NATIONWIDE MONITORING OF PER- AND POLYFLUOROALKYL SUBSTANCES IN WATER IN SOUTH AFRICA

Volume I: Development, optimisation and validation of an LC-MS method for detection and quantification of per- and polyfluoroalkyl substances in water

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WATER RESEARCH COMMISSION

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Volume I: Development, optimisation and validation of an LC-MS method for detection and quantification of per- and polyfluoroalkyl substances in water

Report to the
Water Research Commission

by

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This report forms part of a set of three reports, viz:
- *Nationwide Monitoring of Per- and Polyfluoroalkyl Substances in Water in South Africa*. Volume I: Development, optimisation and validation of an LC-MS method for detection and quantification of per- and polyfluoroalkyl substances in water (this report)
- *Nationwide Monitoring of Per- and Polyfluoroalkyl Substances in Water in South Africa*.Volume II: Provincial data on the presence, levels and sources of per- and polyfluoroalkyl substances (PFAS) in water sources (WRC report no. TT 931/2/23)
- *Nationwide Monitoring of Per- and Polyfluoroalkyl Substances in Water in South Africa*.Volume III: Summary report on distribution, sources and health effects of per- and polyfluoroalkyl substances in water (WRC Report No. TT 931/3/23)

EXECUTIVE SUMMARY

BACKGROUND

Increased knowledge of the potential risks associated to exposure to per- and polyfluoroalkyl substances (PFASs) in the environment has led to higher demand of its monitoring in waters resources. PFASs are synthetic chemicals used in textiles, packaging, papers, carpets and building and construction materials. Other usage includes, but not limited to, cosmetic formulation, insecticides, paints, non-stick cookware, firefighting foams, hydraulic fluids, waxes and others. Their widespread usage is because of their unique thermal stability and excellent surfactant capacity. During usage or disposal of products treated with PFASs, these chemicals can be released from products into the environment. Other routes of releases into the environment include, among others, during production, military and firefighting operations, discharge of treated effluent and sludge, as well as leachate from landfills. The presence of polyfluoroalkyl substances (PFASs) in water resources is of concern because water treatment processes do not effectively remove these chemicals. Additionally, these chemicals are bio-accumulative, persistent, have long range transport characteristics as well as toxic. Their presence in the environment, particularly water, therefore, needs to be monitored.

PROJECT AIMS

The overall aims of the project were to:

1. Monitor the concentrations of legacy and emerging PFASs in different water sources in pre-selected cities and towns from all the nine provinces in South Africa;
2. Use appropriate model to identify the PFASs sources and assess the amounts of pollution by resolving the measured mixture of chemical species into the contributions from the individual source types;
3. Develop a nationwide database on PFASs concentrations in different water sources from different parts of the country, and
4. Apply a test battery of bioassays covering a range of endpoints commonly responsive to drinking water to monitor water quality of source and drinking water.

To achieve the aforementioned aims, it was necessary to first develop, optimize, and validate an analytical method to determine the presence and concentrations of PFASs in various water sources in South Africa. Thus, the current report (**Volume I**) only addresses aspects related to the development, optimisation and validation of methods for the detection and quantification of PFASs in water samples.

The results obtained under Aims 1-4 are presented in **Volumes II and III** of this set of reports.

DETECTION AND QUANTIFICATION OF PFASs IN WATER

Targeted and non-targeted approaches were employed in this nationwide PFASs monitoring initiative. The targeted analysis approach provides an unparalleled level of specificity and sensitivity for the quantitative analysis. However, for new and emerging compounds, this approach may not be effective in detecting species that may be of interest, regardless of their chemistry or concentration. Non-targeted analysis leverages the power of high-resolution modern mass spectrometers to analyse both targeted and undiscovered chemicals.

For non-targeted analysis of PFASs, water samples collected from the Gauteng province of South Africa in clean high-density polyethylene bottles were used. Gauteng province water samples were used as a benchmark for identifying and detecting PFASs in water samples as this province is the most industrialized compared to the others in the country. Native PFASs standards and isotopically labelled internal (MPFDA_$^{13}C_2$, MPFHxA_$^{13}C_2$ and M2PFOA_$^{13}C_2$) and surrogate (MPFNA) standards (50 mg/L in methanol), purchased from Wellington Laboratories (Guelph, Ontario, Canada) were used for instrument calibration, limit of detection (LOD), limit of Quantification (LOQ), recovery tests and multiple reaction monitoring (MRM). Water extraction method was developed using Milli-Q water. Solid Phase Extraction (SPE) USEPA Method 537.1 was employed in the extraction of PFASs from water samples. After extraction, the extracts were reconstituted to 1 mL and internal standard M2PFOA_$^{13}C_2$, MPFDA_$^{13}C_2$, and MPFHxA_$^{13}C_2$, prior instrument analysis.

Analysis was conducted using Chromatograph TripleTOF 6600, SCIEX with Luna Omega 3 µm polar C18 100Å LC column 100 x 2.1 mm, Phenomenex, flow rate of 0.50 mL/min; and 2 mM ammonium acetate, 0.1% formic acid and 100% methanol for mobile phases A and B respectively.

For the targeted analysis of PFASs in water, the methods described above for non-targeted analyses were also used for targeted analysis, albeit with differences in instrumental analysis. The extracts after reconstitution and addition of internal standards were analysed using Liquid Chromatography tandem mass spectrometry (Shimadzu LC-MS 8030 triple quadrupole system, Tokyo, Japan). Following the identification of emerging PFASs compounds using non-targeted analysis, more PFASs standards including the sulphonates and alcohol telomers were added to the pool. Four chromatographic methods were developed to ensure good separation of PFASs compounds. These methods are:

- **Method A** – This method consisted of the following analytes: MPFNA, PFUdA, PFHxA, PFPeA, 4:2 FTS, 6:2 FTS, 8:2 FTS, PFHpA, PFNA, PFDoA, PFODA and PFHxDA;
- **Method B** – This method consisted of the following analytes; MPFUdA, MPFHxS, L-PFBS, L-PFHxS, L-PFOS, PFHpS, PFOA, and L-PFDS;
- **Method C** – This method consisted of the following analytes; FHEA, 6:2 FTS, FOET and FHET, and
- **Method D** – This method only catered for PFBA.

For all 4 methods, multiple reaction monitoring (MRM) chromatograms and calibration curves with 10 points of final concentrations ranging from 1-2000 ng/L for all PFASs analytes were created from diluting a stock solution of 50 mg/L of individual PFASs in methanol.

Validation of the methods developed was determined using the spiking method. Further validation was also conducted using Certified Reference Material, i.e. PFASs in drinking water reference samples (CRM IRMM-428). Field blanks were prepared during sample collection following the same procedure used for environmental samples. During the analyses of samples, solvent and laboratory blanks were prepared and analysed in between samples after every tenth sample to avoid carry over and a 100 ng/L standard was analysed after every 20th sample in the batch.

SUMMARY AND CONCLUSIONS

All isomers calibration curves showed linearity, based on correlation coefficients (r) and correlation of determination (r^2) that were greater than 0.99 with good precision of the internal standard. The chromatograms were well separated. The percentage recoveries of the labelled surrogate standards were within the acceptable range of 50-150 ng/L. Calibration curves for PFUdA, PFDoA, PFHxA, PFNA, PFPeA, PFBA, MPFNA, 4:2 FTS, 6:2 FTS, 8:2 FTS, FHET, FHEA and FOET ranged from 1-1000 ng/L, while that of L-PFBS, L-PFOS, PFOA, L-PFHpS, and L-PFHxS ranged from 100-1000 ng/L and that of MPFUdA, MPFHxS, L-PFDS, PFODA and PFHxDA ranged from 100-2000 ng/L were maintained. Furthermore, the LOD and LOQ values that ranged from 0.0033-0.29 and 0.018-0.67 ng/L respectively.

Based on these results, the following can be concluded:

- Non-targeted and targeted analytical methods for identification and quantification of PFASs in various water sources were successfully developed;
- The developed method was optimized and validated using spiking method and certified reference material;
- The developed method was applied to extract PFASs from the following water sample types; wastewater, drinking water, groundwater (boreholes), surface water (rivers and dams), bottled water and rainwater.
- High percentage recoveries obtained indicated high accuracy and sensitivity of the developed method
- Both grab and passive sampling approaches can be used for PFASs monitoring in water environments
- Both analytical measurements and multivariate analyses are necessary to establish an understanding of the sources, levels, transport and fate of PFAS compounds within water environments.

ACKNOWLEDGEMENTS

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ACRONYMS & ABBREVIATIONS

AFFF	Aqueous Film Forming Foam
ASTM	Association of Standardized Test Method
ATSDR	Agency for Toxic Substances and Disease Registry
BFRs	Brominated Flame Retardants
CCD	Charge-Coupled Devices
DCM	Dichloromethane
DEA	Department of Environmental Affairs
DWS	Department of Water & Sanitation
EDC	Endocrine disrupting compound
EFSA	European Food Safety Authority
EtOH	Ethanol
FIA	Flow Injection Analysis
FTCA	Fluorotelomer Carboxylic Acids
FTOH	Fluorotelomer Alcohol
GC-MS	Gas Chromatography-Mass Spectrometer
HDPB	High Density Polyethylene Bottles
HPLC	High Pressure Liquid Chromatography
L-FABP	Liver Fatty Acid-Binding Protein
LC-MS	Liquid Chromatography-mass spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MeOH	Methanol
MRLs	Minimum Risk Levels
MRM	Multiple Reaction Monitoring
MTBE	Methyl Tert-Butyl Ether
NTMP	National Toxicant Monitoring Programme
PCA	Principal Component Analysis
PCBs	Polychlorinated Biphenyls
PCF	Propyl Chloroformate
PES	Polyethersulfone
PFAAs	Per- fluoroalkyl acids
PFASs	Per- polyfluorinate Alkyl substances
PFCAs	Per- fluoroalkyl Carboxylic Acids
PFHxS	Per- fluohexane Sulfonic Acid
PFNA	Per- fluorononanoic Acid
PFOSE	Per- fluorooctane Sulfonamidoethanol
POPs	Persistent Organic Pollutants
POSF	Per- fluorooctanesulfonyl Fluoride
PP	Polypropylene

PPARs	Activate Peroxisome Proliferator-Activated Receptors
R^2	Correlation Coefficients
ROS	Reactive Oxygen Species
RQS	Resource quality services
RSD	Relative Standard Deviation
SPE	Solid Phase Extraction
St	Saint
STP	Sewage Treatment Plants
TCM	Trichloromethane
TDIs	Tolerable Daily Intakes
TFE	Tetrafluoroethylene
TIC	Total Ion Chromatogram
ToF-HRMS	Time of flight- High Resolution Mass Spectrometry
TUT	Tshwane University of Technology
USEPA	United States Environmental Protection Agency
WAX	Weak Anion Exchange
WTPs	Water Treatment Plants
WVDEP	West Virginia Department of Environmental Protection
WWTPs	Wastewater Treatment Plants

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CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are synthetic chemicals used in textiles, packaging, papers, carpets and building and construction materials. They are used in cosmetic formulation, insecticides, paints, firefighting foams, hydraulic fluids and waxes. Their widespread usage is because of their unique thermal stability and excellent surfactant capacity. During usage or disposal of products treated with PFASs, these chemicals can leach into the environment. They can also be released during production, military and firefighting operations, discharge of treated effluent and sludge, as well as landfill leachates.

The presence of PFASs in source waters is, in most cases, not removed by conventional water treatment processes due to the design and treatment processes. Water users and consumers can, therefore, be exposed unintentionally to PFASs with their concomitant toxic effects in such instances. It is for these reasons that monitoring of PFASs in South African source waters are particularly important. Conducting a large-scale monitoring programme that would provide a nationwide inventory of the concentrations of PFASs in South Africa source waters is a step in the right direction to safeguard public health. In addition, this exercise would contribute towards critically reviewing the current drinking water guidelines in order to address the challenges that may be posed by the presence of PFASs in South African source waters. Data generated on PFASs will contribute towards the National Toxicant Monitoring Programme (NTMP).

The design of monitoring the occurrence of chemicals of emerging concern such as PFASs compounds is particularly complex. Consequently, efforts were geared towards devising cost-effective means of monitoring of such pollutants. Development of a testable analytical method ensures that the results generated from any analysis is reliable and accurate. Therefore, it was deemed necessary to 1) develop appropriate and testable analytical method to determine the presence and concentrations of PFSAs in various water sources and 2) optimize and validate the method before sample collection, treatment and analysis. Generally, analytical method development involves the use of analyte standards in order to determine analytical instrument response to the analyte standards. In this report, the analytical method was developed using PFASs standards for both calibration and multiple reaction monitoring.

Pre-treatment and extraction methods were also optimized because they have a great impact on the determination of PFASs in different matrices. Depending on the type of sample, centrifugation and filtration methods, sample pre-treatment was applied in some of the samples, particularly samples from wastewater treatment plants in order to minimize blockage in the subsequent extraction process and in the instrument.

Two approaches were employed in this nationwide PFASs monitoring programme namely, **targeted** and **non-targeted**. Targeted provides an unparalleled level of specificity and sensitivity for the quantitative analysis. However, for new and emerging compounds, this approach is not effective in detecting species that may be of interest, regardless of their chemistry or concentration. Non-targeted analysis leverages the power of high-resolution modern mass spectrometers to analyse both targeted and undiscovered chemicals.

1.2 PROJECT AIMS

The objectives of the overall project were to:

1. Monitor the concentrations of legacy and emerging PFASs in different water sources in pre-selected cities and towns from all the nine provinces in South Africa;
2. Use appropriate model to identify the PFASs sources and assess the amounts of pollution by resolving the measured mixture of chemical species into the contributions from the individual source types;

3. Develop a nationwide database on PFASs concentrations in different water sources from different parts of the country and
4. Apply a test battery of bioassays covering a range of endpoints commonly responsive to drinking water to monitor water quality of source and drinking water.

1.3 SCOPE OF THIS REPORT

To achieve the aforementioned aims, it was necessary to first develop, optimize, and validate an analytical method to determine the presence and concentrations of PFASs in various water sources in South Africa. Thus, the current report (**Volume I**) only addresses aspects related to the development, optimisation and validation of methods for water sample extraction and analysis for the detection and quantification of PFASs.

The results obtained under Aims 1-4 are presented in V**olumes II and III** of this set of reports.

CHAPTER 2: A REVIEW OF PER- AND POLYFLUOROALKYL SUBSTANCES

2.1 CLASSIFICATION OF PER- AND POLYFLUOROALKYL SUBSTANCES

As the start of the third industrial revolution ushered in the use of technology and many other forms of digital advancements in the 1950s, so did the manufacturing trade discover its own breakthrough in the use of new chemicals for various lucrative commercial applications (Kissa, 2001). Per- and/or polyfluoroalkyl substances became the ground-breaking new chemicals that were widely popular for the various uses with which they could be applied (Smart, 1994).

The term PFASs refers to perfluoroalkyl and polyfluoroalkyl substances, a large group of manmade chemicals with the distinguishing structure of a chain of carbon atoms (forming an 'alkyl') that has at least one fluorine atom bound to a carbon. Perfluoroalkyl substances are fully fluorinated molecules in which every hydrogen atom bonded to a carbon in the alkane backbone (carbon chain) is replaced by a fluorine atom, except for the carbon at one end of the chain that has a charged functional group attached. The carbon-fluorine bond is extremely strong and renders these chemicals highly resistant to complete degradation. The basic chemical structure of perfluoroalkyl substances can be written as CnF2n+1−R, where ' CnF2n+1' defines the length of the perfluoroalkyl chain tail with $n > 2$, and ' R' represents the attached functional group head. The general chemical structures of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs) bear the functional groups -COOH and $-SO_3H$ respectively. The chemical structures of commonly detected perfluoroalkyl substances, including perfluorooctane sulfonic acid (PFOS), perfluorooctane carboxylic acid (PFOA), perfluohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) are all presented in Figure 2-1 to 2-6.

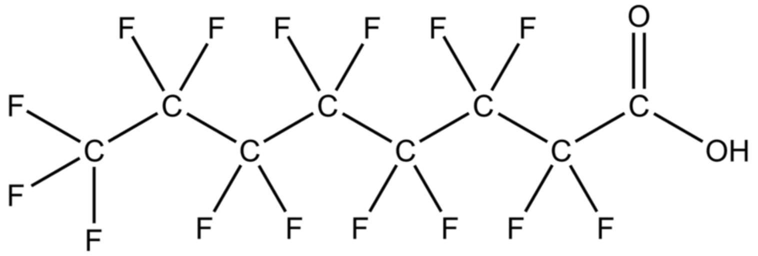

Figure 2.1: Chemical structure of perfluorooctanoic acid (PFOA)

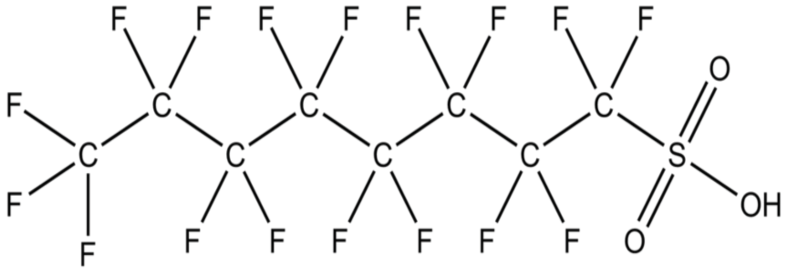

Figure 2.2: Chemical structure of perfluorooctanoic acid (PFOS)

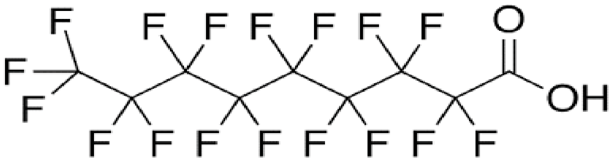

Figure 2.3: Chemical structure of perfluorooctanoic acid (PFNA)

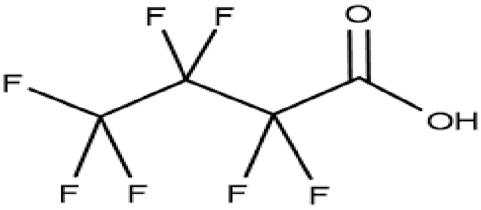

Figure 2.4: Chemical structure of perfluorooctanoic acid (PFBA)

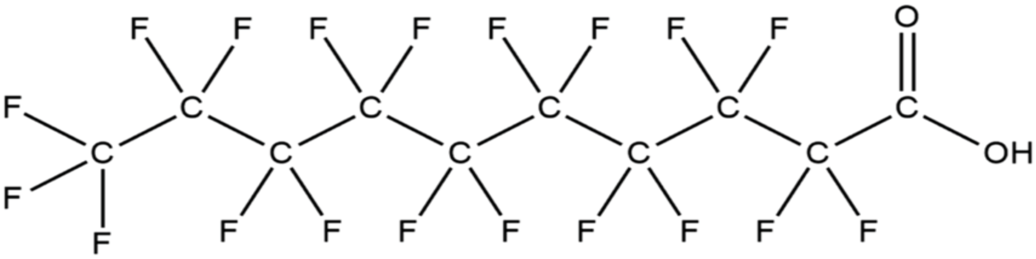

Figure 2.5: Chemical structure of perfluorooctanoic acid (PFDA)

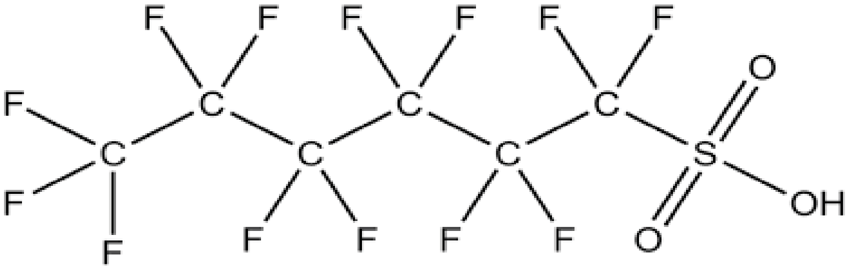

Figure 2.6: Chemical structure of perfluorooctanoic acid (PFHxS)

PFOA and PFOS make up the so-called C8 compounds and they have been the most extensively produced and studied PFAS homologues. Perfluoroalkyl acids (PFAAs) are some of the most basic PFASs molecules and are essentially non-degradable. PFAAs contain three major groups on the basis of the functional group at the end of the carbon chain: perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and perfluoroalkyl phosphonates (PFPAs) or perfluoroalkyl phosphinates (PFPiAs).

Polyfluoroalkyl substances differ from perfluoroalkyl substances by the degree of fluorine substitution in the alkane backbone: at least one carbon must not be bound to a fluorine atom and at least two carbons must be fully fluorinated. The fluorotelomer substances are a subset of polyfluoroalkyl substances because they are oligomers with low molecular weight produced by a telomerisation reaction. Some important examples of fluorotelomer substances are fluorotelomer alcohol (FTOH) and perfluorooctane sulfonamidoethanol (FOSE).

Since polyfluoroalkyl substances have a carbon that is lacking fluorine substitution, this weaker bond increases potential for degradation (Buck et al., 2011). For example, FTOH and FOSE can be transformed biologically or abiotically to PFOA and PFOS. In addition, PFASs can also exist as polymers. These PFASs polymers are large molecules formed by joining many identical small PFAS monomers. Current information indicates that the non-polymer PFASs constitute the greatest risk for environmental contamination and toxicity, although some PFASs polymers can be degradable to basic PFASs.

2.2 SYNTHESIS OF PER- AND POLYFLUOROALKYL SUBSTANCES

According to Lehmler (2005), PFOA, PFOS, and other PFASs are man-made chemicals that are primarily produced by two methods namely Simons Electrochemical Fluorination and Telomerization of tetratfluroethylene as shown in Equations 2.1 and 2.2.

$$C_n\,H_{2n+1}\,COCl + (2_n+2)\,HF \longrightarrow C_n\,F_{2n+1}\,COF + HCl + \text{by products} \qquad \text{(eq. 2.1)}$$

$$C_n\,H_{2n+1}\,SO_2Cl + (2_n+2)\,HF \longrightarrow C_n\,F_{2n+1}\,COF + HCl + \text{by products} \qquad \text{(eq. 2.2)}$$

The Simons Electrochemical Fluorination process is based on the fluorination of different fluorinated organic compounds such as alkanesulfonyl acid chloride (equation 2.1), carboxylic acid chloride (equation 2.2) in the presence of anhydrous hydrogen fluoride (Loganathan and Lam, 2012). An electrical current fuels these reactions causing all the hydrogen atoms on the carbon backbone to be replaced by fluorine atoms (Lau *et al*., 2007). The resulting products yielded are perfluorinated sulfonyl and carbonyl fluorides and compounds such as perfluorooctane sulfonyl fluoride (POSF, $C_8F_{17}SO_2F$). Fluorinated molecules of various carbon chain lengths and a mixture of linear, branched, and cyclic isomers are produced during this process, as fragmentation and rearrangement of the carbon skeleton can occur. The telomerization of tetrafluoroethylene (TFE) method involves units that yields straight-chain alcohols ($F(CF_2CF_2)_nCH_2CH_2OH$) that can be converted into final products for commercial application. The process involves the fluoroiodination of TFE in the presence of telogens to produce pentafluoroiodoethane (Equation 2.3), which is then reacted with TFEs and yields a mixture of perfluoroalkyl iodides (Equation 2.4). PFCAs are then produced through the oxidation of pentafluoroiodoethane and perfluoroalkyl iodides (Equation 2.5) are produced by the reaction of pefluoroalkyl iodides with ethylene (Loganathan and Lam, 2012).

$$5CF_2 - CF_2 + 2I_2 + IF_5 \longrightarrow 5F\,(CF_2CF_2)\,I \qquad \text{(eq. 2.3)}$$

$$F\,(CF_2CF_2)\,I + nCF_2 - CF_2 \longrightarrow F(CF_2CF_2)_{n+1}\,I \qquad \text{(eq. 2.4)}$$

$$F\,(CF_2CF_2)_n\,I + CH_2 \longrightarrow F(CF_2CF_2)_{n+1}\,CH_2CH_2I \qquad \text{(eq. 2.5)}$$

The iodides can then be converted to other intermediates (e.g. alcohols) to produce PFASs. Thus, PFASs found in the environment are composed of a family of target compounds as well as by-products of various chain lengths and isomers (Lau *et al*., 2007). The large-scale production, consumption and adverse effects in human health made PFOS and PFOA the most important representatives of the group of PFASs, which resulted to their being phased out in 2000 by 3M Company (Lindstrom *et al*., 2011). In addition, the production of PFOS and other perfluorooctyl products was phased out in the USA and Europe in 2000-2002 (OECD, 2002), but production has continued in other countries (Wang *et al*., 2009), because of the great demand on high-performance materials (Zushi *et al*., 2012). Some PFASs, C ≥ 8, are listed in the national and international regulations because they are bioaccumulative and toxic (OECD, 2002).

2.3 PROPERTIES OF PER- AND POLYFLUOROALKYL SUBSTANCES

2.3.1 Chemical and physical properties

The strength of the carbon/fluorine bond makes the molecules chemically very stable and highly resistant to biological degradation. Per- and polyfluoroalkyl substances (PFASs) are highly persistent to natural degradation due to the high electronegativity of fluorine. They are also resistant to heat and hydrolysis (Taniyasu *et al*., 2013). Per- and polyfluoroalkyl substances (PFASs) are all anthropogenic organic chemicals (Kissa, 2001; Lindstrom *et al*., 2011). Some of their properties include water, oil and grease repellency. Due to the carboxylic or sulfonic acid groups, PFASs have high water solubility and can be transported across long distances via water (Yamashita *et al*., 2005; Ahrens, 2011). Per- and polyfluoroalkyl substances (PFASs) bioaccumulate and biomagnify (Martin *et al*., 2003) and some studies have confirmed toxic and bioaccumulative effects of two representative PFASs namely, PFOS and PFOA (Lau *et al*., 2007; EPA, 2009). PFASs are highly persistent due to their resistance to photolysis, pyrolysis and biotransformation (Kissa, 2001). Due to their persistence and abiotic degradation properties, they are used widely in industrial and commercial applications (OECD, 2002, OECD, 2005; Washburn *et a*l, 2005; Fromme *et al*, 2009).

