM transitions thus allowing more scans. With those chromatographic parameters, all peaks had at least 10 scans across them allowing good integration. Co-elution of the analytes should be minimized, to reduce the probability of suppression and enhancement effect[76]. Amongst the 10 compounds analysed PFNA and L-PFOS were eluting at 5.50 and 5.55 min respectively (Table 3.3). L-PFOS had intensities an order of magnitude lower than the PFNA for each injection volume tested (Table 3.2). Having a longer analytical column would allow a better separation of those two molecules and could contribute to a better sensitivity for L-PFOS without changing gradient elution.

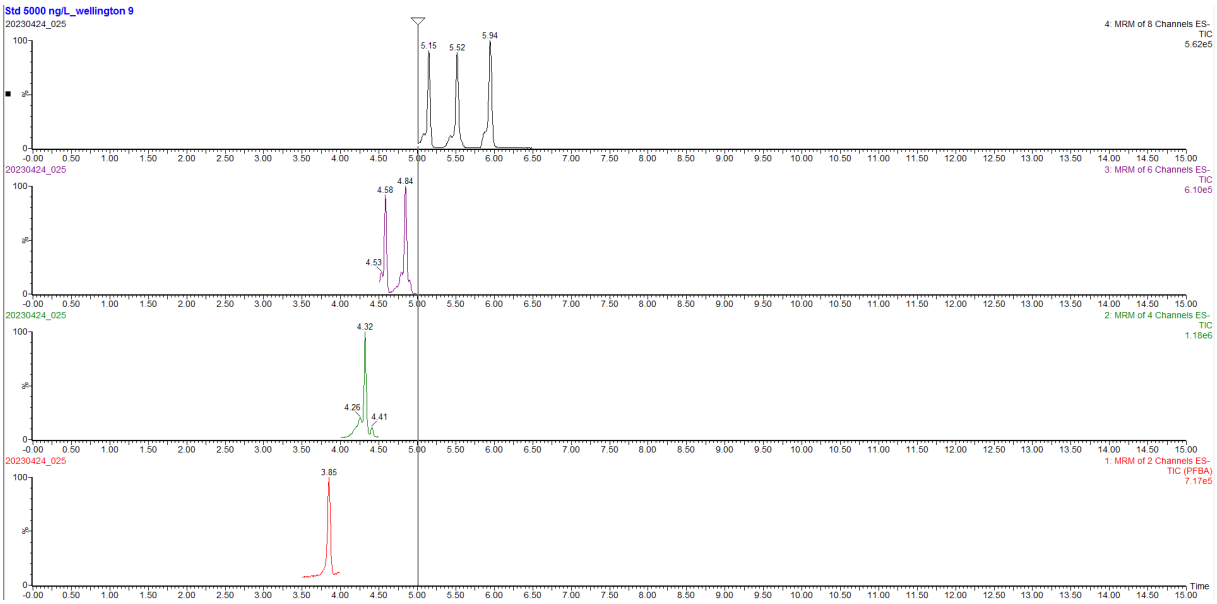


Figure 3. 8. TIC . TIC chromatograms of the 10 PFAS analysed in this work. Here PFAS are regrouped in different retention windows as defined in section 2.4, table 2.5. The column used was a BEH C18 (2.1 x 100 mm, 1.7 µm) with a HSST3 (2.1 x 5 mm, 1.8µm) as the precolumn. Mobile phase A was milliQ water + 10 mM NH_4Ac. Mobile phase B was a mix of MeOH:ACN (80:20, v:v) + 10 mM NH_4Ac. The flow was constant at 0.3 mL/min. Gradient started at 1% of phase B and remained constant until 0.5 min. From 0.5 to 2 min, phase B reached 70 %. Phase B ramped up to 95 % after 8 min and remained at 95 % until 9 min. Then phase B decreased to 1 % and remained at that level until the end of the gradient, after 12 min.

3.1.5 Diluent solvent

Up to this stage in the development of the method, the PFAS mixture was diluted with MeOH to the desired injection concentration. Elution gradient starts with 99 % of mobile phase A (milliQ water + 10 mM NH_4Ac) and 1 % of mobile phase B (MeOH:ACN + 10 mM NH_4Ac, 80:20, v:v). It was decided to test a diluent matching mobile phase A composition. Peak shape was improved when dilutions were made in milliQ water + 10 mM NH_4Ac (Fig. 3.9.a) in comparison to dilutions made in MeOH (Fig 3.9.b). There was less noise when using milliQ water + 10 mM NH_4Ac as a diluent. With milliQ water + 10 mM NH_4Ac as a diluent, sample diluent is similar in elution strength to the initial mobile phase. Doing so, peak broadening is reduced[77]. When sample diluent is higher in elution strength (MeOH at the beginning of method development), it can lead to peak deformation (broadening, tailing or fronting). [78]. One can easily see that using MeOH as diluent (fig 3.9.b) causes fronting in comparison to when diluent composition is close to initial mobile phase (fig 3.9.a). In this study, weaker diluent improves peak shape. But one must pay attention to avoid solvent creating phase separation of the sample[79]. But PFAS have amphiphile properties and are (at

different levels) soluble in water[3]. So, PFAS are in solution when milliQ water + 10 mM NH_4Ac is used.

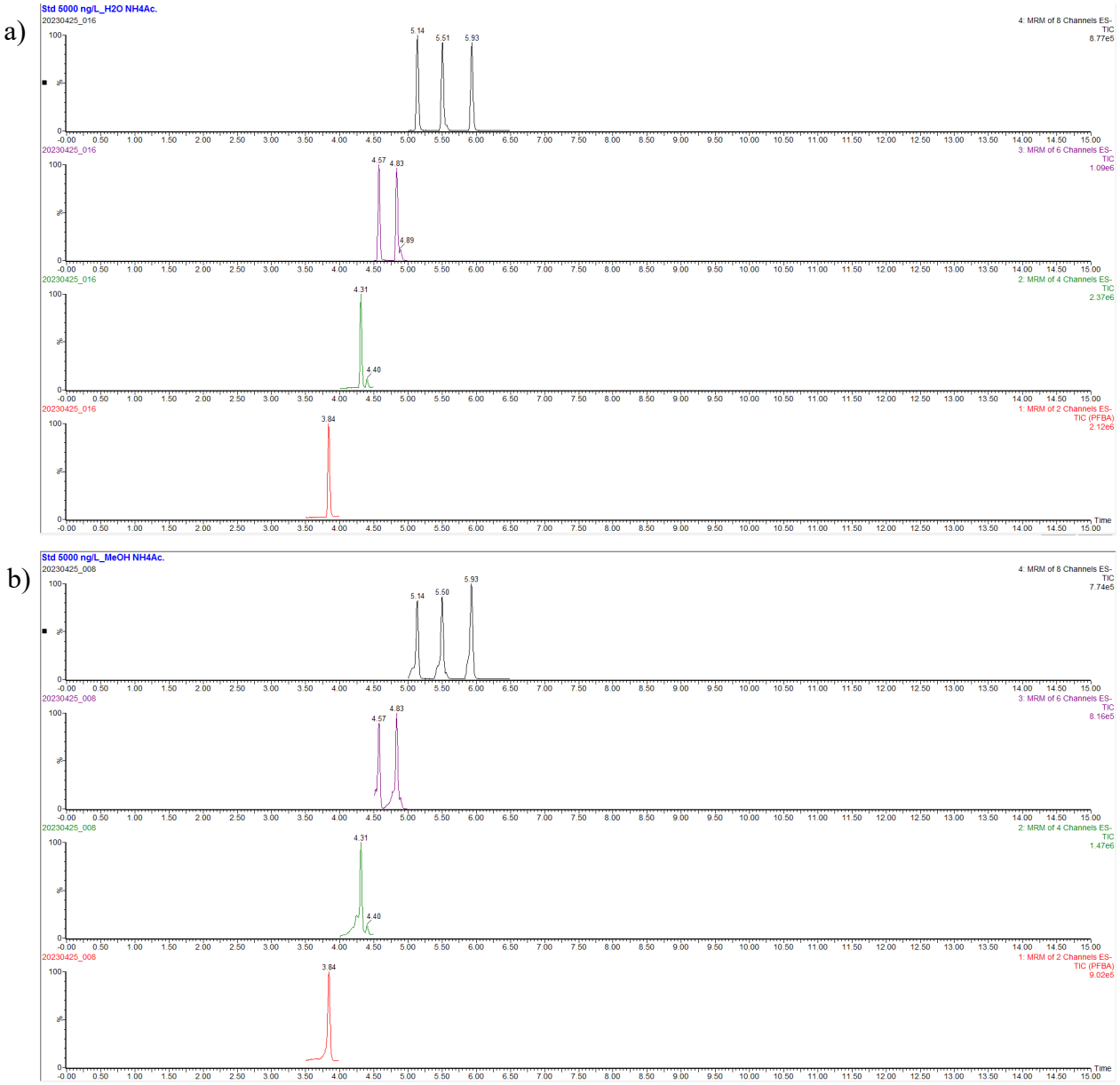


Figure 3. 9. Comparison between 2 different diluents: a) H_2O + 10 mMNH_4Ac and b) MeOH + 10 mMNH_4Ac. Analytes were regrouped in four time functions. The column used was a BEH C18 (2.1 x 100 mm, 1.7 µm) with a HSST3 (2.1 x 5 mm, 1.8µm) as the precolumn. Mobile phase A was milliQ water + 10 mM NH_4Ac. Mobile phase B was a mix of MeOH:ACN (80:20, v:v) + 10 mM NH_4Ac. The flow was constant at 0.3 mL/min. Gradient started at 1% of phase B and remained constant until 0.5 min. From 0.5 to 2 min, phase B reached 70 %. Phase B ramped up to 95 % after 8 min and remained at 95 % until 9 min. Then phase B decreased to 1 % and remained at that level until the end of the gradient, after 12 min.

3.1.6 Injection volume

Table 3. 2. Comparison of the intensity at 1000 ng/L of the 10 PFAS analysed in this work in function of the injection volume.

	1000 ng/L				
	10 µL	25 µL	50 µL	100 µL	200 µL
PFBA	2.95 E+05	6.78 E+05	1.18 E+06	1.65 E+06	2.19 E+06
PFPeA	3.75 E+05	8.49 E+05	1.69 E+06	2.81 E+06	5.42 E+06
L-PFBS	3.21 E+04	8.03 E+04	1.51 E+05	2.62 E+05	4.93 E+05
PFHxA	2.02 E+05	5.34 E+05	1.05 E+06	1.74 E+06	3.34 E+06
PFHpA	1.57 E+05	4.42 E+05	9.01 E+05	1.49 E+06	2.72 E+06
L-PFHxS	1.64 E+04	4.91 E+04	9.32 E+04	1.53 E+05	3.02 E+05
PFOA	1.28 E+05	3.59 E+05	7.19 E+05	1.15 E+06	2.23 E+06
PFNA	1.19 E+05	3.37 E+05	6.64 E+05	1.06 E+06	2.13 E+06
L-PFOS	6.62 E+03	1.64 E+04	3.27 E+04	6.04 E+04	1.15 E+05
PFDA	9.25 E+04	2.86 E+05	5.19 E+05	8.34 E+05	1.62 E+06

Intensity at same concentration injected increase with the injection volume, because total PFAS quantity increase (Table 3.2). It is then not necessary to inject higher concentration than 1000 ng/L. Injecting a larger volume also allows injections to be made at lower concentrations. This makes calibration lines with points of lower concentrations possible. Also, higher concentrations could result in calibration curves that no longer behave linearly.

When SPE is used as the sample preparation method, usual injection volumes are 5, 10 and 20 µL[41,52]. In this study, the sample preparation method was filtration followed by direct injection. External calibration curves ranged began at 10 ng/L for the lowest point concentration. To reach sufficient sensitivity for all analytes at 10 ng/L, 200 µL has been chosen as the injection volume. When injection volume of 5 µL with direct injection, LOQ ranged from 23.3 to 70.0 ng/L for PFBS and PFHpA respectively[57]. For an injection volume of 30 µL, LOQ ranged from 48.1 ng/L for PFBA to 4820 ng/L for PFBS[80]. In this work, instrumental LOQ ranged from 3 ng/L for PFHxA to 53 ng/L for L-PFHxS (Table 3.7). With an higher injection, 200 µL against 5 and 30 µL, this method achieved comparable LOQ.

