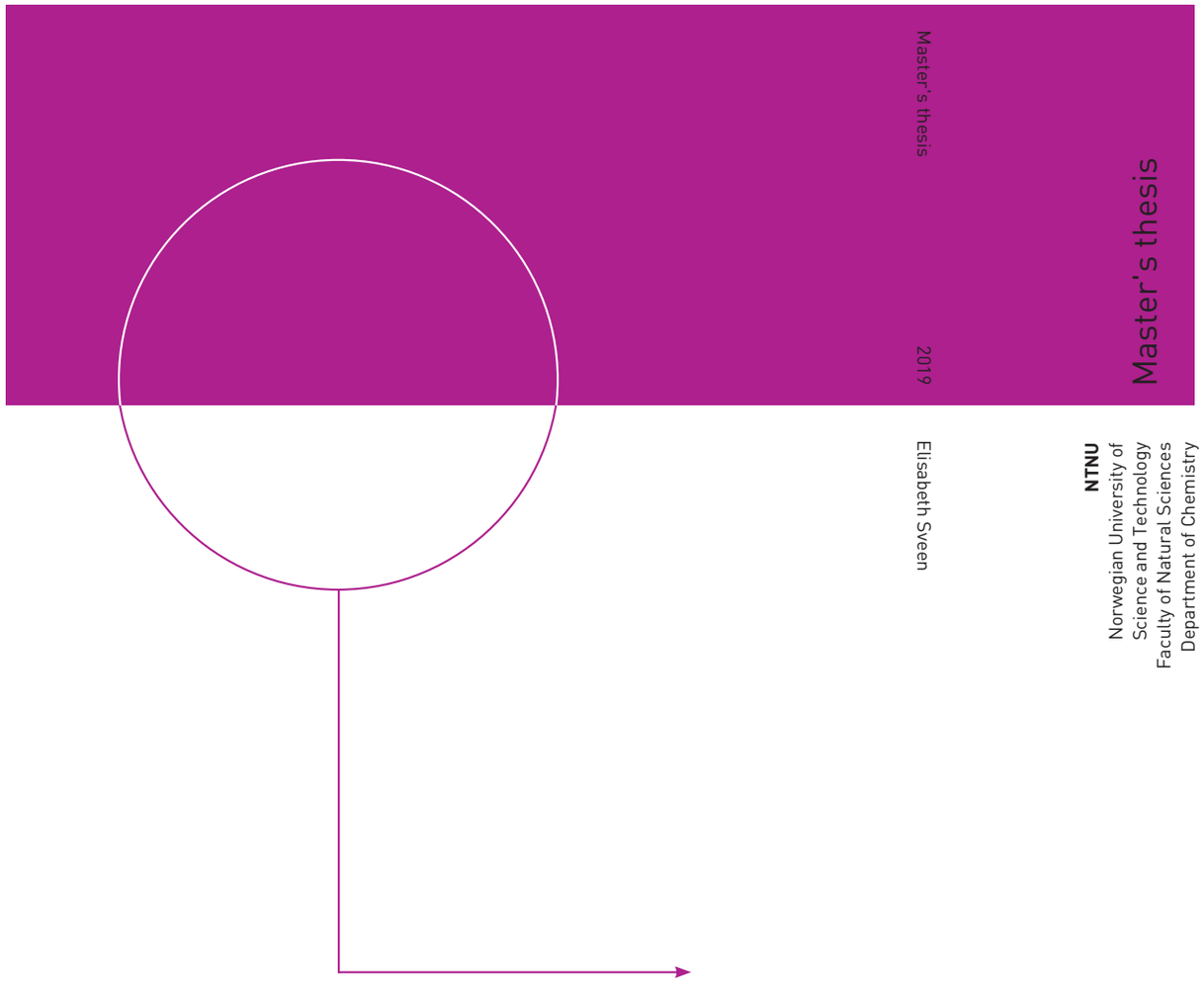

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Master's thesis in Analytical Chemistry with Teacher Education
Submission date: December 2019
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Abstract

Feathers and blood samples from a variety of bird species were collected in Norway and Greece (2015-2018) to assess the occurrence and profiles of selected trace elements and selected organic contaminants. Selected trace elements included the essential elements Copper (Cu), Cobalt (Co), Manganese (Mn) and Zinc (Zn), and the nonessential elements Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb). Trace elements are ubiquitous in the environment, and a deficit in essential elements, as well as too high doses of both essential and nonessential/toxic elements can cause severe adverse effects in both humans and animals. In the present study, inductively coupled plasma mass spectrometry (ICP-MS) was used for determination of eight trace elements in feathers and blood from different bird species, including Scopoli's shearwater *(Calonectris diomedea)*, six passerine Saharan migratory birds and White-tailed eagles *(Haliaeetus albicilla)*. The quantification levels (QL) ranged from 0.0007 µg/g (Cd, Hg and Pb) to 0.07 µg/g (Cu) in feathers depending on the amount of sample analysed. For blood samples the QL ranged from 0.0001 µg/g (Cd, Hg and Pb) to 0.002 µg/g (Cu). Determined maximum essential trace element concentrations in the were determined up to 11.6 µg/g (Mn) and 129 µg/g (Zn) in feathers, and 1.30 µg/g (Cu) and 57.1 µg/g (Zn) in blood. Determined maximum nonessential trace element concentrations were 1.18 µg/g (Pb) and 33.6 µg/g (Hg) in feathers, and 0.04 µg/g (Cd), 9.71 µg/g (Hg) and 9.95 µg/g (As) in blood.

Perfluorinated compounds (PFCs) were selected organic compounds in the present study. PFCs are known chemicals used in a variety of industrial and consumer products. Many of the compounds have been found in the environment as contaminants. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for separation and determination of ten perfluoroalkyl carboxylate acids (PFCAs), two perfluoroalkyl sulphonamides (PFSAs), three perfluoroalkyl sulphonates (PFASs) (15 PFCs in total) and one non-fluorinated similar target compound in bird feathers. The feather samples were washed, extracted by a solid-liquid extraction (SLE) and ultra-sonication procedure, and thereafter concentrated. The internal standards perfluorooctanoic acid (^{13}C8) and perfluorooctanesulfonate (^{13}C8) secured an overview of variations in the extraction and instrument analysis. The lower levels of quantification (LLOQ) ranged from 0.0002 ppb (PFOSA) to 1.84 ppb (Sulf) in the feather samples. The washing procedures showed high efficiency and the methods were applied successfully. PFC concentrations in the feathers were determined up to 66.5 ng/g (PFOS), 597 ng/g (PFPA) and 29.4 ng/g (PFHxA). PFC concentrations from external contamination was

determined by analysing hexane from the washing procedure, and PFC concentrations on the feathers were determined up to 8657 ng/g (PFOS), 5893 ng/g (PFPA) and 1698 ng/g (PFOA). Determined maximum concentrations for the non-fluorinated compound DecaS were 49.3 ng/g in the feathers and 28329 ng/g on the feathers.

For the inorganic analysis, significant differences and different trace element patterns were found for primary feather one (P1) and primary feather ten (P10) feathers in Scopoli's shearwaters which are grown in the breeding- and wintering areas, respectively, which was one of the main aims of the study. All perfluorinated compounds analysed was detected in at least one feather sample, for the six passerine bird species.

Sammendrag (Norwegian abstract)

Fjær- og blodprøver fra en rekke fuglearter ble samlet i Norge og Hellas (2015-2018) for å vurdere forekomst og se på profiler til utvalgte spormetaller og organiske forurensninger. De utvalgte essensielle sporstoffene inkluderte Kobber (Cu), Kobolt (Co), Mangan (Mn) og Zink (Zn), og de ikke-essensielle sporstoffene inkluderte Arsen (As), Kadmium (Cd), Kvikksølv (Hg) og Bly (Pb). Disse sporstoffene forekommer naturlig i miljøet, og et underskudd av essensielle sporstoffer, så vel som for høye doser av ikke-essensielle/giftige sporstoffer, kan forårsake alvorlige skadelige effekter i mennesker og dyr. I denne studien ble ICP-MS benyttet for bestemmelse av konsentrasjonen av 8 utvalgte sporelementer i fjær- og blodprøver fra fugl, inkludert artene Gulnebblire *(Calonectris diomedea),* seks spurvefugler som migrerer over Sahara og Havørn *(Haliaeetus albicilla)*. Laveste konsentrasjon for kvantifisering (QL) varierte fra 0,0007 µg/g (Cd, Hg og Pb) til 0,07 µg/g (Cu) i fjær, avhengig av mengden prøve som ble analysert. For blodprøver varierte QL fra 0,0001 µg/g (Cd, Hg og Pb) til 0,002 µg/g (Cu). Maksimal konsentrasjon av essensielle spormetaller var 11,6 µg/g (Mn) og 129 µg/g (Zn) i fjær, og 1,30 µg/g (Cu) og 57,1 µg/g (Zn) i blod. Maksimal konsentrasjon av ikke-essensielle sporstoffer var 1,18 µg/g (Pb) og 33,6 µg/g (Hg) i fjær, og 0,04 µg/g (Cd), 9,71 µg/g (Hg) og 9,95 µg/g (As) i blod.

Perfluorerte forbindelser (PFC-er) var utvalgte organiske forbindelser i dette studiet. PFC-er er kjente kjemikalier som brukes i en rekke produkter, både i industri og hos forbrukere. Mange av disse forbindelsene er funnet i miljøet og betegnes som miljøgifter. Væskekromatografi med tandem massespektrometri (LC-MS/MS) ble brukt til separasjon og bestemmelse av ti perfluoralkyl karboksylsyrer (PFCA-er), to perfluoralkyl sulfonamider (PFSA-er), tre perfluoralkyl sulfonater (PFAS-er) (totalt 15 PFC-er) og ett ikke-fluorisert liknende stoff i fuglefjær. Fjærprøvene ble vasket, ekstrahert med en kombinasjon av fast-væskeekstraksjon (SLE) og ultralydkar, og tilslutt konsentrert. De interne standardene perfluorert oktansyre (^{13}C8) og perfluorert oktansulfonat (^{13}C8) sikret et innblikk i variasjoner etter ekstraksjon og analyse. Laveste konsentrasjon for kvantifisering (LLOQ) varierte fra 0,0002 ppb (PFOSA) til 1,84 ppb (Sulf) i fjærprøvene. Vaskeprosedyrene viste høy effektivitet og analysemetoden ble vellykket anvendt. PFC-konsentrasjoner i fjærene ble målt opp til 29,4 ng/g (PFHxA), 66,5 ng/g (PFOS) og 597 ng/g (PFPA). PFC-konsentrasjoner fra ekstern forurensning ble bestemt ved analyse av heksan fra vaskeprosedyren. Utvendige PFC-konsentrasjoner på fjærene ble målt opp til 8 657 ng/g (PFOS), 5 893 ng/g (PFPA) og 1 698 ng/g (PFOA). Den bestemte maksimale

konsentrasjonen til den ikke-fluorerte forbindelsen DecaS var 49,3 ng/g i fjærene og 28 329 ng/g på fjærene.

For uorganisk analyse ble signifikante forskjeller og ulike profiler av spormetaller funnet for Primærfjær en (P1) og Primærfjær ti (P10) i Gulnebblire, som vokser ut i hver sine områder (hekke- og overvintringsområder). Dette var ett av målene å undersøke i dette studiet. Alle de perfluorerte stoffene som det ble analysert for ble kvantifisert i minst en fjær for de seks spurvefuglartene.

Table of contents

List of figures

List of tables

Table C.4: Target analytes, internal standards and analyte specific MS/MS parameters using a Kinetex C18 (50 x 2.1 mm) column. Table including primary and secondary transitions, retention times (RT) and relative retention times (RRT (ISTD)) in analysis of 38 feather samples in group 2

Table E.1: Quantification limits (QL) in µg/g, mean detected concentration (µg/g) and RSD% values for group 1 feather samples – trace element analysis

Table E.2: Quantification limits (QL) in µg/g, mean detected concentration (µg/g) and RSD% values for group 3 feather samples – trace element analysis

Table E.3: Quantification limits (QL) in µg/g, mean detected concentrations (µg/g) and RSD% values for group 2 feather samples – trace element analysis

Table E.4: Quantification limits (QL) in µg/g, mean detected concentrations (µg/g) and RSD% values for group 1 blood samples – trace element analysis

Table E.5: Calculated concentrations (ng/g) of 8/16 chosen perfluorinated compounds in 38 feathers from birds. Concentrations detected below LOD are not included in the table. Results from external contamination is represented as “hexane” from the washing process

Table E.6: Calculated concentrations (ng/g) of the next 8/16 chosen perfluorinated compounds in 38 feather samples from birds. Concentrations detected below LOD are not included in the table. Results from external contamination is represented as “hexane” from the washing process

List of abbreviations

Table 0.1: A list of abbreviations

As	Arsenic
Cd	Cadmium
Co	Cobalt
Cu	Copper
ESI	Electrospray ionization
Hg	Mercury
ISTD	Internal standards
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLOQ	Lower level of quantification
LOD	Limit of detection
MeOH	Methanol
Mn	Manganese
MS/MS	Tandem mass spectrometry
m/z	Mass-to-charge
Pb	Lead
PCA	Principal Component Analysis
PFA	Perfluoroalkoxy
PFC	Perfluorinated compounds
POPs	Persistent organic pollutants
PP	Polypropylene
P1	Primary feather one (innermost)
P10	Primary feather ten (outermost)
RT	Retention time
SLE	Solid-liquid extraction
TA	Target analytes
UHPLC	Ultra-high-performance liquid chromatography
QL	Quantification levels
Zn	Zinc

1 Introduction

Birds are useful bioindicators because they are found at the top of the food chain in many cases and collection of samples are not too difficult (Burger & Gochfeld, 2004). Marine birds are highly exposed to a lot of different chemical compounds as they mainly live in the aquatic environment. Contaminants move faster in water compared to terrestrial environments, which also increases the danger of exposure for marine living organisms (Schreiber & Burger, 2001). Marine birds therefore reflect changes in the marine environment regarding pollutants and are proven to be good indicators of environmental trace element pollution (Lodenius & Solonen, 2013; Voulgaris et al., 2019). The samples in this study are collected from one of the largest colonies of Scopoli's shearwaters *(Calonectris diomedea)* at Strofades Islands in Greece (Voulgaris et al., 2019), from six passerine birds from Strofades Islands, and from White-tailed eagles *(Haliaeetus albicilla)* from Steigen archipelago and Smøla archipelago in Norway. Feathers can explain breeding populations by wintering area conditions (Szép, Møller, Vallner, Kovács, & Norman, 2003).

The present study combined inorganic and organic analysis in feather and blood samples from several different bird species. The focus was on four essential elements Copper (Cu), Cobalt (Co), Manganese (Mn) and Zinc (Zn), and four nonessential elements Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb). All are naturally occurring elements, but their concentrations are impacted by anthropogenic input (Voulgaris et al., 2019). The organic compounds analysed were 16 perfluorinated compounds (PFCs). These included ten perfluoroalkyl carboxylate acids (PFCAs C5-C14), two perfluoroalkyl sulphonamides (PFSAs) and three perfluoroalkyl sulphonates (PFASs). In addition, a non-fluorinated compound with the similar properties was included as well.

In the present study, four questions were raised, aiming to look at the given data from various perspectives: i) Can differences in trace element concentrations in Primary feather one (P1) and Primary feather ten (P10) feathers from Scopoli's shearwater be found? Blood samples were also collected for further comparison. ii) Can differences in trace element concentrations in six passerine sub-Saharan migratory species be found? iii) Are there any clear differences in trace element concentrations in birds from the Norwegian coast compared to Mediterranean migratory birds? iv) Which PFCs can be found in bird feathers and can differences in PFC concentrations between the 6 passerine sub-Saharan migratory species be found?

2 Theoretical background

Firstly, theoretical background for the chosen trace elements and perfluorinated compounds are presented. The elements and compounds, anthropogenic sources and health effects are described and results from previous studies are presented at the end. In total, 8 bird species are described, and their feather moulting pattern are thoroughly studied. Sample preparation methods and analytical techniques for both inorganic and organic analysis are described, and considerations regarding quantification and quality assurance are clarified in the end.

2.1 Target trace elements

Many elements occur in biological tissues in low concentrations, so low that early studies were unable to measure some elements precisely with the available methods. These elements were described as occurring in "traces", which lead to the expression "trace elements" used today, when not talking about so-called major elements. Today, trace elements can be detected and measured precisely and with great accuracy with modern detection methods (Underwood, 1977, p. 1). Metals are unique when considering them as pollutant toxicants, as they all occur naturally and are ubiquitous to some degree in the environment (Klaassen, 2019, p. 1108).

Essential elements

Many trace elements are necessary for biological life, as they play a substantial role in growth, development and metabolic processes (Klaassen, 2019, p. 39). These trace elements are categorised as essential elements. Essential elements are naturally occurring in the environment and they are necessary for organisms in proper quantities. If the element is not present in the organism's environment, the organism fails to grow normally due to deficiency responses. In higher concentrations above a specific threshold, essential elements will induce adverse effects similar to nonessential elements (Roselli, Desideri, Meli, Fagiolino, & Feduzi, 2016). The effect of deficiency or excess is illustrated in **figure 2.1**.

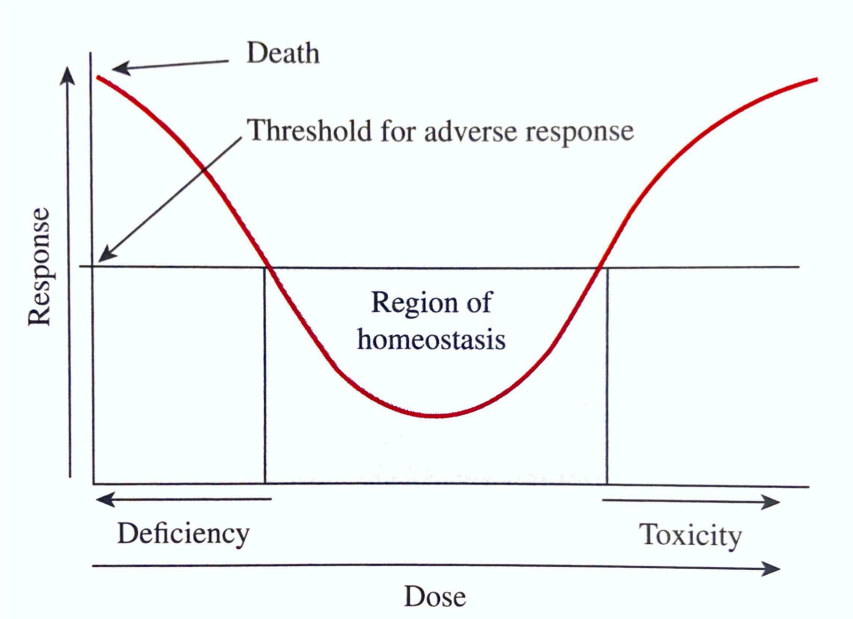


Figure 2.1: Individual dose-response relationship for essential trace elements (retrieved from (Klaassen, 2019, p. 40)

There are 9 elements defined as essential trace elements for animals. Those are Iron (Fe), Iodine (I), Zinc (Zn), Manganese (Mn), Copper (Cu), Cobalt (Co), Molybdenum (Mo), Chromium (Cr) and Selenium (Se) (Klaassen, 2019, p. 1107; Poulsen, 2005; Prasad, 1976). In the present study, the 4 essential trace elements Co, Cu, Mn and Zn were investigated. Cu and Zn are considered essential trace elements, and Cu especially is important for feather formation (Underwood, 1977). Cu is often part of redox enzymes used in defence against oxidative damage, and Zn is a part of many different enzymes playing important roles in reproduction and sexual maturation (Klaassen, 2019). Mn and Co are essential trace elements with low toxicity (Metcheva et al., 2010), and Co as an example is a part of the core of vitamin B12, important for the formation of blood cells (Klaassen, 2019).

Nonessential elements

Many elements are necessary for the function of biological life, but there are also many that are considered nonessential or toxic elements. Nonessential elements are toxic even at low concentrations. Aluminium (Al), Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg), Nickel (Ni), Titanium (Ti) and Uranium (U) are examples of toxic elements (Klaassen, 2019, p. 1107; Roselli et al., 2016). Nonessential elements with physical properties similar to the essential ones, can exploit the transport- and coordination system to cause toxicity

in biological systems (Klaassen, 2019, p. 1109). In the present study, the four toxic trace elements As, Cd, Hg, Pb were investigated. As, Cd, Hg and Pb are considered nonessential or toxic trace elements, and they have no reported physiological function as well as they are contributing to a high health risk (Roselli et al., 2016).

Arsenic is a toxic and carcinogenic metalloid existing in many different forms. The most typical forms are inorganic compounds like sodium arsenate or arsenic acid, and also several methylated forms produced by inorganic biotransformation (Klaassen, 2019, p. 1115). Cadmium is a toxic transition metal, widely used in industrial work and products, where it is produced as a by-product from Zn- and Pb-ore melting. Ores of Zn, Pb and Cd are also environmental sources of Cd (Klaassen, 2019, p. 1119). Mercury has been known and used since ancient times, and it exists in many forms. Hg vapor is more hazardous than the liquid form, which is found in room temperature, and Hg can also exist as inorganic salts and organometallic compounds. Methylmercury (MeHg) is the most important form from a toxicology perspective, as it is produced by biota through biomethylation, and enters the food chain (Klaassen, 2019, pp. 1126-1127). Studies have found that up to a 100% of ingested dose of MeHg are absorbed intestinally, compared to only a few percent of Hg^{2+} (salts) (Scheuhammer, 1987, p. 274). Lead is a ubiquitous metal, widely used by humans for thousands of years. Pb exists in a variety of forms, as metallic, inorganic, and organolead compounds. Environmental Pb mainly originates from human activity (Klaassen, 2019, p. 1123). Metals such as Zn, Pb, Mn, Hg, Cu and Co accumulate in marine animals, including zooplankton and fish (Klaassen, 2019, p. 1110).

Anthropogenic sources and contribution

The metals enter the environment through natural processes, but also from anthropogenic activities since they are great natural resources to human life (Klaassen, 2019, p. 1109; Manahan, 2010). The aquatic ecosystem receives metals from the geosphere through mining and from the anthrosphere through recycling (Manahan, 2010, p. 494). Many of the target elements are widely used in industrial products, such as Pb used in batteries and Cu used in electrical wires and pipes (Manahan, 2010, p. 498). The anthropogenic activity influences the concentration and speciation of the different elements, also referred to as the bioavailability (the proportion of the element available for uptake). In this way, anthropogenic activity also

influences the toxicity, by making nonessential elements bioavailable in the environment (Klaassen, 2019).

Health effects

Metals are non-degradable atomic species not possible to detoxify by the organism's metabolism. This impacts the metals bioabsorption, biotransformation and toxicity. The physical properties of the metals can still be changed, depending on factors such as oxidation/reduction of the metal or anabolic metabolism that renders metals inert for biological reactions (Klaassen, 2019, p. 1109). As and Hg are good examples were the toxicity are dependent on the speciation and physical properties of the metal. MeHg are slowly metabolised with low excretion rate, and due to is chemical stability and lipophilicity it readily can penetrate the blood-brain barrier causing toxic effects like spinal cord degeneration in birds. Symptoms of MeHg poisoning in birds can be reduced food intake, weight loss, decreasing egg production, weakness in wings and legs causing difficulties for flying, walking and standing and even inability to coordinate movements in muscles. Generally, MeHg can induce physical and reproductive toxic effects in birds (Scheuhammer, 1987, pp. 274-275).

A deficiency in the essential elements Ca, Fe or Zn from the diet result in increased Cd uptake by intestines and enhanced Cd toxicity. Cd is known to accumulate through time bound to the sulphydryl-rich protein metallothionein (MT), and the induction of MT by Cd have been reported for some experimental birds. Nephrotoxicity in seabirds has been found linked to increased concentrations of Cd in the kidneys (Cd 100-200 μg/g dry weight kidney). MT can be studied to look at effects from metal exposure (Scheuhammer, 1987).

Previous studies

Trace element concentration in feathers can be affected by many different factors, and the concentration can vary based on which specie and which metal we are looking at. In adult bird feathers it is possible to see clear differences in elemental concentrations based on what food the bird ingested when the feather was formed (Dmowski, 1999; Solonen & Lodenius, 1990). Previous studies by Bernhard (2016); Renzoni, Focardi, Fossi, Leonzio, and Mayol (1986) have shown higher concentrations of Hg in the Mediterranean area compared to other locations. In fact, 50-55 % of the world's mercury resources are located in the area of the Mediterranean,

and the turnover in this sea is lower than in others. This causes a natural higher concentration of Hg, which can be seen analysing organisms living there (Renzoni, Zino, & Franchi, 1998).

The findings in a field experiment with free-living great tits *(Parus major)* showed that the concentrations of the metals increased proportionally with the age of the feather. The concentration of certain trace elements in feathers, e.g. Zn and Hg, are mainly due to deposition endogenously. External contamination may on the other hand be a source of other elemental contaminants, like Cd and Pb (Jaspers et al., 2004; Leonzio, Bianchi, Gustin, Sorace, & Ancora, 2009). A study comparing internal and external contamination in peregrine falcon *(Falco peregrinus)* and Eurasian sparrow hawk *(Accipiter nisus)* found Pb contamination to be both internal and external, and Zn, Cd and Cu contamination are more typical internal (Ek, Morrison, Lindberg, & Rauch, 2004). Dmowski (1999) also found that external contamination causes higher concentrations in older feathers than in younger feathers.

Feathers consists, among other things, of the sulphur containing protein keratin. The essential elements Zn and Cu, and the nonessential elements Hg, Cr, As and Se have stronger affinity to keratin than other metals (Dmowski, 1999). When we look at the effects of these elements in elevated concentrations in feathers, the results are varying. Eisler (1987) found that Hg present in feathers at concentrations 5-40 µg/g were associated with effects such as impaired reproduction, while Bowerman, Evans, Giesy, and Postupalsky (1994) found that Hg and Se do not affect reproduction in bald eagles *(Heliaeetus leucocephalus).* Investigating metal pollution in feathers is important, but since the metal in feathers are not metabolically active this should be taken into count when considering toxic effects in the organism (Lodenius & Solonen, 2013). According to Dmowski (1999), the metals will after food ingestion and metabolism be transported into the blood stream and further deposited into the feathers as a part of elimination from the body. This can be considered as a detoxification mechanism of trace metals (Naccari, Cristani, Cimino, Arcoraci, & Trombetta, 2009). Dietary exposure to MeHg in birds can be monitored using bird feathers. Hg, and other trace metals, are deposited in the feathers from the blood during formation. When the metals are incorporated in the feather keratin structure, they are resistant to leaching (Dauwe, Bervoets, Blust, Pinxten, & Eens, 2000; Leonzio et al., 2009). Especially MeHg are strongly bound to the keratin structure of feathers (Leonzio et al., 2009). An exposure experiment with ducks (*Anas rubripes*), exposing them with low-dietary levels of MeHg (0.5-3 ppm) showed significant dose-dependent deposition of MeHg in primary feathers. It is estimated that 60-70% of the total body burden of Hg may be present in the plumage (Scheuhammer, 1987, p. 278).

When comparing the tissue distribution of Cd in peregrine falcon, a strong accumulation is found in the kidneys and low concentrations are found in feathers. The same was found for Cu, where a strong accumulation was seen in the kidneys and liver, and the concentration in the feathers were medium. The Pb concentrations were found to be low in feathers compared to higher concentration in faeces (Ek et al., 2004). Another study by Martinez-Lopez et al. (2004) found a significant correlation between blood- and feather concentration of Cd in three Spanish raptors.

In 1986, a study comparing the concentration of toxic trace elements in Scopoli's shearwaters from the Atlantic and the Mediterranean was conducted, confirming earlier studies showing high-mercury contaminated fish in the Mediterranean. The focused trace elements were Hg, Se, Cd, Pb and Zn (Renzoni et al., 1986). The Hg concentration in the eggs sampled in the Mediterranean were 2.5-3.5 times higher than for the eggs sampled in the Atlantic. Liver samples showed the same trend where the concentrations from the Mediterranean samples were 4-6 times higher than for the Atlantic samples. The same trend was also shown for kidney and muscle samples. The results regarding Cd confirmed a trend from other studies that the local concentration in the bird are higher in tissues and organs, compared to in eggs (Renzoni et al., 1986). No feathers were analysed in this study.

A study performed in 2003 with the bird Sand martin *(Riparia riparia),* measured trace element concentrations in tail feathers grown in breeding areas compared to wintering areas. For Cd, Mn, Co and Pb the concentrations were significantly higher in the feathers grown in the winter, while for Zn the concentration were significantly higher in the feathers grown in the breeding area (Szép et al., 2003). Results from previous studies by (Abbasi, Jaspers, Chaudhry, Ali, & Malik, 2015), (Dauwe et al., 2000), (Voulgaris et al., 2019) and (Burger & Gochfeld, 2004) are presented in **table 2.1**, where single values represent mean concentrations, single values with * represent median concentrations, and concentrations given in intervals are the range of determined concentrations. Burger and Gochfeld (2004) sums up the levels associated with adverse effects for Pb, Hg and Cd to be: Pb > 4.00 µg/g, Hg > 5.00 µg/g and Cd > 2.00 µg/g. The study determined concentrations of Hg in Shearwaters ranging from 0.2 to 30.7 µg/g.

Table 2.1: Previous studies determining trace element concentrations, given as mean, median and range concentrations in μg/g.

Specie	As	Cd	Co	Cu	Mn	Pb	Zn	Reference
Various	-	0.33-1.43	0.24-2.33	1.62-5.93	0.82-3.31	1.01-4.01	53.3-122	(Abbasi et al., 2015)
Great tits	2.55	0.05	-	5.78	-	0.51	127	(Dauwe et al., 2000)
(reference								
Great tits	3.00	0.007	-	6.16	-	4.83	97.9	(Dauwe et al., 2000)
(polluted site)								
Blue tits	6.44	-	-	4.90	-	0.48	157	(Dauwe et al., 2000)
(reference								
Blue tits	5.24	0.07	-	5.14	-	3.68	119	(Dauwe et al., 2000)
(polluted site)								
Scopoli's	-	0.01*	0.18*	3.41*	0.29*	0.24*	22.9*	(Voulgaris et al., 2019)
shearwaters								
Shearwaters	-	0.07-0.95	-	-	-	0.10-40.8	-	(Burger & Gochfeld, 2004)

2.2 Perfluorinated compounds

Perfluorinated compounds (PFCs) are of great concern due to their characteristics, which is similar to many persistent organic pollutants (POPs) (Arvaniti et al., 2014). Typical characteristics for such compounds are possibility for bioaccumulation, persistency in the environment and global distribution (Arvaniti et al., 2014; Giesy & Kannan, 2002). The toxicity of PFCs is not fully investigated, which makes them potentially harmful compounds. Fluorinated compounds produced by nature contain only one fluorine atom, compared to synthetic fluorinated compounds containing more than one or are fully fluorinated. PFCs are one of the groups of fluorinated compounds that are categorised as stable in the environment, and they can degrade to persistent perfluoroalkyl carboxylate acids (PFCAs), perfluoroalkyl sulfonates (PFASs) and perfluoroalkyl sulphonamides (PFSAs) (Arvaniti et al., 2014; Houde, Martin, Letcher, Solomon, & Muir, 2006). Houde et al. (2006) expressed that there is no evidence that these groups of compounds can be biodegraded. Development of new methods for investigating PFCs in a variety of matrixes is required to understand their presence in the environment (Giesy & Kannan, 2002). Due to a variety of methods used, there is a lack of confidence in the existing data, but studies can give information on sources, exposure transport mechanisms, bioaccumulation potential and trends on global distribution (Houde et al., 2006). This study will encourage to validate a new method for PFC analysis in feather samples from birds.

Sources and anthropogenic contribution

PFCs are widely used because of its special oleophobic and hydrophobic properties much similar to a surfactant (KEMI, Swedish Chemicals Agency). The hydrophobic part will repel water, and the oleophobic part will repel oils and fats, which makes the compounds amphiphilic (Briels, 2019). This is a property widely used in household products, such as the perfluorinated compound PFTE (Teflon). The compounds are used in lubricants, adhesives, repellents, coatings, ski waxes, pharmaceuticals and fire-fighting foams to mention some (Briels, 2019; Giesy & Kannan, 2002; Houde et al., 2006). Some of these products can lead to direct emission to the environment, but precursor PFCs degraded into PFASs, PFCAs and PFSAs are also important indirect emission to the environment (Briels, 2019). PFCs contains a carboxylic acid or a sulfuric acid group, and the backbone of the structure is fluorinated in some degree. This group of compounds have been produced for more than 50 years, but the global total production

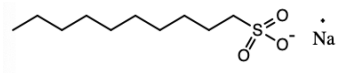
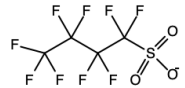
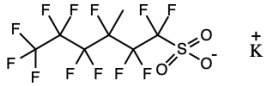
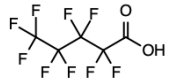
today is unknown (Giesy & Kannan, 2002). After discovering presence of PFOS and PFOA in wildlife and human blood, concerns about PFCs were raised. PFOS was added to the list of regulated persistent organic pollutants (POPs) by the Stockholm Convention in 2009. PFOA and PFHxS has also been proposed for listing (Briels, 2019). It is estimated that there exist about 5000 different PFCs on the global market (Miljødirektoratet, 2019).

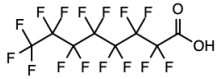
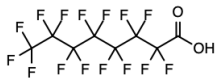
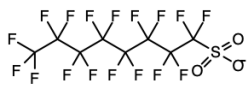
Health effects

A report by Giesy and Kannan (2001) claims to have shown that PFOS can bioaccumulate to higher trophic levels of the food chain. Biomonitoring of PFCs is important for understanding their fate and behaviour in the environment, and their behaviour in organisms if the compounds enter this network. Studies exposing rodents and monkeys to PFCs associated the compounds with several adverse health effects, like hepatotoxicity, cardiotoxicity, alteration of the thyroid hormone status, immunosuppression and delayed growth (Lau et al., 2003; Seacat et al., 2002; Yang et al., 2002). Studies performed on chickens found PFC exposure to be a source of decreased pipping success and developmental cardiotoxicity (Jiang, Lust, Strynar, Dagnino, & DeWitt, 2012; O'brien, Carew, Chu, Letcher, & Kennedy, 2009). Chronic or repeating exposure to PFCs have also shown adverse effects on liver and birth defect in mammals, and some indicators seem to show carcinogenic effects (Miljødirektoratet, 2019).

In the present study, 15 PFCs and one non-fluorinated compound were chosen as target analytes. Information about the compounds such as names, most common abbreviation, PFC type, molar mass, chemical structure, supplier and purity are listed in **table 2.2**. DecaS is not a perfluorinated compound but has a similar structure as many of the perfluorinated target analytes. DecaS was included to open the possibility to compare determined concentrations.

Table 2.2: The 16 target compounds and 2 internal standards chosen for this study, with their full name, abbreviation, PFC type, molar mass, chemical structure, supplier and purity. The table continues on the next page.

