An UHPLC-MS/MS Quantitative Method for Trace Analysis of Per- and Polyfluoroalkyl Substances (PFASs) in Environmental Media from Alabama Estuaries

by

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Abstract

Per- and polyfluoroalkyl substances (PFASs) comprise a large group of anthropogenic industrial compounds that are highly persistent due to their chemical structure. Because of the wide use of PFASs in textile manufacturing, surfactant applications, coatings, and aqueous film–forming foams (AFFF) since the 1950s, some PFASs are ubiquitous in the environment and often found in environmental matrices. Analytical studies of PFASs are challenging due to the sophisticated analytical systems required to perform these studies, the lack of quantitative, validated analytical methods, and the limited availability of standards for quantitation of some PFASs. Standards for the quantitation of legacy PFASs are widely available, thus legacy PFASs are well studied. Emerging PFASs, however, remain understudied due to limited standard availability. The aim of this study was to develop a UHPLC-MS/MS analytical method to quantitate a selection of both legacy and emerging PFASs in a single experimental run. After chromatographic conditions were optimized, full scan, single ion monitoring (SIM) scan, product ion (PI) scan, and multiple reaction monitoring (MRM) scan were conducted to optimize MS conditions. The developed and optimized method is amenable to analysis of 23 target analytes including 13 perfluoroalkyl carboxylic acids (PFCAs), 8 perfluoroalkane sulfonates (PFSAs), and 2 perfluoropolyethers (PFPEs). The method demonstrated acceptable mean percent recoveries of 90.46% ± 4.09% and 103.94% ± 3.49% at two spiking levels (2 ng/L and 10 ng/L) of standard mixtures. Slight carry over issues were

observed but can be avoided in future work. A sample extraction and cleanup strategy was designed and optimized using Oasis PRiME HLB 6cc extraction cartridges during sample purification. The developed quantitative method was applied to surface water samples collected from the Perdido Bay estuary. Eight out of twenty-three compounds were detected, with average total PFAS concentrations of 12.75 ng/L and 12.84 ng/L in July 2017 and October 2017, respectively.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADONA	3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]
AFFF	Aqueous film–forming foams
CE	Collison energy
CEC	Contaminants of emerging concern
CLC	Capillary liquid chromatography
EDC	Endocrine disrupting chemical
ESI	Electron ionization source
FV	Fragmentor voltage
FUSLE	Focused ultrasound solid-liquid extraction
GIWW	Gulf Intracoastal Waterway
HFPO-DA	2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid
LC	Liquid Chromatograph
LC/MS	Liquid Chromatograph/Single Quadrupole Mass Spectrometer
LC/MS/MS	Liquid Chromatograph-tandem Mass Spectrometer
LOD	Limit of detection
LOQ	Limit of quantitation
MAE	Microwave assisted extraction
MPFHxA	Perfluoro-[1,2-13C2]hexanoic acid
MS	Mass Spectrometer
PAP	Polyfluoroalkyl phosphoric acid ester
PEEK	Polyetheretherketone

PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonate
PFCA	Perfluoroalkyl carboxylic acid
PFDA	Perfluorodecanoic acid
PFDoA	Perfluorododecanoic acid
PFDoS	Perfluorododecanesulfonic acid
PFDS	Perfluorodecane sulfonate
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptane sulfonate
PFHxA	Perfluorohexanoic acid
PFHxDA	Perfluorohexadecanoic acid
PFHxS	Perfluorohexane sulfonate
PI	Product ion
PFNA	Perfluorononanoic acid
PFNS	Perfluorononylsulfonate
PFOA	Perfluorooctanoic acid
PFODA	Perfluorooctadecanoic acid
PFOS	Perfluorooctylsulfonate
PFPeA	Perfluoropentanoic acid
PFPE	Perfluoropolyether
PFPeS	Perfluoropentylsulfonate
PFPiA	Perfluoroalkyphosphinic acid
PFSA	Perfluoroalkane sulfonate
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid

PFUnDA	Perfluoroundecanoic acid
PLE	Pressurized liquid extraction
PTFE	Polytetrafluoroethylene
SEME	Solid-phase microextraction
SIM	Single ion monitoring
SPE	Solid phase extraction
UHPLC-MS/MS	Ultrahigh-performance liquid chromatography-tandem mass spectrometry

Chapter 1: Introduction

1.1 Background

Estuaries are shallow transitional areas between fresh water and seawater with highly dynamic physicochemical conditions. Approximately 50% of the world's human population live or work adjacent to estuaries (Costanza, Kemp, & Boynton, 1993). Release of contaminants from municipal, industrial, and agriculture sources are increasing because of rapid population growth near estuaries, potentially affecting wildlife and humans living in or near estuarine systems (Vidal-Dorsch Doris et al., 2012). Many organic micropollutants in surface water are contaminants of emerging concern (CECs) at very low concentrations. Some CECs are also known or suspected endocrine disrupting chemicals (EDCs), including pesticides, natural and synthetic steroid hormones, alkylphenols, polychlorinated biphenyls, and some per- and polyfluoroalkyl substance (PFASs). EDCs can affect endocrine and reproductive functions in organisms (Mulabagal, Wilson, & Hayworth, 2017).

PFASs are synthetic organic chemicals that have fully or partially fluorinated hydrophobic alkyl chains ranging from C4 to C14 with polar end groups such as carboxylates, sulfonates, or phosphonates; PFASs can be anionic, zwitterionic, cationic, and neutral. The hydrophilic end functional groups significantly reduce the surface tension of PFASs. These structural characteristics impart thermal and chemical stability to PFASs.

These unique properties have led to the use of PFASs for a wide range of industrial applications (Lin et al., 2016; Onghena et al., 2012).

1.2 Study Area

Estuarine water samples used in this study were collected from 16 locations in the Perdido Bay system. (Figure 1.1). Perdido Bay covers approximately 50 mi^2 with 1250 mi^2 drainage area in Florida and Alabama (Handley, Altsman, & DeMay, 2007). Perdido Bay is approximately 33 mi in length, 3 miles in width, and 10 ft in depth. Perdido Bay is connected to the Gulf of Mexico through Perdido Pass, and the Gulf Intracoastal Waterway (GIWW) passes through the southern portion of the bay (The Nature Conservancy, 2014). The primary source of freshwater to the bay is the Perdido River, which supplies more than

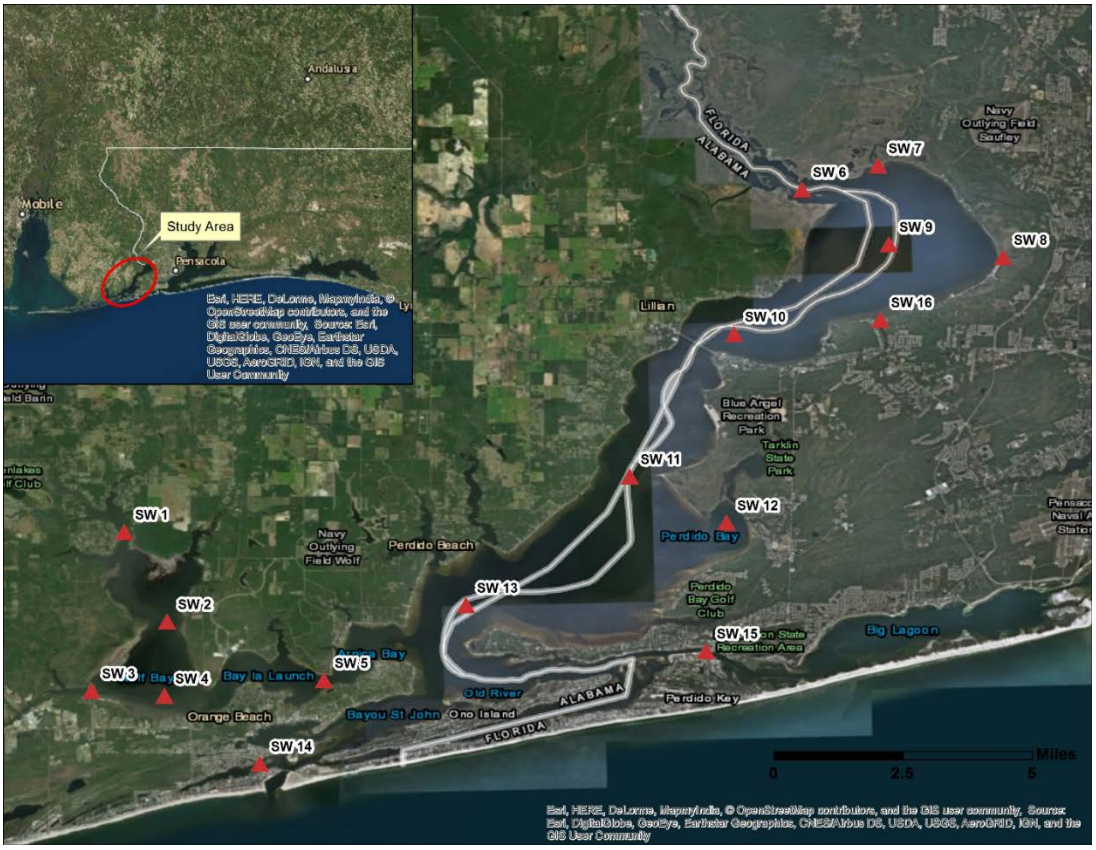


Figure 1.1: Study area (Perdido Bay system) with 16 sampling locations.

70% of freshwater inflow at around 2200 ft^3/s. The Perdido River is approximately 70 miles long, and forms the border between Florida and Alabama. In Alabama, the Perdido River and tributaries Dyas Creek, Hollinger Creek, Styx River, and Blackwater River constitute the major surface water features (The Nature Conservancy, 2014). As a consequence of its small size relative to the size of its watershed, Perdido Bay experiences rapid changes in water quality in response to rainfall, wind, and tides (Macauley, Engle, Summers, Clark, & Flemer, 1995).

1.3 Overview of PFASs

The term "PFASs" is used to collectively describe all fluorinated hydrocarbons (USEPA, 2018a). PFASs comprise a variety of chemicals including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonates (PFSAs), perfluoropolyethers (PFPEs), polyfluoroalkyl phosphoric acid esters (PAPs), perfluoroalkyl phosphinic acids (PFPiAs), and others (Ahrens & Bundschuh, 2014). Several properties of PFASs, including their dielectric constant, resistance to heat and chemical agents, and low surface energy and friction, make them useful industrial chemicals world-wide (OECD, 2013; Turner & Millward, 2002). For example, PFASs have been utilized as flame-retardants for nearly half a century. The term "legacy PFASs" describes compounds that were predominately used prior to 2000. Increasing regulatory attention and action since then has led to the replacement of many legacy PFASs with new "emerging PFASs" (Sun et al., 2016).

1.3.1 History of PFASs

The industrial production of PFASs in the United States was estimated to be nearly 100,000 tons from 1970 to 2002, and the total demand for PFASs in one year (2004) was

between 50,000 and 100,000 metric tons (USEPA, 2009). PFASs are used in a variety of industrial processes and commercial products (Kissa, 2001). PFASs can be found in fire-fighting foam, food containers, coatings of nonstick cookware, waterproof clothing, stain-resistant furniture and painting materials. Due to their high resistance to thermal and chemical decomposition, these substances can remain intact in the environment for decades. Significant attention has been focused on PFOA and PFOS, (a legacy PFSA and PFSA, respectively) which represent the majority of PFASs identified in the environment, although these two compounds were phased-out of commercial production in the U.S. in 2002 (Corsini, Luebke, Germolec, & DeWitt, 2014). Figure 1.2 summarizes some of the important events of PFASs in the United States.

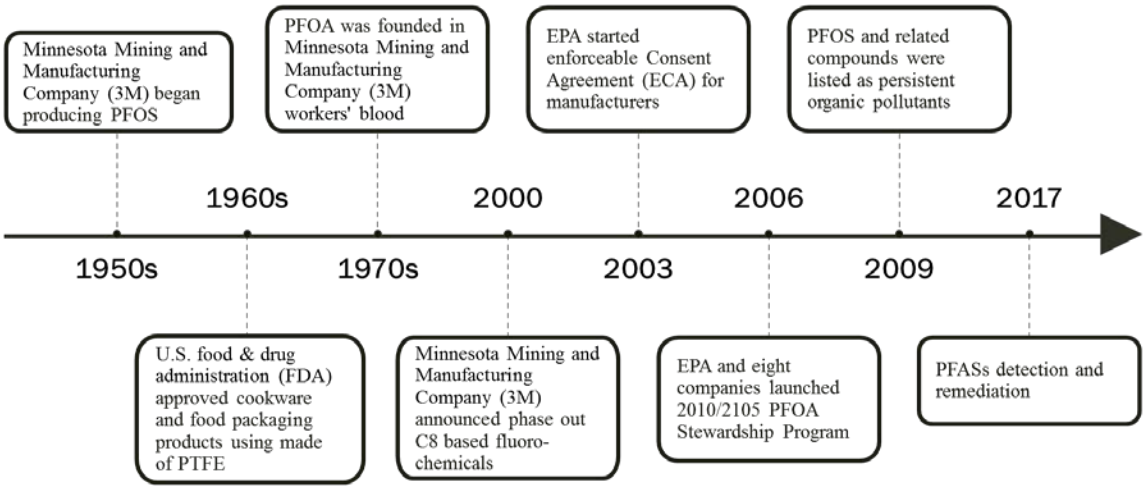


Figure 1.2: Important events in the history of PFASs in the U.S. (Lindstrom, Strynar, & Libelo, 2011).

1.3.2 Legacy PFASs

Many scientific studies have focused on PFCAs and PFSAs, which are two major classes of legacy PFASs (Rahman, Peldszus, & Anderson, 2014; Rayne & Forest, 2009; Vestergren, Herzke, Wang, & Cousins, 2015). PFCAs and PFSAs share the same chemical skeleton, consisting of a partially or fully fluorinated alkyl chain with different functional

groups (carboxylic and sulfonic acids attached to PFCAs and PFSAs, respectively). Legacy PFASs (such as PFOA, and PFOS) have been used extensively since 1940s (Webster, 2010). Both groups exhibit hydrophobic characteristics; PFCAs are stronger acids, while PFSAs are more polar due to their sulfonic acid or sulfonate groups. PFOA and PFOS are the most studied legacy PFASs, since they have a relatively long history of substantial use and are common degradation products of many precursor PFASs (Webster, 2010).

1.3.2.1 PFOS and PFOA

In 2006, concern over the potential risk legacy long-chain PFASs pose to humans and environmental health led eight major companies to take action to reduce the production of PFOA, PFOS and their precursor chemicals (USEPA, 2016b). In an investigation by the EPA in 2014, 11% of lakes and rivers studied nation-wide had PFOS tissue concentrations exceeding 40 ppb. This discovery led the EPA to recommend limiting human consumption (USEPA, 2016a). In 2016, the EPA released drinking water guidance of 40 ng/L lifetime exposure for the sum of PFOS and PFOA (USEPA, 2016b).

1.3.3 Emerging PFASs

Since 2000, shorter chain, emerging PFASs began replacing phased out PFASs (such as PFOS and PFOA) (Sun et al., 2016; USEPA, 2016b). For example, PFPEs are emerging alternatives to PFCAs and PFSAs, comprised of repeating perfluorinated methyl-, ethyl- or isopropyl-ether units and various end-groups (Lorenzo, 2012). Although novel PFASs were manufactured for their supposed degradation potential, these emerging PFASs may also be persistent in the environment (Gomis, Wang, Scheringer, & Cousins, 2015). Manufacture of emerging PFASs continues to grow as industrial users focus on the

economic feasibility of these replacement PFASs rather than their potential environmental effects (Danish EPA, 2015).

1.4 PFASs in Environment

PFASs have been found world-wide in sediment, soil, surface water, wastewater, sewage sludge, and biota (Bečanová, Komprdová, Vrana, & Klánová, 2016; Buck et al., 2011; Campo, Masia, Pico, Farre, & Barcelo, 2014; Chen et al., 2016; Gomez, Vicente, Echavarri-Erasun, Porte, & Lacorte, 2011; Martinez-Moral & Tena, 2013; Myers et al., 2012; Pan, Zhao, et al., 2014). For example, a selection of recently reported concentrations of PFASs in natural environmental samples is shown in Table 1.1. As an example from Table 1.1, high concentrations of PFHpA and PFOA were detected in fish from a retail fish market (Gosetti et al., 2010). This finding is significant because these fish are available for human purchase and consumption.

1.5 Toxicity of PFASs

Although the potential adverse effects of PFASs on wildlife and humans are poorly understood, available evidence suggests some PFASs can interrupt normal endocrine activity (Attina et al., 2016; Bergman et al., 2013; Slotkin, MacKillop, Melnick, Thayer, & Seidler, 2008). Exposure to PFASs may induce immunosuppressive effects (Impinen et al., 2018), interfere with metabolism of fatty acids (Yu et al., 2016), change cholesterol levels (Corsini et al., 2014), influence gene expression (Wei et al., 2008), impact fecundity (Fei, McLaughlin, Lipworth, & Olsen, 2009), modify membrane integrity (Rodea-Palomares et al., 2015), and disrupt thyroid functions (Ren et al., 2015). Studies have shown that the toxicokinetics of PFASs can be species and gender-specific (Gribble et al., 2015).

Table 1.1: A selection of recently reported PFASs in natural water systems.

Analytes		Sample types										
Compound name	Acronym	River, ng/L	Groundwater, ng/L	Tap Water, ng/L	Well Water, ng/L	Rain, ng/L	Surface Water, ng/L	Soil, ng/g	Sediment, ng/g	Biota, ng/g	Fish, ng/kg	Serum, ng/L
Perfluorobutyric acid	PFBA	n.a.-0.8	<0.01-9.15	-	-	n.a.	9.7	-	-	-	-	-
Perfluorobutane sulfonate	PFBS	-	<1.11-4.87	<0.30	<0.30	0.0231-0.058	13	n.a.	<0.05	0.2	n.a.	n.a.
Perfluoropentanoic acid	PFPeA	n.a.	<0.44-2.15	-	-	<0.01	12	-	n.a.	n.a.	n.a.	n.a.
Perfluorohexanoic acid	PFHxA	n.a.	0.8-4.9	<0.52	<0.52	0.008-0.079	20	n.a.-12.4	0.06	0.2	-	-
Perfluorohexane sulfonate	PFHxS	n.a.-8.6	<0.017-0.08	<0.07–0.11	<0.07–4.35	<0.002-0.014	5	n.a.-1.39	0.1	28	n.a.	641
Perfluoroheptanoic acid	PFHpA	n.a.-6.2	0.46-3.46	<0.45-0.90	<0.45	0.006-0.284	5.1	n.a.-79.1	0.03	0.3	3106-3118	718
Perfluoroheptane sulfonate	PFHpS	n.a.	n.a.	-	-	-	0.34	-	0.03	2.7	-	-
Perfluorooctanoic acid	PFOA	n.a.-11	<0.051-0.76	<0.37-0.48	<0.37-5.48	0.003-0.072	15	0.764-31.7	<0.07	1.5	9082	n.a.
Perfluorooctylsulfonate	PFOS	n.a.-171	<0.01-1.13	<0.03	<0.03–1.42	0.022-0.120	-	n.a.-10.1	4.3	2811	n.a.	4892
Perfluorononanoic acid	PFNA	n.a.	<0.027-0.22	<0.06-0.22	<0.06-1.10	<0.002	0.55	n.a.-0.609	0.05	1	-	-
Perfluorodecanoic acid	PFDA	n.a.-1.2	<0.0161-0.1	<0.03	<0.03	<0.002-0.020	0.69	n.a.-2.03	0.3	132	-	-
Perfluorodecane sulfonate	PFDS	n.a.	<0.0184-0.06	<0.11	<0.11	-	n.a.	n.a.	0.12	10	-	-
Perfluoroundecanoic acid	PFUnDA	n.a.	n.a.	<0.06	<0.06	<0.005	0.15	n.a.-1.31	0.29	120	-	-
Perfluorododecanoic acid	PFDoA	n.a.	<0.025-0.05	<0.04	<0.04	-	n.a.	n.a.-3.94	1.7	419	-	-
Perfluorotridecanoic acid	PFTrDA	n.a.	<0.015-0.02	<0.05	<0.05	-	n.a.	n.a.-4.01	0.3	62	-	-
Perfluorotetradecanoic acid	PFTeDA	n.a.	n.a.	<0.06	<0.06	-	n.a.	n.a.-2.24	0.86	5.6	n.a.	n.a.
Perfluorohexadecanoic acid	PFHxDA	n.a.	-	-	-	-	-	-	-	-	-	-
Perfluorooctadecanoic acid	PFODA	n.a.-7.4	-	-	-	-	-	-	-	-	n.a.	n.a.
References		Onghena, Moliner-Martinez, Picó, Campíns-Falcó, & Barceló, 2012	Sharma et al., 2016	Lam, Cho, Kannan, & Cho, 2017		Yeung, Stadey, & Mabury, 2017	Munoz et al., 2017	Strynar, Lindstrom, Nakayama, Egeghy, & Helfant, 2012	Labadie & Chevreuil, 2011		Gosetti et al., 2010	

Moderate acute toxicity of PFOA and PFOS has been found in animal models (USEPA, 2017). Additionally, mixtures of PFOA, PFOS and perfluorononanoic acid (PFNA) have been shown to induce higher toxicity in zebrafish embryos and human macrophage cell lines than any individual compounds (Rainieri et al., 2017). Studies have also shown that long-chain PFASs, which include long-chain PFCAs with eight or more carbons and PFSAs with six or more carbons, can induce systemic effects in laboratory animals and wildlife, while short-chain PFASs appear less detrimental (USEPA, 2018b). As noted previously, increasing concern regarding human and ecological health risks has led to the phasing-out of some legacy PFASs (Corsini et al., 2014). However, little is known regarding the environmental and human health risk of replacement, emerging PFASs, since many companies manufacturing or using these substances list ingredients as "proprietary fluorosurfactant mixtures" (Mejia-Avendaño, Munoz, Sauvé, & Liu, 2017).

1.6 Analytical Methods for Quantitating PFASs

Nuclear magnetic resonance spectroscopy (NMR), gas chromatography with single or tandem mass spectrometry (GC-MS, GC-MS/MS), and liquid chromatography with single and tandem mass spectrometry (LC-MS, LC-MS/MS) have been used to detect and quantify PFASs (Dufková, Čabala, Maradová, & Štícha, 2009; Moody, Kwan, Martin, Muir, & Mabury, 2001; Niisoe et al., 2015; Pitarch et al., 2016; Portolés, Rosales, Sancho, Santos, & Moyano, 2015). As detection technology advances, our capacity to identify and quantify these compounds in biological and environmental matrices increases.