In this work, 200 µL has been chosen as the injection volume. It is because one did not have prior knowledge of the instrumental LOQ during method development. To prevent

overload effect, injection volume should be less than 15 % of peak volume (calculated as peak base width x flow rate) of the first eluting peak to avoid overload effect[I]. PFBA was the first eluting peak, its peak volume was 75 µL which is below 200 µL. But peak shape of early eluting compounds might be improved by increasing injection volume[42].

3.1.7 Retention time

Table 3. 3. Analytes retention times after method parameters have been set. The column used was a BEH C18 (2.1 x 100 mm, 1.7 µm) with a HSST3 (2.1 x 5 mm, 1.8µm) as the precolumn. Mobile phase A was milliQ water + 10 mM NH_4Ac. Mobile phase B was a mix of MeOH:ACN (80:20, v:v) + 10 mM NH_4Ac. The flow was constant at 0.3 mL/min. Gradient started at 1% of phase B and remained constant until 0.5 min. From 0.5 to 2 min, phase B reached 70 %. Phase B ramped up to 95 % after 8 min and remained at 95 % until 9 min. Then phase B decreased to 1 % and remained at that level until the end of the gradient, after 12 min.

Analyte	Rt [min]	Area
PFBA	3.84	94814
PFPeA	4.31	96558
L-PFBS	4.40	10788
PFHxA	4.57	55708
PFHpA	4.83	46725
L-PFHxS	4.89	6690
PFOA	5.14	40603
PFNA	5.50	35757
L-PFOS	5.55	2556
PFDA	5.93	29786

Since that the LC-MS/MS parameters are fixed, the analytes were identified according to their retention time. EPA method 537.1 explains that identifying PFAS can be achieved by comparing obtained Rt to Rt acquired from calibration standards with same LC-MS/MS parameters[55].

As in this work, LC-MS/MS PFAS analytical methods often use a C18 reversed stationary phase and two mobile phases, one of which is aqueous and the other organic. So, similar elution order can be expected. EPA method 533 and 537.1 had the same elution order for the same compounds analysed in this work[42,55]. Both EPA methods could not separate efficiently PFNA and L-PFOS, with a difference in Rt of 0.02 min and 0.01 min for method 533 and

[I] https://www.crawfordscientific.com/chromatography-blog/post/sample-diluent-effects-in-hplc

method 537.1 respectively. In this work, PFNA and L-PFOS were separated by 0.05 min (Table 3.3). They were better separated in this study than in EPA methods 533 and 537.1. One crucial difference is that columns used in method 533 and 537.1 have particle size of 3 and 5 µm respectively. In this method particle size was 1.7 µm. EPA method 8327 used a column with particle size of 1.8 µm (RBAX RRHD Stable Bond C18, 2.1 x 100 mm) and achieved Rt of 7.68 and 8.6 min for PFNA and L-PFOS respectively[43]. EPA method 8327 achieved a better L-PFOS/PFNA separation than in this work. But the analytical column was longer, 100 and 50 mm for EPA method 8327 and this study respectively. Also the organic mobile phase was constituted of pure ACN in EPA method 8327[43]. PFNA and L-PFOS have very similar chemical structure (Table 2.1), explaining why they elute closely. The detected intensity of L-PFOS is low, this could be due to the PFNA eluting close to the L-PFOS causing a suppression effect[55]. Rosnack et al. (2019) had an elution gradient lasting for 22 min and used a BEH C18 column of 100 mm (ACQUITY UPLC BEH C18 2.1 x 100 mm, 1.7 µm). In that application note, PFNA and L-PFOS Rt were both 12.3. So here, column length did not seem to have an impact on PFNA/L-PFOS separation at the opposite of particle size.

The relative polarity of ACN is 0.46[I] and the relative polarity of MeOH is 0.762[II]. When classifying organic phase by decreasing polarity this order appears: Rosnack et al (MeOH + 2mM NH4Ac), this method (MeOH:ACN, 80:20, v:v, + 10 mM NH4Ac), EPA method 8327 (pure ACN). One can notice that the more the organic phase is apolar, the better PFNA and L-PFOS are separated. In this work PFNA and L-PFOS are, relatively, the less polar PFAS (long chains). To elute them, relatively strong mobile phase is needed since a reversed stationary phase is used.

3.1.8 SPE and filtration

Rosnack et al. (2019) used SPE to concentrate PFAS from water samples. Their method provided a sample EF of 250. In this work, samples were completely evaporated, and a volume of 5 mL has been added (section 2.5.4). Rosnack et al. (2019) used an injection volume of 10 µL. Injection volumes used during SPE tests in this study were 25 and 50 µL.

[I] https://study.com/learn/lesson/acetonitrile.html#:~:text=Acetonitrile%20is%20classified%20as%20a,carbon.

[II] https://www.researchgate.net/figure/Some-physical-properties-of-solvents-used_tbl1_326566608

As seen in the previous section if injection volume increases, injected concentration can be lower to achieve similar sensitivity (Table 3.2). So, an EF as high as in Rosnack et al. (2019) was not necessary.

Two sample preparation methods have been tested after spiking samples: SPE and filtration through 0.7 µm glass fibre filter (section 2.5.4 and 2.5.1). For the first test, milliQ water samples have been spiked at 100 and 500 ng/L (Table A.4). SPE %Rec showed poor results at the opposite of filtration. SPE %Rec for spiking at 100 ng/L ranged from 22.7 % (PFDA) to 46.8 % (PFBA) and for 500 ng/L it ranged from 42.4 % (PFDA) to 51.3 % (PFBA) (Table A.4). Filtration %Rec were overall closer to 100 % than SPE %rec, only L-PFOS and PFDA had %Rec outside the 100 ± 30 % range (Table A.1). The following application note from Macherey-Nagel also tested SPE in water samples, the same stationary phase than this study was used[73]. For the same 10 PFAS analysed in this work, %Rec were ranging from 81.1 % (PFBA) to 90.9 % (PFDA). Longer chain PFAS such as PFDoA (C12) had lower %Rec, this could be explained by solubility problem in water samples and adsorption on material surfaces[73]. So, long chain PFAS adsorption on material surfaces might be a source to PFAS loss. In this SPE protocol, SPE eluate was evaporated to dryness which could partially explain low %Rec[51].

SPE end consisted to elution after rinsing the cartridge. The rinse was directly sent into the waste. Solvent used for the rinse and the elution were close, MeOH and MeOH + 0.5 % ammonia respectively. Poor %Rec were perhaps due to the proximity of the composition of these two solvents, causing potential elution of PFAS into the waste with the rinse phase. According to EPA method 533, MeOH:H_2O (80:20, v:v) was the optimal solution for eluting PFAS from SPE cartridges[1]. Here pure MeOH, a stronger eluent than MeOH:H_2O (82:20, v:v), was used to rinse SPE cartridges. Therefore, the rinse could partially elute out PFAS from SPE cartridge. Table 3.4 shows the comparison of PFAS retrieved (%Rec) in the rinse and elution phases during SPE after spiking milliQ water at 100 ng/L. PFDA was the PFAS which the biggest %Rec in the rinse phase (%Rec = 0.668 %) (Table 3.4). In the eluate, %Rec ranged from 29.1 % to 96.7 % (Table 3.4). So, it can be noted that the analytes were not eluted during the rinse step. In this test filtration spike at 100 ng/L has also been repeated and no PFAS had %Rec outside the 100 ± 30 % range in comparison to results in Table 3.3.

Table 3. 4. Comparison of the recovery percentage (%Rec) of the 10 PFAs spiked at 100 ng/L by filtration (n = 2), in the SPE eluate (n = 2) and the SPE rinse phase (n = 2). Spikes were made in milliQ water.

	%Rec				
	PFBA	PFPeA	L-PFBS	PFHxA	PFHpA
Filtration	103	113	114	117	115
SPE - Rinse	0.208	0.048	0	0	0.168
SPE - Eluate	96.7	87.5	98.4	85.8	84.6
	%Rec				
	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA
Filtration	109	102	97.1	105	87.0
SPE - Rinse	0	0	0	0	0.668
SPE - Eluate	89.0	74.2	47.7	43.4	29.1

When we look at the results in tables A.1 and 3.4, we can see a trend in the %Rec of SPEs. The longer the PFAS chain is, the lower the %Rec will be. As said before, this could be explained by solubility problem and adsorption on material surfaces[73]. These last two tests showed that direct injection after filtration on glass fibre filter gave better and probably, more repeatable results. Mottaleb et al. (2021) developed a direct injection analysis for PFAS in water samples. Water samples were filtered through acrodisc filters and the method gave reproducible results[57]. Like the method developed in this work, it was faster and cheaper than doing cartridge SPE extraction. Mottaleb et al. (2021) added that the direct injection analysis after filtration was suitable for routine PFAS analysis in surface water[57]. When SPE was repeated, only 7 out of 10 PFAS had %Rec improved. When Table 3.4 results are compared to %Rec of Macherey-Nagel, PFNA, L-PFOS and PFDA still much lower %Rec for SPE in this method[73]. Thus, SPE has been set aside in favour of filtration.

In this study three different water sample types were analysed: surface water, leachates of waste and underground water (sections 2.5.1 and 2.5.2). To validate a method, it is preferable to have a blank matrix. Environmental water samples were all contaminated with the 10 PFAS, except Orneau-Escaille and Orneau-INASEP for which L-PFOS has not been detected (Table 3.5). Lix 82281 was less contaminated than lix 82282 except for PFHpA, L-PFHxS and L-PFOS. Orneau Escaille was less contaminated than Orneau INASEP except for PFBA with 11.6 and 9.15 ng/L respectively. Therefore, Lix 82281 and Orneau Escaille are chosen as sample to be spiked during method validation.

Table 3. 5. Comparison of the concentrations of the 10 analysed PFAS found in waste leachates samples (Lix 82281 and Lix 82282) and water samples sampled in the river Orneau. For the river Orneau, sampling sites are explained in section 2.5.1 of this work. All modalities had n = 2.

	ng/L				
	PFBA	PFPeA	L-PFBS	PFHxA	PFHpA
lix 82281	139	7.6	19.6	12.8	3.55
lix 82282	1230	10.2	36.2	94.4	1.8
Orneau - Escaille	11.6	1.25	1.05	2.25	0
Orneau - INASEP	9.15	2.75	2.3	3.9	0.55
	ng/L				
	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA
lix 82281	11.4	11.3	2.8	17.4	7.2
lix 82282	8.25	16.5	2.7	16.2	7.75
Orneau - Escaille	0.9	3.25	2.8	0	6.25
Orneau - INASEP	1.25	3.6	3.1	0	7.05

Waste leachate samples have already been filtered (section 2.5.2). Orneau samples must be filtered to 0.7µm, before or after spiking. Orneau river samples have been spiked at 100 ng/L after and before filtration (Table 3.6). No clear differences emerged for PFBA, PFPeA, L-PFBS, PFHxA, PFHpA and L-PFHxS. The highest %Rec difference found between spike first and filtration first was for L-PFOS, with 73.7 and 109 % respectively. Overall, %Rec were closer to 100 % when samples were filtered before spiking. 0.7µm pore size glass fibre can be used to retain particulate suspended matter[81]. It is known that PFAS can adsorb on particulate matter[33]. So, if there is still suspended particulate matter before spiking, this could lead to a drop in %Rec. It was then preferred to filter samples to 0.7 µm first for method validation. In this experiment spiked samples were put at pH ≤ 3 after spiking. PFAS shows an increasing affinity with isolated organic matters fractions at decreasing pH[82,83]. Experiment in section 3.3.2 showed no clear differences between spiking after or before setting pH ≤ 3 in waste leachate samples.

Table 3. 6. Comparison of recovery percentages (%Rec) of spike at 100 ng/L of the 10 PFAS analysed in this study. Spikes have been made before ("Spike then filtration") (n = 2) or after ("Filtration then spike") filtration (n = 2).