Chemical	Abbreviation	PFC type	Molar mass	Chemical structure	Supplier	Purity
Sodium 1-decanesulfonate sodium salt	DecaS	Non-fluorinated	221.34		Sigma Aldrich	98%
Tetrabutylammonium nonafluorobutane sulfonate	NonaFBS	C4-PFAS	299.09		Sigma Aldrich	98%
Tridecafluorohexane-1-sulfonic acid potassium salt	TriDeFHxSA	C6-PFAS	399.11		Sigma Aldrich	≥ 98%
Tetrabutylammonium hepta-decafluorooctane sulfonate	PFOS	C8-PFAS	499.12		Sigma Aldrich	≥ 95%
Perfluorooctane sulphonamide	PFOSA	C8-PFSA	499.14		Sigma Aldrich	-
Sulfluramid	Sulf	C8-PFSA	527.20		Sigma Aldrich	≥ 98%
Perfluoropentanoic acid	PFPA	C5-PFCA	264.05		Sigma Aldrich	97%
Perfluorohexanoic acid	PFHxA (UnFHxA)	C6-PFCA	314.05		Sigma Aldrich	97%
Perfluoroheptanoic acid	PFHeA	C7-PFCA	364.06		Sigma Aldrich	99%

Perfluorooctanoic acid	PFOA	C8-PFCA	414.07		Sigma Aldrich	96%
Perfluorononanoic acid	PFNA (PFNonDeA)	C9-PFCA	464.08		Sigma Aldrich	97%
Perfluorodecanoic acid	PFDA	C10-PFCA	514.09		Sigma Aldrich	95%
Perfluoroundecanoic acid	PFUnA	C11-PFCA	564.09		Sigma Aldrich	98%
Perfluorododecanoic acid	PFDoDeA (TricoFDoDeA)	C12-PFCA	614.10		Sigma Aldrich	95%
Perfluorotridecanoic acid	PFTriDe	C13-PFCA	664.11		Sigma Aldrich	97%
Perfluorotetradecanoic acid	PFTetDeA	C14-PFCA	714.12		Sigma Aldrich	96%
Perfluorooctanoic acid 13C8	PFOA-13C8	13C8-PFCA	421		Sigma Aldrich	99%
Perfluorooctanesulfonate 13C8	PFOS-13C8	13C8-PFAS	506		Sigma Aldrich	99%

Previous studies

Studies have shown that PFOS, PFOSA, PFOA and perfluorohexane sulfonate (PFHS) are represented in several living organisms (Houde et al., 2006; Kannan et al., 2002; Nakayama et al., 2008; Pedersen, Letcher, Sonne, Dietz, & Styrishave, 2016). PFOSA, PFOA and PFHS are PFC production intermediates. The compound perfluorooctanesulfonyl fluoride (POSF) is used as a surfactant and surface protector of carpets, leather, paper, packaging and fabric, and its ultimate degradation product is perfluorooctane sulfonate (PFOS) (Giesy & Kannan, 2002). A study performed by Kannan et al. (2002) analysing several matrices (liver and blood) from fish, bird and cetaceans in the Mediterranean, found PFOS to be the most predominant PFC in the tissues analysed.

Houde et al. (2006) sums up many studies of PFCs and the different degradation products in a review and found that PFOS was detected in the highest amounts in industrial areas, while oceanic birds had the lowest PFOS concentrations. The review also states that not many studies have assessed a wide range of PFCs, as PFOA and PFOS are the most common analysed. Bird eggs has also been analysed for PFCs with a positive result, indicating oviparous transfer to offspring (Houde et al., 2006).

Results from previous studies by (Meyer, Jaspers, Eens, & de Coen, 2009), (Gómez-Ramírez et al., 2017) and (Løseth et al., 2019) are presented in **table 2.3** as the range of concentrations determined. Løseth et al. (2019) determined PFC concentrations in feathers and plasma, concluding that plasma was the preferred matrix for monitoring internal concentrations of PFCs with higher detection frequency and better results.

Table 2.3: Previous studies determining PFC concentrations, given as range concentrations in ng/g.

Specie	PFOSA	PFOS	PFHxA	PFOA	PFNA	PFDA	PFUnA	PFDoDeA	PFTriDe	PFTetDeA	Reference
White-tailed eagles	0.23-6.52	0.22-90.2	0.02-0.33	0.11-0.91	0.05-0.45	0.07-0.46	0.05-1.07	0.07-0.78	0.11-2.02	-	(Løseth et al., 2019)
White-tailed eagles	0.46-2.61	1.89-16.4	-	0.10-0.61	<2.91	0.14-1.12	0.63-1.99	0.17-0.40	0.71-2.32	<0.19	(Gómez-Ramírez et al., 2017)
Grey Heron	-	66.2-1489	-	<7.3	7.6-17.5	-	-	-	-	-	(Meyer et al., 2009)
Herring Gull	-	52.3-677	-	7.3-28.2	7.6-16.9	-	-	-	-	-	(Meyer et al., 2009)
Eurasian Sparrowhawk	-	47.6-775	-	7.3-15.9	7.6-8.5	-	-	-	-	-	(Meyer et al., 2009)
Eurasian Magpie	-	8.5-37.1	-	3.2-6.7	<7.6	-	-	-	-	-	(Meyer et al., 2009)
Eurasian Collared Dove	-	2.50-39.5	-	<7.3	<7.6	-	-	-	-	-	(Meyer et al., 2009)

2.3 Study population

Study group 1

The first group of samples were feathers from live Scopoli's shearwaters *(Calonectris diomedea)*. The Scopoli's shearwaters is a pelagic migratory seabird spending a lot of its time over the oceans, and its food sources are mainly pelagic or mesopelagic organisms (Renzoni et al., 1986; Voulgaris et al., 2019). Scopoli's shearwaters breeding areas are the Mediterranean (mostly in the areas from Spain to Turkey) and their winter habitat is tropical and south Atlantic, more specific Canary and north Benguela currents, and western Africa (coast areas of Senegal), where they take advantage of the natural upwelling system (Oliveira, Nunes, Marques, & Bugoni, 2019). The bird is a secretive type, with nests difficult to access (González-Solís, Croxall, Oro, & Ruiz, 2007; Roscales, González-Solís, Muñoz-Arnanz, & Jiménez, 2011). Voulgaris et al. (2019) summed up the five major threats of Scopoli's shearwaters in breeding areas to be: i) predation of eggs and chicks by invasive mammals; ii) accidental entrapment (by-catch) in fishery gears; iii) human disturbance such as light pollution; iv) marine pollution; and v) plastic debris.

Study group 2

The second group of samples were wings of freshly dead passerine birds, originating from six different species sampled in the same area at the island Strofades in Greece during spring migration (April 2018). The six species were Wood warbler (*Phylloscopus sibilatrix)*, Sedge warbler *(Acrocephalus schoenobaenus)*, Garden warbler (*Sylvia borin)*, Great reed warbler (*Acrocephalus arundinaceus)*, Sand martin (*Riparia riparia)* and Spotted flycatcher (*Muscicapa striata)*. All six are trans-Saharan migratory birds with wintering areas in Africa.

The Wood warbler is a long-distance migratory bird with a decreasing population over the last 20 years, and due to this, studies are performed investigating the wintering and breeding areas (Awa, Evaristus, Whytock, Guilain, & Mallord, 2018; Weisshaupt & Rodríguez-Pérez, 2017). The Wood warbler winter in sub-Saharan Africa and breeds in south Europe and Asia (Del Hoyo, Elliott, & Christie, 2006). The Sedge warbler is a migratory bird, wintering in sub-Saharan Africa and breeds in West Europe (Bibby & Green, 1981). The Garden warbler is one of the most common trans-Saharan migratory birds widely studied in terms of collecting knowledge on winter and summer regions, as well as regions used to prepare for crossing the

Sahara Desert (Barboutis, Henshaw, Mylonas, & Fransson, 2011; Bayly & Rumsey, 2010). The Garden warbler winters in central and south-eastern Africa, and breeds in the temperate sone in Eurasia (Barboutis et al., 2011), similar to the Wood warbler. The Great reed warbler is a migratory bird specie wintering in sub-Saharan Africa and breeding in Palearctic areas, especially the Iberian peninsula and all the way east to the Himalayas. The breeding areas are particularly wetlands, and due to this the specie is one of the most vulnerable for extinction, as wetland species are threathened (Horns et al., 2016). Studying the wetland species can identify possible factors for the decreasing population. The Sand martin is the smallest, long-distance migratory bird breeding in Europe. Its wintering areas are covering major parts of the African continent as it varies between western, central, eastern and southern Africa (Szép et al., 2003). The Spotted flycatcher is also a migratory bird, breeding in Europe and west and central Asia, and winter in coastal west and south Africa (B. Taylor et al., 2016). During the time period 1994-2007 the Spotted Flycatcher population in the United Kingdom were surveyed and the decrease was found to be over 50 % making it a priority specie in the UK (Risely, Noble, & Baillie, 2008). All the six species undergo full feather moult in the wintering areas, which all are in the African continent.

Study group 3

The third group of samples were feathers from alive White-tailed eagles (*Haliaeetus albicilla*). The White-tailed eagle are birds of prey, widely distributed in the northern hemisphere, all the way from Greenland and Iceland in the west to the Pacific coast and Japan in the east. Its breeding habitats are coastal and freshwater regions, both in the Arctic and sub tropic areas (Hailer et al., 2007). The advantages of using the seabird White-tailed eagle as a monitoring bird for pollutants is many, including the size of the bird, and the fact that it is long-lived, easily observed and wide-ranging (Burger & Gochfeld, 2004).

2.3.1 Bird feathers as biomonitors

Feathers are a non-destructive matrix, since they are easy to collect, store and transport. Feathers can even be sampled without affecting the fitness of the bird to any damaging degree (Abbasi et al., 2015; Furness, 1993). During the time a feather grows, the metals in the blood accumulate in the feathers proportional to the blood concentration. The accumulation continues

until the blood vessels atrophy from the feather, and the feather will reflect the metal concentrations in the bird at that specific period of time (Abbasi et al., 2015; Leonzio et al., 2009). In a study performed in 1985, Cd and Pb concentrations in a various of matrices from birds were measured. The study of Goede and de Voogt (1985) concluded that using feathers for monitoring exposure to Cd was of doubtful value. Cd levels were hardly ever measured in feathers, even when other tissue levels were very high. The study also found it not to recommend feathers for monitoring of Pb, since the high concentrations found was more likely to reflect external contamination than dietary Pb levels during feather formation (Goede & de Voogt, 1985). On the other hand, a study published in 2015 (Abbasi et al.) successfully determined concentrations of nine trace elements in feathers (Pb, Cd, Cr, Ni, Co, Cu, Fe, Zn and Mn). According to Burger and Gochfeld (2004), feathers are useful indicators of trace element pollution, because the metals are isolated in the feathers and a relatively high proportion of the body burden of certain metals is stored in the feathers. There is proven to be a correlation between ingested Hg from diet and the Hg levels in the feathers (Burger & Gochfeld, 2004). A study by Jaspers, Covaci, Herzke, Eulaers, and Eens (2019) sums up the possible uses of bird feathers as monitors, and conclude that feathers are useful as biomonitors for POPs and several metals, given that appropriate conditions for sampling, storage, preparation and analysis, with its respective QA/QC protocols, are taken into account. Recently, feathers have also been investigated as a potential matrix for analysing and monitoring perfluorinated compounds (Løseth et al., 2019). The physio-chemical properties of PFCs makes them often present in preen oil, were the contamination may originate from external sources (Jaspers et al., 2019).

2.3.2 Moulting patterns

Moulting is a natural process where the bird loose old feathers for new feathers to grow, and the process goes on during the winter season in most cases (Ramos, Militão, González-Solís, & Ruiz, 2009). As shown in **figure 2.1**, the feathers on the wings are numbered based on if they are primary (P) or secondary (S) feathers. The outermost feather is the primary feather ten (P10), and the following feathers are numbered down to primary feather one (P1). The remaining feathers of the wing are secondary feathers, starting at S1 next to the P1 feather. Since moulting in most cases takes place in the non-breeding season, most seabirds are

unavailable for investigation during the moulting process. Dead specimens found in different locations can give reliable and detailed information about the moult pattern, even outside the breeding season (Ramos et al., 2009).

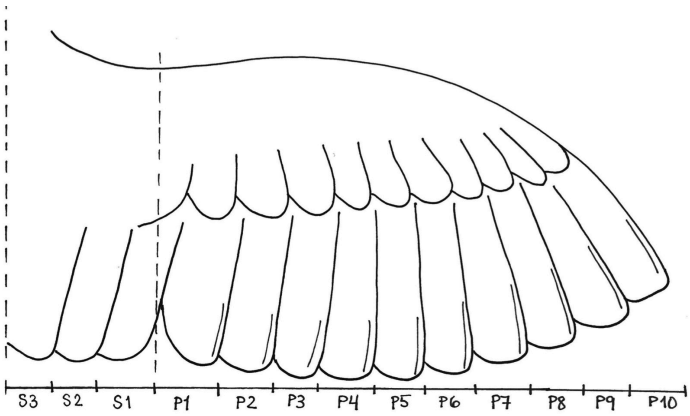


Figure 2.2: The numbering of the 10 outermost feathers on a bird wing

A study looking at the primary moult of the Spotted flycatcher, found that the primary feather nine (P9) is the first feather in the moulting process (Salewski, Bairlein, & Leisler, 2002). This is the opposite as earlier findings for Passeriformes, where the moult was found to start at the P1 feather (Diesselhorst, 1961). Salewski et al. (2002) studied the beginning of the moult process in Spotted Flycatchers and found it to vary between late October to the start of February. For the Great Reed warbler moulting habitats are found in the non-breeding season (Yohannes, Bensch, & Lee, 2008), which means that the feathers can describe the winter area conditions.

A study by Ramos et al. (2009) performed on Cory's shearwater, also referred to as *Calonectris diomedea* in earlier literature (Oliveira et al., 2019), showed that the moulting strategy for the primary feathers starts at the P1 and ends with the P10 feather. Depending on which environment these feathers grow in, they contain different trace element concentrations, and can give an overview of the element concentration in a specific environment. To be able to use this information, a good understanding of the moulting pattern is important. For Cory's shearwaters, P1 is considered to grow in the breeding area, and P10 is considered to grow in wintering area, as the moult goes on over a long period of time (Ramos et al., 2009). This special case where the P1 is grown in the breeding area is called a moult-breeding overlap, which is an exception in moulting pattern seen in seabirds. For Cory's shearwater it is found that moulting pattern has a moult-breeding overlap (Bridge, 2006).

2.4 Sample preparation for inorganic analysis

When performing trace analysis of elements in a variation of matrices, there is a long list of challenges to keep in mind. In order to analyse the matrices containing elements, and not external surface contamination, a good washing procedure is the key. Further, a good method for digestion of the samples is important, including the correct dilution to obtain the wanted concentration.

2.4.1 Washing procedure

The washing procedure contains 5 steps, sequentially washing with acetone, nitric acid and milli-Q water. The goal of the washing is to remove external contamination before trace element analysis of feathers. External contamination may be trace elements from liquids, gases or dust that sticks to the feather's special structure. It is important to have in mind that no cleaning procedure exclusively remove all external contamination from feathers (Dmowski, 1999). Due to a high risk of contamination when working with trace elements, all procedures are performed with plastic equipment and wearing gloves. The samples are delivered in air-tight plastic zip-bags to avoid contamination during transport and storage, and they are transferred to suitable washing vials (new polypropylene vials, 45 mL) using plastic tweezers (Jaspers et al., 2019).

2.4.2 Sample digestion

The analytical technique ICP-MS and other modern instrumental techniques require samples in a homogeneously distributed solution. A conversion from the original sample form to solution is necessary prior to analysis. A complete digestion of the sample is performed, involving mineralization of organic content and a conversion of inorganic content to solution. Residues in the sample that interfere the detection must be removed prior to analysis. The digestion procedure should be simple and preferably not require any complicated apparatuses, and the systematic errors should be minimized if possible. Clean vessels of an inert material is important, in addition to a small amount of high-purity reagents and the reaction chambers should be as small as possible (Baranowska, 2016). The UltraCLAVE digestion system is a high-pressure and high-temperature system, using microwaves for heating. Systems using microwaves achieve quality results with faster digestion procedure, reproducibility and no pollution from the environment or the laboratory interferes (Thermo Fisher Scientific, 2019).

Nitric acid (HNO_3) is a strong oxidant dissolving all metals, except from aluminium and chromium (Skoog, West, Holler, & Crouch, 2014).

2.5 Sample preparation for organic analysis

Reliable results from trace organic analysis depend on factors such as concentration of the analyte compared to other components in the matrix, contamination from other reagents, apparatus and laboratory environment, loss of analyte by adsorption, degradation during analytical operations, interfering compounds in the matrix and available suitable reference materials (Bedson, 1996). To reduce these errors, a good washing procedure and preparation of the sample is important.

2.5.1 Washing procedure

The washing procedure developed by Jaspers et al. (2007) is a detailed description of how to remove external contamination before organic analysis of feathers. External contamination may be organic contaminants from liquids, gases or dust that sticks to the feathers special structure. It may also be contamination from equipment used during the sampling process. Similar to the trace element washing procedure, no cleaning procedure exclusively remove all external contamination from feathers (Dmowski, 1999). All procedures are performed with glass or stainless-steel equipment and no gloves are worn when handling the feathers directly. Glassware, tweezers and scissors used during the sample preparation are precleaned with methanol (MeOH) and thoroughly rinsed between each sample (Jaspers, Covaci, Van den Steen, & Eens, 2007). The feathers are washed in two sequences, first with distilled water and second with *n*-hexane (Jaspers, Herzke, Eulaers, Gillespie, & Eens, 2013). Earlier studies have shown that *n*-hexane wash do not remove any internal PFCs from feathers (Løseth et al., 2019). The *n*-hexane is analysed by the same protocol as the feathers, to validate the success of the washing procedure and to evaluate external contamination.

2.5.2 Solid-liquid extraction

Solid-liquid extraction (SLE) is a classic extraction technique for extracting target organic compounds from a solid sample matrix into a liquid phase (Ballesteros, Teixeira, & Mussatto,

2014). The technique is also an important step for removing interfering compounds to minimize errors in the analytical procedure (Skoog et al., 2014). Different organic solvents (MeOH, ethanol, acetone etc.) or distilled water can be used as the liquid phase, removing the soluble compounds from the solid phase into the liquid phase. The process is performed by dissolving the solid sample in the preferred solvent, where the target analytes are soluble. The choice of the extraction solvent is important to obtain the most efficient extraction, and factors such as solvent concentration, solvent to solid sample ratio, temperature and time of contact are critical for a good result (Ballesteros et al., 2014). Ultrasound can function as a great help in pre-treatment of solid samples, increasing the efficiency of solid-liquid extraction, due to its benefits such as accelerating and facilitating extraction processes. The extraction process is more efficient as the ultrasound accelerates the mass heat transfer between phases, such as solid to liquid phase, because of its intensity (Baranowska, 2016).

2.6 Quantitative analytical techniques

2.6.1 ICP-MS

Inductively coupled plasma-mass spectrometry (ICP-MS) is an analytical technique for characterization of virtually any material. The technique is well suited for chemical analysis, with its ability to precisely identify and quantitate all elements in the periodic table, even efficiently determining composition of multielement matrices. Another benefit with the technique is its ability to detect and determine concentrations at very low levels (1-10 ng/L elemental concentration in solution) and even non-metal elements can be determined. ICP-MS gives high accuracy and precision, and contributes to minimal interfering components, which makes it a powerful analytical tool (Skoog et al., 2014, p. 778; H. E. Taylor, 2001, pp. 1-2).

ICP-MS is an atomic spectroscopic technique, and these techniques require atomization of the samples before analysis. Samples usually enter the atomizer as a solution, where the target compounds in the sample are converted to ions in gas-phase (Skoog et al., 2014, p. 776). The sample is often introduced as an aerosol into the system, and the most common introduction is by a nebulizer.

Plasma is an electrically neutral gas consisting of positive ions and free electrons. It is a continuous atomizer, which means that the ions in gas-phase are introduced in a steady continuous stream (Skoog et al., 2014, p. 776). Its high energy is enough to atomize, ionize and excite virtually all the elements, which are introduced into the plasma for elemental chemical analysis. Typical inert gases used to sustain plasmas are Argon (Ar), Helium (He), Nitrogen (N) and air, due to their ionization properties and availability. Ar especially has the advantage of minimal reactivity with other analyte species, which is an undesirable effect. Ar ions in plasma are capable of absorbing enough power from an external source to maintain temperature levels at which further ionization sustains the plasma indefinitely. Due to this, temperatures up to 10 000 K are achieved (Skoog et al., 2014, pp. 776-779). One of the power sources available for argon plasma spectroscopy, is the inductively coupled plasma source, presented in **figure 2.3** on the next page.

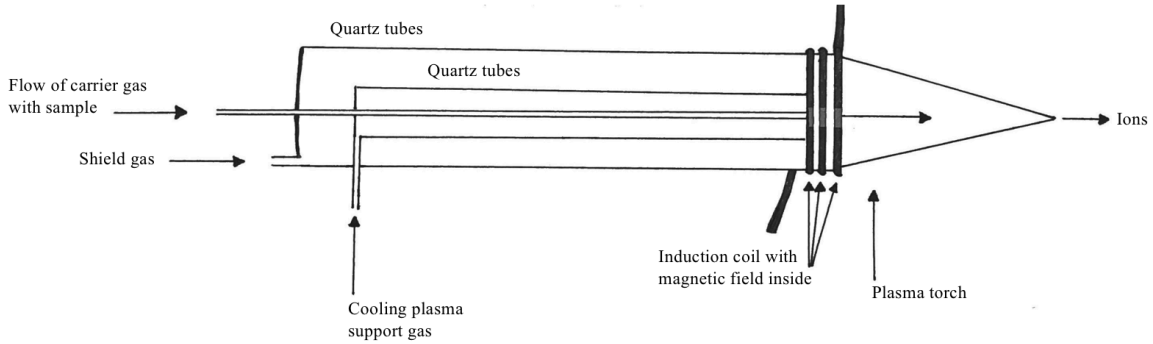


Figure 2.3: The inductively coupled plasma source with its explained components. The source is made of 3 concentric quartz tubes (inspired by Skoog et al. (2014, p. 776)).

The inductively coupled plasma (ICP) has shown the most useful properties to serve as an ion source for the detection method mass spectrometry (H. E. Taylor, 2001, p. 15).

Mass Spectrometry

Mass spectrometry (MS) is a useful detection method for both inorganic (atomic mass spectrometry) and organic (molecular mass spectrometry) compounds, due to the instruments high sensitivity, low detection limits, speed and diversity (Fifield & Haines, 2000; Skoog et al., 2014, pp. 808-812). The ions introduced to the mass spectrometer undergo fragmentation to ions of lower masses. Then these fragments are firstly separated based on their mass-to-charge ratio *(m/z)* and secondly, directed to a transducer converting the number of ions (abundance) to an electrical signal. The ion abundance plotted against the *m/z* gives a mass spectrum (Gross, 2006, p. 3; Skoog et al., 2014, p. 802).

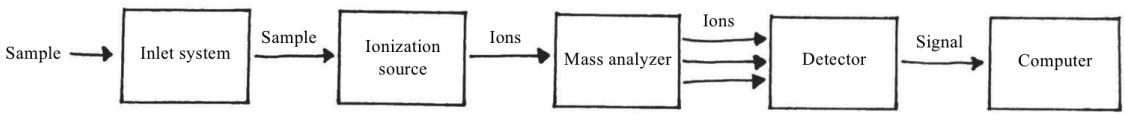


Figure 2.4: A basic diagram illustrating the principal components of a mass spectrometer (inspired by Skoog et al. (2014, p. 808)).

A mass spectrometer consists of the basic principal components given in **figure 2.4**. The sample is introduced through the sample inlet system, and further introduced to the ionization step where ions and fragments are produced. The ions are accelerated into the mass analyser and are

separated by their *m/z* ratio. The detector collects the *m/z* values and convert them to a signal which is further processed in a computer system. The resolution of a mass spectrometer is defined as the ability to separate close *m/z* regions in the mass spectrums and separating the elements from interferences (Skoog et al., 2014, pp. 804-805).

When combining the inductively coupled plasma (ICP) source with mass spectrometry (MS), the ICP served both as an atomizer and an ionizer, introducing ions straight into the mass analyzer, often a quadrupole, where the ions are sorted according to their *m/z* ratio and detected as described in **figure 2.4** (Skoog et al., 2014, pp. 808-809). Quadrupole mass analyzers function as a filter, where only ions with a certain *m/z* ratios pass through (Skoog et al., 2014, p. 806).

2.6.2 LC-MS/MS

High-performance liquid chromatography (HPLC) with electrospray ionization (ESI) and tandem mass spectrometry (MS/MS) has earlier been used as the analytical method for detecting perfluorinated compounds (PFCs) (Arvaniti et al., 2014; Kannan et al., 2002).

Liquid Chromatography

An HPLC instrument can separate almost any dissolved mixture. The instrument separates the sample solution based on a gradient, and a mass spectrometer ionize each compound and provide a fragmentation pattern based on the molecular weight of each fragment. HPLC combined with MS, can identify and quantify all compounds introduced by comparing the fragmentation pattern to a standard database for organic compounds (McMaster, 2005, p. 1).

The LC/MS system has a chromatographic pump and inlet system, and a column (McMaster, 2005, p. 4; Skoog et al., 2014). An overview of the system is illustrated in **figure 2.5** on the next page. The samples are introduced into the system in a sample manager, which will control the samples chosen for analysis at any time. The chosen solvents (mobile phase) are connected to the system through a pump, pumping a mix of the solvents into the injector. At this point the sample is introduced and dissolved in the mobile phase. The mobile phase with the sample continues into the column for separation, and after elution the different compounds are detected, possibly by a MS detector as described earlier (Lundanes, Reubsaet, & Greibrokk, 2013).

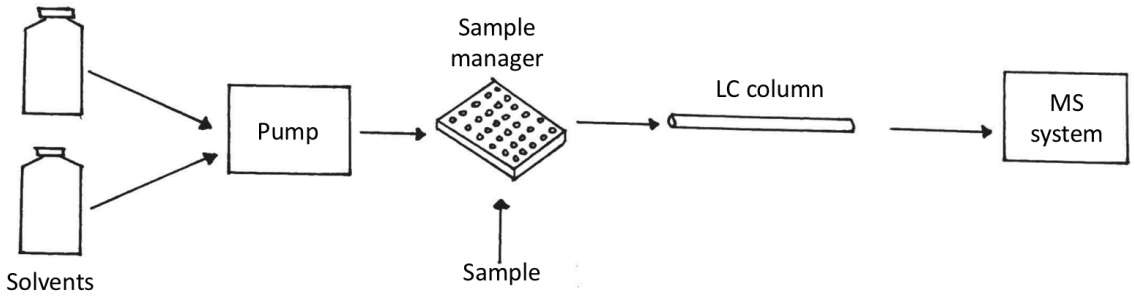


Figure 2.5: A schematic diagram illustrating the principal components of a liquid chromatography system linked to an MS instrument (inspired by McMaster (2005, p. 5)).

To be able to successfully analyse compounds using a LC system, the following conditions must be suitable for the target compound; type of column packing and mobile phase, the length and diameter of the column, mobile-phase flowrate, separation temperature and sample size to mention some (Snyder & Kirkland, 1979, p. 16). The column is the most important part of the system, as it separates the components of the sample. The components are separated based on their interaction with the stationary phase in the column, leading to different retention times since the compounds migrate through the column at different speed. The retention time (RT) of a component is defined as the time from injection of the sample to the signal of the component (signal maximum) is recorded in the detector (Lundanes et al., 2013, p. 2).

Electrospray Ionization

Electrospray Ionization (ESI) is a ionization technique available for a wide range of liquid-phase samples, and has become the most successful interface for LC/MS analysis (Dass, 2007, pp. 48-49). ESI is a desorption source, meaning it converts the sample directly from liquid state to gaseous ions (Skoog et al., 2014). The process produces a fine spray of droplets that are converted to gas-phase ions as the solvent evaporates.

The ESI technique is based on sending the sample in a suitable solvent continuously through a capillary tube, while applying a strong electrical field to the tube. A high potential is applied to the capillary tube tip, and an opposite potential to a counter-electrode, causing an accumulating of charged ions in the end of the tube. The sample is dispersed into a fine mist of charged droplets, leaving the tube tip. A flow of hot nitrogen will encourage evaporation of the charged droplets as they pass through a short distance, before some ions will reach the high vacuum of

the mass analyzer (Dass, 2007, p. 49). A schematic explanation of the process is given in **figure 2.6**. ESI as the ionization technique is the first step in the mass spectrometric part of the analysis (see **figure 2.7**).

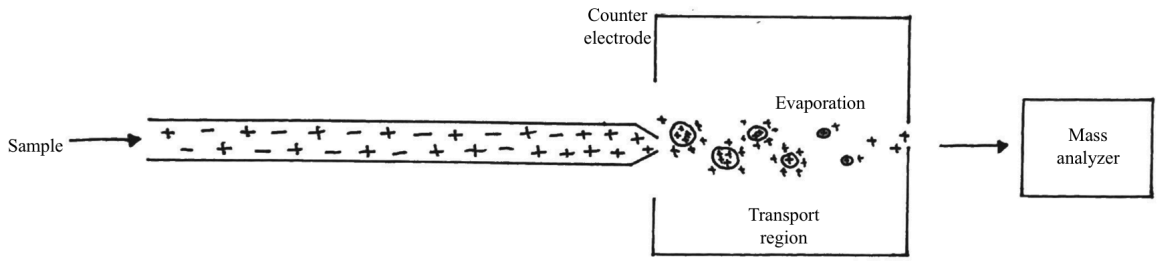


Figure 2.6: A schematic presentation of the basic components of electrospray ionization (reproduced from Dass (2007, p. 49))

Tandem Mass Spectrometry

Tandem mass spectrometry (MS/MS) is a detection technique for organic molecules. Its principal components are similar to **figure 2.4**, but the tandem principal is illustrated in **figure 2.7**. Tandem MS involves two MS systems, where each mass analyzer perform different tasks (Dass, 2007, p. 119). The charged molecular ion is accelerated into the first mass analyzer, where the precursor ion is isolated and fragmented. The fragments, also called product ions, are further accelerated into the second mass analyzer where they are separated by their *m/z* and the process continues as described in **section 2.6.1** (Skoog et al., 2014, pp. 814-815).

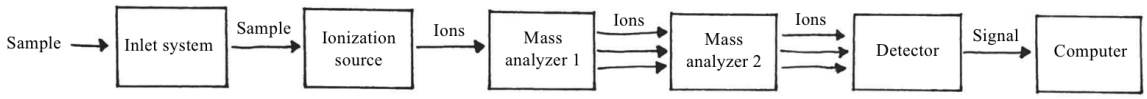


Figure 2.7: A basic diagram illustrating the principal components of a tandem mass spectrometer (inspired by Skoog et al. (2014, p. 808)).

MS/MS instruments can be classified in two categories: tandem in-space and tandem in-time. A triple-quadrupole MS instrument is a tandem in-space type of instrument, meaning that three quadrupoles are arranged sequentially (Dass, 2007, p. 129). A quadrupole mass analyzer consist of four parallel metal rods, precisely matched, functioning as electrodes. A direct-current (dc) and radio-frequency (rf) are applied to the electrodes creating a high-frequency electric field

causing stable vibration motions of the ions (Dass, 2007, p. 75). The ions will accelerate towards and against the metal rods, causing retention in the field in varying degree depending on their *m/z* ratio. By changing the dc and rf potentials, but keeping the ratio constant, a mass spectrum for each compound can be obtained (Dass, 2007, p. 76).

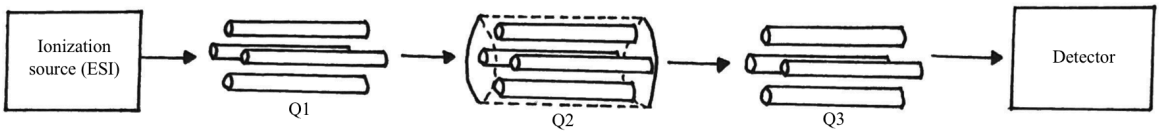


Figure 2.8: A basic diagram illustrating the principles of a triple-quadrupole mass spectrometer (reproduced from Dass (2007, p. 132))

A triple-quadrupole instrument consists of three sequentially separate quadrupole devices with four metal rods, as shown in **figure 2.8**. The first quadrupole (Q1) and the third quadrupole (Q3) operate as normal mass analysers, transmitting ions with specific *m/z* values. Q1 transmit all precursor ions of a chosen *m/z* value. The second quadrupole (Q2) function as a radio-frequency (rf)-only quadrupole and a collision cell. The ions undergo a collision-induced dissociation (CID) process in Q2, where the precursor ions are excited to higher energy states by collision with inert gas atoms, which leads to fragmentation. The fragments continues to Q3 that transmit only the *m/z* value for a chosen product ion (Dass, 2007, p. 132).

2.7 Quantification and Quality Assurance

Applying analytical methods to real-world problems is not straight-forward as good quality of the results is not guaranteed. Also the performance of the instruments used must be evaluated constantly, in order to secure a good quality control (Skoog et al., 2014, p. 188). In this section, and the following (2.8), all activities involved in the quality assurance are described, such as quality control, validation and statistics.

2.7.1 Inorganic analysis

Quantification limits and precision

The quantification limits (QL) for the inorganic trace element analysis was defined according to the relative standard deviation (RSD%) value (Skoog et al., 2014, p. 155). The RSD % values and detected concentrations were plotted for each target element, and the concentration equal to the 25% RSD-value was set as the QL for that specific element, uncorrected of the baseline. The QL value was calculated back to per g sample, using average amount of sample used in the analytical procedure. RSD% values were calculated for all detected elements to enable assessment of precision.

Accuracy and reproducibility

Accuracy and reproducibility were ensured by including standard reference material and blank samples during all analytical processes. Reference samples included a reference material with all target elements in known concentrations and was treated like an ordinary sample. All blank samples were containing no material but were treated similar to ordinary samples and reference samples (Skoog et al., 2014, pp. 90-91).

2.7.2 Organic analysis

Internal standard method and method errors

The internal standard method is a supportive method for quantitative analysis, correcting the varying responses from run to run. A known and constant amount of internal standard is added to all samples, blanks and standard solutions (Dass, 2007, p. 489; Skoog et al., 2014, p. 182).

The internal standards chromatographic characteristics should be similar to the target analytes, but the molar mass must be different, in order to elute it in the similar area as the target analytes (Dass, 2007, p. 490). The similarity is also important in order to respond similar to the sample preparation steps such as extraction. Blank samples containing no material were also included throughout the whole protocol (Skoog et al., 2014, pp. 90-91).

The internal standard method is also based on a calibration curve for each target analyte. The calibration curve is made of all target analytes at different concentrations, and a fixed concentration of the internal standard is added to all calibration curve samples. The calibration curve is made by using the ratio of the analyte response to a specific internal standard response from a measured standard solution (e.g. matrix match samples) and plotting it against the concentration of the spiked analyte. This ratio is the basis of all calculations of validation, such as reproducibility and accuracy, when using the internal standard method (Asimakopoulos, Wang, Thomaidis, & Kannan, 2014; Skoog et al., 2014, p. 182). The calculation of the ratio of the analyte signal to the internal standards signal is explained in the section **relative response**.