New analytical methods for detecting and quantifying novel PFASs are continuing to be developed (Avataneo, Navarrini, De Patto, & Marchionni, 2009; Gebbink, van Asseldonk, & van Leeuwen, 2017; Newton et al., 2017). Approaches for more efficient

and rapid quantitative analysis of PFASs have followed several strategies. Some liquid chromatographic methods reduced particle size or the inner diameter of the liquid chromatography (LC) column (Onghena et al., 2012; Y. Wu et al., 2012). For example, Capillary liquid chromatography (CLC) using a C18 column coupled with MS or MS/MS has been used to provide efficient analysis for some PFASs at conventional flow rates (µl/min). Ultrahigh-performance liquid chromatography- tandem mass spectrometry (UHPLC–MS/MS) using a C18 column, has been shown to produce rapid resolution of some PFASs at higher column pressures and linear velocities (Onghena et al., 2012). Both methods have demonstrated reliable, reproducible results with shortened analysis time (Onghena et al., 2012). Challenges remain, however, in improving instrument sensitivity and specificity (resulting in quantitation of trace amounts of PFASs), increasing the number of PFASs detected and quantitated in a single analytical run, working with varied sample matrices, and developing new and more efficient sample preparation strategies (Boone et al., 2014; Llorca, Farre, Pico, & Barcelo, 2009).

1.7 LC-MS

LC-MS is an analytical technique that combines the ability to separate individual compounds in a mixture (LC) with the ability to quantify the amount of each individual compound present in a sample (MS). In LC, mixtures of chemicals are injected (liquid mobile phase) under pressure into a capillary column and are separated as they move through the column based on differing physicochemical interactions between the column materials (stationary phase) and mobile liquid phase. Chemicals migrate through the column at rates determined by their molecular size, charge, hydrophobicity, specific binding interactions, or a combination of these factors.

Mass spectrometry measures the characteristics of individual molecules by converting them to ions in an ion source. Due to magnetic field deflection, ions can be moved and manipulated according to their mass to charge ratio value (m/z) in the mass analyzer. In the detector, the separated ions are measured. Tandem mass spectrometry (MS/MS) provides the ability to perform selected ion monitoring (SIM) to obtain reliable confirmation with less matrix interference (Rogatsky & Stein, 2005).

LC-MS/MS and UHPLC-MS/MS demonstrate the most effective analytical capability for detection and quantitation of PFASs as a result of their sensitivity, accuracy, and rapidity (Dufková et al., 2009; Lindstrom et al., 2011; Pitarch et al., 2016). In particular, UHPLC-MS/MS is both effective and efficient, reducing analysis time and solvent consumption by minimizing column particle size and column dimensions.

1.8 Goals and Objectives

As explained in Section 1.3, legacy PFASs are the most studied compounds in our environment due to their extensive production and consumption since the 1950s, whereas emerging PFASs, created as substitutes for legacy PFASs, are much less studied. The goal of the work reported here was to develop an analytical method to identify and quantify both legacy and emerging PFASs in a single experimental run.

As previously discussed in Section 1.4, there are several challenges regarding quantitative analysis of PFASs in environmental samples, including trace quantitation of multiple PFAS compounds, and limitations with respect to instrument resolution, sensitivity, and sample variability. To address these challenges and develop an efficient and reliable UHPLC-MS/MS-based analytical method for quantifying PFASs in estuarine water, the following objectives were met: development of an efficient UHPLC-MS/MS

quantitative analytical method for trace analysis of three classes of PFASs including PFCAs, PFSAs, and PFPEs (Section 2.2); validation of the developed quantitative method (Section 2.3); and application of this method to surface water samples collected in the Perdido Bay estuarine system (Section 3.2).

1.9 Organization

This thesis is divided into 5 chapters. Chapter 1 introduces PFASs and summarizes what is known regarding their chemical properties, environmental detection, toxicity, and the methods currently used to detect and quantitate PFASs. Chapter 1 also states the goals and objectives of this study. Chapter 2 presents the process and results of the UHPLC-MS/MS quantitative method developed during this study, including materials, standard preparation, and optimization of chromatographic and mass spectrometry conditions. Chapter 3 details the method validation process. Chapter 4 presents results from the application of the developed and validated UHPLC-MS/MS method to estuarine surface water samples collected from multiple locations within Perdido Bay. Chapter 5 summarizes the results of this study, discusses limitations of the method, and provides recommendations for future work.

Chapter 2: Development of an UHPLC-MS/MS Method for Detection and Quantitation of PFASs

2.1 Introduction

Chapter 1 notes that UHPLC–MS/MS methods generally have shorter analysis times with improved linearity and sensitivity compared to other LC-MS/MS methods (Onghena et al., 2012). Although there are existing analytical methods for some PFASs using LC-MS or LC-MS/MS, these methods are applicable to a limited number of analytes, and generally have low recoveries and detection limits (Boone et al., 2014). Therefore, this study focuses on analyzing three groups of PFASs (PFCAs, PFSAs, and PFPEs) using UHPLC-MS/MS. The optimal analytical method provides trace quantitation of twenty-three PFASs in a single eight-minute run. Four types of MS/MS scans were used to develop the method: full scan, SIM scan, PI scan, and MRM scan. The process and results for each scan mode are discussed in the following sections.

Method validation was necessary prior to its application to complex environmental samples (Kruve et al., 2015). Method validation was critical to the success of this study. Although there are many published studies related to PFAS detection and quantitation in environmental matrices, most published analytical methods were not validated, and thus data accuracy cannot be confirmed (William K. Reagen, 2009).

Awareness of the criticality of UHPLC-MS/MS method validation in the scientific community has led to the development of several method validation guidelines including United States Pharmacopoeia (USP), International Conference on Harmonization (ICH), and U.S. Food and Drug Administration (FDA) (Kruve et al., 2015). Because both of the recent USP and FDA guidelines refer to the ICH guideline, the ICH guideline was utilized to validate the UHPLC-MS/MS method developed in this study (Breaux, Jones, & Boulas, 2003). The ICH guidelines specify eight parameters in the method validation process (system suitability, specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness. Together, these parameters characterize the accuracy and reproducibility of method results (Panchumarthy Ravisankar, 2015). Since 2008, method validation studies have become an integral part of published LC-MS methods (Kruve et al., 2015). A summary of published PFAS analytical methods for different types of studies from 2001 to 2008 is presented in Figure 2.1. For this study, quantitative analysis, duplicates of experiments, and recovery experiments were conducted to complete method validation.

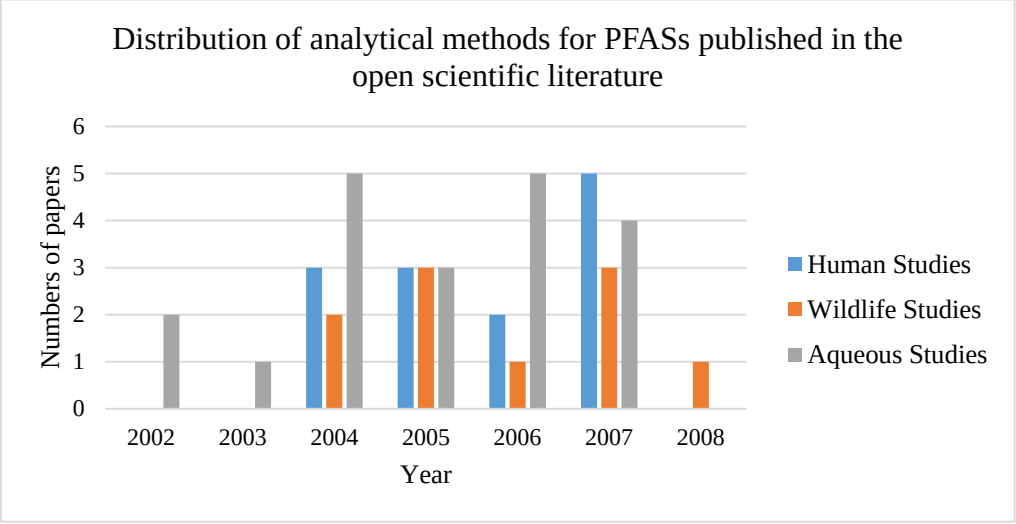


Figure 2.1: Summary of published analytical methods for PFASs from 2002 to 2008.

2.2 Analytical Method

2.2.1 Chemicals, Reagents, and Other Materials

Twenty-three PFASs and one internal standards (>99% purity) were used in this study. These PFASs, listed in Table 2.1, include 13 PFCAs, 8 PFSAs, 2 PFPEs and 1 internal standard. PFHxDA, PFTeDA, PFDoS, PFDoA, PFDS, PFBA, PFBS, PFPeA, PFTrDA, PFUnDA, PFNS, PFDA, PFOS, PFNA, PFHpS, PFOA, PFHxS, PFHpA, PFPeS, PFHxA, PFODA, ADONA, HFPO-DA, and MPFHxA were purchased from Wellington Laboratories (Canada).

LC grade solvents (methanol, acetonitrile, and water), reagent grade ammonium formate, analytical UHPLC columns (Rapid Resolution HD, 2.1×100 mm, 1.8-Micron, p/n 959758-902; InfinityLab Poroshell 120 Bonus-RP, 2.1 x 100 mm, 2.7 µm, p/n 861768-901; InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1 x 100 mm, 2.7 µm, p/n 695775-912), and a guard column (ZORBAX Eclipse Plus C18 2.1×5 mm, 1.8Micron, p/n 821725-901) were purchased from Agilent Technologies (Wilmington, DE). Glass Fiber/Nylon syringe filters (0.2 µm) were purchased from Sigma Aldrich (St. Louis, MO). The Oasis PRiME HLB 6cc, 200 mg sorbent extraction cartridges and a 20-Position vacuum manifold were supplied by Waters Corporation (Milford, MA, USA).

Table 2.1: Chemical structures of target PFASs used in this study.

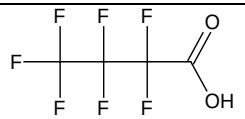
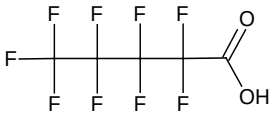
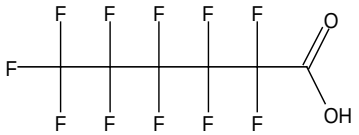
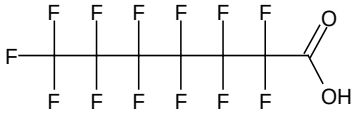
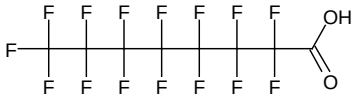
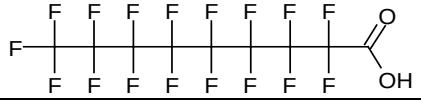
	Analyte	Acronym	Molecular weight	Chemical structure
Perfluoroalkyl carboxylic acids (PFCAs)				
1	Perfluorobutanoic acid	PFBA	213.9	
2	Perfluoropentanoic acid	PFPeA	263.9	
3	Perfluorohexanoic acid	PFHxA	312.9	
4	Perfluoroheptanoic acid	PFHpA	363.9	
5	Perfluorooctanoic acid	PFOA	413.9	
6	Perfluorononanoic acid	PFNA	463.9	

Table 2.1 (continued).

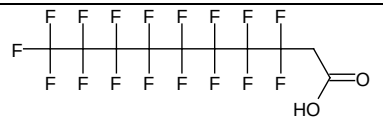
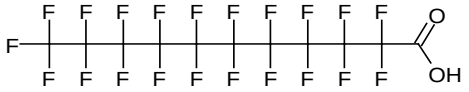
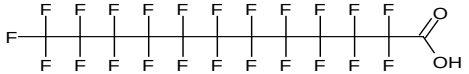
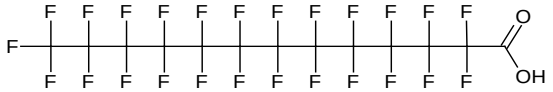
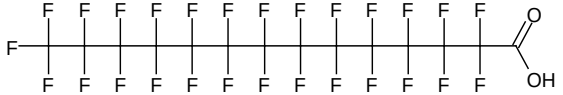
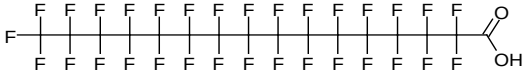
	Analyte	Acronym	Molecular weight	Chemical structure
7	Perfluorodecanoic acid	PFDA	513.9	
8	Perfluoroundecanoic acid	PFUnDA	563.9	
9	Perfluorododecanoic acid	PFDoA	613.9	
10	Perfluorotridecanoic acid	PFTrDA	663.9	
11	Perfluorotetradecanoic acid	PFTeDA	713.8	
12	Perfluorohexadecanoic acid	PFHxDA	813.9	

Table 2.1 (continued).

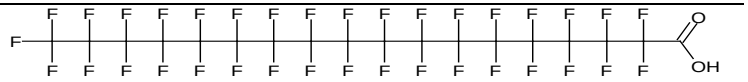
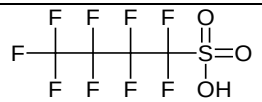
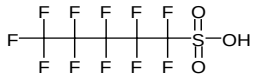
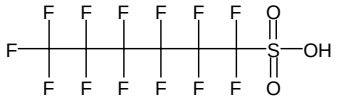
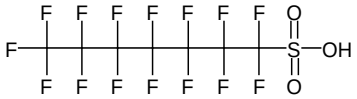
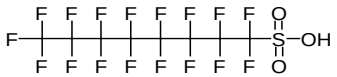
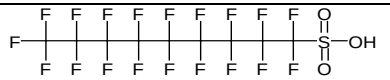
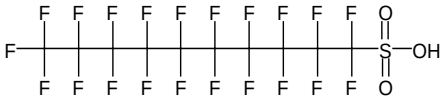
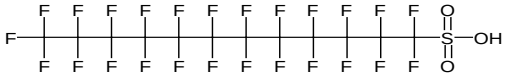
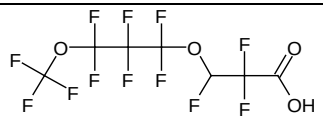
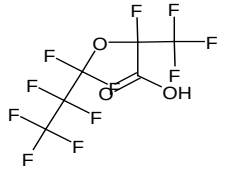
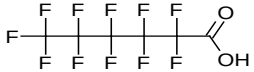
	Analyte	Acronym	Molecular weight	Chemical structure
13	Perfluorooctadecanoic acid	PFODA	913.8	
Perfluoroalkane sulfonates (PFSAs)				
14	Perfluorobutane sulfonate	PFBS	300	
15	Perfluoropentylsulfonate	PFPeS	349.9	
16	Perfluorohexane sulfonate	PFHxS	399.8	
17	Perfluoroheptane sulfonate	PFHpS	449.9	
18	Perfluorooctylsulfonate	PFOS	499.9	

Table 2.1 (continued).

	Analyte	Acronym	Molecular weight	Chemical structure
19	Perfluorononylsulfonate	PFNS	550.1	
20	Perfluorodecane sulfonate	PFDS	599.9	
21	Perfluorododecanesulfonic acid	PFDoS	699.8	
Perfluoropolyethers (PFPEs)				
22	3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]	ADONA	377.9	
23	2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	HFPO-DA	329.9	
Internal Standard				
	Perfluoro-[1,2-13C2]hexanoic acid	MPFHxA	316.1	

2.2.2 Standard Preparation

Two solvent mixtures were examined for preparation of standard solutions: methanol and water (9:1, v/v; 8:2, v/v; 7:3, v/v), and acetonitrile and water (9:1, v/v). Methanol and water (9:1, v/v) provided the best chromatographic response and thus was selected for standard preparation. Primary stock solutions for each target analyte (0.1 and 0.01 µg/mL for PFASs) were prepared independently in methanol and water (9:1, v/v) for use in both method development and validation studies. A seven-point calibration curve was developed by diluting stock solution to achieve concentrations ranging from 0.1 ng/mL to 10 ng/mL, with spiking levels at 1 ng/mL internal standard (MPFHxA).

2.2.3 Chromatographic Conditions

The method developed for quantitation of the 23 target PFASs shown in Table 2.1 employed an UHPLC-MS/MS system consisting of an Agilent 1290 Infinity II high speed pump (model G7120A) coupled to a triple quadrupole (model G6460C) mass spectrometer and Jet-Stream Electrospray Ionization source (Agilent Technologies Inc., Santa Clara, CA, USA). Figure 2.2 shows the instrument used for this study.

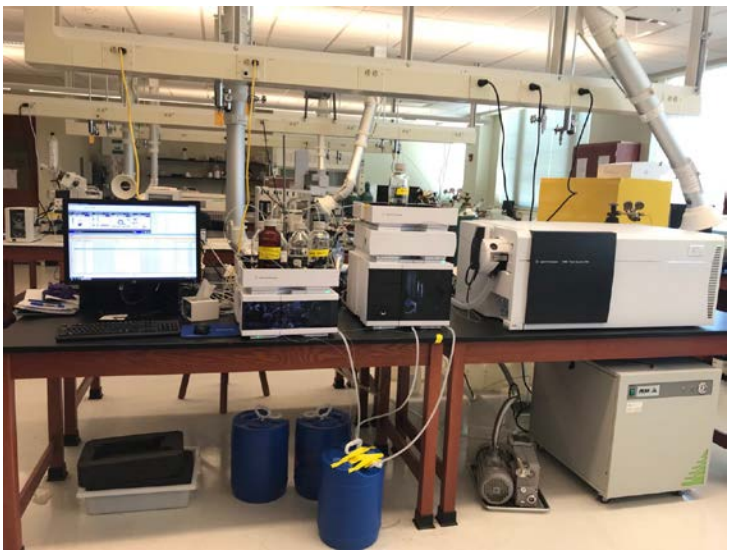


Figure 2.2: UHPLC-MS/MS instrument.

Four Agilent C18 columns (InfinityLab Poroshell 120 Bonus-RP, 2.1 x 100 mm, 2.7 µm; InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1 x 100 mm, 2.7 µm; ZORBAX Eclipse Plus, 2.1 x 50 mm, 1.8 µm; and ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm) were compared for chromatographic separation.

Development of the mobile phase method required selection of a modifier to control the extent to which analytes will ionize. The chosen modifier controls the pH of the mobile phase when analyzing compounds that are ionizable by the reversed phase (Waters., 2018). The pH range for mobile phase modifiers vary. For example, for ammonium acetate, pH ranges from 3.8-5.8; for ammonium formate, pH ranges from 2.8-4.8; and for formic acid (0.1%), pH is approximately 2.7 (Dolan, 2016). Stationary phase, C18 columns are the most versatile non-polar stationary reverse-phase for avoiding ionizable impurities (Mannur, Patel, Mastiholimath, & Shah, 2011).

To conduct and optimize chromatographic experiments with acceptable peak shapes, several combinations of modifiers (ammonium acetate, ammonium formate, formic acid) infused into different combinations of solvents (water, methanol, acetonitrile, and methanol/acetonitrile mixture) were tested. Additionally, different flow rates (0.15 to 0.3 mL/min) and column temperatures (20-60 ºC) were tested to optimize chromatographic experiments. Details related to the development of the mobile phase are given in Table 2.2, showing that 9 combinations of mobile phases with different solvent and buffer concentrations were tested to determine optimal analyte solubility and signal response.

Table 2.2: Information of mobile phases tested during analysis.

	Mobile Phase A	Mobile Phase B
1	5 mM ammonium formate in water	5 mM ammonium formate in methanol
2	10 mM ammonium formate in water	10 mM ammonium formate in methanol
3	5 mM ammonium acetate in water	5 mM ammonium acetate in methanol
4	5 mM ammonium formate in water	5 mM ammonium formate in acetonitrile
5	10 mM ammonium formate in water	10 mM ammonium formate in acetonitrile
6	5 mM ammonium acetate in water	5 mM ammonium acetate in acetonitrile
7	0.1% formic acid in water	methanol
8	5 mM ammonium formate in water	methanol
9	5 mM ammonium formate in water	acetonitrile

2.2.4 Mass Spectrometry Conditions

To achieve favorable fragmentation conditions for PFASs detection and quantification, MS/MS studies including full scan, single ion monitoring scan (SIM), and product ion scan (PI) were performed in negative mode using an Agilent Jet-Stream ion source. Source parameters (gas temperature, gas flow, sheath gas temperature, sheath gas flow, fragmentor voltages (FV), collision energies (CE), nebulizer pressure, nozzle voltage, and capillary voltage conditions) were also evaluated. Qualitative data were processed for MRM data acquisition parameters using Agilent Mass Hunter software version B. 07.1.

2.2.4.1 Full Scan Analysis

Full scan analysis was conducted separately for different groups of compounds using 0.1 µg/mL as the analyte concentration. The full scan was run from 150 to 1000 m/z for PFCAs; 200 to 800 m/z for PFSAs; and 250 to 400 m/z for PFPEs. The negative scan mode was selected for this study based on previous published works which considered molecule polarity to select scan mode (positive or negative) (Dolman & Pelzing, 2011; Herzke, Olsson, & Posner, 2012; Llorca, Farre, Pico, & Barcelo, 2011; Martin et al., 2016;

Yamamoto et al., 2014). The results of this study confirmed negative scan mode effectiveness.

2.2.4.2 SIM Scan Analysis

SIM scan analysis was also performed separately for three groups of PFASs mixtures at a concentration of 0.1 µg/mL: PFCAs, PFSAs, and PFPEs. Fragment voltage values ranging from 60 to 200 V were tested to optimize peak-shape profiles. In addition, the appropriate dwell time ranging from 20 to 50 seconds was determined by optimizing peak-shape profiles. The results presented accurate retention time with optimized fragment voltage and dwell time.

2.2.4.3 PI Scan Analysis

PI scanning, a qualitative assessment involving the application of different CE (from 0 eV to 70 eV), was used to determine which CE obtained the greatest amount of product ions from each precursor ion.

2.2.4.4 MRM Analysis

MRM analysis combines selected dwell time with optimized peak-shape profiles, precursor ions with optimized fragment voltage, and the most abundant set of product ions with optimized collision cell energy for each analyte. This analysis was made to obtain method with high sensitivity. The analysis time was determined by choosing the time that the samples were either sent to waste or to the MS/MS based on retention time (determined from the SIM scan) for each analyte.

2.3 Method Validation

2.3.1 Experimental Design

This study employed the internal standard method to minimize possible matrix effects from environmental samples. MPFHxA was selected as the internal standard for the target analytes because of its median carbon chain length and structural similarity. Seven levels of calibration solutions were prepared by diluting the stock solution. Each of the 7 calibration solutions were then spiked with 1 ng/mL internal standard before UHPLC-MS/MS analysis. Three experimental runs were performed for each PFAS calibration solution (23 analytes plus internal standard) using analyte concentrations ranging from 0.05 to 10 ng/mL, to examine method linearity. Seven-point calibration curves were developed based on the relative response ratios obtained from the instrument which is proportional to the known concentration of each analyte. Method linearity was quantified by the R^2 value of the linear regression curve. Calibration curves showing acceptable linearity were used to calculate PFAS concentrations in real surface water samples (Chapter 3).

Additionally, LOQ for each PFAS analyte was determined. LOQ is the lowest analyte concentration that can be precisely measured by the method (Armbruster & Pry, 2008). For target analytes, LOQ was estimated based on the signal-to-noise ratio approach using equations (1), where δ is the standard deviation of calibration curve intercepts, and S is the mean of the slopes of the calibration curves (Panchumarthy Ravisankar, 2015).

$$LOQ = 10 \times \frac{\delta}{S} \qquad (1)$$

After validating quantitative method, the reliability of the designed sample extraction protocol was evaluated by conducting recovery experiments. Running HPLC-MS/MS experiments with known concentrations of PFAS mixtures ensures that the contents of each

sample are recovered completely when analyzed. Two recovery experiments were performed by spiking four surface water samples (one liter each) with known concentrations of PFASs at two levels (2 and 10 ng/mL). The experimental process is shown in Figure 2.3.