2.3.2 Transport of PFASs

PFASs can travel long distances in air and water current due to their chemical structure. The aquatic ecosystem has been found to be an important and major medium for PFASs transportation, since these chemicals have been found often detected in environmental waters and strongly proved to accumulate in aquatic biotas (Prevedouros *et al*., 2006). Rivers are an important pathway for transport of contaminants from land to oceans, and PFASs levels in rivers are up to thousands of ngL^{-1} (Skutlarek *et al*., 2006).

2.4 APPLICATIONS OF PER- AND POLYFLUOROALKYL SUBSTANCES

PFASs are man-made chemicals which have been commercially produced since the 1960s (Ahrens *et al*., 2015). A number of perfluorinated compounds have been used for household and industrial applications. These applications can be separated into three categories as follows:

2.4.1 Surface Treatment Applications

Surface treatment applications provide soil, oil and water resistance to personal apparel and home furnishings (OECD, 2002). The applications are used for the protection of apparel and leather, fabric/upholstery and carpet. Industries that use these applications include textile mills, leather tanneries, finishers, fibre producers and carpet manufactures.

2.4.2 Paper Protection Applications

Paper protection applications provide grease, oil and water resistance to paper and paperboard as part of sizing agent information (OECD, 2002). The applications are used in food contact applications that include plates, food containers, bags and wraps and non-food contact applications such as folding cartons, containers, carbonless forms and masking papers. Industries that use these applications include paper mills, manufacturers of bags, wraps and other products from paper and paperboard.

2.4.3 Performance Chemical Applications

Performance chemical applications are specialized industrial, commercial and consumer applications. These applications are used in firefighting foams, mining and oil well surfactants, acid mist suppressants for metal plating, electronic etching baths, photolithography, electronic chemicals, hydraulic fluid additives, alkaline cleaners, floor polishes, photographic films, denture cleaners, shampoos, chemical intermediates, coating additives, carpet cleaners and as an insecticide in bait stations (3M Company, 2000).

2.5 DETECTION AND QUANTIFICATION OF PER- AND POLYFLUOROALKYL SUBSTANCES

2.5.1 Overview

Careful attention and precautionary measures are taken when it comes to the determination of PFASs, from sample collection; sample preparation until sample analysis. Since PFASs can potentially adsorb to the surface of the glassware and can be found in commonly used laboratory supplies and equipment, such as PTFE products, liquid chromatograph solvent lines, methanol, aluminium foil, and solid phase extraction (SPE) sample transfer lines. Therefore, PFAS standards; extracts; samples should not come into contact with any glass containers or pipettes and polypropylene containers should be used instead. The materials need to be routinely demonstrated to be free of interferences through laboratory reagent blanks (USEPA 2009). During samples collection, water samples are collected in clean polypropylene (PP) bottles rinsed with methanol and sediments in polypropylene plastic bags (Wang *et al*., 2011).

2.5.2 Water sample preparation for PFASs

A wide variety of SPE methods have been reported for the sample extraction and clean-up of water samples and columns such as C_{18}, Oasis HLB and weak anion exchange (WAX) have been used (van Leeuwen and Boer, 2007). Taniyashu *et al*. (2005) evaluated the Oasis HLB and Oasis Wax columns for the extraction of PFASs and the recoveries found were between 70-100% for most of the compounds, short-chain PFCAs were efficiently trapped by the Oasis Wax column, and recoveries for long-chain PFCAs ($\geq C_{11}$) were less than 70% on both columns. From Taniyashu *et al*. (2005) findings, both columns were found to be comparable. Yamashita *et al*. (2004) also developed a very sensitive method for seven PFASs using Oasis HLB column for seawater samples, but the method was found to be less accurate compared to the method developed by Taniyashu *et al*. (2005) (van Leeuwen and Boer, 2007).

Gonzalez-Barreiro *et al*. (2006) optimized two extraction methods, an SPE and liquid-liquid extraction (LLE) method for the analysis of eleven PFASs by LC-ESI-MS-MS. For SPE, several cartridges were tested and because all of them resulted in similar recoveries the C_{18} cartridge was selected due to its low costs. In addition, acidic conditions (pH 4) for PFCAs with carbon chains more than 10 and basic conditions (pH 11) for PFOA, PFNA, PFDA, PFDS and PFOSA were used (Gonzalez-Barreiro *et al*., 2006). However, long-chain PFASs could not be extracted with the SPE methods. A liquid-liquid extraction (LLE) method was then optimized, with three solvents tested, namely, n-hexane, MTBE and trichloromethane (TCM), methyl tert-butyl ether (MTBE) was the best extraction solvent selected (Gonzalez-Barreiro *et al*., 2006). Recoveries for PFCAs with carbon chains ≥8 and PFSAs and fluoroalkyl sulphonamides were 80-93% (Gonzalez-Barreiro *et al*., 2006). Short-chain PFCAs, however, could not be extracted by this method making it less efficient compared to SPE method (van Leeuwen and Boer, 2007).

Due to the efficiency of solid phase extraction (SPE), it is the widely used extraction method to extract PFASs and was used in the current project for the extraction of water samples. The extraction procedure followed in this project was as described by Wang *et al*. (2011). Briefly an aliquot of water sample is passed through thoroughly rinsed sample transfer lines and preconditioned SPE cartridge. The cartridge is preconditioned with a selection of solvents such as acetonitrile, methanol followed by ultra-pure water. The samples are allowed to pass through at a rate of one drop a second; the analytes of interest are then trapped on the SPE cartridge while the water goes through to waste. After all the sample has passed through, the cartridge is vacuum dried for one hour and the analytes are eluted. During the elution stage, an amount of a selected solvent is poured onto the cartridge to elute the target compounds and collected in a particular size PP tube usually 10 mL or 15 mL. Then the eluate is reduced to 1 mL under a gentle stream of nitrogen gas and transferred into a vial for analysis.

2.5.3 Analysis of PFASs with LC-MS

Liquid Chromatography-Mass Spectrometer (LC-MS) replaced Gas Chromatography-Mass Spectrometer (GC-MS) for the determination of PFASs because of its higher sensitivity (Dufková *et al*., 2012) and omission of multiple derivatization steps (Shafique *et al*., 2017). Yamashita *et al*. (2004) developed a method for the analysis of PFASs at parts per quadrillion levels in seawater using liquid chromatography tandem mass spectrometry. Major sources of background contamination of PFOA were found in LC tubing and internal LC parts in study. These parts were then replaced with stainless steel, which significantly decreased the contamination. In addition, LOQs in the low pg L^{-1} range were achieved and the method deemed suitable for samples containing very low PFASs levels.

LC coupled to ion-trap MS-MS, ToF-HRMS, and quadrupole MS-MS which are types of mass spectrometers were compared by Berger *et al*. (2004) for the determination of PFASs. Time of flight- High Resolution Mass Spectrometry (ToF-HRMS) was found to the superior methodology with high selectivity and optimal sensitivity in the study. Wille *et al.* (2010) also validated an analytical method for the determination of PFASs in surface, sea and sewage water using LC coupled to ToF-MS. The method was reported to have resulted in a highly selective MS-technique for the detection of PFASs in complex aqueous matrices, which confirmed Berger *et al.* (2004) findings. However, due to the low distribution of the instrument in analytical laboratories, quadrupole MS-MS is used most frequently (Jahnke and Berger, 2009).

2.5.4 Analysis of PFASs using GC

According to Shafique *et al*. (2017) per- and polyfluoroalkyl substances, particularly PFCAs cannot be determined by GC directly. However, to be able to determine PFCAs using a GC, derivatisation is required to convert the polar functional group to a non-polar derivative prior to injection in GC (Shafique *et al*., 2017). Usually esters are used as the derivatives and a number of reagents can be used to react with PFCAs for the derivatisation such as diazomethane (CH_2N_2), propyl chloroformate (PCF) in the presence of pyridine and propanol at pH 2.5 and 2,4-Difluoroanilides. The derived compounds are then injected into the GC column where they are separated according to their boiling points and vapour pressures. Various detectors such as FID; ECD; EI-MS; NCI-MS and NCI-MS^2 are used after separation to determine the PFCA concentrations in the sample.

GC-NCI-MS is one of the mostly used methods to analyse PFASs. Dufková *et al*. (2012) developed a GC-NCI-MS method for the determination of ultra-trace concentrations of perfluoroalkyl carboxylic acids in river water. The method employed sample pre-treatment by SPE followed by a derivatisation procedure, which involved mixing the PFCAs dissolved in acetonitrile with isobutylchloroformate (IBCF) and pyridine forming isobutyl esters. The isobutyl esters were then extracted for the determination of the PFCAs by GC-NCI-MS. The method was applied to river samples and concentrations in the pg mL^{-1} order of units were obtained. The method was reported to exhibit low detection limits, comparable results with those of LC-MS-MS instrumentation and sensitivity increased with increasing length of analyte chain.

However, due to the major drawbacks associated with the use of this method to determine PFASs such as, small range of analytes, long analysis time and laborious derivatization prior to chromatographic separation not much studies use GC for analysis of PFASs (Shafique *et al.*, 2017).

2.6 SUMMARY

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have garnered significant attention due to their widespread presence in the environment and potential health risks. Monitoring PFAS in water is crucial for understanding their sources and extent of contamination, as well as for protecting public health. Below are some of the important considerations for monitoring PFASs in water:

- Sampling: Samples from various water sources, including groundwater, surface water, and drinking water supplies should be collected, and sampling locations should be strategically chosen to assess potential contamination sources.
- Sample Preparation: Before analysis, water samples are typically filtered to remove particulate matter, and in other instances, the pH may need to be adjusted to ensure accurate measurements. Solid-phase extraction (SPE) or liquid-liquid extraction (LLE) techniques are often employed to concentrate PFAS from large water volumes.
- Detection and quantification: Several methods can be used for the detection and quantification of PFASs in water. Liquid Chromatography-Mass Spectrometry (LC-MS) is one of the most commonly used methods for PFAS detection and quantification. It involves separating PFAS compounds based on their chemical properties and then quantifying them using mass spectrometry. Gas Chromatography-Mass Spectrometry (GC-MS) is used for specific PFAS compounds, particularly volatile ones. It separates and identifies these compounds based on their vaporization and ionization properties. High-Performance Liquid Chromatography (HPLC) is typically used in tandem with mass spectrometry or other detectors to analyse specific PFAS compounds by separating them based on their chemical properties. To quantify PFAS concentrations accurately, standards of known PFAS concentrations are used to calibrate the analytical instruments.
- Quality Control: Rigorous quality control procedures are essential to ensure the reliability and accuracy of the results. This includes the use of blank samples, duplicates, and certified reference materials for validation.
- Data Analysis: Once the PFAS content in water samples is measured, data analysis is conducted to determine the concentration of each individual PFAS compound present. The results are often reported in nanograms per litre (ng/L) or parts per trillion (ppt).
- Reporting: The final results are reported to relevant authorities and stakeholders, such as environmental agencies, water treatment plants, and the public. Where available, action levels or regulatory limits are used to assess the potential risks associated with detected PFAS levels.

Detection and quantification of PFAS in water are crucial for understanding the extent of contamination and protecting public health. As our understanding of PFAS and their effects continues to evolve, so too will the methods and technologies for their analysis. Ongoing research is exploring new and innovative technologies for PFAS detection, including biosensors and advanced mass spectrometry techniques, to enhance sensitivity and reduce analysis time.

CHAPTER 3: DEVELOPMENT, OPTIMISATION AND VALIDATION OF AN LC-MS METHOD FOR THE ANALYSIS OF PFASS IN WATER

3.1 INTRODUCTION

Development of a testable LC-MS method ensures that the results generated from any analysis is reliable and accurate. Therefore, it was deemed necessary to 1) develop appropriate and testable analytical method to determine the presence and concentrations of PFASs in various water sources and 2) optimize and validate the method before sample collection, treatment and analysis. In the current project, LC-MS triple quadrupole system instrumentation was used after sample extraction to analyse the target analytes. The instrumentation was chosen because triple quadrupole mass spectrometer plays very important role in trace analysis of complex matrices such as residual pesticide in foods, contaminants in environment, drug concentration in blood and screening of abused drugs (Shimadzu, 2013).

LC-MS delivers the fastest MRM acquisition times available today and works with the most challenging samples, delivering robust and high-sensitivity detection using ESI, APCI or our dual probe ionization interface. It also provides unmatched qualitative and quantitative analysis, increased productivity, and accelerated workflows for high throughput data analysis (Shimadzu, 2013). In addition, the use of LC-MS does not require the multiple derivatisation steps required with the use of GC-MS which saves time during sample preparation. The use of LC-MS also solves the problem experienced with GC-MS with the analysis of perfluorinated sulfonates as these compounds do not form stable, volatile derivatives, making analysis using GC-MS difficult (Saito *et al*., 2004).

3.2 LC-MS/MS METHOD DEVELOPMENT AND OPTIMISATION FOR HIGH CONCENTRATION SAMPLES

Developing an LC-MS method for the analysis of PFASs involves several critical steps to ensure accurate and precise results. This is because samples with a high concentration of PFAs can lead to issues like ion suppression, detector saturation, and increased noise, and thus optimizing the method is crucial. The key elements involved in the optimisation of the LC-MS method for high concentration samples are detailed in the sub-sections below.

3.2.1 Selection of PFAS Standards for Calibration

For the purposes of this study, twenty-eight (28) PFASs standards were purchased in methanol from Wellington Laboratories (Ontario, Canada). These standards included legacy and emerging PFASs, as well as labelled PFASs internal standards (Table 3.1). Calibration curves were prepared by diluting a stock solution of 2000 ng/mL of PFASs mixture in methanol. A 10-point calibration curve was constructed with ranges from 0.1-1000 ng/L for all PFASs analytes. LOD and LOQ were calculated from formula 3σ and 10σ of the response/slope of calibration curve, respectively.

Table 3.1: PFAS standards for LC-MS method development for high concentrations

Name of compound	Acronym
MPFHxA_$^{13}C_2$	MPFHxA_$^{13}C_2$
MPFNA_$^{13}C_5$	MPFNA_$^{13}C_5$
MPFDA_$^{13}C_2$	MPFDA_$^{13}C_2$
Sodium perfluoro-1-hexanesulfonate	L-PFHxS
Perfluoro-n-dodecanoic acid	PFDoA
Perfluoro-n-heptanoic acid	PFHpA
Perfluoro-n-hexanoic acid	PFHxA
Perfluoro-n-hexadecanoic acid	PFHxDA
Perfluoro-n-nonanoic acid	PFNA
Perfluoro-n-octadecanoic acid	PFODA
Perfluoro-n-pentanoic acid	PFPeA
Perfluoro-n-tetradecanoic acid	PFTeDA
Perfluoro-n-tridecanoic acid	PFTrDA
Perfluoro-n-undecanoic acid	PFUdA
Potassium perfluoro-1-butanesulfonate	L-PFBS
Sodium perfluoro-1-decanesulfonate	L-PFDS
Sodium perfluoro-1-heptanesulfonate	L-PFHpS
Sodium perfluoro-1-nonanesulfonate	L-PFNS
Sodium perfluoro-1-octanesulfonate	L-PFOS
Perfluoro-n-butanoic acid	PFBA
Perfluoro-n-decanoic acid	PFDA
Perfluoro-n-octanoic acid	PFOA
Sodiumperfluoro-1-dodecanesulfonate	L-PFDoS
Sodium perfluoro-1-pentanesulfonate	L-PFPeS
Labelled PFAS internal standards	
perfluoro-n-[1,2-$^{13}C_2$] octanoic acid	M2PFOA
perfluoro-n-[1,2-$^{13}C_2$] decanoic acid	MPFDA
perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid	MPFHxA
Surrogate standards	
perfluoro-n-[1,2,3,4, 5-$^{13}C_5$] nonanoic acid	MPFNA

3.2.2 Optimization of Chromatographic and Mass Spectrometry Conditions

LC-MS-MS grade water, methanol, acetonitrile and ammonium acetate were purchased from Sigma-Aldrich (Aston Manor, South Africa). LC-MS grade water, methanol and ammonium acetate were purchased from Sigma-Aldrich (Aston Manor, South Africa). Supelco ENVI-18™ SPE cartridges (500 mg, 6 mL) were purchased from Sigma-Aldrich (Aston Manor, South Africa).

Multiple Reaction Monitoring (MRM) transitions were optimized using Flow Injection Analysis (FIA) for all compounds, bypassing the analytical column. A high concentration standard of 1000 ng/L containing a mixture of all the PFASs compounds was used for optimization of MRM conditions. The mixture was then run under optimized LC-MS/MS conditions times to obtain retention times of each method analyte.

Ten microlitre (10 µL) of the standards were injected and analysed using the Liquid Chromatography tandem mass spectrometry (Shimadzu LC-MS 8030 triple quadrupole system, Tokyo, Japan). The instrument was equipped with an electrospray ionization (ESI) source and the target compounds were separated on an

InertSustain C18 (3 µm, 2.1 i.d. x 150 mm) HPLC column (Tokyo, Japan). The instrument conditions for targeted analysis of PFASs on LC-MS-8030 triple quadrupole system and the non-targeted analysis of PFASs using TripleTOF 6600, SCIEX are shown in Tables 3.2 and 3.3, respectively. The quantitation of the target compounds was based on internal standard method calibration with concentrations ranging from 1.0-1000 ng/L. An R^2=0.99 was achieved in all the calibrations with good precision of the internal standard. The method was then applied to spiked water samples.

Table 3.2: Instrument conditions for the targeted analysis of PFASs

LC-MS/MS instrument	Shimadzu, LC-MS-8030 triple quadrupole system
Analytical column	Kinetex® 2.6 µm XB-C18 100 Å, LC Column 50 x 4.6 mm
Column temperature	40°C
Injection volume	10.00 µL
Flow rate	0.3000 mL/min
Mobile Phases	A 20 mM Ammonium Acetate B 50:50 Methanol: Acetonitrile
Gradient conditions	
Time (min)	% Mobile phase B
1	20
4	90
7	20
12	0
Acquisition time	12 min

Table 3.3: Instrument conditions for non-target PFASs identification using TOF-MSW

Instrument name	TripleTOF 6600, SCIEX
Analytical column	Luna Omega 3 µm polar C18 100Å LC column 100 x 2.1 mm, Phenomenex
Column temperature	40°C
Injection volume	10.00 µL
Flow rate	0.50 mL/min
Mobile Phases	A 2 mM Ammonium Acetate, 0.1% Formic Acid B 100% Methanol
Gradient conditions	
Time (min)	% Mobile phase B
1	5.0
16	95
20	5.0
26	0
Acquisition	Information Dependent Acquisition
Acquisition time	26 min

3.2.3 Chromatogram of standards

Figure 3.1 shows the chromatogram of overlaid peaks of mixed PFASs standard solution at 10 ng/mL.

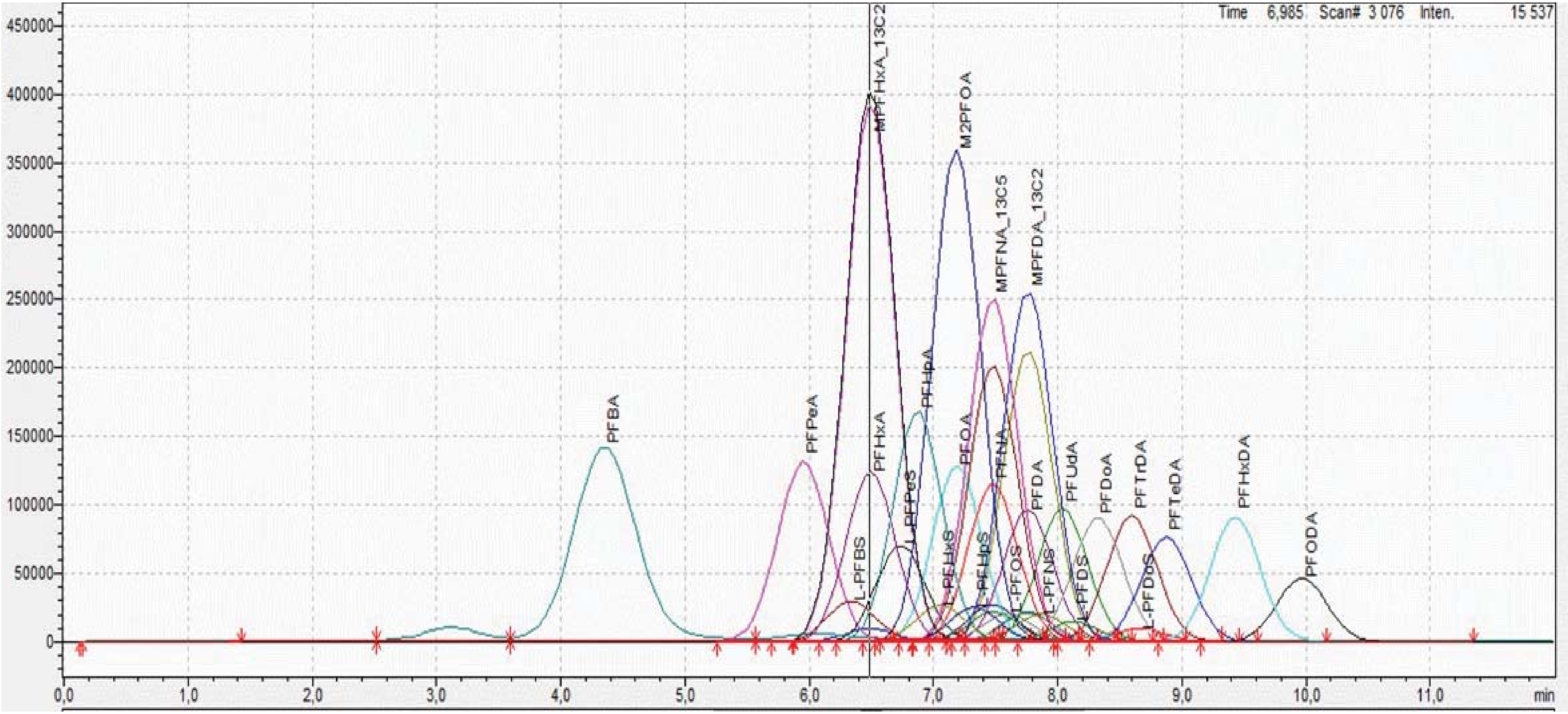

Figure 3.1: Chromatogram of overlaid peaks of mixed PFASs standard solution at 10 ng/mL

3.2.4 Instrument method development and optimization

The calibration curves and total ion chromatography (TIC) of the internal standards and surrogate are shown in Figures 3.2 to 3.4; and the target compounds in Figures 3.5 to 3.22. All isomers calibration curves showed linearity, based on correlation coefficients (R) and correlation of determination (R^2) that were greater than 0.99 with good precision of the internal standard.

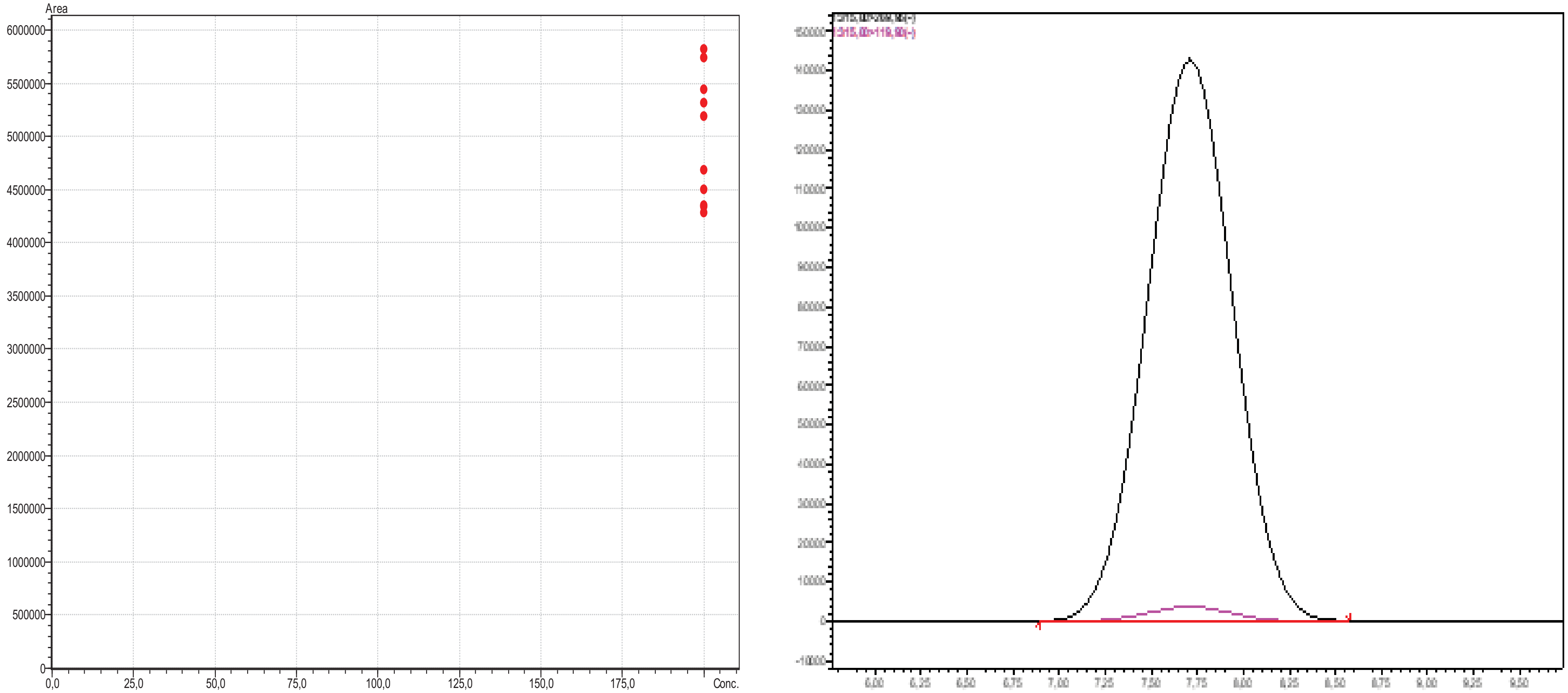

Figure 3.2: Perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid (MPFHxA_$^{13}C_2$) calibration curve (left) and total ion chromatograph (TIC) (right).