Retention time and relative retention time

The retention time (RT) of a compound is the measured time from injection of a compound in the chromatographic system to detection after passing through the chromatographic column. The RT can vary, due to its dependence on the chromatographic system and conditions. There are several factors that can cause a change in the retention time, for the column; instability of the temperature, degradation and length differences, and for the mobile phase; instability in the flow rate and air bubbles (Skoog et al., 2014). A comparison of the RTs can therefore give an unclear picture. Calculating the relative retention time (RRT) gives more true values, since also internal standards are affected by the same factors during passing through the column (Asimakopoulos et al., 2014). RRT is calculated by dividing the analytes RT by the internal standards RT, using **equation 2.1** (Ettre, 1980).

$$\mathrm{RRT} = \frac{\text{RT analyte}}{\text{RT internal standard}} \tag{2.1}$$

Relative response

The relative response (RR) is a ratio used for compensating to variations in the signal intensity for a target analyte. The observed variations in the responses can occur due to variations during the sample preparation (e.g., loss of sample) and variations in the chromatographic system. As similar to the RRT, a ratio compensating for these variations can be calculated based on the response of the internal standard. The relative response ratio is calculated using **equation 2.2** (Asheim, 2018).

$$RR = \frac{\text{Response analyte}}{\text{Response internal standard}} \tag{2.2}$$

Ion ratio

The ion ratio (IR%) is a confirmation parameter for the target analytes, as it gives a unique specific ratio for each compound in the sample matrix. The ion ratio is calculated by dividing the peak area of the confirmation ion by the peak area of the quantification ion as shown in **equation 2.3** (Asheim, 2018).

$$IR\ \% = \frac{\text{Area of confirmation ion}}{\text{Area of quantification ion}} \text{ x } 100\% \tag{2.3}$$

Repeatability and reproducibility

Precision of the method can be measured in repeatability and reproducibility, and the parameters give information about variability in the measurements made under identical conditions (Wood, 1999). The precision of an analytical technique can be determined by measuring replicates of the same sample with the same amount of analyte added. Repeatability applies to intra-laboratory variations under constant circumstances, where measurements are carried out under controlled conditions in a short period of time. Reproducibility applies to inter-laboratory variations under different circumstances, where measurements are carried out in different time and place, but following the same method (Fifield & Haines, 2000; Wood, 1999). This data is normally represented as standard deviations or relative standard deviations, calculated by **equation 2.5** and **2.6**. The mean of the data set is calculated by equation **2.4**

$$\bar{x} = \sum_{i=1}^{n} x_i \tag{2.4}$$

$$STD = \sqrt{\frac{\Sigma_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \quad (2.5)$$

where $\mathbf{X_1, X_2 \ldots X_n}$ are observed values, $\bar{\mathbf{x}}$ is the experimental mean value of the data, **n** is the number of samples and **n-1** is the degree of freedom. The relative standard deviation (RSD%) can give a more correct picture of the data quality than the standard deviation (STD). The RSD% is calculated by **equation 2.6** (Skoog et al., 2014, pp. 99-100)

$$RSD\% = \frac{STD}{\bar{x}} \times 100\ \% \quad (2.6)$$

where STD is the standard deviation and the $\bar{\mathbf{x}}$ is the experimental mean value of the data (Skoog et al., 2014, p. 109).

Absolute and relative recovery

Recovery is a measure of the accuracy of the analytical method, and especially a measure on the efficiency of the sample preparation steps. The recovery of the method represents the actual amount of compound obtained after the analytical procedure. A way of measuring the recovery of an analyte through an analytical method can be by adding known and equal amounts of target analytes to a sample with a similar matrix as the original samples (control samples). The absolute and relative recovery for each target analyte were calculated using the **equations 2.7** and **2.8** (Asimakopoulos et al., 2014)

$$\text{Absolute recovery } \% = \frac{(A_{Ssp}) - (A_{RB})}{(A_{MM}) - (A_{RB})} \times 100\% \quad (2.7)$$

$$\text{Relative recovery } \% = \frac{\frac{(A_{Ssp}) - (A_{RB})}{(ISTD_{Ssp})}}{\frac{(A_{MM}) - (A_{RB})}{(ISTD_{MM})}} \times 100\% \quad (2.8)$$

where $\mathbf{A_{Ssp}}$ is the peak area of the pre-extraction spiked sample, $\mathbf{A_{RB}}$ is the peak area of the blank sample, and $\mathbf{A_{MM}}$ is the peak area of the post-extraction spiked sample (matrix match sample). The $\mathbf{ISTD_{Ssp}}$ is the peak area of the internal standard pre-extraction spiked sample, and the $\mathbf{ISTD_{MM}}$ is the peak area of the internal standard post-extraction spiked sample (matrix match sample) (Asimakopoulos et al., 2014). The absolute recovery is typically of higher uncertainty as for its corresponding relative recovery, as the relative recovery compensate for

the analyte loss during the sample preparation (Caban, Migowska, Stepnowski, Kwiatkowski, & Kumirska, 2012).

Instrumental limit of detection and lower level of quantification

The limit of detection (LOD) is the smallest amount of an analyte possible to detect, that is significantly different from a blank sample (Dass, 2007, p. 491). The lower level of quantification (LLOQ) is the smallest amount of an analyte possible to measure with a reasonable accuracy (Asheim, 2018). In this study, the LOD was calculated as 3 times the noise detected in the chromatogram of the lowest concentration in the calibration curve. Using these values, the LLOQ was calculated by the **equation 2.9**:

$$\text{LLOQ} = 3 \text{ x LOD} \tag{2.9}$$

Matrix effects

Matrix effects (ME%) is important to consider when working with LC-MS/MS, due to the effect of possible coeluting matrix compounds. Coeluting compounds can cause an interference with the target compounds signal, causing an unwanted ionic attenuation or enhancement (Asimakopoulos et al., 2014; Caban et al., 2012). The matrix factor (MF), which is considered as the effect on the response from matrix interferences for each target analyte, were calculated according to **equation 2.10** (Asimakopoulos et al., 2014).

$$\text{MF} = \frac{(\text{A}_{\text{MM}}) - (\text{A}_{\text{RB}})}{(\text{A standard solvent solution})} \tag{2.10}$$

The $\mathbf{A_{MM}}$ is the area of the matrix match sample and the "A standard solvent solution" is the area of the standard solution sample with the same target analyte concentration. $\mathbf{A_{RB}}$ is the area from the reagent blank peak. From **equation 2.10** the matrix effect percentage can be calculated using **equation 2.11** (Asimakopoulos et al., 2014).

$$\text{ME \%} = (\text{MF} - 1) \text{ x } 100\% \tag{2.11}$$

A ME% value > 0 indicates ionization enhancement, and a ME% value < 0 indicates ionization attenuation (Asimakopoulos et al., 2014).

2.8 Statistics

Descriptive statistics

It is important to establish the distribution of the data, since the majority of statistical tests assumes normal distribution (Fifield & Haines, 2000). A confirmation of a normal distributed dataset can be achieved by looking at histograms or by performing a Shapiro-Wilk test (Shapiro & Wilk, 1965). The mean values in two groups can be compared using a student T-test, primarily for normally distributed data, and for non-normally distributed data, a f-test can be performed (Bower, 2013; Weaver, Morales, Dunn, Godde, & Weaver, 2017). If more than two datasets or groups are to be compared, an analysis of variance (ANOVA) test can be performed, checking for differences between groups for normally distributed data, and a Tukey HSD post-hoc test can be used to find the specific pair of groups that are significantly different (Weaver et al., 2017).

Correlation

Correlation is a measure of relationship strength between to variables and it is described as the correlation coefficient (Pearson correlation) (r). The correlation coefficient value can range from -1 to 1, where -1 is a strong negative correlation, 0 is no correlation and 1 is a strong positive correlation (Asheim, 2018; Fifield & Haines, 2000; Rian, 2019).

Principal component analysis

Principal component analysis (PCA) is a multivariate tool for pattern of similarity analysis in complex datasets, presented as points in maps (score plots) (Abdi & Williams, 2010). The goals of applying PCA can be simplification or reduction of data, detection of outliers, classification or prediction. A large dataset can in many cases be challenging to comprehend, and by simplifying the data and reducing its complexity a clearer picture can be obtained (Wold, Esbensen, & Geladi, 1987). The PCA compute new variables called principal components, which explains the variation of the data by a small number of linear combinations of the variables. Correlation between variables and components in PCA is called loading, and the loadings can be plotted as coordinates in a PCA biplot. A PCA biplot is a plot showing the variables as arrows indicating relative loadings on the two principal compartments (Abdi & Williams, 2010; Crawley, 2012).

3 Materials and methods

The samples used in this study were collected at the islands Strofades (Greece), Smøla and Steigen (Norway). The samples from Greece were brought from the Technological Educational Institute (TEI) of Ionian Islands to the Norwegian University of Science and Technology (NTNU) by Associate Professor Alexandros Asimakopoulos in September 2018, after confirmation from the Norwegian Food Safety Authority. The samples from Norway were brought from Smøla and Steigen to NTNU in 2015 and 2016. Sample preparation and analysis for the present study started September 2018 and finished October 2019.

3.1 Sampling and sample population

3.1.1 Sample group 1

The samples in group 1 of the study population are feathers and blood from Scopoli's shearwaters. The majority of the sampled birds were breeders and a few of them were prospectors. The sample collection consisted of 30 pieces of P1 feathers, 30 pieces of P10 feathers, 29 blood samples in ethyl alcohol and 28 blood samples in heparin. Sampling took place from June to July in 2018 and was performed as previous field samplings by Karris, Xirouchakis, Maina, Grivas, and Kavadas (2018) and Voulgaris et al. (2019). Each feather was stored in a separate sealed plastic bag to avoid contamination.

3.1.2 Sample group 2

The samples in group 2 of the study population consisted of 38 wings from dead migratory birds. The samples came from six different seabird species; Wood warbler, Sedge warbler, Garden warbler, Sand martin, Spotted flycatcher and Great reed warbler. The samples were collected during spring migration, where Strofades island was the first possible stop after crossing the Saharan desert and the Mediterranean Sea. Each wing was stored in a separate sealed plastic bag to avoid contamination.

3.1.3 Sample group 3

The samples in group 3 of the study population consisted of 69 feathers from White-tailed eagles collected at Smøla and Steigen islands. Sampling was performed according to the field sampling by Løseth et al. (2019). Each feather was stored in a separate sealed plastic bag to avoid contamination.

3.2 Inorganic analysis

3.2.1 Chemicals and materials

Multielement calibration solution (Elemental Scientific, Omaha, NE, U.S.) containing 73 elements; Ag, Al, As, Au, B, Ba, Be, Bi, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Hg, Ho, I, In, Ir, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, Os, P, Pd, Pb, Pr, Pt, Re, Rb, Rh, Ru, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn and Zr for ICP-MS.

Concentrated HNO_3 (Ultrapure grade, distilled by Milestone SubPur unit), Milli-Q water and acetone (technical grade) from VWR Chemicals (Rue Carnot, Fontenay-sous-Bois, France) were used for the different steps in the inorganic analysis.

The certified reference materials were obtained from the Institute of Nuclear Chemistry and Technology Warszawa, Poland (Polish Virginia Tobacco Leaves, INCT-PVTL-6) and Seronorm Trace elements whole blood L-1, from Sero (Billingstad, Norway), Lot 1406263, REF 210105, density of the blood set to 1.06.

3.2.2 Sample preparation

Samples

A total of 224 samples were analysed by ICP-MS, 167 feather samples and 57 blood samples. Feather samples (n=60) from group 1 were transferred to 50 mL polypropylene (PP) vials and weighed (3.9 – 15.1 mg). The feathers from group 2 (n=69) were cut to remove any tissue before weighed into 50 mL PP vials (200 – 768 mg). The samples from group 3 (n=38) were obtained by pulling out the P10 feather from the 38 wings before weighed into 50 mL PP vials (7.8 – 24.9 mg). The feathers were washed in 5 steps using a laboratory shaker as followed: i) Acetone wash (5 minutes), flushed with water twice; ii) Milli-Q ultrapure water wash (5 minutes); iii) Acetone wash (5 minutes), flushed with water twice; iv) Ultrapure 0.64 M nitric acid wash (5 minutes), flushed with water twice; v) Milli-Q ultrapure water wash (5 minutes). As much liquid as possible were removed from the samples, before samples were freezed at -20ºC. Blood samples required no specific preparation but kept in the freezer at -20ºC.

Reference material

A certified reference material was included in the analytical process for both feather and blood samples. For feather samples the certified reference material was a powder containing all target trace elements (INCT-PVTL-6). For blood samples the certified reference material was a Seronorm L-1 material. Information regarding amount of reference material analysed is described in **table A.1** in **Appendix A**.

3.2.3 Freeze drying, decomposition and dilution

Feather samples were freeze dried overnight (min. 24 hours) until no moisture was left in the samples. The vials were closed and stored until the next preparation step started.

Dried feather samples from PP vials and reference material were added into 18 mL perfluoroalkoxy (PFA) vessels, the samples were weighed and acidified by 1.5 ml 50 % v/v concentrated HNO_3 (Ultra-Pure grade). Samples, blanks and reference material were digested using a high-pressure digestion unit UltraCLAVE (Milestone). The cooled down samples were diluted with distilled water to a total volume of 17 mL in PP vials and weighed before elemental composition was analysed using an HR-ICP-MS instrument.

Blood samples were mixed by a vortex mixer before a maximum of 500 mg sample were poured and weighed into PFA vessels. There was added 0.5 mL 65 % concentrated HNO_3 (Ultra-Pure grade) to the vessels. Samples and blanks were digested using a high-pressure digestion unit UltraCLAVE (Milestone). The cooled down samples were diluted with distilled water to a total volume of 13 mL in PP vials and weighed before elemental composition was analysed using an HR-ICP-MS instrument.

Due to various amounts of blood in the samples obtained from the two different vials (containing: i) blood + ethyl alcohol and ii) blood + heparin), the dry weight of the remaining sample was calculated. The samples were weighed into a plastic container, and dried for 48 hours, before weighed again. A dry-weight percentage factor was obtained and used in the calculations of the determined concentrations after analysis.

3.2.4 Analysis (ICP-MS)

High resolution coupled plasma mass spectrometer (HR-ICP-MS) analysis were performed with a Thermo Finnigan model Element 2 instrument (Bremen, Germany). Samples were introduced to the ICP-MS system using a SC2 DX autosampler (with ULPA filter dust cover) combined with a PrepFAST flow injection analysis system (ESI, Elemental Scientific, Inc. Omaha, NE) with a total flow of 200 µL/min. The instrument is equipped with a PFA-ST nebulizer, spray chamber (ESI), quartz demountable torch, standard quartz injector, Aluminium sample cone and an Aluminium X-skimmer cone. Methane gas was used in a splitting of sample gas in addition to argon to lower oxides and increase sensitivity for Se and As. More details on the instrument are given in **Appendix A**.

The precision of the ICP-MS was verified using certified calibration solutions. In this analysis, two calibration solutions from two independent producers were used, one as a calibration solution (CS) and one as a quality solution (QS), both delivered by ESI. Both solutions are made out of two solutions to cover all the elements, which are a PS-70 solution and a PS-ClBrI solution. PS-70 contains 70 elements (listed in **section 3.2.1**), and the matrix consists of HCl, HNO_3 and HF. PS-ClBrI contains chlorine, bromine and iodine and it is delivered in a separate solution to avoid interferences with the HCl matrix in PS-70.

3.2.5 Quantification limit and data treatment

The quantification limit (QL) were calculated based on the RSD% value at approximately 25% from the data, as described in **section 2.7.1 (see figure 4.1)**. At this point in the graph, and towards higher concentration, the RSD are decreasing. Data treatment and statistical calculations was performed mainly using Excel (Microsoft Office, 2019). The statistical software R were used to calculate principal component analysis (PCA), descriptive statistical analysis and correlations.

3.3 Organic analysis (PFCs)

3.3.1 Chemicals and materials

Analytical standards for 15 perfluorinated compounds, and one non-fluorinated similar compound, of interest were obtained from Sigma-Aldrich (St. Louis MO, US). All standards were given at concentration 100 ppm in methanol (dilution for each compound is described in **appendix C, table C.2**). Standards include: tetrabutylammonium nonafluorobutane sulfonate (NonaFBS; C4), sodium 1-decanesulfonate (DecaS), tridecafluorohexane-1-sulfonic acid potassium salt (TriDeFHxSA; C6), sulfluramid (Sulf; C8), tetrabutylammonium hepta-decafluorooctane sulfonate (PFOS; C8), perfluorooctane sulphonamide (PFOSA; C8), perfluoropentanoic acid (PFPA; C5), perfluorohexanoic acid (PFHxA/UnFHxA; C6), perfluoroheptanoic acid (PFHeA; C7), perfluorooctanoic acid (PFOA; C8), perfluorononanoic acid (PFNA; C9), perfluorodecanoic acid (PFDA; C10), perfluoroundecanoic acid (PFUnA; C11), perfluorododecanoic acid (PFDoDeA/TricoFDoDeA; C12), perfluorotridecanoic acid (PFTriDe; C13) and perfluorotetradecanoic acid (PFTetDeA; C14).

Internal standards (both given at concentration 50 ppm in methanol): perfluorooctanoic acid (PFOA-^{13}C8) and perfluorooctanesulfonate ^{13}C8 sodium salt (PFOS-^{13}C8).

Milli-Q ultrapure distilled water were used for sample wash. N-hexane, sodium hydroxide (NaOH) and hydrochloric acid (37%) for sample preparation and extraction were obtained from VWR chemicals (Rue Carnot, Fontenay-sous-Bois, France). Also, for sample preparation steps, Supelclean^{TM} ENVI-carb^{TM} SPE bulk pack from Sigma-Aldrich (St. Louis MO, US) and Acetic acid (glacial, 100%) from Merck (Billerica MA, US) were used. Methanol (MeOH) (hypergrade for LC-MS) were obtained from Merck (Billerica MA, US) and was employed for preparation of standard solutions and extraction.

3.3.2 Sample preparation

Samples

The samples for the organic analysis were feathers from sample group 2 (N=38). All feathers left were pulled out from the wing and checked for any tissue left at the calamus. The total length of the feathers were measured (64.4-109.4 cm) and the following washing procedure was applied: All feathers from one wing were placed in a petri dish with distilled water (Milli-Q ultrapure) and metal tweezers were used to cover the feathers with water and to separate the

barbs to wash thorough. The feathers were further put in aluminium foil trays covered with clean tissue paper for drying (minimum 24 hours).

The following day, all feathers in each sample were cut in small pieces (2-3 mm) and capped in aluminium foil. Cutting of feathers is crucial for effective extraction of contaminants like PFCs (Jaspers et al., 2019). The samples were transferred to 50 mL metal free PP tubes and weighed (0.049-0.159 g). All samples and blanks were washed with 20 mL hexane in an ultrasonic bath for 10 minutes. Hexane were taken out of the vials and concentrated in 15 mL metal free PP vials down to 1 mL in a water bath at 25°C under a gentle stream of nitrogen (TurboVap® LV automated evaporation system). This 1 mL solution was saved for external PFC analysis. The feather samples and blanks were left to dry covered with aluminium foil.

Control samples for spiked samples and matrix match samples were obtained from a pooled sample consisting of feathers from eagles. These feathers were treated the same way as all feather samples until this part of the protocol.

Internal standards

Perfluorooctanoic acid (PFOA-13C8, 50 ppm) and perfluorooctanesulfonate 13C8 sodium salt (PFOS-13C8, 50 ppm) were used as internal standards (ISTD). A mix of 1.5 mL 1 ppm ISTD solution were made by adding 30 µL of the two standards to a glass vial (LC-MS) using a 100 µL Eppendorf pipette, and diluting the mix by adding 1440 µL methanol (MeOH) using a 1000 µL Eppendorf pipette. A total amount of 20 µL 1 ppm ISTD mix was added to dried feather samples, blanks and spiked samples before extraction. 20 µL 1 ppm ISTD mix was added to matrix match samples after extraction.

Target analytes standards

Spiked samples and matrix match samples were spiked by a 1 ppm target analyte (TA) mix, which is a mix of all the 16 target compounds (NonaFBS, PFTetDeA, DecaS, PFTriDe, PFNA, PFDoDeA, PFHeA, PFHxA, PFPA, PFUnA, PFOA, PFDA, TriDeFHxSA, Sulf, PFOSA, PFDoDA). A mix of 1 mL 1 ppm TA mix were made by adding 10 µL 100 ppm of each standard to a glass vial (LC-MS) using a 100 µL Eppendorf pipette, and diluting the mix by adding 840 µL MeOH using a 1000 µL Eppendorf pipette. Spiked samples and matrix match samples were

spiked as followed: 10 µL TA mix was added to 10 ppb spiked samples and 50 µL TA mix was added to 50 ppb spiked samples, prior to extraction. 10 µL TA mix was added to 10 ppb matrix match samples and 50 µL TA mix was added to 50 ppb matrix match samples, after extraction.

3.3.3 Solid-liquid extraction and pre-concentration

Feather samples were weighed (0.197-0.717 g) and spiked with 20 µL ISTD, before NaOH in MeOH (2 mL 200 mM) was added to the samples. The samples were mixed by a vortex mixer and left soaking for 60 minutes. 10 mL MeOH was added to the PP vials and the solution was vortex mixed before extracted with ultrasonication for 10 minutes. The extract was added into a 50 mL PP vial and a new 10 mL MeOH was added to the sample. The SLE was repeated 3 times in total and were left to soak overnight. MeOH were chosen as extraction solution, to resolve the PFCs bound to proteins in the feathers.

To the extract, 200 µL 2M HCl in MeOH was added and then mixed by a vortex mixer. The extract was treated with ultrasonication for 10 minutes before centrifuged 5 minutes at 2000 rpm for sedimentation. The extract was transferred to a 15 mL PP vial and evaporated down to 1 mL in a water bath at 25°C under a gentle stream of nitrogen (TurboVap® LV automated evaporation system). The rest of the extract was added during the evaporation process. The 50 mL PP vial was rinsed by 2 mL MeOH, vortex mixed and centrifuged for 10 minutes at 2000 rpm and the remaining supernatant was added to the concentrated sample vial. The final eluent concentrated samples were refrigerated at 3°C until further preparation.

Eppendorf centrifuge tubes (1.5 mL) were prepared by adding 25 mg ENVI-carb (Supelclean™ ENVI-carb™), and 50 µL glacial acetic acid (added right before the sample). 1 mL sample from the 15 mL PP vials was added in the tube. The Eppendorf centrifuge tube with sample was mixed with a vortex mixer and centrifuged for 10 minutes at 10 000 rpm. Exact 0.5 mL supernatant was taken out and added into an autoinjector vial for LC/MS. TA mix and ISTD was added to the matrix match samples at this point. Finally, 2 mM NH_4OAc was added to the vials to achieve a total volume of 1 mL (500 µL to samples, blanks and spiked samples, 470 µL to 10 ppb matrix match samples and 430 µL to 50 ppb matrix match samples).

3.3.4 Analysis (LC-MS/MS)

The analytical method is extrapolated from Waters method (Silcock, Karrman, & van Bavel, 2014). The chromatographic separation of the 16 target compounds was carried out using a Waters Acquity UHPLC Thermo system with column manager, flow through needle sample manager, and binary solvent manager (Waters, Milford, US). The column used was a Kinetex C18 column (50 x 2.1 mm, 1.3 µm, 100Å Phenomenex) serially connected to a Phenomenex guard column (C18). The separation was carried out using a mobile phase mixture of water with 2 mM ammonium acetate and an organic phase (methanol). The flow rate was set to 0.4 µL/min, injection volume of the sample was 4 µL and the total run time per sample was 5 minutes. The gradient elution program is presented in **table 3.1**.

Table 3.1: Gradient elution program with Kinetex C18 (50 x 2.1 mm) column, using a mobile phase mixture of milli-Q water (with 2 mM ammonium acetate) and methanol. Constant flow rate of 0.4 µL/min.

Time (min)	Water (%)	Methanol (%)
Init	90	10
0.2	90	10
3.0	0	100
4.0	0	100
4.1	90	10
5.0	90	10

The LC/MS system used was the tandem mass spectrometric system Xevo TQ-S, triple-quadrupole mass analyzer with ZSpray ESI in a negative ionization mode (Waters, Milford, US). The ESI voltage was applied at 1.8 kV. The cone gas (N_2) flow rate was set at 150 L/Hr, and the collision gas flow at 0.15 mL/min. The desolvation gas (N_2) flow rate was set to 900 L/Hr and the desolvation temperature was set to 450°C. The source temperature was set to 150°C. The parent and fragmentation ions for the target analytes are shown in **table C.3** (**Appendix C**).

3.3.5 Calibration curve

A calibration curve was made by making ten standard solutions with concentrations 0.05, 0.10, 0.20, 0.50, 1, 2, 5, 10, 20 and 50 ppb of the 16 TA. Internal standards were added to all calibration solutions (20 µL ISTD), and a master mix of 50/50 NH_4OAc and MeOH were used to dilute the standards to the given concentrations. Calibration curves for all 16 TAs, based on both absolute and relative areas, are presented in **figures C.1-C.16 (Appendix C)**.

3.3.6 Limits of detection and data treatment

Limits of detection (LOD) and lower level of quantification (LLOQ) was calculated for each TA using the **equation 2.9** (section 2.7.2). The LC-MS/MS data was processed using the software package MassLynx and TargetLynx (version 4, Waters, US). Data treatment and statistical calculations was performed with Excel (Microsoft Office, 2019) and the statistical software R. Concentration in blank samples were subtracted from all sample concentrations, which was calculated based on relative areas using the internal standard with the most similar retention time as the TA. The software R were applied for obtaining PCA biplots and correlation heat maps.

3.4 Quality Control

When working with trace elements, quality assurance on the equipment used is important. All equipment such as tubes and tweezers were based on plastic, all equipment was cleaned with acetone prior to use, and gloves were used during all washing procedures.

When working with organic pollutants, quality assurance is important in order to avoid contamination by plastic additives from plastic equipment. Glass and metal were used as material for all containers and equipment during the process, except from the polypropylene vials that was approved by the supervisor. All glassware and metal tweezers are washed thoroughly with methanol. Plastic containers were avoided if possible, and gloves were not used during the washing procedure.

The amount of feather sample was not big enough to do duplicates, so a pooled feather control sample was obtained to do spiked samples and matrix matched samples in order to control loss and interferences during the method. ISTD was added to all samples prior to extraction to control losses during extraction and analysis. ISTD was also added to matrix matched samples

after extraction to control losses only during analysis. TA was added to spiked samples prior to extraction to control losses, and TA was also added to the matrix matched samples after extraction and prior to analysis.

The results were considered acceptable when the values were in the range of the calibration curve, that was made the day prior to the analysis. The calibration curve was accepted with a coefficient of determination (R^2) equal to or higher than 0.99.

4 Results and discussion

4.1 Quantification and Quality Assurance

To evaluate the efficiency and performance of the sample preparation protocol and analysis (described in **section 3.2** and **3.3**), method validation and quality assurance was performed based on precision and accuracy for the inorganic analysis, and recovery, precision, limits of detection, lower levels of quantifications, matrix effects and ion ratios for the organic analysis.

4.1.1 Inorganic analysis

Precision

The precision of the elemental analysis was good for most of the target elements. Only Cd had RSD% over 25% in the feather samples. Quantification limits (QL) and RSD% values for the target trace elements are given in **table 4.1**. QLs for all sample groups was calculated to per g values based on average sample amount during analysis. For group 1 average sample weight was 6.8 mg, for group 2: 11.7 mg and group 3: 47.0 mg. For blood samples the average sample weight was 180 mg.

Most elements were detected above the QL, except from Cd were the detection frequency varied. The detection frequency for Cd in feather samples was very low (5.99%), compared to blood samples where the detection frequency was high (91.2%). In general, Cd had the highest RSD% value for all sample groups (**see table 4.1**).

Table 4.1: Quantification limits (QL) and RSD% for target trace elements with ICP-MS in bird feathers and blood (µg/g).

	Feathers group 1		**Feathers group 2**		**Feathers group 3**		**Blood samples**	
	QL (µg/g)	**RSD%**	**QL (µg/g)**	**RSD%**	**QL (µg/g)**	**RSD%**	**QL (µg/g)**	**RSD%**
As	0.06	14.8	0.04	9.40	0.009	7.60	0.0007	2.51
Cd	0.005	34.2	0.003	32.3	0.0007	60.0	0.0001	19.6
Co	0.01	22.4	0.006	8.90	0.001	21.8	0.0003	15.8
Cu	0.07	3.60	0.04	2.70	0.01	2.20	0.002	6.02
Hg	0.005	1.40	0.003	4.60	0.0007	1.20	0.0001	2.00
Mn	0.01	7.70	0.009	2.60	0.002	5.30	0.0004	6.73
Pb	0.005	6.20	0.003	3.30	0.0007	6.10	0.0001	8.70
Zn	0.06	5.30	0.04	2.50	0.009	2.20	0.002	4.52

Full tables with all detected elements with ICP-MS and their QLs, RSD% and mean values are given in **Appendix E (tables E.1-E.4)**.

Calculations for RSD% values are described in **section 3.3.6**, and **figure 4.1** is a graph illustrating the concentrations plotted against RSD% values where the QL is set at RSD at 25%.

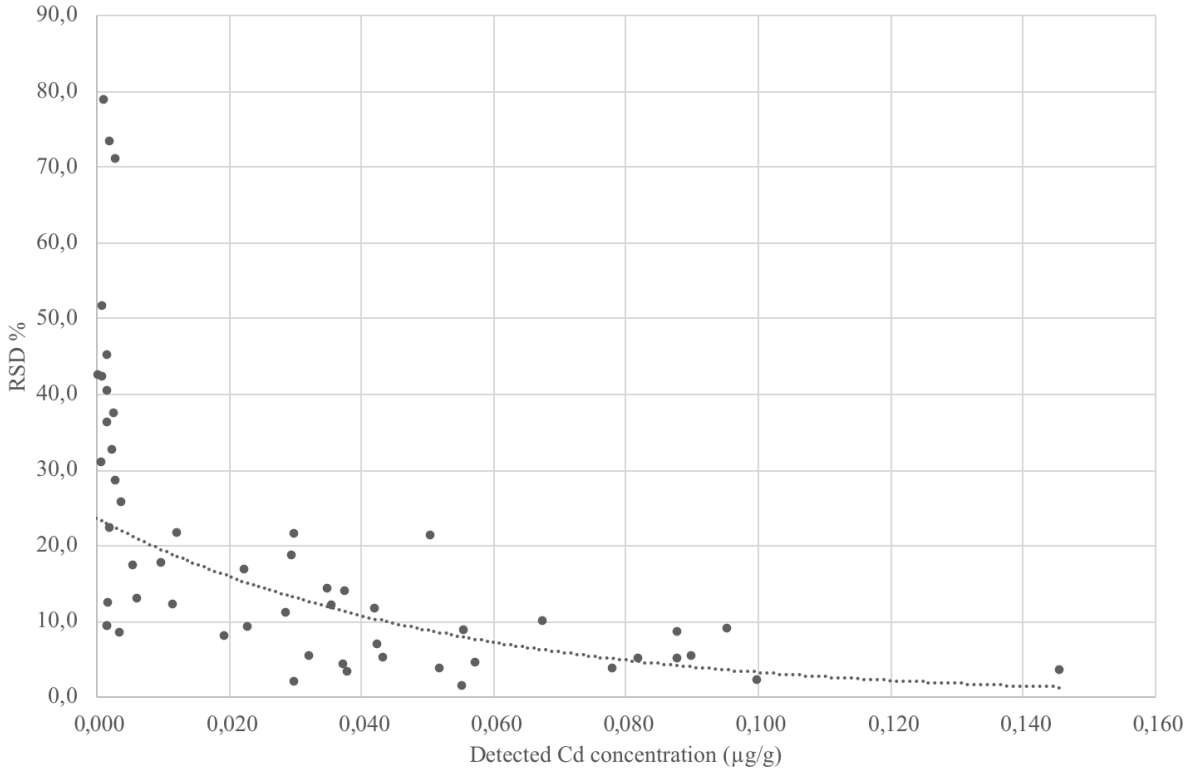


Figure 4.1: An example of QL calculations, where RSD% values are plotted against detected concentrations. This example is Cd from sample group 2.

Accuracy

The accuracy of the method was evaluated analysing certified reference material for both feathers and blood samples. Accuracy for all target trace elements for feather samples are shown in **table 4.2** and accuracy for blood samples are shown in **table 4.3**. All target elements showed satisfactory accuracy (87.0-134%), except from Hg (blood) with 166 % and As (blood) with 39.0 %.

Table 4.2: Average amount of reference material for feathers analysed was 0.12 g.

Element	Analysed values	Certified values	Analysed/Certified (%)
As	0.13	0.14	96.0 %
Cd	2.72	2.23	122 %
Co	0.15	0.15	97.0 %
Cu	4.87	5.12	95.0 %
Hg	0.03	0.02	134 %
Mn	155	136	114 %
Pb	0.85	0.97	87.0 %
Zn	44.6	43.6	102 %

Table 4.3: Average amount of reference material for blood analysed was 0.25 g.

Element	Analysed values	Certified values	Analysed/Certified (%)
As	1.77	4.6	39.0 %
Cd	0.30	0.28	108 %
Co	0.27	0.20	134 %
Cu	556	640	87.0 %
Hg	2.45	1.48	166 %
Mn	16.7	18.4	91.0 %
Pb	9.60	9.90	97.0 %
Zn	4327	4300	101 %

4.1.2 Organic analysis

Recoveries

All target analytes demonstrated absolute recoveries in the range of 17.3-29.5 %, denoting a high affinity to organic matter. When calculating recoveries based on internal standards (relative recoveries) the recoveries ranged of 66.9-114 %. All absolute and relative recoveries for spiked samples 50 ppb are given in **table 4.4.** See **section 2.7.2** for calculations.

Table 4.4: Absolute and relative recoveries (%) for the 16 target compounds in bird feathers in group 2. Spiked and matrix match samples used to calculate recoveries are 50 ppb.

	Absolute recovery (%)	**Relative recovery (%)**
DecaS	20.1	71.8
NonaFBS	24.9	89.8
TriDeFHxSA	25.0	90.5
PFOS	24.9	96.6
PFOSA	23.9	92.8
Sulf	17.3	66.9
PFPA	23.6	85.2
PFHxA	26.1	94.3
PFHeA	27.3	98.8
PFOA	27.0	97.6
PFNA	29.5	114
PFDA	26.3	102
PFUnA	25.9	101
PFDoDeA	24.7	95.9
PFTriDe	21.3	82.5
PFTetDeA	19.7	75.9

The absolute recoveries of all the TA were relatively low, indicating loss of sample during sample extraction or other preparation steps, but the relative recoveries were acceptable.

Repeatability and reproducibility

The precision of the method is demonstrated through repeatability and reproducibility calculations. The results, presented in **table 4.5**, showed satisfactory precision for the feather protocol, with the majority of TA presenting RSD < 15%. Only spiked sample (10 ppb) for DecaS had higher RSD% values (19.6%). Correction based on internal standards gave RSD < 15% for all TA.

Table 4.5: Statistics for perfluorinated compounds in triplicates of samples spiked prior to sample preparation. Concentrations in spiked samples are 10 ppb (Ssp_{10}) and 50 ppb (Ssp_{50}). Table including mean and median area, standard deviation (SD) and relative standard deviation (RSD%) for absolute and relative values.