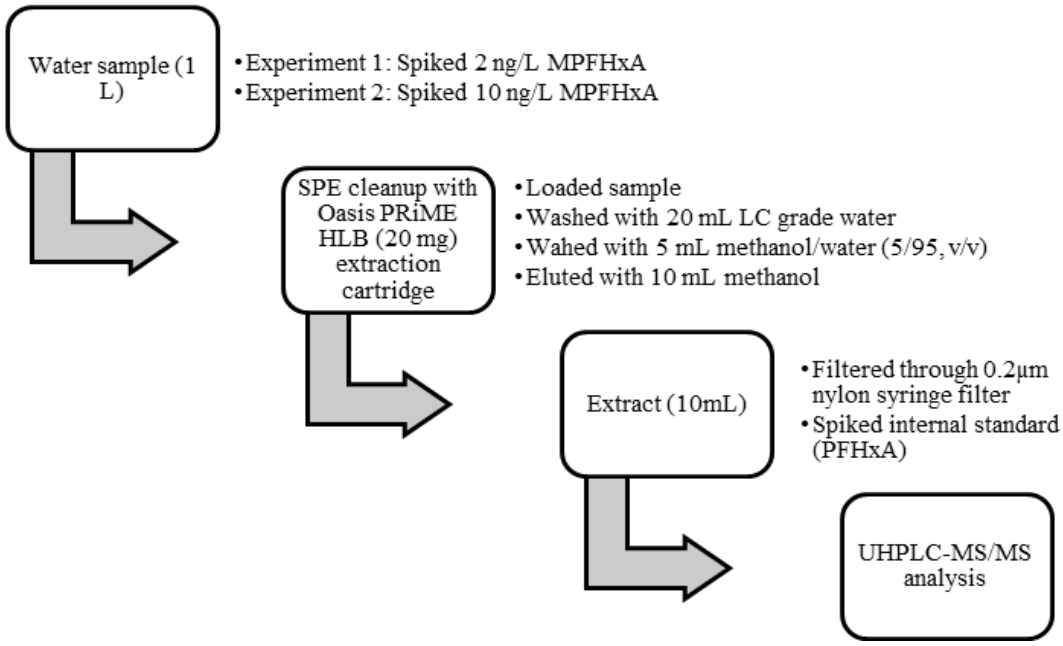


Figure 2.3: Experimental process for method validation.

Results of the recovery experiments were compared with the known concentrations of the spiked samples to determine the accuracy, as percent recovery (%R), and precision, as standard deviation of %R, of the procedure. %R was calculated using equation (2), where AR means analyte response:

$$\%R = \frac{AR\ spiked\ sample - AR\ unspiked\ sample}{Spiked\ standard\ concentration} \times 100 \qquad (2)$$

Finally, solvent blanks were analyzed between each run to test specificity of method (a means of determining method reliability).

2.4 Results and Discussions

2.4.1 Optimization of Chromatographic Conditions

Methanol and water (9:1, v/v) was selected as the suitable solvent for standard analyte preparation due to its effective solubility for all analytes and acceptable chromatographic response. An Agilent ZORBAX RRHD Eclipse Plus C18 column (2.1 x 100 mm, 1.8 µm) was selected among four C18 columns tested because it produced excellent peak resolution. An Agilent ZORBAX Eclipse Plus C18 guard column (2.1 x 5 mm, 1.8 µm) was connected to the analytical column to ensure retention time reproducibility during the analysis.

Among the combinations of mobile phase solvents evaluation, optimum chromatography was achieved with a mobile phase consisting of 5 mM ammonium formate in water (mobile phase A) and methanol (mobile phase B). Gradient method conditions, flow rate, and column temperature giving the optimum peak shape, peak response, and resolution are presented in Table 2.3.

T able 2.3: Optimized UHPLC conditions.

UHPLC Conditions		
Pump	Agilent Infinity 1290 II	
Mobile Phase	A. 5mM ammonium formate in water	
	B. Methanol	
Gradient Method Conditions	Time (min)	B%
	0.00	30
	0.20	30
	0.50	70
	4.00	85
	5.50	95
	6.50	99
	8.00	30
	Post run: 3 min	
Flow rate	0.25 mL/min	
Total run time analysis	11 min	
Column temperature	40 ºC	
Injection volume	5 µL	
Injection wash solvent	Methanol/acetonitrile/water (40:40:20, v/v/v)	

The total run time for the method was 8 minutes. From 1.5 minutes to 7.5 minutes, samples were sent to the detector, thus actual analysis time for 23 PFASs analytes (plus internal standard) was 6 minutes.

2.4.2 Optimization of Mass Spectrometry (MS) Conditions

Optimized mass spectrometry conditions were developed in previous study and applied for the entire analysis which are shown in Table 2.4.

Table 2.4: MS conditions.

MS Conditions	
Ion source	Agilent Jet-stream ESI
MS analyzer	QqQ MS
Gas temperature	225 ºC
Gas flow	10 L/min
Nebulizer	45 Psi
Sheath gas temperature	350 ºC
Sheath gas flow	11 L/min
Capillary voltage	+3600
Nozzle voltage	-1500
Delta EMV Cell acceleration voltage	400 v 4 v
MS1 and MS2 resolution	Unit

2.4.3. Results of Qualitative Scan Analysis

Prior to MRM method development, a series of experiments (full, SIM, and PI scans) were conducted to optimize suitable ionization conditions for each target analyte. Full scan analysis (in negative mode) produced the observed precursor ions for each analyte shown in Table 2.5. The chromatograms for each analyte are presented in Figures A1, A2, and A3. The chromatograms for SIM and PI analysis are provided in Figure A4 to Figure A13. During the ionization process, perfluorocarbon chain fragmentation was observed with PFCAs. PFSAs resulted in characteristically different product ions due to loss of -SO_3 and -FSO_3 groups.

Results of the full, SIM, and PI scan experiments were combined to perform MRM analysis. Optimized target analyte MRM transitions were setup in the data acquisition system to conduct quantitative experiments. Details of further MRM condition optimization are presented in Table 2.5.

Table 2.5: MRM data acquisition parameters.

Target analytes		Retention time (Rt, min)	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (eV)
1	PFBA	2.026	212.9	169	70	5
2	PFPeA	2.249	262.9	218.8	70	5
3	PFHxA	2.471	312.9	268.8	70	5
4	PFHpA	2.758	362.9	319/168.9	70	5/15
5	PFOA	3.111	413.1	369.1/169.1	70	5/15
6	PFNA	3.534	463.1	419/169.1	80	5/20
7	PFDA	4.023	513.1	469.1/169	90	5/20
8	PFUnDA	4.513	563.1	518.9/169	70	5/25
9	PFDoA	5.002	612.9	569/169	70	5/20
10	PFTrDA	5.448	662.9	619/169	100	10/30
11	PFTeDA	5.824	712.8	669/169	110	5/40
12	PFHxDA	6.381	812.9	768.8/169	100	15/40
13	PFODA	6.736	912.8	868.9/169	100	15/40
14	PFBS	2.248	299	99/80	70	35/35
15	PFPeS	2.468	348.9	99/80	70	35/35
16	PFHxS	2.733	398.8	99/80	70	40/40
17	PFHpS	3.088	448.9	99/80	70	45/45
18	PFOS	3.511	498.9	99/80	70	50/50
19	PFNS	3.978	549.1	99/80	70	55/55
20	PFDS	4.467	598.9	99/80	70	55/55
21	PFDoS	5.38	698.8	99/80	70	60/60
22	ADONA	2.778	376.9	250.9/84.9	80	10/30
23	HFPO-DA	2.536	328.9	284.9/169	70	5/15
Internal standard	MPFHxA	2.471	315.1	270	70	5

The extracted UHPLC-MRM chromatograms of each target analyte, presented in Figure 2.4, display excellent peak shapes and PFASs separation. These results also indicate short run times and reproducible retention times.

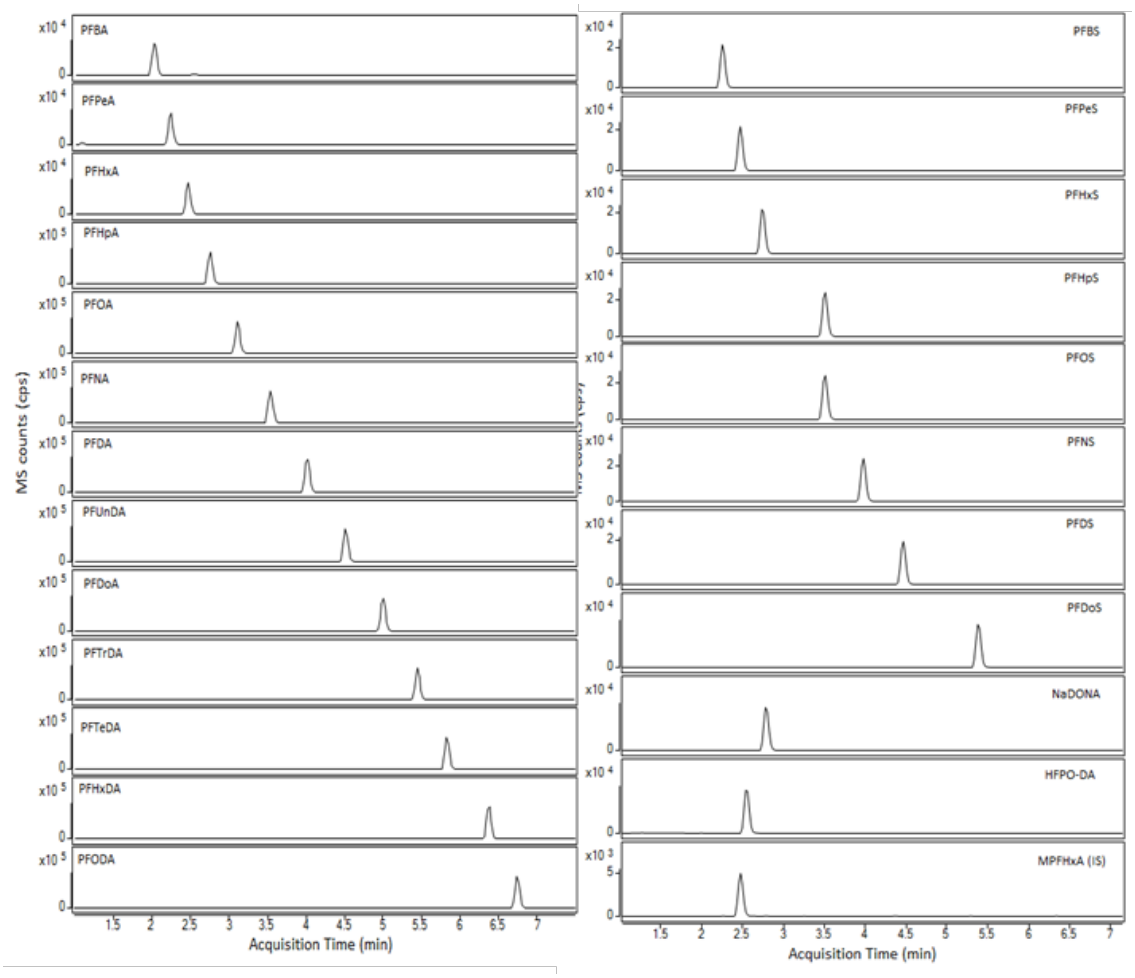


Figure 2.4: UHPLC-MRM chromatograms of PFAS.

2.4.4 Method Validation

As shown in Figures 2.5, 2.6, and 2.7, the obtained calibration curves indicate acceptable linearity, with R^2 values greater than 0.997, which would be used for PFASs detections in real surface water samples (Section 3.4).

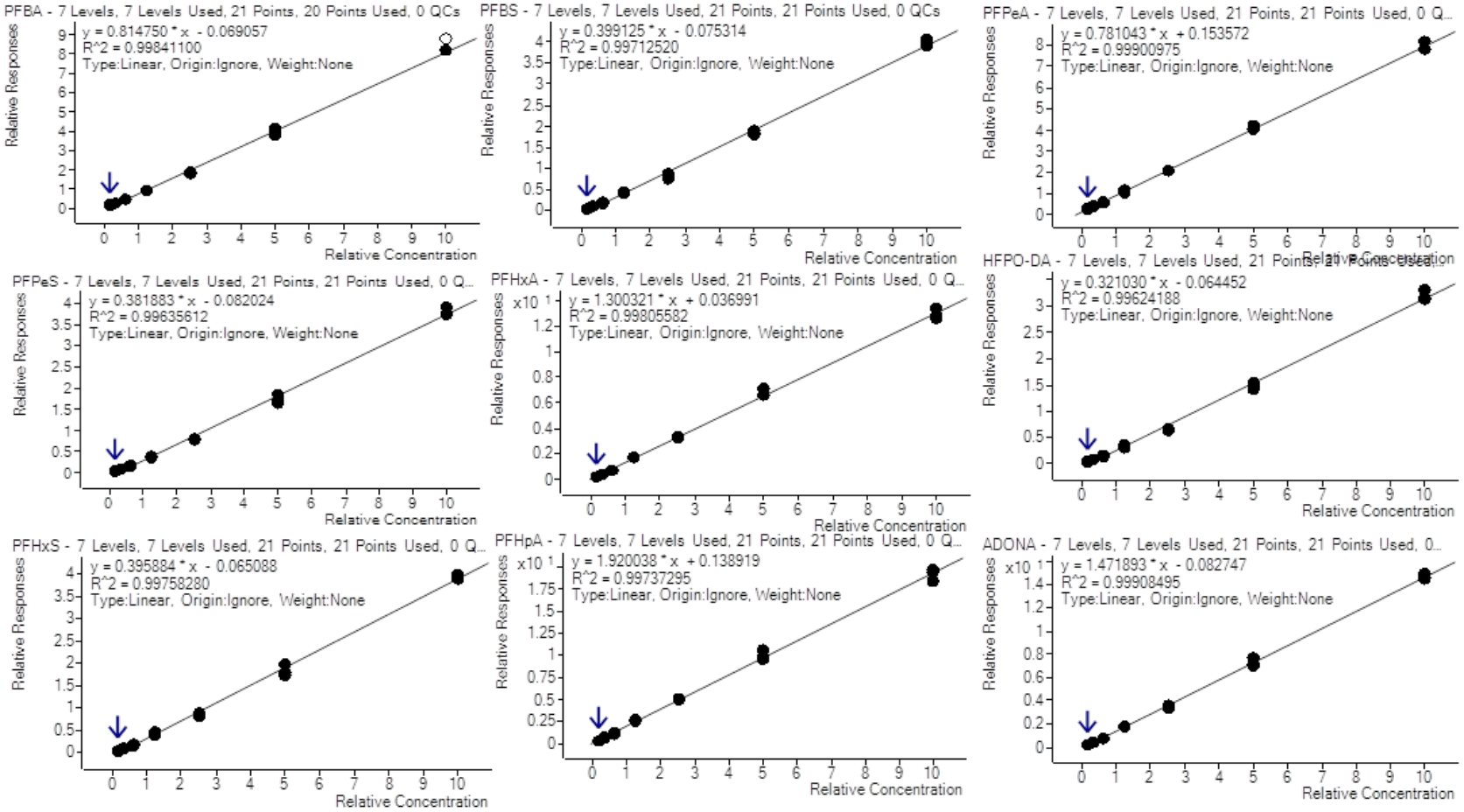


Figure 2.5: Calibration curves for PFBA, PFBS, PFPeA, PFPeS, PFHxA, PFHxS, PFHpA, HFPO-DA, and ADONA.

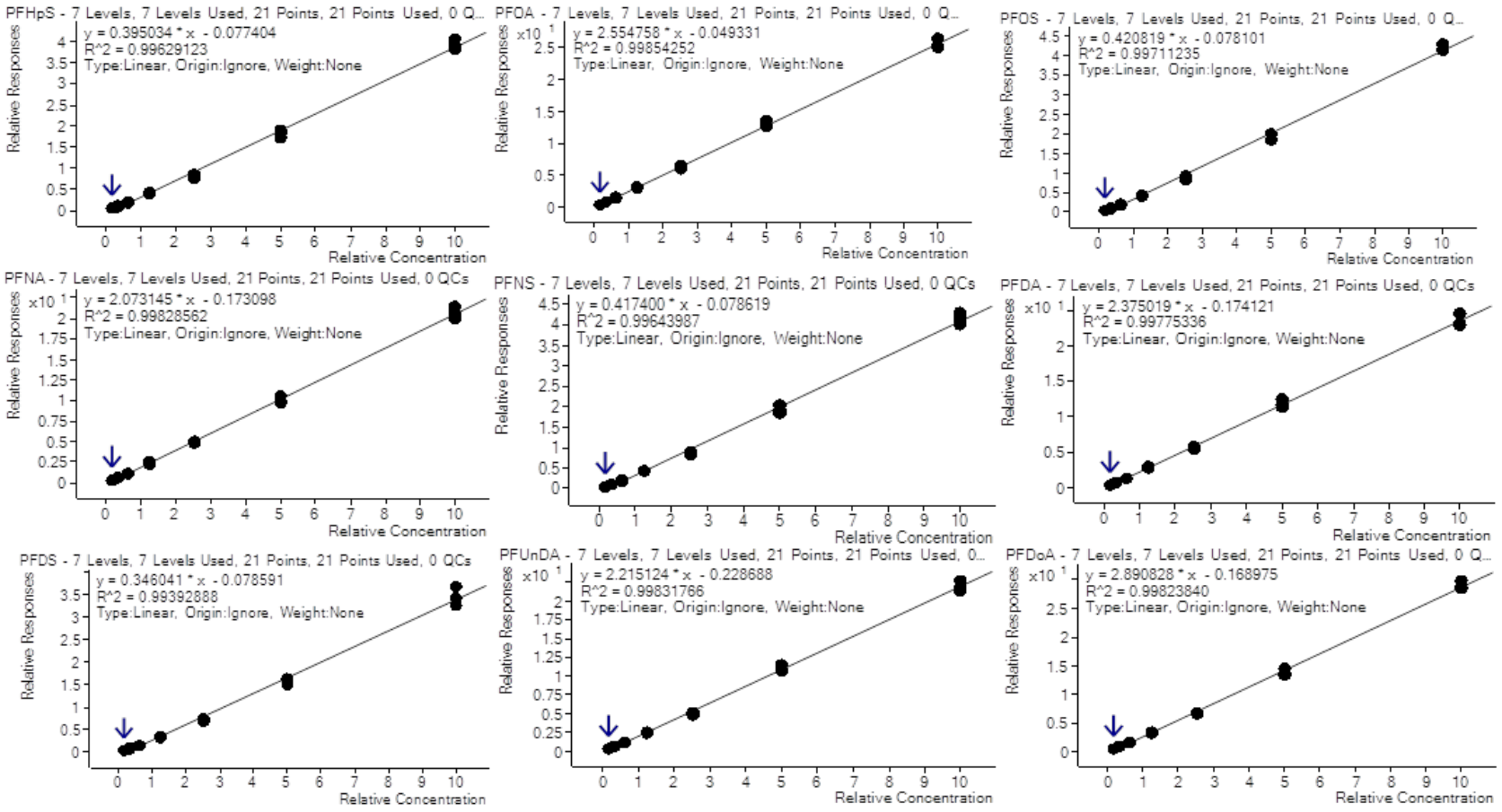


Figure 2.6: Calibration curves for PFHpS, PFOA, PFOS, PFNA, PFNS, PFDA, PFDS, PFUnDA, and PFDoA.

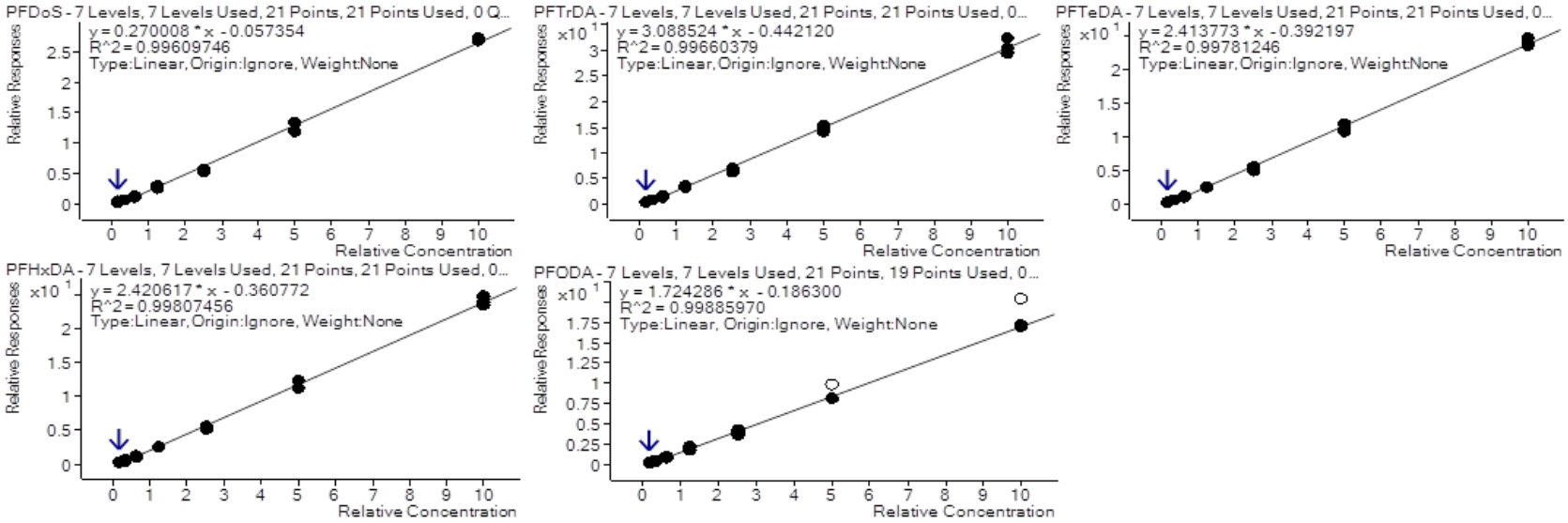


Figure 2.7: Calibration curves for PFDoS, PFTrDA, PFTeDA, PFHxDA, and PFODA.

Although LOD values remain to be calculated in future work for maximum reliability, LOQ values were determined for each PFAS analyte and are presented in Table 2.6. Results of the recovery study are presented in Table 2.7 (%R ± standard deviation (SD)).

Table 2.6: LOQ for PFASs.

Target analytes		LOQ (pg/injection)
1	PFBA	0.78
2	PFPeA	0.39
3	PFHxA	0.20
4	PFHpA	1.56
5	PFOA	0.39
6	PFNA	1.56
7	PFDA	0.78
8	PFUnDA	0.39
9	PFDoA	0.78
10	PFTrDA	0.39
11	PFTeDA	0.78
12	PFHxDA	0.78
13	PFODA	0.78
14	PFBS	1.56
15	PFPeS	0.78
16	PFHxS	0.78
17	PFHpS	0.78
18	PFOS	0.78
19	PFNS	0.78
20	PFDS	0.78
21	PFDoS	0.78
22	ADONA	0.20
23	HFPO-DA	0.10

Table 2.7: Percent recoveries of PFASs (%R).