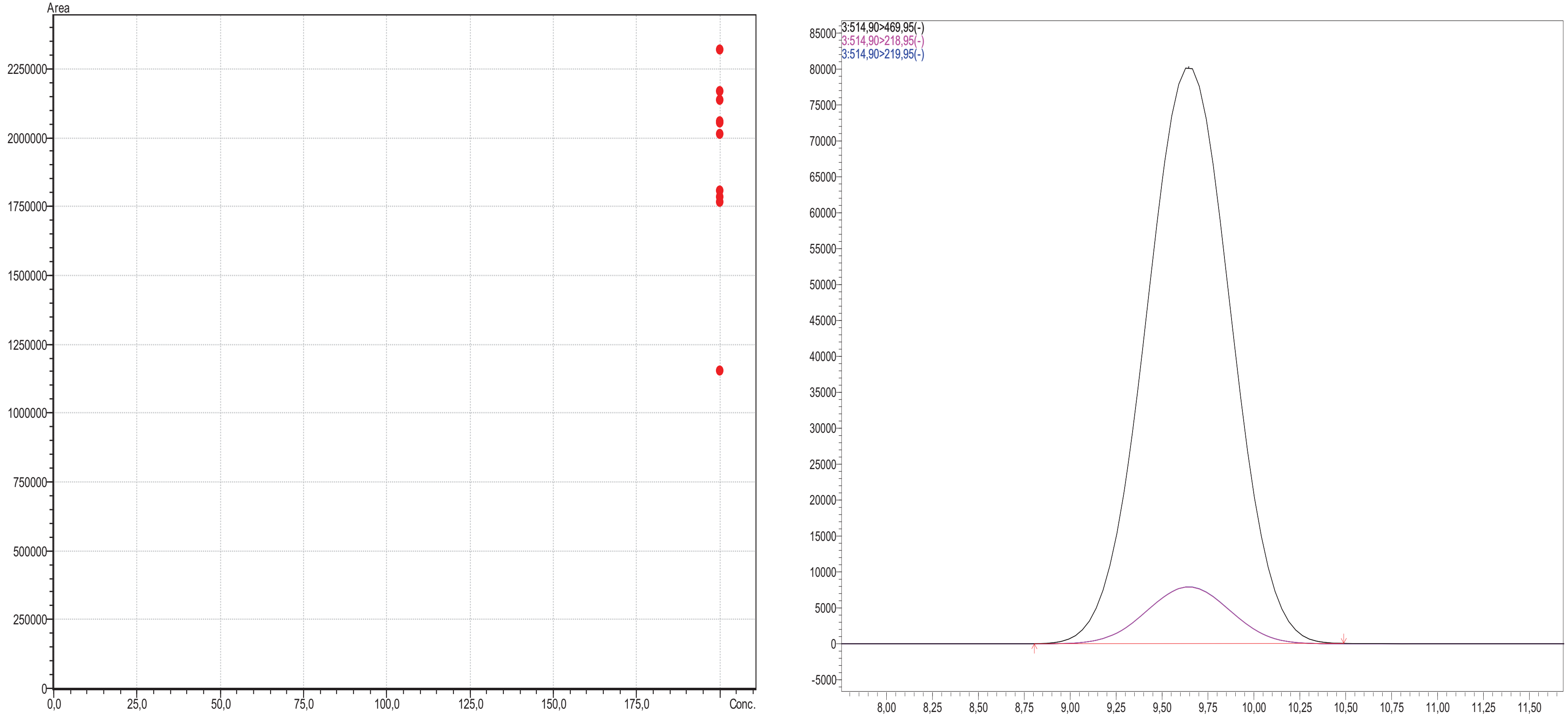

Figure 3.3: Perfluoro-n-[1,2-$^{13}C_2$] decanoic acid (MPFDA_$^{13}C_2$) calibration curve (left) and TIC (right).

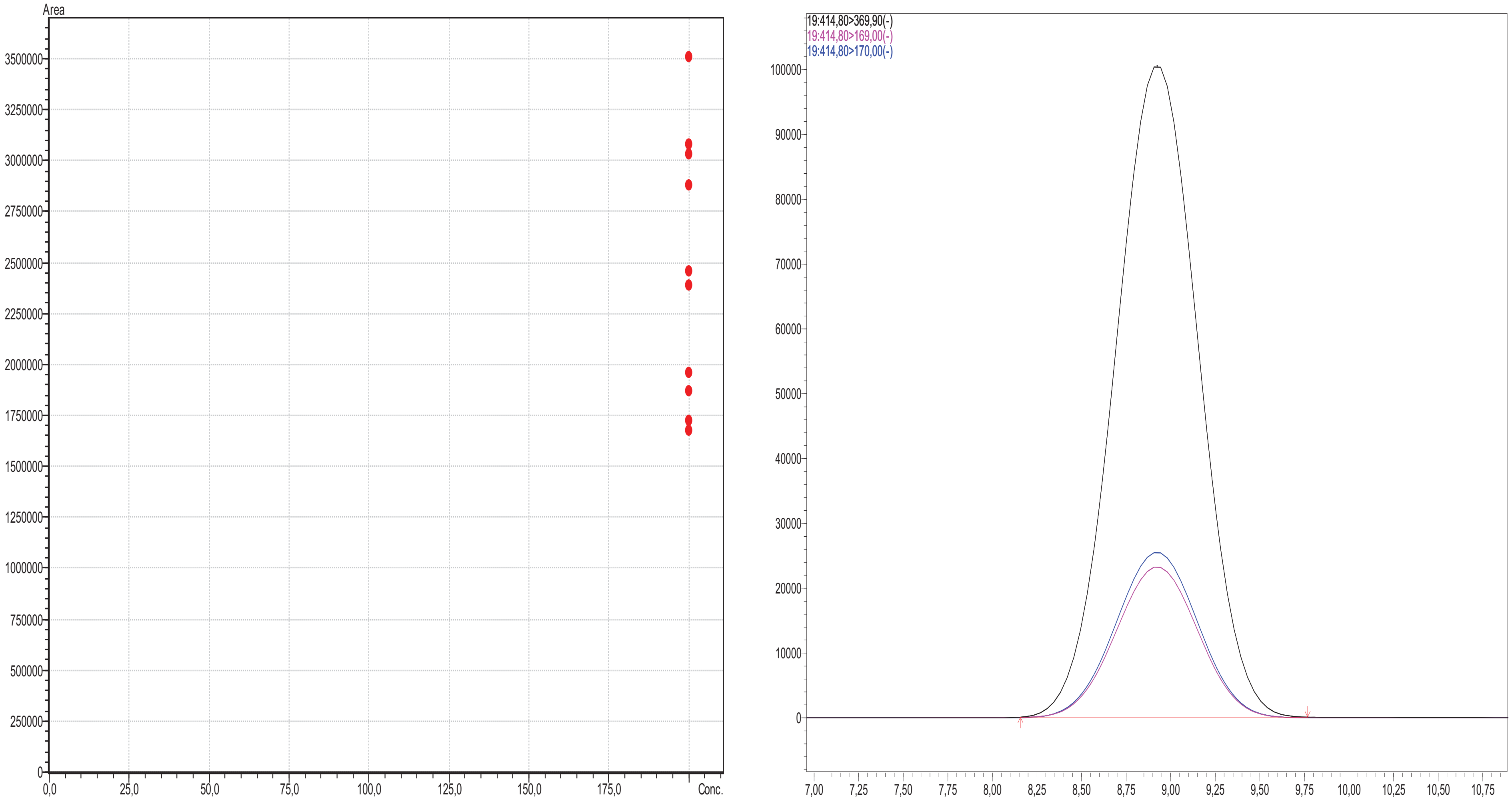

Figure 3.4: Perfluoro-n-[1,2-$^{13}C_2$] octanoic acid M2PFOA calibration curve (left) and TIC (right).

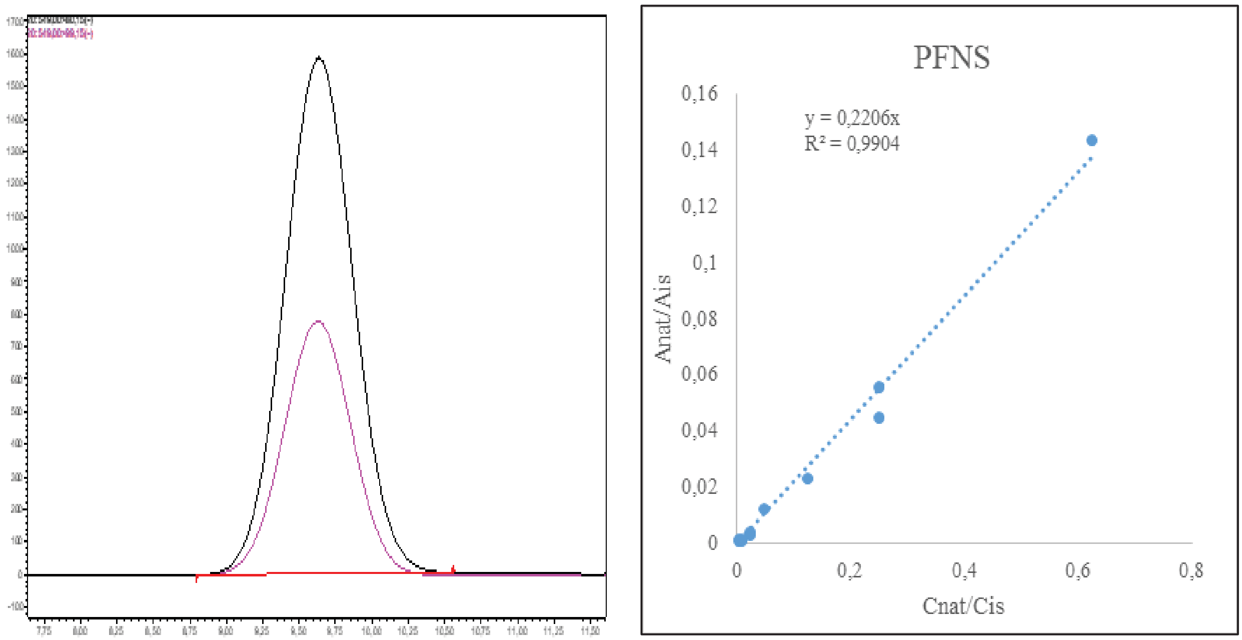

Figure 3.5: Perfluoro-n-nonanesulfonic acid (PFNA) with TIC (left) and calibration (right).

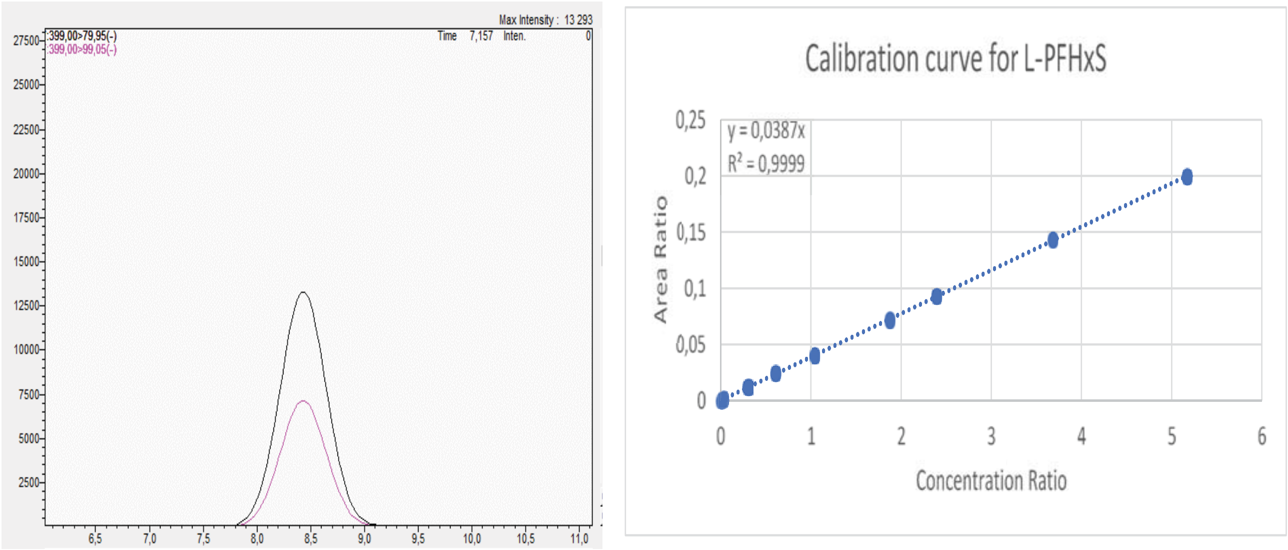

Figure 3.6: Total ion chromatogram (left) and calibration (right) for LPFHxS).

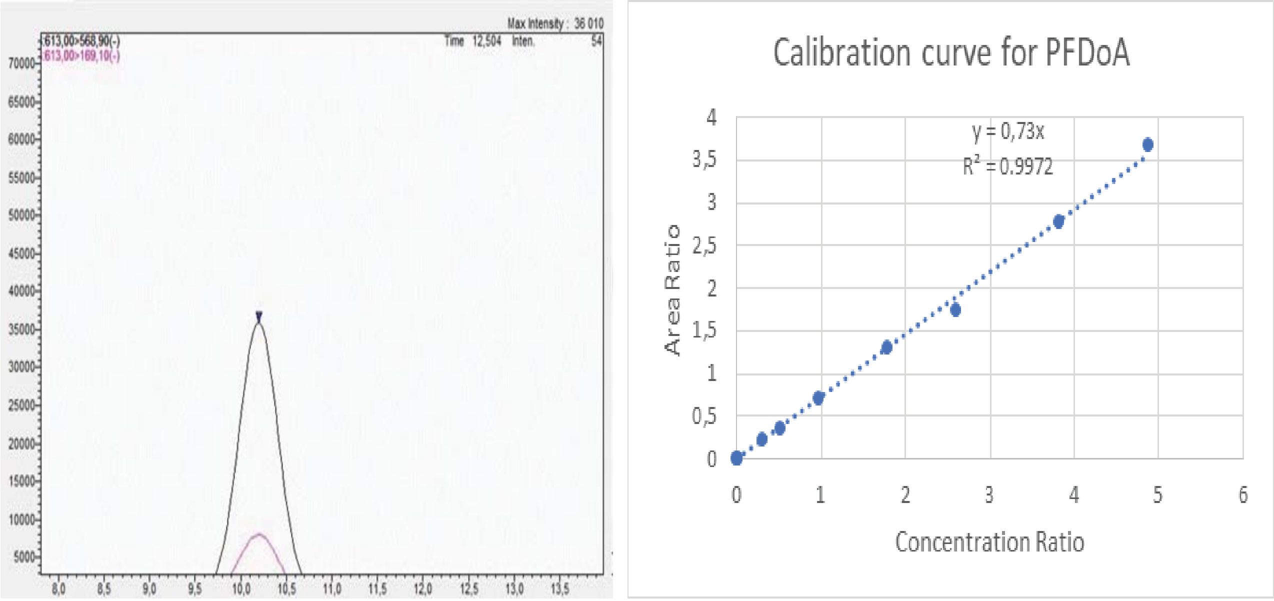

Figure 3.7: Total ion chromatogram (left) and calibration (right) for PFDoA

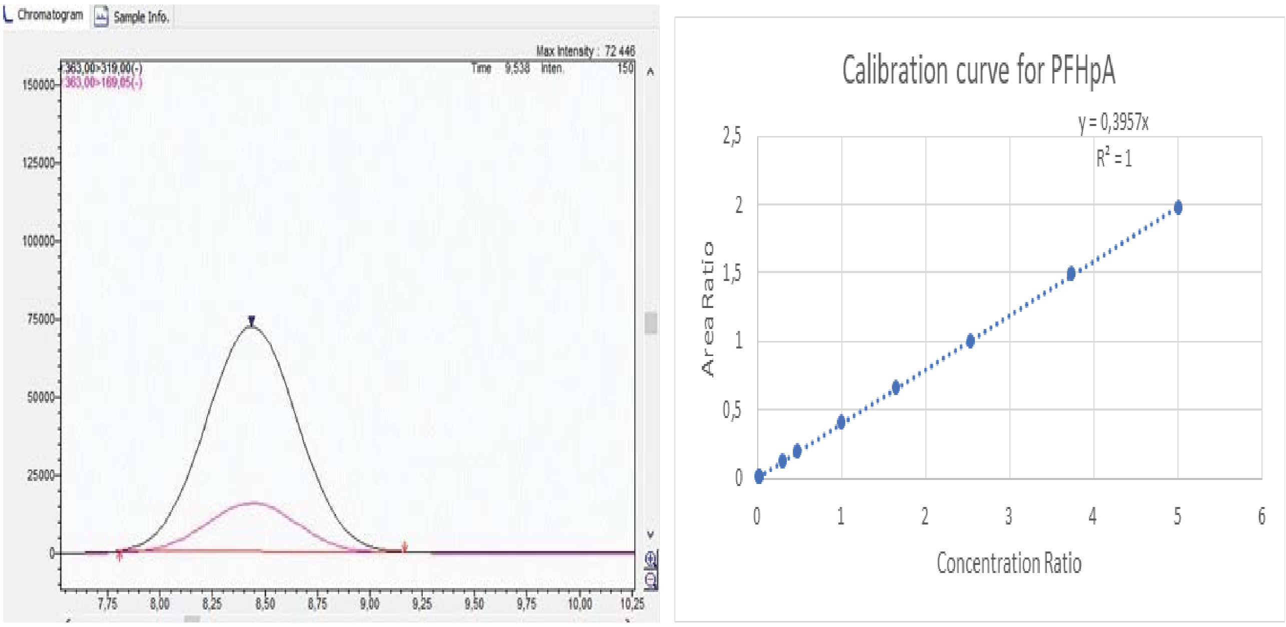

Figure 3.8: Total ion chromatogram (left) and calibration (right) for PFHpA.

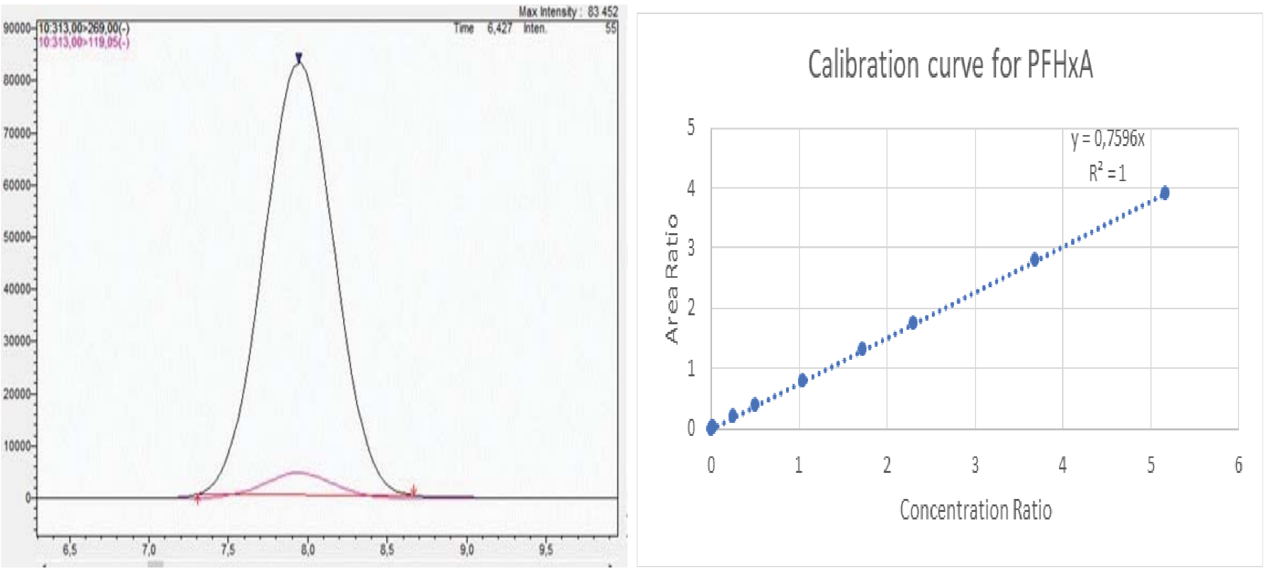

Figure 3.9: Total ion chromatogram (left) and calibration (right) for PFHxA.

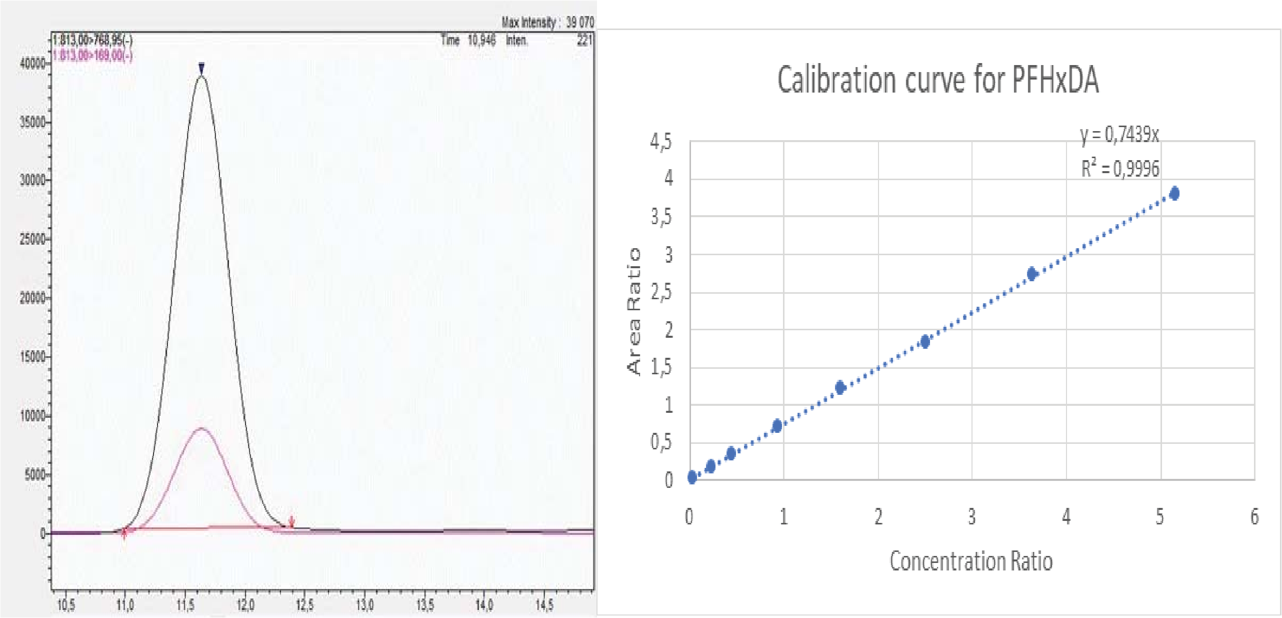

Figure 3.10: Total ion chromatogram (left) and calibration (right) for PFHxDA.

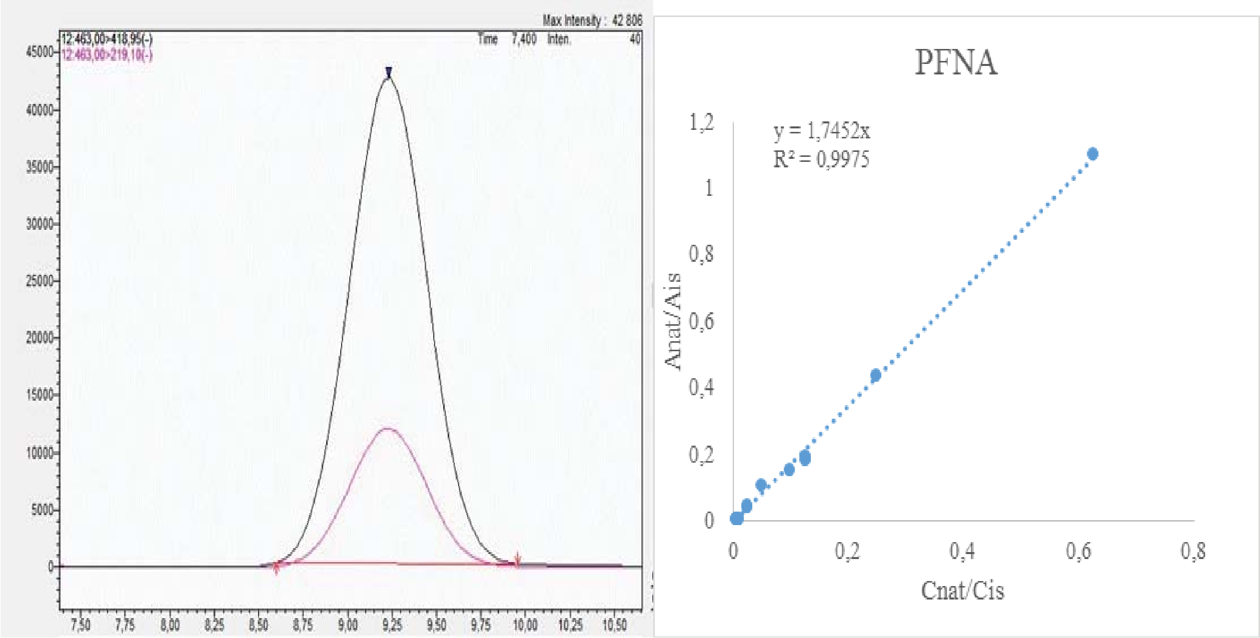

Figure 3.11: Total ion chromatogram (left) and calibration (right) for PFNA.