		Absolute values				**Relative values**			
		Mean	Median	SD	RSD%	Mean	Median	SD	RSD%
DecaS	Ssp_{10}	5231	5245	376	7.18	0.98	0.94	0.10	10.3
	Ssp_{50}	24754	24882	4855	19.6	4.64	4.61	0.66	14.1
NonaFBS	Ssp_{10}	26499	26155	796	3.01	4.94	5.05	0.17	3.38
	Ssp_{50}	134049	133307	6271	4.68	25.4	26.3	2.06	8.12
TriDeFHxSA	Ssp_{10}	10982	10979	280	2.55	2.05	2.08	0.10	4.89
	Ssp_{50}	51893	51839	2008	3.87	9.85	10.1	0.77	7.82
PFOS	Ssp_{10}	12622	12937	709	5.62	1.69	1.76	0.19	11.4
	Ssp_{50}	68298	69046	2480	3.63	9.10	8.54	0.96	10.6
PFOSA	Ssp_{10}	40012	39759	884	2.21	5.34	5.32	0.39	7.35
	Ssp_{50}	195822	196003	6941	3.54	26.1	24.6	2.56	9.84
Sulf	Ssp_{10}	24743	25605	1463	5.91	3.31	3.49	0.39	11.6
	Ssp_{50}	128046	130322	5785	4.52	17.0	17.1	1.03	6.08
PFPA	Ssp_{10}	6418	6560	225	3.50	1.20	1.19	0.07	5.72
	Ssp_{50}	28927	28972	1908	6.60	5.47	5.46	0.26	4.67
PFHxA	Ssp_{10}	14105	14359	978	6.93	2.62	2.58	0.12	4.38
	Ssp_{50}	55403	55246	242	0.44	10.5	10.3	0.63	6.03
PFHeA	Ssp_{10}	8396	8368	213	2.54	1.57	1.54	0.10	6.27
	Ssp_{50}	40982	41538	1317	3.21	7.78	7.69	0.70	8.99
PFOA	Ssp_{10}	30227	30230	489	1.62	5.64	5.66	0.25	4.36
	Ssp_{50}	152509	152588	7242	4.75	28.9	29.9	2.45	8.48
PFNA	Ssp_{10}	7491	7316	293	3.91	1.00	1.00	0.09	9.39
	Ssp_{50}	37059	37218	748	2.02	4.94	4.74	0.51	10.3
PFDA	Ssp_{10}	39859	39950	683	1.71	5.32	5.53	0.39	7.33
	Ssp_{50}	194642	194275	2140	1.10	26.0	25.3	2.96	11.4
PFUnA	Ssp_{10}	44826	44815	851	1.90	5.98	6.24	0.44	7.37
	Ssp_{50}	220002	226945	10401	4.73	29.3	27.0	3.55	12.1
PFDoDeA	Ssp_{10}	90923	91988	2707	2.98	12.1	12.5	1.08	8.89
	Ssp_{50}	425926	427757	13807	3.24	56.7	53.7	5.74	10.1
PFTriDe	Ssp_{10}	32433	33055	1207	3.72	4.32	4.35	0.21	4.88
	Ssp_{50}	155648	149520	10908	7.01	20.6	20.0	1.45	7.02
PFTetDeA	Ssp_{10}	12336	12809	774	6.28	1.65	1.76	0.20	11.8
	Ssp_{50}	66003	63905	5772	8.74	8.72	8.65	0.30	3.40

Limits of detection and lower level of quantification

The limit of detection (LOD) and lower level of quantification (LLOQ) were calculated for each analyte based on the noise in the chromatograms, as described in **section 2.7.2**. LODs were in the range from 0.00006 (PFOSA) to 0.61 (PFPA), and the LLOQs were in the range of 0.0002 (PFOSA) to 1.84 (PFPA). All LODs and LLOQs are given in **table 4.6**.

Table 4.6: Limit of detection (LOD) and lower level of quantification (LLOQ) of PFCs in bird feathers given in ppb.

	LOD	LLOQ
NonaFBS	0.02	0.06
DecaS	0.16	0.48
TriDeFHxSA	0.07	0.22
Sulf	0.006	0.02
PFOS	0.22	0.07
PFOSA	0.00006	0.0002
PFPA	0.61	1.84
PFHxA	0.41	1.22
PFHeA	0.19	0.58
PFOA	0.23	0.70
PFNA	0.04	0.12
PFDA	0.07	0.21
PFUnA	0.12	0.36
PFDoDeA	0.04	0.12
PFTriDe	0.10	0.29
PFTetDeA	0.12	0.37

Matrix effects

Matrix effects (ME %) are presented for all target compounds in **table 4.7**. Ion suppression occurs when ME % < 0 and ion enhancement occurs when ME % > 0. Ion enhancement was demonstrated for most target compounds, except from the smallest PFCA (PFPA) and the non-fluorinated compound DecaS, both with negative ME % values. Quantification of PFCs based on the internal standard method together with a pre-extraction protocol, matched the calibration standard mandatory for the accomplishment of accurate measurements.

Table 4.7: Matrix effect (%) for the 16 target compounds in bird feathers in group 2.

Matrix effect (ME %)	
DecaS	-50.6
NonaFBS	97.8
TriDeFHxSA	29.9
PFOS	59.1
PFOSA	65.9
Sulf	48.2
PFPA	-8.34
PFHxA	47.7
PFHeA	77.2
PFOA	38.3
PFNA	43.7
PFDA	42.7
PFUnA	43.2
PFDoDeA	44.4
PFTriDe	43.3
PFTetDeA	1.55

Ion ratio (IR%) of all PFCs (except from PFPA with only one transition ion), retention times (RT) and relative retention (RRT) times are presented in **table C.3** and **C.4** in **Appendix C**. Standard calibration curves for all TA were obtained with concentrations from 0.05 to 50 ppb, with a coefficient of determination defined as satisfactory if $R^2 > 0.99$ for all absolute and relative areas. All calibration curves are presented in **figures C.1-C.16** in **Appendix C**.

The main issue of PFC analysis is background contamination (Arvaniti et al., 2014). The effect of the background contamination was minimized as much as possible by using materials not containing PFCs, especially fluorinated polytetrafluoroethylene (PTFE), which is a typical additive to plastic materials and a possible contaminant leaching out from containers etc. (Arvaniti et al., 2014). Reagent blank samples were regularly analysed between every tenth sample or so, to measure instrument background PFC levels.

4.2 Occurrence of target trace elements in analysed matrices

4.2.1 Trace element analysis for sample group 1

The trace element concentrations found in the bird feathers are compared to concentrations found in previous studies, as well as comparison of P1 and P10 feather trace element concentrations. **Table 4.8** and **4.9** shows the concentrations of the chosen target trace elements detected in P1 and P10 feather samples from group 1. A full table of mean concentrations of all elements detected for group 1 feather samples can be found in **table E.1** in **Appendix E.**

Table 4.8: Results from trace element analysis in feather samples from sample group 1. Results from P1 feathers (N=30) from Scopoli's shearwaters are given in µg/g.

	N (detected above LOD)	Detection frequency (%)	Mean	Median	Min.	Max.
As	28	93.3	0.09	0.08	0.07	0.16
Cd	2	6.67	0.006	0.006	0.006	0.007
Co	26	86.7	0.03	0.03	0.01	0.06
Cu	30	100	4.22	3.71	1.94	7.06
Hg	30	100	13.9	13.6	5.24	33.6
Mn	30	100	0.16	0.15	0.07	0.35
Pb	30	100	0.08	0.08	0.02	0.15
Zn	30	100	7.57	6.79	4.38	18.3

All the eight target trace elements were detected in the P1 feathers in group 1, but with a varying degree of detection frequency from Cd (with 6.67 %, N=30) to Cu, Hg, Mn, Pb and Zn (with 100 %, N=30). The rank order of mean concentrations in P1 feathers was Hg > Zn > Cu > Mn > As > Pb > Co > Cd. The highest mean concentration was observed for Hg with 13.9 µg/g (range; 5.24-33.6 µg/g) followed by Zn with 7.57 µg/g (range; 4.38-18.3 µg/g). The lowest detected mean concentration was observed for Cd with 0.006 µg/g (range; 0.006-0.007 µg/g). The concentrations determined for Hg in all the P1 feathers ranged above the level of Hg associated with adverse effects (> 5.00 µg/g) (Burger & Gochfeld, 2004). None of the determined concentrations for Cd and Pb ranged above the levels associated with adverse effects (2.00 µg/g and 4.00 µg/g, respectively) (Burger & Gochfeld, 2004). The determined concentrations for Cd are 50-200 times lower than detected by Abbasi et al. (2015). The determined concentrations for Co, Mn, Pb and Zn are lower, but closer to the values of Abbasi et al. (2015), and the determined concentrations for Cu are ranging in the similar area.

Table 4.9: Results from trace element analysis in feather samples from sample group 1. Results from P10 feathers (N=30) from Scopoli's shearwaters are given in µg/g.

	N (detected above LOD)	Detection frequency (%)	Mean	Median	Min.	Max.
As	24	80.0	0.09	0.08	0.06	0.18
Cd	11	36.7	0.008	0.007	0.005	0.01
Co	23	76.7	0.02	0.02	0.01	0.05
Cu	28	93.3	1.88	1.87	0.92	3.35
Hg	28	93.3	6.62	5.67	3.07	12.1
Mn	28	93.3	0.15	0.13	0.06	0.31
Pb	28	93.3	0.07	0.06	0.02	0.16
Zn	28	93.3	13.3	12.0	2.94	44.1

All the eight target trace elements were detected in the P10 feathers in group 1, but with a varying degree of detection frequency from Cd (with 36.7 %, N=30) to Cu, Hg, Mn, Pb and Zn (with 93.3 %, N=30). The rank order of mean concentrations in P10 feathers was Zn > Hg > Cu > Mn > As > Pb > Co > Cd. The highest mean concentration was observed for Zn with mean concentration 13.3 µg/g (range; 2.94-44.1 µg/g) followed by Hg with 6.62 µg/g (range; 5.673.07-12.1 µg/g), which is above the level of Hg associated with adverse effects (> 5.00 µg/g) (Burger & Gochfeld, 2004). The lowest detected mean concentration was observed for Cd with 0.008 µg/g (range; 0.005-0.01 µg/g). Similar to the P1 feathers, none of the determined concentrations for Cd and Pb ranged above the levels associated with adverse effects (2.00 µg/g and 4.00 µg/g, respectively) (Burger & Gochfeld, 2004). The determined concentrations for Cd were 50-150 times lower than detected by Abbasi et al. (2015). Determined concentrations for Co, Mn, Pb and Zn were also lower, but not as much as for Cd, and determined concentration for Cu was similar as for Abbasi et al. (2015).

Table 4.10 shows the concentrations of the chosen target trace elements detected in blood samples from group 1. A full table of mean concentrations of all elements detected for group 1 blood samples can be found in **table E.4** in **Appendix E.** All the eight target trace elements were detected in the blood samples in group 1, but with a varying degree of detection frequency from Pb (with 56.1 % total detection frequency, N=57) to As, Co, Cu, Mn, and Zn (with 93.0 % total detection frequency, N=57). The highest mean concentration was observed for Zn with 23.0 µg/g (range; 0.58-57.1 µg/g, ethyl alc.) followed by Hg with 4.24 µg/g (range; 2.06-9.17 µg/g, ethyl alc.). The lowest detected mean concentration was observed for Cd with 0.005 µg/g (range; 0.0006-0.02 µg/g, heparin). Due to varying condition of the blood stored in heparin, concentrations from the blood samples in stored in ethyl alcohol are considered when evaluating the results.

Table 4.10: Results from trace element analysis in blood samples from sample group 1. Results from 29 samples in ethyl alcohol and 28 samples in heparin from Scopoli's shearwater are given in µg/g.

	N (detected above LOD)	Detection frequency (%)	Mean	Median	Min.	Max.
Blood samples stored in ethyl alcohol (n = 29)						
As	29	100	4.00	3.91	2.12	8.26
Cd	29	100	0.02	0.01	0.007	0.04
Co	29	100	0.07	0.07	0.03	0.15
Cu	29	100	0.99	0.98	0.77	1.27
Hg	29	100	4.24	3.97	2.06	9.17
Mn	29	100	0.12	0.11	0.06	0.18
Pb	20	69.0	0.02	0.007	0.0005	0.15
Zn	29	100	23.0	22.4	19.1	32.5
Blood samples stored in Heparin (n = 28)			**Mean**	**Median**	**Min.**	**Max.**
As	24	85.7	1.59	0.94	0.20	9.95
Cd	23	82.1	0.005	0.003	0.0006	0.02
Co	24	85.7	0.02	0.02	0.001	0.09
Cu	24	85.7	0.42	0.39	0.005	1.10
Hg	19	67.9	1.61	0.78	0.23	9.70
Mn	24	85.7	0.10	0.06	0.01	0.44
Pb	12	42.9	0.01	0.008	0.0004	0.05
Zn	24	85.7	12.7	6.11	0.58	57.1

A comparative table with all mean values from the detected target trace elements is made to evaluate the differences in group 1, presented in **table 4.11**.

Table 4.11: Comparative table for detected mean values in feathers and blood from sample group 1 given in µg/g.

	Tissue	As	Cd	Co	Cu	Hg	Mn	Pb	Zn
P1 (µg/g)	Feather	0.09	0.006	0.03[a]	4.22[b]	13.9[b]	0.16	0.08[a]	7.57[a]
P10 (µg/g) *	Feather	0.09	0.008	0.02[a]	1.88[b]	6.62[b]	0.15	0.07[a]	13.3[a]
Ethyl alcohol (µg/g) *	Blood	4.00	0.02	0.07	0.99	4.24	0.12	0.02	23.0
Heparin (µg/g)	Blood	1.59	0.005	0.02	0.42	1.60	0.10	0.01	12.7

Feather groups: [a)] Significantly different ($p < 0.05$), [b)] Significantly different ($p << 0.05$)
Blood in ethyl alcohol compared to P10 feathers: *Significantly different ($p < 0.05$)

All groups of data for each target element in feathers were tested for normal distribution by using a Shapiro-Wilk test ($p > 0.05$ indicating normal distribution of the data), and further testing for significance between the two groups (P1 and P10) for each target element were done using a t-test or f-test ($p < 0.05$ indicated significant difference between two groups).

For As, both P1 and P10 datasets were found to be not normally distributed (p=0.003 and p=0.0002, respectively), and therefore a f-test were applied testing for significance between the two groups. The p-value was found to be 0.35, concluding that there is no significant difference between P1 and P10 feathers regarding As concentrations. For Cd, the P1 dataset did not include enough data to test for normal distribution, while P10 was found to be normally distributed (p=0.17). A t-test was performed concluding that there was no significant difference between P1 and P10 feathers regarding Cd concentrations (p=0.11). For Co, the data for P1 feathers were found to be normally distributed (p=0.23), and the data for P10 feathers was not (p=0.001). T-test concluded that the P1 and P10 concentrations was significantly different (p=0.01). For Cu, both P1 and P10 datasets were found to be normally distributed (p=0.05 and p=0.06, respectively). A t-test also concluded that the datasets were clearly significantly different ($p=2.5*10^{-9}$). The determined mean concentration for Cu is 2.2 times higher in P1 feathers than in P10 feathers, which can imply a greater availability of the essential trace element Cu in breeding areas for Scopoli's shearwaters.

For Hg, the datasets of P1 and P10 showed no normal distribution (p=0.005 and 0.02, respectively). The two datasets were compared by a t-test, confirming a clear significant difference ($p=4.1*10^{-6}$). As described by Ramos et al. (2009), the P1 feather concentrations can describe conditions in the breeding areas for the Scopoli's shearwaters (Mediterranean areas), and the P10 feather described the wintering areas (west African or Atlantic coast areas). This study confirm earlier findings that Hg concentrations are found at higher levels in the Mediterranean areas, compared to the Atlantic areas (Renzoni et al., 1986). The determined mean concentration for Hg is 2.1 times higher in the P1 feathers compared to the P10 feathers, implying a greater availability of the toxic trace element Hg in the breeding areas for Scopoli's shearwaters.

For Mn, the data for P1 feathers were found to be normally distributed (p=0.06), and the data for P10 was not (p=0.01). A t-test performed concluded with no significant difference between the groups (p=0.35). For Pb, the data for both P1 and P10 feathers were found normally distributed (p=0.14 and p=0.05, respectively). A t-test concluded that the two datasets were significantly different (p=0.03). For Zn, both P1 and P10 datasets were found to be not normally distributed ($p=4.3*10^{-5}$ and p=0.0009, respectively). A t-test concluded that the datasets were significantly different (p=0.002). The determined mean concentration for Zn is 1.8 times higher

in the P10 feathers, compared to the P1 feathers, which can imply a greater availability of the essential trace element Zn in the wintering areas for Scopoli's shearwaters.
The determined mean concentrations in blood samples (in ethyl alcohol) were tested for significance compared to the P10 feathers for each target element using a t-test. All tests showed significantly different concentrations in blood and P10 feathers for Scopoli's shearwaters.

The PCA analysis demonstrates a fingerprint of the samples, where the samples groups or separates based on variations in the samples. The samples are grouped together based on the same relationship between the components analysed. The PCA analysis showed that the trace elements Cu, Mn and Pb were associated with the first axis which explained 40.0 % of the variance, while Hg and Zn were more inclined towards the second axis, which explained 25.9 % of the variance in the data (**figure 4.2**).

In the PCA comparing P1 and P10 sample groups, there is a clear separation of P1 and P10 samples. It can therefore be concluded that the P1 and P10 feathers are different in composition. This supports the earlier findings by Ramos et al. (2009) that the feathers grown in different areas will have different trace element composition. The trend shows a higher concentration of the nonessential trace elements Hg and Pb in the breeding areas around the Mediterranean Sea. Also, higher concentrations of the essential trace elements Cu and Co are found here. A clear trend is that the concentration of the essential trace element Zn is found in higher concentrations in the wintering areas around the west African or Atlantic coast. A correlation plot for the trace elements detected in group 1, both feathers and blood can be found in **figure F.1** and **F.2** in **Appendix F**.

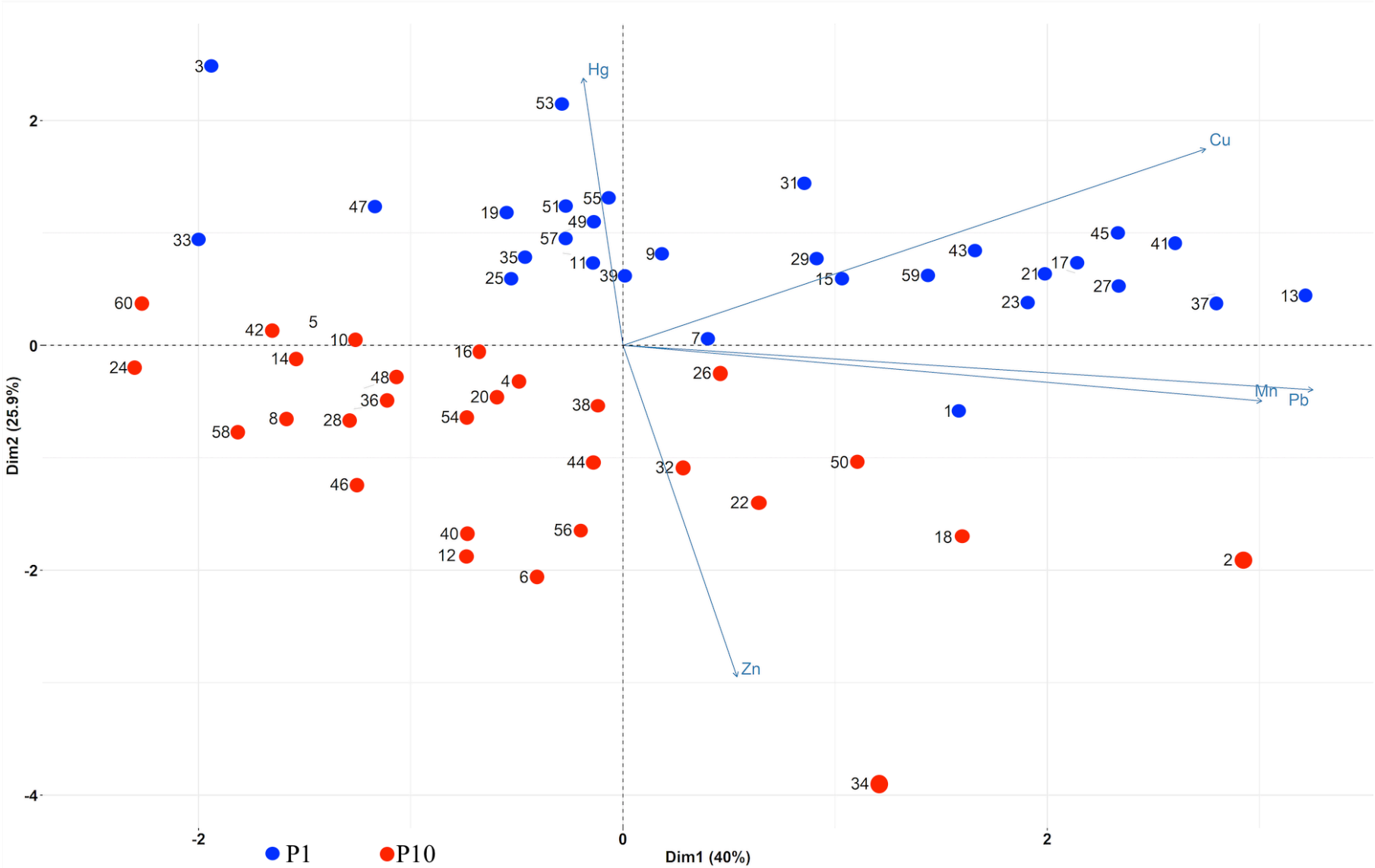


Figure 4.2: Results from PCA biplot for group 1. X-axis shows component 1 and y-axis shows component 2. The arrows indicate the distribution of the tested metals. P1 feathers are represented in blue and P10 feathers are represented in red. (The numbers plotted represent the sample number found in **Appendix A**).

4.2.2 Trace element analysis for sample group 2

Table 4.12 shows the concentrations of the chosen target trace elements detected in feather samples from group 2. A full table of mean concentrations of all elements detected for group 2 feather samples can be found in **table E.2** in **Appendix E.**

Table 4.12: Results from trace element analysis in group 2 feather samples, obtained from six passerine bird species. Results from P10 samples (N=38) are given in µg/g.

	N (detected above LOD)	Detection frequency (%)	Mean	Median	Min.	Max.
As	34	89,5	0.10	0.08	0.05	0.19
Cd	9	23.7	0.006	0.005	0.003	0.008
Co	38	100	0.19	0.15	0.05	0.59
Cu	38	100	5.65	5.43	2.92	10.6
Hg	38	100	0.49	0.26	0.02	4.27
Mn	38	100	3.93	3.20	0.10	11.6
Pb	38	100	0.21	0.17	0.04	0.87
Zn	38	100	61.0	59.2	36.0	129

All the eight target trace elements were detected in the P10 feathers in group 2, but with a varying degree of detection frequency from Cd (with 23.7%, N=38) to Cu, Co, Hg, Mn, Pb and Zn (with 100 %, N=38). The highest mean concentration was observed for Zn with 69.4 µg/g (range; 36.0-129 µg/g). The lowest detected mean concentration was observed for Cd with 0.004 µg/g (range; 0.003-0.008 µg/g). None of the concentrations determined for Cd, Hg and Pb ranged above the level associated with adverse effects (> 2.00 µg/g, 5.00 µg/g and 4.00 µg/g, respectively) (Burger & Gochfeld, 2004). The determined concentrations for Cd are 100-200 times lower than detected by Abbasi et al. (2015). The determined concentrations for Co and Pb are lower, but closer to the values of Abbasi et al. (2015), and the determined concentrations for Cu, Mn and Zn are ranging in the similar area.

A comparative table with all mean values from the detected target trace elements is made to evaluate the differences in group 2, presented in **table 4.13**. The 6 species compared are Wood warbler, Sedge warbler, Garden warbler, Great reed warbler, Sand marti and Spotted flycatcher.

Table 4.13: Comparative table for detected mean values of target elements in group 2 feather samples given in µg/g, sorted after the six passerine species.

	Wood warbler	Sedge warbler	Garden warbler	Great reed warbler	Sand martin	Spotted flycatcher
N=	13	6	12	1	3	3
As	0.12[a]	0.10	0.07[a]	0.10	0.10	0.07
Cd	0.006	-	-	0.008	0.003	0.004
Co	0.27[a,b,c]	0.14[a]	0.15[b]	0.23	0.16	0.09[c]
Cu	5.35	4.18[a,b]	6.59[b,d]	8.22	7.64[a,c]	3.29[c,d]
Hg	0.30	0.80[a]	0.14[a]	4.27	0.86	0.43
Mn	4.19	6.80[a,b]	2.97[a]	4.83	1.84[b]	2.66
Pb	0.33[a,b]	0.11[a]	0.16[b]	0.17	0.14	0.13
Zn	69.4	57.3	56.6	45.8	51.7	64.0

[a,b,c,d)] Significantly different in pairs ($p < 0.05$)

All groups of data for each target element were tested for significance between the six groups (6 species) using ANOVA, and Tukey HSD post-hoc test for testing which pairs of groups with significant difference ($p < 0.05$). The groups were tested in pairs for each target element. It is important to note that the dataset of Great reed warbler, Sand martin and Spotted flycatcher contains a low number of data.

For As, a significant difference in the dataset between Wood warbler and Garden warbler feathers was found ($p=0.04$). For Cd, no significant differences were found between any of the data. For Co, significant differences were found between the dataset of Wood warbler and three different groups, Sedge warbler ($p=0.05$), Garden warbler ($p=0.02$) and Spotted flycatcher ($p=0.04$). For Cu, a significant difference was found between four groups of data. The Sedge warbler was significantly different from Garden warbler ($p=0.005$) and Sand martin ($p=0.005$), and the Spotted flycatcher was significantly different from Garden warbler ($p=0.003$) and Sand martin ($p=0.002$). For Hg, a significant difference was found for the Sedge warbler and Garden warbler ($p=0.05$). For Mn, significant differences were found between Sedge warbler and two different groups, Garden warbler ($p=0.007$) and Sand martin ($p=0.02$). For Pb, significant differences were found between Wood warbler and two different groups, Sedge warbler ($p=0.03$) and Garden warbler ($p=0.05$). For Zn, no significant differences were found between any of the data.

The Garden warbler had the most significant different mean concentrations (7) compared to the other species. For the nonessential elements As, Hg and Pb the concentrations were all the lower in the pair compared, and Cd was not detected. This indicated a total lower toxicity than other

species, and none of the nonessential element concentrations ranged above levels associated with adverse effects (Burger & Gochfeld, 2004). The essential elements Co and Mn were lower compared to the data significantly different, and Zn had no significant difference. The essential element Cu was the only trace element significantly higher in the compared groups for the garden warbler.

The PCA analysis showed that the trace elements Co, Mn and Pb were associated with the first axis which explained 34.4 % of the variance, while Cu, Hg and Zn were more inclined towards the second axis, which explained 21.7 % of the variance in the data (**figure 4.3**). The sample groups of Great reed warbler, Sand martin and Spotted flycatcher is separated in the PCA biplot, but since the sample groups are small (N=1,N=3 and N=3, respectively) no conclusion can be drawn from this. There is a small trend that the samples from Wood warbler are grouped different than Garden warbler, but with no clear separation there can not be drawn any conclusion regarding differences in trace element composition in the feathers from these two species. In general, there seem to be a similar composition of trace elements in several of the species.

A correlation plot for the trace elements detected in group 2 can be found in **figure F.3** in **Appendix F**.

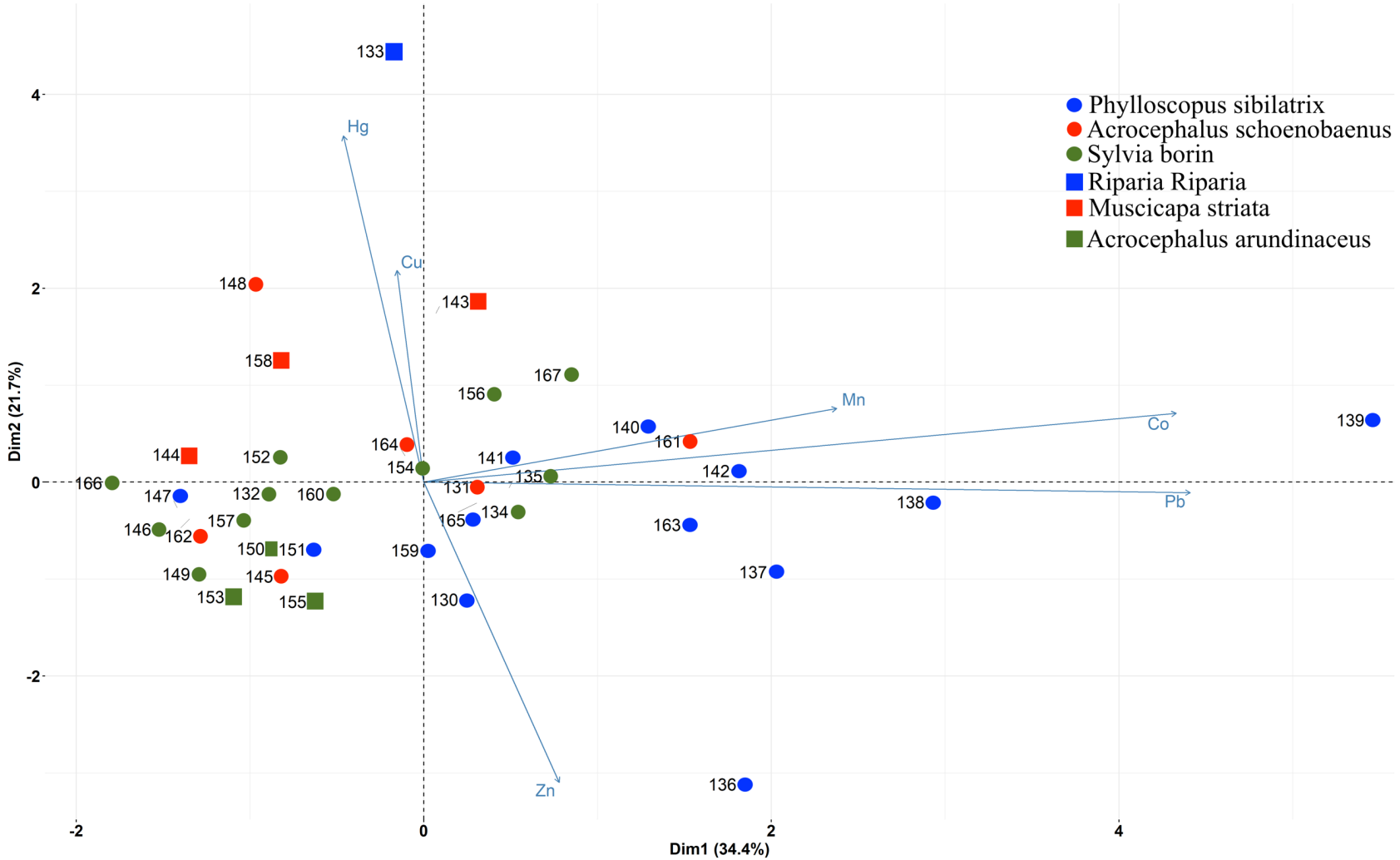


Figure 4.3: Results from PCA biplot for group 2. X-axis shows component 1 and y-axis shows component 2. The arrows indicate the distribution of the tested elements. Feathers from the different species are represented with different colour coding. (The numbers plotted represent the sample number found in **Appendix A**). Wood warbler (*Phylloscopus sibilatrix*), Sedge warbler (*Acrocephalus schoenobaenus*), Garden warbler (*Sylvia borin*), Great reed warbler (*Riparia riparia*), Sand martin (*Muscicapa striata)* and Spotted flycatcher (*Acrocephalus arundinaceus*).

4.2.3 Trace element analysis for sample group 3

Table 4.14 shows the concentrations of the chosen target trace elements detected in feather samples from group 3. A full table of mean concentrations of all elements detected for group 1 blood samples can be found in **table E.3** in **Appendix E.**

Table 4.14: Results from trace element analysis in feather samples from sample group 3. Results from feathers from White-tailed Eagles (N=69) are given in µg/g.

	N (detected out of 69)	Detection frequency (%)	Mean	Median	Min.	Max.
As	69	100	0.04*	0.03	0.02	0.08
Cd	20	29.0	0.0009*	0.0008	0.0007	0.002
Co	67	97.1	0.004*	0.002	0.001	0.05
Cu	69	100	1.78	1.77	0.94	3.01
Hg	69	100	3.30*	2.91	1.76	6.51
Mn	69	100	0.11	0.04	0.01	3.58
Pb	69	100	0.06	0.01	0.003	1.18
Zn	69	100	8.55*	8.13	4.65	13.5

*Significantly different from dataset of P10 feathers in group 1 ($p < 0.05$).

None of the concentrations determined for Cd and Pb ranged above the level associated with adverse effects (> 2.00 µg/g and 4.00 µg/g, respectively). The highest determined concentration for Hg (6.51 µg/g) did range above this level, set to be > 5.00 µg/g for Hg, but the mean concentration were below this level (3.30 µg/g) (Burger & Gochfeld, 2004). All groups of data for each target element were tested for normal distribution by using a Shapiro-Wilk test ($p > 0.05$ indicating normal distribution of the data), and compared to the P10 feather for Scopoli's shearwaters in group 1, to look for significant differences between species in the two different areas (t-test with 95% confidence interval, $p < 0.05$). The data from group 3 were only normally distributed for Cu ($p=0.51$) and Zn ($p=0.15$). The t-test for the groups showed significant differences between the P10 feather for Scopoli's shearwaters and the white-tailed eagles for As ($p=1*10^{-8}$), Cd ($p=2.7*10^{-6}$), Co ($p=2.8*10^{-9}$), Hg ($p=9*10^{-7}$), and Zn ($p=0.006$). The determined mean concentrations for the White-tailed eagles were significantly lower for As, Cd, Co and Hg, and significantly higher for Zn.

The PCA analysis showed that the trace elements Cu, Hg and Zn were associated with the first axis which explained 33.3 % of the variance, while Mn were more inclined towards the second axis, which explained 29.7 % of the variance in the data (**figure 4.4**). A correlation plot for the trace elements detected in group 3 can be found in **figure F.4** in **Appendix F**.