Analyte	1A	2A	3A	4A	1B	2B	3B	4B
PFBA	90.45 ± 5.37	83.86 ± 4.24	90.51 ± 3.76	93.89 ± 3.67	100.09 ± 3.93	108.83 ± 2.42	112.61 ± 4.35	101.80 ± 4.47
PFBS	90.69 ± 3.72	80.84 ± 5.38	90.86 ± 5.77	93.76 ± 5.79	97.50 ± 3.48	104.73 ± 2.44	109.92 ± 4.12	98.30 ± 4.09
PFPeA	99.9 4± 3.59	91.90 ± 6.72	99.71 ± 5.48	105.07 ± 5.40	96.47 ± 2.35	105.00 ± 1.46	111.17 ± 4.99	99.52 ± 6.13
PFPeS	91.02 ± 4.81	83.20 ± 4.10	89.63 ± 5.28	92.44 ± 5.16	99.18 ± 2.39	107.46 ± 3.51	110.82 ± 3.00	100.23 ± 4.10
PFHxA	98.76 ± 4.49	90.08 ± 4.79	99.49 ± 3.86	102.78 ± 4.92	96.42 ± 1.07	104.34 ± 3.88	107.74 ± 4.56	96.98 ± 4.15
HFPO-DA	90.56 ± 2.70	84.61 ± 4.62	91.39 ± 3.42	92.55 ± 4.13	102.29 ± 0.78	110.73 ± 2.03	113.56 ± 3.49	102.17 ± 5.25
PFHxS	90.21 ± 3.31	83.26 ± 4.45	89.82 ± 5.55	93.24 ± 3.10	99.17 ± 2.87	107.26 ± 2.82	110.85 ± 5.07	99.81 ± 4.84
PFHpA	101.58 ± 2.94	94.02 ± 4.98	102.15 ± 4.73	104.96 ± 5.95	96.63 ± 1.95	106.23 ± 2.89	110.32 ± 3.67	97.96 ± 5.74
ADONA	95.00 ± 3.46	86.93 ± 4.30	95.71 ± 3.67	97.83 ± 4.11	97.09 ± 2.26	105.71 ± 2.16	110.78 ± 4.54	98.61 ± 4.20
PFHpS	86.27 ± 4.14	82.02 ± 6.03	85.17 ± 4.03	89.37 ± 4.71	98.17 ± 1.75	104.31 ± 2.39	109.51 ± 5.23	98.66 ± 5.39
PFOA	96.48 ± 3.81	87.38 ± 4.58	95.57 ± 4.62	100.06 ± 5.57	97.69 ± 1.92	105.27 ± 2.71	110.03 ± 3.75	98.26 ± 4.88
PFOS	89.04 ± 4.81	81.54 ± 5.67	89.25 ± 3.85	90.85 ± 3.98	98.81 ± 2.62	108.68 ± 4.22	111.38 ± 3.93	101.21 ± 3.53
PFNA	95.02 ± 4.16	86.95 ± 4.80	95.68 ± 5.43	97.85 ± 4.38	97.67 ± 2.31	105.15 ± 2.69	109.78 ± 4.77	98.98 ± 4.03
PFNS	86.93 ± 2.23	82.65 ± 2.87	89.08 ± 2.81	91.46 ± 4.21	100.63 ± 1.66	107.94 ± 3.58	111.17 ± 4.54	101.75 ± 4.43
PFDA	92.11 ± 3.79	84.97 ± 4.12	92.69 ± 2.77	96.78 ± 4.20	97.74 ± 2.00	105.09 ± 2.76	110.31 ± 4.32	99.20 ± 4.13
PFDS	85.87 ± 3.31	78.54 ± 4.06	87.00 ± 2.92	89.38 ± 3.74	98.90 ± 1.84	105.06 ± 3.24	110.88 ± 4.54	99.32 ± 5.89
PFUnDA	91.40 ± 3.98	83.67 ± 3.28	90.85 ± 3.15	93.94 ± 3.67	98.47 ± 2.22	107.12 ± 2.62	111.59 ± 3.45	100.16 ± 4.47
PFDoA	90.04 ± 3.25	81.56 ± 4.33	88.92 ± 4.44	92.03 ± 3.12	99.07 ± 2.24	105.92 ± 2.33	111.40 ± 3.65	100.55 ± 4.36
PFDoS	88.02 ± 4.88	81.50 ± 5.83	86.60 ± 5.45	89.61 ± 3.91	98.58 ± 2.14	104.88 ± 4.34	110.81 ± 4.28	98.85 ± 5.85
PFTrDA	91.24 ± 2.85	83.08 ± 3.24	90.95 ± 3.98	93.14 ± 2.41	99.31 ± 1.96	108.10 ± 2.68	111.83 ± 5.32	101.07 ± 4.96
PFTeDA	88.77 ± 2.31	81.86 ± 3.81	87.85 ± 4.27	90.99 ± 3.42	99.37 ± 1.84	107.10 ± 1.94	110.74 ± 4.40	99.58 ± 4.53
PFDOA	88.71 ± 1.40	80.95 ± 3.20	87.01 ± 2.77	90.15 ± 3.31	99.76 ± 1.77	107.53 ± 1.93	111.14 ± 4.47	100.53 ± 5.64
PFHxDA	90.11 ± 1.96	81.90 ± 4.27	87.70 ± 2.81	91.46 ± 3.55	99.89 ± 1.52	107.39 ± 1.98	111.05 ± 5.05	101.26 ± 4.51

The mean recoveries obtained from samples spiked at 2 ng/mL and 10 ng/mL for twenty-three analytes were 90.46% ± 4.09% and 103.94% ± 3.49%, respectively. For 2 ng/mL, recovery ranged from 86.37% to 94.55%; for 10 ng/mL, recovery ranged from 107.43% to 100.46%. Recovery results greater than 100% are indicative of analyte carry over from multiple analytical runs. All target analytes had lower recovery at 2 ng/mL compared to recovery at 10 ng/mL. Recoveries between 80-120% are considered satisfactory based on past published method development studies (Lin et al., 2016).

Analysis of 6 solvent blanks were used to demonstrate method specificity. The chromatograms for the 6 blanks are shown in Figure 2.8, which demonstrate that were no interfering peaks with retention times corresponding to any PFAS analytes.

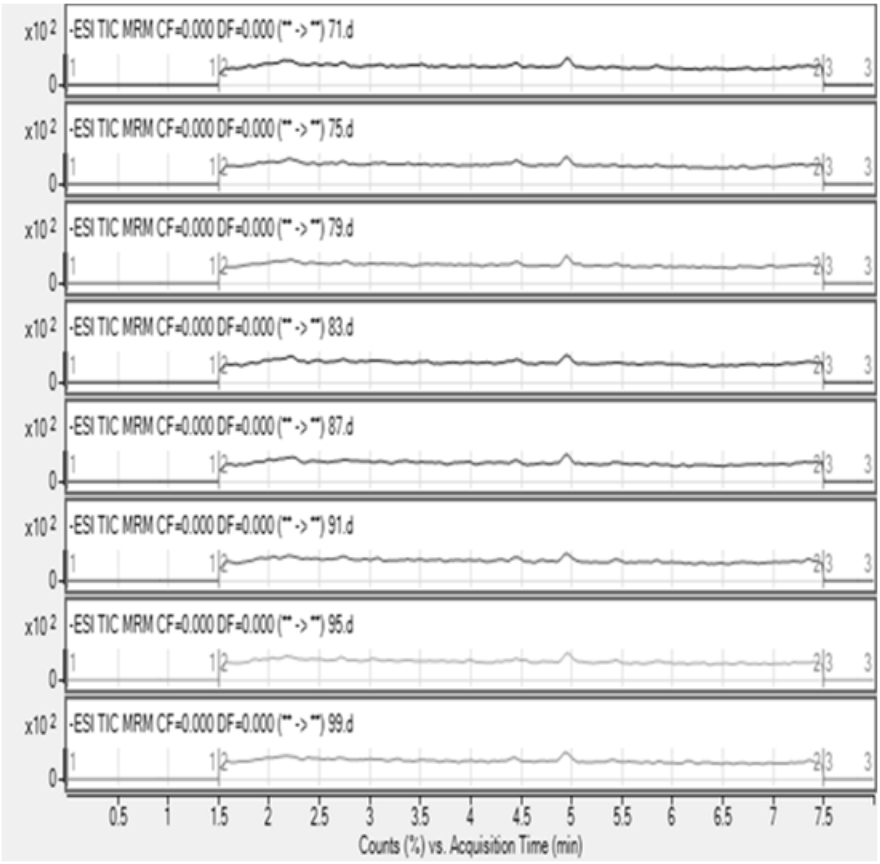


Figure 2.8: Chromatograms for solvent blanks between each PFAS analytical run.

Chapter 3: Application of a Quantitative Method for Per- and Polyfluoroalkyl Substances (PFASs) in Environmental Media Using UHPLC-MS/MS

3.1 Introduction

Aqueous phase PFAS detection and quantitation can be affected by several factors, including cross contamination, matrix interferences, and the presence of branched isomers (Trojanowicz & Koc, 2013). Samples can be contaminated by improper sampling practices, such as use of unsuitable sampling devices and storage containers. Additionally, selection of appropriate materials for sample preparation and analysis is critical, as improper materials may leach or sorb substances and skew analytical results. Sample cleanup prior to extraction is necessary to reduce matrix effects, optimize PFAS extraction, and maintain instrument accuracies (Van Leeuwen & de Boer, 2007).

The advantages and limitations of a variety of combined methods for sample cleanup and PFAS extraction were considered to determine the optimal method for this analysis. Combined cleanup/extraction methods include solid phase extraction (SPE), dispersive graphitized carbon, destructive methods, and (fluorous) silica column chromatography. Pressurized liquid extraction (PLE), microwave assisted extraction (MAE), and solid-phase microextraction (SEME) have also been applied for sample cleanup/extraction since 2009 (Llorca et al., 2009).

In 2013, PFAS concentrations in sewage sludge following PLE and focused ultrasound solid-liquid extraction (FUSLE) methods were compared to each other, and no differences were reported with the exception of lower PFHpA and PFNA concentrations for FUSLE (Martinez-Moral & Tena, 2013). Both PLE and MAE are equally efficient with respect to time and solvent consumption for varied environmental samples (Beser, Pardo, Beltrán, & Yusà, 2011). Three micro extraction method coupled to LC/MS were developed and optimized for PFASs analysis in environmental water samples with similar recoveries and LODs (Pan, Ying, et al., 2014). Recently, Turbulent Flow Chromatography (TFC) was used for water and sediment sample cleanup/extraction with minimum manipulation, high efficiency, and low contamination, but this technique demonstrates limited diffusion capability and analyte selectivity (Mazzoni, Polesello, Rusconi, & Valsecchi, 2016).

Among cleanup/extraction strategies, SPE is suitable for most PFASs and the most commonly used (Zhang, Wang, Tang, Nie, & Xu, 2018). SPE is widely applicable due to its efficiency with minimum degradation (Zhao, Cheng, & Lin, 2014). Off-line SPE strategies including ion-pair and HLB cartridges have been employed for sludge extraction and PFASs analysis (Yoo, Washington, Jenkins, & Libelo, 2009). On-line SPE strategies (loading samples into pre-concentration columns before sample analysis) have also been used for PFAS analysis (Castiglioni et al., 2015). In recent years, polymeric sorbents have been widely used as an optimized SPE method for sample cleanup/extraction prior to PFAS analysis due to their ion exchange characteristics (Zhang et al., 2018). Generally, SPE method performance varies depending on sample volume and elution conditions (Zhang et al., 2018). Although some cleanup/extraction methods work well when using high-grade

laboratory water, their performance decreases for water from natural systems (Boone et al., 2014) and for samples having trace analyte concentrations (Wille et al., 2012).

3.2 Sample Collection

The optimized and validated UHPLC-MS/MS method for PFASs analysis was applied to surface water samples collected from the Perdido Bay estuarine system on two different dates (7/25/17 and 10/31/17). During each sampling event, samples were collected from 16 locations within the study area using a stainless steel Kemmerer Bottle water sampler (1 L) (Figure 3.1). Water samples were collected in duplicate (2, 4 L samples) at ½ the total water depth at each location. All sample containers were made of high-density polyethylene (HDPE). Immediately following collection, all samples were stored in coolers on ice (approximately 4 ºC), transported to the laboratory, and stored at -20 ºC until analyzed.

Figure 3.1: Sample collection.

3.3 Sample Preparation

Prior to sample cleanup and extraction, water samples were removed from storage and allowed to thaw naturally to room temperature. The sample protocol validated in Section 2.3.1 was applied to the water samples. Each 4 L sample was passed through a Whatman 1.6 μm glass microfiber filter to remove suspended solids using micro-filtration under vacuum. Filtered water samples were pre-concentrated using SPE by passing each sample through a Waters Oasis PRiME HLB 6cc extraction cartridge under vacuum. Target analytes retained by the sorbent in cartridges were recovered by flushing under vacuum with 20 mL of water and 5 mL of methanol/water (5/95, v/v) for sample cleanup to minimize environmental matrix effects. The target analytes were then eluted with 10 mL methanol and filtered through 0.2 μm Agilent Captive premium nylon glass fiber syringe filters. Samples were spiked with 1 ng/mL internal standard and stored at 4 ºC until analyzed by the developed UHPLC-MS/MS method.

3.4 Application of UHPLC-MS/MS Method

The developed UHPLC-MS/MS MRM method described in this thesis was used to identify the 23 PFASs target analytes in the aforementioned surface water samples collected from the Perdido Bay estuarine system. After UHPLC-MS/MS analysis, the concentration of each compound was determined using the same calibration curves developed in Section 2.4.4 by using Agilent Mass Hunter software version B. 07.1. Details describing setup of the UHPLC-MS/MS control and data acquisition systems are shown in Figures A14 through A19. The concentration for each compound detected in a sample injected into the UHPLC-MS/MS system was calculated using a linearly regressed, seven-point calibration curves relating sample concentration to instrument response (Figure 2.5,

2.6, and 2.7). The actual concentration for each detected PFAS analyte was calculated using equation (3), where y is the final concentration in the surface water sample (ng/L), x is the concentration in the injected sample (ng/mL), v_1 is the volume of the injected sample (mL), and v_2 is the volume of the surface water sample (L):

$$y = \frac{c \times v_1}{v_2} \qquad (3)$$

3.5 Statistical Significance of Seasonal Differences in PFAS Concentrations

A statistical analysis was performed to determine the significance of seasonal effects on the presence of PFASs in surface water. The average cumulative PFASs concentrations at each location were used to present the samples collected during summer (07/26/17) and fall (10/31/17). During each of the two sampling events, surface water samples were collected from the same 16 geographic locations, allowing for statistical comparison of PFAS concentrations at each sample location. To evaluate seasonal effects, the paired t test was performed to compare the mean total PFAS concentration in July with October. Four criteria must be met to perform the paired t test: continuity, normality, no outliers, and independence. Although the PFAS concentrations (dependent variable) were continuous with no outliers and the observations were independent of each other, the normality of the differences between July and October were uncertain. Thus, the Shapiro test was performed to check normality. The null and alternative hypotheses are shown below.

$$H_0: The\ data\ is\ normally\ distributed.$$

$$H_1: The\ data\ is\ not\ normally\ distributed.$$

Because the results of the Shapiro-Wilk test (shown in Section 3.6) indicated normality, the paired t test was then used, comparing the average concentrations (ng/L) of PFASs in surface water samples collected in the two different months. To perform the paired t test, two hypotheses were tested as shown below, where μ_d is true mean difference:

$$H_0: \mu_d = 0$$

$$H_1: \mu_d \neq 0$$

The statistical significance of the difference was identified based on the p-value generated from the paired t test. The statistical process described herein is described in Appendix B.

3.6 Results and Discussion

Two surface water samples were collected at sixteen locations within the Perdido Bay estuarine system on two different dates (7/25/17 and 10/31/17) and analyzed for 23 target PFAS analytes using the developed and validated UHPLC-MS/MS method. Eight PFASs out of the twenty-three analytes were detected in all surface water samples. The total concentrations of PFBS, PFHxA, PFHxS, PFHpA, PFOA, PFOS, PFNA, and PFDA detected in surface water samples collected in July 2017 and October 2017 ranged from 4.48 to 25.08 ng/L and 8.09 to 24.44 ng/L, respectively. The average detections of total PFASs were 12.73 ng/L and 12.87 ng/L from water samples collected in July 2017 and October 2017, respectively. These values are comparable to the average value of 6.02 ng/L reported for surface water collected from the Altamaha River, GA (Brad J. Konwick, 2009).

For PFASs observed in July and October, PFOS concentrations ranged from 0.83 to 4.33 ng/L and 1.35 to 4.13 ng/L, respectively, accounting for nearly half of all PFASs. PFOA concentrations also accounted for nearly half of all observed PFASs, ranging from

0.71 to 9.69 ng/L and 1.94 to 10.31 ng/L in July and October, respectively. The abundance of PFOA and PFOS compared to other PFASs is not surprising due to their extensive historical production and consumption (Moody et al., 2001; Munoz et al., 2017; USEPA, 2016b). The total concentrations of PFOA and PFOS at locations SW7 (Mouth of Elevenmile Creek) and SW8 (Mouth of Bayou Marcus) were dramatically higher compared to other sampling locations in both July and October; however, no locations exceeded the combined Health Advisory Level (70 ng/L) issued by EPA in 2016 (USEPA, 2016b). The average PFASs concentrations detected in Perdido Bay surface water at each location are given in Tables 3.1 and Table 3.2, and presented in Figures 3.2 and Figure 3.3.

Neither of the two emerging PFASs amenable to quantitation with the developed method were detected in any samples. This result is consistent with findings from several recent studies, and could be explained by the higher solubility and biodegradation potential reported for many emerging compounds compared to their legacy counterparts (Munoz et al., 2017; Onghena et al., 2012).

Table 3.1: Distribution of PFASs in surface water samples collected from Perdido Bay on July 25, 2017.

Surface Waters	Sample Location	PFASs (ng/L)								
		PFBS	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA	Total PFASs
7/26/2017										
SW1	Moccasin Bayou	1.76 ± 0.19	1.74 ± 0.15	1.31 ± 0.11	0.84 ± 0.06	2.56 ± 0.09	2.05 ± 0.37	0.60 ± 0.05	0.46 ± 0.04	11.31
SW2	Middle Wolf	1.88 ± 0.11	1.74 ± 0.15	1.01 ± 0.07	0.76 ± 0.12	2.31 ± 0.09	1.58 ± 0.27	0.54 ± 0.06	0.44 ± 0.08	10.25
SW3	Low Wolf	2.75 ± 0.13	2.25 ± 0.13	0.95 ± 0.06	1.00 ± 0.09	2.44 ± 0.13	1.82 ± 0.09	0.64 ± 0.05	0.46 ± 0.06	12.30
SW4	GIWW to Wolf Bay	2.15 ± 0.14	1.65 ± 0.22	0.95 ± 0.09	0.76 ± 0.15	2.09 ± 0.18	1.64 ± 0.20	0.57 ± 0.04	0.45 ± 0.05	10.26
SW5	Middle Wolf	1.57 ± 0.15	1.30 ± 0.15	0.82 ± 0.09	0.55 ± 0.11	1.59 ± 0.27	1.32 ± 0.16	0.47 ± 0.03	0.42 ± 0.06	8.02
SW6	Perdido River	0.84 ± 0.10	2.19 ± 0.29	0.83 ± 0.05	1.69 ± 0.16	3.68 ± 0.21	2.01 ± 0.19	0.90 ± 0.09	0.97 ± 0.07	13.10
SW7	Mouth of Elevenmile Creek	1.43 ± 0.19	3.22 ± 0.30	2.86 ± 0.61	1.87 ± 0.17	9.69 ± 1.75	4.33 ± 0.95	0.88 ± 0.10	0.80 ± 0.04	25.08
SW8	Mouth of Bayou Marcus	2.21 ± 0.23	4.61 ± 0.58	2.13 ± 0.15	2.08 ± 0.21	5.88 ± 0.62	2.87 ± 0.14	0.89 ± 0.07	0.86 ± 0.06	21.52
SW9	Upper Perdido Bay	1.14 ± 0.18	2.78 ± 0.21	1.51 ± 0.18	1.93 ± 0.23	6.10 ± 0.47	2.76 ± 0.32	0.96 ± 0.08	1.01 ± 0.11	18.19
SW10	Middle Perdido Bay	0.99 ± 0.05	2.33 ± 0.20	1.14 ± 0.07	1.81 ± 0.39	4.45 ± 0.47	2.20 ± 0.35	0.87 ± 0.11	0.89 ± 0.22	14.68
SW11	Lower Perdido Bay	1.06 ± 0.09	1.84 ± 0.32	1.02 ± 0.16	1.24 ± 0.30	2.96 ± 0.50	1.69 ± 0.12	0.63 ± 0.08	0.61 ± 0.06	11.05
SW12	Tarkiln Bayou	1.03 ± 0.05	1.76 ± 0.20	0.95 ± 0.06	1.16 ± 0.26	3.02 ± 0.24	1.72 ± 0.12	0.69 ± 0.07	0.64 ± 0.03	10.98
SW13	Deepest point (Ross Point)	1.19 ± 0.13	1.93 ± 0.24	0.98 ± 0.09	1.06 ± 0.14	3.01 ± 0.34	1.71 ± 0.21	0.65 ± 0.06	0.65 ± 0.03	11.17
SW14	Cotton Bayou	0.92 ± 0.07	0.51 ± 0.07	0.64 ± 0.03	0.18 ± 0.05	0.71 ± 0.05	0.83 ± 0.06	0.39 ± 0.02	0.30 ± 0.02	4.48
SW15	Dolphin Pass	0.98 ± 0.07	0.93 ± 0.14	0.75 ± 0.05	0.42 ± 0.15	1.24 ± 0.07	1.13 ± 0.09	0.47 ± 0.04	0.39 ± 0.02	6.32
SW16	Heron Bayou	1.40 ± 0.11	2.37 ± 0.21	1.34 ± 0.14	1.50 ± 0.09	4.41 ± 0.18	2.39 ± 0.29	0.85 ± 0.08	0.76 ± 0.14	15.01

Table 3.2: Distribution of PFASs in surface water samples collected from Perdido Bay on October 31, 2017.