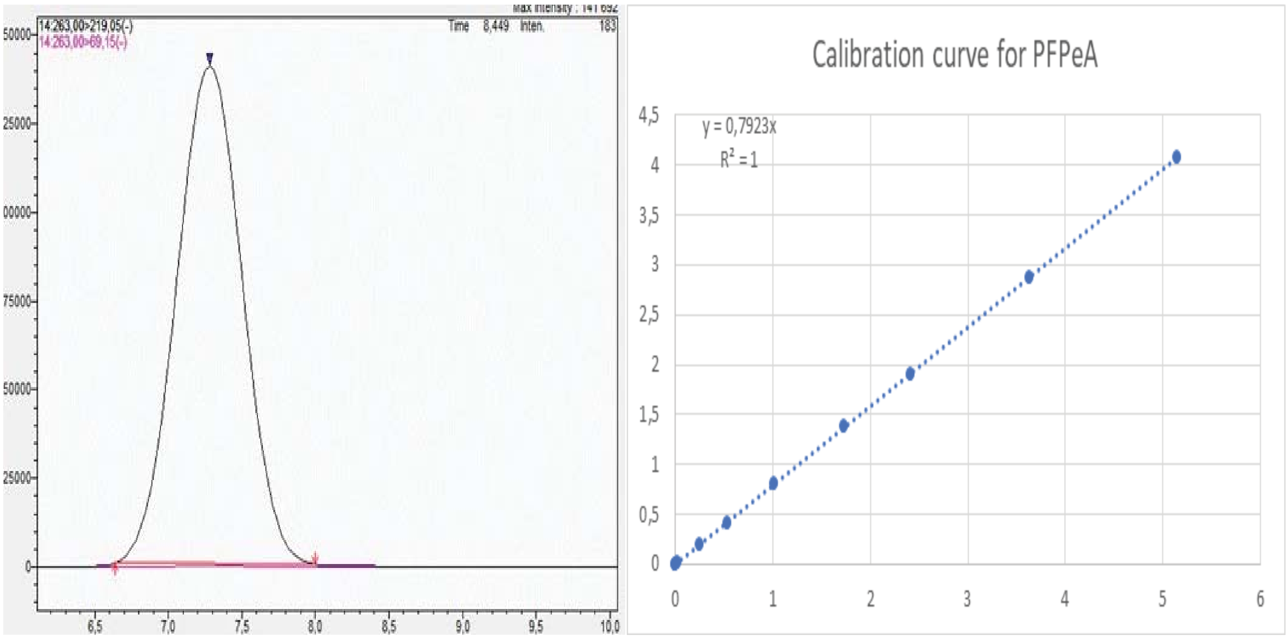

Figure 3.12: Total ion chromatogram (left) and calibration (right) for PFPeA.

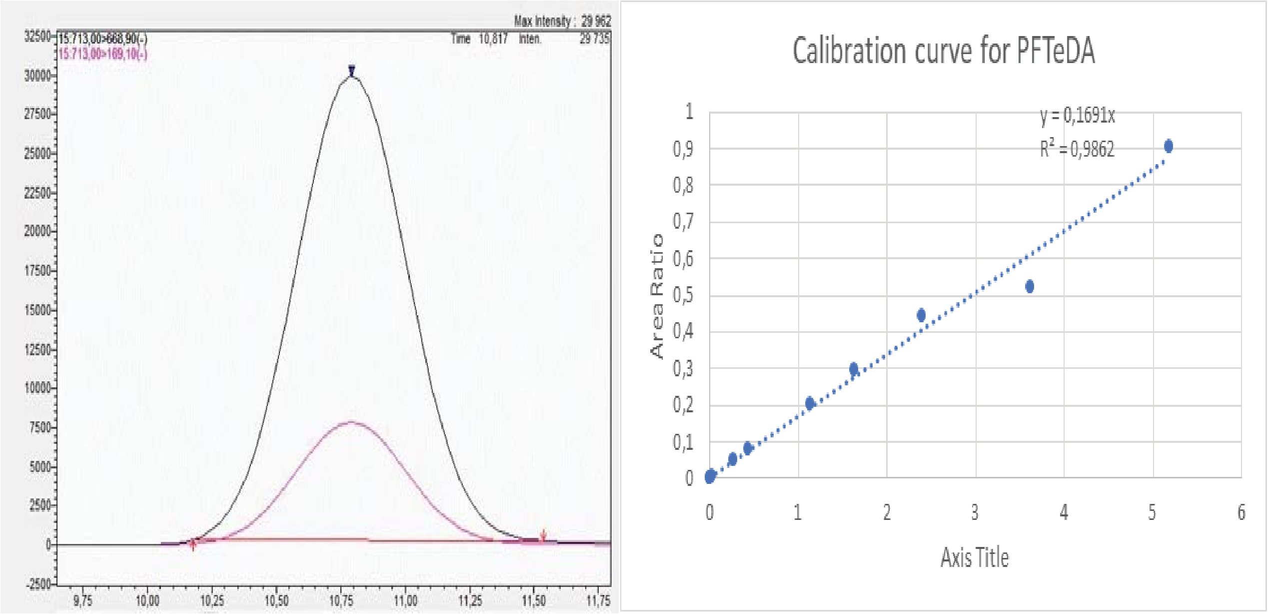

Figure 3.13: Total ion chromatogram (left) and calibration (right) for PFTeDA.

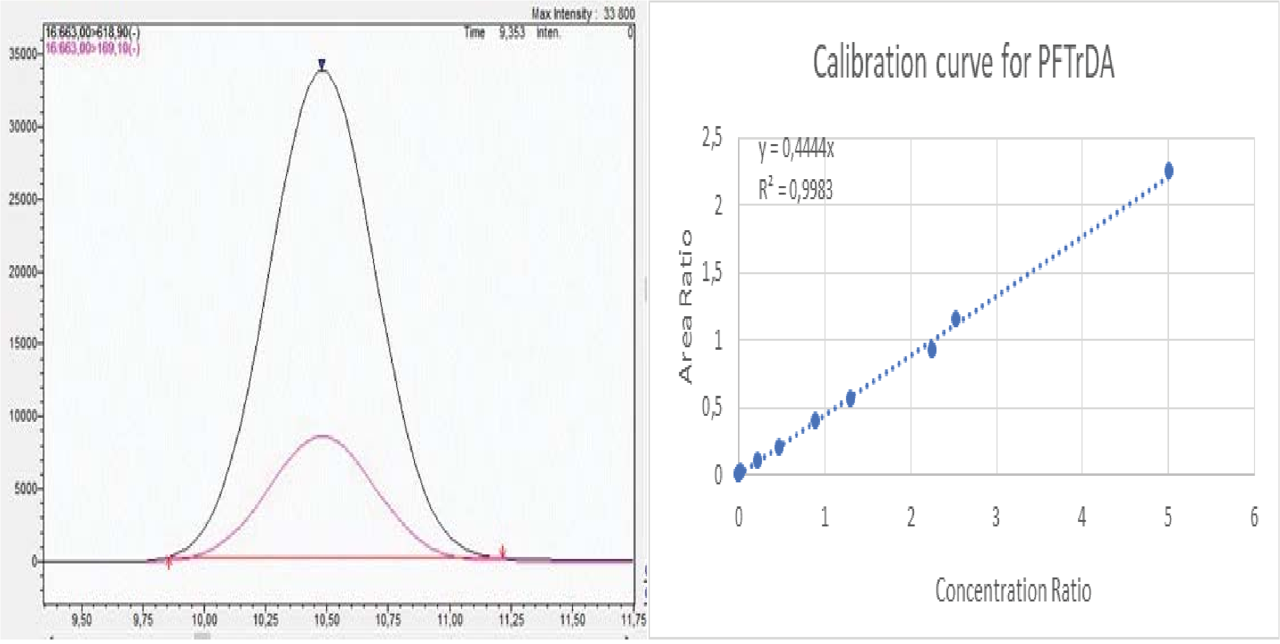

Figure 3.14: Total ion chromatogram (left) and calibration (right) for PFTrDA.

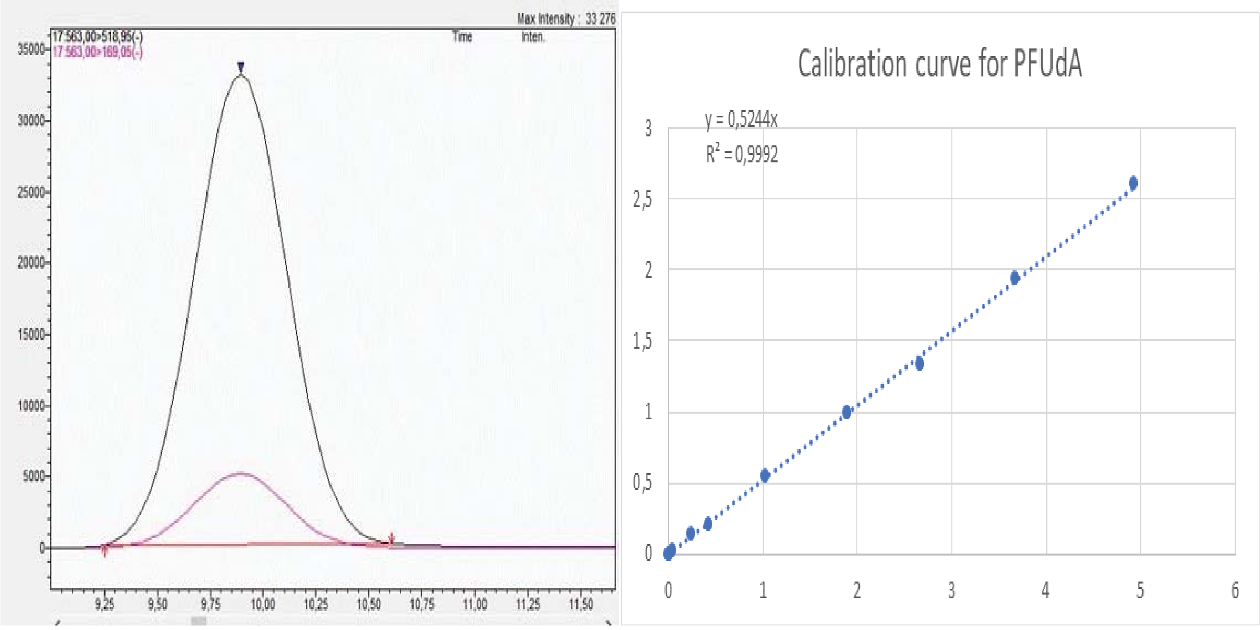

Figure 3.15: Total ion chromatogram (left) and calibration (right) for PFUdA.

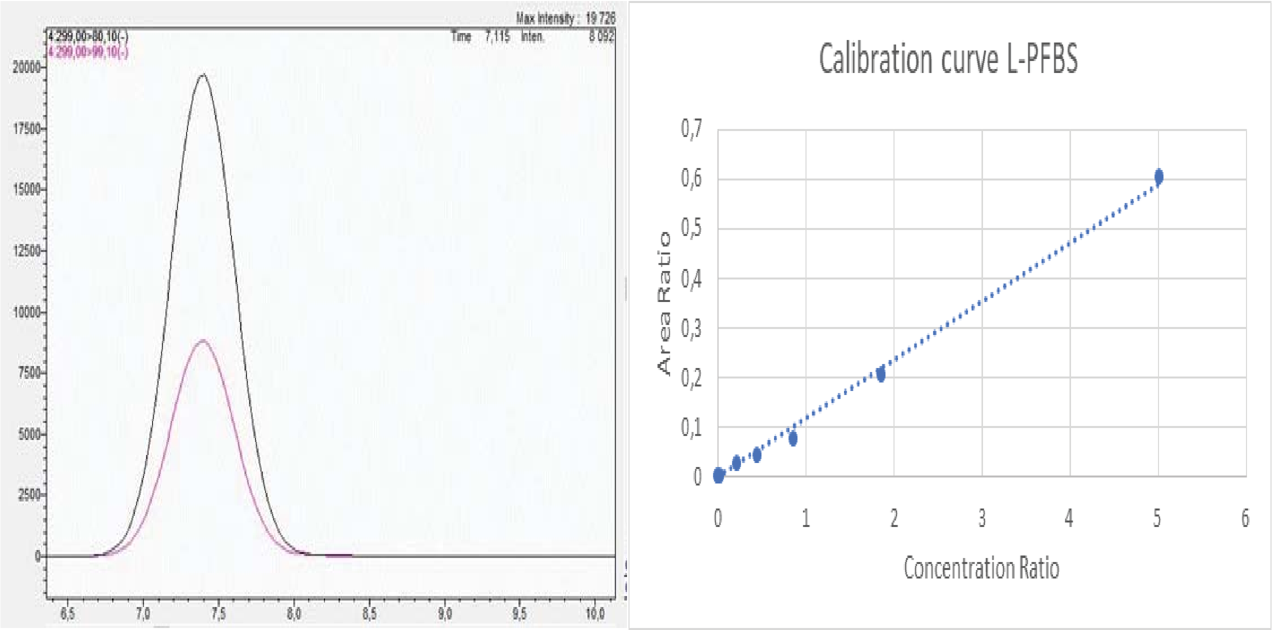

Figure 3.16: Total ion chromatogram (left) and calibration (right) for L-PFBS.

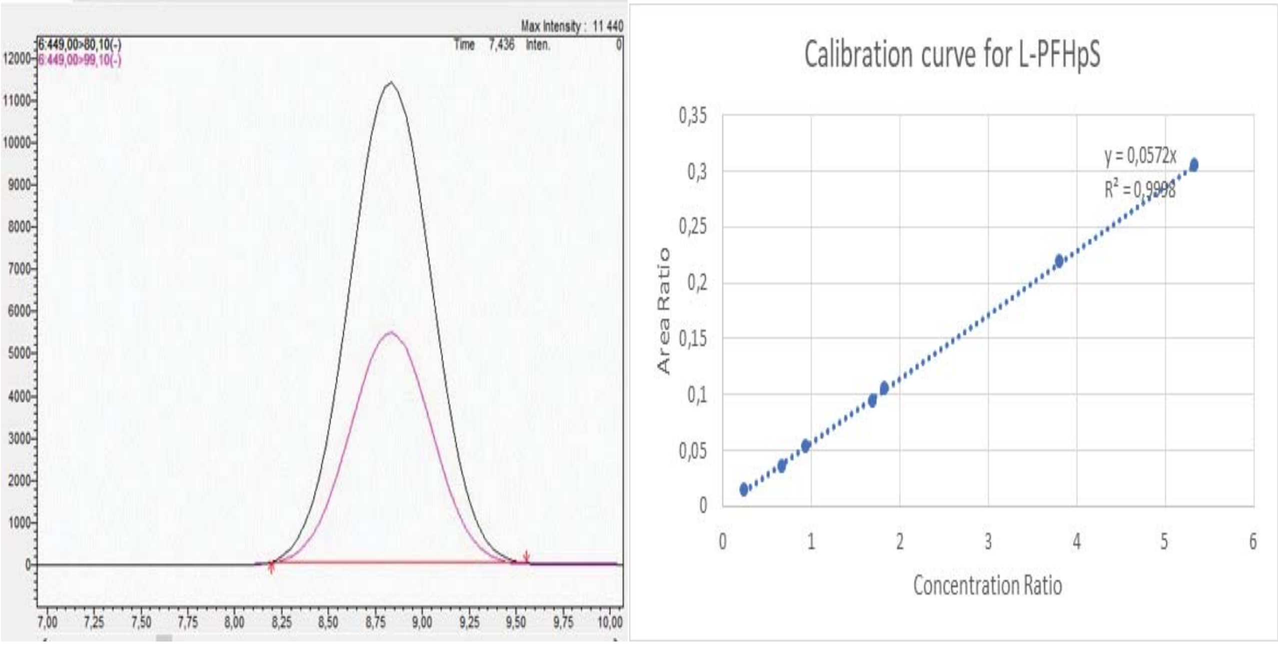

Figure 3.17: Total ion chromatogram (left) and calibration (right) for L-PFHpS.

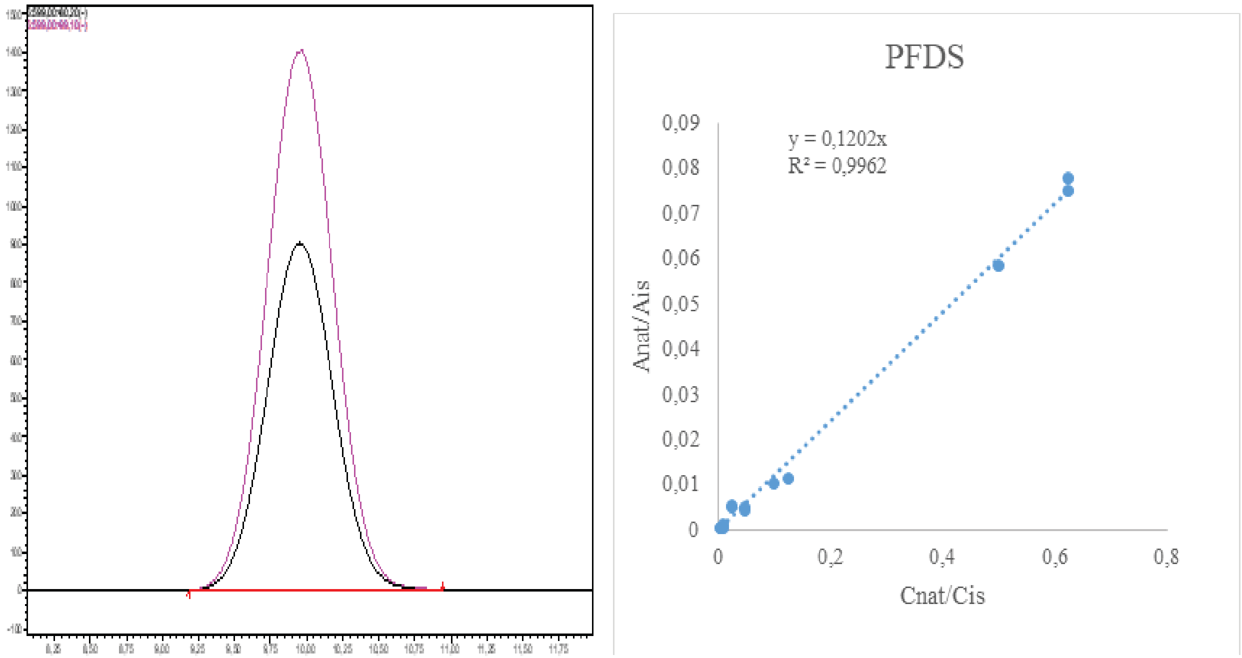

Figure 3.18: Total ion chromatogram (left) and calibration (right) for L-PFDS.

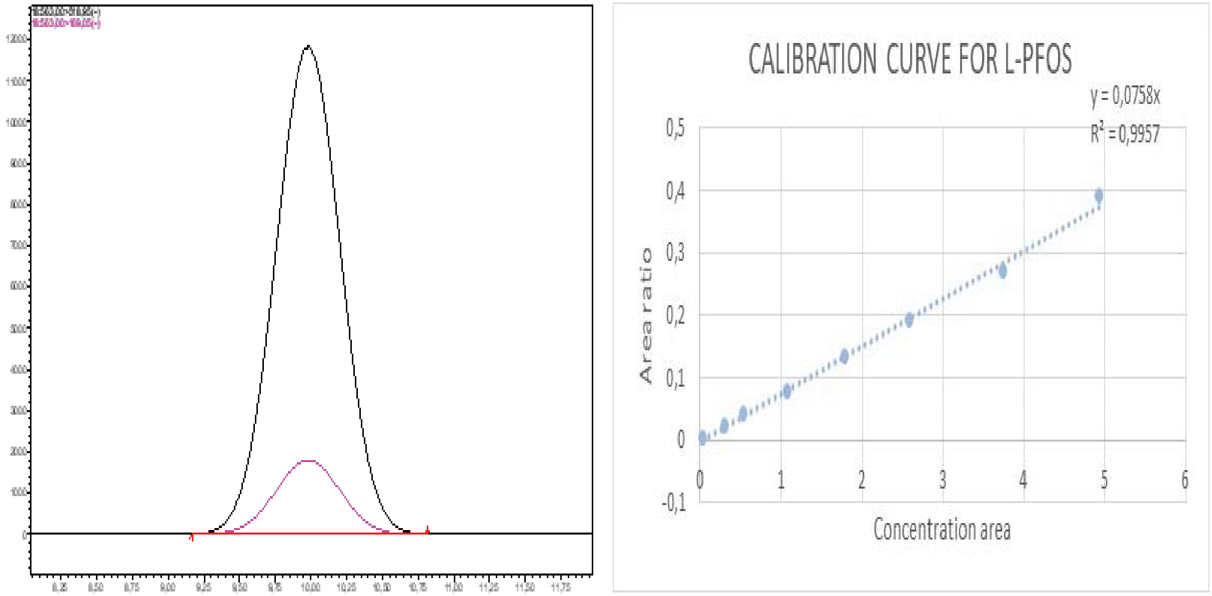

Figure 3.19: Total ion chromatogram (left) and calibration (right) for L-PFOS.

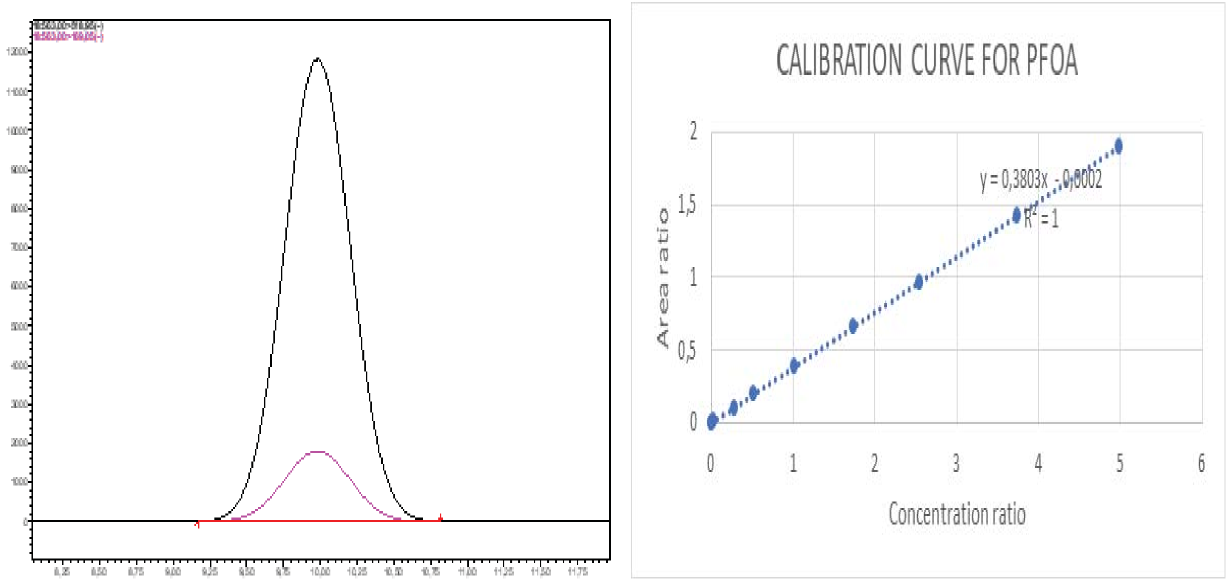

Figure 3.20: Total ion chromatogram (left) and calibration (right) for PFOA.

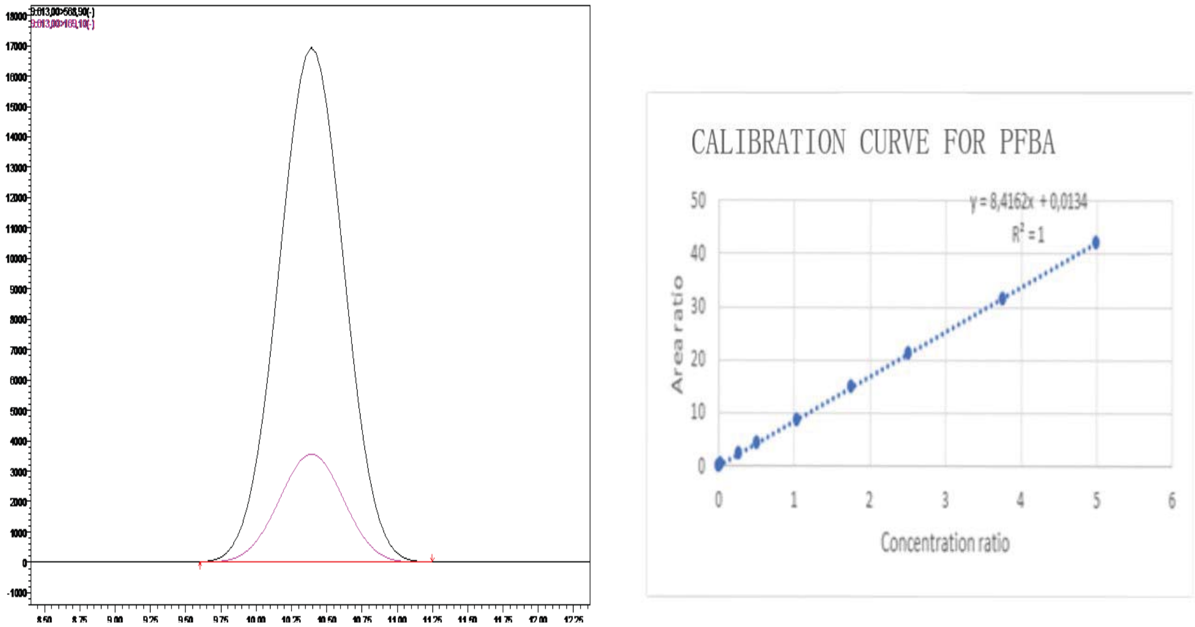

Figure 3.21: Total ion chromatogram (left) and calibration (right) for PFBA.

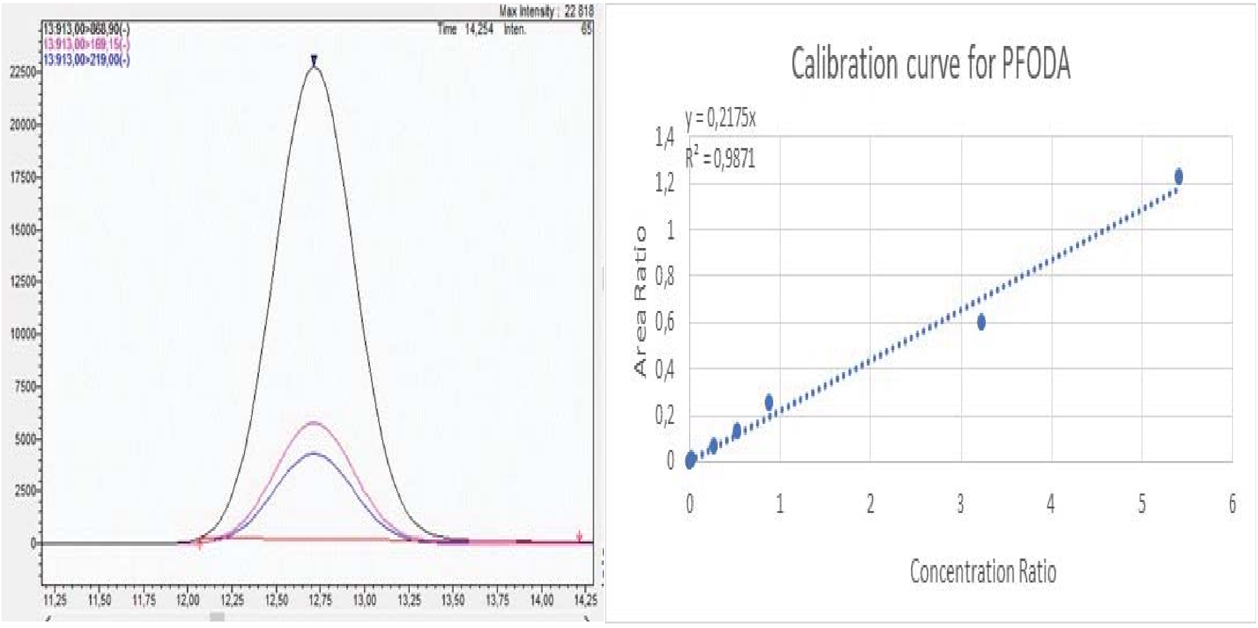

Figure 3.22: Total ion chromatogram (left) and calibration (right) for PFODA.