	%Rec				
	PFBA	PFPeA	L-PFBS	PFHxA	PFHpA
Filtration then spike	85.9	105	111	104	111
Spike then filtration	86.9	106	116	108	108
	%Rec				
	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA
Filtration then spike	109	107	103	109	95.3
Spike then filtration	103	96.8	79.2	73.7	64.6

3.2 Validation – calibration Curves

3.2.1 Validation criteria

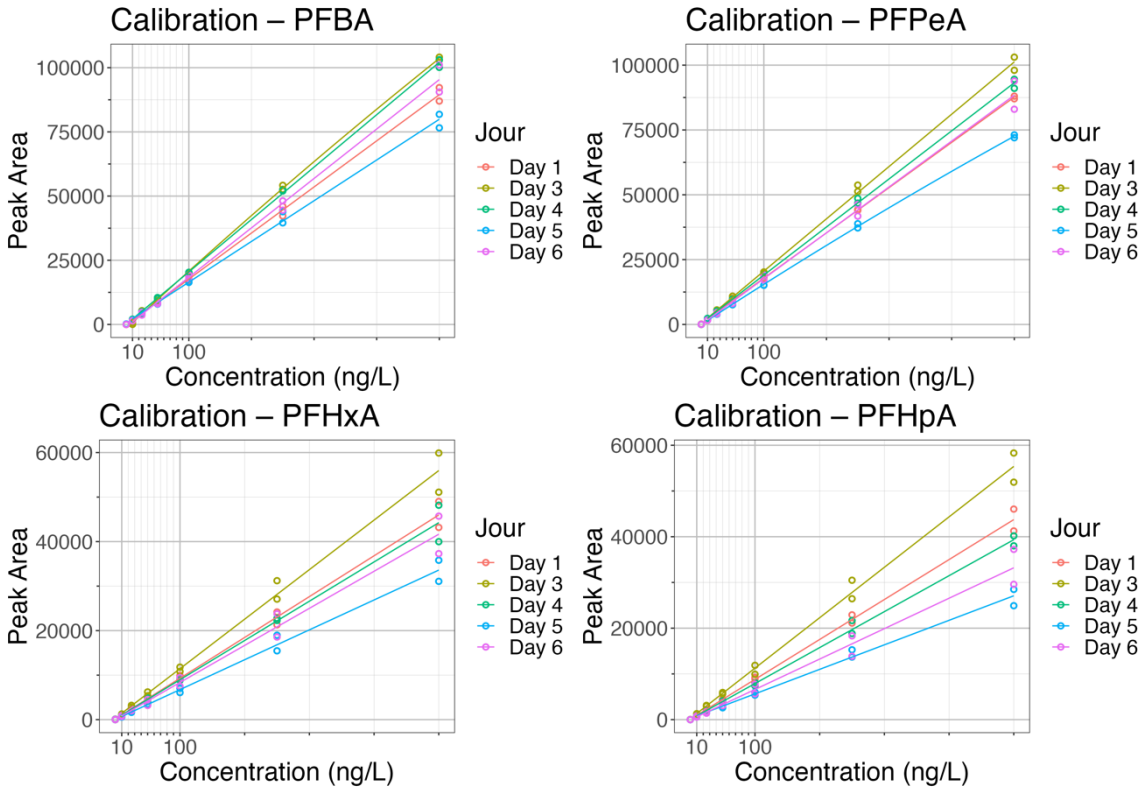
Some validation criteria applied in this work are dependent of LOD and LOQ. Estimates of instrumental LOD and instrumental LOQ in this work are given in Table 3.7. In Rosnack et al. (2019) the highest LOQ for the molecules analysed in this study, was 0.13 ng/L for PFBA, PFPeA, PFHxA, PFNA and PFDA. LOQ in this work are higher than those encountered in Rosnack et al. (2019). That may be cause background PFAS noise was not eliminated by using delay column or PEEK tubing.

Table 3. 7. Instrumental limits of detection (LOD) and limits of quantification (LOQ) for each compound analysed in this work. The values are written as LOD – LOQ and the unit is ng/L.

Analyte	LOD – LOQ [ng/L]
PFBA	12 – 37
PFPeA	6 – 20
PFHxA	1 – 3
PFHpA	1 – 4
PFOA	6 – 18
PFNA	4 – 13
PFDA	4 – 12
L-PFBS	4 – 13
L-PFHxS	17 – 53
L-PFOS	5 – 16

3.2.2 Daily calibration

Initial calibration curves ranged from 0 to 1000 ng/L and were repeated each day. Calibrations curves were made with eight points which meet recommendations[84]. After inspection of the method validation results, it was decided to withdraw validation day 2 and the point at 1000 ng/L (all days) from the validation dataset. When all the data were kept, the daily calibration curves were more often quadratic (Table A.1) than when validation data set had been truncated (Table A.3). For concentration ranges similar to those in this study, and with a triple quadrupole mass spectrometer, calibration curves for the same analysed PFAS showed linear response[41,52,85,86]. Within the present experimental conditions, the analytical system had a better linear response over concentration range from 0 to 500 ng/L (Table A.3). Aberrant results were observed in the calibration curves for validation day 2. Withdrawing validation day 2 and 1000 ng/L data, has also made it possible to eliminate aberrant results. Furthermore, legislation will set a limit of 100 ng/L for the sum of the ten PFAS analysed in this study. It is an order of magnitude lower than the maximum concentration used in this method validation. In this study, the instrumental LOQs for these 10 PFASs were below this 100 ng/L limit, with a maximum of 53 ng/L for L-PFHxS (Table 3.7). The daily calibration curves obtained can be seen below (Fig. 3.10).



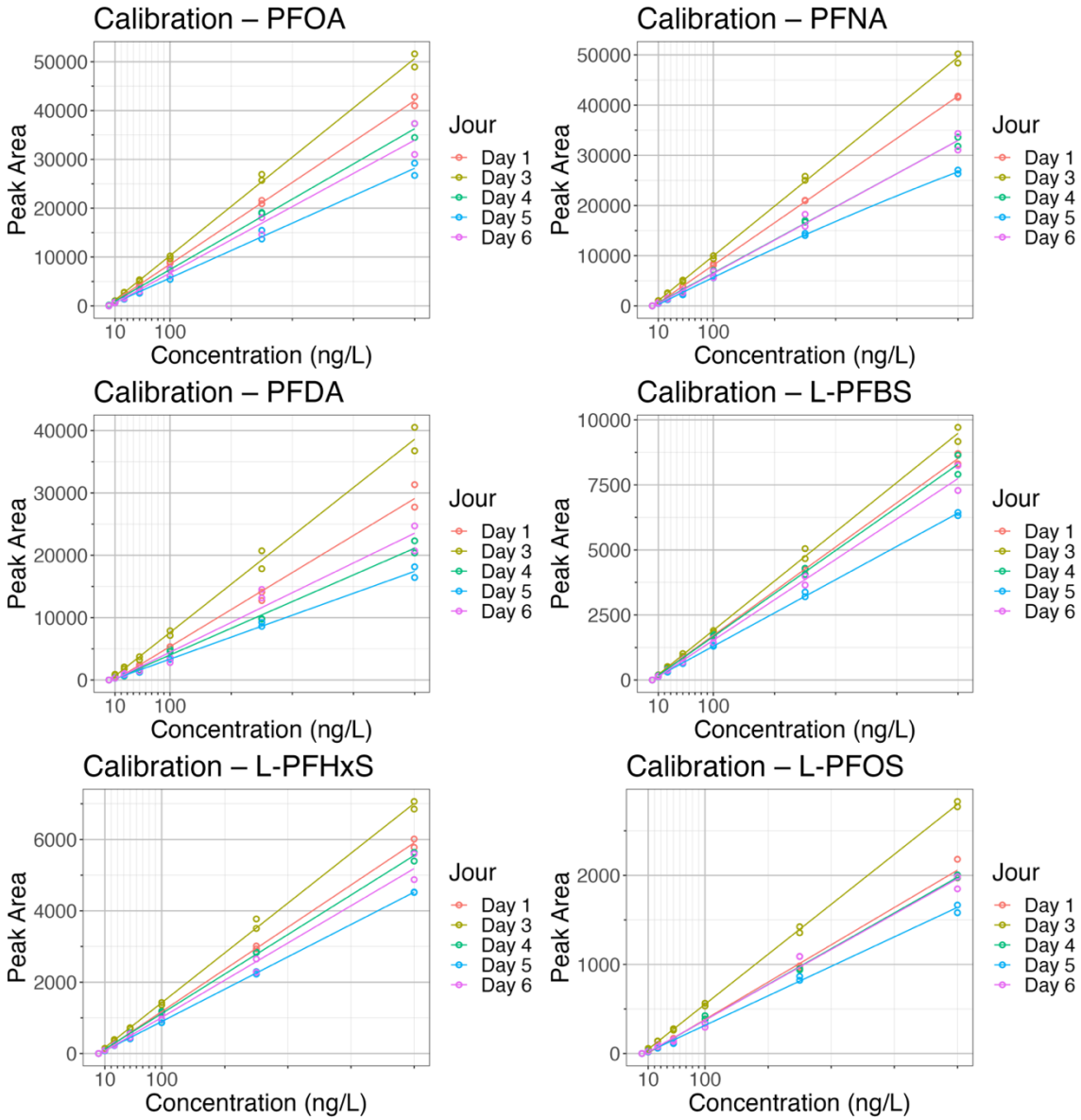


Figure 3. 10. Daily calibration curves obtained during method validation after excluding validation day 2 and 1000 ng/L (all days) from validation dataset.

The coefficient of determination (R^2) was also subject to a validation criterion (Table 2.11). When 1000 ng/L and validation day 2 were excluded, only some calibration curves for PFHxA, PFHpA PFOA and PFDA had $R^2 < 0.99$ (Table A.3).

Olomukoro et al. (2021) used calibration range from 0 to 1000 ng/L. Doing so allowed them to have a large linear dynamic range. As in this study, it allowed to quantify above concentrations of highly contaminated samples[52]. When the calibration range was going from 5 to 200 ng/L all R^2 were > 0.99[85]. This study achieved similar R^2 for most calibration

curves. Also, LOQ must be higher than the lowest calibration point[43]. The calibration range selected in this study ranged from 0 to 500 ng/L. And all analytes instrumental LOQ were > 3 ng/L (Table 3.7). Using this working range was adequate and respected EPA requirement. Lowest concentration was below the lowest instrumental LOQ, and highest concentration was higher than the most contaminated validation sample.

The calibration curve slope can also be used as a proxy for the average sensitivity of the instrumental system. For each compound, the slopes of the daily calibration curves varied relatively slightly (Table A.3). L-PFBS was the compound with the greatest relative variation for the slopes of the calibration curve, with a minimum and maximum slope value of 12.8 and 50.6 (Table A.3). For the other compounds the slopes stayed within the same order of magnitude. The maximum slope value did not exceed 3.9 times the minimum slope value for each analyte (Table A.3).

The %Rec of calibration solutions were also subjected to a validation criterion. %Rec of a calibration point at the LLOQ must be 50 % to 150 % and 70 % to 130 % for all the other concentrations (Table 2.11). The analyte instrumental LOQ must be considered to apply the adequate validation requirement. Only some spikes at 10 ng/L for PFBA, PFHpA, PFNA, PFDA and L-PFOS, had %Rec outside 100 ± 30 % (Fig. 3.11). PFBA instrumental LOQ was 37 ng/L (Table 3.7), so for PFBA 10 ng/L point cannot be considered. Instrumental LOQ of PFHpA was 4 ng/L and 10 ng/L is two times bigger. Then it can be considered that calibration point %Rec is satisfying for PFHpA. PFNA, PFDA and L-PFOS had instrumental LOQ ranging from 12 ng/L to 16 ng/L (table 3.7). The 10 ng/L cannot be considered, as it is lower than their instrumental LOQ. For those five compounds, all higher calibration points (25 to 500 ng/L), presented %Rec between 70 and 130 % (Fig. 3.11.c, d and e). 25 to 500 ng/L calibration points respected validation requirement. It can be said that calibration point presented overall a satisfactory %Rec for concentrations higher than instrumental LOQs.