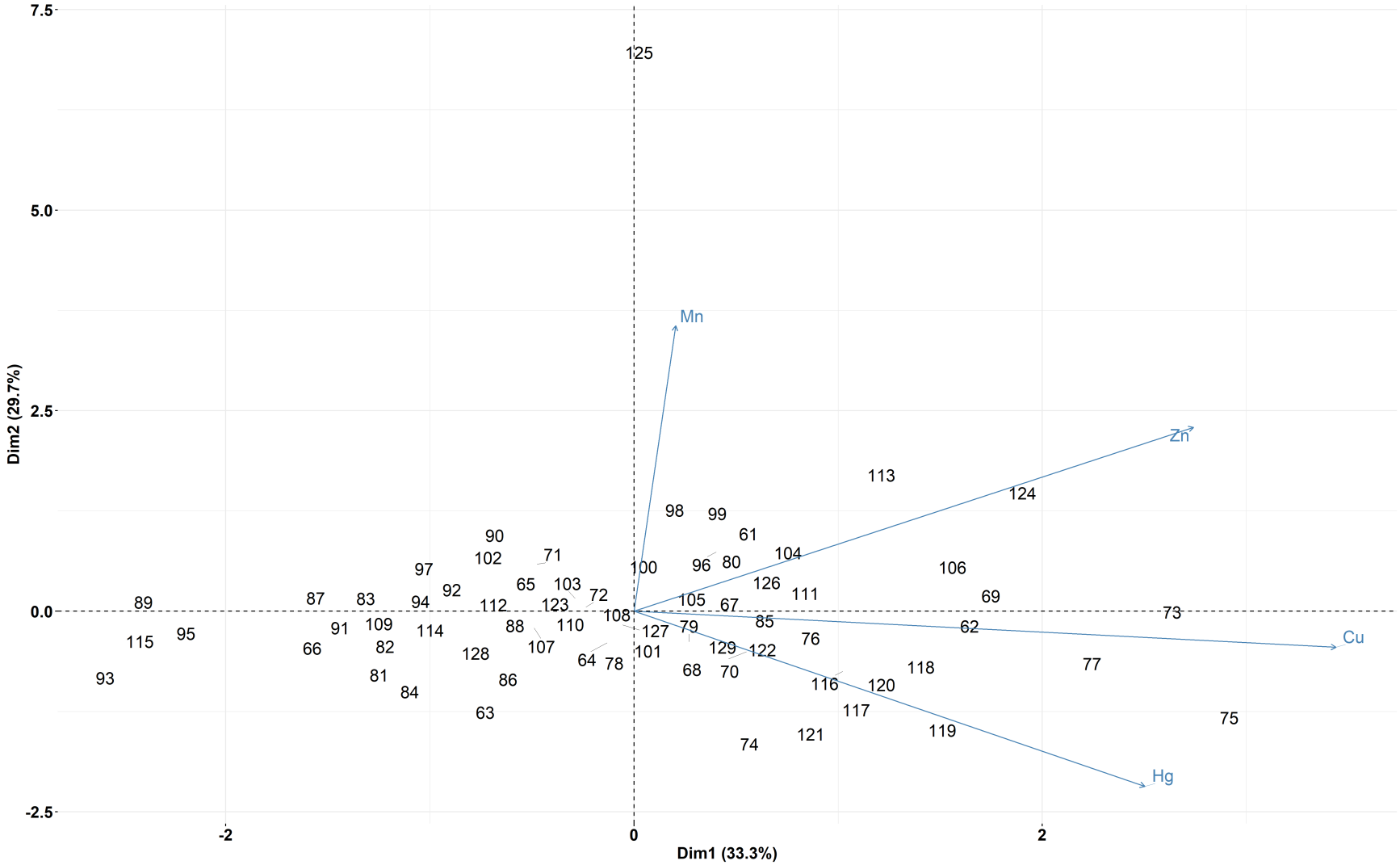


Figure 4.4: Results from PCA biplot for group 3. X-axis shows component 1 and y-axis shows component 2. The arrows indicate the distribution of the tested elements. (The numbers plotted represent the sample number found in **Appendix A**).

4.2.4 Common discussion for group 1, 2 and 3

One of the aims of this study was to look at differences in trace element concentrations in P1 and P10 feathers from Scopoli's shearwaters. The P1 feather is considered to grow in the breeding area, representing the conditions for trace element intake and exposure deposited in the feathers during growth (Dmowski, 1999; Solonen & Lodenius, 1990). The breeding areas for Scopoli's shearwaters are mainly in the Mediterranean area. The P10 feather is considered to grow in the wintering area, mainly located in the south Atlantic and western Africa (Oliveira et al., 2019). Previous studies have shown that due to high Hg resources in the Mediterranean areas and good conditions for natural higher concentrations, fish and birds in the area are found to be more contaminated when compared to Atlantic fish and birds (Bernhard, 2016; Renzoni et al., 1986; Renzoni et al., 1998). The findings in this study are confirming this, as the levels of Hg found in the P1 feathers were significantly higher than for concentrations found in the P10 feather. All the determined concentrations in P1 feathers ranged above the level associated with causing adverse effects (> 5.00 µg/g) (Burger & Gochfeld, 2004; Eisler, 1987), possibly causing effects like spinal cord degeneration, and other physical and reproductive toxic effects (Scheuhammer, 1987). It should be taken in count that metals in feathers are not metabolically active when considering the toxicity (Lodenius & Solonen, 2013). Some of the Hg concentrations determined in the P10 feather also ranged above this level, including the mean concentration, indicating bioavailable Hg in both wintering and breeding areas. The Hg blood concentration was found to be significantly lower than in the P10 feathers, indicating that feathers can give a picture of contamination for a specific time or location, as the blood gives the current contamination (Abbasi et al., 2015; Leonzio et al., 2009).

When considering the determined concentration for Cd in both P1 and P10 feathers, all concentrations were quite low, and the total detection frequency was low (21.7 %) due to many concentrations below the quantification limit. The concentrations in the two groups were not significantly different. Previous studies suggested that feathers were not the best matrix for Cd analysis, as the main proportion of the toxic trace element were found in the kidneys and liver of peregrine falcon (Ek et al., 2004; Goede & de Voogt, 1985). The element is not considered as one with high affinity to keratin in feathers (Dmowski, 1999), even though it is considered to be mainly an internal contaminant (Ek et al., 2004). The concentrations determined in this study are similar to or lower than what is found in previous studies (Abbasi et al., 2015; Dauwe et al., 2000).

The As and Pb concentrations determined in both the feathers and blood samples were low and lower than determined in previous studies (Abbasi et al., 2015; Dauwe et al., 2000; Voulgaris et al., 2019). All determined Pb concentrations ranged below the level associated with adverse effects in birds (> 2.00 µg/g) (Burger & Gochfeld, 2004). Previous studies also Pb hard to detect in feathers, as it often represents exogenous contamination (Goede & de Voogt, 1985; Jaspers et al., 2004; Leonzio et al., 2009). Since the concentrations in this study is found to be very low for Pb, the washing procedure may be considered successful, but further investigation should confirm this, as well as Pb analysis of different tissues may be relevant.

The essential elements Co, Cu, Mn and Zn were successfully determined in the feathers, with good detection frequencies 96.7 % (Cu, Mn and Zn) and 81.7 % (Co), and in the blood samples (91.2 %) for all the essential elements. The determined concentrations ranged in the similar area as previous studies investigating feathers, both for the P1 and P10 feathers (Abbasi et al., 2015; Voulgaris et al., 2019). For Co and Zn, the determined blood concentrations were significantly higher than in the P10 feathers. For Cu and Mn, the blood concentration were significantly lower than in the feather, indicating an accumulation in the feather keratin structure as the feathers are formed, as stated for Cu in previous studies (Dmowski, 1999).

For the six passerine species studied in group 2, the P10 feather analysed can represent the contamination conditions in the wintering area, since the full moult takes place there for all the six passerine species analysed. The exact location for the wintering areas of the six species is varying to some degree, but they all winter on the African continent (Barboutis et al., 2011; Bibby & Green, 1981; Del Hoyo et al., 2006; Horns et al., 2016; Szép et al., 2003; B. Taylor et al., 2016). For the nonessential elements Cd, Hg and Pb, none of the determined concentration were found above the level associated with adverse effects (Burger & Gochfeld, 2004; Eisler, 1987). All concentrations, including As were determined to be lower than earlier concentrations found in birds by (Abbasi et al., 2015; Dauwe et al., 2000). All the essential elements (Co, Cu, Mn and Zn) were determined in all feathers (detection frequency 100 %), and the concentrations found are similar to concentrations found in birds in previous studies (Abbasi et al., 2015; Dauwe et al., 2000). A study performed on Sand martin found the concentrations of Cd, Co, Mn and Pb to be higher in feathers grown in the wintering areas, and the concentrations of Zn where highest in feathers grown in the breeding areas. The concentrations determined for Cd and Pb in this study were low and representing contamination in the wintering areas, possibly indicating even lower concentrations in the breeding areas, but further investigation must be performed.

For group 3, all determined concentrations were determined at low levels, except from Hg were a few samples had levels above > 5.00 µg/g, associated with causing adverse effects (Burger & Gochfeld, 2004). The trace element concentrations found in group 3 feathers from White-tailed eagles were compared to the findings in group 1 for P10 feathers from Scopoli's shearwaters. These two species represent very different environments, as the feathers represent contamination in Artic and sub tropic areas (White-tailed eagles (Hailer et al., 2007)) and south-western areas of Africa (Wintering areas of Scopoli's shearwaters). The trace elements As, Cd, Hg, Co and Zn were found at significantly lower concentrations in White-tailed eagles, indicating that the wintering areas in Africa and the natural upwelling system there provide more of the essential (Co and Zn) and the nonessential trace elements (As, Cd and Hg) to the environment. The Hg concentrations determined for the P1 feathers, representing the breeding areas in the Mediterranean for Scopoli's shearwaters were even more different (higher) than for the P10 concentrations. The concentrations for Co and Zn were found in significant lower concentrations for the P1 feathers (group 1), indicating a more similar pattern with the White-tailed eagle concentrations, but no statistical tests were performed to confirm this. No clear correlations were found for group 3 (see **figure F.4** in **Appendix F**). Previous studies have analysed differences in location (Steigen vs. Smøla) and sampling year (2015 vs. 2016) for these exact sampled birds, but in blood samples (Marcinekova, 2019).

4.3 Occurrence of PFCs in analysed matrices

4.3.1 Organic analysis for sample group 2

The method applied for PFC analysis were successfully tested, and all 16 target compounds was detected in the feather samples, with a varying degree of detection frequency. The highest concentration in the feathers were determined for PFPA (597 ng/g), but this high level stood out compared to all other detected concentrations, which had maximum detected values ranging from 0.28 ng/g (Sulf) to 49.3 ng/g (DecaS). **Table 4.15** shows the mean, median, minimum and maximum concentrations of the chosen target PFCs detected in feather samples from group 2. A full table of all detected concentrations can be found in **table E.5** and **E.6** in **Appendix E.**

Table 4.15: Results from 16 target PFC analysis in group 2 feather samples (N=38+2 replicates).

	N (detected out of" are given per	Detection frequency (%)	Mean	Median	Min.	Max.
DecaS	38	95.0	24.9	22.9	3.91	49.3
NonaFBS	38	95.0	3.19	2.87	0.74	9.58
TriDeFHxSA	13	32.5	4.96	2.86	0.80	27.5
Sulf	13	32.5	0.12	0.11	0.05	0.28
PFOS	40	100	18.1	14.4	2.67	66.5
PFOSA	38	95.0	0.23	0.05	0.002	4.20
PFPA	36	90.0	41.9	15.2	2.30	597
PFHxA	35	87.5	11.9	10.2	2.41	29.4
PFHeA	12	30.0	2.99	2.44	1.15	8.37
PFOA	19	47.5	4.78	4.81	1.50	8.36
PFNA	33	82.5	2.11	1.76	0.22	8.21
PFDA	21	52.5	1.89	1.22	0.27	7.31
PFUnA	1	2.50	5.66	5.66	5.66	5.66
PFDoDeA	34	85.0	1.28	0.88	0.39	6.45
PFTriDe	6	15.0	2.22	2.01	1.05	3.99
PFTetDeA	1	2.50	4.70	4.70	4.70	4.70

The detection frequency varied from PFUnA and PFTetDeA (with 2.50 %, N=40) to PFOS (with 100 %, N=40). The highest mean concentration was observed for PFPA with 41.9 ng/g (range; 2.30-597 ng/g), also including the highest maximum concentration measured. The lowest detected mean concentration was observed for Sulf with 0.12 ng/g (range; 0.05-0.28 ng/g).

Table 4.16 shows the concentrations of the chosen target PFCs detected external to the feather samples from group 2. A full table of all detected concentrations can be found in **table E.5** and **E.6** in **Appendix E.**

Table 4.16: Results from PFC analysis on exterior contamination on group 2 feather samples given in ng/g (N=38).

	N (detected out of" are given per	Detection frequency (%)	Mean	Median	Min.	Max.
DecaS	13	34.2	7.41*	4.14*	800	28.3*
NonaFBS	5	13.2	28.7	22.1	11.7	53.3
TriDeFHxSA	4	10.5	106	55.5	31.6	283
Sulf	24	63.2	30.5	7.83	2.11	303
PFOS	26	68.4	1.21*	563	101	8.66*
PFOSA	18	47.3	27.2	6.84	0.25	157
PFPA	7	18.4	3.03*	2.68*	140	5.82*
PFHxA	4	10.5	517	474	212	909
PFHeA	3	7.89	310	77.8	56.0	795
PFOA	9	23.7	552	357	181	1.70*
PFNA	22	57.9	182	133	7.87	1.12*
PFDA	6	15.8	101	57.6	11.2	341
PFUnA	13	34.2	369	148	30.1	2.47*
PFDoDeA	16	42.1	185	132	24.8	1.14*
PFTriDe	16	42.1	320	172	24.7	2.22*
PFTetDeA	0	-	-	-	-	-

*Concentrations given in µg/g

The detection frequency varied from PFTetDeA (with 0 %, N=40) to PFOS (with 68.4 %, N=40). The highest mean concentration was observed for DecaS with 7407 ng/g (range; 800-28329 ng/g). The lowest detected mean concentration was observed for PFOSA with 27.2 ng/g (range; 0.25-157 ng/g). The highest maximum concentration was observed for PFOS with 8657 ng/g.

A comparative table with all mean values from the detected target trace elements is made to evaluate the differences in PFC concentrations in group 2, presented in **table 4.17**. The 6 species compared are Wood warbler, Sedge warbler, Garden warbler, Great reed warbler, Sand martin and Spotted flycatcher and also values for external contamination for each specie are included.

Table 4.17: Comparative table for detected mean values in ng/g of 16 PFCs in feathers samples from six species in group 2. Measured PFCs in feathers are marked as "int." (internal) and measured PFCs from external contamination on the feathers are marked as "ext." (external).

	Wood warbler		**Sedge warbler**		**Garden warbler**		**Great reed warbler**		**Sand martin**		**Spotted flycatcher**	
	Int.	Ext.	Int.	Ext.	Int.	Ext.	Int.	Ext.	Int.	Ext.	Int.	Ext.
DecaS	29.8[a]	2471	22.9	7878	18.1[a,b]	6763	49.3[b]	4676	29.9	17859	17.5	2500
NonaFBS	4.05[a]	17.1	2.79[b]	53.3	2.06[a,c]	16.9	2.61	39.1	7.26[b,c,d]	-	0.98[d]	-
TriDeFHxSA	6.99	31.6	2.38	283	1.78	55.5	5.38	-	-	-	-	-
Sulf	0.15	25.9	0.12	103	0.15	12.1	-	-	0.07	4.07	0.06	27.2
PFOS	23.6	1206	16.8	2654	9.07	525	11.0	-	34.9	492	15.2	1118
PFOSA	0.43	37.6	0.23	6.41	0.08	18.9	0.29	-	0.01	7.51	0.05	2.81
PFPA	61.4	138	17.4	2675	10.7	3858	5.48	-	178	3420	9.38	-
PFHxA	15.9[a]	-	10.8	-	7.43[a]	376	8.56	909	17.0	-	7.27	409
PFHeA	-	66.9	-	795	3.37	-	2.53	-	-	-	1.54	-
PFOA	5.40	586	6.69[a,b]	1698	3.40[a]	345	2.10	533	4.87	-	1.50[b]	181
PFNA	3.15[a]	146	2.41	507	0.82[a]	78.0	0.67	-	2.21	196	1.02	182
PFDA	2.59	183	1.88	91.3	1.09	44.6	0.99	-	1.02	-	0.84	11.2
PFUnA	5.66	180	-	2472	-	133	-	-	-	-	-	284
PFDoDeA	1.80	237	1.57	-	0.81	108	0.61	-	0.61	-	0.50	87.3
PFTriDe	2.57	408	1.99	186	1.05	126	-	-	-	65.2	-	-
PFTetDeA	4.70	-	-	-	-	-	-	-	-	-	-	-

[a,b,c)] Pairs significantly different ($p < 0.05$)

All groups of data for each compound (internal concentration) were tested for significance between the any of the six groups (six species) using ANOVA, and Tukey HSD post-hoc test for testing which pairs of groups with significant difference ($p < 0.05$). For PFPA, PFHeA, TriDeFHxSA, PFOSA, PFOS, PFDA, Sulf, PFDoDeA and PFTriDe no significant differences in the groups were found. PFUnA and PFTetDeA was not tested due to a low number of data.

For **DecaS**, significant different mean concentrations were found for Garden warbler compared to two groups, Wood warbler ($p=0.04$) and Great reed warbler ($p=0.03$). For **NonaFBS**, significant different mean concentrations were found for four groups. The dataset of Wood warbler was significantly different from Garden Warbler ($p=0.01$). The dataset of Sand martin was significantly different from three other groups, Sedge warbler ($p=0.007$), Garden warbler ($p=0.0005$) and Spotted flycatcher ($p=0.002$) where Sand martin had the higher mean concentrations in all comparisons. For **PFHxA**, a significant difference was found between the datasets of Wood warbler and Garden warbler ($p=0.006$). For **PFOA**, significant different datasets were found for Sedge warbler compared to two groups, Garden warbler ($p=0.03$) and

Spotted flycatcher (p=0.04). For **PFNA**, a significant difference was found between the datasets for Wood warbler and Garden warbler (p=0.05).

The six passerine birds investigated for PFCs, all undergo complete feather moult in the wintering areas, which is in the African continent. The results from this study can therefore be stated to represent the pollution from PFCs in this area. The results in this study follows the trend found by Kannan et al. (2002) that PFOS is the most predominant PFC, as it was found in all feathers (det. frequency 100 %). It was also the most common PFC detected in the hexane samples where external contamination was analysed (det. frequency 68.4 %). PFOS is the only PFC on the list of the regulated POPs by the Stockholm convention where it was included in 2009 (Briels, 2019).

The concentrations found in this study can be compared to several previous studies, but none studies were found analysing the same species, to my far knowledge. When comparing the result with the study by Løseth et al. (2019) the concentrations for PFOSA, PFOS and PFTriDe range in the similar area. For the compounds PFHxA, PFOA, PFNA, PFDA, PFUnA and PFDoDeA the concentrations determined in this study are ranging above what is found by Løseth et al. (2019). PFHxA and PFOA stand out, with approximately 100 times higher concentrations. When comparing the results from this study with the results from Gómez-Ramírez et al. (2017), the concentrations for PFOSA and PFTriDe are found in the similar range, but concentrations for PFOS, PFOA, PFNA, PFDA, PFUnA, PFDoDeA, PFTetDeA are higher in this study, but not very different. Lastly, the results can be compared to the results by (Meyer et al., 2009), where the concentrations of PFOA and PFNA is found to range in the similar area. For PFOS the concentrations were similar for two of the species investigated by Meyer et al., the Eurasian Magpie and the Eurasian Collared Dove. For the other three species, Grey Heron, Herring Gull and Eurasian Sparrowhawk, the lowest concentration determined was equal to the highest concentration determined in this study.

The external contamination was determined to get a broader perspective, as the contamination in the feather may be deposited during growth over a short period of time, and the external contamination may stick to the feathers and represent a broader area. But even if high external contamination was found, very few conclusions can be made drawn from the present study. The washing procedure with hexane can on the other hand be evaluated to make a big difference, when studying the mean concentrations for internal and external contamination in **table 4.17**.

The removed amount of contamination is high, but patterns regarding internal and external contamination should be analysed further to evaluate the efficiency of this washing step.

DecaS was investigated as a non-fluorinated compound with similar structure as many of the perfluorinated target analytes. DecaS were detected with a high frequency in the feathers (95.0 %) and was determined to have the second highest mean concentration of all the target analytes. The detection frequency in the hexane samples on the other hand, had low detection frequency (34.2 %). It is clear that DecaS is accumulated and stored in the feathers to a similar or higher degree than the PFCs, making it a possible interesting compound for further investigation. DecaS owned the highest external contamination value, which was determined to be 28300 ng/g.

5 Conclusions

In this master thesis, four questions are answered as the aims of the study. Can differences in trace element concentration in P1 and P10 feathers from Scopoli's shearwater be found? The P1 and P10 feathers are grown in two different areas, and the PCA biplots indicated a noticeable pattern in trace element composition in the two feathers. The P1 feathers contained a significant higher Hg-concentration than P10 feathers. The Hg levels in the blood were significantly lower, indicating that feathers are a good matrix for tracing certain metals, supported by other studies (Abbasi et al., 2015; Goede & de Voogt, 1985; Jaspers et al., 2019). The detection frequency and detected concentrations for Cd indicated that feathers were not the best matrix for Cd determination. As and Pb were found in low concentrations in both P1, P10 and blood samples. Co and Zn were determined in significantly higher concentrations in blood, compared to feathers. For Cu and Mn, the trend was opposite, indicating feathers to be a good matrix for Cu and Mn determination. These results supports Lodenius and Solonen (2013) and Voulgaris et al. (2019), that marine birds are good trace element pollution indicators.

Can differences in trace element concentration in six passerine sub-Saharan migratory species be found? Differences between the six passerine species in trace element composition were found to some degree, but no definite conclusion can be drawn from it due to a low number of samples for several of the species. The nonessential elements As, Cd, Hg and Pb were found in low concentrations in all samples, and below levels associated with causing adverse effects in birds. The essential elements Co, Cu, Mn and Zn were all determined in the feather samples of the six species and found in similar concentrations as previous studies. These samples trace element composition represent the wintering areas in Africa. For further work, a higher number of samples for each specie will be an advantage.

Are there any clear differences in trace element concentrations in birds from the Norwegian coast compared to birds from the Mediterranean area? The trace elements As, Cd, Hg, Co and Zn were found in significantly lower concentrations in feathers from White-tailed eagles, compared to feathers from Scopoli's shearwaters. The White-tailed eagle feathers represent environments in the northern hemisphere, and Scopoli's shearwater feathers represent environments in Western Africa.

Which PFCs are found in bird feathers and can differences in PFC concentration between the six passerine sub-Saharan migratory species be found? All 16 target compounds, 15 PFCs and

one nonfluorinated compound, were detected in the feather samples. PFOS was detected in all samples and was determined in the highest concentrations, similar to previous studies. The PFOS concentration varied among the six species, with concentrations ranging from 9.07 ng/g (Garden warbler) to 34.9 ng/g (Sand martin). The external contamination of PFOS displayed a greater variance, with concentrations ranging from not detected (Great reed warbler) to 2654 ng/g (Sedge warbler). The nonfluorinated compound DecaS emerged as the compound with the highest concentrations, possibly an interesting perspective for further work.

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Appendices

Appendix A

Sample information – 262 feather and blood samples

A.1 Reference material – trace element analysis

Table A.1: Information on reference material for trace element analysis. Including sample name and number, reference material for type of tissue and sample weight (mg).

Reference material	For sample type	Wet weight analysed (mg)
PVTL-6-1	Feather	13.3
PVTL-6-2	Feather	11.0
PVTL-6-3	Feather	11.4
Seronorm-L1-1	Blood	0.215
Seronorm-L-1-2	Blood	0.265
Seronorm-L-1-3	Blood	0.157
Seronorm-L-1-4	Blood	0.304
Seronorm-L-1-5	Blood	0.326

A.2 Group 1 feather samples – trace element analysis

Table A.2: Sample information for feather samples in group 1. Including sample number, sample weight (mg), date of sampling, specie, sex, sample ID and weight after freeze drying. The table continues on the next page.

Sample number	Sample weight (mg)	Date of sampling	Specie	Sex	Sample ID	Sample weight after freeze drying (mg)
1	7.5	26.07.2018	*Calonectris diomedea*	M	P004501 / P1	4.2
2	7.6	26.07.2018	*Calonectris diomedea*	M	P004501 / P10	3.7
3	5.2	26.07.2018	*Calonectris diomedea*	M	P004502 / P1	5.8
4	10.2	26.07.2018	*Calonectris diomedea*	M	P004502 / P10	10.0
5	6.8	26.07.2018	*Calonectris diomedea*	F	P004503 / P1	7.4
6	15.1	26.07.2018	*Calonectris diomedea*	F	P004503 / P10	14.2
7	8.0	26.07.2018	*Calonectris diomedea*	F	P004504 / P1	8.7
8	11.6	26.07.2018	*Calonectris diomedea*	F	P004504 / P10	11.9
9	7.8	26.07.2018	*Calonectris diomedea*	M	P004505 / P1	7.9
10	9.2	26.07.2018	*Calonectris diomedea*	M	P004505 / P10	8.8
11	6.4	26.07.2018	*Calonectris diomedea*	-	P004506 / P1	6.5
12	8.6	26.07.2018	*Calonectris diomedea*	-	P004506 / P10	8.8
13	5.0	26.07.2018	*Calonectris diomedea*	-	P004507 / P1	5.6
14	6.8	26.07.2018	*Calonectris diomedea*	-	P004507 / P10	7.8
15	5.6	26.07.2018	*Calonectris diomedea*	-	P004508 / P1	4.7
16	7.9	26.07.2018	*Calonectris diomedea*	-	P004508 / P10	7.5
17	6.1	26.07.2018	*Calonectris diomedea*	-	P004509 / P1	6.0

18	5.7	26.07.2018	*Calonectris diomedea*	-	P004509 / P10	5.9
19	5.7	26.07.2018	*Calonectris diomedea*	-	P004510 / P1	5.9
20	6.6	26.07.2018	*Calonectris diomedea*	-	P004510 / P10	7.1
21	7.8	28.07.2018	*Calonectris diomedea*	-	P004511 / P1	8.3
22	10.6	28.07.2018	*Calonectris diomedea*	-	P004511 / P10	10.6
23	5.4	28.07.2018	*Calonectris diomedea*	-	P004512 / P1	6.5
24	6.1	28.07.2018	*Calonectris diomedea*	-	P004512 / P10	6.5
25	6.3	26.07.2018	*Calonectris diomedea*	-	P004513 / P1	6.4
26	8.2	26.07.2018	*Calonectris diomedea*	-	P004513 / P10	8.4
27	7.2	26.07.2018	*Calonectris diomedea*	-	P004514 / P1	7.8
28	9.1	26.07.2018	*Calonectris diomedea*	-	P004514 / P10	5.9
29	5.8	26.07.2018	*Calonectris diomedea*	-	P004515 / P1	8.3
30	6.0	26.07.2018	*Calonectris diomedea*	-	P004515 / P10	0.0
31	6.2	26.07.2018	*Calonectris diomedea*	-	P004516 / P1	6.0
32	6.8	26.07.2018	*Calonectris diomedea*	-	P004516 / P10	7.3
33	8.6	28.07.2018	*Calonectris diomedea*	-	P004517 / P1	8.6
34	7.8	28.07.2018	*Calonectris diomedea*	-	P004517 / P10	8.0
35	7.4	28.07.2018	*Calonectris diomedea*	-	P004518 / P1	7.0
36	6.8	28.07.2018	*Calonectris diomedea*	-	P004518 / P10	7.7
37	8.6	28.07.2018	*Calonectris diomedea*	-	P004519 / P1	7.6
38	8.3	28.07.2018	*Calonectris diomedea*	-	P004519 / P10	8.1
39	5.5	28.07.2018	*Calonectris diomedea*	-	P004520 / P1	4.7
40	12.3	28.07.2018	*Calonectris diomedea*	-	P004520 / P10	11.9
41	6.2	28.07.2018	*Calonectris diomedea*	-	P004521 / P1	5.2
42	5.0	28.07.2018	*Calonectris diomedea*	-	P004521 / P10	5.0
43	10.2	28.07.2018	*Calonectris diomedea*	-	P004522 / P1	8.1
44	6.3	28.07.2018	*Calonectris diomedea*	-	P004522 / P10	2.5
45	6.0	28.07.2018	*Calonectris diomedea*	-	P004523 / P1	5.5
46	6.4	28.07.2018	*Calonectris diomedea*	-	P004523 / P10	6.5
47	3.9	26.07.2018	*Calonectris diomedea*	F	P003913 / P1	3.5
48	10.3	26.07.2018	*Calonectris diomedea*	F	P003913 / P10	10.0
49	8.1	27.07.2018	*Calonectris diomedea*	M	P003917 / P1	8.2
50	8.8	27.07.2018	*Calonectris diomedea*	-	P003917 / P10	8.5
51	5.3	27.07.2018	*Calonectris diomedea*	-	P003918 / P1	4.6
52	7.5	27.07.2018	*Calonectris diomedea*	-	P003918 / P10	0.0
53	7.2	27.07.2018	*Calonectris diomedea*	-	P003919 / P1	4.3
54	7.6	27.07.2018	*Calonectris diomedea*	-	P003919 / P10	7.2
55	5.4	28.07.2018	*Calonectris diomedea*	-	P003944 / P1	3.0
56	6.1	28.07.2018	*Calonectris diomedea*	-	P003944 / P10	5.9
57	8.7	28.07.2018	*Calonectris diomedea*	-	P003985 / P1	7.7
58	9.8	28.07.2018	*Calonectris diomedea*	-	P003985 / P10	8.3
59	6.5	26.07.2018	*Calonectris diomedea*	M	P004165 / P1	1.8
60	7.4	26.07.2018	*Calonectris diomedea*	M	P004165 / P10	6.9

A.3 Group 3 feather samples – trace elements analysis

Table A.3: Sample information for feather samples in group 3. Including sample number, Sample weight (mg), date of sampling, specie, sex and sample ID. The table continues on the next page.

Sample number	Sample weight (mg)	Date of sampling	Specie	Sex	Sample ID	Sample weight after freeze drying (mg)
61	62.8	2015	*White-tailed*	-	15.ST.1.1	58.6
62	23.4	2015	*White-tailed*	-	15.ST.2.1	21.6
63	46.9	2015	*White-tailed*	-	15.ST.3.1.	40.9
64	49.1	2015	*White-tailed*	-	15.ST.4.1	45.5
65	39.2	2015	*White-tailed*	-	15.ST.4.2	36.0
66	37.6	2015	*White-tailed*	-	15.ST.5.1	32.4
67	58.1	2015	*White-tailed*	-	15.ST.6.1	53.9
68	41.9	2015	*White-tailed*	-	15.ST.6.2	39.4
69	50.2	2015	*White-tailed*	-	15.ST.7.1	47.3
70	60.1	2015	*White-tailed*	-	15.ST.7.2	56.6
71	43.0	2015	*White-tailed*	-	15.ST.8.1	37.0
72	41.6	2015	*White-tailed*	-	15.ST.8.2	33.3
73	47.9	2015	*White-tailed*	-	15.ST.9.1	42.3
74	57.9	2015	*White-tailed*	-	15.SM.1.1	53.9
75	23.7	2015	*White-tailed*	-	15.SM.1.2	22.1
76	58.6	2015	*White-tailed*	-	15.SM.2.1	54.3
77	36.6	2015	*White-tailed*	-	15.SM.2.2	32.9
78	49.0	2015	*White-tailed*	-	15.SM.3.1	45.8
79	49.2	2015	*White-tailed*	-	15.SM.4.1	44.6
80	54.9	2015	*White-tailed*	-	15.SM.5.1	51.1
81	61.5	2015	*White-tailed*	-	15.SM.6.1	52.9
82	45.6	2015	*White-tailed*	-	15.SM.7.1	41.1
83	54.5	2015	*White-tailed*	-	15.SM.7.2	49.9
84	20.0	2015	*White-tailed*	-	15.SM.8.1	18.5
85	61.4	2015	*White-tailed*	-	15.SM.9.1	57.5
86	42.8	2015	*White-tailed*	-	15.SM.10.1	38.7
87	46.2	2016	*White-tailed*	-	16.ST.1.1	41.3
88	46.4	2016	*White-tailed*	-	16.ST.2.1	42.9
89	73.6	2016	*White-tailed*	-	16.ST.3.1	54.8
90	74.9	2016	*White-tailed*	-	16.ST.3.2	48.3
91	59.3	2016	*White-tailed*	-	16.ST.4.1	53.1
92	51.8	2016	*White-tailed*	-	16.ST.5.1	46.7
93	49.6	2016	*White-tailed*	-	16.ST.5.2	46.5
94	35.9	2016	*White-tailed*	-	16.ST.6.1	32.7
95	53.3	2016	*White-tailed*	-	16.ST.7.1	49.7
96	48.6	2016	*White-tailed*	-	16.ST.8.1	45.9
97	49.8	2016	*White-tailed*	-	16.ST.9.1	46.6
98	47.7	2016	*White-tailed*	-	16.ST.10.1	44.7
99	61.2	2016	*White-tailed*	-	16.ST.10.2	49.9
100	61.5	2016	*White-tailed*	-	16.ST.11.1	58.8
101	54.8	2016	*White-tailed*	-	16.ST.12.1	48.9
102	62.8	2016	*White-tailed*	-	16.ST.13.1	58.8
103	54.7	2016	*White-tailed*	-	16.ST.14.1	48.3

104	57.3	2016	*White-tailed*	-	16.ST.14.2	52.6
105	45.8	2016	*White-tailed*	-	16.ST.15.1	42.9
106	63.0	2016	*White-tailed*	-	16.ST.15.2	62.0
107	59.8	2016	*White-tailed*	-	16.ST.15.3	57.3
108	60.1	2016	*White-tailed*	-	16.SM.1.1	55.1
109	54.7	2016	*White-tailed*	-	16.SM.1.2	51.0
110	53.4	2016	*White-tailed*	-	16.SM.2.1	47.3
111	45.6	2016	*White-tailed*	-	16.SM.3.1	33.3
112	40.7	2016	*White-tailed*	-	16.SM.3.2	32.6
113	62.3	2016	*White-tailed*	-	16.SM.4.1	54.7
114	61.6	2016	*White-tailed*	-	16.SM.4.2	55.6
115	58.6	2016	*White-tailed*	-	16.SM.5.1	54.3
116	46.4	2016	*White-tailed*	-	16.SM.6.1	42.5
117	48.7	2016	*White-tailed*	-	16.SM.7.1	41.2
118	76.8	2016	*White-tailed*	-	16.SM.7.2	62.2
119	58.4	2016	*White-tailed*	-	16.SM.8.1	42.6
120	51.7	2016	*White-tailed*	-	16.SM.8.2	47.7
121	63.1	2016	*White-tailed*	-	16.SM.8.3	50.1
122	63.5	2016	*White-tailed*	-	16.SM.9.1	59.0
123	67.8	2016	*White-tailed*	-	16.SM.10.1	58.0
124	69.3	2016	*White-tailed*	-	16.SM.11.1	63.4
125	58.9	2016	*White-tailed*	-	16.SM.11.2	55.3
126	59.9	2016	*White-tailed*	-	16.SM.12.1	55.1
127	33.2	2016	*White-tailed*	-	16.SM.13.1	30.3
128	53.7	2016	*White-tailed*	-	16.SM.14.1	49.6
129	50.7	2016	*White-tailed*	-	16.SM.14.2	48.3

A.4 Group 2 feather samples – trace element analysis

Table A.4: Sample information for P10 feather samples in group 2. Including sample number, sample weight (mg), date of sampling, specie, sex and sample ID.