Surface Waters	Sample Location	PFASs (ng/L)								
		PFBS	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA	Total PFASs
10/31/2017										
SW1	Moccasin Bayou	1.07 ± 0.10	1.00 ± 0.20	1.58 ± 0.12	1.12 ± 0.27	2.96 ± 0.15	2.05 ± 0.18	0.75 ± 0.08	0.66 ± 0.04	11.17
SW2	Middle Wolf	0.95 ± 0.14	0.77 ± 0.22	1.10 ± 0.18	0.64 ± 0.28	1.94 ± 0.78	1.46 ± 0.39	0.64 ± 0.15	0.59 ± 0.09	8.09
SW3	Low Wolf	1.07 ± 0.07	0.81 ± 0.13	1.30 ± 0.08	0.80 ± 0.15	2.35 ± 0.14	1.72 ± 0.10	0.72 ± 0.05	0.59 ± 0.03	9.34
SW4	GIWW to Wolf Bay	1.09 ± 0.10	0.91 ± 0.19	1.27 ± 0.10	0.83 ± 0.17	2.44 ± 0.11	1.69 ± 0.12	0.68 ± 0.07	0.63 ± 0.03	9.55
SW5	Middle Wolf	1.15 ± 0.12	1.31 ± 0.40	1.34 ± 0.09	1.08 ± 0.12	2.79 ± 0.27	1.88 ± 0.15	0.76 ± 0.06	0.66 ± 0.06	10.97
SW6	Perdido River	0.72 ± 0.34	0.45 ± 0.11	0.92 ± 0.06	1.40 ± 0.37	3.28 ± 0.23	1.89 ± 0.12	1.01 ± 0.09	1.04 ± 0.07	10.72
SW7	Mouth of Elevenmile Creek	0.99 ± 0.23	1.02 ± 0.24	3.11 ± 0.15	1.63 ± 0.31	10.31 ± 0.34	4.13 ± 0.34	0.95 ± 0.13	0.89 ± 0.10	23.03
SW8	Mouth of Bayou Marcus	1.71 ± 0.24	1.58 ± 0.23	3.36 ± 0.31	2.83 ± 0.13	8.94 ± 0.17	3.94 ± 0.26	1.15 ± 0.05	0.94 ± 0.06	24.44
SW9	Upper Perdido Bay	0.81 ± 0.07	0.74 ± 0.23	1.12 ± 0.06	1.27 ± 0.13	3.98 ± 0.37	2.10 ± 0.14	1.01 ± 0.11	1.06 ± 0.06	12.09
SW10	Middle Perdido Bay	0.82 ± 0.07	0.64 ± 0.13	1.22 ± 0.12	1.82 ± 0.19	5.21 ± 0.43	2.48 ± 0.19	1.21 ± 0.07	1.26 ± 0.10	14.66
SW11	Lower Perdido Bay	0.90 ± 0.08	0.79 ± 0.20	1.18 ± 0.11	1.56 ± 0.23	4.26 ± 0.45	2.07 ± 0.21	1.01 ± 0.09	1.16 ± 0.08	12.93
SW12	Tarkiln Bayou	0.84 ± 0.08	0.69 ± 0.08	1.16 ± 0.10	1.18 ± 0.24	3.43 ± 0.09	1.98 ± 0.09	0.93 ± 0.06	1.05 ± 0.05	11.25
SW13	Deepest point (Ross Point)	0.94 ± 0.03	0.86 ± 0.21	1.22 ± 0.11	1.56 ± 0.35	3.92 ± 0.59	2.24 ± 0.36	1.01 ± 0.16	1.08 ± 0.17	12.83
SW14	Cotton Bayou	1.12 ± 0.10	0.93 ± 0.12	1.76 ± 0.07	1.05 ± 0.18	2.71 ± 0.18	2.09 ± 0.22	0.84 ± 0.07	0.76 ± 0.03	11.25
SW15	Dolphin Pass	0.93 ± 0.06	0.90 ± 0.07	1.10 ± 0.10	0.82 ± 0.11	1.98 ± 0.08	1.35 ± 0.13	0.63 ± 0.04	0.60 ± 0.04	8.32
SW16	Heron Bayou	1.15 ± 0.13	0.96 ± 0.11	1.82 ± 0.29	1.33 ± 0.17	4.32 ± 0.75	3.76 ± 0.12	1.06 ± 0.21	0.92 ± 0.21	15.30

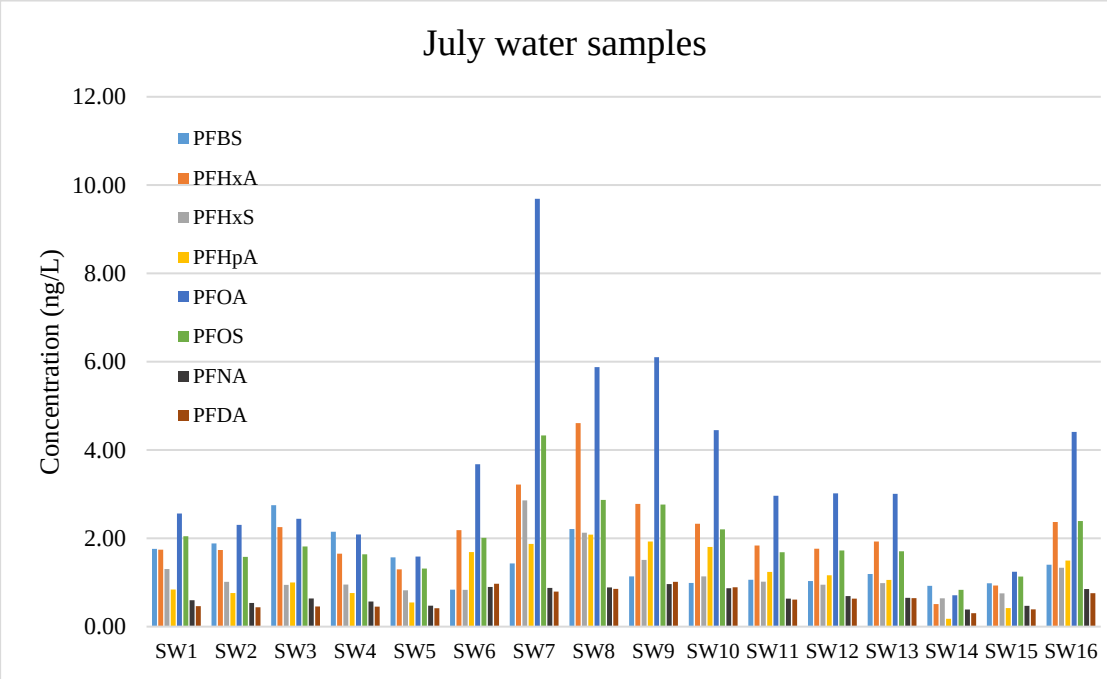


Figure 3.2: PFASs concentrations (July 2017 surface water samples).

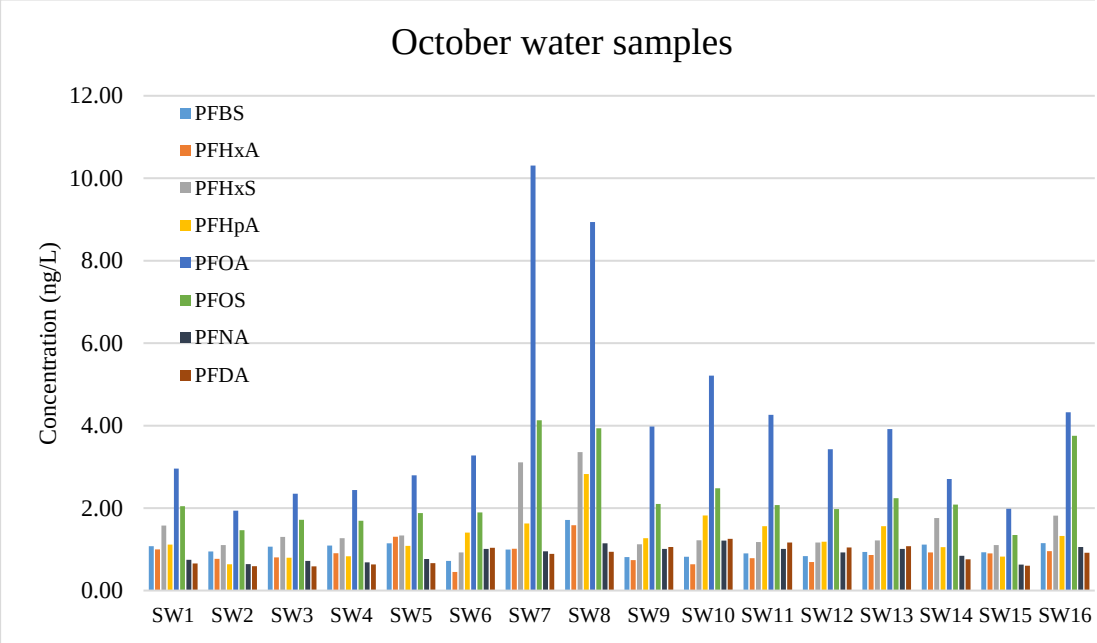


Figure 3.3: PFASs concentrations (October 2017 surface water samples).

The average cumulated PFAS concentrations in surface water samples at each location collected on different dates are presented in Figure 3.4, where SW 1a to SW 16a represent sampling locations in July 2017, SW 1b to SW 16b represent sampling locations in October 2017.

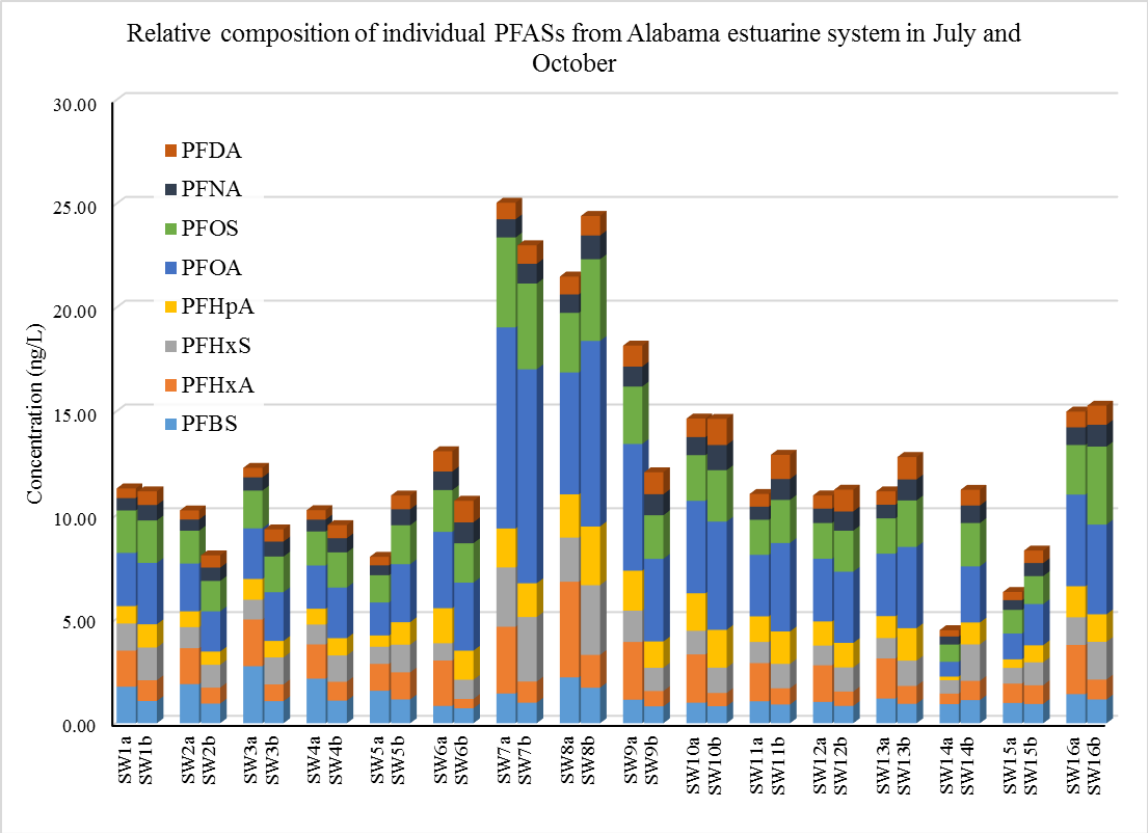


Figure 3.4: Relative composition of individual PFASs in surface water samples.

The average concentrations of PFASs at each location were higher at SW7, SW8, and SW9, which are located at the upstream of Perdido Bay, the possible sources of PFASs contamination could be wastewater treatment plants, industries, and landfill sites. The specific potential sources of contamination can not be identified due to the complex

dynamic estuarine conditions. The cumulative PFASs distribution at different sampling locations from July 2017 and Octorber 2107 are shown in Figure 3.5.

Figure 3.5: Distribution of total PFASs at all sampling locations.

To compare potential seasonal effects resulting from different sample collection dates (summer and fall), a paired t-test analysis was performed using the cumulative PFAS dataset. The p-value of the Shapiro-Wilk normality test was 0.3013, which was greater than 0.05. Thus, the null hypothesis (H_0) is not rejected, the data are normally distributed, and a paired t-test analysis is appropriate for this dataset. P-values from the paired t-test were greater than 0.05, meaning that the null hypothesis is not rejected (mean difference is equal to zero). Thus, there was no significant difference (p-value = 0.7895) between the mean concentrations of PFASs for samples collected during the two different events at each location. Therefore, it is reasonable to conclude that the observed PFAS concentrations are not seasonably dependent.

Chapter 4: Conclusions, Limitations, and Recommendations

4.1 Conclusions

At the time of this publication, Alabama had the second-most number of drinking water systems known to be contaminated with PFAS (Hu et al., 2016; Sheets, 2017). Because the problem of PFAS contamination is likely to continue to grow, it is critical that scientists and engineers understand the risks PFAS compounds pose to human health and the environment and begin to develop technologies to mitigate those risks. A necessary component of understanding these risks is continued development of efficient, validated analytical methods for detection and quantitation of PFAS compounds in a variety of environmental media. This study focused on developing, optimizing, and validating a method for simultaneous, trace analysis of 23 PFASs in estuarine surface water. Development of the method described herein considered various options for optimization with respect to chromatographic and MS conditions. Chromatographic optimization focused on selecting appropriate solvents for standard preparation, suitable solvents for mobile phases, and appropriate analytical column and guard column selection to achieve optimum peak profiles and resolution in UHPLC-MS/MS analysis. MS optimization included a sequence of scan analyses conducted to produce an MRM method. The complete optimized method was validated, and sample preparation methods were developed to optimize sample cleanup and extraction prior to analyzing real surface water samples.

The developed method was used to detect and quantitate target PFAS analytes in water samples collected from the Perdido Bay estuarine system in July and October, 2017. Out of 23 target PFAS compounds, only 8 were detected in surface water samples (PFBS, PFHxA, PFHxS, PFHpA, PFOA, PFOS, PFNA, and PFDA), among which PFOA and PFOS accounted for the majority of total PFAS concentrations. Total detected PFASs concentrations were at ng/L level, and the concentrations of PFOA and PFOS were considerably higher in the northern portion of Perdido Bay compared to other sampling locations. There was no statistically significant seasonal effect observed when the cumulative PFAS concentration dataset was considered.

4.2 Limitations

Although the results of percent recovery are acceptable and reasonable, with values ranging from 80 to 120%, recoveries exceeding 100% suggest that some target PFAS compounds were carried over during multiple sample analysis. Additional method development, including additional equipment modifications, is required to reduce or eliminate possible sources of carry-over contamination.

4.3 Recommendations

To improve method accuracy and reproducibility, instrument performance over multiple analyses must be optimized. A previous study recommends replacement of polytetrafluoroethylene (PTFE) solvent frits with stainless steel frits and the use of polyetheretherketone (PEEK) tubing to eliminate contamination (M. Wu et al., 2017; Yamashita et al., 2004). In addition, an adequate numbers of blanks between sample analyses, and the installation of a PFAS trapping column are recommended to eliminate carry over issues. Because the sample extraction and cleanup procedure developed in this

work is time consuming, development of more efficient sample preparation methods is necessary for improving overall method efficiency.

PFAS concentrations observed in Perdido Bay surface water were higher at sampling locations SW7, SW8, and SW9, which suggests a potential source for these compounds may be present in this area. Further investigation of potential source areas is justified in order to determine the origin of observed PFAS detections.

Although comparison of PFAS concentrations from July 2017 and October 2017 sampling events did not differ statistically, long-term data collection and analysis (sample collection and HPLC-MS/MS analysis throughout the year) is recommended to fully investigate potential seasonal effects.

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Appendix A: PFASs Analysis Details

Appendix A displays the procedure of qualitative analysis of PFASs using MassHunter Qualitative Analysis Version B.07.00, and explains the process of quantitative analysis of PFASs using MassHunter Quantitative Analysis Version B.07.00 for QQQ.

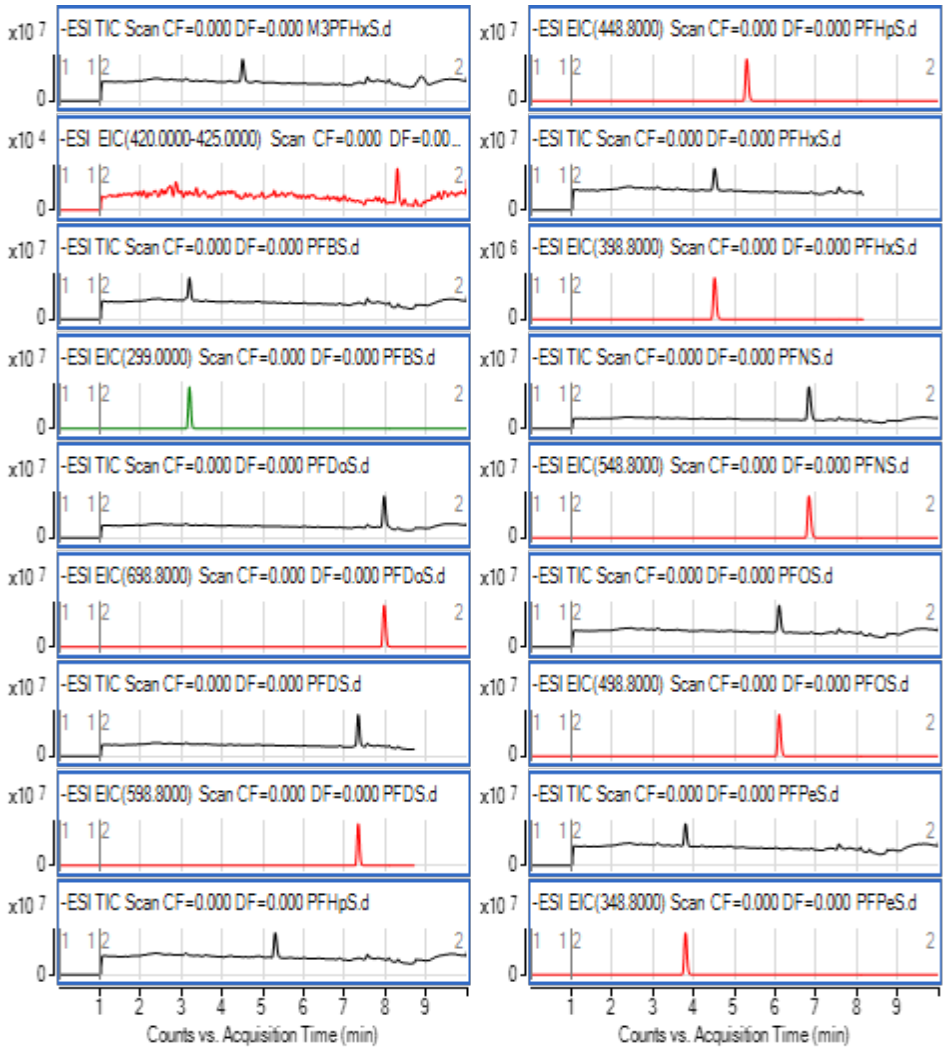

Figure A.1: Full scan analysis for PFSAs.

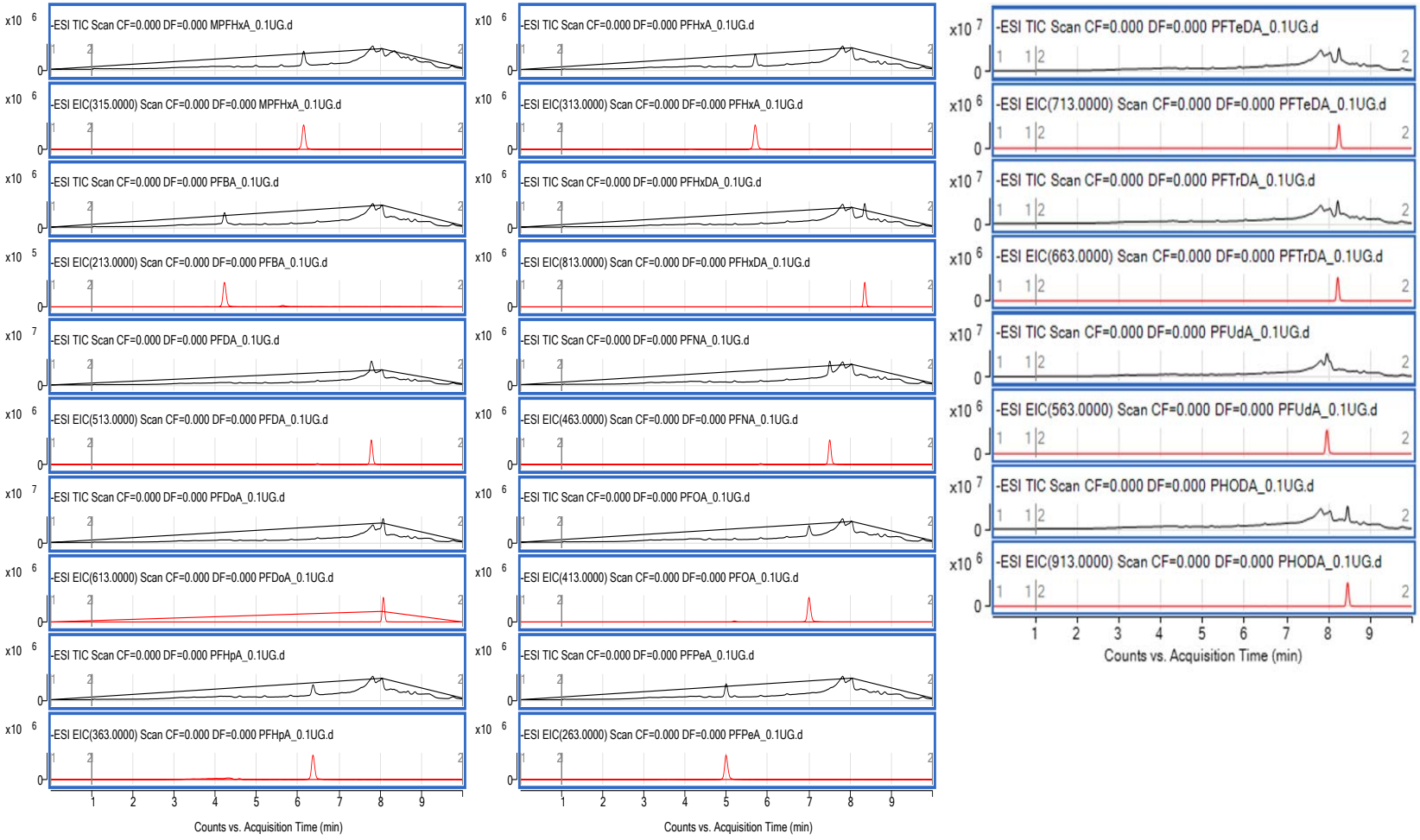

Figure A.2: Full scan analysis for PFCAs.