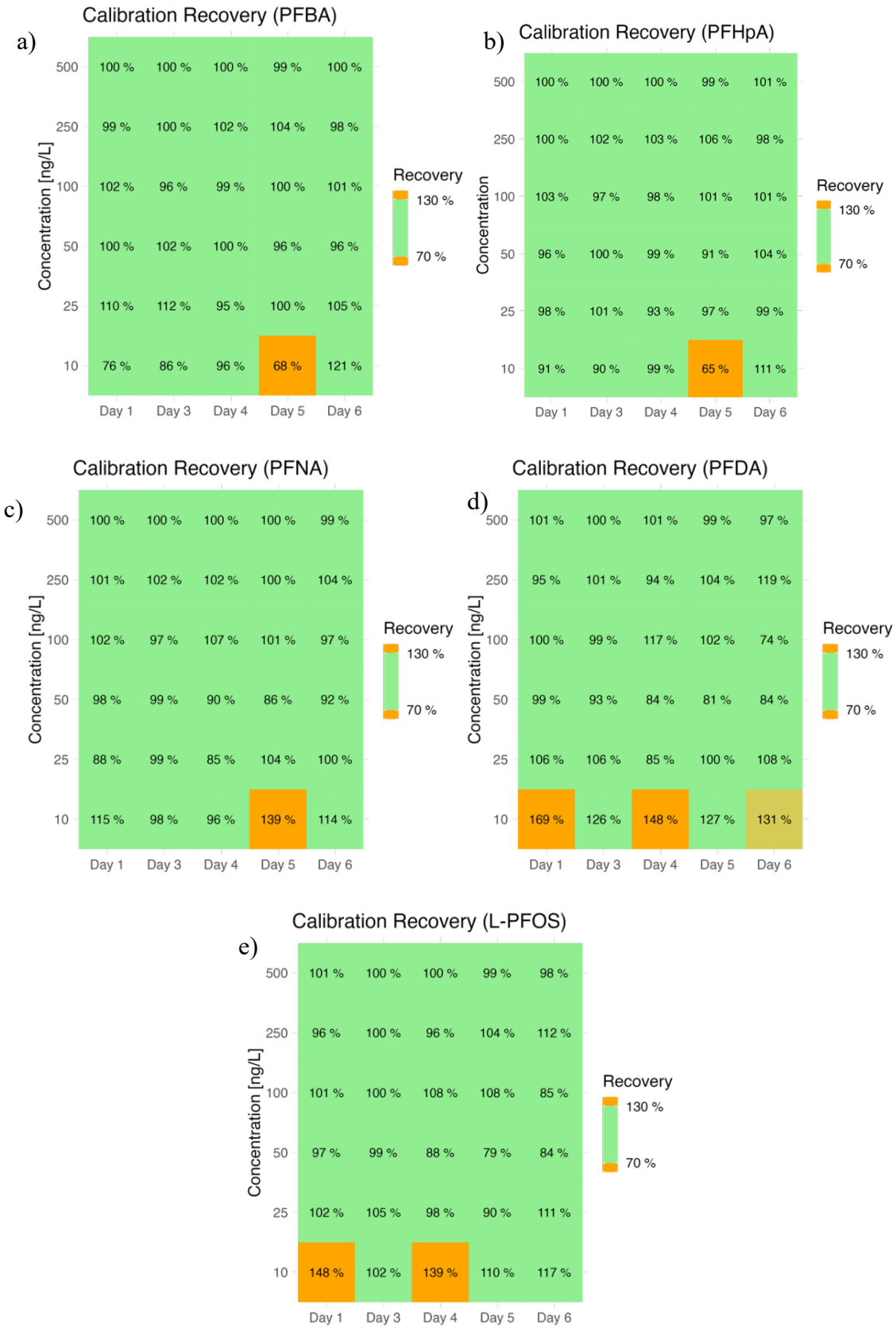


Figure 3. 11. Recovery percentage (%Rec) of calibration points per analyte, per concentration and per validation day. %Rec outside the 70 – 130 % window are underlined in orange and do not satisfy validation requirement. %Rec within that range are underlined in green. a) PFBA, b) PFHpA, c) PFNA, d) PFDA and e) L-PFOS. For each concentration, each compound and each day, n = 2.

3.3 Validation – Samples

In the previous section validation day 2 and 1000 ng/L calibration point data were excluded from validation dataset. The analysis of spiked samples is then based on the truncated dataset from day 2 of validation and the 1000 ng/L calibration point.

3.3.1 Trueness (Recovery from spiked samples (%Rec))

The three different matrices were spiked at 40, 100 and 200 ng/L. The highest instrumental LOQs were for L-PFHxS and PFBA (53 and 37 ng/L respectively). The next highest instrumental LOQ was 20 ng/L for PFPeA. Consequently, the lowest spike concentration 40 ng/L was at least two times higher than the analyte instrumental LOQ, except for PFBA and L-PFHxS. So, %Rec needs to be within 70 to 130 % range to meet validation requirement. PFOA and L-PFBS were the only two analytes for which all three matrices, all 3 spiking concentrations and all validation days showed %Rec within validation limits.

For PFBA, PFPeA, PFHxA, PFHpA and L-PFHxS only spiked waste leachate samples did not entirely meet %Rec validation criterion (Fig 3.12.a, 3.13.d, 3.12.d and 3.12.c respectively). For PFHpA and PFPeA all waste leachate samples had a %Rec lower than 70 % (Fig 3.12.c and 3.13.d respectively). PFHxA only had one validation day meeting %Rec requirement (Fig. 3.12.d). PFBA spikes had %Rec < 70 when %Rec criterion was not met (Fig. 3.12.a). For PFBA, 40 ng/L can be considered close enough to its instrumental LOQ (37 ng/L). Thus, for PFBA the lowest %Rec at 40 ng/L (%Rec = 52 %) meet %Rec validation criterion, because it is included in the 50 to 150 % range (Table 2.10). L-PFHxS had spiking concentrations not respecting %Rec requirements only in waste leachate samples for every validation day (Fig 3.13.a). For L-PFHxS, the highest %Rec in waste leachate samples was 84 % (day 1 for 40 ng/L spike) (Fig. 3.13.a).

PFNA had spiked samples not meeting %Rec limits in lysimeter and waste leachate samples (Fig. 3.13.b). PFDA and L-PFOS had %Rec outside validation limits for the three matrices (Fig. 3.12.b and 3.13.c respectively). For Orneau water samples, PFDA and L-PFOS only had validation day 1 spiked samples that did not meet validation requirements.

It was in lysimeter samples that PFDA had the lowest and the highest %Rec (Fig 3.11.b). For L-PFOS in lysimeter samples, the lowest %Rec value was 60 % and the highest value was 146 % (Fig. 3.13.c). For PFDA and L-PFOS, waste leachates spiked samples %Rec were as good as Orneau water samples (Fig. 3.12.b and 3.13.c).

Regarding %Rec, waste leachate was the matrix with the biggest occurrence of spiked samples not meeting validation requirements. In this work the waste leachate spiked samples came from the first leaching of a sample from a customer (c.f. section 2.7.2). No information was available on the matrix composition. Influence of matrix effect on %Rec cannot be excluded. Waste leachate samples differed from the Ormeau and lysimeter samples in terms of initial pH. Initial pH value of waste leachate samples was 11.3, which is much higher than initial pH value of lysimeter and Orneau samples (around 7). pH value of 11.3 are not met in normal environmental conditions. It could be hypothesized that a pH as high as 11.3 somehow influences the stability of PFAS.

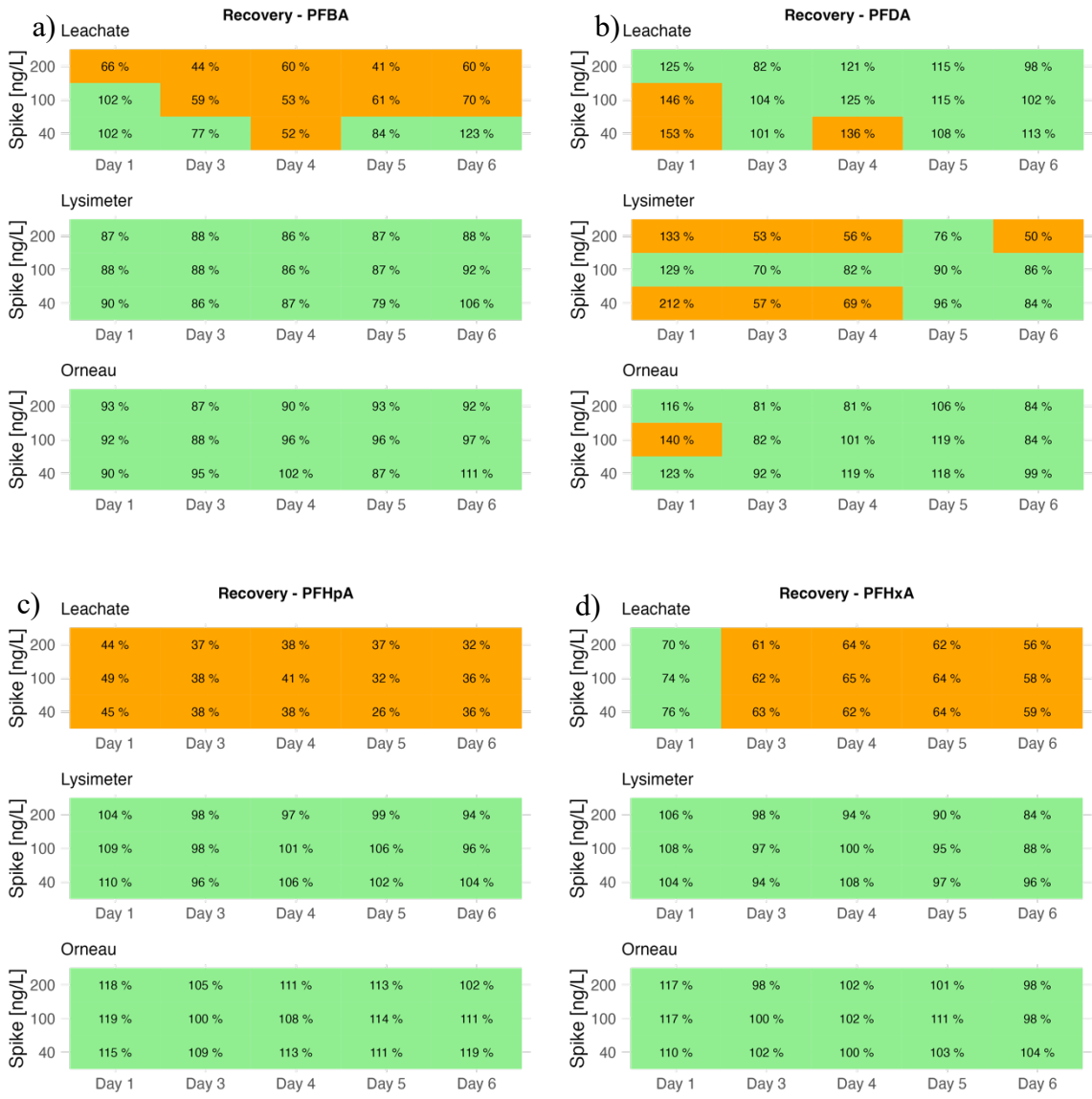


Figure 3. 12. Recovery percentage (%Rec) of spiked samples per analyte, per concentration and per validation day. Samples were spiked at 40, 100 and 200 ng/L. %Rec outside the 70 – 130 % window are underlined in orange and do not meet vaildation requirement. %Rec within that range are underlined in green. a) PFBA, b) PFDA, c) PFHpA and d) PFHxA. For each concentration, each compound and each day, n = 2.