Sample number	Sample weight	Date of sampling	Specie	Sample ID	Sample weight after freeze drying (mg)
130	8.1	23.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 1 – P10	8.1
131	8.2	23.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 2 – P10	8.3
132	13.9	24.04.2018	*Sylvia borin*	Strofades Greece 3 – P10	14.1
133	24.9	25.04.2018	*Riparia riparia*	Strofades Greece 4 – P10	25.0
134	13.8	25.04.2018	*Sylvia borin*	Strofades Greece 5 – P10	13.5
135	13.2	26.04.2018	*Sylvia borin*	Strofades Greece 6 – P10	13.2
136	9.5	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 7 – P10	9.7
137	8.2	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 8 – P10	8.0
138	8.8	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 9 – P10	9.4
139	8.4	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 10 – P10	8.0
140	8.7	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 11 – P10	8.5
141	9.7	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 12 – P10	9.7
142	9.6	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 13 – P10	9.4
143	11.6	26.04.2018	*Muscicapa striata*	Strofades Greece 14 – P10	12.2
144	13.5	26.04.2018	*Muscicapa striata*	Strofades Greece 15 – P10	13.7
145	9.5	26.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 16 – P10	9.5
146	11.5	27.04.2018	*Sylvia borin*	Strofades Greece 17 – P10	11.7
147	8.3	27.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 18 – P10	8.5
148	8.7	27.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 19 – P10	8.9
149	12.6	27.04.2018	*Sylvia borin*	Strofades Greece 20 – P10	12.9
150	22.1	27.04.2018	*Acrocephalus arundinaceus*	Strofades Greece 21 – P10	22.2
151	7.8	28.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 22 – P10	8.1
152	13.8	28.04.2018	*Sylvia borin*	Strofades Greece 23 – P10	14.0
153	21.8	28.04.2018	*Acrocephalus arundinaceus*	Strofades Greece 24 – P10	22.0
154	11.7	28.04.2018	*Sylvia borin*	Strofades Greece 25 – P10	11.6
155	23.2	28.04.2018	*Acrocephalus arundinaceus*	Strofades Greece 26 – P10	23.5
156	11.3	28.04.2018	*Sylvia borin*	Strofades Greece 27 – P10	12.0
157	11.6	28.04.2018	*Sylvia borin*	Strofades Greece 28 – P10	12.1
158	14.8	28.04.2018	*Muscicapa striata*	Strofades Greece 29 – P10	14.7
159	9	28.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 30 – P10	8.5
160	13.7	28.04.2018	*Sylvia borin*	Strofades Greece 31 – P10	13.4
161	8.2	28.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 32 – P10	8.4
162	9.5	29.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 33 – P10	10.0
163	9.8	29.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 34 – P10	9.4
164	8.9	30.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 35 – P10	9.5
165	8.6	01.05.2018	*Phylloscopus sibilatrix*	Strofades Greece 36 – P10	8.9
166	13.5	01.05.2018	*Sylvia borin*	Strofades Greece 37 – P10	14.0
167	11.9	01.05.2018	*Sylvia borin*	Strofades Greece 38 – P10	12.0

A.5 Group 1 blood samples – trace element analysis

Table A.5: Sample information for group 1 blood samples for trace element analysis. Samples 168-196 are samples in ethyl alcohol and 197-224 are samples in heparin. Table including sample number, sample dry weight (mg), date of sampling, specie, sex and sample ID. The table continues on the next page.

Sample number	Sample dry weight (mg)	Date of sampling	Specie	Sex	Sample ID
168	7.5		*Calonectris diomedea*	-	P003913
169	18.1		*Calonectris diomedea*	-	P003917
170	1.1		*Calonectris diomedea*	-	P003918
171	22.9		*Calonectris diomedea*	-	P003919
172	8.3		*Calonectris diomedea*	-	P003944
173	20.1		*Calonectris diomedea*	-	P003985
174	20.6		*Calonectris diomedea*	-	P004165
175	7.0		*Calonectris diomedea*	M	P004502
176	11.4		*Calonectris diomedea*	F	P004503
177	23.9		*Calonectris diomedea*	F	P004504
178	19.7		*Calonectris diomedea*	M	P004505
179	21.0		*Calonectris diomedea*	-	P004506
180	20.9		*Calonectris diomedea*	-	P004507
181	6.6		*Calonectris diomedea*	-	P004508
182	2.7		*Calonectris diomedea*	-	P004509
183	20.5		*Calonectris diomedea*	-	P004510
184	21.4		*Calonectris diomedea*	-	P004511
185	20.2		*Calonectris diomedea*	-	P004512
186	20.8		*Calonectris diomedea*	-	P004513
187	14.9		*Calonectris diomedea*	-	P004514
188	21.5		*Calonectris diomedea*	-	P004515
189	21.0		*Calonectris diomedea*	-	P004516
190	21.9		*Calonectris diomedea*	-	P004517
191	4.2		*Calonectris diomedea*	-	P004518
192	20.4		*Calonectris diomedea*	-	P004519
193	10.3		*Calonectris diomedea*	-	P004520
194	13.1		*Calonectris diomedea*	-	P004521
195	5.2		*Calonectris diomedea*	-	P004523
196	21.3		*Calonectris diomedea*	-	unknown
197	-		*Calonectris diomedea*	-	P003917
198	0.8		*Calonectris diomedea*	-	P003918
199	0.2		*Calonectris diomedea*	-	P003919
200	1.2		*Calonectris diomedea*	-	P003944
201	1.0		*Calonectris diomedea*	-	P003985
202	2.1		*Calonectris diomedea*	M	P004501
203	1.9		*Calonectris diomedea*	M	P004502
204	0.2		*Calonectris diomedea*	F	P004503
205	-		*Calonectris diomedea*	F	P004504
206	-		*Calonectris diomedea*	M	P004505
207	0.6		*Calonectris diomedea*	-	P004506
208	1.3		*Calonectris diomedea*	-	P004507
209	0.2		*Calonectris diomedea*	-	P004508

210	0.5	*Calonectris diomedea*	-	P004509
211	1.0	*Calonectris diomedea*	-	P004510
212	-	*Calonectris diomedea*	-	P004511
213	0.3	*Calonectris diomedea*	-	P004512
214	1.8	*Calonectris diomedea*	-	P004513
215	0.4	*Calonectris diomedea*	-	P004514
216	0.6	*Calonectris diomedea*	-	P004515
217	-	*Calonectris diomedea*	-	P004516
218	0.3	*Calonectris diomedea*	-	P004517
219	1.2	*Calonectris diomedea*	-	P004518
220	0.5	*Calonectris diomedea*	-	P004519
221	1.3	*Calonectris diomedea*	-	P004520
222	-	*Calonectris diomedea*	-	P004521
223	1.0	*Calonectris diomedea*	-	P004522
224	0.6	*Calonectris diomedea*	-	P004523

A.6 Group 2 feather samples – organic analysis

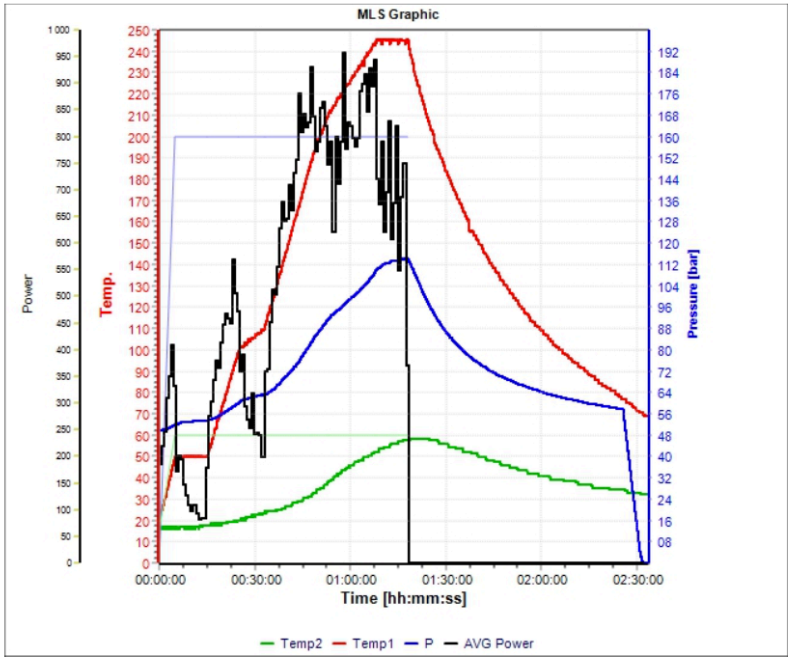
Table A.6: Sample information for group 2 feather samples for organic analysis. Sample length is the total length of all feathers from one bird wing, after P10 feather is removed. Table including sample number, sample weight (mg), sample length (cm), date of sampling, specie and sample ID.

Sample number	Sample weight (mg)	Sample length (cm)	Date of sampling	Specie	Sample ID
1	61.2	77.3	23.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 1
2	63.0	76.1	23.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 2
3	93.0	95	24.04.2018	*Sylvia borin*	Strofades Greece 3
4	122.0	78.5	25.04.2018	*Riparia riparia*	Strofades Greece 4
5	127.2	88.9	25.04.2018	*Sylvia borin*	Strofades Greece 5
6	91.1	89.2	26.04.2018	*Sylvia borin*	Strofades Greece 6
7	72.1	78.5	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 7
8	60.8	77.6	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 8
9	72.4	81.1	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 9
10	76.6	79.1	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 10
11	49.0	79.2	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 11
12	74.7	83.1	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 12
13	69.7	82.6	26.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 13
14	88.9	91.8	26.04.2018	*Muscicapa striata*	Strofades Greece 14
15	92.6	95.9	26.04.2018	*Muscicapa striata*	Strofades Greece 15
16	64.0	76.6	26.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 16
17	88.4	78.5	27.04.2018	*Sylvia borin*	Strofades Greece 17
18	67.1	80.5	27.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 18
19	65.6	74	27.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 19
20	109.2	94	27.04.2018	*Sylvia borin*	Strofades Greece 20
21	136.5	108.5	27.04.2018	*Acrocephalus arundinaceus*	Strofades Greece 21
22	52.6	64.4	28.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 22
23	102.5	88.7	28.04.2018	*Sylvia borin*	Strofades Greece 23
24	158.0	109.4	28.04.2018	*Acrocephalus arundinaceus*	Strofades Greece 24
25	95.7	87.2	28.04.2018	*Sylvia borin*	Strofades Greece 25
26	159.5	105.3	28.04.2018	*Acrocephalus arundinaceus*	Strofades Greece 26
27	95.0	84	28.04.2018	*Sylvia borin*	Strofades Greece 27
28	84.1	86.2	28.04.2018	*Sylvia borin*	Strofades Greece 28
29	114.8	97	28.04.2018	*Muscicapa striata*	Strofades Greece 29
30	63.6	79.2	28.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 30
31	93.8	81.2	28.04.2018	*Sylvia borin*	Strofades Greece 31
32	61.1	72	28.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 32
33	69.8	76.2	29.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 33
34	56.8	81.9	29.04.2018	*Phylloscopus sibilatrix*	Strofades Greece 34
35	65.9	76	30.04.2018	*Acrocephalus schoenobaenus*	Strofades Greece 35
36	63.0	81	01.05.2018	*Phylloscopus sibilatrix*	Strofades Greece 36
37	277.6	93.1	01.05.2018	*Sylvia borin*	Strofades Greece 37
38	264.9	85.9	01.05.2018	*Sylvia borin*	Strofades Greece 38

Appendix B

Conditions for inorganic analysis

Sample digestion by high-pressure digestion unit UltraCLAVE (Milestone)



Parameter

```
Signature name  :
Signature date  :
Signature func. :
Operator        :Administrator
Date            :11.12.2018
14:14:40
Method filename
:Stand-profile-245C-10-min-oper
Create run      :Administrator
Microwave Power :pulse
Load pressure   :50.0 bar
Release temp.   :78.0 °C
Release pressure:10.0 bar/min
Cooling         :ON
Auto open       :OFF
Cooling on Temp.:40.0 °C
Ground load     :300 30 2
Ventilation time:01:15:23
Rotor           :40 positions
Vessel-Type     :18ml teflon and
quarts
```

Figure B.1: A diagram from digestion procedure and parameters given in the UltraCLAVE digestion.

MW Program

Step	Time [hh:mm:ss]	Temp 1 [°C]	Temp 2 [°C]	Press [bar]	Engery [Watt]
1	00:05:00	50	60	160	1 000
2	00:10:00	50	60	160	1 000
3	00:10:00	100	60	160	1 000
4	00:08:00	110	60	160	1 000
5	00:15:00	190	60	160	1 000
6	00:05:00	210	60	160	1 000
7	00:15:00	245	60	160	1 000
8	00:10:00	245	60	160	1 000

Figure B.2: Information regarding the steps in UltraCLAVE digestion

ICP-MS parameters

Table B.1: Specifications for ICP-MS, Element 2 from Thermo Scientific

Instrumental part	Specification
Autosampler	SC2 DX equipped with a dustcover with ULPA filter
Sample injector	PrepFAST
Nebulizer	PFA-ST with approx. volume range 50-700 µL/min
Spray chamber	Quartz baffled micro cyclonic with a dual gas inlet type (ESI – ES-3452-111-11)
Cooling	PC^{3x} – Peltier cooling and heated inlet system
Torch	Quartz Demountable with o-rings
Injector	Quartz 2.5 mm with o-rings (ES-1024-0250)
Sample cone	Aluminium (ES-3000-18032)
Skimmer cone	Aluminium type X-skimmer (ES-3000-1805 X)
Radio frequency (RF)-power	1350 W

Table B.2: Gas flow settings for ICP-MS

Type of gas	Flow (L/min)
Cool gas	15.5
Auxiliary gas	1.1
Sample gas 1 (nebulizer)	0.75
Sample gas 2 (T-connection)	0.55
Additional gas (10% methane in Argon)	0.0004, corresponding to approx. 0.04% in the sample

Appendix C

Conditions for organic analysis

Table C.1: Weights (mg) of feather control samples from a pooled feather sample. Control samples were used as triplicates for method validation. Internal standards were added to all samples (20 µL).

Sample	Weight (mg)
Control 1, 20 µL ISTD	99.3
Control 2, 20 µL ISTD	102
Spiked sample 1, 10 ppb TA, 20 µL ISTD	101
Spiked sample 2, 10 ppb TA, 20 µL ISTD	98.4
Spiked sample 3, 10 ppb TA, 20 µL ISTD	96.4
Spiked sample 1, 50 ppb TA, 20 µL ISTD	97.4
Spiked sample 2, 50 ppb TA, 20 µL ISTD	102
Spiked sample 3, 50 ppb TA, 20 µL ISTD	102
Matrix match 1, 10 ppb TA, 20 µL ISTD	102
Matrix match 2, 10 ppb TA, 20 µL ISTD	100
Matrix match 1, 50 ppb TA, 20 µL ISTD	95.0
Matrix match 2, 50 ppb TA, 20 µL ISTD	98.6
Total number of samples: 12 + 2 reagent blanks	(no tissue added)

Table C.2: Accurate concentrations in target analytes (TA) before dilution to 100 ppm.

Compound	ppm	TA (µL)	Solvent	Final conc. (ppm)
DecaS	970	103	897	99.91
NonaFBS	1050	95	905	99.75
TriDeFHxSA	870	115	885	100.05
PFOS	100	-	-	100
PFOSA	1123	89	911	99.95
Sulf	830	121	879	100.43
PFPA	2750	73	927	100.375
PFHxA	2460	41	959	100.86
PFHeA	1670	06	940	100.2
PFOA	2650	75	925	99.375
PFNA	2150	93	907	99.975
PFDA	1030	97	903	99.91
PFUnA	970	103	897	99.91
PFDoDeA	1020	98	902	99.96
PFTriDe	1610	62	938	99.8
PFTetDeA	1060	94	906	99.64

Table C.3: Mean ion ration (IR) % (RSD%) based on the 8 highest calibration points (N=8).

Compound	IR % (RSD%)	Quantification ion	Confirmation ion
DecaS	14.5 (29.5) [a)]	220 > 80*	221 > 221
NonaFBS	237 (7.80)	299 > 80*	299 > 299
TriDeFHxSA	139 (7.63)	399 > 399*	399 > 80
PFOS	119 (5.69)	499 > 99*	499 > 80
PFOSA	5.26 (37.2)	498 > 78*	498 > 478
Sulf	69.2 (7.99)	526 > 169*	526 > 219
PFPA	-	263 > 219*	-
PFHxA	4.02 (37.7)	313 > 269*	313 > 119
PFHeA	383 (15.6)	363 > 169*	362 > 319
PFOA	30.8 (10.5)	413 > 369*	413 > 169
PFNA	505 (14.9)	463 > 219*	463 > 419
PFDA	12.6 (12.1)	512 > 468*	512 > 269
PFUnA	14.6 (15.6)	562 > 518*	562 > 269
PFDoDeA	12.2 (6.38)	612 > 568*	612 > 169
PFTriDe	12.5 (19.2)	662 > 618*	662 > 169
PFTetDeA	11.0 (39.4)	712 > 668*	712 > 169

*Quantification ion, [a)] N=6

Table C.4: Target analytes, internal standards and analyte specific MS/MS parameters using a Kinetex C18 (50 x 2.1 mm) column. Table including primary and secondary transitions, retention times (RT) and relative retention times (RRT (ISTD)) in analysis of 38 feather samples in group 2.

Compound	Molar mass	RT (min)	RRT (ISTD)	Primary transition	Secondary transition
DecaS	221.34	2.10	1.00 (PFOA-13C)	221 > 221	220 > 80*
NonaFBS	299.09	1.71	0.82 (PFOA-13C)	299 > 299	299 > 80*
TriDeFHxSA	399.11	1.98	0.95 (PFOA-13C)	399 > 399*	399 > 80
PFOS	499.12	2.20	1.00 (PFOS-13C)	499 > 99*	499 > 80
PFOSA	499.14	2.44	1.11 (PFOS-13C)	498 > 478	498 > 78*
Sulf	527.20	2.69	1.23 (PFOS-13C)	526 > 219	526 > 169*
PFPA	264.05	1.67	0.80 (PFOA-13C)	263 > 219*	
PFHxA	314.05	1.85	0.89 (PFOA-13C)	313 > 269*	313 > 119
PFHeA	364.06	1.98	0.95 (PFOA-13C)	362 > 319	363 > 169*
PFOA	414.07	2.10	1.00 (PFOA-13C)	413 > 369*	413 > 169
PFNA	464.08	2.20	1.00 (PFOS-13C)	463 > 419	463 > 219*
PFDA	514.09	2.29	1.05 (PFOS-13C)	512 > 468*	512 > 269
PFUnA	564.09	2.38	1.10 (PFOS-13C)	562 > 518*	562 > 269
PFDoDeA	614.10	2.46	1.12 (PFOS-13C)	612 > 568*	612 > 169
PFTriDe	664.11	2.54	1.16 (PFOS-13C)	662 > 618*	662 > 169
PFTetDeA	714.12	2.61	1.19 (PFOS-13C)	712 > 668*	712 > 169
			Internal standards		
PFOA-13C	422	2.09	1 (PFOA-13C)	421 > 223*	421 > 172
PFOS-13C	507	2.19	1 (PFOS-13C)	507 > 172*	507 > 80

* Quantification ion.

Calibration curves

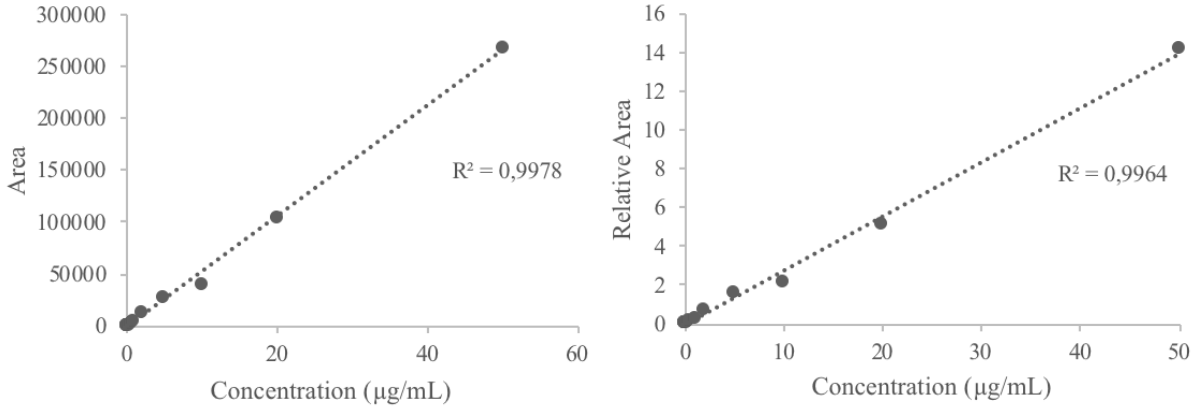


Figure C.1: Calibration curves for DecaS based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOA-13C.

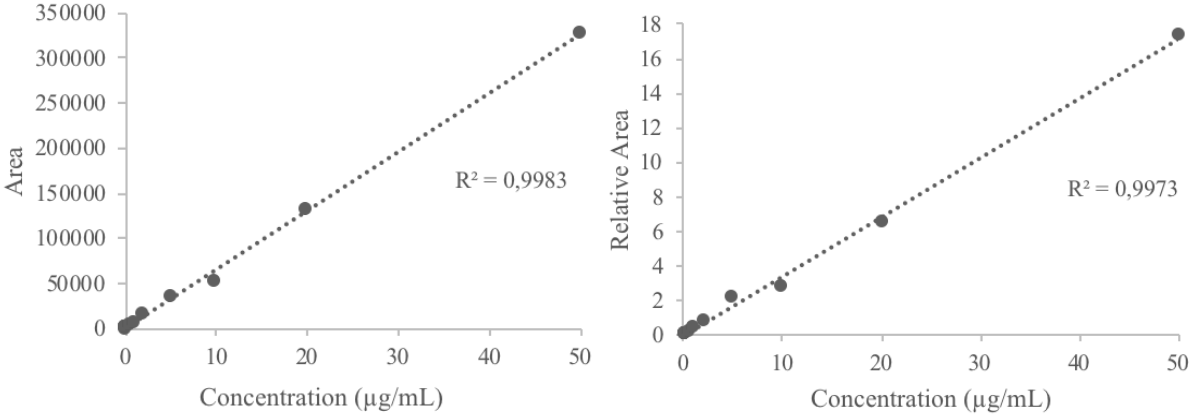


Figure C.2: Calibration curves for NonaFBS based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOA-13C.

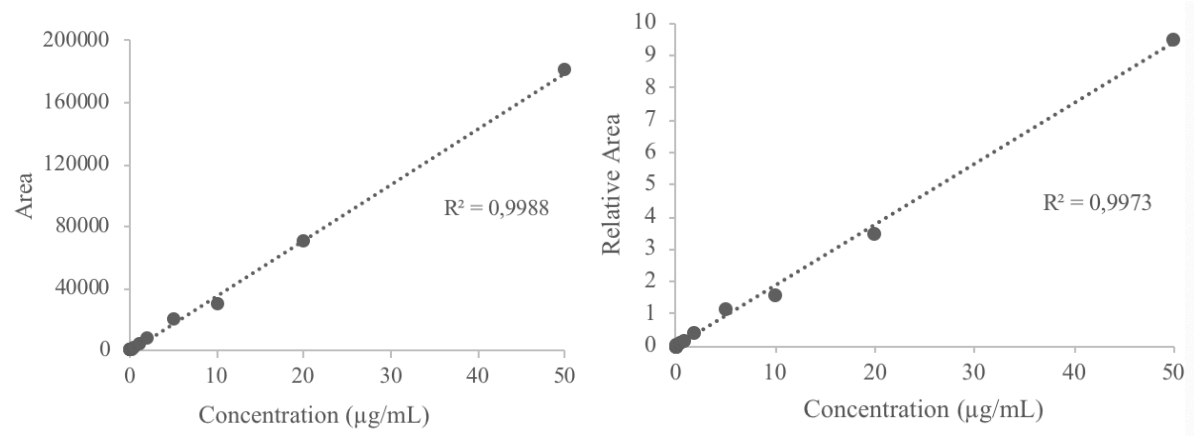


Figure C.3: Calibration curves for TriDeFHxSA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOA-13C.

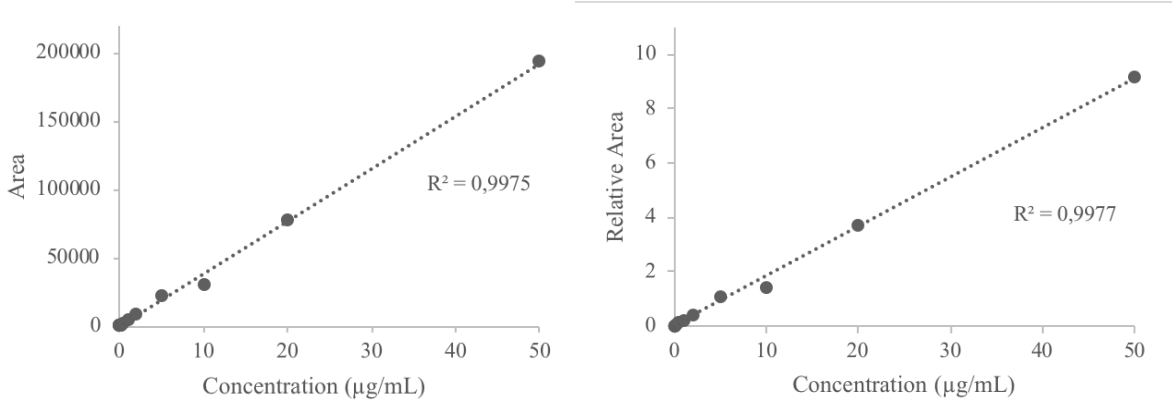


Figure C.4: Calibration curves for PFOS based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

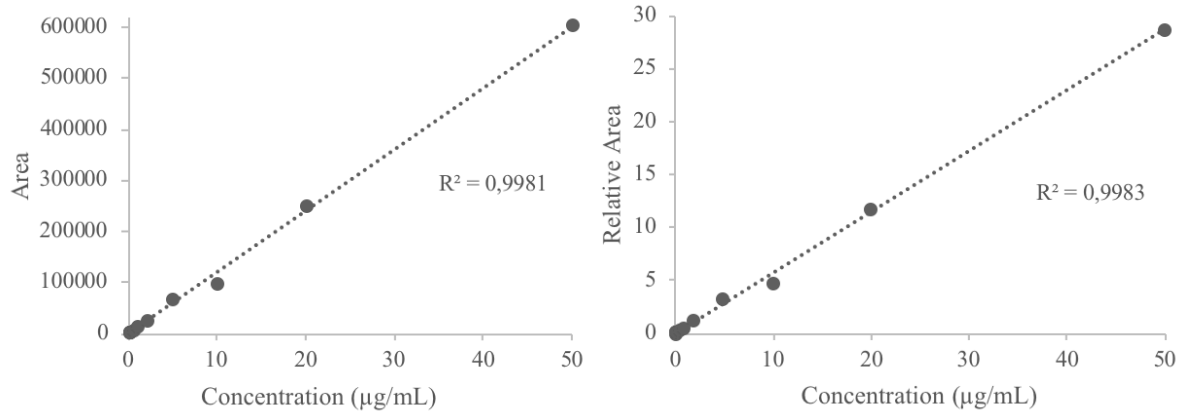


Figure C.5: Calibration curves for PFOSA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

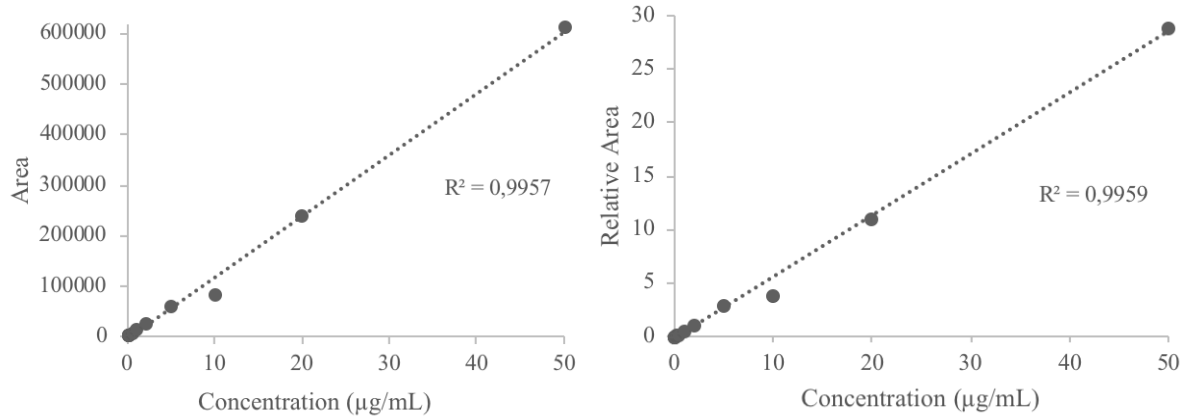


Figure C.6: Calibration curves for Sulf based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

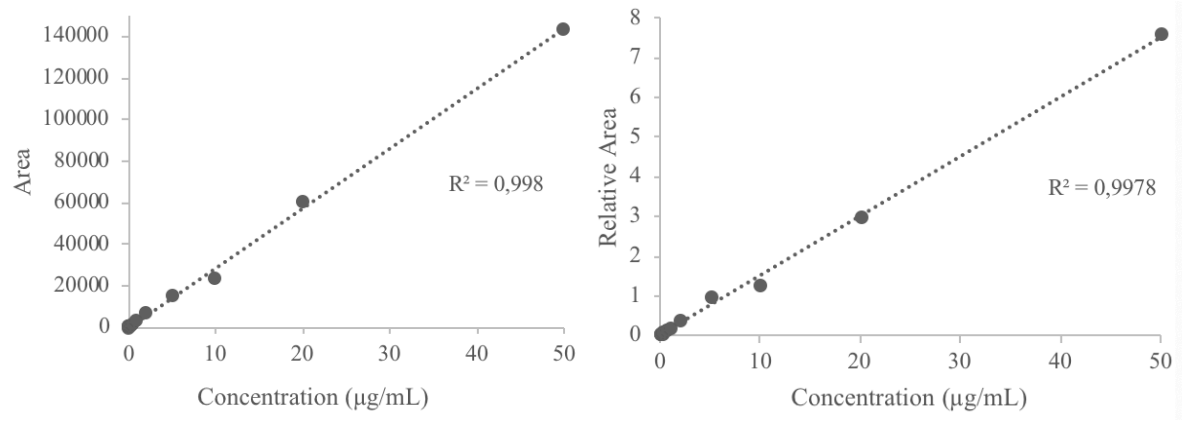


Figure C.7: Calibration curves for PFPA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOA-13C.

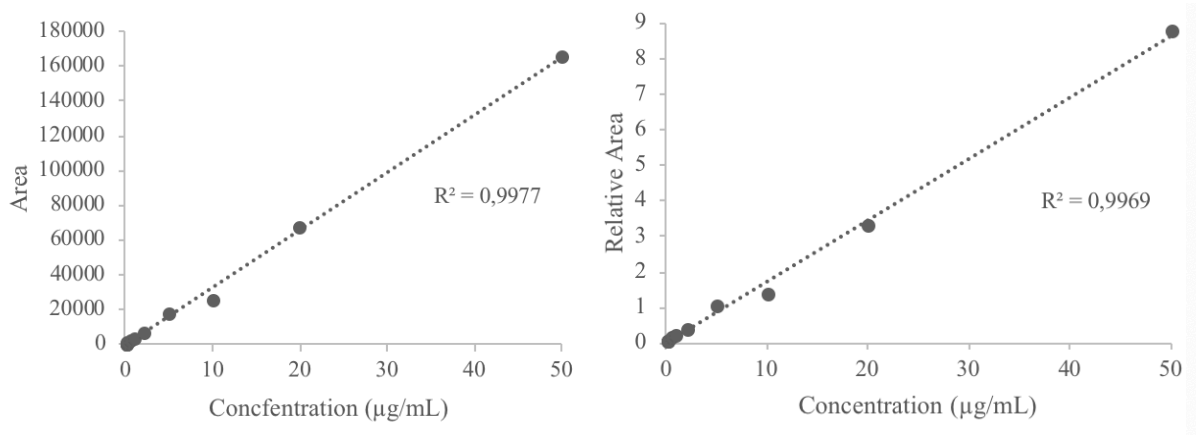


Figure C.8: Calibration curves for PFHxA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOA-13C.

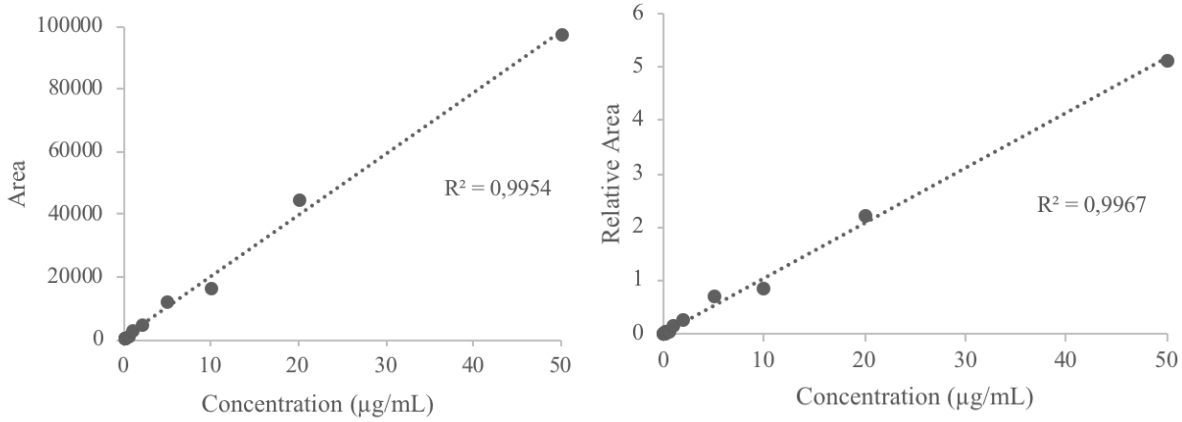


Figure C.9: Calibration curves for PFHeA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOA-13C.

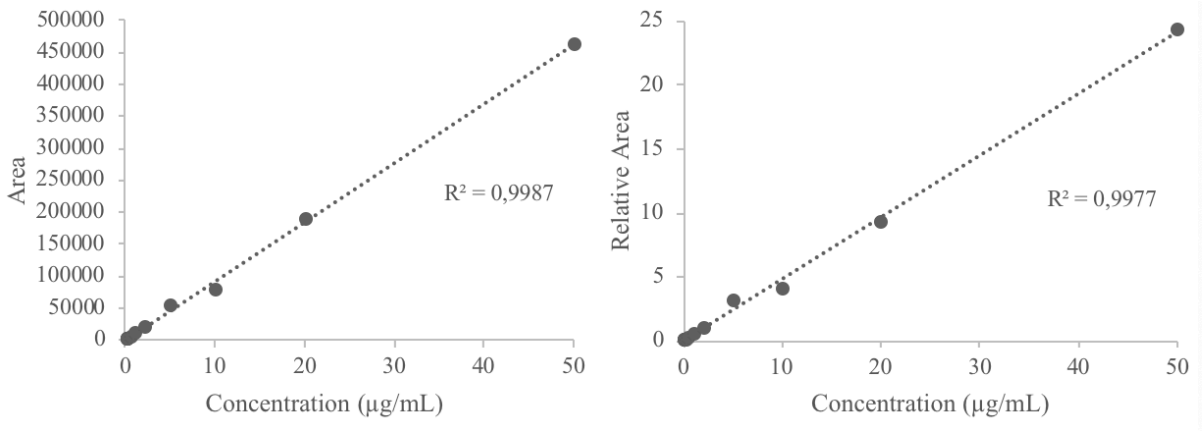


Figure C.10: Calibration curves for PFOA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOA-13C.