3.2.5 Multiple reaction monitoring (MRM)

During the MRM selection, a full scan of ions was done. Ions corresponding to the compounds of interest were targeted followed by the fragmentation of the targeted ions producing a range of daughter ions. The ions corresponding to the compounds of interest were then selected and isolated from other ions within the mass spectrometer to quantitate the method. The multiple reaction monitoring results are shown in Table 3.4.

Table 3.4: MRM of precursor and product ions

Compounds	Abbreviation	Precursor ion (m/z)	Product ion (m/z)	Retention time (min)
MPFHxA $^{13}C_2$	MPFHxA $^{13}C_2$	315.00	269.95	6.46
MPFNA $^{13}C_5$	MPFNA $^{13}C_5$	467.90	423.00	7.51
MPFDA $^{13}C_2$	MPFDA $^{13}C_2$	514.90	469.95	7.76
Sodium perfluoro-1-hexanesulfonate	L-PFHxS	399.00	79.95	7.06
Perfluoro-n-dodecanoic acid	PFDoA	613.00	568.90	8.33
Perfluoro-n-heptanoic acid	PFHpA	363.00	319.00	6.88
Perfluoro-n-hexanoic acid	PFHxA	313.00	269.00	6.49
Perfluoro-n-hexadecanoic acid	PFHxDA	813.00	768.95	9.43
Perfluoro-n-nonanoic acid	PFNA	463.00	418.95	7.50
Perfluoro-n-octadecanoic acid	PFODA	913.00	868.90	9.97
Perfluoro-n-pentanoic acid	PFPeA	263.00	219.05	5.96
Perfluoro-n-tetradecanoic acid	PFTeDA	713.00	668.90	8.87
Perfluoro-n-tridecanoic acid	PFTrDA	663.00	618.90	8.60
Perfluoro-n-undecanoic acid	PFUdA	563.00	518.95	8.04
Potassium perfluoro-1-butanesulfonate	L-PFBS	299.00	80.10	6.34
Sodium perfluoro-1-decanesulfonate	L-PFDS	599.00	80.20	8.13
Sodium perfluoro-1-heptanesulfonate	L-PFHpS	449.00	80.10	7.34
Sodium perfluoro-1-nonanesulfonate	L-PFNS	549.00	80.15	7.87
M2PFOA	M2PFOA	414.80	369.90	7.20
Sodium perfluoro-1-octanesulfonate	L-PFOS	499.00	80.15	7.60
Perfluoro-n-butanoic acid	PFBA	213.00	169.05	4.34
Perfluoro-n-decanoic acid	PFDA	513.00	468.90	7.777
Perfluoro-n-octanoic acid	PFOA	413.00	368.95	7.186
Sodiumperfluoro-1-dodecanesulfonate	L-PFDoS	699.00	80.20	8.675
Sodium perfluoro-1-pentanesulfonate	L-PFPeS	349.00	79.90	6.754

3.2.6 Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ are shown in Table 3.5, and accordingly, the LOD and LOQ values ranged from 0.005-0.395 and 0.016-1.197 ng/L respectively.

Table 3.5: LOD and LOQ values (ng/L) of the targeted standards

COMPOUND	LOD	LOQ
L-PFPeS	0.046	0.139
PFOA	0.005	0.016
PFDA	0.016	0.048
PFBA	0.012	0.036
L-PFOS	0.395	1.19

COMPOUND	LOD	LOQ
L-PFHpS	0.020	0.063
L-PFDS	0.008	0.026
L-PFBS	0.023	0.070
L-PFdUA	0.015	0.047
PFTrDA	0.056	0.171
PFTeDA	0.286	0.868
PFPeA	0.067	0.204
PFNA	0.025	0.076
PFODA	0.034	0.106
L-PFHxDA	0.178	0.541
PFHxA	0.015	0.046
PFHpA	0.039	0.120
PFDoA	0.097	0.290
PFHxS	0.202	0.613

3.3 LC-MS/MS METHOD DEVELOPMENT AND OPTIMISATION FOR LOW CONCENTRATION SAMPLES

Analysis of low PFASs concentration samples with LC-MS can present challenges related to signal-to-noise ratios and detection limits, and requires specific considerations to maximize sensitivity and accuracy. Analysis of PFASs in different environmental matrices requires developing a suitable analytical method due to their occurrence at lower concentrations. The method should be optimized in a sense that LC and MS/MS conditions meet the highest sensitivity and throughput for the analysis of analytes of interest. For the purpose of this project, LC-MS/MS (LCMS-8030, Shimadzu), was optimized and used. The key elements involved in the optimisation of the LC-MS method for low concentration samples are detailed in the sub-sections below.

3.3.1 Selection of PFAS Standards for Calibration

Table 3.6 shows the list of PFAS standards used for LC-MS method development for low concentrations. Fifty milligram per litre (50 mg/L (in methanol) individual native standards (1.2 mL) of PFUdA, PFHxA, PFPeA, 4:2 FTS, 8:2 FTS, PFHpA, PFNA, PFDoA, PFODA, PFHxDA, L-PFBS, L-PFHxS, L-PFOS, PFHpS, PFOA, L-PFDS, FHEA, 6:2 FTS, FOET, FHET and PFBA and isotopically labelled internal standards (MPFDA_$^{13}C_2$, MPFHxA_$^{13}C_2$ and M2PFOA) and surrogate standards (MPFNA, MPFUdA, and MPFHxS) standard were purchased from Wellington Laboratories (Guelph, Ontario, Canada). LC-MS Ultrapure Methanol and Acetonitrile were purchased from Aqualytic (Pty) Ltd, and ammonium acetate and ammonium formate were purchased from Sigma Aldrich Ltd. LC grade water was purchased from Labchem (Pty) Ltd.). Calibration curves were created from diluting a stock solution of 50 mg/L of individual PFASs in methanol. A 10-point calibration curve was constructed with ranges from 1-2000 ng/L for all PFASs analytes. Calibration curve for PFUdA, PFDoA, PFHxA, PFNA, PFPeA, PFBA, MPFNA, 4:2 FTS, 6:2 FTS, 8:2 FTS, FHET, FHEA and FOET ranged from 1-1000 ng/L, while that of L-PFBS, L-PFOS, PFOA, L-PFHpS, and L-PFHxS ranged from 100-1000 ng/L and that of MPFUdA, MPFHxS, L-PFDS, PFODA and PFHxDA ranged from 100-2000 ng/L. The Limit of Detection (LOD) was set as instrument detection limit, and this was different for each compound.

Table 3.6: PFAS standards for LC-MS method development for low concentrations

Name of compound	Acronym
Sodium 1H,1H,2H,2H-perfluorohexane sulfonate(4:2)	4:2 FTS
Sodium 1H,1H,2H,2H-perfluorodecane sulfonate(8:2)	8:2 FTS
Sodium 1H,1H,2H,2H-perfluorooctane sulfonate(6:2)	6:2 FTS
2-Perfluorohexyl ethanoic acid (6:2)	FHEA
2-Perfluorooctyl ethanol (8:2)	FOET
2-Perfluorohexyl ethanol (6:2)	FHET
Sodium perfluoro-1-hexanesulfonate	L-PFHxS
Perfluoro-n-dodecanoic acid	PFDoA
Perfluoro-n-heptanoic acid	PFHpA
Perfluoro-n-hexanoic acid	PFHxA
Perfluoro-n-hexadecanoic acid	PFHxDA
Perfluoro-n-nonanoic acid	PFNA
Perfluoro-n-octadecanoic acid	PFODA
Perfluoro-n-pentanoic acid	PFPeA
Perfluoro-n-undecanoic acid	PFUdA
Potassium perfluoro-1-butanesulfonate	L-PFBS
Sodium perfluoro-1-decanesulfonate	L-PFDS
Sodium perfluoro-1-heptanesulfonate	L-PFHpS
Sodium perfluoro-1-octanesulfonate	L-PFOS
Perfluoro-n-butanoic acid	PFBA
Perfluoro-n-octanoic acid	PFOA
Isotopically labelled internal standards	
perfluoro-n-[1,2-$^{13}C_2$] octanoic acid	M2PFOA
MPFHxA_$^{13}C_2$	MPFHxA_$^{13}C_2$
MPFDA_$^{13}C_2$	MPFDA_$^{13}C_2$
Surrogate standards	
perfluoro-n-[1,2,3,4, 5-$^{13}C_5$] nonanoic acid	MPFNA
perfluoro-n-[1,2-$^{13}C_2$]undecanoic Acid	MPFUdA
sodium perfluoro-1-hexane[18O2]sulfonate	MPFHxS

3.3.2 Optimization of Chromatographic and Mass Spectrometry Conditions

Multiple Reaction Monitoring (MRM) transitions were optimized using Flow Injection Analysis (FIA) for all compounds, bypassing the analytical column. High concentration standards of 1000 ng/L containing a mixture of all the PFASs compounds was used for optimization of MRM conditions. The mixture was then analysed under optimized LC-MS/MS conditions to obtain retention times of each method analyte. Due to the high variations in the K_{ow} values of the standards, the separation of all the standards using a single method was extremely poor. Therefore, it was necessary to develop four different methods that can ensure good separation of the standards, and these are shown in Table 3.7.

- **Method A** consisted of the following analystes: MPFNA, PFUdA, PFHxA, PFPeA, 4:2 FTS, 8:2 FTS, PFHpA, PFNA, PFDoA, PFODA and PFHxDA
- **Method B** comprised MPFUdA, MPFHxS, L-PFBS, L-PFHxS, L-PFOS, PFHpS, PFOA, and L-PFDS.
- **Method C** included FHEA, 6:2 FTS, FOET and FHET, and
- **Method D** catered for PFBA.

Table 3.7: Instrument and optimization conditions for targeted analysis of PFASs

LC-MS/MS instrument	Shimadzu, LCMS-8030
Analytical column	Kinetex 2.6 um Polar C18 100 A LC Column 100 x 2.1 mm, Unit
Column temperature	40°C
Injection volume	10.00 µL
Flow rate	0.3000 mL/min
	Method A
Mobile Phases	A: 10 mM Ammonium Formate
	B: 20:80 Methanol: Acetonitrile
	Gradient Conditions
Time (min)	Mobile Phase B (%)
1	45
3	50
4	60
4.5	70
5	65
5.5	68
6	80
7.5	70
10	0
16	Stop
	Method B
Mobile Phases	A: 10 mM Ammonium Formate
	B: 50:50 Methanol: Acetonitrile
	Gradient conditions
Time (min)	Mobile Phase B (%)
1	
4	20
6.5	55
7	75
7.2	95
9	0
10	20
12	Stop
	Method C
Mobile Phases	A: 20 mM Ammonium Acetate
	B: 95:5 Methanol: Water
	Gradient Conditions
Time (Min)	Mobile Phase B (%)
1	20
2	75
3	85
4	70
6	95
7.5	100

10	90
16	Stop

	Method D
Mobile Phases	A: 10 mM Ammonium Formate
	B: 20:80 Methanol: Acetonitrile
	Gradient Conditions
Time (Min)	Mobile Phase B (%)
1	20
2	55
3.5	70
4	0
5	Stop

3.3.3 Chromatogram of standards

Figures 3.23 to 3.26, corresponding to methods A to D, show the chromatograms of overlaid peaks of mixed PFASs standard solutions.

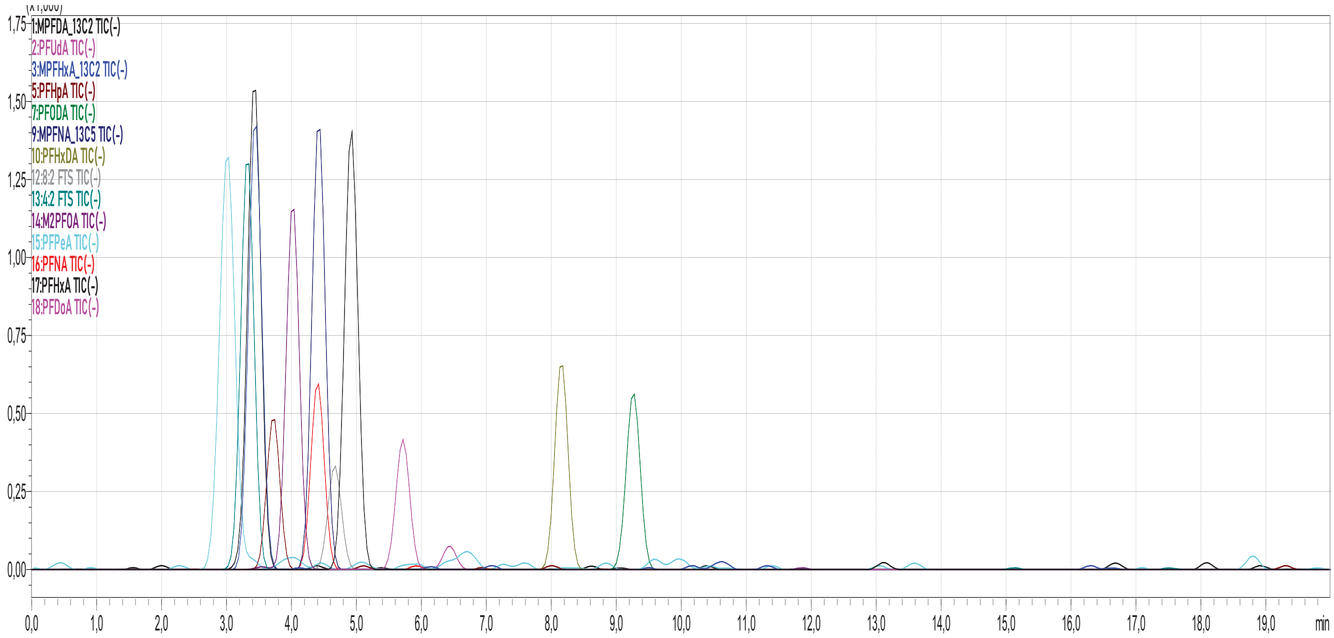

Figure 3.23: Chromatogram of mixed standards (method A).

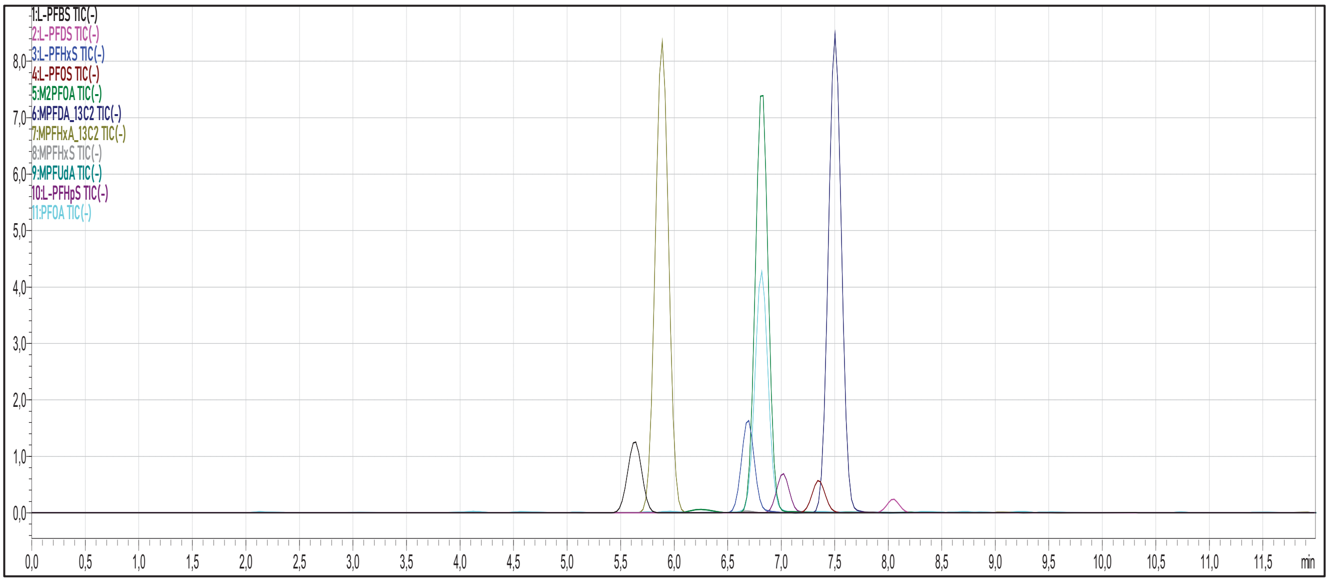

Figure 3.24: Chromatogram of mixed standards (method B)

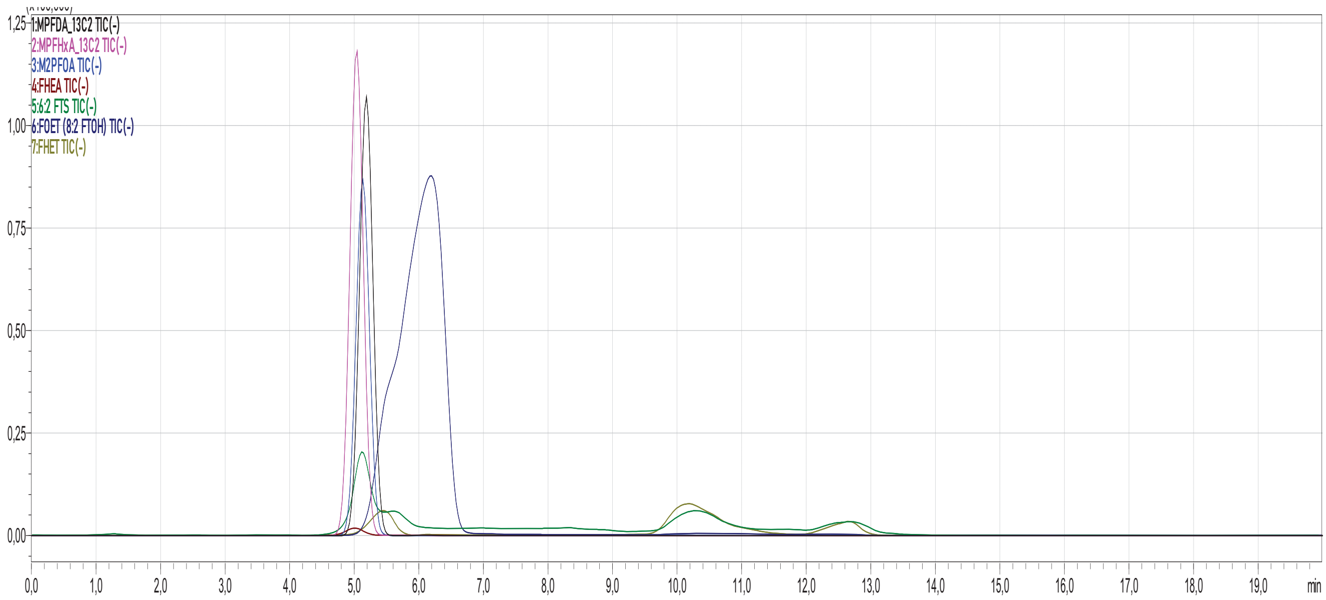

Figure 3.25: Chromatogram of mixed standards (method C)

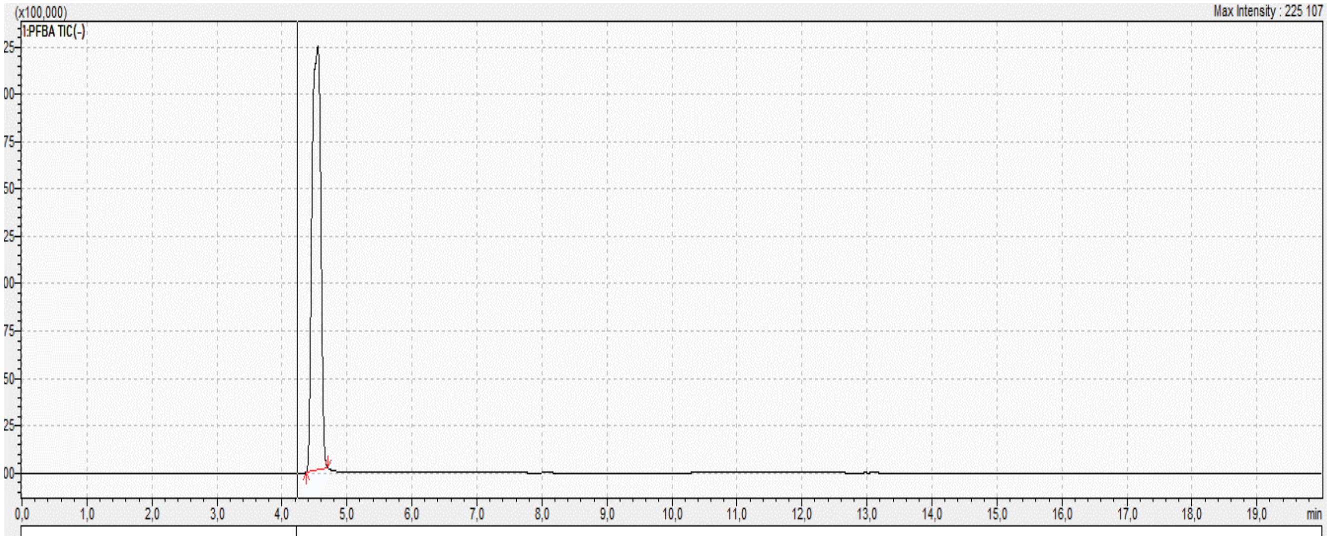

Figure 3.26: Chromatogram of PFBA standard (method D)

3.3.4 Instrument method development and optimization

The calibration curves and total ion chromatography (TIC) of the internal standards and surrogate are shown in Figures 3.27 to 3.31; and the target compounds in Figures 3.32 to 3.51. All isomers calibration curves showed linearity, based on correlation coefficients (R) and correlation of determination (R^2) that were greater than 0.99 with good precision of the internal standard.