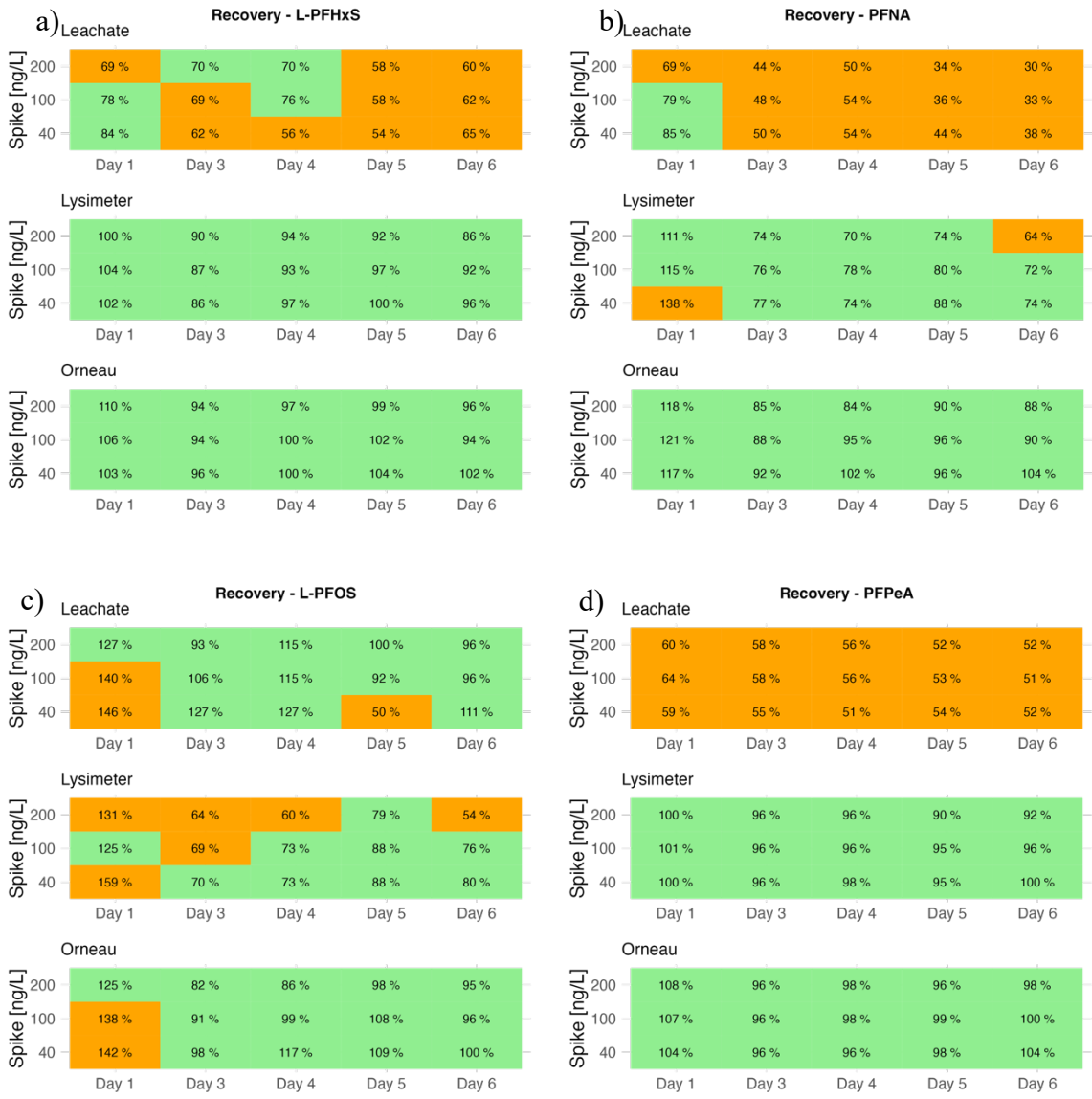


Figure 3. 13. Recovery percentage (%Rec) of spiked samples per analyte, per concentration and per validation day. Concentration unit is ng/L on the y axis. Samples were spiked at 40, 100 and 200 ng/L. %Rec outside the 70 – 130 % window are underlined in orange and do not meet validation requirement. %Rec within that range are underlined in green. a) L-PFHxS, b) PFNA, c) L-PFOS and d) PFPeA. For each concentration, each compound and each day, n = 2.

3.3.2 Influence of Samples' pH

During sample preparation, the pH of samples was adjusted to ≤ 3 after spiking them with the PFAS mix. It implies that each matrix had a different pH from another. Amongst the three different water types, waste leachates samples from sample 82281 had shown an outstanding pH. Their pH values were at 11.3. %Rec of analytes in waste leachates were lower than validation criteria in most samples (Table 3.8). The pH value can change PFAS

speciation and their electro-statical processes in real samples. The impact of adjusting samples pH to ≤ 3 before or after spiking has been tested. To test that hypothesis waste leachate samples spiked at 40 ng/L (5 spiked before and 5 spiked after setting pH value at 3) were analysed. No conclusive differences were found (Table 3.8). For PFBA it seemed that means were different. But further inspection of PFBA boxplot of "Spike first" and "pH first" showed no clear differences (Fig. A.2). It can be said that samples, even if they present pH value higher than 11, can be prepared with the current protocol without degrading spiked PFAS.

Table 3. 8. Comparison of setting waste leachate samples pH at pH ≤ 3 after ("spike first") or before ("pH first") spiking at 100 ng/L. The mean and standard deviation (SD) were obtained from 5 replicates (n = 5).

	PFBA		PFPeA		L-PFBS		PFHxA		PFHpA	
	Spike first	pH first	Spike first	pH first	Spike first	pH first	Spike first	pH first	Spike first	pH first
Mean	1268	2635	5043	4976	1127	1109	2633	2550	918	811
SD	898	1161	39.2	52.6	26.9	14.1	100	165	61.4	51.2
	L-PFHxS		PFOA		PFNA		L-PFOS		PFDA	
	Spike first	pH first	Spike first	pH first	Spike first	pH first	Spike first	pH first	Spike first	pH first
Mean	439	447	3236	3176	1111	1089	264	288	2139	2243
SD	14.7	29.0	75.7	187	44.0	62.9	12.4	30.0	157	235

3.3.3 Precision

Precision for repeatability (%RSD_r) and for reproducibility (%RSD_R) have been calculated. Both for repeatability and reproducibility, %RSD must be < 20 % to meet validation requirement (table 3.1). Spiked concentrations were 40, 100 and 200 ng/L and %RSD_r for analytes and the three water sample types are presented in Fig. 3.13.

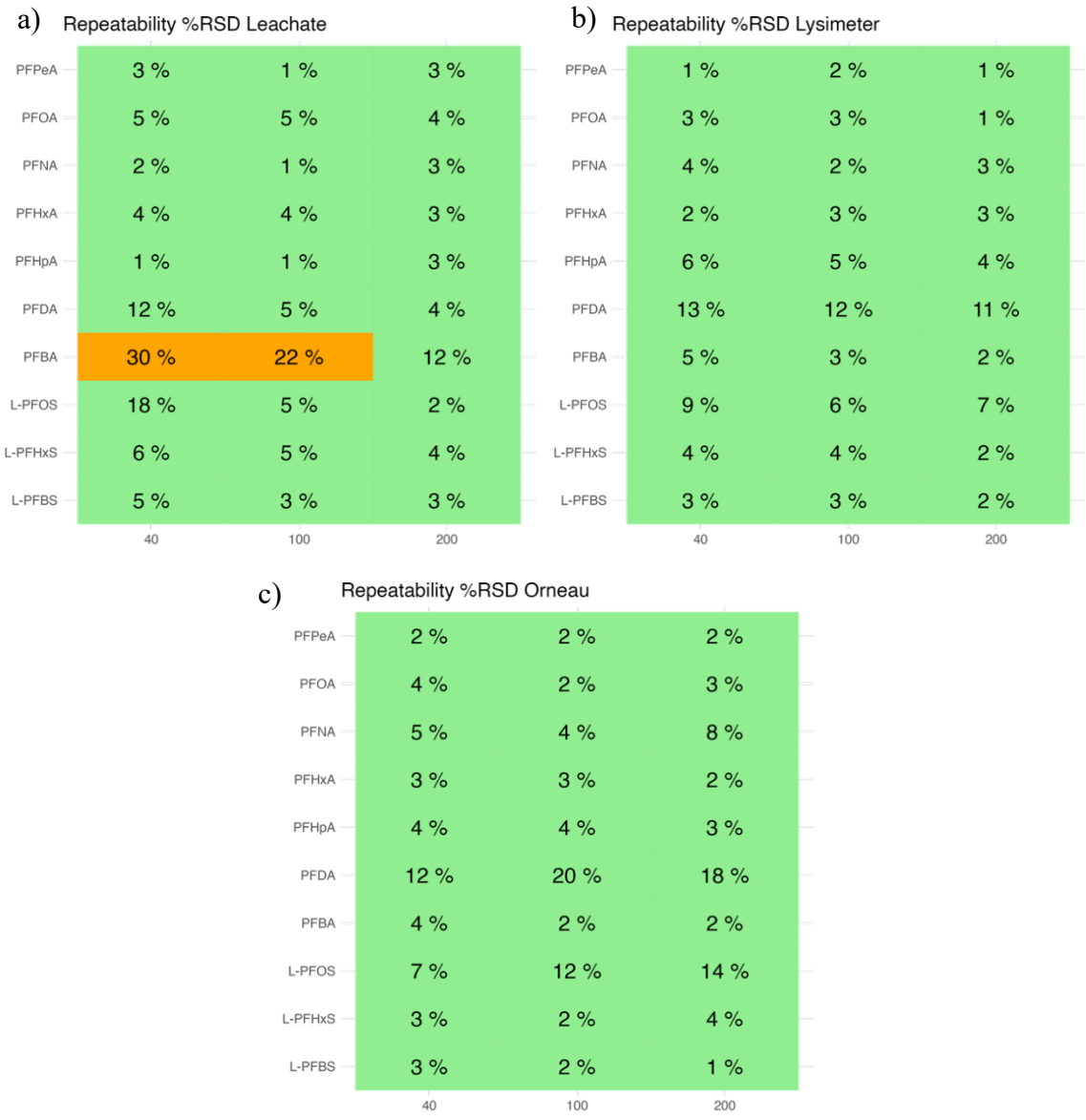


Figure 3. 14. Repeatability expressed in %RSD calculated for spike concentration at 40, 100 and 100 ng/L, in a) waste leachate samples, b) in lysimeter samples and c) in Orneau samples.

For Orneau and Lysimeter samples, all spiked concentrations and analytes had $\%RSD_r \leq 20$ % (Fig. 3.14.b and 3.14.c). Waste leachate is the only sample type with %RSD not meeting validation requirement. PFBA was the only compound in waste leachate spiked samples having %RSD > 20 % with a maximum of 30 % (Fig. 3.14.a). In a study using filtration, 2 to 26 $\%RSD_r$ for spiked water samples were achieved[57]. EPA 533 spiked groundwater and surface water at 10 and 80 ng/L, for the same PFAS analysed in this study $\%RSD_r$ were < 20 %[42]. In Yang et al. (2018), $\%RSD_r$ for L-PFOS and PFOA were within 0.6 – 14 % for spiking at 2.5, 40 and 200 ng/L[40]. In this study, maximum $\%RSD_r$ for L-PFOS was 18 % at 40 ng/L in waste leachate samples. Hunt and Hindle (2018) reached $\%RSD_r$ from 1 to 6 %, for tap water quality samples[51]. The method developed in

this work achieved higher $\%RSD_r$ than Hunt and Hindle (2018) but for environmental water samples, probably because the present method did not rely on isotopic internal standard. And yet it respected validation requirements. Another study analysed PFAS in non-potable waters, spiked $\%RSD_r$ were 18 % (for PFNA at 10 ng /L) and 16.3 % for PFOS[85]. Those percentages are similar to those in this study. Initially, samples preparation was based on Rosnack et al. (2019). Rosnack et al. (2019) achieved $\%RSD_r$ of 12.5 % at the highest, for the 10 PFAS analysed in this study in environmental water samples[41].