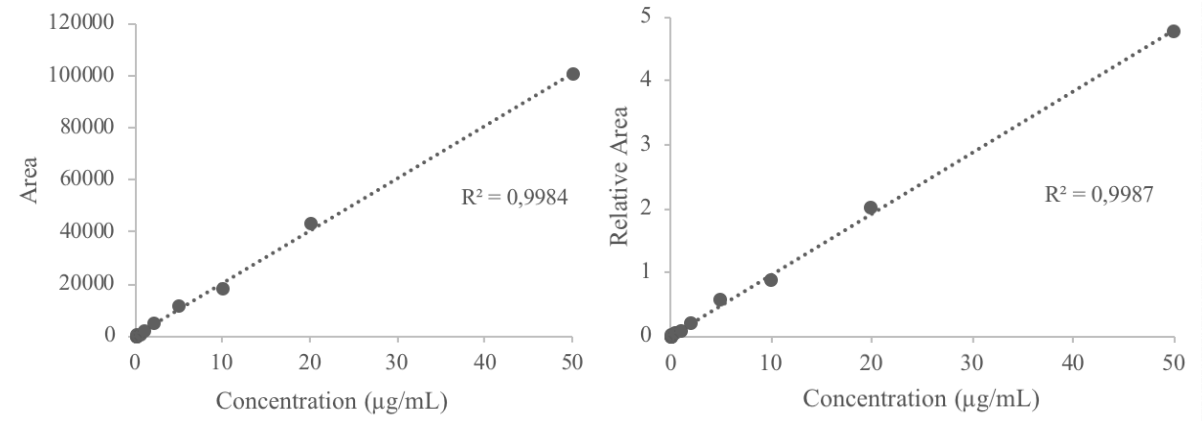


Figure C.11: Calibration curves for PFNA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

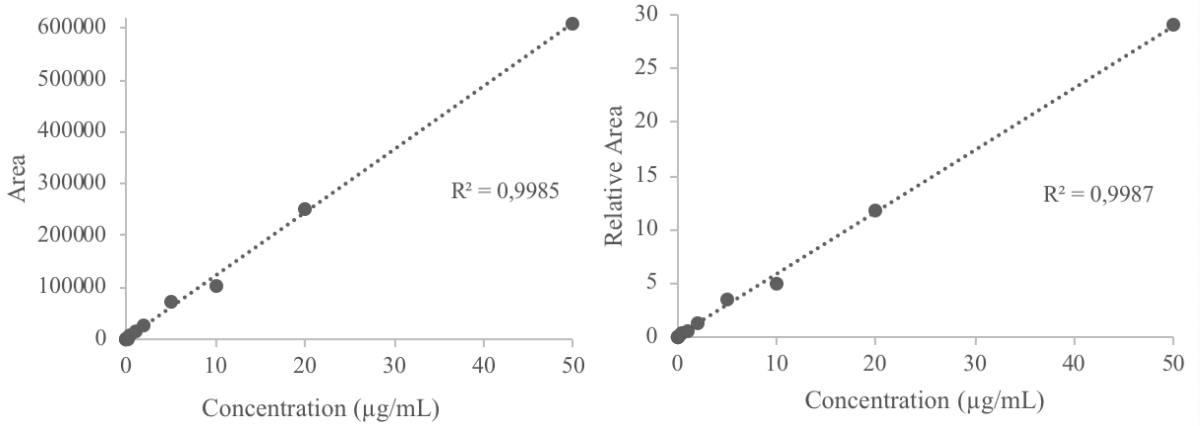


Figure C.12: Calibration curves for PFDA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

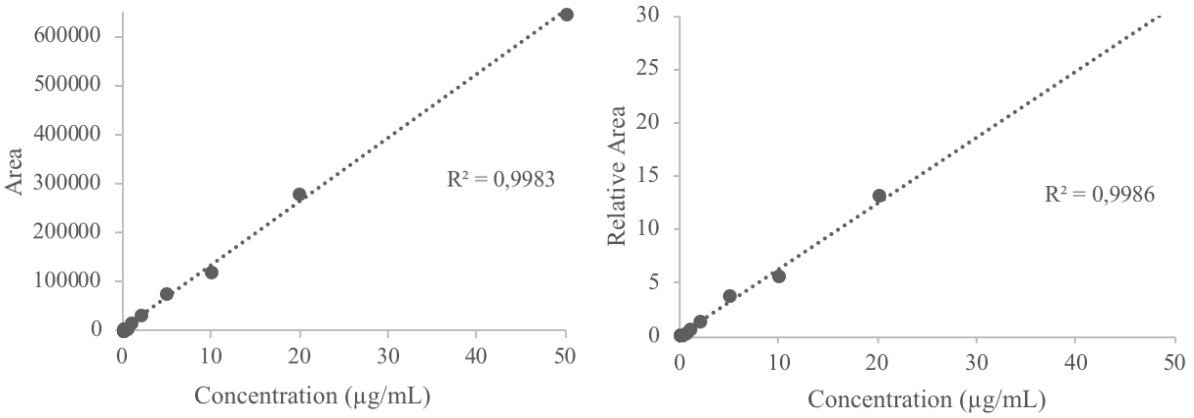


Figure C.13: Calibration curves for PFUnA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

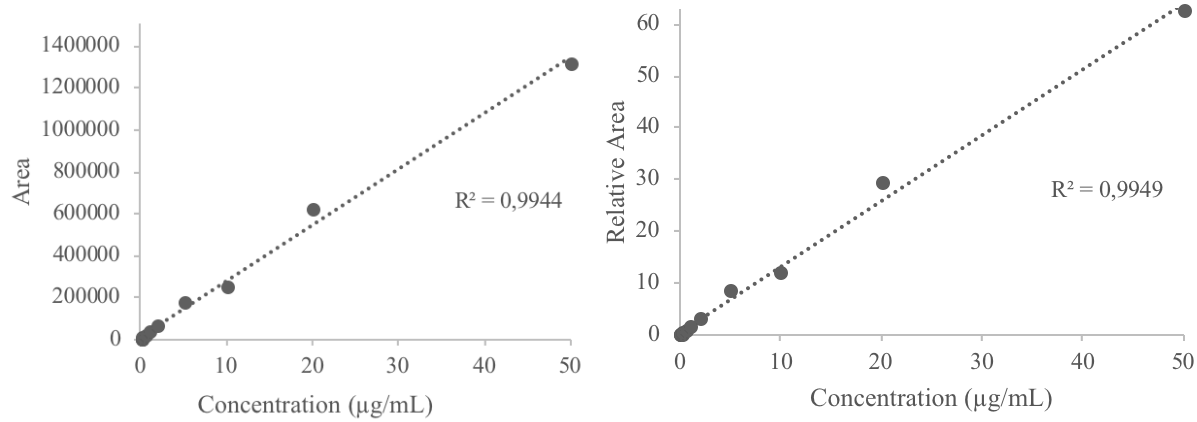


Figure C.14: Calibration curves for PFDoDeA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

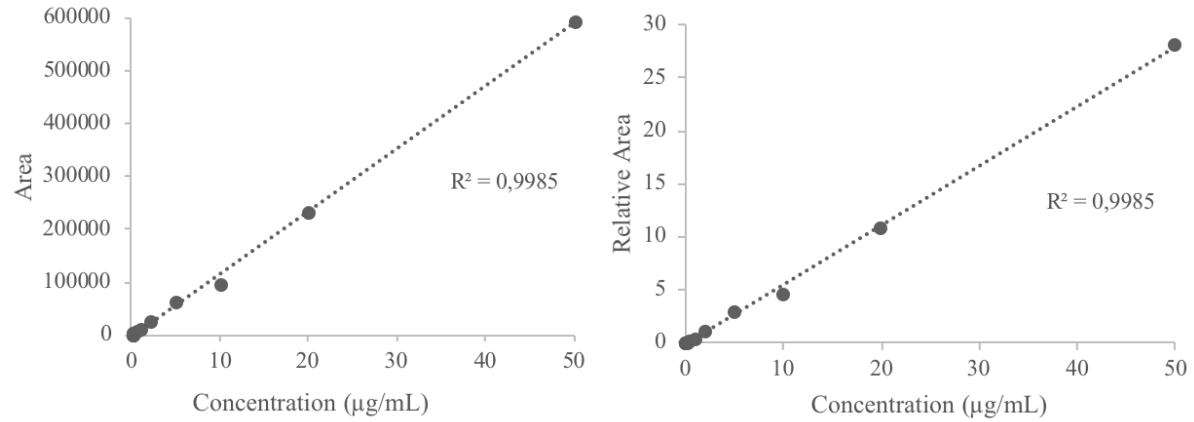


Figure C.15: Calibration curves for PFTriDe based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

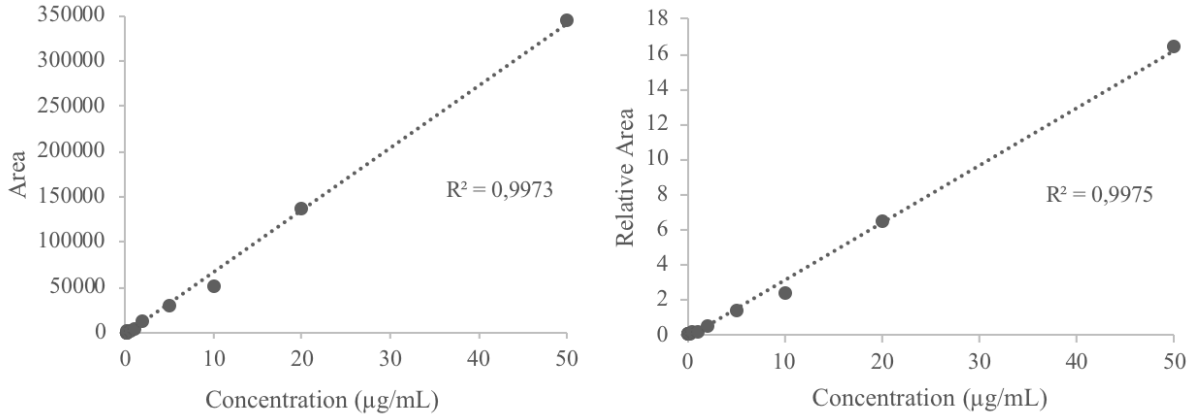


Figure C.16: Calibration curves for PFTetDeA based on absolute and relative areas, respectively. Internal standard used for calculating relative area was PFOS-13C.

Appendix D Chromatograms

All figures in Appendix D are MRM chromatogram for spiked sample with 20 ppb ISTD and 50 ppb TA.

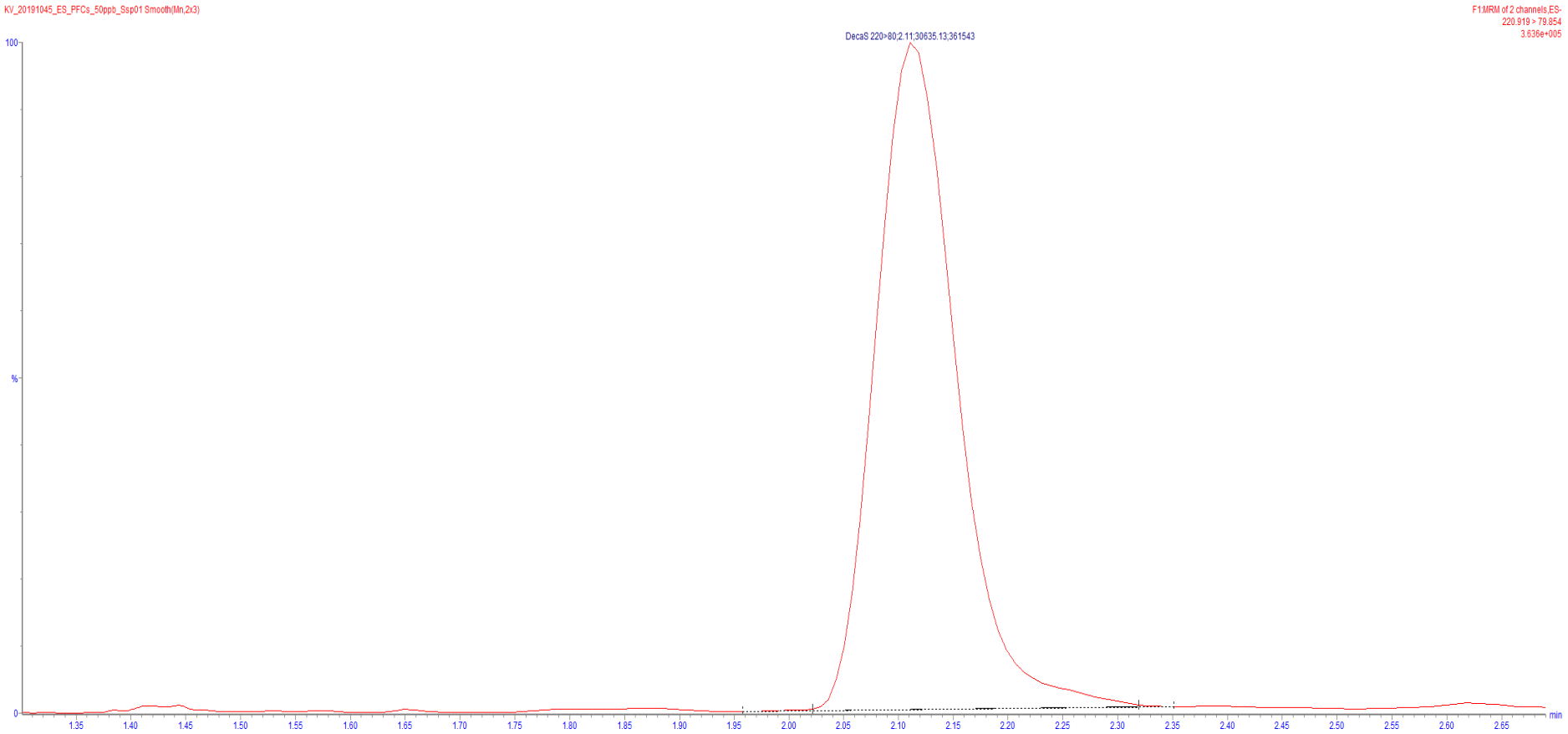


Figure D.1: Secondary transition only, for DecaS.

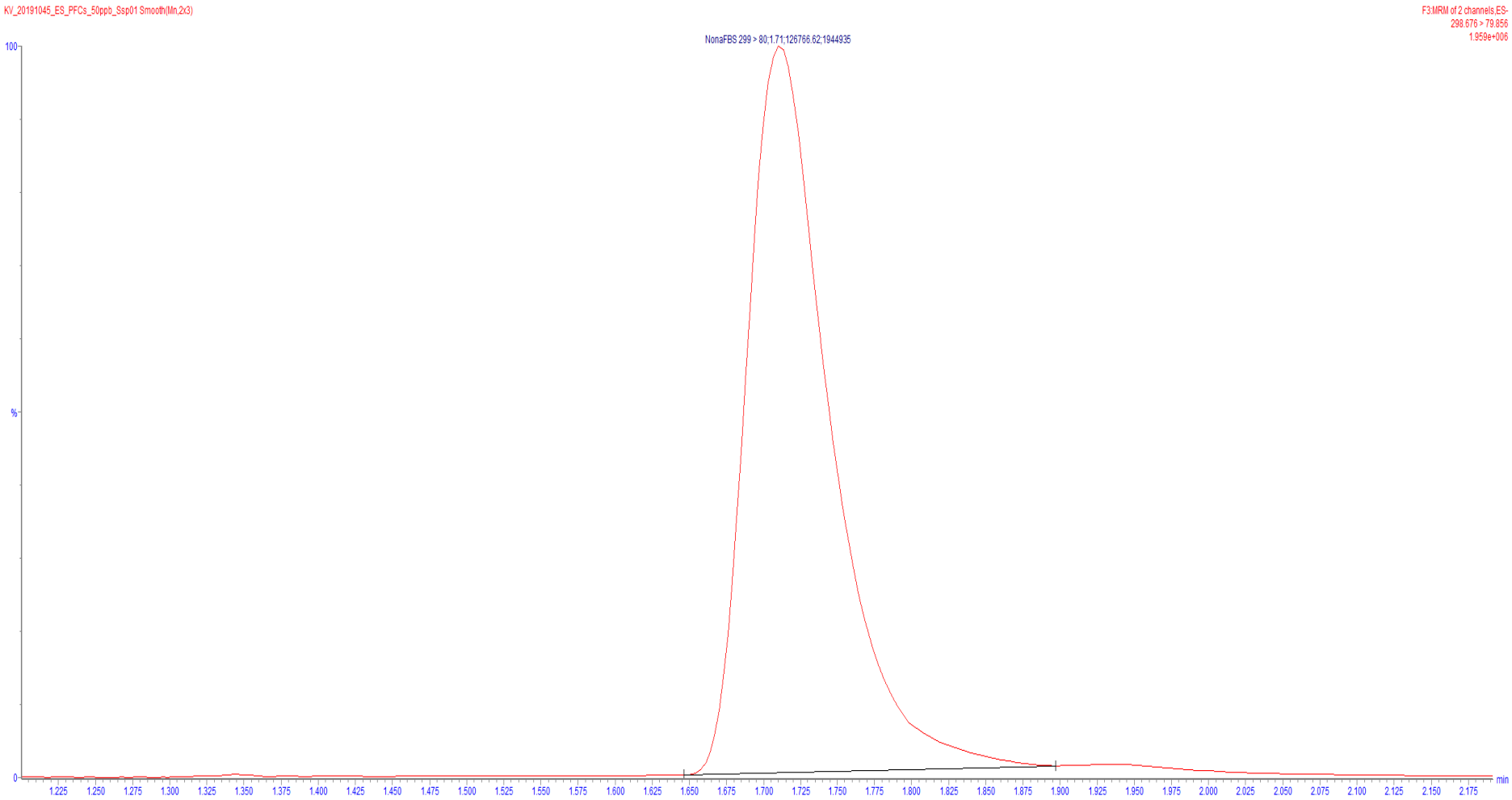


Figure D.2: Secondary transition for NonaFBS.

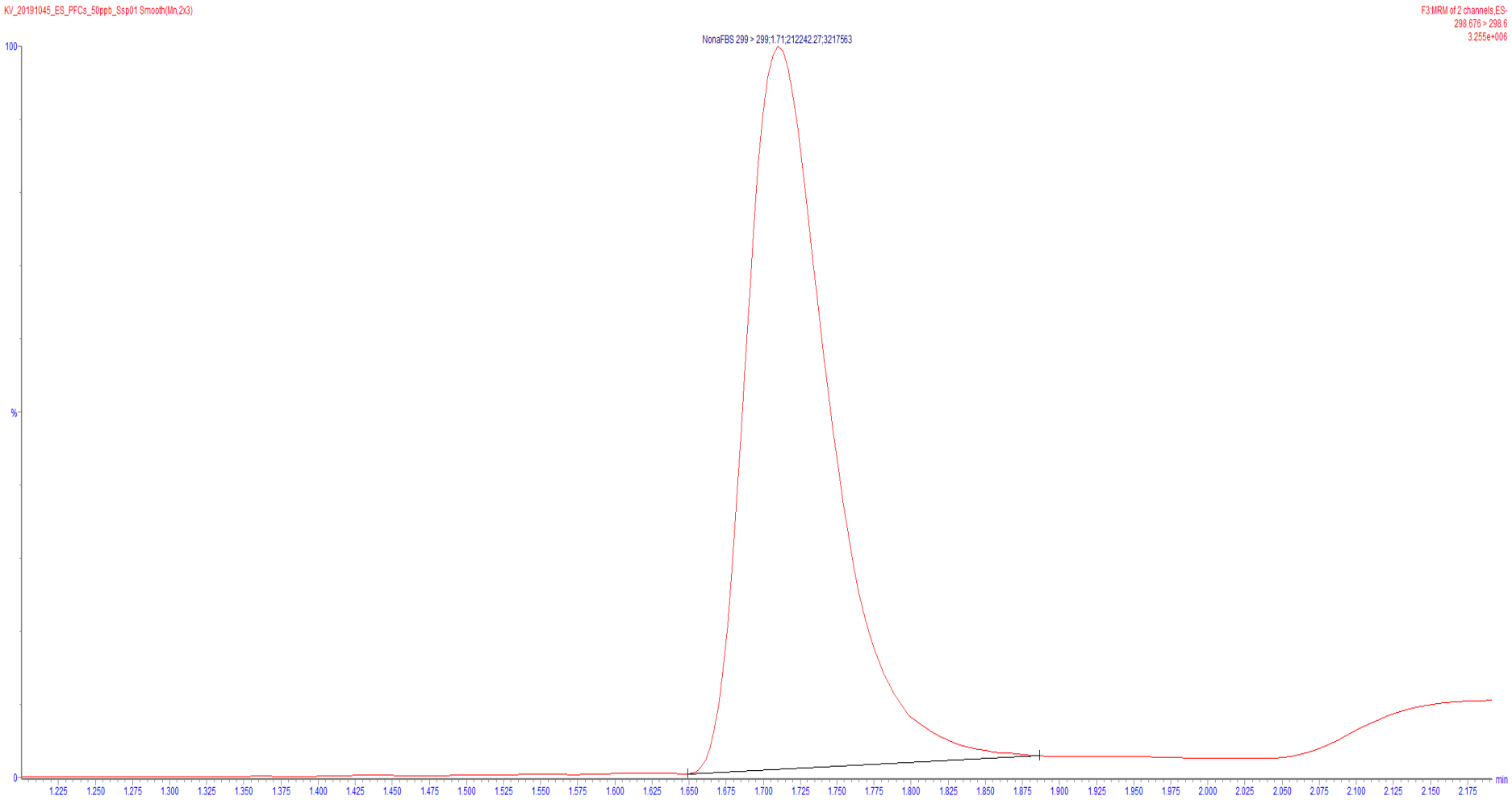


Figure D.3: Primary transition for NonaFBS.

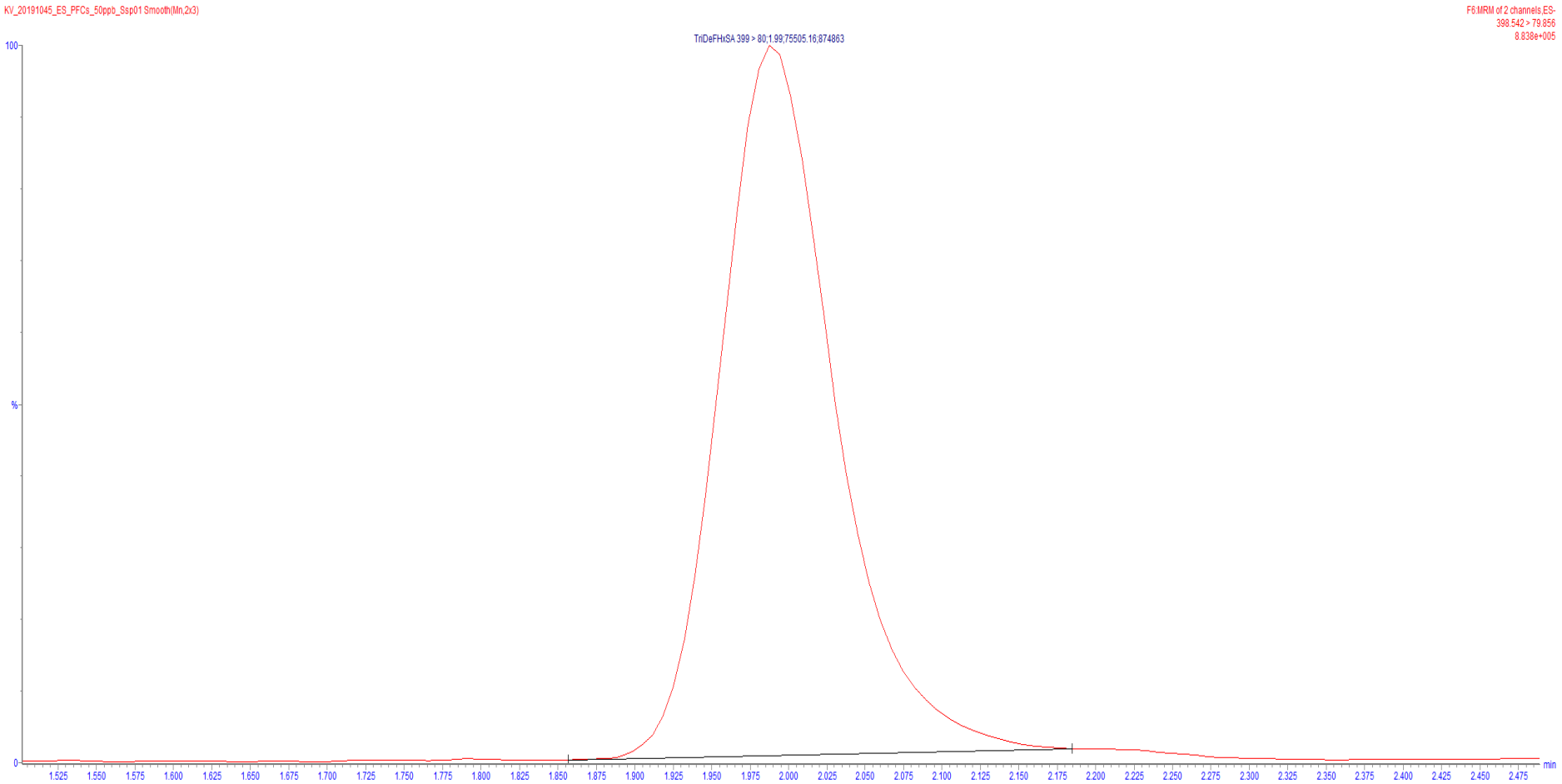


Figure D.4: Secondary transition for TriDeFHxSA.

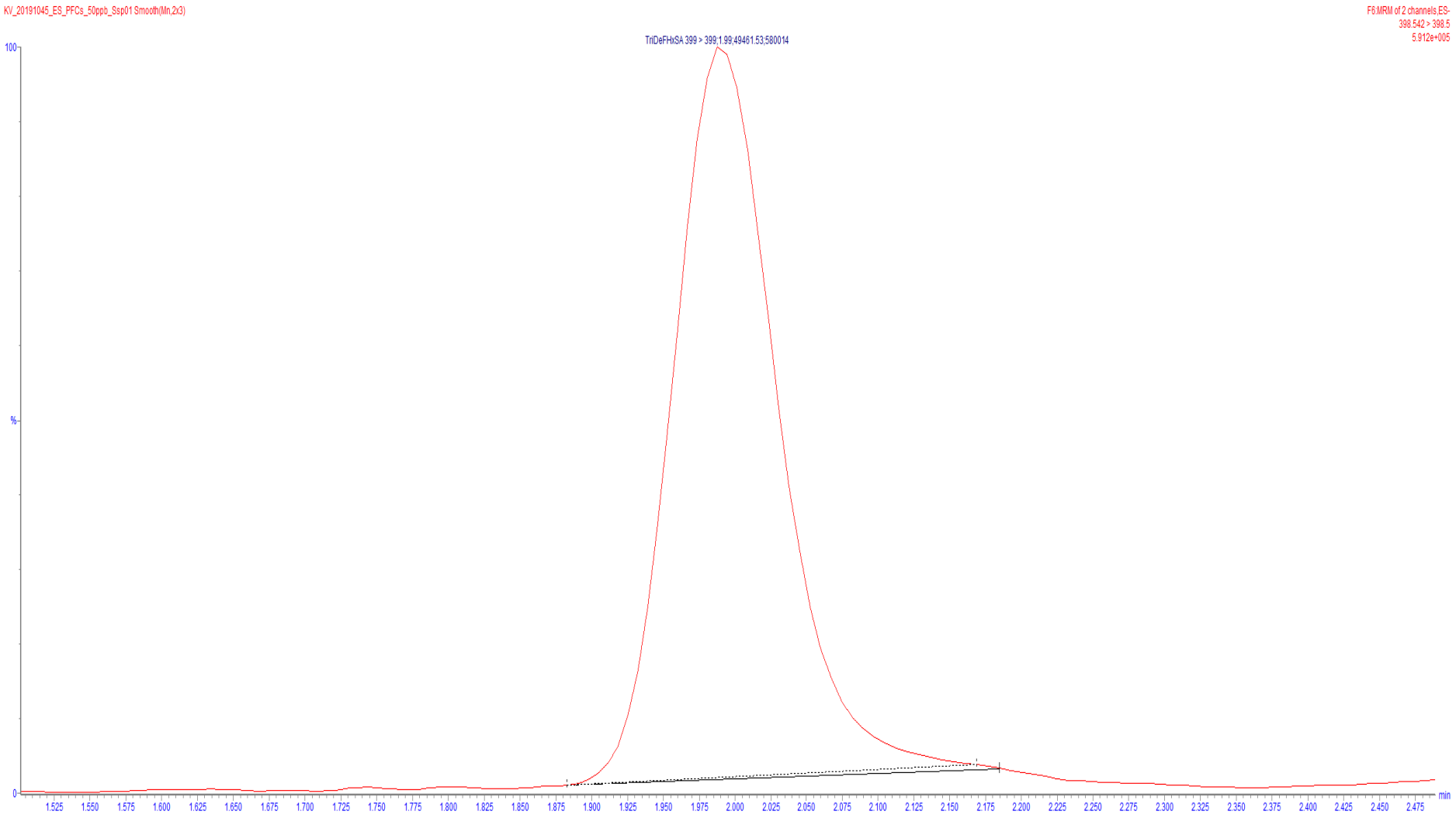


Figure D.5: Primary transition for TriDeFHxSA.

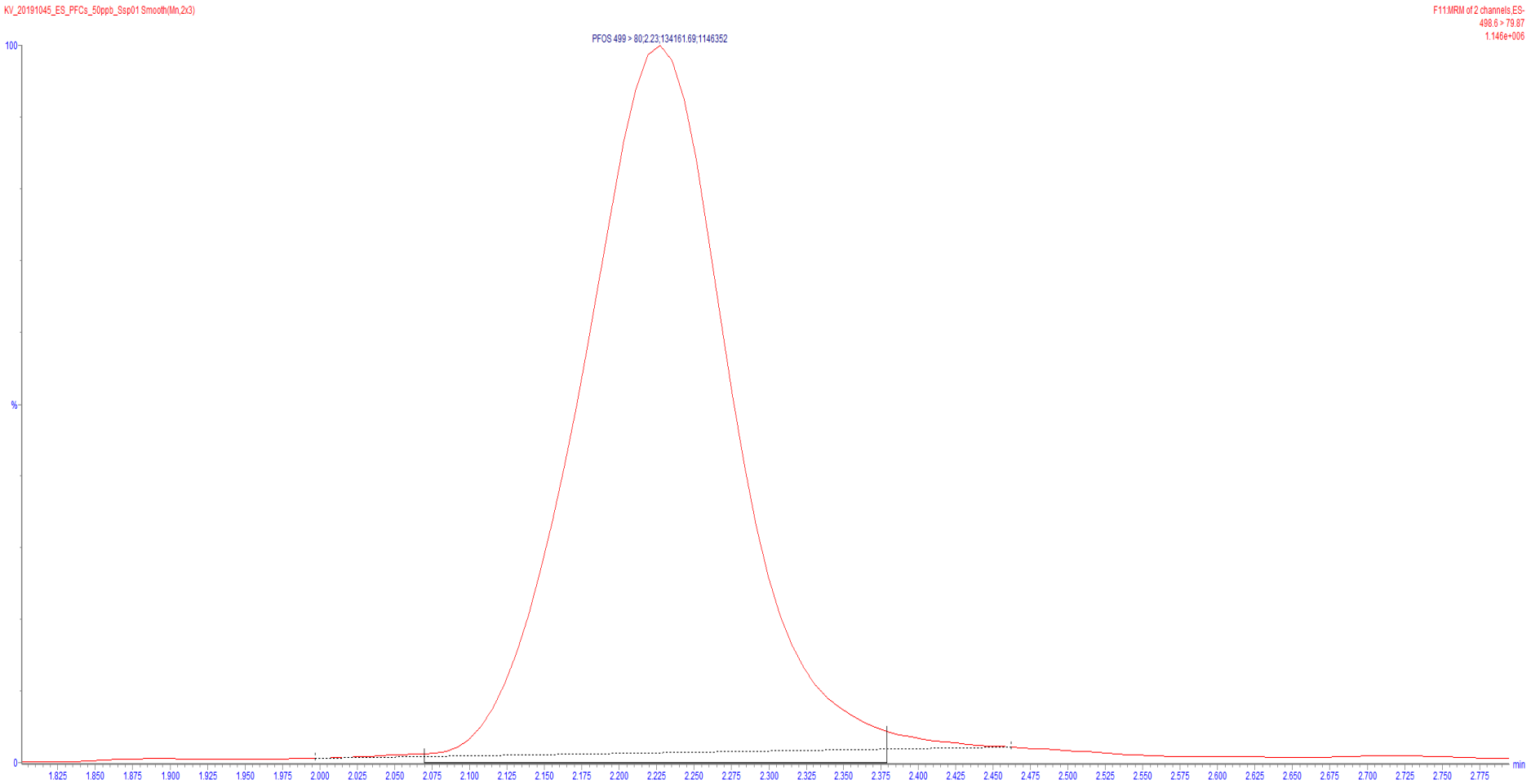


Figure D.6: Secondary transition for PFOS.

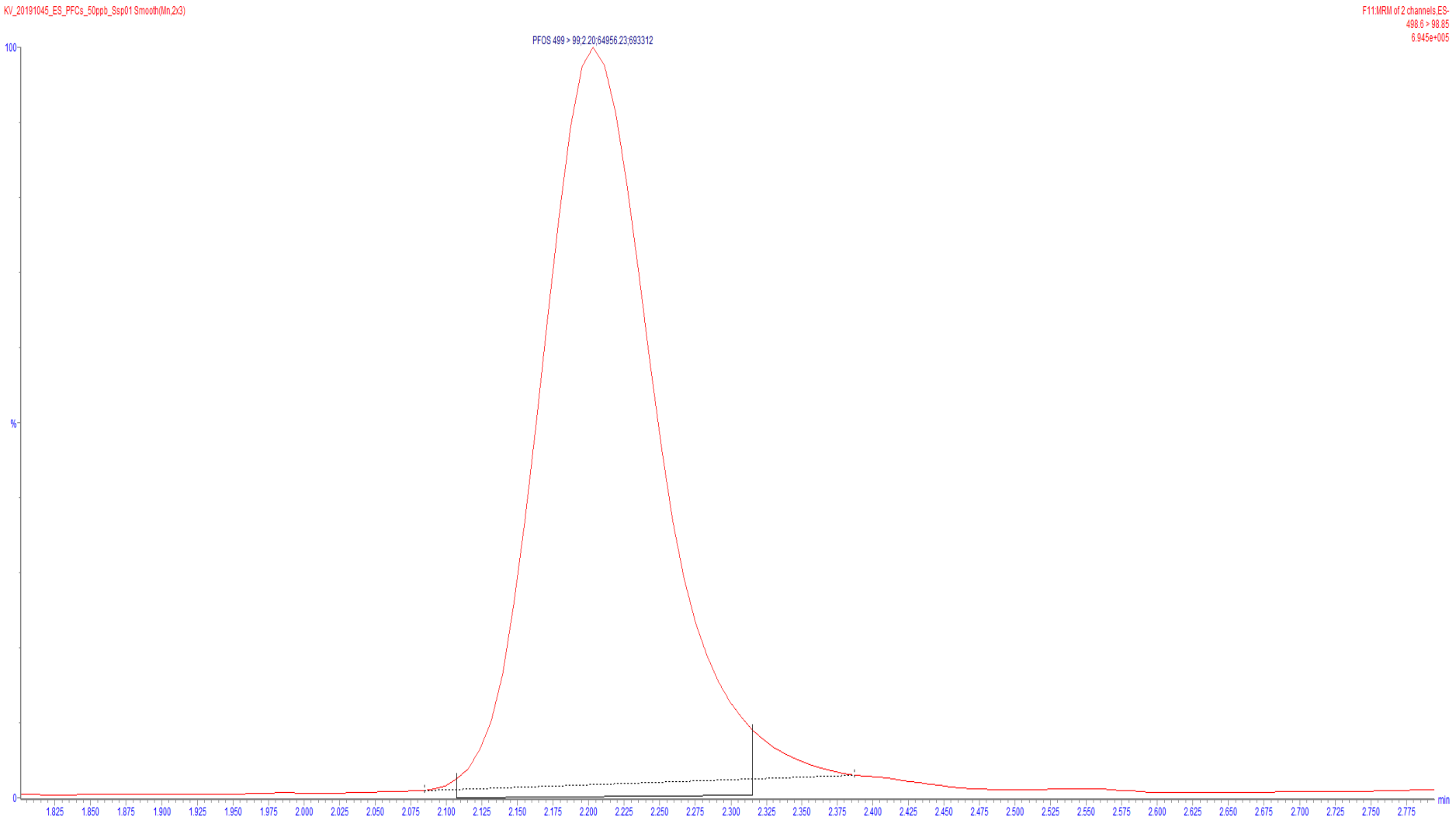


Figure D.7: Primary transition for PFOS.