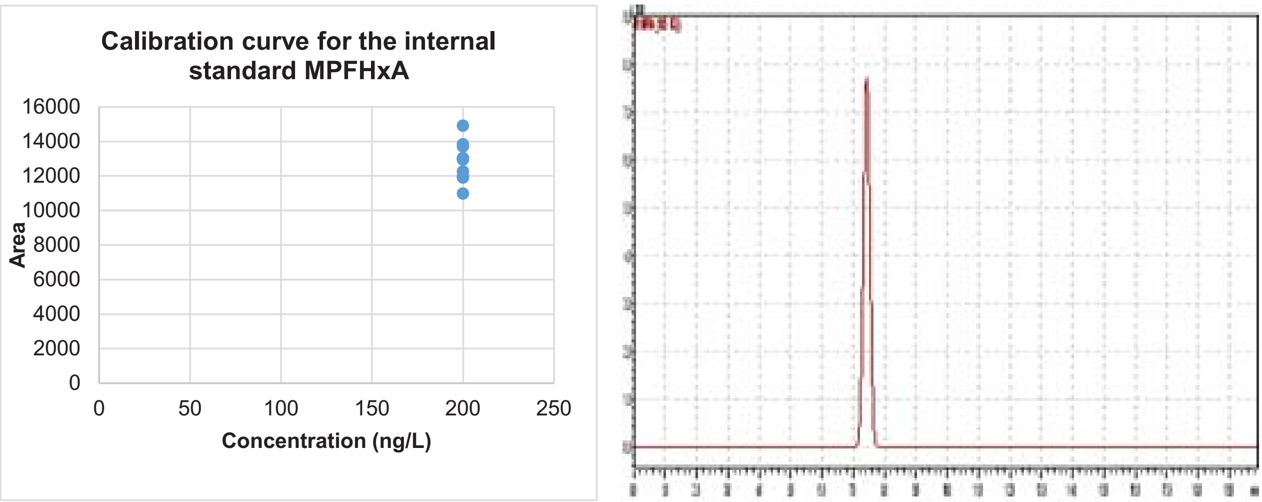

Figure 3.27: Perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid (MPFHxA_$^{13}C_2$) calibration curve (left) and TIC (right)

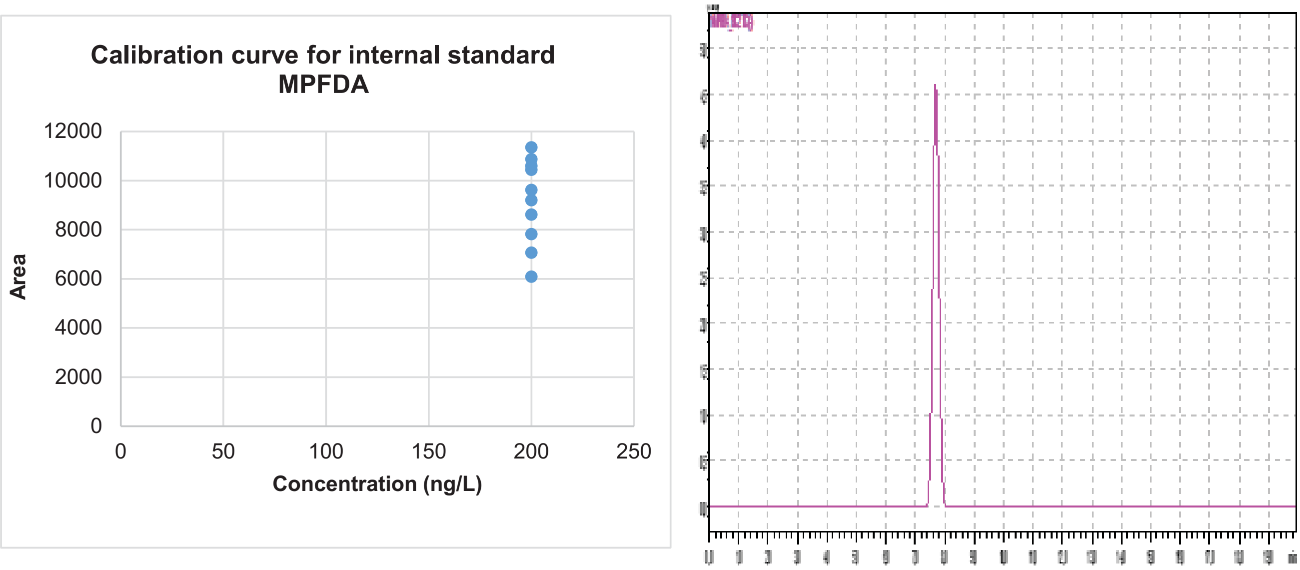

Figure 3.28: Perfluoro-n-[1,2-$^{13}C_2$] decanoic acid (MPFDA_$^{13}C_2$) calibration curve (left) and TIC (right)

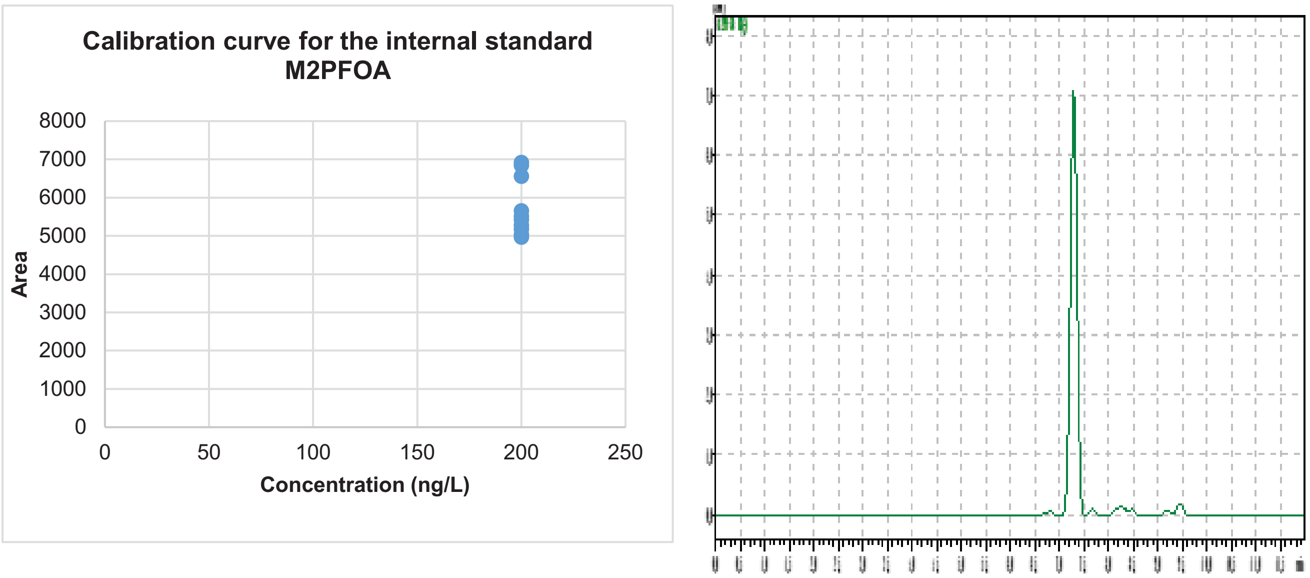

Figure 3.29: Perfluoro-n-[1,2-$^{13}C_2$] octanoic acid M2PFOA calibration curve (left) and TIC (right)

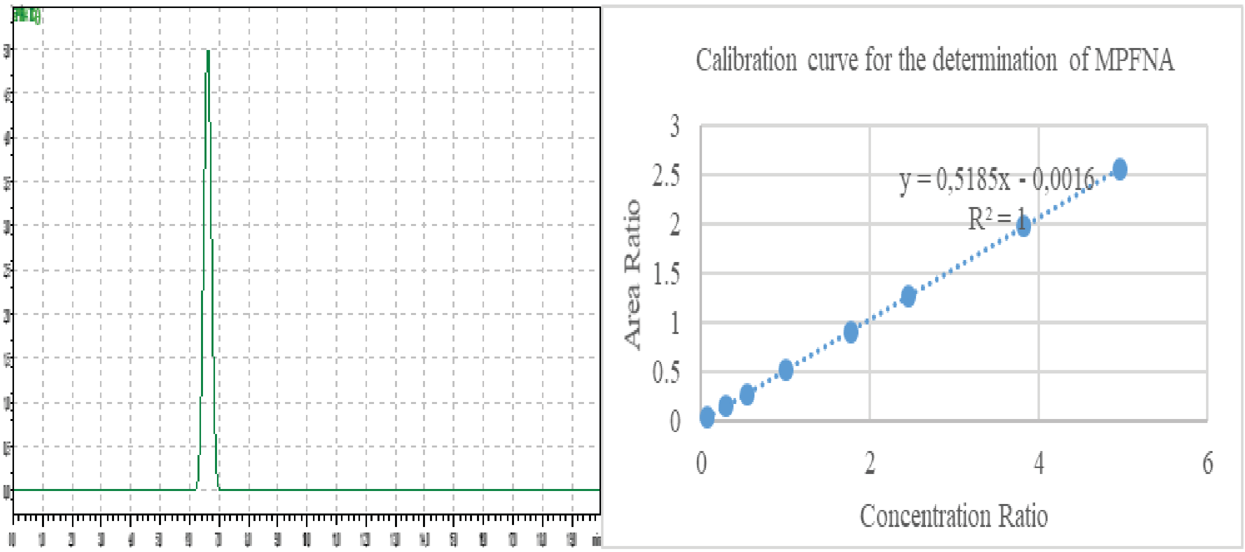

Figure 3.30: Perfluoro-n-nonanesulfonic acid (PFNA) with TIC (left) and calibration (right).

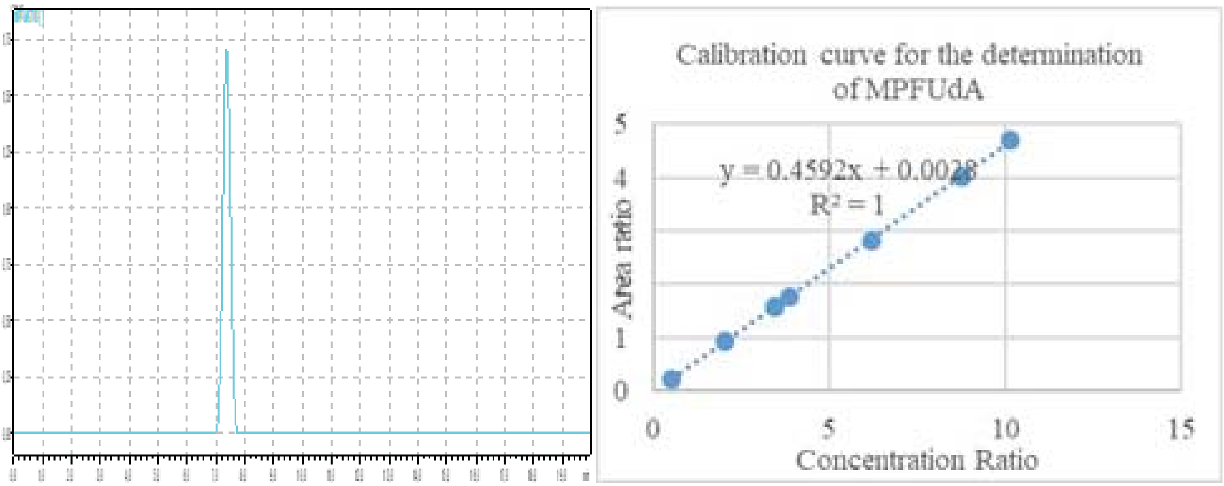

Figure 3.31: Total ion chromatogram (left) and calibration (right) for MPFUdA.

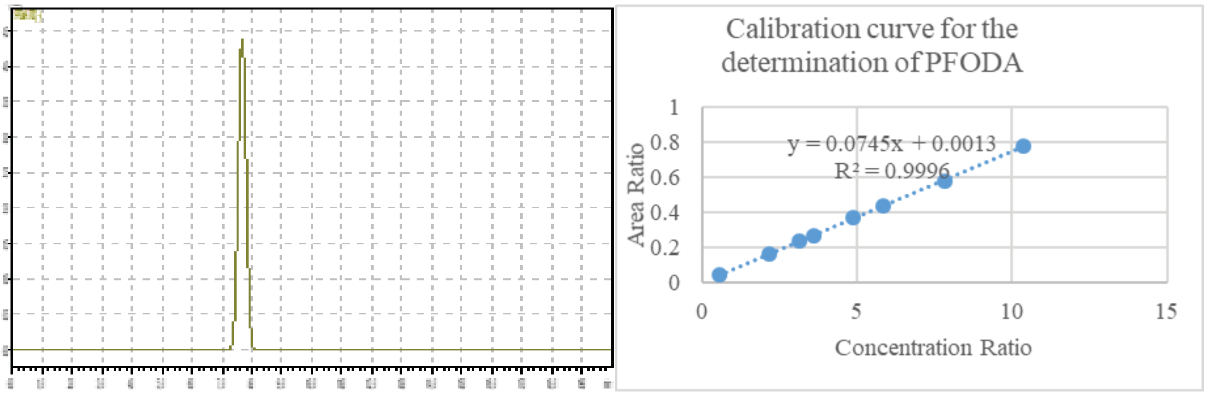

Figure 3.32: Total ion chromatogram (left) and calibration (right) for PFODA

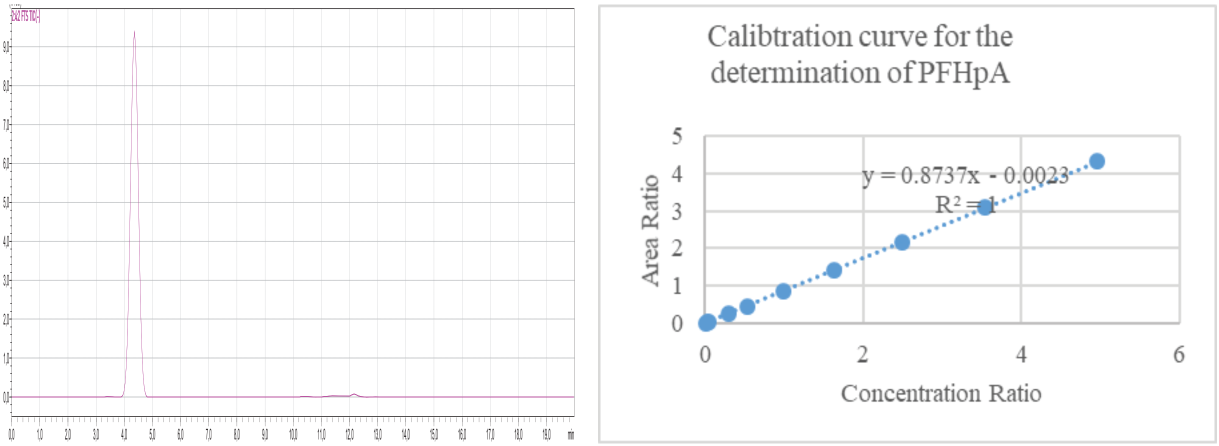

Figure 3.33: Total ion chromatogram (left) and calibration (right) for PFHpA.

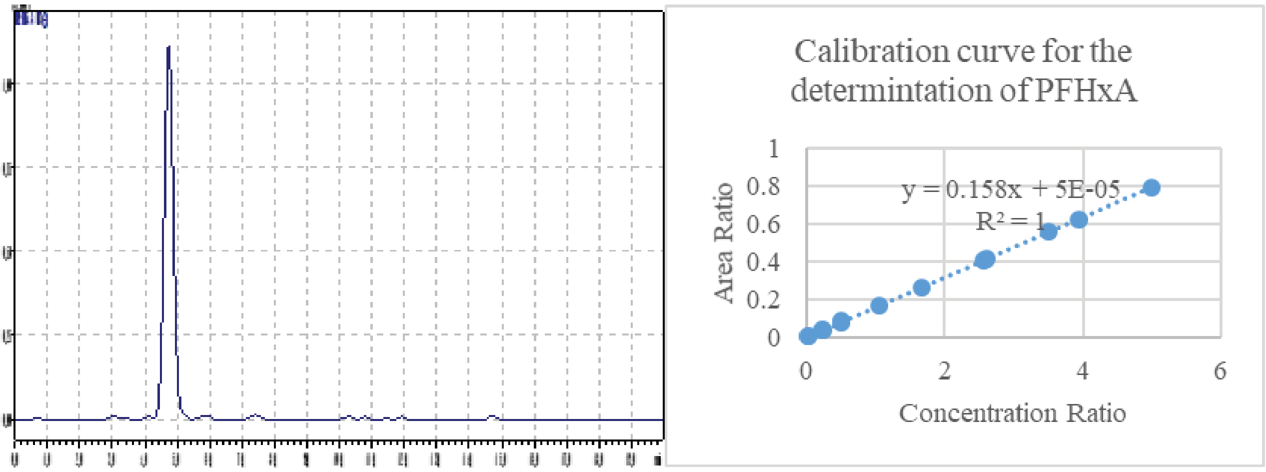

Figure 3.34: Total ion chromatogram (left) and calibration (right) for PFHxA.

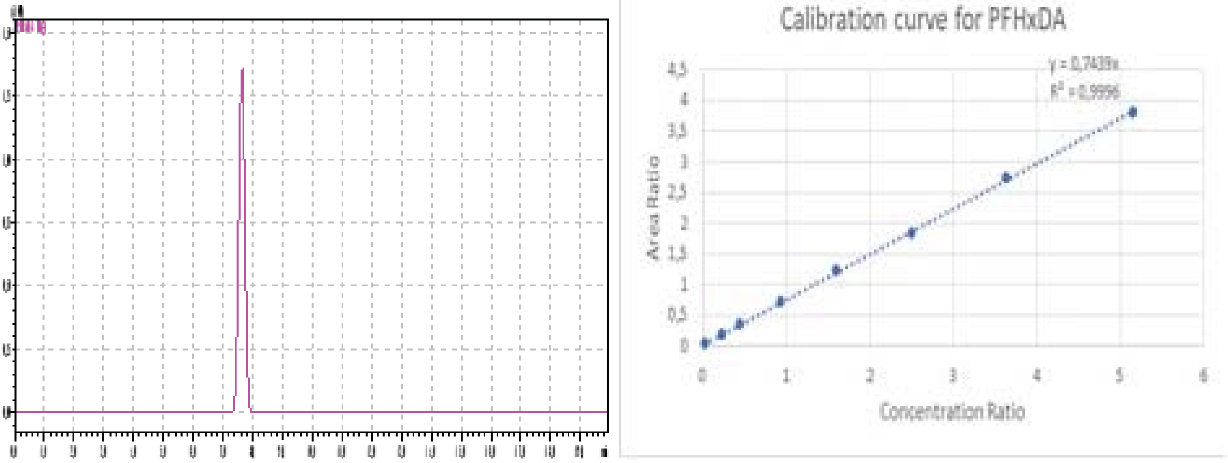

Figure 3.35: Total ion chromatogram (left) and calibration (right) for PFHxDA

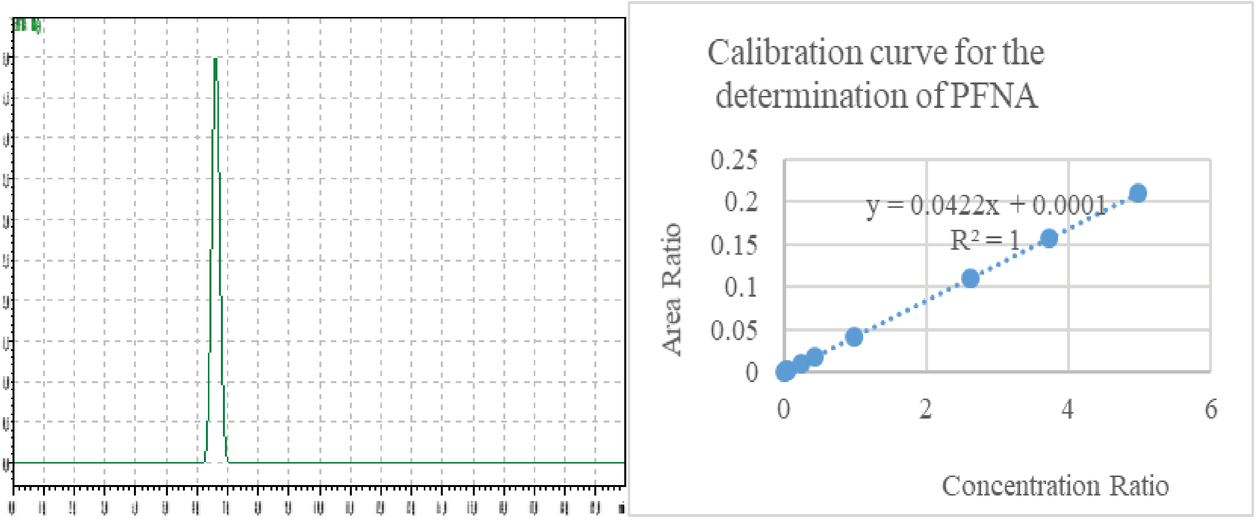

Figure 3.36: Total ion chromatogram (left) and calibration (right) for PFNA.

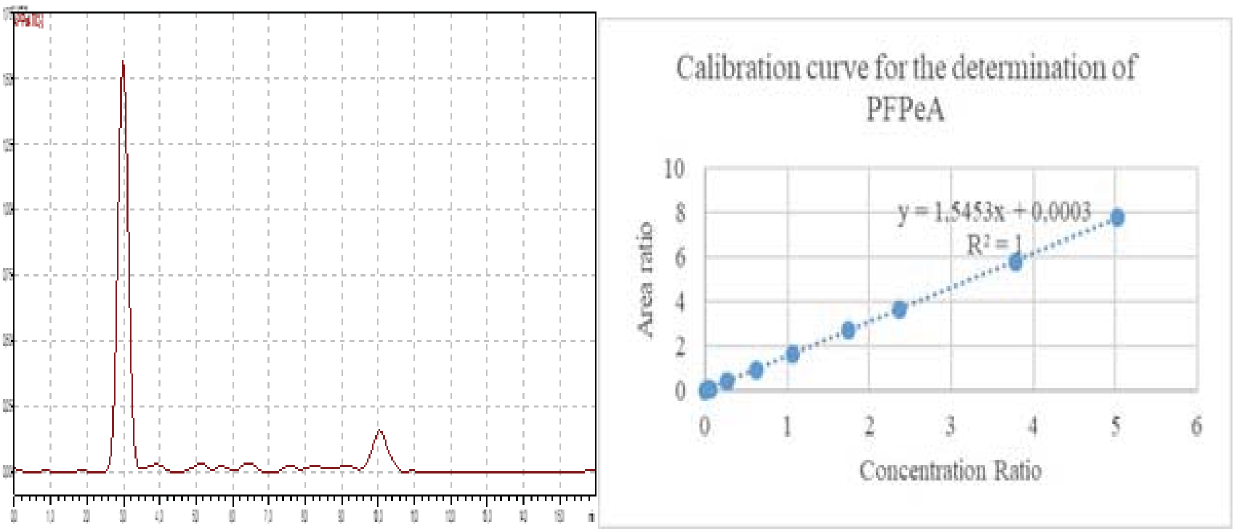

Figure 3.37: Total ion chromatogram (left) and calibration (right) for PFPeA

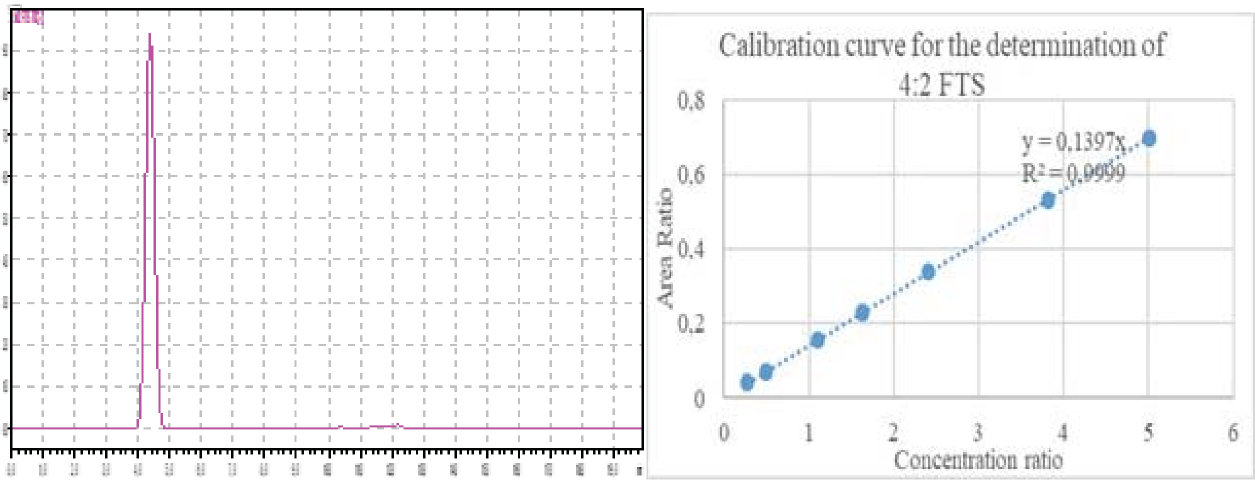

Figure 3.38: Total ion chromatogram (left) and calibration (right) for 4:2 FTS.

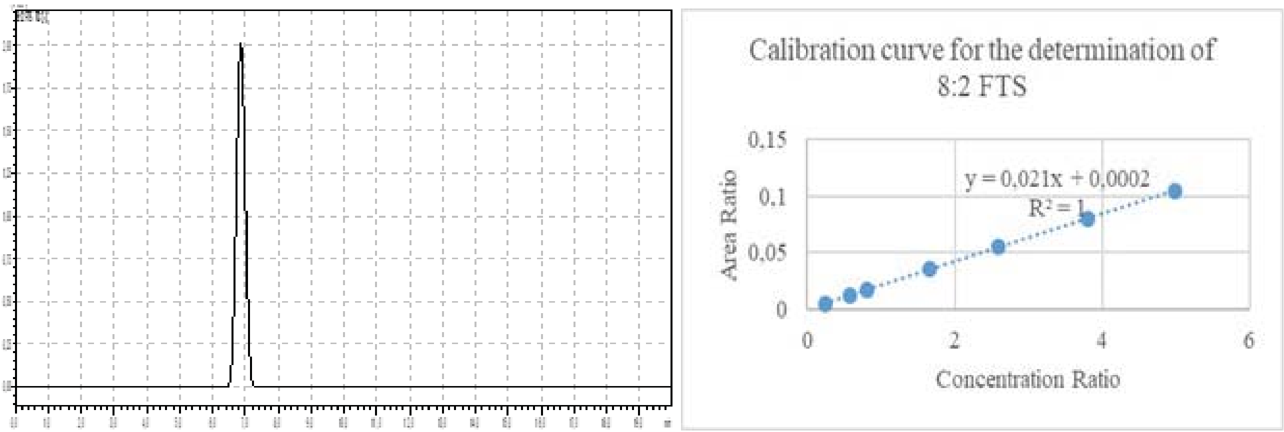

Figure 3.39: Total ion chromatogram (left) and calibration (right) for 8:2 FTS.

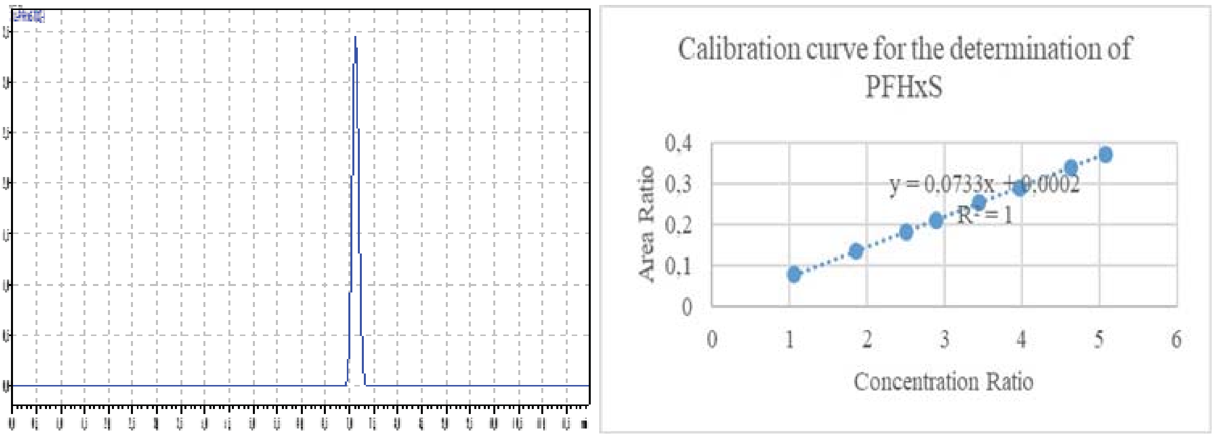

Figure 3.40: Total ion chromatogram (left) and calibration (right) for PFHxS.