At the opposite of repeatability, reproducibility presented worse results regarding $\%RSD_R$. For the three matrices (Orneau, lysimeter and waste leachates), when comparing all compounds per concentration, PFDA, PFNA and L-PFOS had the highest $\%RSD_R$. For those three molecules $\%RSD_R$ were at minimum 32 % (Orneau) and at the maximum 2300 % (lysimeter) (Fig. 3.15. a and c, respectively). $\%RSD_R$ is the reproducibility relative standard deviation. Then if average response is low and vary between days it will lead to a higher $\%RSD_R$ in reproducibility. L-PFOS had low response signal in the MS/MS system (Table 3.2). Therefore, a slight variation between days will drastically increase $\%RSD_R$. PFDA had the worst $\%RSD_R$ in all three matrices, except for PFBA in waste leachate matrix. Munoz et al. (2016) analysed PFAS in sediments and the extraction was based on ultrasonic method. $\%RSD_R$ was always better than 23 % for all analytes, including those in this study[88]. Kobayashi et al. (2022) used SPE to extract PFAS from drinking water samples. For PFAS analysed in this study, Kobayashi et al. (2022) achieved at worst $\%RSD_R$ of 61 % and 56 % for PFHxA at 1 and 10 ng/L respectively[89]. In this study, $\%RSD_R$ for PFHxA in surface water were 4 %, 63 % and 115 % at 40 and 100 and 200 ng/L respectively (Fig. 3.15). In this work, the worst $\%RSD_R$ for surface water samples was 400 % for PFDA at 100 ng/L (Fig. 3.15). In Taniyasu et al. (2022), PFAS were extracted by SPE from water samples. In Taniyasu et al. (2022), for drinking water and river water and seawater and wastewater, all $\%RSD_R$ were < 40 % for all ten PFAS. No clearly defined limits for $\%RSD_R$ have been found. The regulation (EU) 2021/808 (although not applicable to PFAS nor environmental analysis) states that within laboratory reproducibility shall not exceed levels calculated by the Horwitz equation[90]. For concentrations below 1 ppb (1000 ng/L), $\%RSD_R$ should not exceed 45 %[91]. The results were mixed and the $\%RSD_R$ values were not clearly below 45% (Fig. 3.15). At such concentrations, the Horwitz equation gives results with excessive uncertainties[90]. The limit of 45% should therefore be used with caution.

Overall, it seems that SPE allowed better $\%RSD_R$ for reproducibility than sample filtration. The addition of isotope-labelled internal standard would also improve reproducibility.

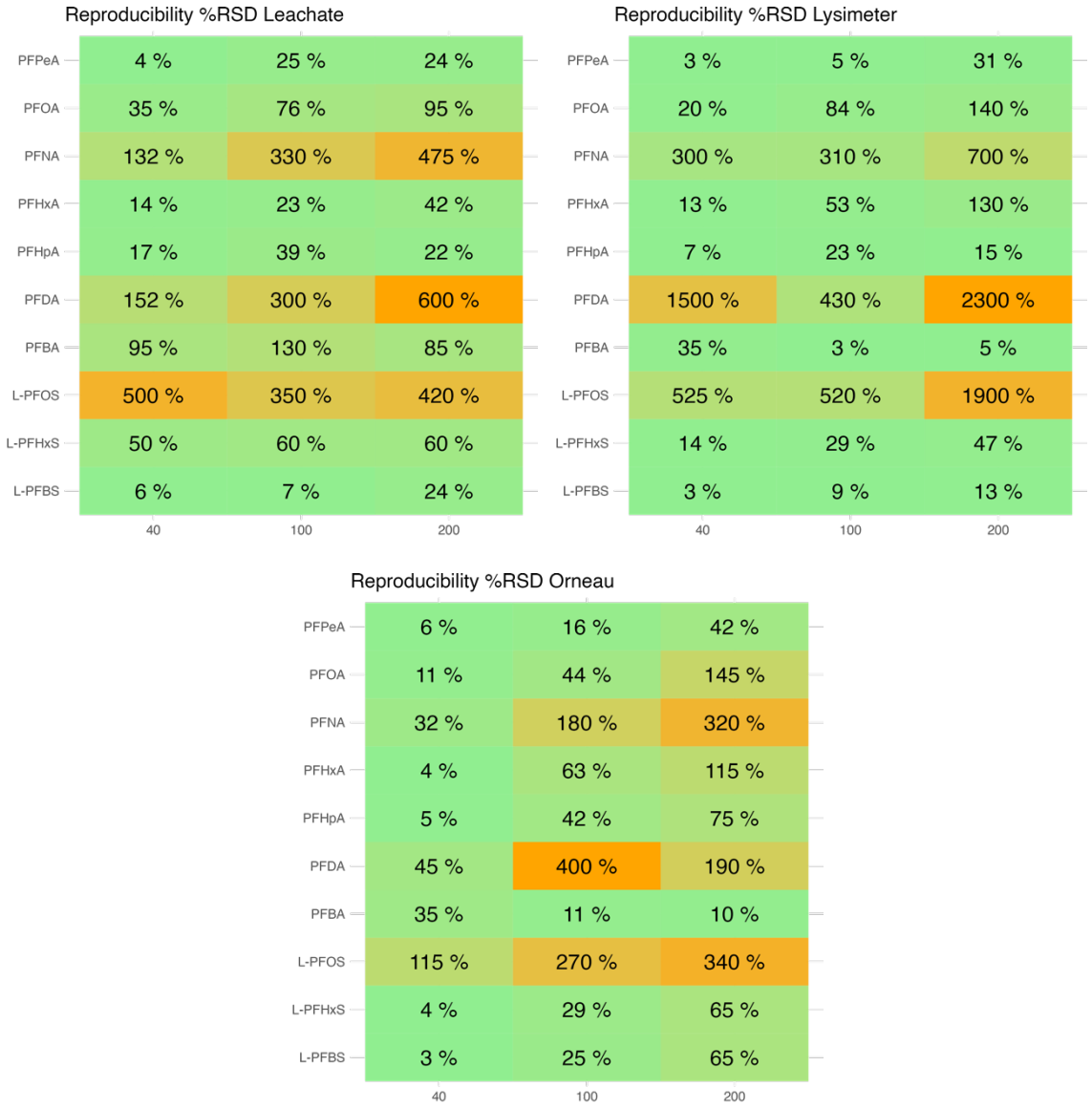


Figure 3. 15. Reproducibility expressed in %RSD calculated for spike concentration at 40, 100 and 100 ng/L, in a) waste leachate samples, b) in lysimeter samples and c) in Orneau samples.

3.4 Standard additions on sample 82588

82588 matrix was discharge water from a waste treatment centre. No additional information about the sample was provided. This sample was also analysed by another laboratory. Unfortunately, no insights about their analytical method have been communicated. Initially, standard additions were realised with calibration points going from 0 to 1000 ng/L. However, it was previously decided to remove point 1000 at ng/L from the dataset. Analyte concentration is obtained by calculating the abscissa at origin. An example of calibration curve for PFBA is given in Fig. 3.16.

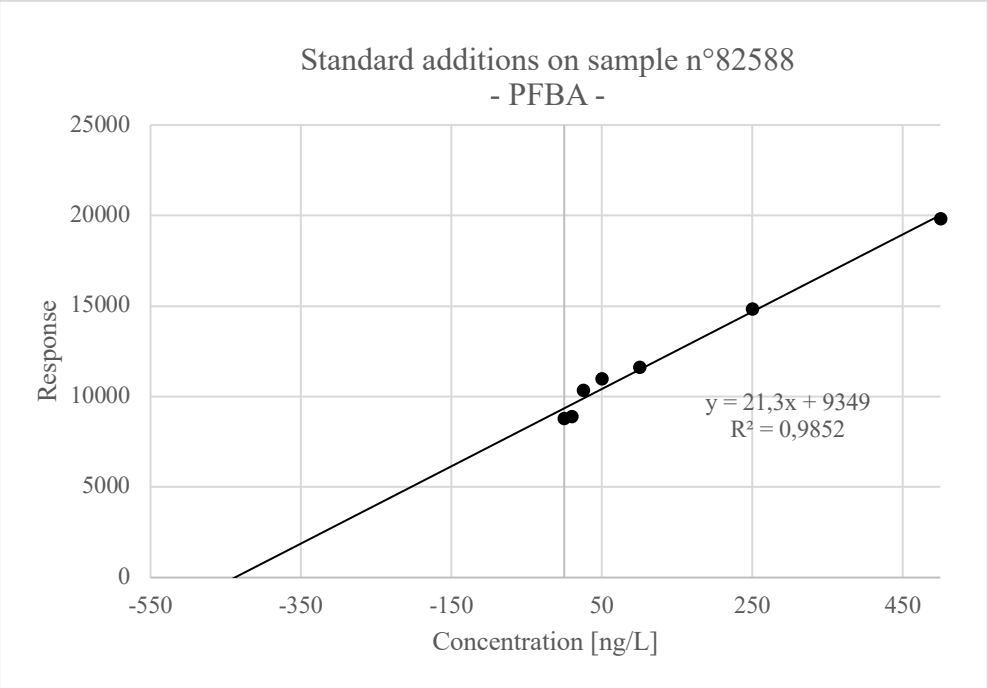


Figure 3. 16. Example of a curve obtained from the standard addition on sample n° 82588 for PFBA.

So, concentrations obtained for 82588 by the method developed in this work (internal results) and those from the other lab (external results) are compared in Fig. 3.17. Four analytes (L-PFHxS, PFOA, PFNA and L-PFOS) on ten were below 50 % of concentrations obtained by the external laboratory (Fig. 3.17). Five compounds on ten (PFBA, PFPeA, PFHxA, PFHpA and PFDA) were within the 50 to 150 % range of their respective concentrations obtained by the external laboratory (Fig. 3.17). L-PFBS is the molecule with the highest ratio (Fig. 3.17). PFAS here are quantified between ppt and the ppb level. Slight contaminations introduced by method can introduce background noise potentially equivalent to PFAS initially present in the sample. Only PFNA had a concentration below its

instrumental LOQ (Table A.4). Calculated concentration for PFDA was at the exact same level than its instrumental LOQ (Table A.4). Despite big differences in ratio internal results over external results, all concentrations were in the same order of magnitude (Table A.4). Lower concentrations can be originated from a lack recovery. Sample preparation and analytical system are likely the origin of the main contamination sources differences. For quantitation, it is recommended to have a working range going up to 200% of the expected concentration. Prior to standard additions no information about were available about sample PFAS content. This method used 500 ng/L as the highest calibration concentration which is lower than highest concentration found in 82588 sample (Table A.4). The purpose of this work was to develop and validate a method for the determination of 10 PFAS, to determine whether samples were within the recommended limits (< 100 ng/L for the sum of the 10 PFAS analysed in this work). So being able to quantify at such high concentrations is not necessary.

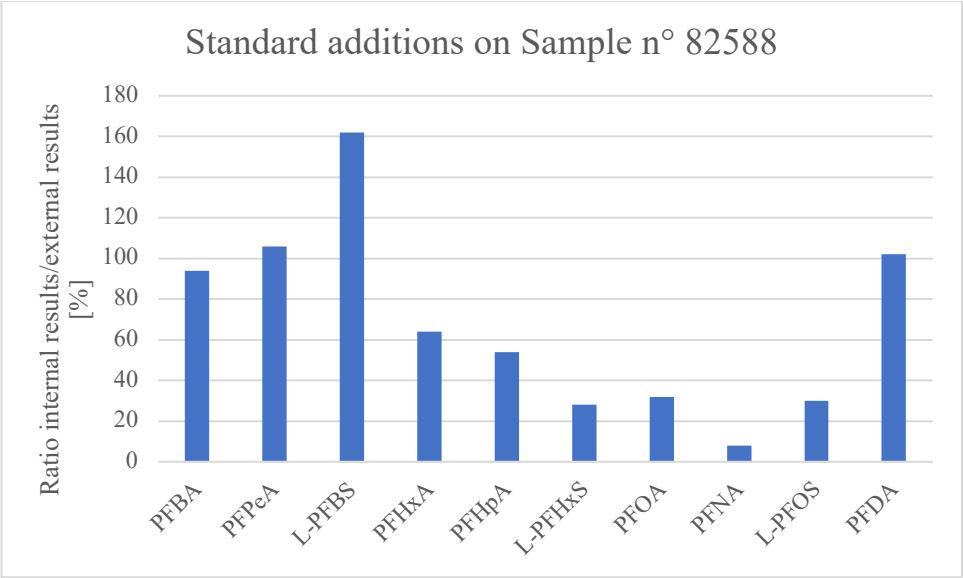


Figure 3. 17. Ratio of concentrations calculated by standard additions (internal results, n = 2) over results obtained by an external laboratory (external results).