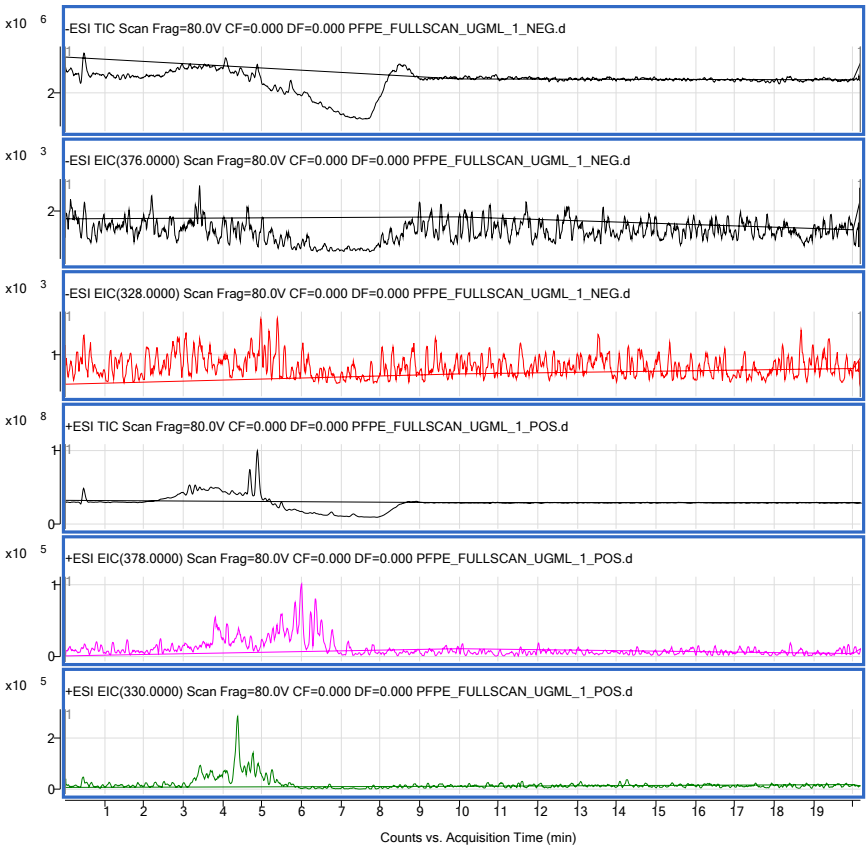

Figure A.3: Full scan analysis for PFPEs.

Table A.1: SIM analysis comparison for PFSAs.

Precursor ion molecular weight (m/z)	Area (FV=70V)	Area (FV=80V)	Area (FV=90V)	Area (FV=100V)	Area (FV=110V)	Area (FV=120V)	Area (FV=130V)	Area (FV=140V)	Area (FV=150V)
698.8	58128986.65	55990847.63	54085213.00	52963724.57	51404742.68	50950224.48	49335765.37	49337590.14	48354629.52
598.8	37549990.31	36092763.76	35192387.33	34560409.44	32904156.39	32364162.36	32281034.17	30766240.93	30551662.74
548.8	34060196.66	32107753.82	31064450.94	30652405.19	29380454.10	29292875.24	27902638.48	27767311.59	26971467.80
498.8	100148294.23	97337983.24	94656060.38	91918574.57	89680123.47	87167055.15	85494558.98	84018551.80	82617547.77
448.8	26353924.29	25032143.88	24529677.43	23409659.32	22604089.23	21860475.34	21240805.78	21065177.29	20843608.29
401.8	20795537.19	19877929.65	19362613.59	18406580.53	17753254.02	17250480.39	16733104.39	16447436.43	16052823.58
398.8	21749941.85	20519633.50	19720693.46	19029573.11	18391367.66	18235205.34	17680513.83	17295493.54	16635273.07
348.8	20429331.03	19531874.99	18800228.40	18456304.52	17481875.45	17316717.62	16510277.75	15923148.62	15656451.61
298.9	17940417.72	17152898.71	16594497.64	15667548.91	15007737.76	14396301.79	13802896.88	13279617.63	12393812.67

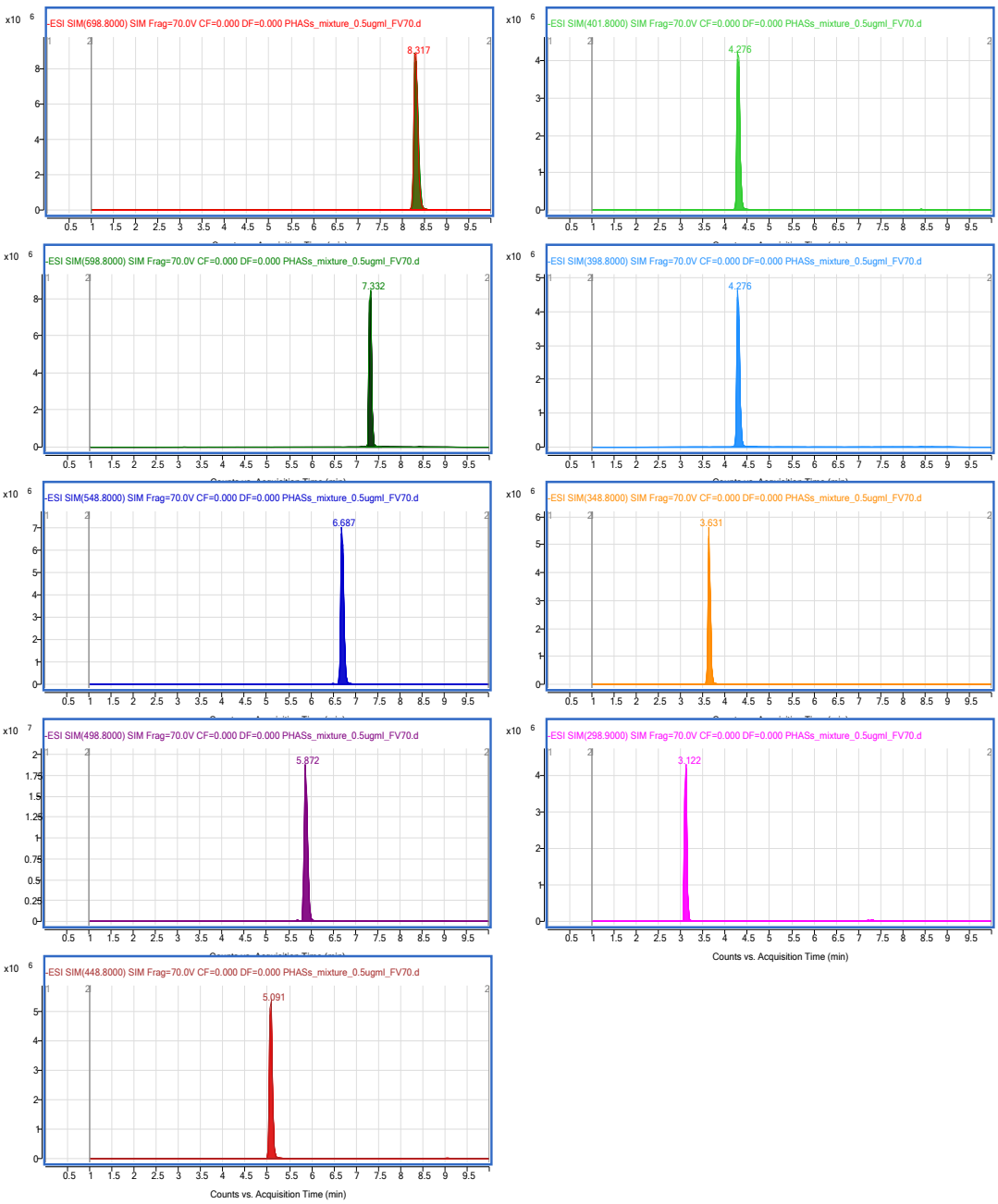

Figure A.4: SIM scan analysis for PFSAs.

Table A.2: SIM analysis comparison for PFSAs.

Precursor ion molecular weight (m/z)	Area (FV=70V)	Area (FV=80V)	Area (FV=90V)	Area (FV=100V)	Area (FV=110V)	Area (FV=120V)	Area (FV=130V)	Area (FV=140V)	Area (FV=150V)
912.8	78132.53	91865.97	88570.32	98602.60	94941.96	97204.02	94612.22	92650.52	91555.42
812.9	411498.25	395081.85	402700.17	419549.60	401631.07	396188.84	385241.05	351442.30	321127.40
712.9	66406.89	75142.59	77423.29	78443.59	84337.97	82087.65	76850.56	68862.12	64231.09
662.9	476569.53	487847.12	488160.66	490644.33	448199.24	424005.52	394900.85	306112.97	190163.86
612.9	703238.58	696787.96	681007.71	697632.48	652253.98	564666.14	475841.25	322409.31	206785.51
562.8	105006.68	93096.22	80174.99	95807.44	73443.45	63209.09	46391.81	31581.76	20233.50
512.9	106945.37	124919.31	134420.98	109698.98	102505.49	73934.50	55379.45	42679.94	32601.16
462.9	135843.56	145595.78	140392.11	134862.98	96497.36	69848.24	42007.23	14330.69	5907.58
412.9	191053.79	187728.63	181537.81	165457.07	113057.31	59472.44	28552.31	16952.69	16161.44
362.9	432265.93	427436.73	379818.18	279929.21	166633.19	77509.70	34506.44	33554.28	21163.67
312.9	369910.01	353886.96	278962.53	178596.67	91666.06	41762.43	24087.09	20279.37	23131.23
262.9	351116.69	310623.79	219409.84	122803.85	60991.20	32905.25	26606.20	27919.69	31066.60
212.9	205171.88	170850.61	109822.63	61918.62	36984.75	30107.87	30254.02	52122.90	69921.67

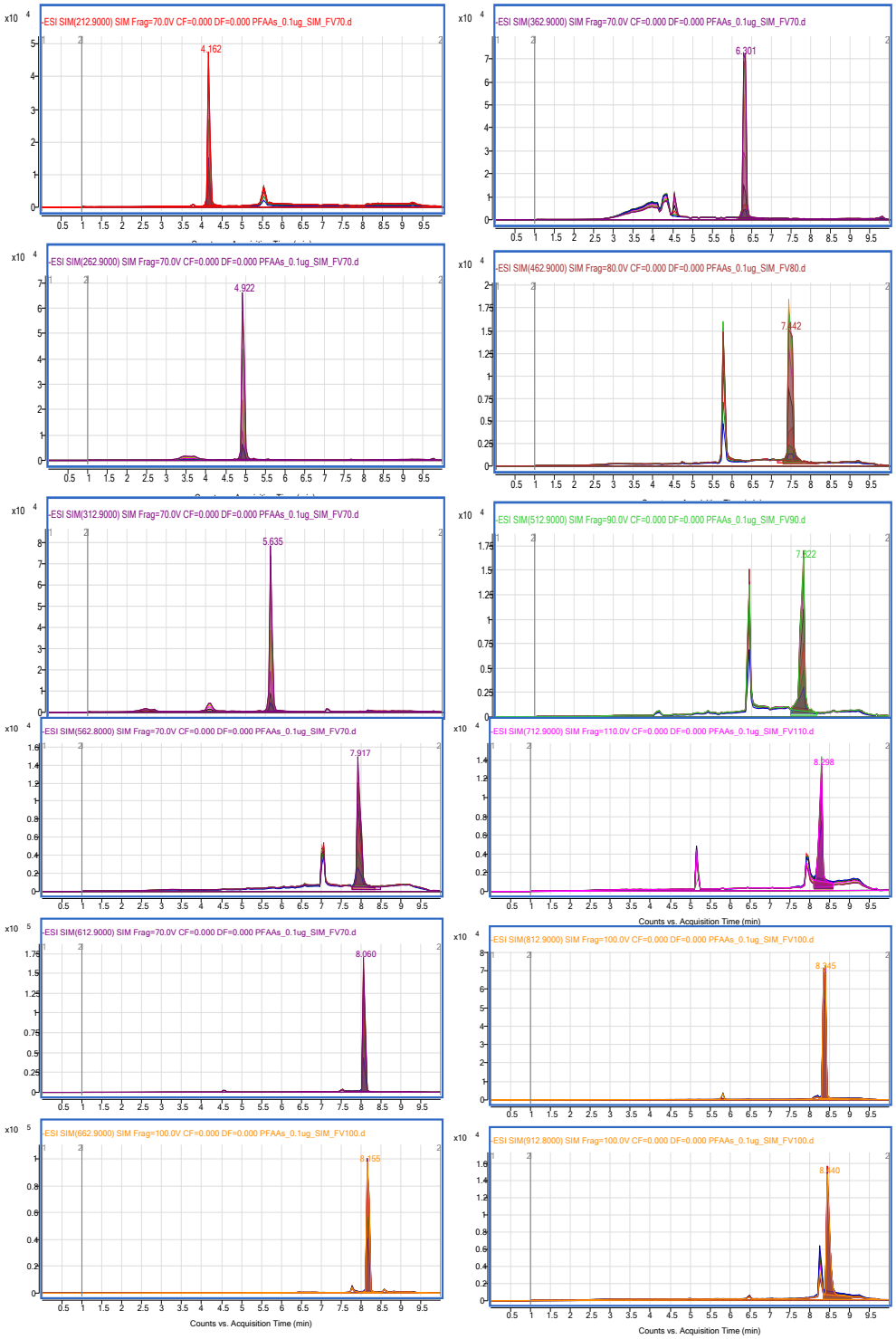

Figure A.5: SIM scan analysis for PFCAs.

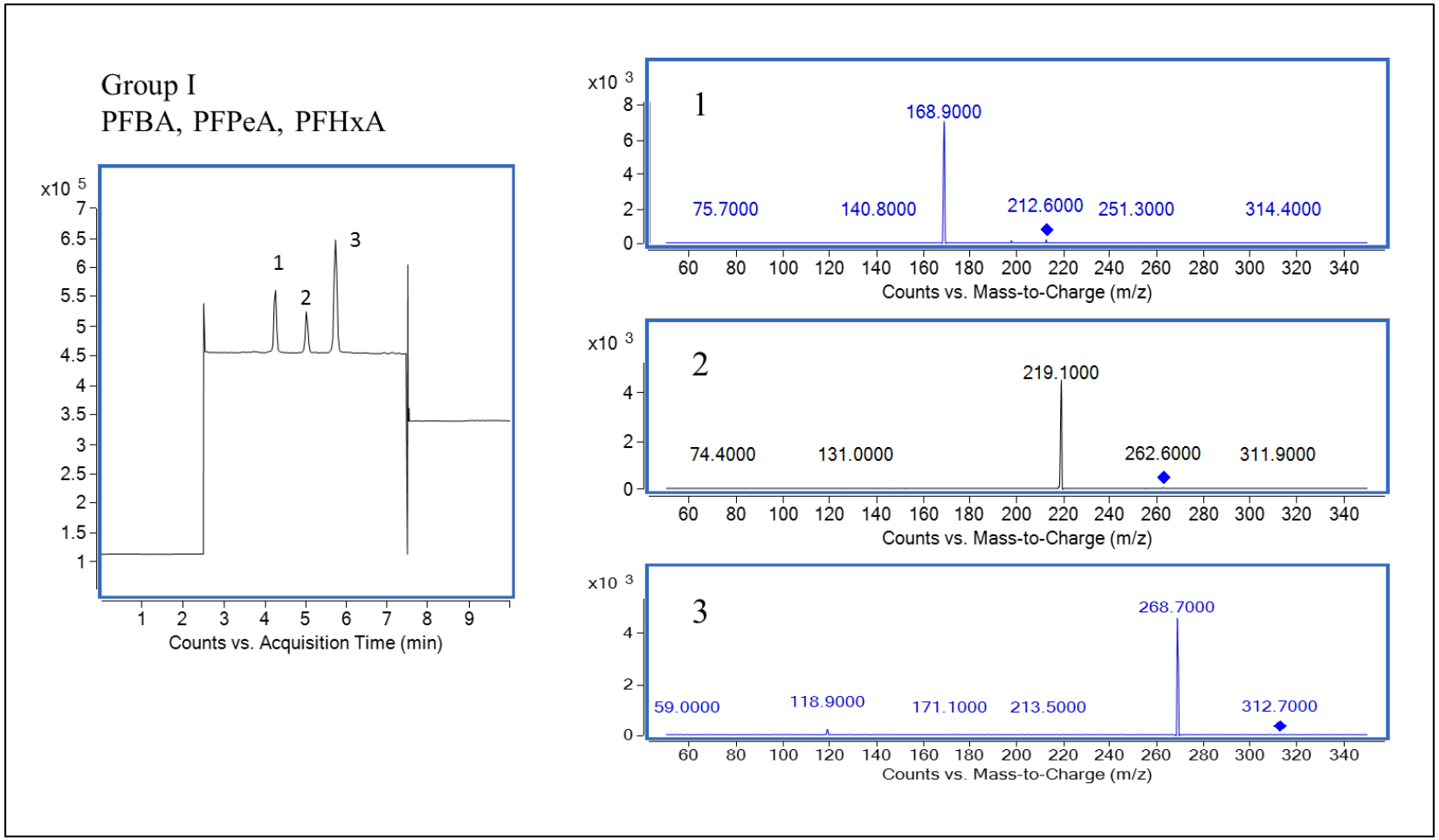

Figure A.6: PI scan analysis for PFCAs (group 1).

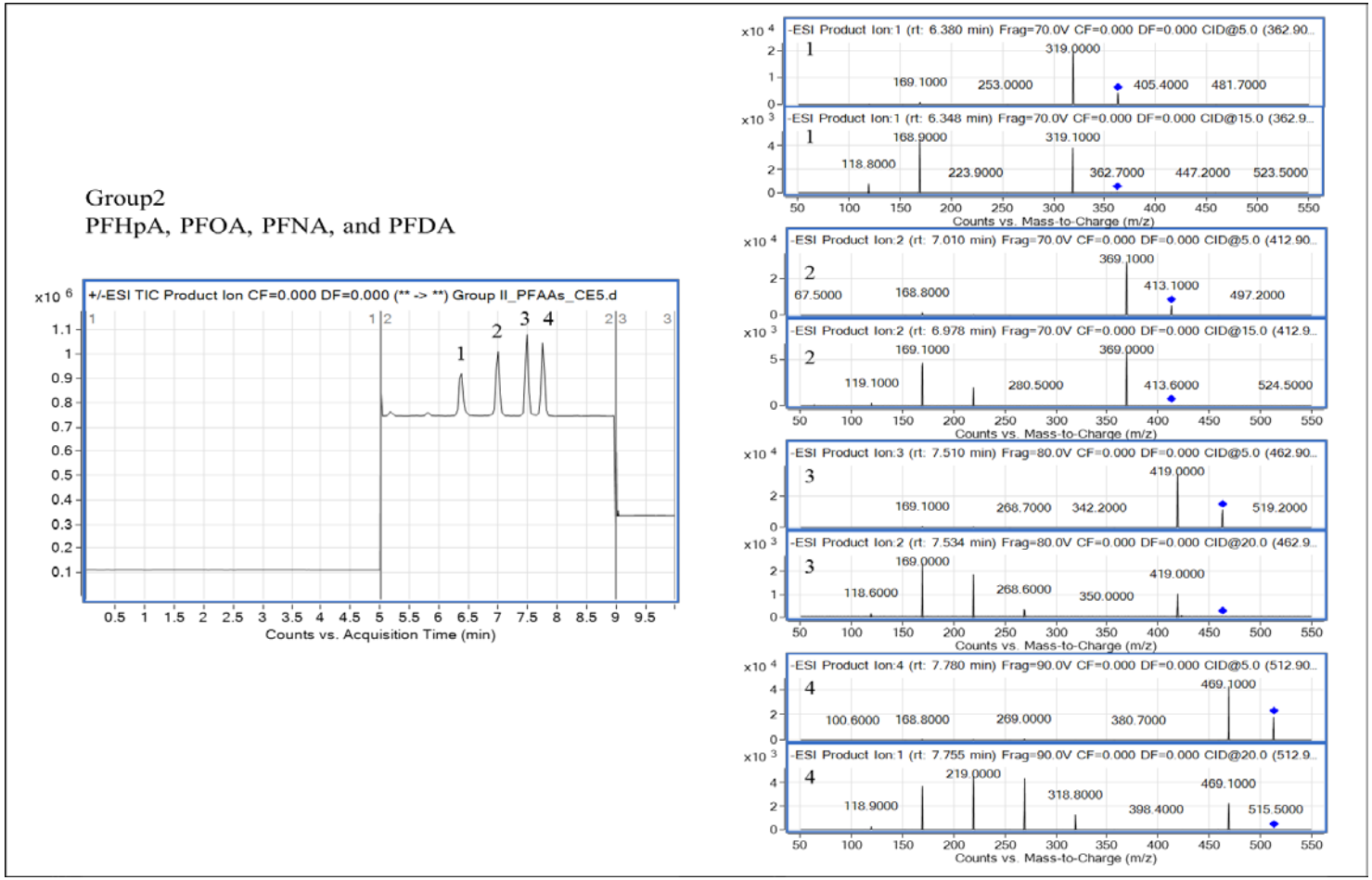

Figure A.7: PI scan analysis for PFCAs (group 2).