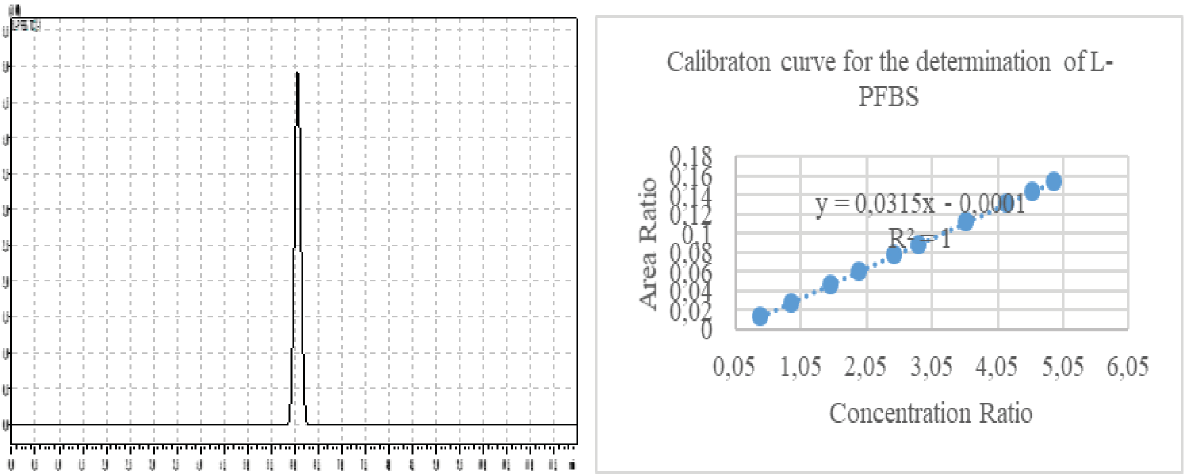

Figure 3.41: Total ion chromatogram (left) and calibration (right) for L-PFBS.

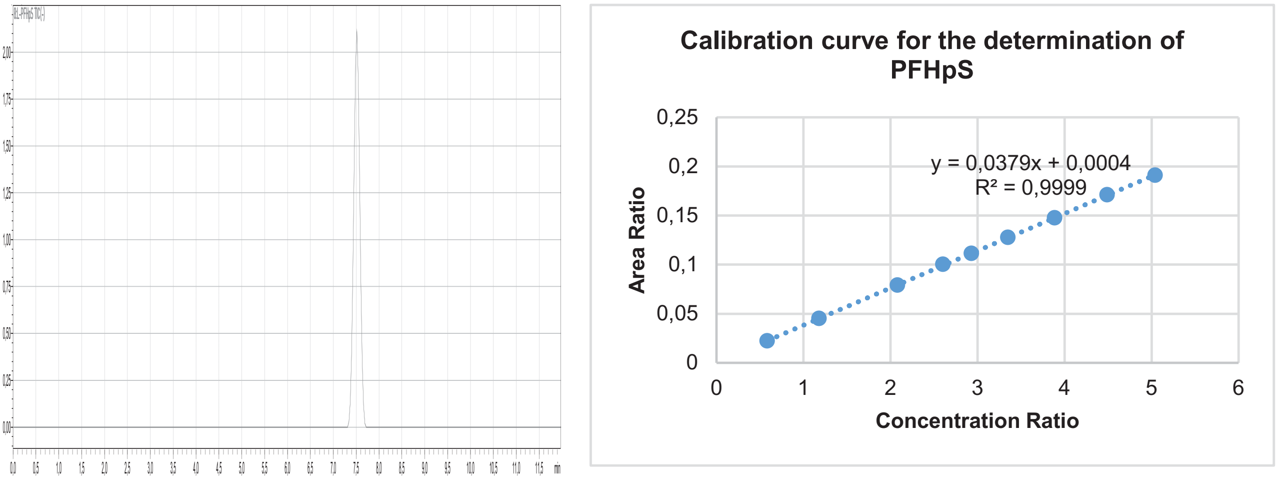

Figure 3.42: Total ion chromatogram (left) and calibration (right) for L-PFHpS.

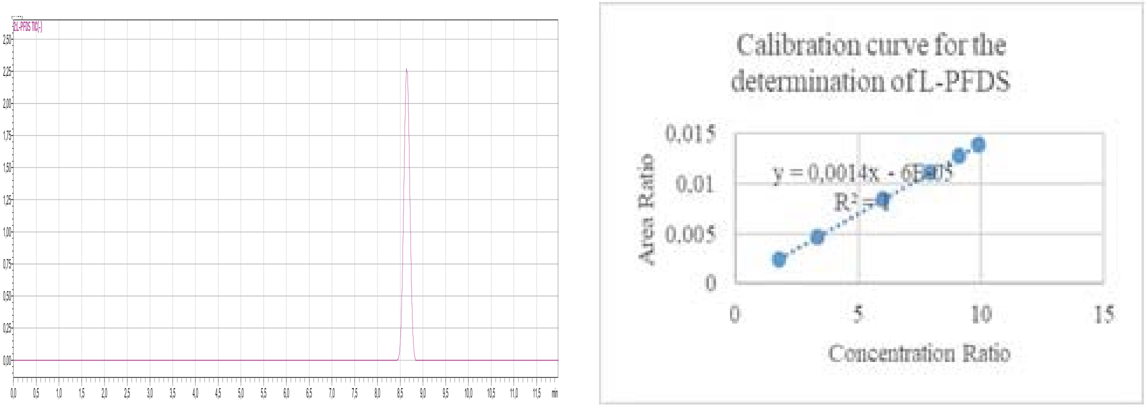

Figure 3.43: Total ion chromatogram (left) and calibration (right) for L-PFDS.

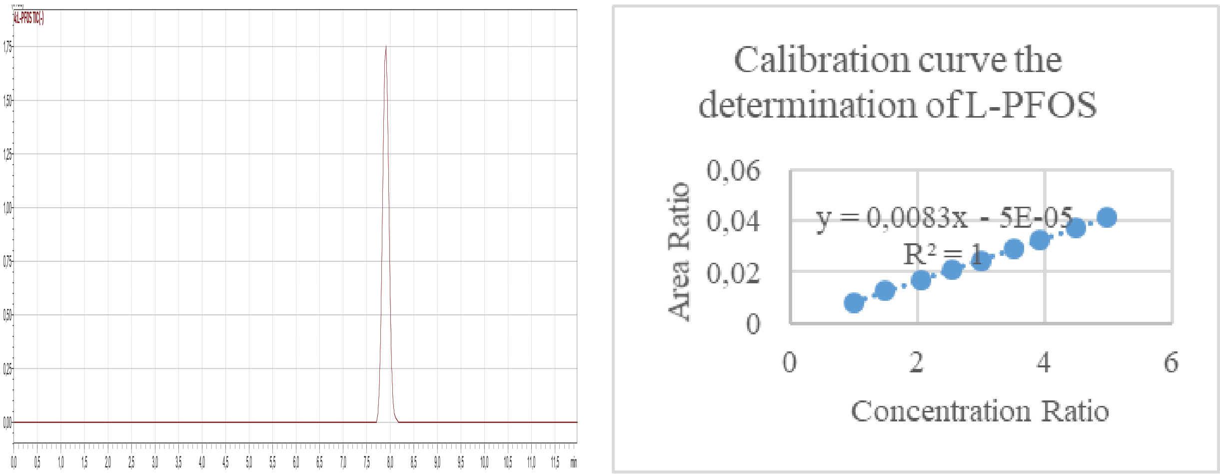

Figure 3.44: Total ion chromatogram (left) and calibration (right) for L-PFOS.

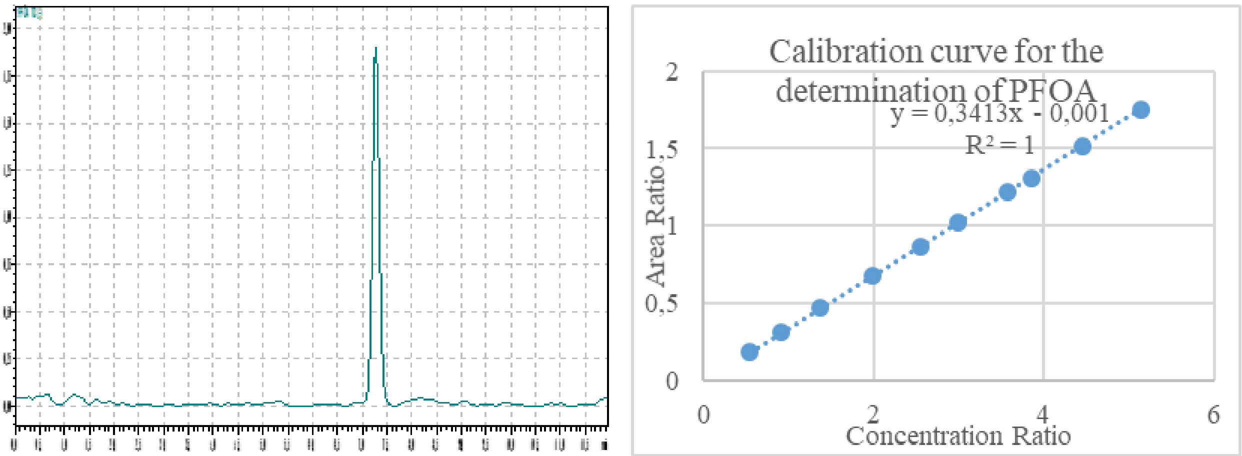

Figure 3.45: Total ion chromatogram (left) and calibration (right) for PFOA.

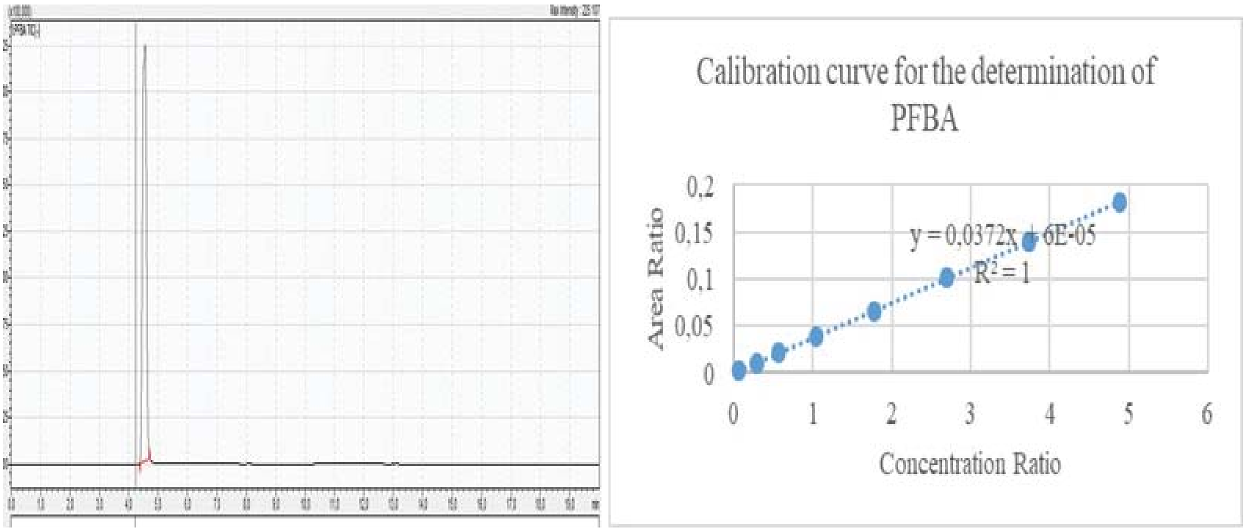

Figure 3.46: Total ion chromatogram (left) and calibration (right) for PFBA.

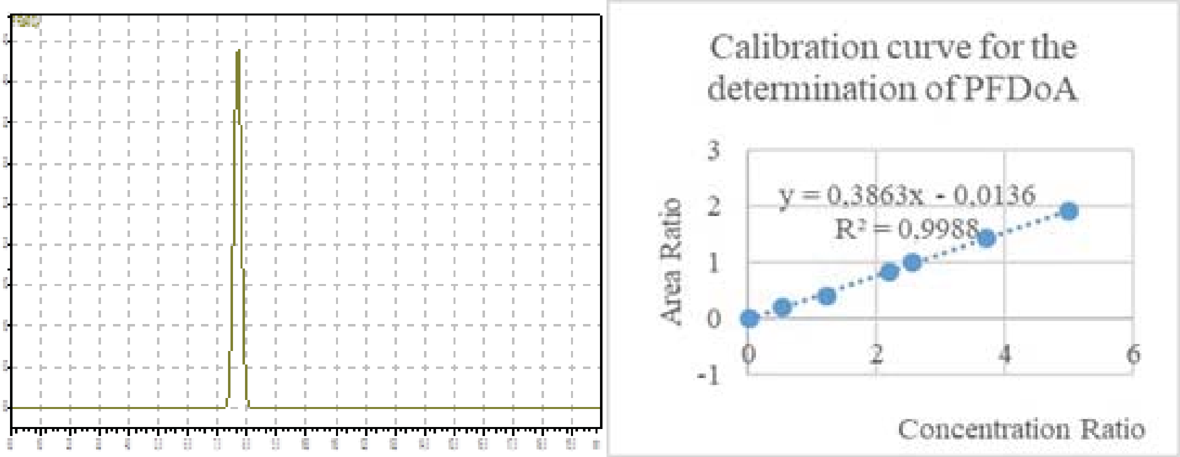

Figure 3.47: Total ion chromatogram (left) and calibration (right) for PFDoA.

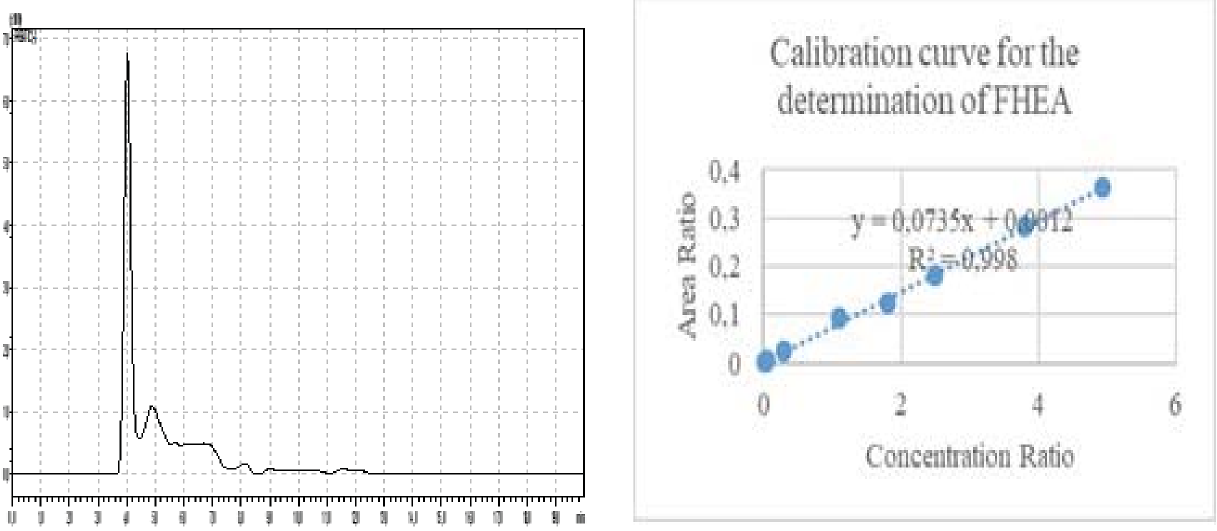

Figure 3.48: Total ion chromatogram (left) and calibration (right) for FHEA.

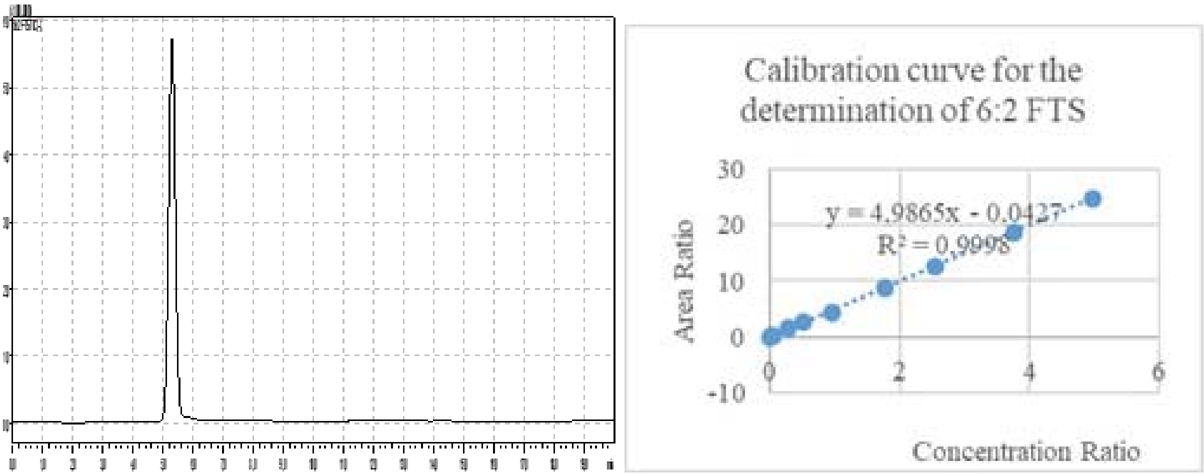

Figure 3.49: Total ion chromatogram (left) and calibration (right) for 6:2 FTS.

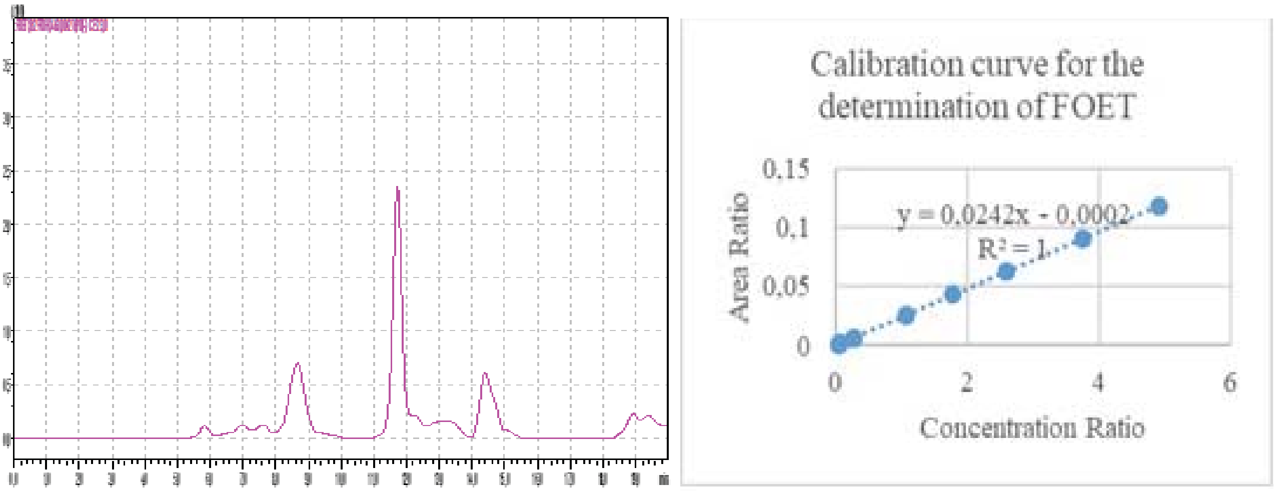

Figure 3.50: Total ion chromatogram (left) and calibration (right) for FOET.

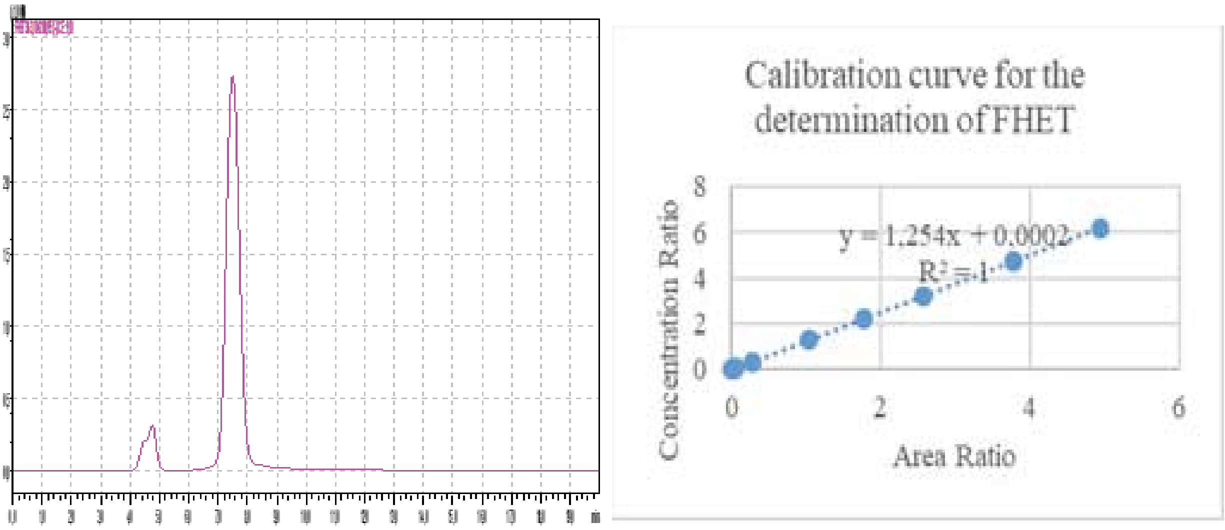

Figure 3.51: Total ion chromatogram (left) and calibration (right) for FHET.

3.3.5 Multiple reaction monitoring (MRM)

During the MRM selection, a full scan of ions was done. Ions corresponding to the compounds of interest were targeted followed by the fragmentation of the targeted ions producing a range of daughter ions. The ions corresponding to the compounds of interest were then selected and isolated from other ions within the mass spectrometer to quantitate the method. The multiple reaction monitoring results are shown in Table 3.8.

Table 3.8: MRM of precursor and product ions

Compounds	Abbreviation	Precursor ion (m/z)	Product ion (m/z)	Retention time (min)		
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid	MPFNA	467.90	423.00	4.4		
Perfluoroundecanoic acid_$^{13}C_2$	MPFUdA	565.00	520.00	8.10		
Sodium perfluoro-1-hexane[18O2]sulfonate	MPFHxS	403.00	83.95	6.60		
Perfluoro-n-undecanoic acid	PFUdA	563.00	518.95	5.72		
Perfluoro-n-hexanoic acid	PFHxA	313.00	269.00	3.43		
Perfluoro-n-pentanoic acid	PFPeA	263.00	219.05	3.03		
Sodium 1H,1H,2H,2H-perfluorohexane Sulfonate	4:2 FTS	327.00	307.05	3.33		
Sodium 1H,1H,2H,2H-perfluorodecane Sulfonate	8:2 FTS	526.90	80.95	4.70		
Perfluoro-n-heptanoic acid	PFHpA	363.00	319.00	3.72		
Perfluoro-n-nonanoic acid	PFNA	463.00	418.95	4.40		
Potassium perfluoro-1-butanesulfonate	L-PFBS	299.00	80.10	5.24		
Sodium perfluoro-1-hexanesulfonate	L-PFHxS	399.00	79.95	6.54		
Sodium perfluoro-1-octanesulfonate	L-PFOS	499.00	80.15	7.39		
Sodium perfluoro-1-heptanesulfonate	PFHpS	449.00	80.10	6.98		
Perfluoro-n-octanoic acid	PFOA	413.00	368.95	6.73		
Perfluoro-n-dodecanoic acid	PFDoA	613.00	568.90	6.46		
Perfluoro-n-octadecanoic acid	PFODA	913.00	868.90	9.25		
Sodium perfluoro-1-decanesulfonate	L-PFDS	599.00	80.20	8.34		
Perfluoro-n-hexadecanoic acid	PFHxDA	813.00	768.95	8.13		
2-Perfluorohexyl ethanoic acid (6:2)	FHEA	376.90	292.90	5.05		
Sodium 1H,1H,2H,2H-perfluorooctane Sulfonate	6:2 FTS	426.90	426.90	5.23		
8:2 fluorotelomer alcohol	FOET	463.00	216.90	14.953		
6:2 fluorotelomer alcohol	FHET	363.10	280.95	10.288		
Perfluoro-n-butanoic acid	PFBA	213.00	169.05	4.328		
Perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid	MPFHxA_$^{13}C_2$	315.00	269.95	3.36	5.56	4.36
Perfluoro-n-[1,2-$^{13}C_2$] decanoic acid	MPFDA_$^{13}C_2$	514.90	469.95	4.99	6.70	5.14
Perfluoro-n-1 2-$^{13}C_2$ octanoic acid	M2PFOA	414.80	369.90	3.99	7.62	5.18

3.3.6 Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ are shown in Table 3.9 values range from 0.0033-0.29 and 0.018-0.67 ng/L, respectively.

Table 3.9: LOD and LOQ values (ng/L) of the targeted standards

Analytes	LOD (ng/L)	LOQ (ng/L)
MPFNA	0.040	0.12
MPFUdA	0.080	0.27
MPFHxS	0.039	0.12
PFUdA	0.0059	0.018
PFHxA	0.0033	0.010
PFPeA	0.034	0.10

Analytes	LOD (ng/L)	LOQ (ng/L)
4:2 FTS	0.032	0.10
8:2 FTS	0.033	0.099
PFHpA	0.017	0.051
PFNA	0.016	0.050
L-PFBS	0.029	0.089
L-PFHxS	0.010	0.030
L-PFOS	0.034	0.10
PFHpS	0.046	0.14
PFOA	0.025	0.077
PFDoA	0.22	0.67
PFODA	0.21	0.65
L-PFDS	0.032	0.097
PFHxDA	0.62	1.9
FHEA	0.29	0.87
6:2 FTS	0.083	0.25
FOET	0.037	0.11
FHET	0.0089	0.027
PFBA	0.013	0.040

3.4 METHOD VALIDATION

3.4.1 Overview

Spiked water samples were used for method validation to assess the performance of the LC-MS method, evaluate its accuracy, and determine its limits of detection and quantification. This validation step helps confirm that the method can produce reliable results when applied to real environmental samples. This is because environmental samples can be complex, with various interfering compounds present. As such, when developing an LC-MS method for environmental analysis, it's crucial to assess and mitigate matrix effects. In this study, water samples were spiked with surrogate standards of interest (see Table 3.1 and 3.4) at known concentrations and then analysed using the developed methods to observe how matrix components affect analyte detection and quantification.

3.4.2 Water Samples Collection

Water samples were collected from all the nine provinces in South Africa in clean high-density polyethylene bottles from various water sources, wastewater treatment plant, rivers and tap water. After collection, the samples were kept in ice and transported to the Environmental-Analytical Research in Chemistry laboratory at Tshwane University of Technology, Pretoria.