4 Conclusion

The objectives of this work were to develop and validate, in UPLC-MS/MS, an analytical method for 10 PFAS in environmental matrices: surface water (Orneau river), groundwater (lysimeter) and waste leachate. The validation of the method has been realised over 6 separate days, on the three matrices at spiking concentrations of 40, 100 and 200 ng/L.

During the development of the method, an HSST3 VanGuard (1.8 µm, 2.1 x 5 mm) as a pre-column was installed before a BEH C18 (1.7 µm, 2.1 x 50 mm) column. The analytical gradient lasted for 12 minutes and used milliQ water + 10 mM NH_4Ac and MeOH:ACN (80:20, v:v) + 10 mM NH_4Ac as solvents. Sample preparation by SPE and direct injection after filtration have been compared. SPE provided %Rec ranging from 29.1 to 98.4 %. Filtration gave better and more stable %Rec than SPE. For filtration, %Rec for L- overall were closer to 100 %. Therefore, filtration was chosen over SPE. Validation day 2 and 1000 ng/L (all days) have been excluded from validation data set as it had aberrant values and too high leverage on calibration curves, respectively. Instrumental LOQ of analytes were ranging from 1 to 53 ng/L. All analytes instrumental instrumental LOQ were below the regulatory limit in groundwater of 100 ng/L. %Rec of calibration points were at the lowest 68 % for PFBA at 10 ng/L and at the highest 169 % for PFDA at 10 ng/L. Most of the calibration points %Rec were within 70-130 %. When samples were spiked, waste leachate was the matrix presenting the biggest occurrence of samples with %Rec outside 70-130 %. For waste leachate, lowest %Rec was PFPeA. For surface and groundwaters %Rec were better. Repeatability was always below 20 %, except for PFBA in waste leachates. Reproducibility had generally $\%RSD_R$ between 4 and 64 %. The most extreme value encountered was 2300% for PFDA in lysimeter samples. On average, the three matrices had similar $\%RSD_R$. But waste leachate was the matrix with the worst %Rec and $\%RSD_r$. The validated method makes it possible to obtain satisfactory results without the use of a delay-column or internal isotopic standards for the surface and groundwater tested. Given the matrix effect, an internal isotopic standard or quantification by dosed additions remains necessary for the waste leachate tested.

The robustness of the method has not been assessed. It would be interesting to do so. This method has been validated by external calibration. Standard addition or isotope-labelled internal standards could be investigated to improve performances if required. This choice should take into account the time required, the consumption of reagents and standards and the cost involved.

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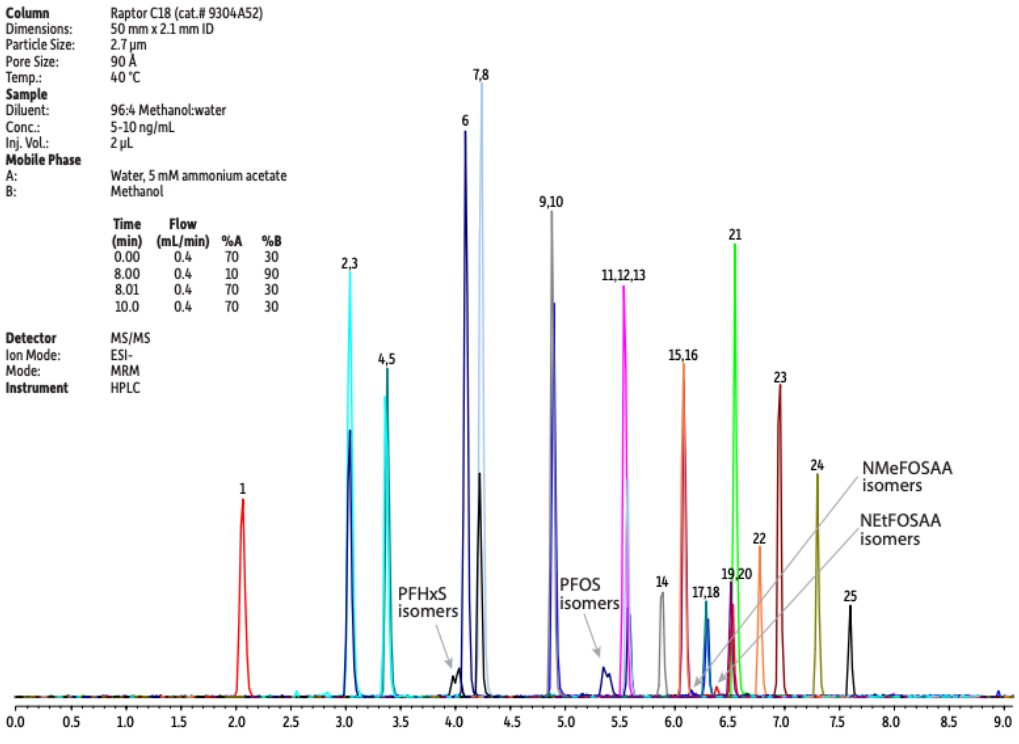
6 Appendices

6.1 Figures

Figure 1: A 50 x 2.1 mm Raptor C18 column is a great PFAS LC column choice; it meets all EPA 537.1 method criteria in a fast 10-minute total cycle time.



	Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1.	Perfluorobutanesulfonic acid (PFBS)	2.06	10	298.8	79.9
2.	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid ($^{13}C_2$-PFHxA)	3.03	5	314.9	270.0
3.	Perfluorohexanoic acid (PFHxA)	3.04	5	312.9	268.7
4.	Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid ($^{13}C_3$-HFPO-DA)	3.36	5	286.8	168.7
5.	Hexafluoropropylene oxide dimer acid (HFPO-DA)	3.38	5	285.0	168.9
6.	Perfluoroheptanoic acid (PFHpA)	4.09	5	362.8	318.8
7.	Perfluorohexanesulfonic acid (PFHxS)	4.22	10	398.8	79.9
8.	4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	4.24	5	376.9	250.7
9.	Perfluoro-[1,2-$^{13}C_2$]octanoic acid ($^{13}C_2$-PFOA)	4.88	5	415.0	370.0
10.	Perfluorooctanoic acid (PFOA)	4.90	5	413.1	368.9
11.	Perfluorononanoic acid (PFNA)	5.54	5	462.9	418.9
12.	Perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonic acid ($^{13}C_4$-PFOS)	5.57	10	503.0	80.0
13.	Perfluorooctanesulfonic acid (PFOS)	5.58	10	498.9	80.0
14.	9-Chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS)	5.88	5	530.8	350.7
15.	Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid ($^{13}C_2$-PFDA)	6.08	5	514.9	469.9
16.	Perfluorodecanoic acid (PFDA)	6.08	5	512.9	469.0
17.	N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid (d3-NMeFOSAA)	6.28	10	572.9	418.8
18.	N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA)	6.30	10	569.8	418.8
19.	N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid (d5-NEtFOSAA)	6.51	10	588.9	418.8
20.	N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)	6.52	10	583.8	418.8
21.	Perfluoroundecanoic acid (PFUnA)	6.55	5	562.9	518.8
22.	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	6.77	5	630.7	451.0
23.	Perfluorododecanoic acid (PFDoA)	6.95	5	612.7	568.9
24.	Perfluorotridecanoic acid (PFTrDA)	7.30	5	662.7	618.8
25.	Perfluorotetradecanoic acid (PFTA)	7.60	5	712.7	668.7

Figure Appendix 1. A 50 x 2.1 mm Raptor C18 column is a great PFAS LC column choice; it meets all EPA 537.1 method criteria in a fast 10-minute total cycle time. https://www.restek.com/en/technical-literature-library/articles/PFAS-LC-column-anatomy-which-phase-dimensions-and-particle-type-are-best/

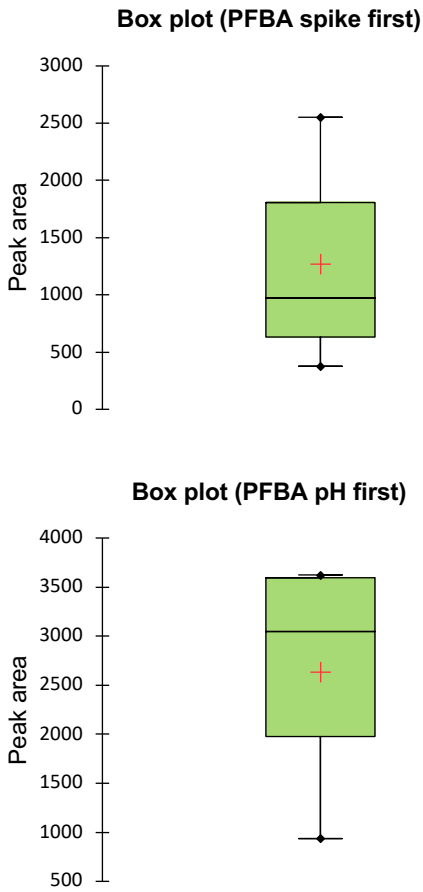


Figure Appendix 2. Box plots comparing peak area for a) spiking waste leachate samples (n = 5) before and b) spiking waste leachate samples after setting pH ≤ 3 (n = 5).

6.2 Tables

Table Appendix 1. Comparison of the recovery percentage (%Rec) by SPE or filtration of spiked milliQ water at 100 and 500 ng/L (n = 2)

	%Rec				
	PFBA	PFPeA	L-PFBS	PFHxA	PFHpA
SPE 100 ng/L	46.8	42.5	47.0	42.0	40.9
SPE 500 ng/L	51.3	47.6	50.5	47.0	45.3
filtration 100 ng/L	70.6	93.9	101	98.8	102
	%Rec				
	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA
SPE 100 ng/L	43.4	38.1	31.1	29.1	22.7
SPE 500 ng/L	48.7	45.3	43.2	45.2	42.4
filtration 100 ng/L	105	98.9	112	138	157

Table Appendix 2. Results of calculated calibration curves per day and per analyte with complete validation dataset.

Calibration per day and per analyte									
		Partial F-Test (order)				**Retained model**			
Analyte	**Day**	**F_{obs}**	**F_{crit}**	**p-value**	**Conclusion**	**b0**	**b1**	**b2**	**R^2**
PFBA	Day 1	0.1	4.8	0.710	Linear	-106.0	178.0		0.9987
PFPeA	Day 1	10.8	4.8	0.007	Quadratic	279.4	177.5	-0.01	0.9999
PFHxA	Day 1	0.0	4.8	0.890	Linear	73.0	92.1		0.9949
PFHpA	Day 1	1.3	4.8	0.280	Linear	711.5	82.0		0.9881
PFOA	Day 1	0.3	4.8	0.608	Linear	272.3	82.9		0.9984
PFNA	Day 1	16.7	4.8	0.002	Quadratic	-142.4	81.1	0.01	0.9997
PFDA	Day 1	30.4	4.8	0.000	Quadratic	80.8	45.4	0.03	0.9963
L-PFBS	Day 1	0.9	4.8	0.357	Linear	58.0	16.6		0.9985
L-PFHxS	Day 1	10.6	4.8	0.008	Quadratic	-27.1	12.2	0.00	0.9997
PFOS	Day 1	26.1	4.8	0.000	Quadratic	-8.1	3.6	0.00	0.9981
PFBA	Day 2	2.8	4.8	0.121	Linear	-949.4	219.1		0.9974
PFPeA	Day 2	21.0	4.8	0.001	Quadratic	371.5	269.7	-0.02	0.9998
PFHxA	Day 2	87.0	4.8	0.000	Quadratic	108.8	216.3	-0.02	0.9999
PFHpA	Day 2	5.7	4.8	0.036	Quadratic	324.6	192.9	-0.02	0.9989
PFOA	Day 2	166.2	4.8	0.000	Quadratic	-1 265.5	182.9	-0.08	0.9979
PFNA	Day 2	29.9	4.8	0.000	Quadratic	-2 560.4	165.9	-0.12	0.9156
PFDA	Day 2	5.0	4.8	0.047	Quadratic	-2 216.6	111.3	-0.07	0.7176
L-PFBS	Day 2	121.0	4.8	0.000	Quadratic	51.9	49.5	0.00	1.0000
L-PFHxS	Day 2	42.0	4.8	0.000	Quadratic	-169.9	35.6	-0.01	0.9964
PFOS	Day 2	13.4	4.8	0.004	Quadratic	-316.1	16.0	-0.01	0.7906
PFBA	Day 3	11.5	4.8	0.006	Quadratic	-1 239.7	219.4	-0.02	0.9995