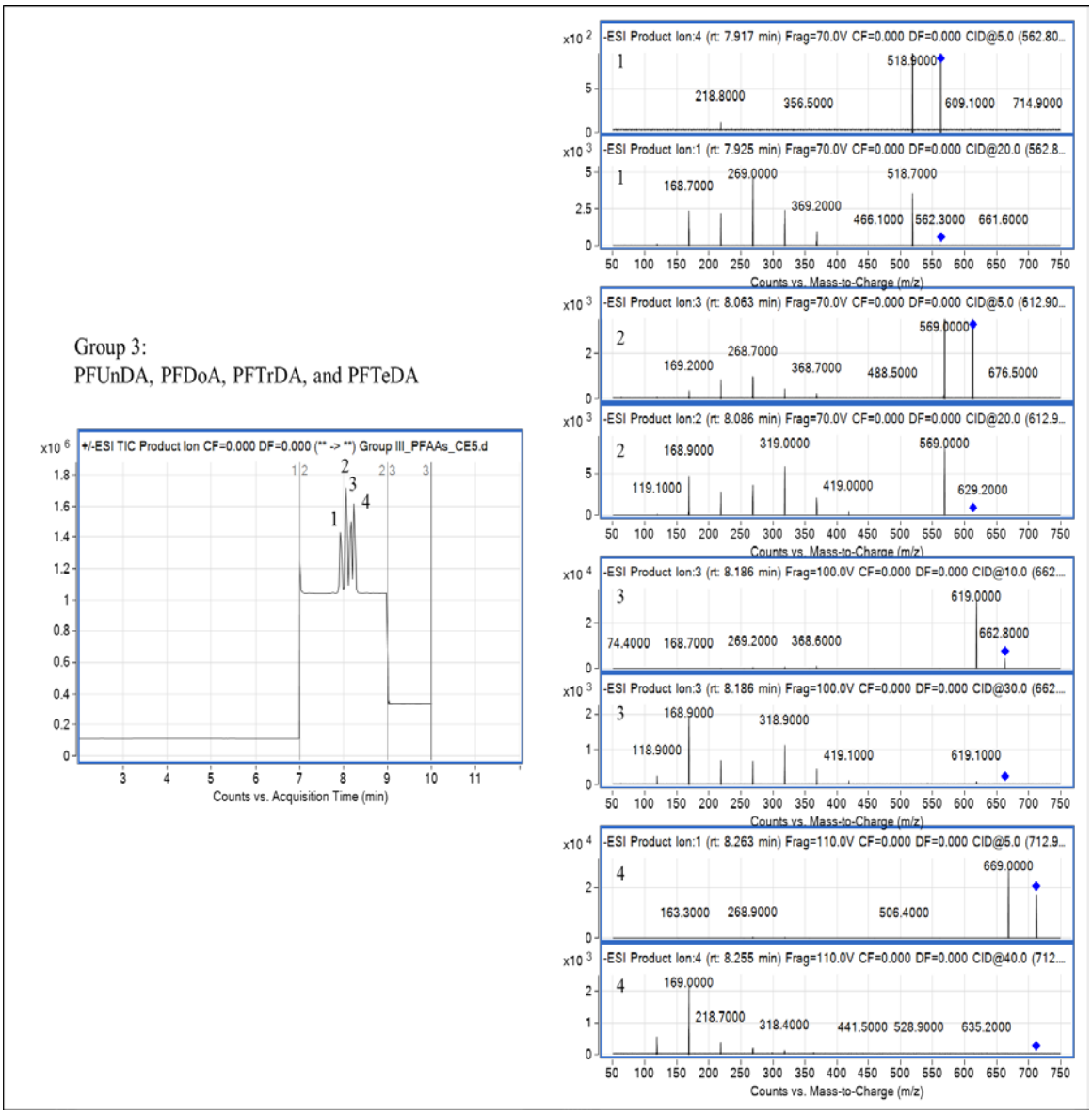

Figure A.8: PI scan analysis for PFCAs (group 3).

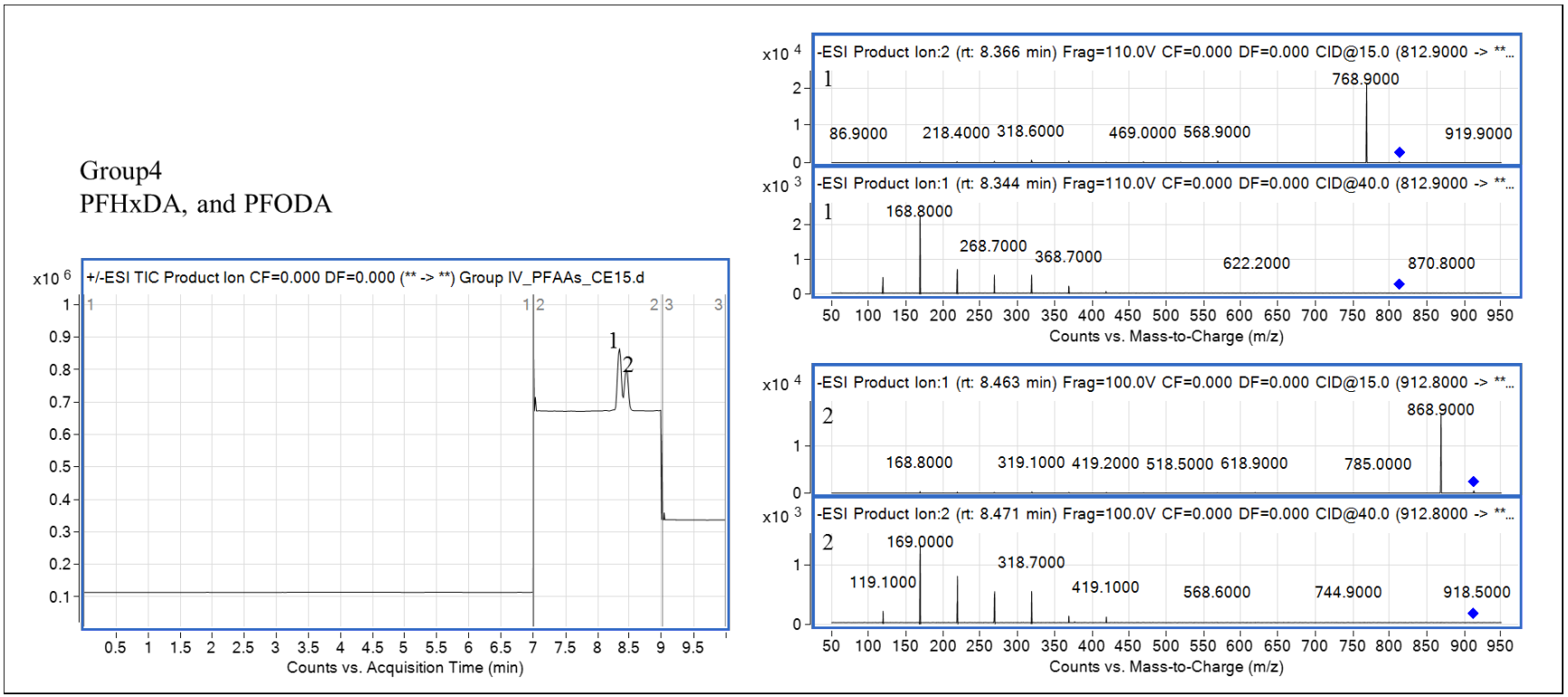

Figure A.9: PI scan analysis for PFCAs (group 4).

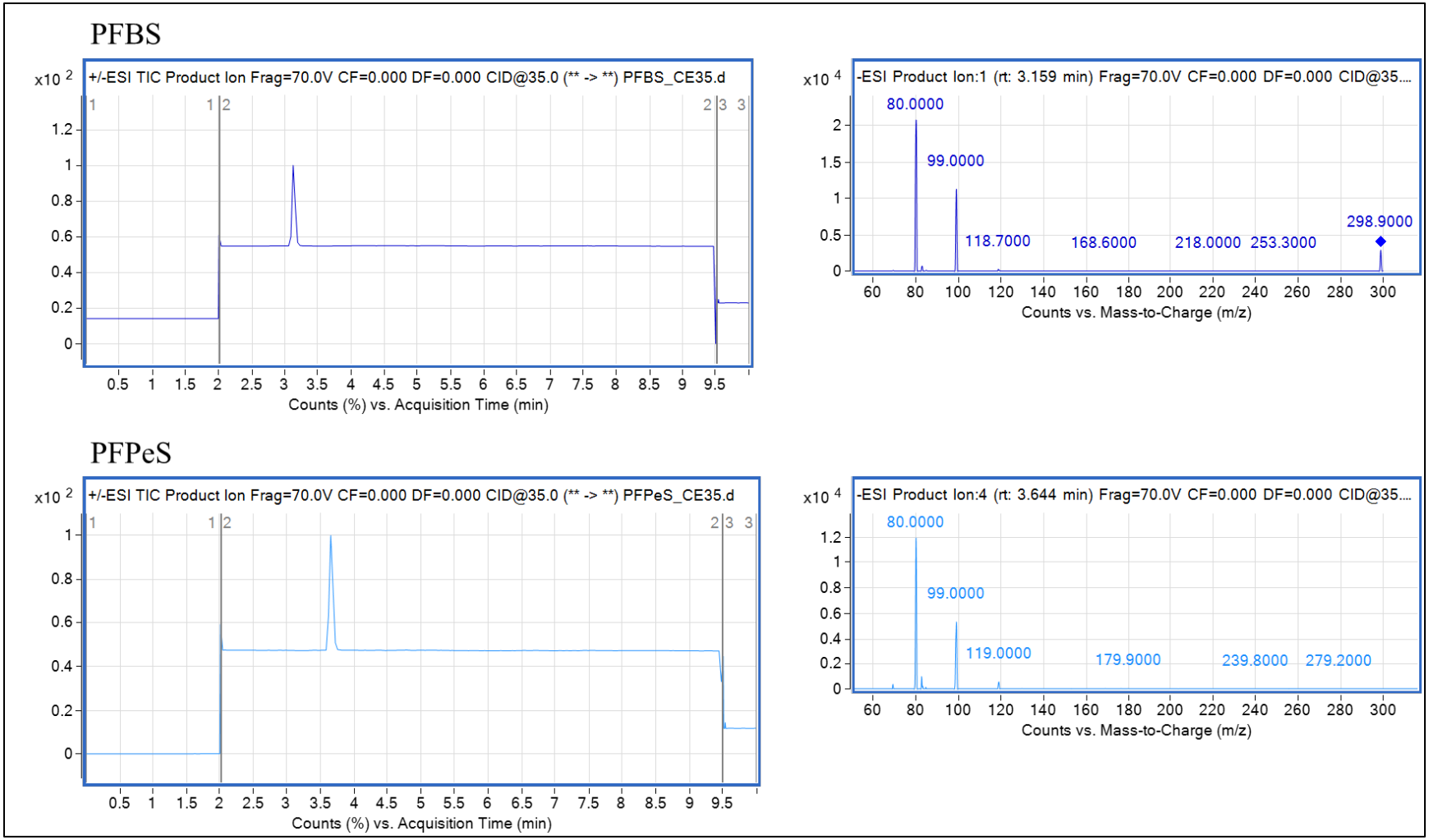

Figure A.10: PI scan for PFBS and PFPeS.

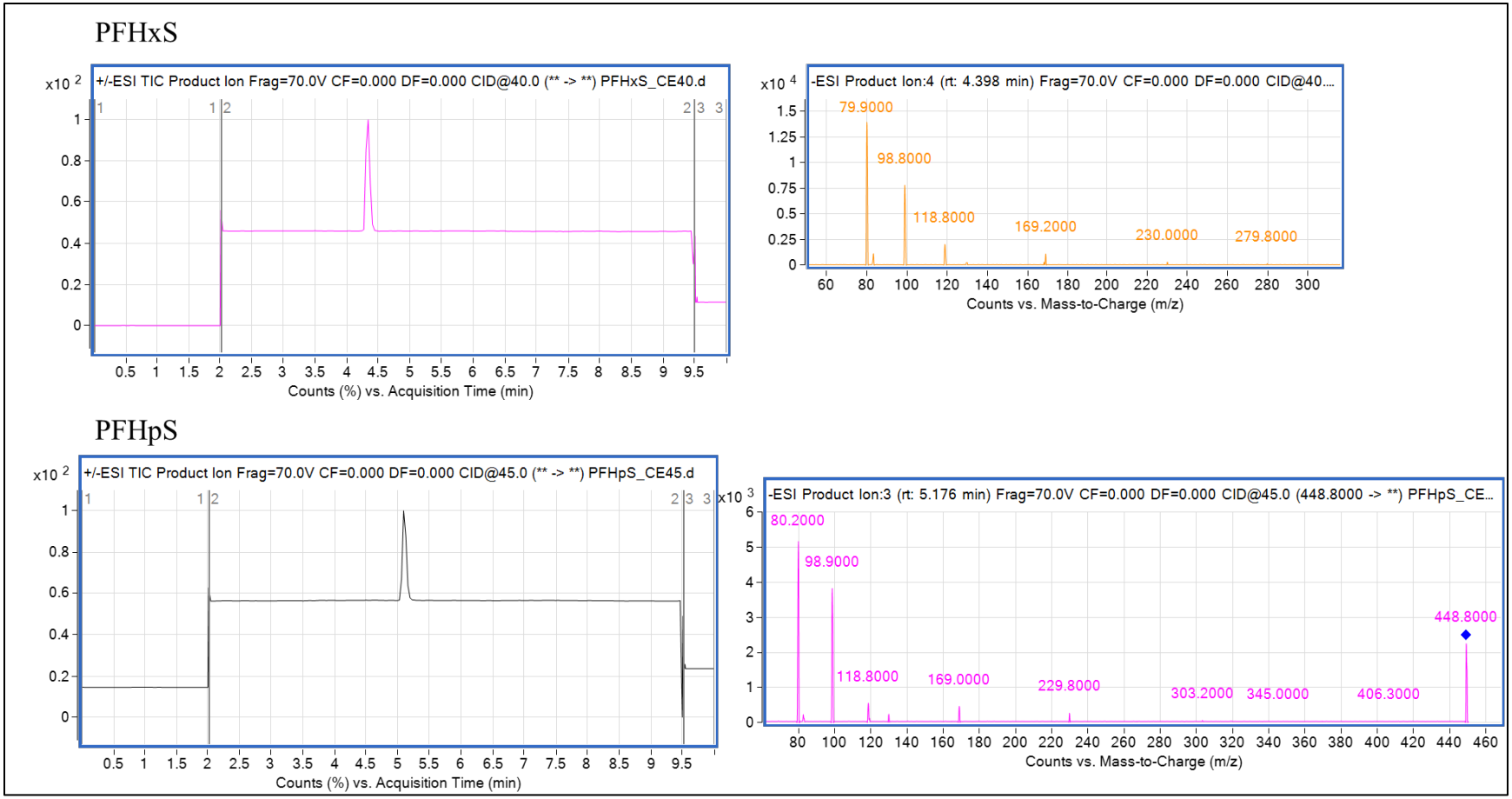

Figure A.11: PI scan for PFHxS and PFHpS.

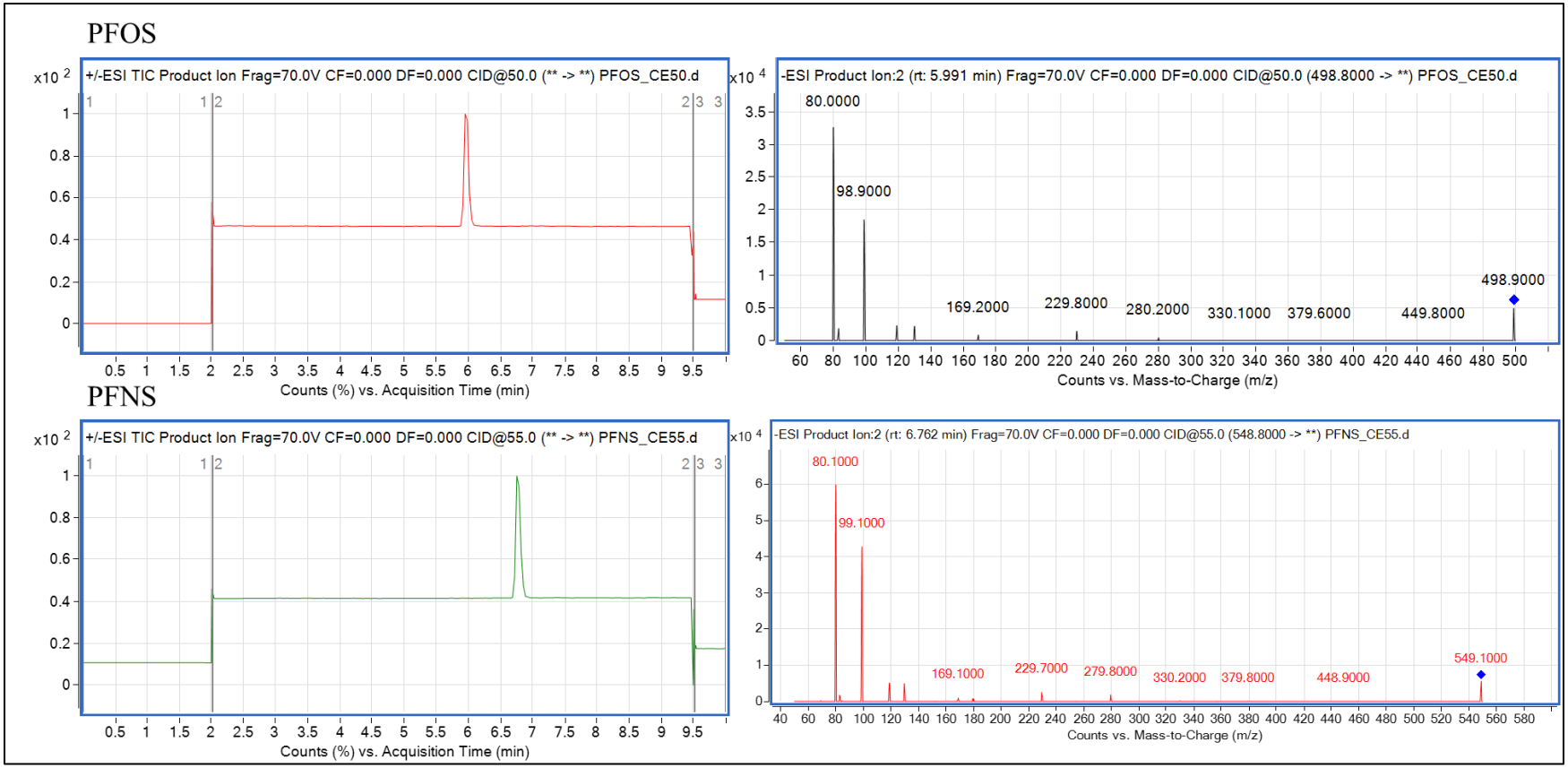

Figure A.12: PI scan for PFOS and PFNS.

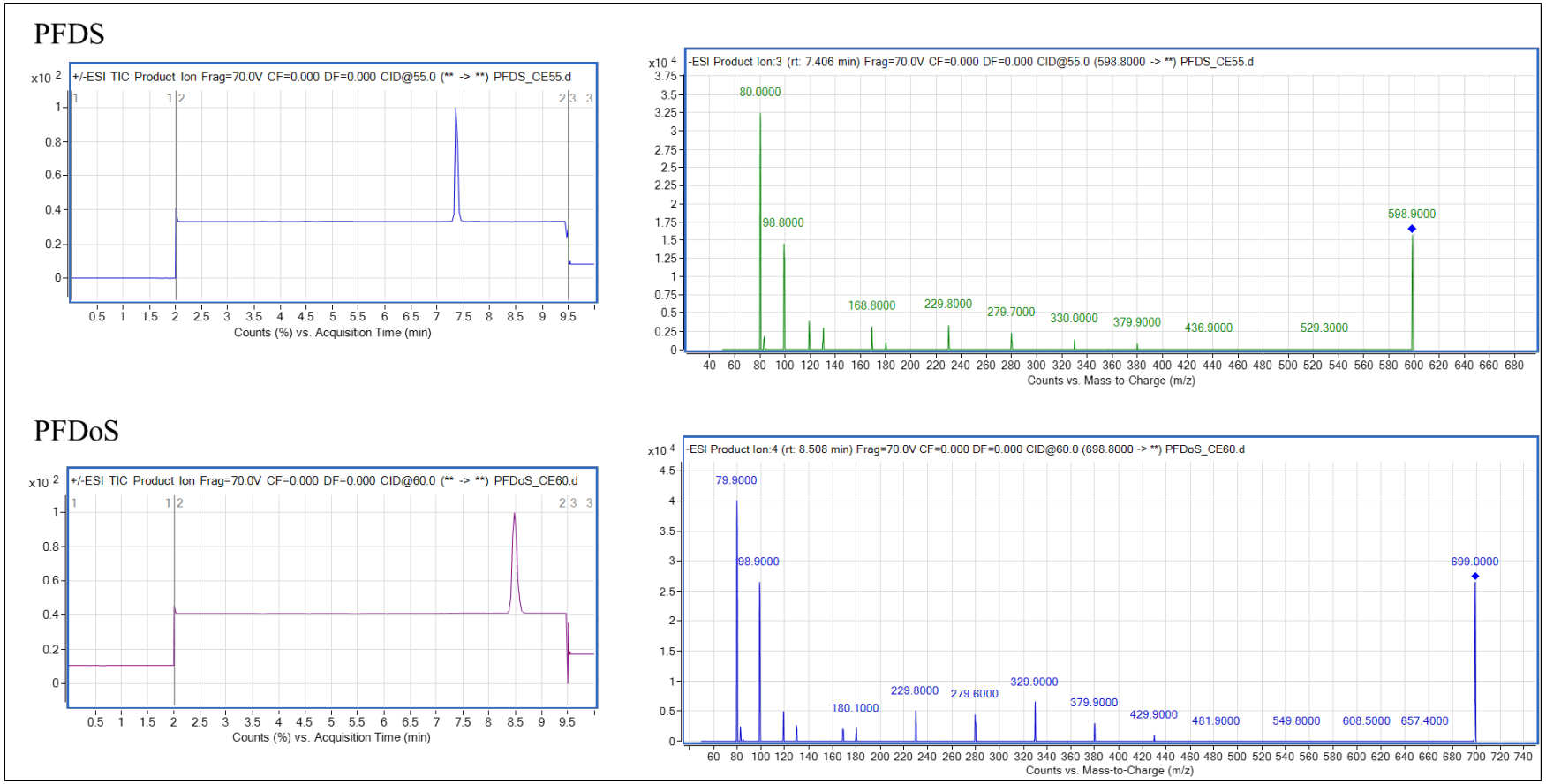

Figure A.13: PI scan for PFDS and PFDoS.

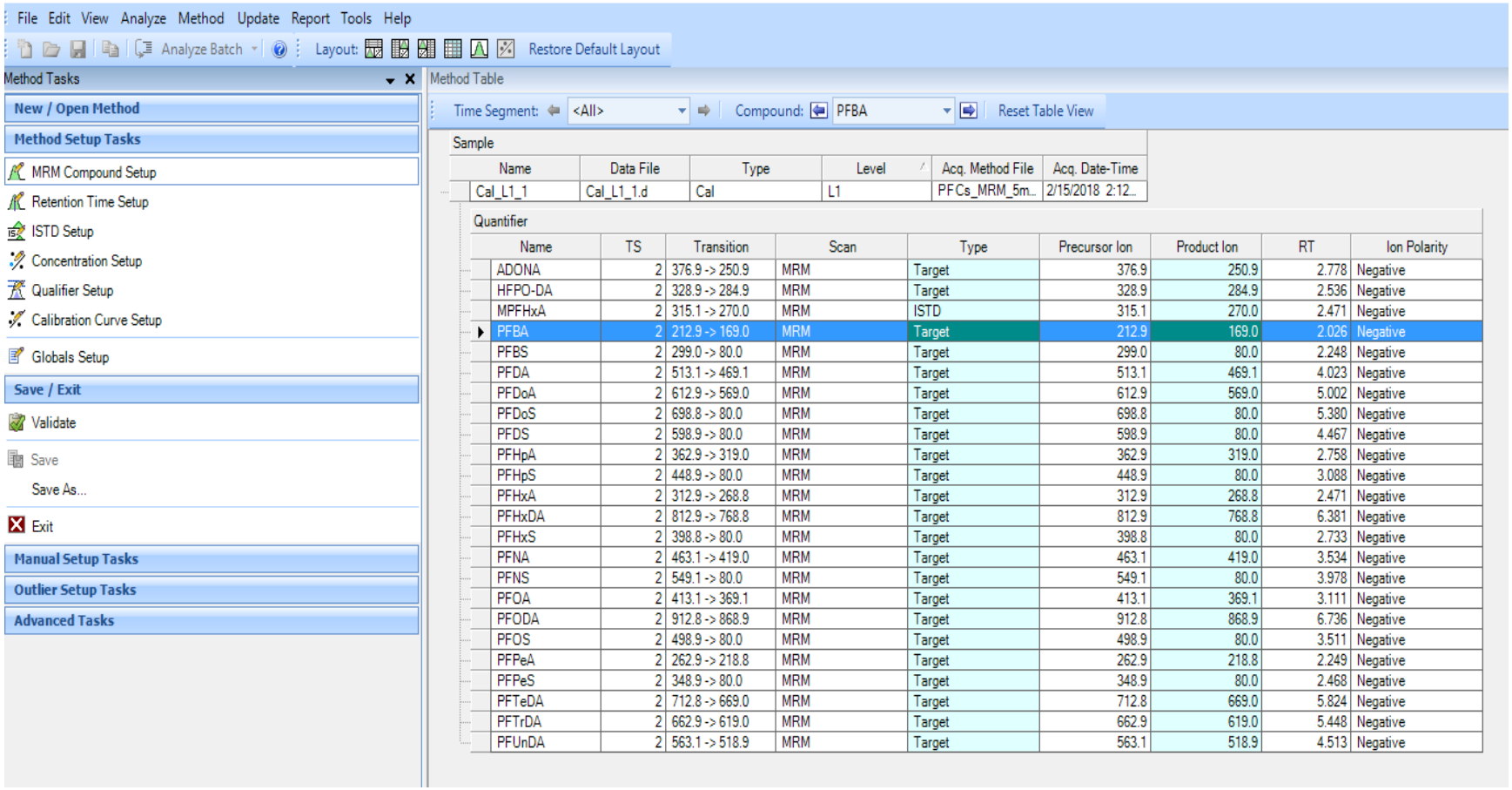

Figure A.14: MRM parameters setup.