3.4.3 Water Samples Extraction

Prior to extraction, 1.2 mL of spiking surrogate standards was added to 200 mL of both water and wastewater samples, including blanks, to make a resulting concentration of 200 ng/L, before passing the solutions through SPE. For wastewater, samples were first filtered using a 0.45 μm glass fibre filter on a vacuum filtration unit before SPE extraction in order to remove suspended matter which tends to block the cartridges if not removed.

The SPE SupelcoTM Envi18 cartridges purchased from SIGMA Aldrich Ltd were used for extraction of PFASs. Cartridges were first conditioned by 5 mL of LC-grade methanol followed by 5 mL of ultra-pure water. Without allowing the cartridges to dry, samples were passed through the cartridges under a vacuum flow rate of 10-15 mL/min. Thereafter, the sample bottles were rinsed with 7 mL of ultra-pure water and the rinse water of each sample was passed through the cartridge. The cartridges were then allowed to dry under vacuum for an 1 h.

During elution step, sample bottles were rinsed using 10.00 mL of methanol and analytes eluted from the cartridge by pulling the 10.00 mL of methanol through the sample transfer tubes and cartridges. The solvent was allowed to exit the cartridge under the force of gravity. The solvent extract was then concentrated under the gentle steam of nitrogen with an aid of sonication at temperatures between 60-70°C. One millimetre of methanol was then added to the dried sample extract and vortexed for 1 min. The reconstituted extract was then transferred to a 2 mL centrifuge tubes, and the extract centrifuged for 5 min. A 950 µL of the extract and a 50.00 µL of 1000 ng/L of internal standard were added to an autosampler vial. A 10.00 µL of the samples was then injected to the LC-MS/MS.

3.4.4 Quantification of PFAs Concentrations using Targeted Analysis

The chromatographic conditions developed were used to calculate the final concentrations of PFASs in the water samples using the following formula:

$$\mathbf{A_{nat}/A_{IS} \times 1/RRT \quad \times \ M_{IS}/SS} \qquad \textbf{(Equation 3.1)}$$

where: A_{nat} = area of surrogate standard; A_{is} = area of internal standard; M_{IS} = mass of internal standard (ng); RRF = slope or gradient in the calibration curves; SS = sample size (mL).

The RRF is obtained when the ratio of response for the unit amount of the contaminant of interest to the response of the IS and is expressed in equation below:

$$\mathbf{RRF= \ A_{NAT}/A_{IS} \ \times \ C_{IS}/C_{NAT}} \qquad \textbf{(Equation 3.2)}$$

where: A_{NAT} is peak area of the native ($^{13}C_2$) compound; A_{IS} is the peak area of the internal standard in the standard. C_{NAT} is the concentration of the native standard; C_{IS} is the internal standard concentration.

3.4.5 Identification of Emerging and Legacy PFASs using Non-targeted Analysis

For non-target identification of PFASs, the MS acquisition was performed and operated in a full-scan TOF-MS using Information Dependent Acquisition (IDA). Figure 3.52 depicts the general workflow employed in identifying PFASs compounds in water samples. The IDA method consisted of the survey scan type (TOF-MS) that was a full-scan mass spectrum between m/z 100-950 and the information dependent acquisition type (MS/MS), a product ion scan in which the system selects ions automatically without any ion predefined by the user.

Both the full-scan TOF-MS and MS/MS mode through IDA were operated within a single-run analysis. The workflow used in this study involved suspect screening and considered evidence reported in the literature to identify legacy and emerging PFASs in different water samples, and such evidence was based on the actual mass, library score of >70%, the presence of fragment ions, homologues mass difference, mass error (mDa) and retention times.

As an example, Figure 3.53 shows the XIC, MS and MS/MS chromatograms of PFOA which showed 100% library score.

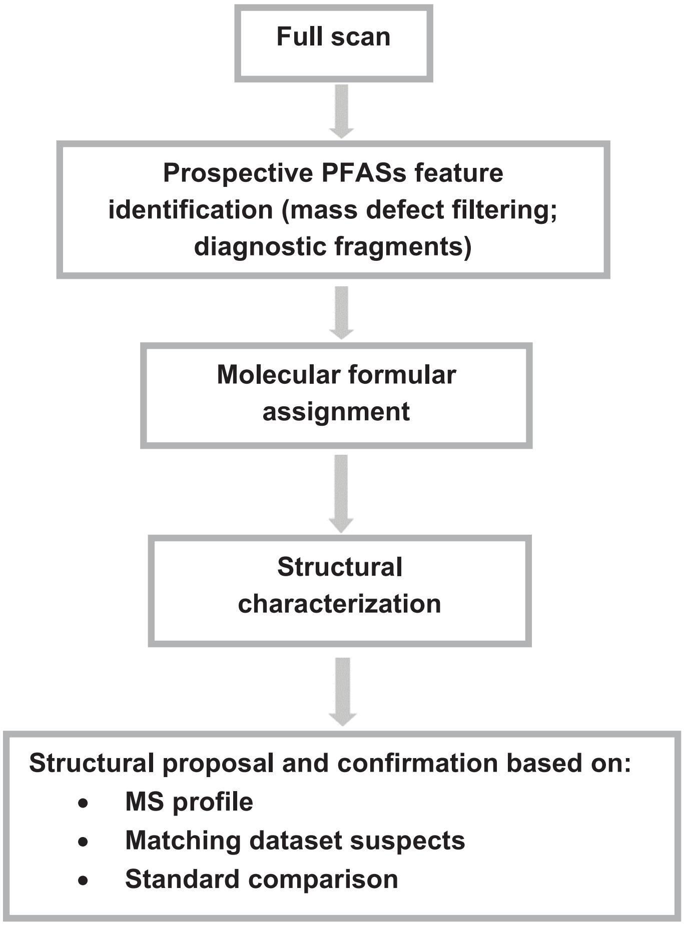

Figure 3.52: General schematic workflow for non-target PFAS by TOF-MS.

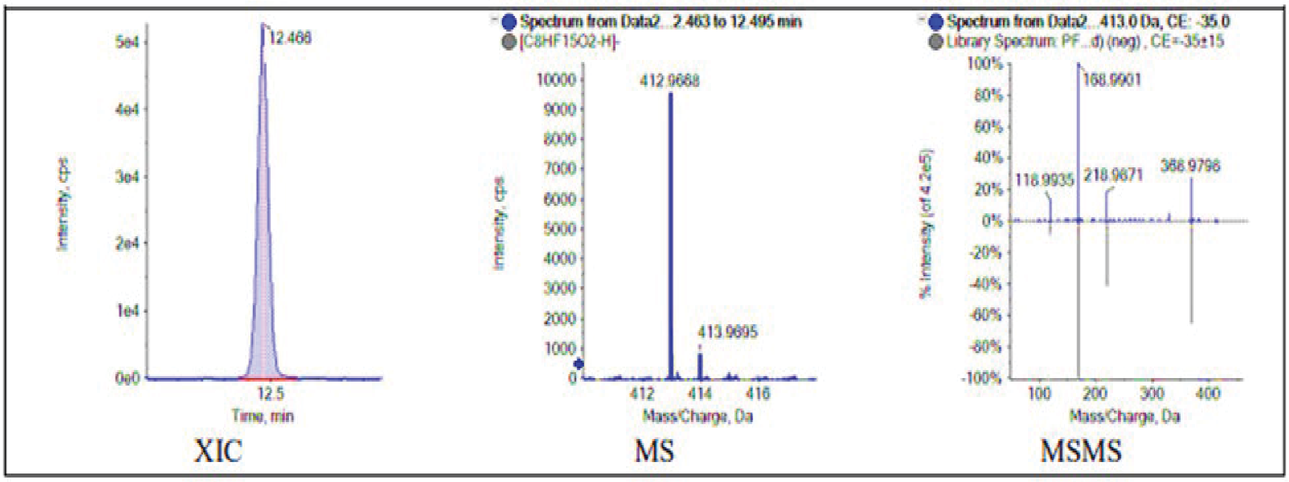

Figure 3.53: PFOA, measured in A 1000 ng/L standard with a library score of 100.0%. (a) Shows an extracted ion chromatography at RT 12.466 min. (b) Shows m/z = 412.9668 for [M-H]- and monoisotopic mass of 413.9695 for [M-H+1]-. (c) Shows fragments observed with m/z 118.9935, 168.9901, 218.9671 and 368.9766.

3.4.6 Quality assurance and method validation

Field blanks were prepared during sample collection following the same procedure used for environmental samples to ensure there was no contamination during sample preparation. During the analyses of samples, solvent and laboratory blanks were prepared and analysed in between samples after every tenth sample to avoid carry over and a 100 ng/L standard was analysed after every 20th sample in the batch. The percentage relative standard deviation (%RSD) was then calculated to determine the precision of the method. The water and blanks samples were spiked with the surrogate standard and the recoveries of each sample calculated. Samples from each matrix groups, including procedural blanks, were used for validation of the optimized method. Each sample matrix, spiked and unspiked, were prepared in quadruple and samples were spiked with analytes (low, high and medium concentrations) of interest within-day to check for recoveries and relative standard deviation (%RSD). A total of 12 procedural blanks were treated in the same manner as the samples. Analyte recoveries were determined from each matrix groups. Recoveries for spiked blanks ranged from 67.5-116.1%. Additionally, Certified Reference Material (European Commission – Joint Centre Institute for Reference Materials and measurements Retieseweg 111, B-2440 Geel (Belgium) (CRM IRMM-428) was used for further validation of the extraction method. Before use, the CRM was re-homogenised by using ultrasonication for 10 min under room temperature. A 100 mL aliquo was subsampled immediately after shaking the CRM. The extraction procedure used for sample extraction described in Section in 3.4.3 was followed. Shown in Table 3.10 are the mean percentage recoveries of PFASs in procedural blanks at low, medium and high spikes.

Table 3.10: Percentage recoveries of PFASs in procedural blanks

	Procedural blanks (n = 4)					
Target analytes	**Low spike (5 ng/L)**		**Medium spike (800 ng/L)**		**High spike (1600 ng/L)**	
	Mean%Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD
PFNA	106.5	5.94	83.6	15.7	116.1	2.3
PFudA	82.6	3.57	99.2	24.4	71.7	2.9
8:2 FTS	103.0	0.46	101.0	2.0	86.4	1.6
PFHpA	113.8	14.99	94.2	14.2	98.1	9.7
4:2 FTS	97.6	20.42	89.2	1.9	85.9	14.4
PFDoA	97.3	17.81	94.2	2.5	95.6	11.9
PFHxA	93.2	21.37	90.9	23.0	95.8	2.16
PFPeA	73.9	13.27	83.4	6.0	86.4	5.6
PFODA	115.4	1.75	73.5	6.8	97.2	0.0
PFBA	104.8	9.74	102.0	13.8	81.9	1.7
PFHxDA	77.5	4.74	77.0	6.6	78.7	0.5
FHET	124.7	3.52	97.	16.5	88.6	2.5
FOET	126.1	1.52	104.3	6.4	94.8	7.4
6:2 FTS	102.7	23.50	110.5	1.0	89.9	7.9
FHEA	116.6	2.75	76.3	3.3	80.1	0.3
PFDS	109.7	22.03	88.7	30.3	86.2	17.1
PFBS	67.5	14.60	103.5	2.8	80.7	4.1
PFHxS	120.5	3.60	97.2	13.2	100.9	6.1
PFOS	107.1	23.08	77.2	3.2	98.3	6.2
PFHps	75.7	3.79	94.5	11.4	90.7	12.4
PFOA	85.0	3.09	105.1	16.1	92.3	1.9

The mean percentage recoveries ranged from 67.5-120.5, 76.3-110.5 and 71.7-116.1 at low, medium and high spiking concentrations respectively. These ranges are within the acceptable range of 50-150 percentage recovery. The percentage relative standard deviations are, in most instances below the acceptable value of 20% except the values for 6:2 FTS (23.50), PFDS (22.03) and PFOS (23.08) all at low spiking concentrations; PFDS (30.3) and PFHxA (23.0) at medium concentrations.

The recoveries observed for drinking, surface and borehole water samples ranged from 69.0-117.1% with %RSD <30% (Table 3.11). For surface water samples, PFDoA exhibited the lowest analyte recoveries of 51.4%. The observed low recovery of PFDoA may have originated from adsorption of PFDoA on solid surfaces since a number of the surface water had some suspended solids. Analyte recoveries observed for borehole samples ranged from 70.0-144.0%. The recoveries of PFASs in influent and effluent of drinking water treatment plant (DWTPs) and these ranged from 59.1-130.1% (Table 3.12).

Table 3.13 shows the recoveries of PFASs from wastewater treatment plants (WWTPs) samples. As can be seen in Table 3.13, the recoveries ranged within the acceptable range of 50-150%. However, lower recoveries of 43.3% and 48.8% in the effluent and influent respectively at high spikes were observed for PFDoA. Percent RSD >30% was observed for PFHxA and PFODA in effluent and influent samples at %RSD of 32.5% and 37.0% respectively, both at low spike. With the exception of PFDoA, high recoveries of other PFASs from the water samples were observed and these high recoveries validated the extraction method used in the present study. However, certified reference material (CRM) has been order and this will be used for further validation of the extraction method.

Table 3.14 shows the recoveries of PFASs compounds in the certified reference water samples. The percentage recoveries and uncertainty range from 78-112% and 0.7-1.7 ng/L respectively. The observed instrument concentrations are generally close to or slightly higher than the certified reference values. The high recoveries obtained with the spiking methods as well as the CRM validate the method used in quantifying PFASs compounds in the water samples.

Table 3.11: Percentage recoveries of spiked PFASs standards from drinking, surface and borehole water samples

Target analytes	Drinking water (n =4)						Surface water (n=4)						Borehole (n=4)					
	Low spike (5 ng/L)		**Medium spike (800 ng/L)**		**High spike (1600 ng/L)**		**Low spike (5 ng/L)**		**Medium spike (800 ng/L)**		**High spike (1600 ng/L)**		**Low spike (5 ng/L)**		**Medium spike**		**High spike**	
	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD
PFNA	76.0	3.3	100.3	10.5	109.6	7.3	84.0	5.5	85.1	8.0	72.0	3.3	93.9	10.9	100.5	9.2	89.0	3.2
PFudA	102.7	15.0	98.8	7.4	104.3	10.7	85.2	20.7	57.1	0.9	77.8	2.0	111.3	16.2	98.6	1.8	83.7	0.1
8:2 FTS	80.9	16.8	98.6	2.2	90.5	23.9	91.0	18.3	87.3	3.3	84.1	1.2	109.1	1.2	101.1	2.1	101.1	1.0
PFHpA	100.5	16.8	68.8	1.4	91.2	6.4	85.5	13.1	77.9	1.2	81.6	2.2	96.2	4.2	83.0	11.8	100.0	3.2
4:2 FTS	117.1	8.4	82.4	4.2	83.6	2.4	113.4	7.5	83.4	2.9	88.1	8.3	110.1	7.9	105.6	2.6	92.0	7.3
PFDoA	104.1	28.5	87.0	18.8	92.0	9.4	**51.4**	11.8	72.5	2.0	96.7	7.4	135.7	5.8	85.1	1.3	87.6	6.1
PFHxA	101.1	1.4	106.4	5.3	89.0	5.0	86.7	14.4	91.8	15.0	99.5	6.2	123.0	6.0	91.1	12.7	102.7	2.1
PFPeA	108.2	5.2	89.1	3.3	74.6	0.4	98.8	1.1	74.7	10.3	88.8	19.1	123.5	5.3	90.4	17.3	91.1	6.3
PFODA	69.0	0.5	85.9	9.1	83.4	16.7	128.6	6.3	99.9	10.5	83.1	16.5	106.6	0.6	94.4	12.4	98.5	0.4
PFBA	101.6	18.7	88.5	8.7	71.0	1.5	98.0	22.0	102.2	7.3	83.8	2.0	80.2	26.0	105.2	4.0	70.0	2.1
PFHxDA	102.5	13.6	77.1	6.7	69.6	1.2	97.6	23.3	77.4	1.7	83.5	23.3	82.5	27.1	104.9	22.7	70.2	1.3
FHET	105.1	9.8	98.7	10.7	86.4	1.0	108.1	16.5	100.8	9.4	75.8	4.2	88.0	25.9	108.8	12.9	94.3	7.3
FOET	106.0	12.4	95.4	13.7	90.1	17.4	116.4	7.4	80.6	0.6	86.0	1.7	78.0	3.5	99.6	6.4	96.3	0.5
6:2 FTS	70.9	9.6	85.9	6.3	69.8	0.7	94.4	3.1	83.9	8.7	92.3	6.4	117.5	6.1	85.4	9.2	93.6	10.6
FHEA	75.2	26.6	76.3	1.4	88.4	12.9	114.3	4.1	90.7	7.5	96.5	13.6	144.0	6.8	119.6	2.6	99.7	1.1
PFDS	106.2	6.4	80.8	6.0	80.2	20.3	103.1	2.8	83.2	8.5	98.0	16.2	84.8	3.2	93.3	0.2	83.1	2.1
PFBS	84.1	5.6	108.9	7.3	90.1	4.1	119.4	4.8	92.9	3.5	89.5	15.1	106.5	6.6	89.1	10.1	80.7	3.3
PFHxS	90.3	17.1	88.2	7.4	78.0	8.2	116.5	16.3	95.6	9.9	89.7	9.1	106.3	10.2	88.5	12.0	85.4	6.4
PFOS	100.9	5.5	99.4	5.3	80.3	2.4	74.8	17.8	82.4	4.9	91.9	0.7	93.4	8.2	105.1	6.5	100.7	0.4
PFHps	109.7	1.6	84.7	11.6	72.7	15.1	103.6	24.5	96.4	1.1	98.8	2.8	100.6	1.8	103.4	8.6	97.2	4.1
PFOA	91.8	8.9	93.1	17.8	92.4	6.9	73.5	3.7	93.5	3.3	88.3	23.8	106.7	24.6	95.2	4.9	86.4	4.5

Table 3.12: Percentage recoveries of spiked PFASs standards from drinking water influent and effluent samples

Target analytes	DWTP-Influent						DWTP-Effluent					
	Low spike		**Medium spike**		**High spike**		**Low spike**		**Medium spike**		**High spike**	
	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD
PFNA	124.4	19.7	97.5	17.7	108.7	6.8	66.5	9.9	92.5	12.3	102.8	7.8
PFudA	92.9	10.7	72.5	11.7	60.1	8.6	110.7	3.1	105.9	0.3	93.2	2.1
8:2 FTS	79.8	13.1	93.2	3.6	94.5	7.4	78.6	33.5	103.1	2.6	107.6	3.3
PFHpA	105.3	2.4	67.8	0.7	78.7	11.8	128.6	12.2	70.1	3.5	85.0	7.1
4:2 FTS	124.7	1.1	108.7	2.9	74.0	2.1	111.1	15.9	90.8	5.4	84.9	13.0
PFDoA	130.1	15.1	88.4	4.6	101.2	15.4	93.9	0.1	113.3	3.6	103.2	11.2
PFHxA	103.2	26.6	74.9	12.9	104.4	8.1	106.4	6.2	107.9	0.1	102.9	8.6
PFPeA	63.5	26.8	78.8	1.7	75.3	6.6	70.3	20.1	76.9	4.8	94.6	10.7
PFODA	77.4	5.5	70.3	17.5	79.2	7.6	64.3	2.1	83.5	0.1	76.8	10.7
PFBA	93.6	19.3	84.9	20.2	96.9	5.2	104.8	3.5	80.2	4.2	84.0	12.1
PFHxDA	73.7	3.3	88.0	6.4	122.8	3.9	119.1	8.3	91.5	6.4	85.1	10.8
FHET	104.2	25.7	103.9	3.7	88.0	4.2	88.7	27.6	74.8	15.2	97.2	11.1
FOET	127.8	22.1	95.0	7.3	76.3	11.4	112.1	3.5	91.2	1.8	80.6	0.0
6:2 FTS	110.9	2.4	75.5	2.6	73.5	1.7	98.5	19.4	93.6	1.1	80.8	3.7
FHEA	125.0	1.8	108.5	6.7	91.6	24.9	112.8	7.2	83.6	8.0	86.2	12.3
PFDS	78.8	24.7	66.8	3.9	89.1	28.4	101.9	13.8	68.0	0.4	88.2	14.4
PFBS	115.2	6.7	92.4	9.2	105.5	4.3	94.2	29.9	90.8	3.1	83.9	2.1
PFHxS	116.2	6.3	107.5	5.4	91.4	4.0	110.2	10.6	89.5	14.4	89.1	9.0
PFOS	124.7	5.1	67.8	12.9	93.5	4.3	97.4	27.8	77.4	0.1	91.5	22.8
PFHps	101.0	16.9	85.9	2.6	97.5	3.9	92.5	30.4	91.2	3.8	93.8	25.2
PFOA	108.4	2.8	93.4	10.6	84.0	7.8	59.3	15.9	94.6	5.6	83.5	14.9

Table 3.13: Percentage recoveries of spiked PFASs standards from wastewater influent and effluent samples

Target analytes	WWTP-Effluent						WWTP- influent					
	Low spike (5 ng/L)		Medium spike (800 ng/L)		High spike (1600 ng/L)		Low spike (5 ng/L)		Medium spike (800 ng/L)		High spike (1600 ng/L)	
	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec.	%RSD	Mean %Rec	%RSD	Mean %Rec	%RSD	Mean %Rec.	%RSD
PFNA	94.5	25.5	95.6	16.9	84.6	0.1	100.4	1.3	107.6	13.0	78.0	4.1
PFudA	71.5	20.1	96.0	2.1	92.2	20.8	93.0	4.1	71.3	2.7	50.4	11.2
8:2 FTS	84.4	20.2	80.2	13.7	83.9	3.3	80.6	29.7	107.7	1.0	108.3	2.5
PFHpA	71.8	2.5	80.1	19.2	89.1	21.4	116.1	20.0	92.3	19.3	82.6	19.6
4:2 FTS	123.0	4.5	109.5	3.0	69.4	5.5	100.5	5.1	95.9	3.9	93.5	25.9
PFDoA	95.7	23.1	100.1	15.3	**43.3**	2.5	86.0	8.7	79.1	5.0	**48.8**	24.9
PFHxA	99.5	**32.5**	104.4	5.0	100.0	1.4	110.4	13.8	111.5	3.6	98.8	5.2
PFPeA	113.1	19.8	99.2	4.9	93.2	11.6	128.6	9.8	95.0	4.3	77.9	8.3
PFODA	58.5	4.3	81.3	25.0	78.9	8.9	84.4	**37.0**	75.4	0.0	76.8	14.9
PFBA	72.2	22.1	87.8	3.8	83.0	2.9	111.5	5.2	93.3	28.9	86.6	1.5
PFHxDA	109.7	9.0	69.9	9.3	79.1	19.4	106.0	14.6	76.3	0.7	73.4	14.9
FHET	95.2	21.9	95.3	5.5	87.7	7.2	83.4	19.0	101.0	0.1	96.8	19.6
FOET	105.0	1.8	90.9	5.5	71.0	16.3	83.4	19.0	101.0	0.1	96.8	19.6
6:2 FTS	98.9	2.4	95.6	4.8	96.1	14.1	87.1	3.4	104.2	12.4	104.7	10.0
FHEA	87.2	21.4	87.0	2.6	93.8	13.5	109.9	25.7	98.3	2.0	82.5	13.0
PFDS	78.0	4.6	86.4	12.0	81.3	7.1	87.6	13.1	110.7	8.4	101.5	7.4
PFBS	123.6	2.3	94.3	15.0	80.9	8.7	83.7	20.0	78.0	3.9	107.6	6.9
PFHxS	81.8	18.3	94.1	10.2	98.6	19.4	96.3	1.9	100.8	3.9	101.6	0.4
PFOS	93.3	12.4	79.7	9.9	94.7	0.4	82.1	10.1	76.8	11.7	80.2	4.7
PFHps	96.4	10.8	91.4	18.0	99.0	5.1	90.5	5.8	95.8	10.2	80.6	0.9
PFOA	111.8	3.2	99.6	5.8	102.0	2.2	79.5	20.9	99.5	12.5	84.4	2.6

Table 3.14: Certified reference material recoveries (CRM IRMM-428)

	Certified value concentration (ng/L)	Uncertainty concentration (Certified value) (ng/L)	Instrument concentration (ng/L)	%RSD	%Recovery
PFBS	5.5	1.4	5.4±1.1	19.5	98
PFHxS	3.6	1.0	4.0±0.7	17.6	112
PFOS	9.6	1.7	11.6±0.7	6.0	121
PFPeA	4.0	1.0	3.1±0.5	15.5	78
PFHxA	7.4	1.0	8.2±1.0	12.5	110
PFHpA	3.7	0.7	3.5±0.4	10.5	97
PFNA	3.9	1.4	4.2±0.5	12.0	109

CHAPTER 4: CONCLUSIONS

4.1 CONCLUSIONS

All isomers calibration curves showed linearity, based on correlation coefficients (r) and correlation of determination (r^2) that were greater than 0.99 with good precision of the internal standard. The chromatograms were well separated. The percentage recoveries of the labelled surrogate standards were within the acceptable range of 50-150 ng/L. Calibration curves for PFUdA, PFDoA, PFHxA, PFNA, PFPeA, PFBA, MPFNA, 4:2 FTS, 6:2 FTS, 8:2 FTS, FHET, FHEA and FOET ranged from 1-1000 ng/L, while that of L-PFBS, L-PFOS, PFOA, L-PFHpS, and L-PFHxS ranged from 100-1000 ng/L and that of MPFUdA, MPFHxS, L-PFDS, PFODA and PFHxDA ranged from 100-2000 ng/L were maintained. Furthermore, the LOD and LOQ values that ranged from 0.0033-0.29 and 0.018-0.67 ng/L respectively. Based on these results, the following can be concluded:

- Non-targeted and targeted analytical methods for identification and quantification of PFASs in various water sources were successfully developed;
- The developed method was optimized and validated using spiking method and certified reference material;
- The developed method was applied to extract PFASs from the following water sample types; wastewater, drinking water, groundwater (boreholes), surface water (rivers and dams), bottled water and rainwater.
- High percentage recoveries obtained indicated high accuracy and sensitivity of the developed method
- Both grab and passive sampling approaches can be used for PFASs monitoring in water environments
- Both analytical measurements and multivariate analyses are necessary to establish an understanding of the sources, levels, transport and fate of PFAS compounds within water environments.

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