PFPeA	Day 3	4.6	4.8	0.055	Linear	1 688.6	191.0		0.9966
PFHxA	Day 3	0.8	4.8	0.382	Linear	976.1	105.8		0.9874
PFHpA	Day 3	1.1	4.8	0.316	Linear	836.9	105.3		0.9924
PFOA	Day 3	2.1	4.8	0.176	Linear	685.6	96.8		0.9964
PFNA	Day 3	0.0	4.8	0.935	Linear	35.3	99.3		0.9991
PFDA	Day 3	11.4	4.8	0.006	Quadratic	345.9	66.9	0.02	0.9959
L-PFBS	Day 3	3.7	4.8	0.080	Linear	137.4	17.9		0.9964
L-PFHxS	Day 3	7.8	4.8	0.018	Quadratic	-13.4	14.8	0.00	0.9987
PFOS	Day 3	8.3	4.8	0.015	Quadratic	11.6	5.2	0.00	0.9980
PFBA	Day 4	2.0	4.8	0.182	Linear	373.1	201.5		0.9996
PFPeA	Day 4	1.9	4.8	0.194	Linear	1 164.7	179.9		0.9980
PFHxA	Day 4	0.0	4.8	0.887	Linear	34.7	89.1		0.9906
PFHpA	Day 4	2.0	4.8	0.184	Linear	558.1	74.2		0.9930
PFOA	Day 4	0.6	4.8	0.449	Linear	-56.2	74.5		0.9961
PFNA	Day 4	41.3	4.8	0.000	Quadratic	465.7	54.2	0.03	0.9981
PFDA	Day 4	210.9	4.8	0.000	Quadratic	1 261.7	8.9	0.07	0.9972
L-PFBS	Day 4	0.3	4.8	0.588	Linear	46.6	16.3		0.9971
L-PFHxS	Day 4	0.1	4.8	0.768	Linear	15.2	11.1		0.9990
PFOS	Day 4	230.0	4.8	0.000	Quadratic	37.7	2.6	0.00	0.9990
PFBA	Day 5	0.3	4.8	0.592	Linear	-47.7	164.1		0.9912
PFPeA	Day 5	0.3	4.8	0.596	Linear	321.3	147.2		0.9988
PFHxA	Day 5	0.1	4.8	0.764	Linear	-138.5	68.6		0.9886
PFHpA	Day 5	0.1	4.8	0.743	Linear	81.9	55.2		0.9867
PFOA	Day 5	0.1	4.8	0.808	Linear	2.3	57.0		0.9937
PFNA	Day 5	15.5	4.8	0.002	Quadratic	283.1	47.6	0.01	0.9973
PFDA	Day 5	63.9	4.8	0.000	Quadratic	611.6	17.6	0.04	0.9945
L-PFBS	Day 5	0.7	4.8	0.414	Linear	-6.1	13.0		0.9991
L-PFHxS	Day 5	2.5	4.8	0.146	Linear	2.9	9.0		0.9998
PFOS	Day 5	94.4	4.8	0.000	Quadratic	30.5	2.3	0.00	0.9980
PFBA	Day 6	0.0	4.8	0.886	Linear	-737.4	191.3		0.9982
PFPeA	Day 6	0.3	4.8	0.596	Linear	338.1	173.8		0.9966
PFHxA	Day 6	0.1	4.8	0.739	Linear	203.7	81.8		0.9898
PFHpA	Day 6	0.1	4.8	0.821	Linear	-162.5	67.4		0.9844
PFOA	Day 6	0.4	4.8	0.540	Linear	-317.2	70.0		0.9914
PFNA	Day 6	10.8	4.8	0.007	Quadratic	90.2	60.3	0.01	0.9978
PFDA	Day 6	14.1	4.8	0.003	Quadratic	149.8	36.1	0.03	0.9906
L-PFBS	Day 6	1.0	4.8	0.345	Linear	46.2	15.0		0.9953
L-PFHxS	Day 6	0.2	4.8	0.699	Linear	-9.8	10.3		0.9971
PFOS	Day 6	28.3	4.8	0.000	Quadratic	5.9	3.3	0.00	0.9972

Table Appendix 3. Results of calculated calibration curves without 1000 ng/L calibration point and without validation day 2.

Calibration per day and per analyte without 1000 ng/L calibration point and without validation day 2									
	Partial F-Test (order)					**Retained model**			
Analyte	**Day**	**F_{obs}**	**F_{crit}**	**p-value**	**Conclusion**	**b0**	**b1**	**b2**	**R^2**
PFBA	Day 1	0.1	5.1	0.817	Linear	-290.1	179.5		0.9978
PFPeA	Day 1	1.4	5.1	0.265	Linear	428.5	174.5		0.9998
PFHxA	Day 1	0.0	5.1	0.853	Linear	124.5	91.7		0.9928
PFHpA	Day 1	0.1	5.1	0.782	Linear	62.0	87.4		0.9951
PFOA	Day 1	1.2	5.1	0.309	Linear	174.2	83.7		0.9991
PFNA	Day 1	4.6	5.1	0.060	Linear	-265.9	84.1		0.9998
PFDA	Day 1	2.3	5.1	0.167	Linear	-577.8	59.4		0.9925
L-PFBS	Day 1	0.1	5.1	0.768	Linear	17.8	17.0		0.9991
L-PFHxS	Day 1	0.3	5.1	0.588	Linear	-9.3	11.8		0.9993
L-PFOS	Day 1	1.4	5.1	0.271	Linear	-36.5	4.2		0.9958
PFBA	Day 3	7.3	5.1	0.025	Quadratic	-1 531.3	225.9	-0.03	0.9996
PFPeA	Day 3	1.9	5.1	0.202	Linear	403.1	201.7		0.9983
PFHxA	Day 3	0.4	5.1	0.556	Linear	314.3	111.3		0.9887
PFHpA	Day 3	0.2	5.1	0.707	Linear	234.5	110.3		0.9928
PFOA	Day 3	1.9	5.1	0.207	Linear	203.6	100.9		0.9982
PFNA	Day 3	1.5	5.1	0.247	Linear	80.6	98.9		0.9991
PFDA	Day 3	0.0	5.1	0.897	Linear	-128.6	77.5		0.9945
L-PFBS	Day 3	0.5	5.1	0.484	Linear	19.4	18.9		0.9980
L-PFHxS	Day 3	3.0	5.1	0.116	Linear	26.0	14.0		0.9986
L-PFOS	Day 3	0.2	5.1	0.629	Linear	-8.1	5.6		0.9996
PFBA	Day 4	3.4	5.1	0.099	Linear	67.1	204.0		0.9995
PFPeA	Day 4	1.7	5.1	0.229	Linear	504.8	185.4		0.9992
PFHxA	Day 4	0.1	5.1	0.770	Linear	145.1	88.2		0.9878
PFHpA	Day 4	0.7	5.1	0.427	Linear	30.8	78.6		0.9966
PFOA	Day 4	2.9	5.1	0.125	Linear	237.2	72.1		0.9969
PFNA	Day 4	3.1	5.1	0.114	Linear	-35.3	66.0		0.9982
PFDA	Day 4	0.6	5.1	0.449	Linear	-265.0	42.8		0.9935
L-PFBS	Day 4	0.1	5.1	0.731	Linear	15.9	16.6		0.9969
L-PFHxS	Day 4	3.9	5.1	0.080	Linear	12.9	11.1		0.9989
L-PFOS	Day 4	1.7	5.1	0.227	Linear	-27.5	4.0		0.9988
PFBA	Day 5	1.8	5.1	0.208	Linear	621.8	158.6		0.9967
PFPeA	Day 5	11.9	5.1	0.007	Quadratic	-7.2	157.2	-0.02	0.9997
PFHxA	Day 5	0.1	5.1	0.755	Linear	35.3	67.1		0.9888
PFHpA	Day 5	1.6	5.1	0.237	Linear	278.7	53.6		0.9908
PFOA	Day 5	0.7	5.1	0.441	Linear	104.5	56.1		0.9951

PFNA	Day 5	13.3	5.1	0.005	Quadratic	-354.3	61.9	-0.02	0.9991
PFDA	Day 5	0.6	5.1	0.452	Linear	-162.8	35.2		0.9952
L-PFBS	Day 5	4.6	5.1	0.060	Linear	20.7	12.8		0.9993
L-PFHxS	Day 5	0.1	5.1	0.826	Linear	-10.5	9.1		0.9998
L-PFOS	Day 5	3.5	5.1	0.096	Linear	-14.7	3.3		0.9973
PFBA	Day 6	0.3	5.1	0.612	Linear	-876.0	192.5		0.9950
PFPeA	Day 6	0.0	5.1	0.978	Linear	-35.1	176.9		0.9936
PFHxA	Day 6	0.0	5.1	0.845	Linear	30.1	83.3		0.9794
PFHpA	Day 6	0.1	5.1	0.780	Linear	-53.7	66.5		0.9741
PFOA	Day 6	0.2	5.1	0.669	Linear	-71.4	68.0		0.9831
PFNA	Day 6	0.4	5.1	0.524	Linear	-154.5	66.2		0.9934
PFDA	Day 6	2.2	5.1	0.172	Linear	-311.5	47.7		0.9713
L-PFBS	Day 6	0.1	5.1	0.780	Linear	-23.0	15.5		0.9937
L-PFHxS	Day 6	0.6	5.1	0.441	Linear	-31.4	10.4		0.9905
L-PFOS	Day 6	3.4	5.1	0.100	Linear	-22.3	4.0		0.9907

Table Appendix 4. PFAS analysis for 82588 sample. The slope, the intercept and the abscissa at origin were obtained by doing dosed additions from 10 to 500 ng/L. The obtained abscissa at origin has already been multiplied by 1.5 to match factor dilution (1 mL of sample)

Analyte	Slope	Intercept [Peak area]	Abscissa at origin [ng/L]	External results [ng/L]	ratio internal results/external lab [%]
PFBA	21.3	9349	657	700	94%
PFPeA	38.1	11256	444	420	106%
L-PFBS	9.40	4873	778	480	162%
PFHxA	26.4	21533	1222	1900	64%
PFHpA	24.0	1453	91	160	57%
L-PFHxS	4.93	1862	567	2000	28%
PFOA	33.6	1575	70	220	32%
PFNA	38.0	-9.72	0.38 *	5 *	8%
L-PFOS	1.06	513	726	2400	30%
PFDA	18.7	152	12 **	12 **	102%

** Concentration below analyte instrumental LOQ determined in this work*

*** Concentration equal to analyte instrumental LOQ determined in this work*

Table Appendix 5. Example of a sample list used during this work. This sample list corresponds to when filtration and SPE were compared in section 3.1.8.

MeOH
MeOH
MeOH
blanc milliQ
Calibration curve (7 to 8 points)
blanc milliQ
filtration blanc_A
blanc milliQ
filtration blanc_B
blanc milliQ
filtration spike 100ng/L_A_sans rinc
blanc milliQ
filtration spike 100ng/L_B_sans rinc
blanc milliQ
filtration spike 100ng/L_A_rinc
blanc milliQ
filtration spike 100ng/L_B_rinc
blanc milliQ
SPE 100 ng/L_A_rinc
blanc milliQ
SPE 100 ng/L_A_elution
blanc milliQ
SPE 100 ng/L_B_rinc
blanc milliQ
SPE 100 ng/L_B_elution
blanc milliQ
SPE blanc rinc
blanc milliQ
SPE blanc elution
blanc milliQ
Calibration curve (7 to 8 points)
blanc milliQ
MeOH_HPLC
MeOH
MeOH