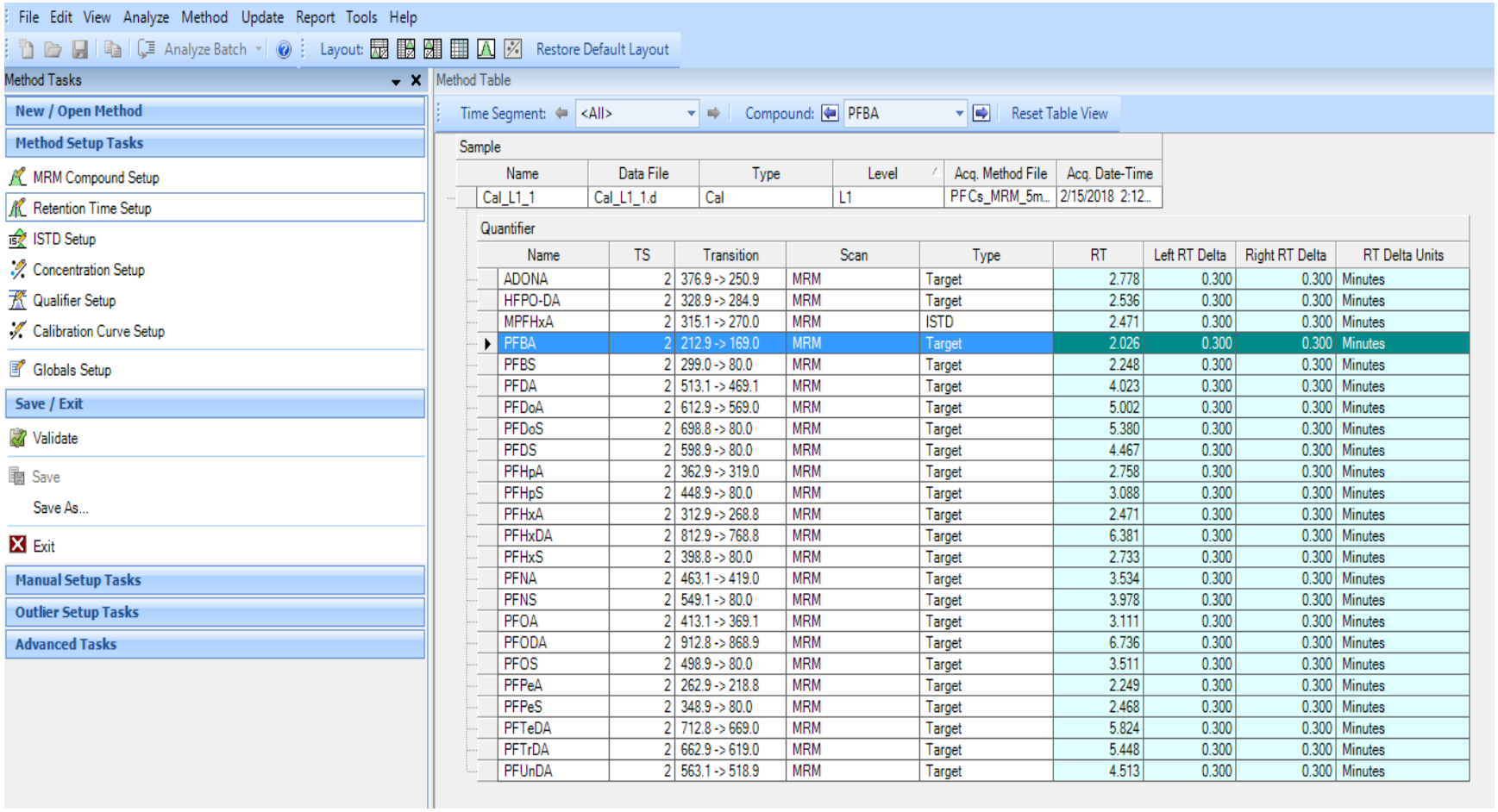

Figure A.15: Retention time setup.

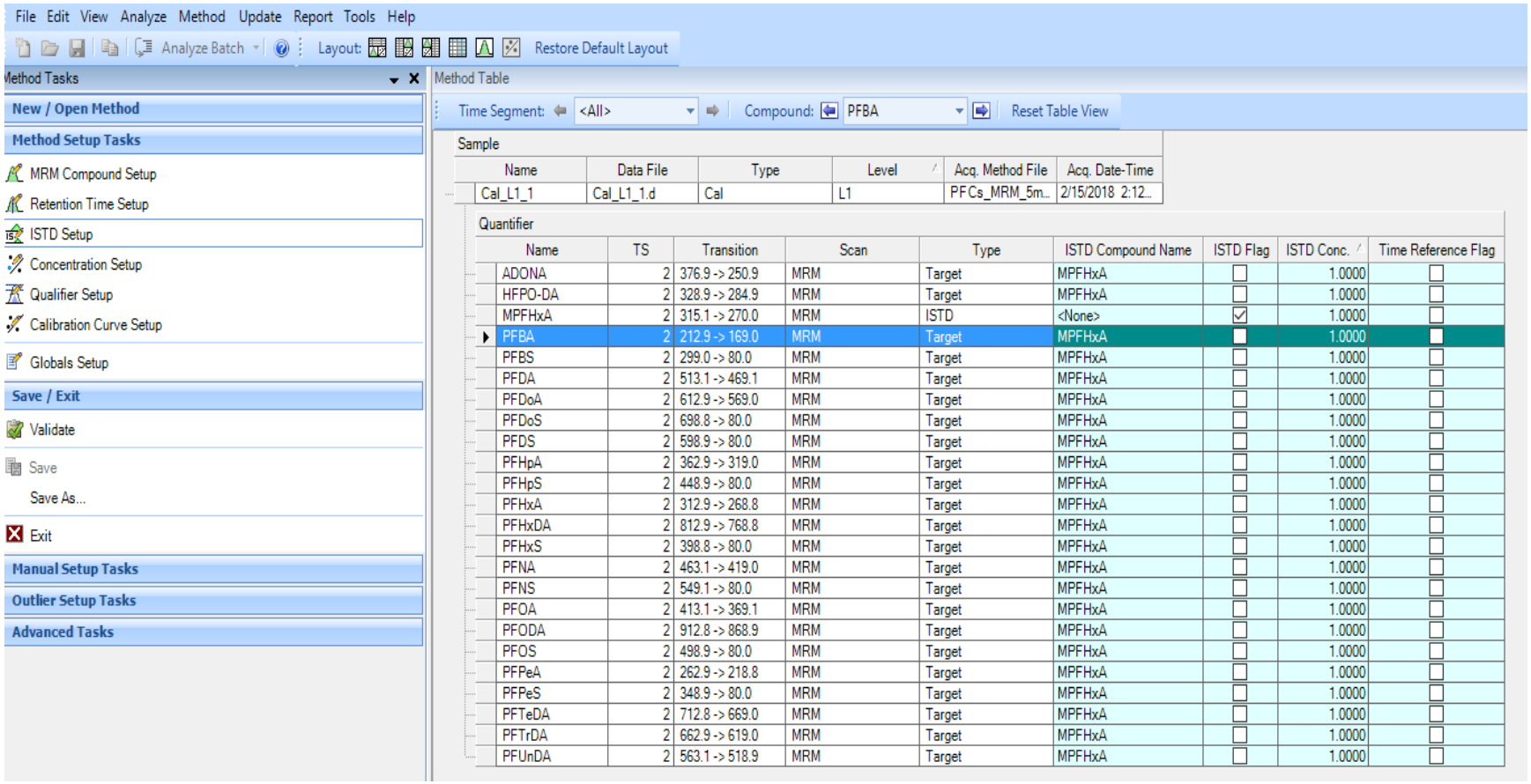

Figure A.16: Internal standard information.

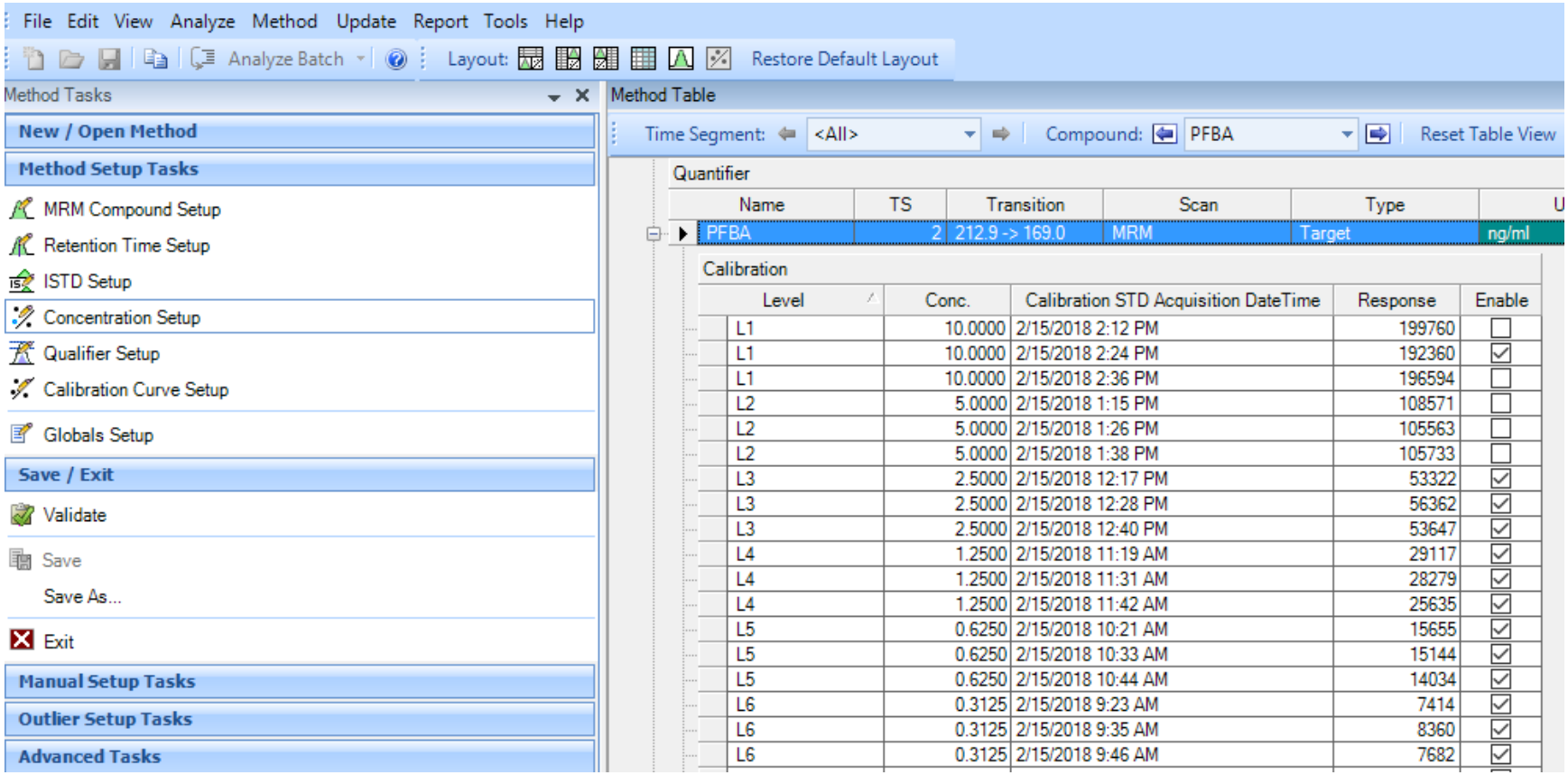

Figure A.17: Concentration setup.

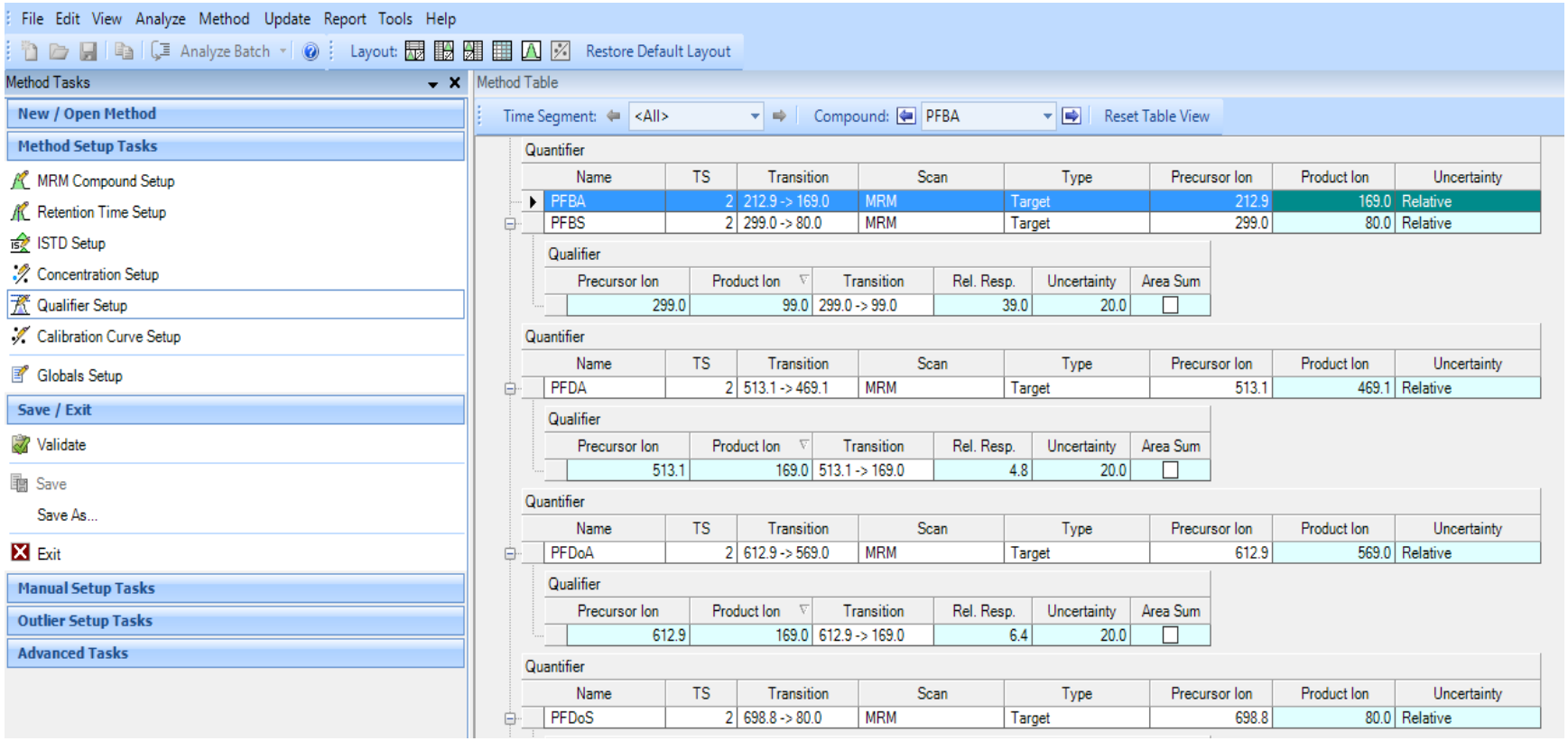

Figure A.18: Qualifier setup.

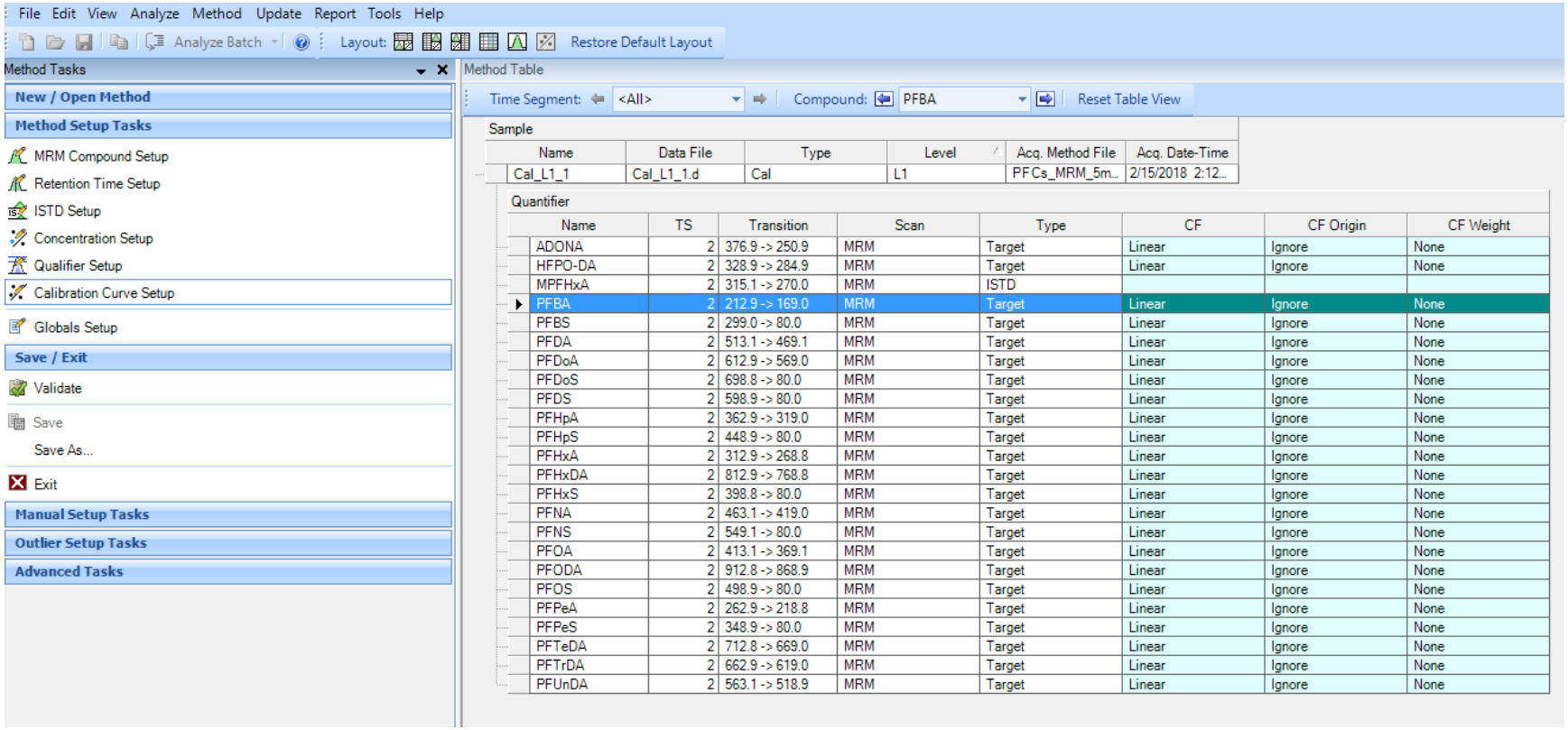

Figure A.19: Calibration curve setup.

Appendix B: Seasonal Analysis of PFASs Contamination

Appendix B provides detailed process about seasonal analysis of PFASs contamination by using R. Seasonal analysis of the short-term (from July to October) PFASs contamination at 16 locations from Alabama estuarine system.

The paired t test is used to compare mean difference. The paired t test has four assumptions: the dependent variable must be continuous; the difference between each dataset should be approximately normally distributed with no outliers; the observations are independent to each other; the dependent variable (concentration) is continuous with no outliers, which are independent variables.

However, we are not sure if this data set is normally distributed. Shapiro test was performed to check normality. The null and alternative hypotheses are shown below, where μd is true mean difference:

H_0: The data is normally distributed

H_1: The data is not normally distributed

The developed R code is displayed below:

```
july = c(11.31, 10.25, 12.3, 10.26, 8.02, 13.13, 25.24, 21.52, 18.19, 14.68, 11.05, 10.98, 1
1.17, 2.28, 6.32, 15.01)
octo = c(11.17, 8.09, 9.34, 9.55, 10.97, 10.72, 23.03, 24.44, 12.09, 14.66, 12.93, 11.25, 1
2.83, 11. 25, 8.32, 14.72)

diffe=july-octo
shapiro.test(diffe)

##
##  Shapiro-Wilk normality test
##
## data:  diffe
## W = 0.93586, p-value = 0.3013
```

The p-value is 0.3013 which is greater than 0.05. We fail to reject H0. The data is normally distributed. And paired t test can be used for this dataset.

After validated all assumptions, the parried t test is performed. The null and alternative hypotheses are shown below, where μd is true mean difference:

$$H_0: \mu d = 0$$

$$H_1: \mu d \neq 0$$

The concentrations (ng/L) of PFASs in surface water samples collected in different month:

July: 23.75, 33.14, 19.19, 18.86, 55.85, 32.04, 11.01, 10.10

October: 16.23, 14.21, 24.59, 20.94, 65.11, 36.02, 14.34, 13.91

```
t.test(july, octo, paired = TRUE, alternative = "two.sided")

##
##  Paired t-test
##
## data:  july and octo
## t = -0.27173, df = 15, p-value = 0.7895
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -2.017557  1.561307
## sample estimates:
## mean of the differences
##              -0.228125
```

P-values is greater than alpha (0.05), which means that we fail to reject the null hypothesis. In conclusion, there is no significant difference (p-value = 0.7895) of the two means.