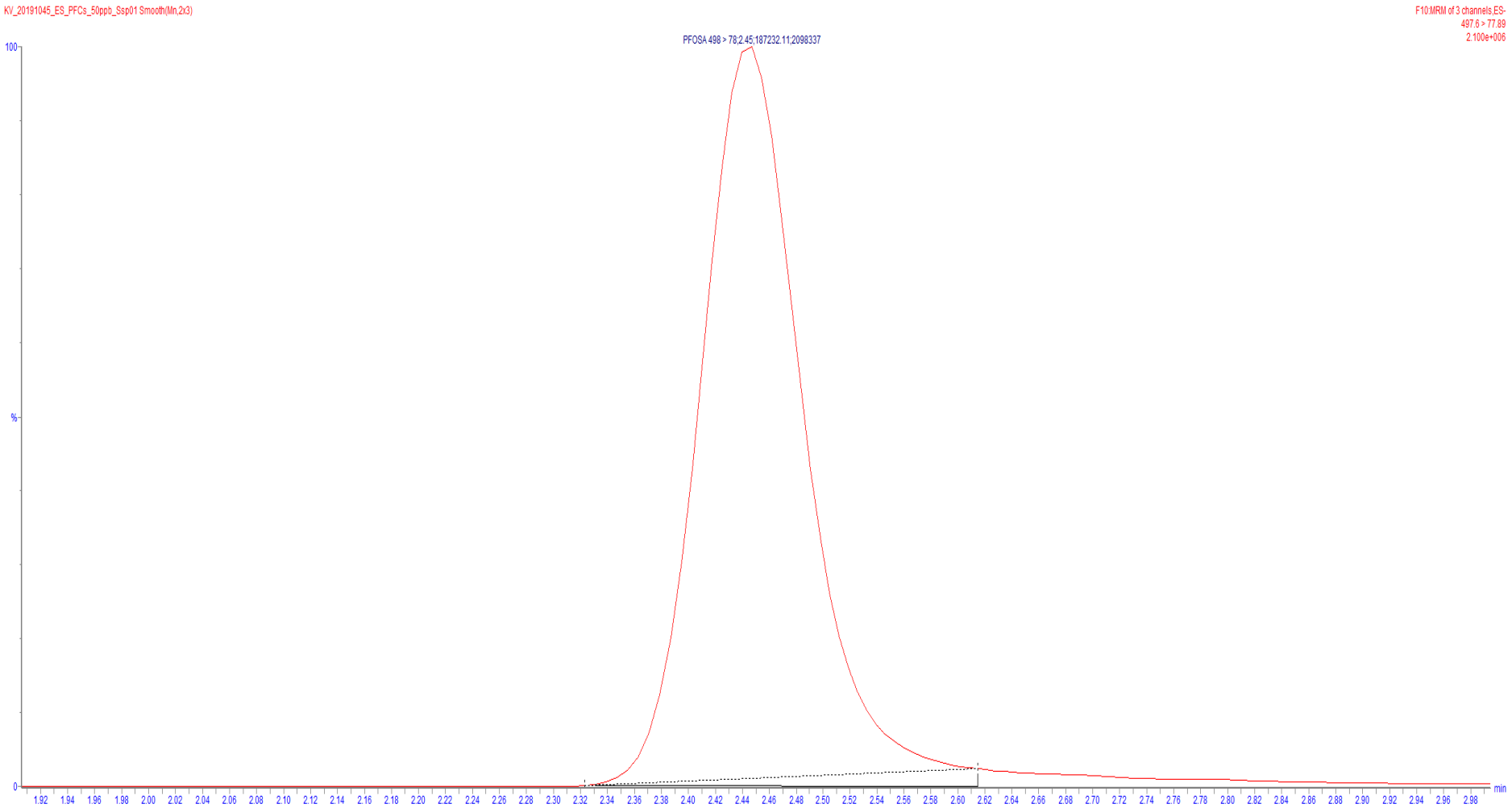


Figure D.8: Secondary transition for PFOSA.

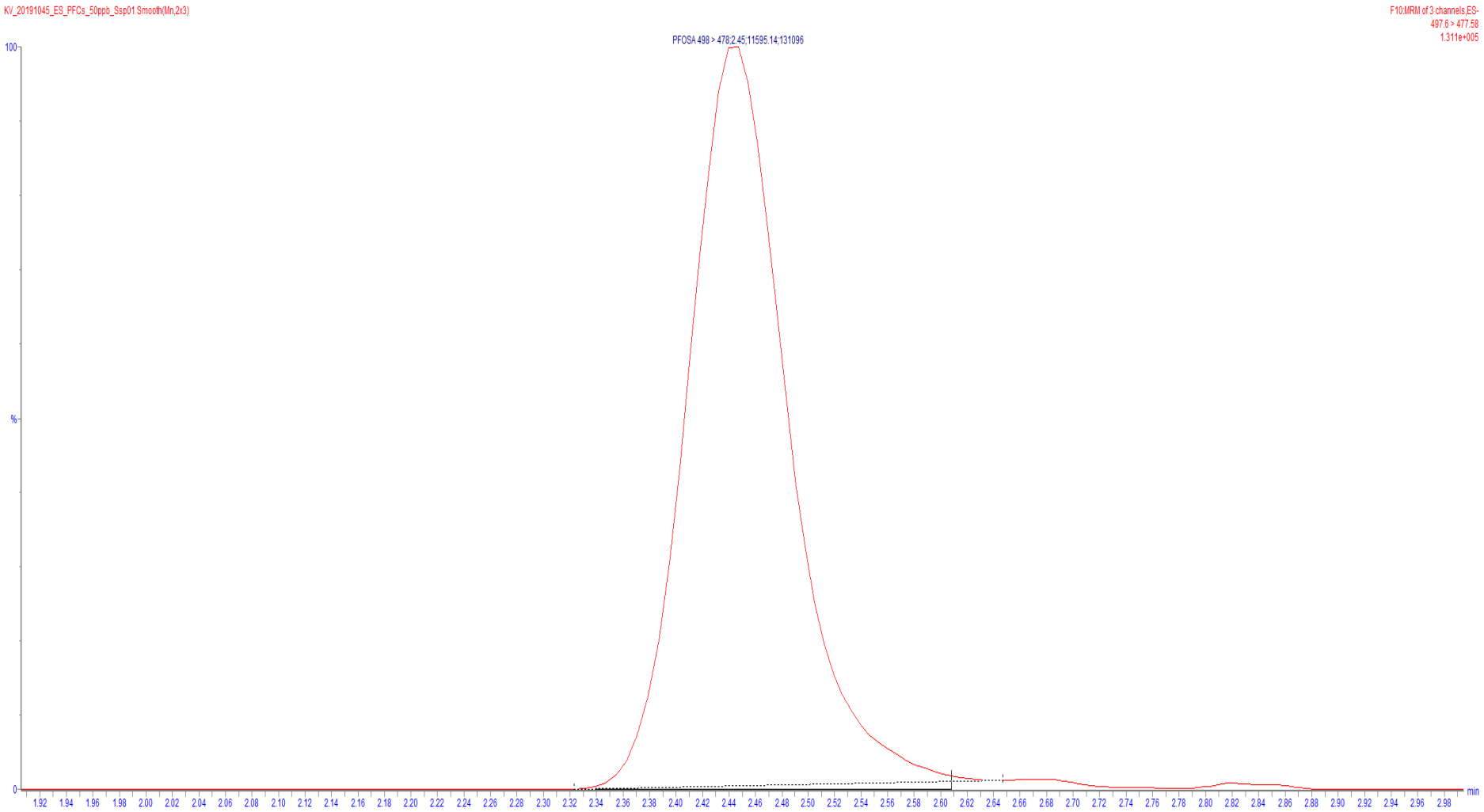


Figure D.9: Primary transition for PFOSA.

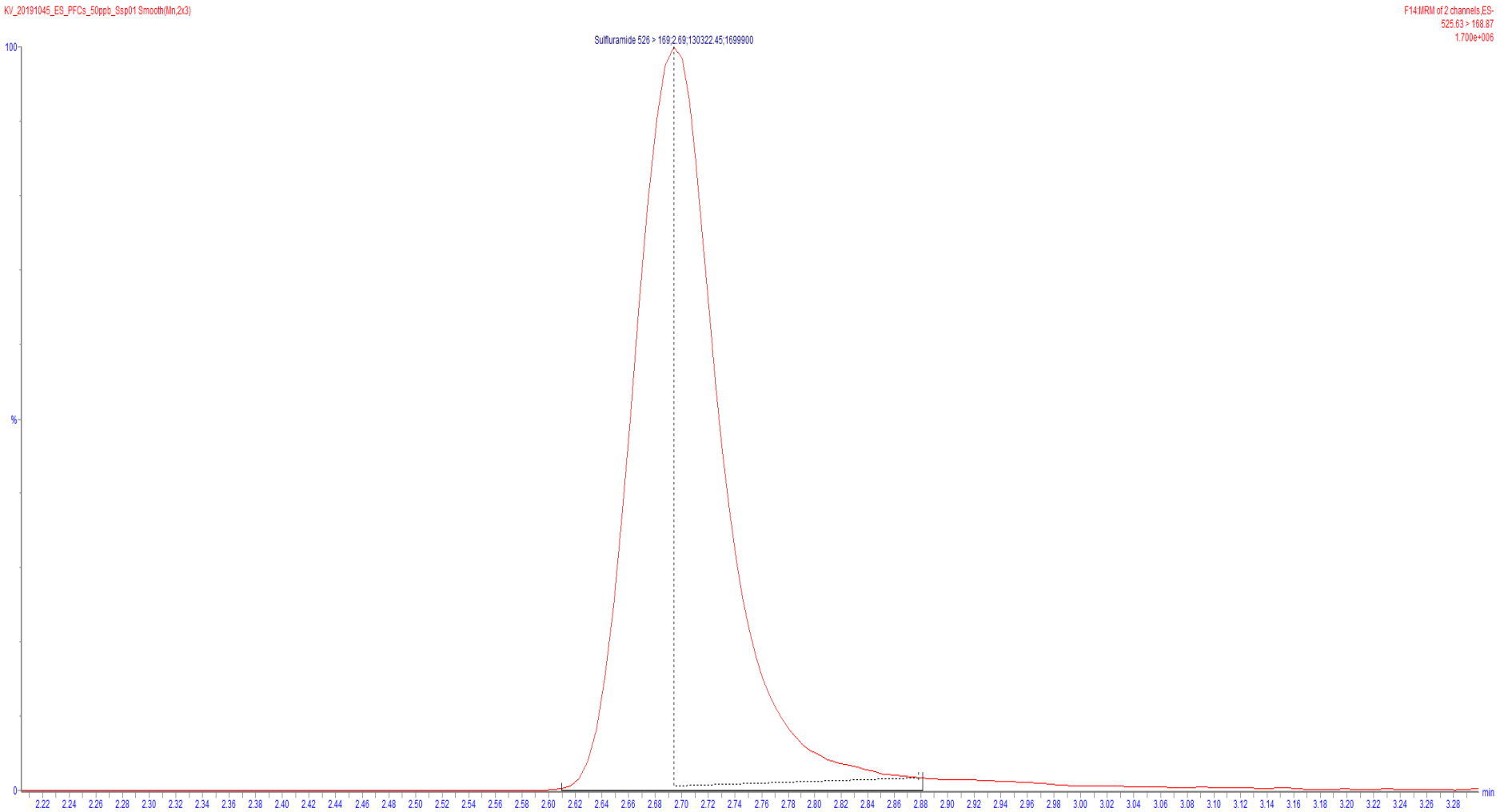


Figure D.10: Secondary transition for Sulf.

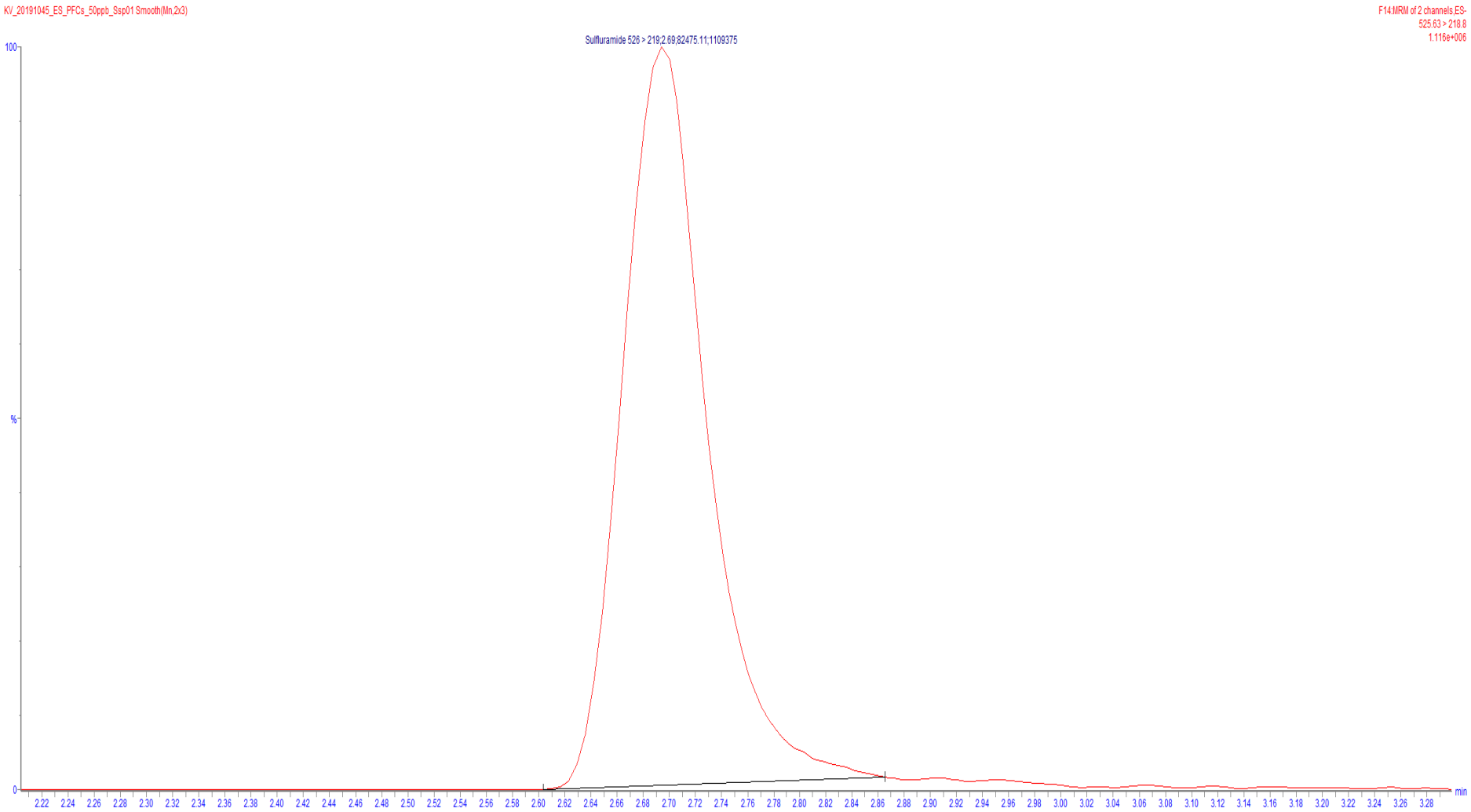


Figure D.11: Primary transition for Sulf.

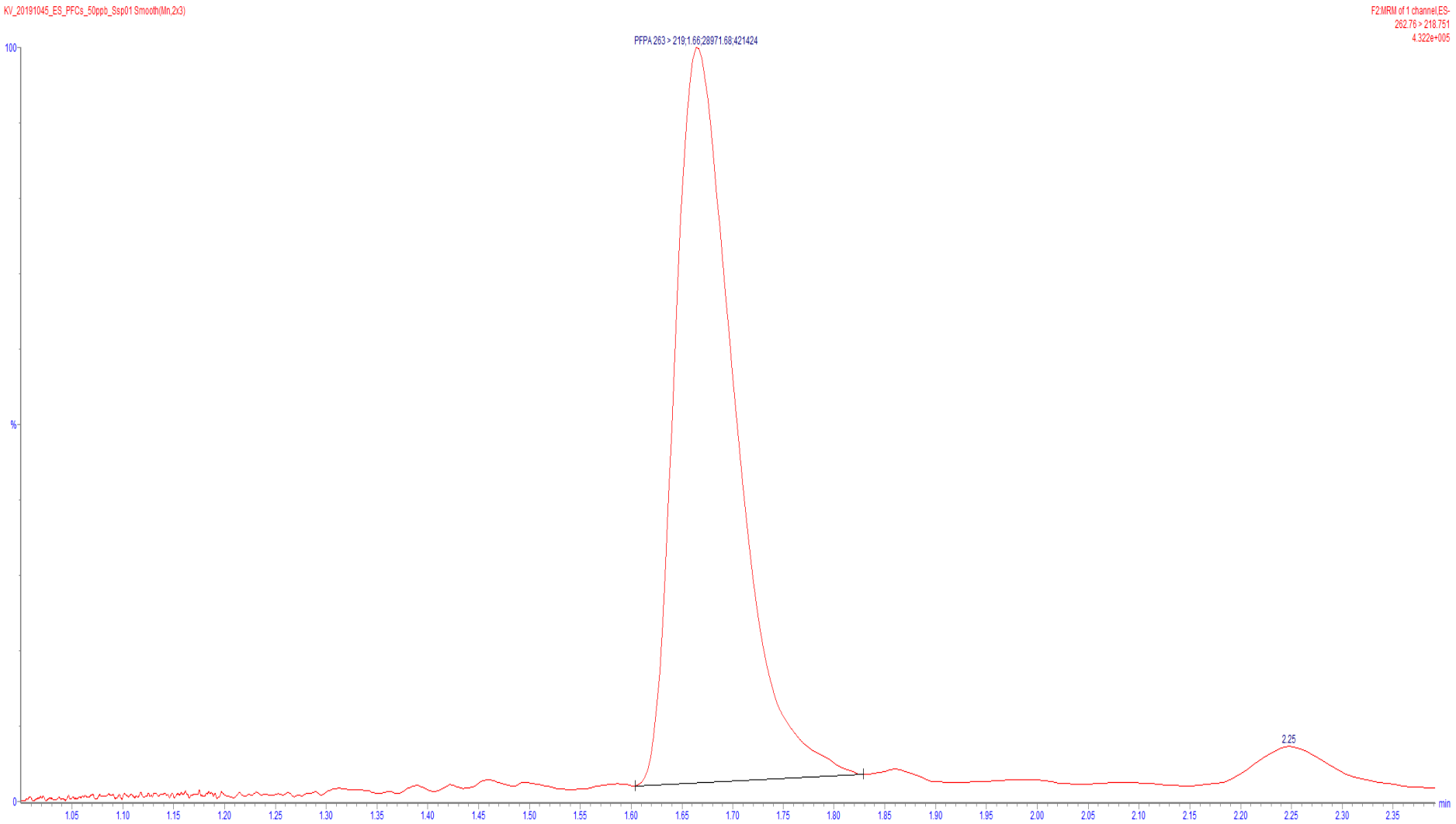


Figure D.12: Primary transition only, for PFPA.

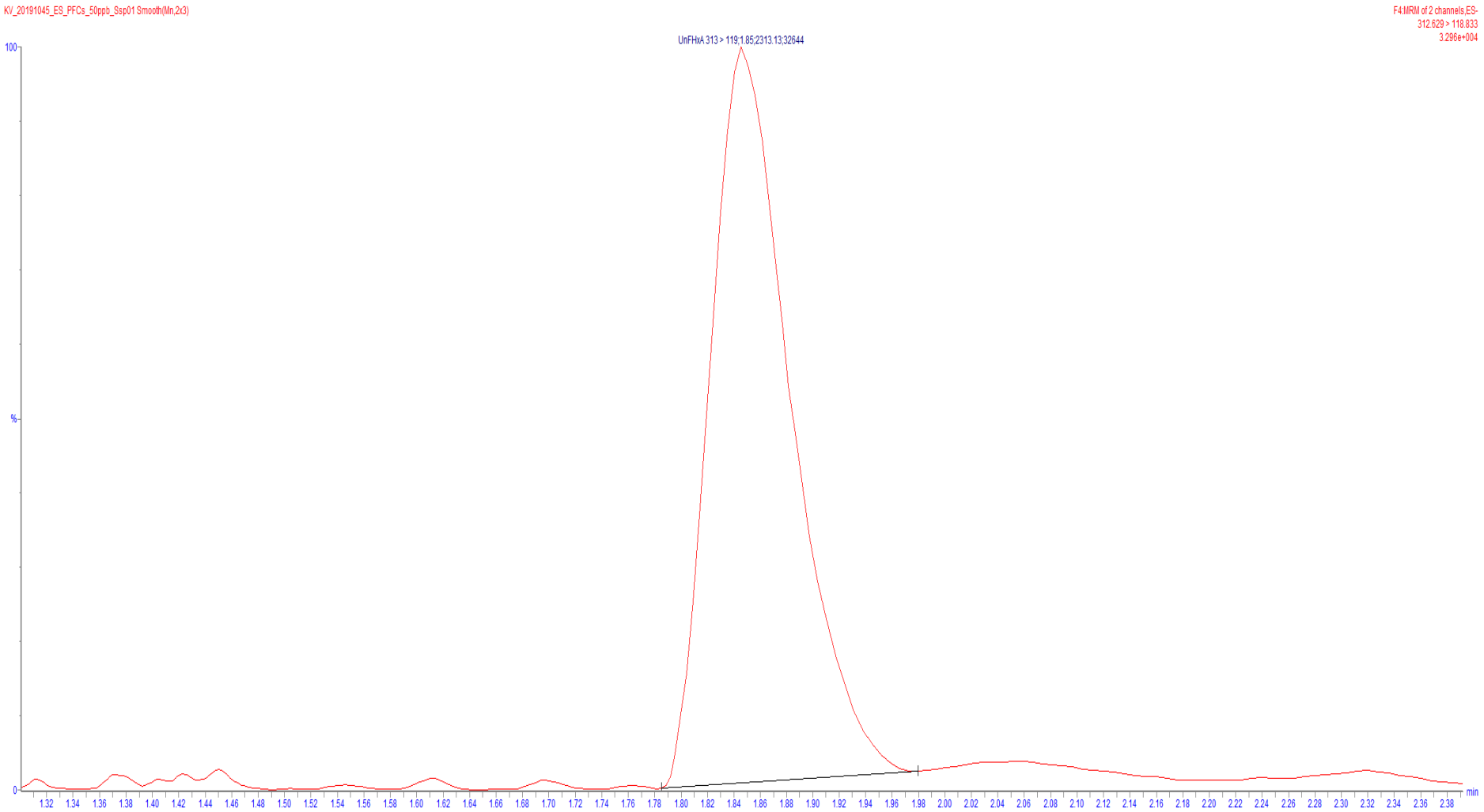


Figure D.13: Secondary transition for PFHxA.

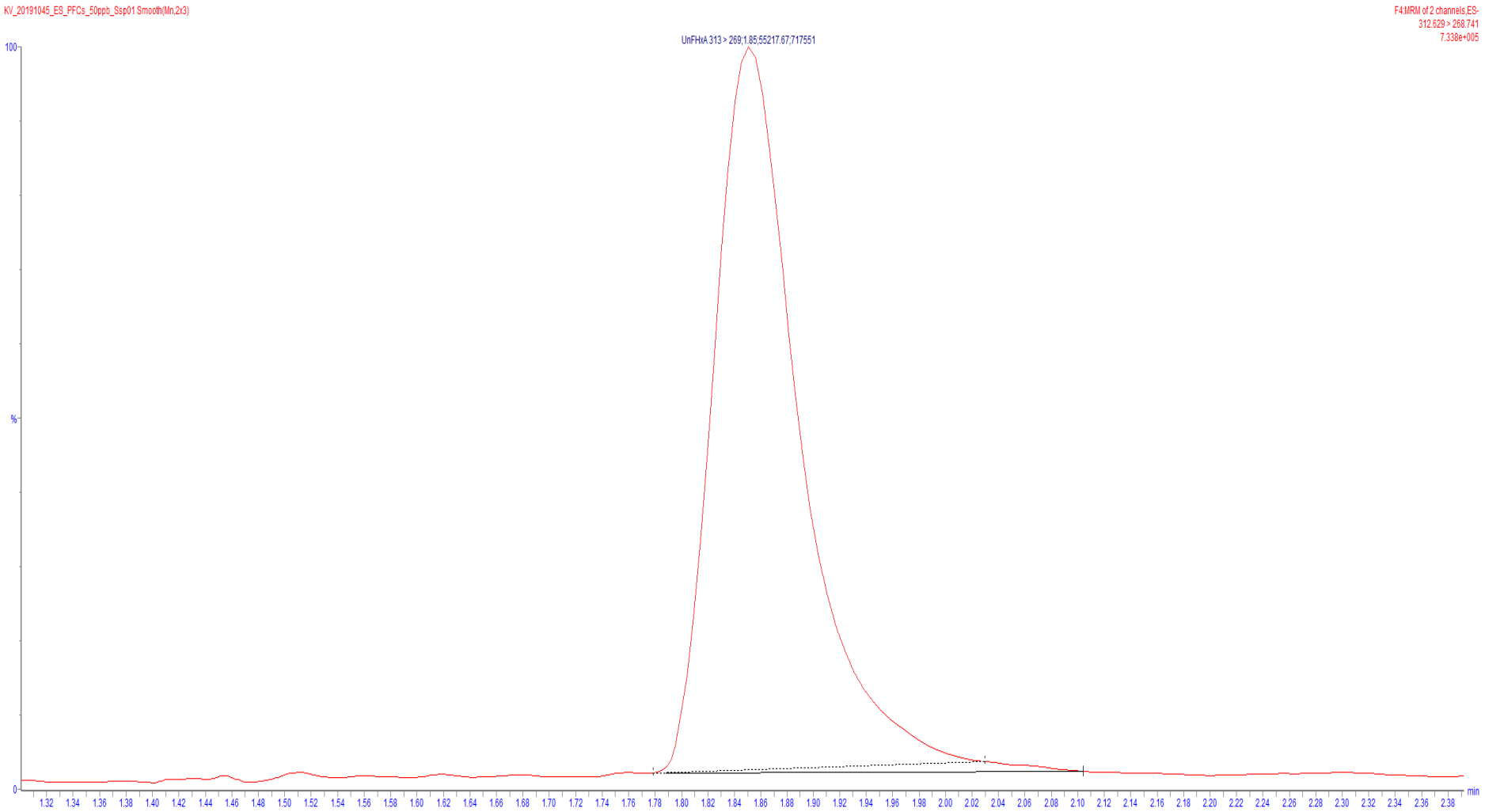


Figure D.14: Primary transition for PFHxA.

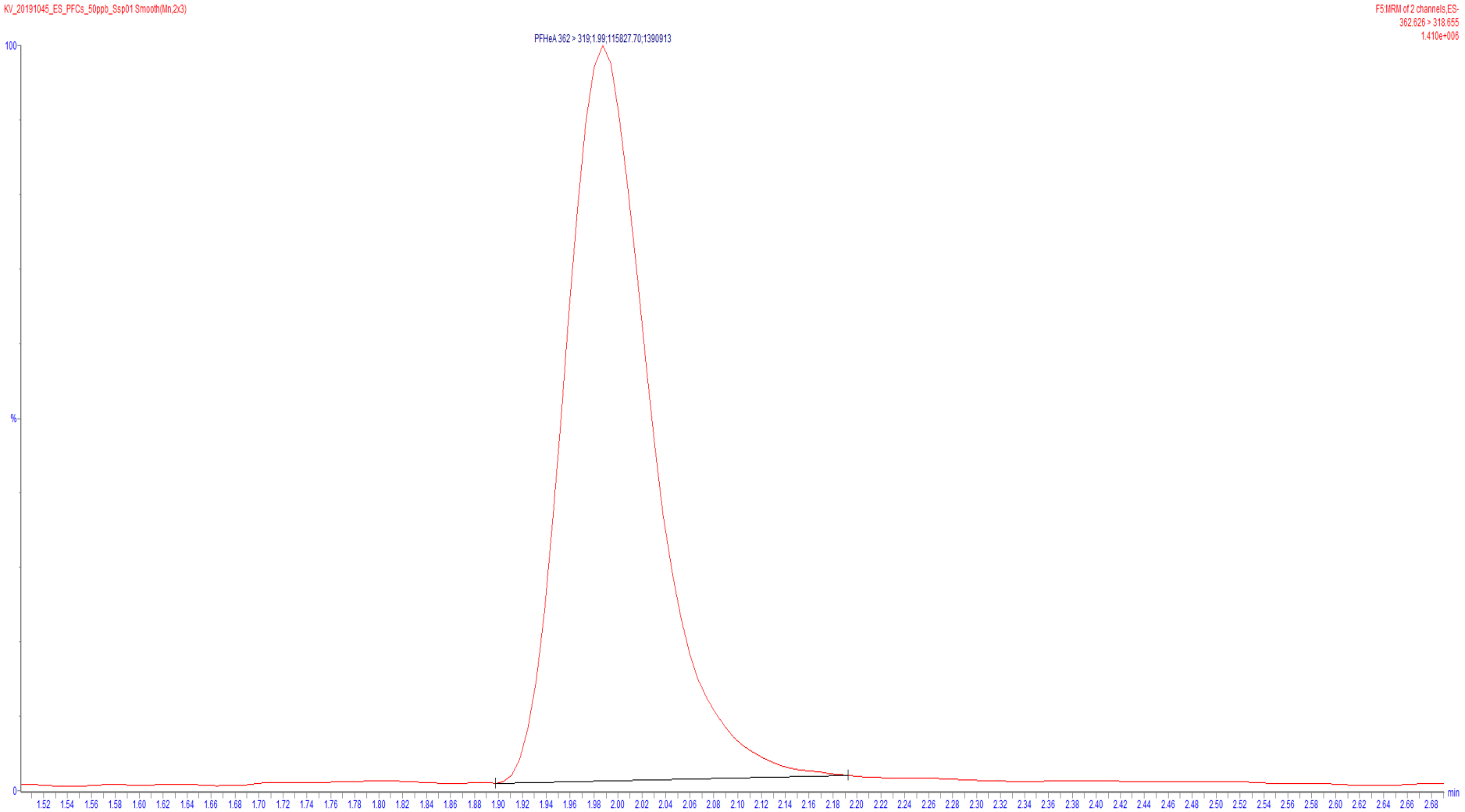


Figure D.15: Primary transition for PFHeA.

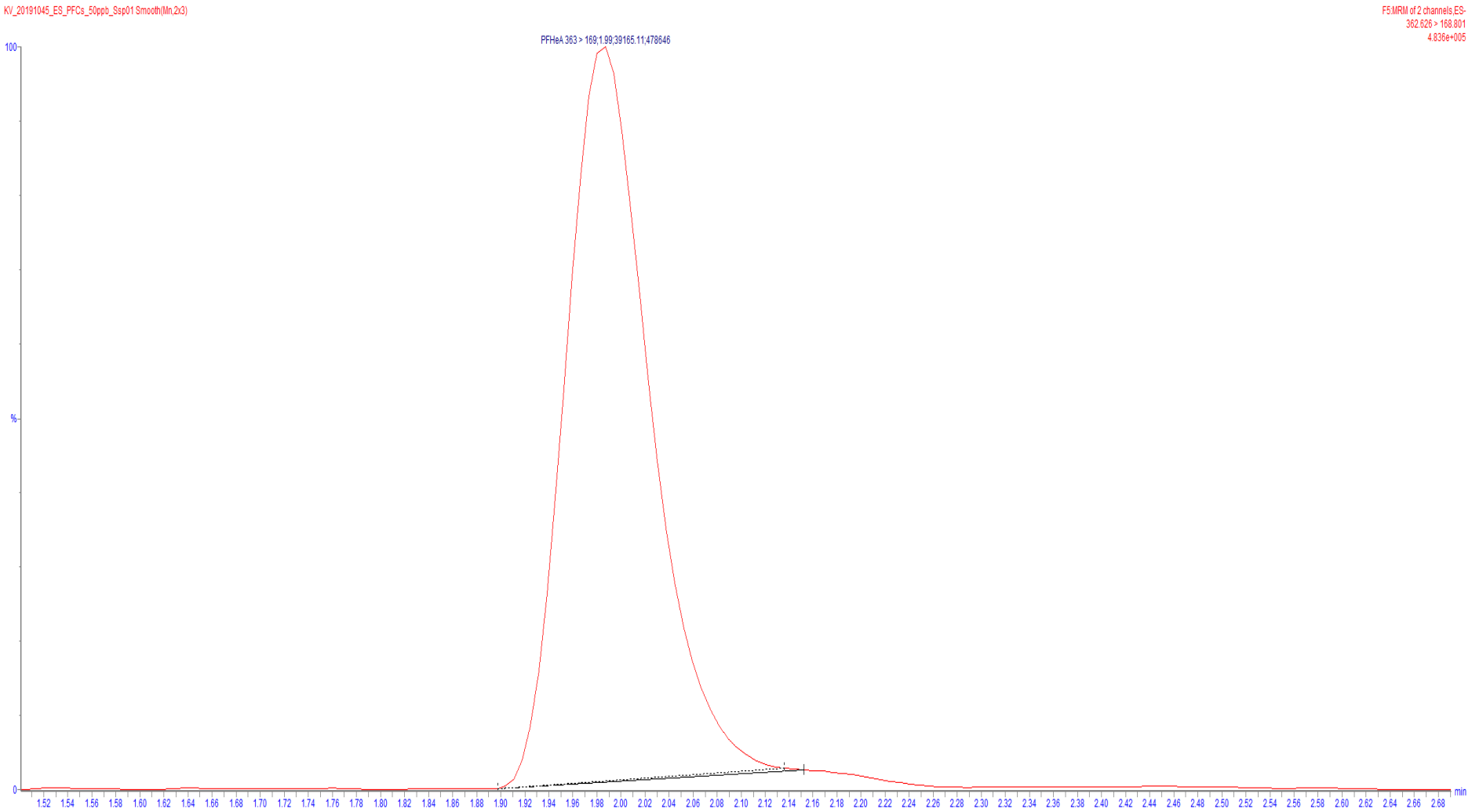


Figure D.16: Secondary transition for PFHeA.

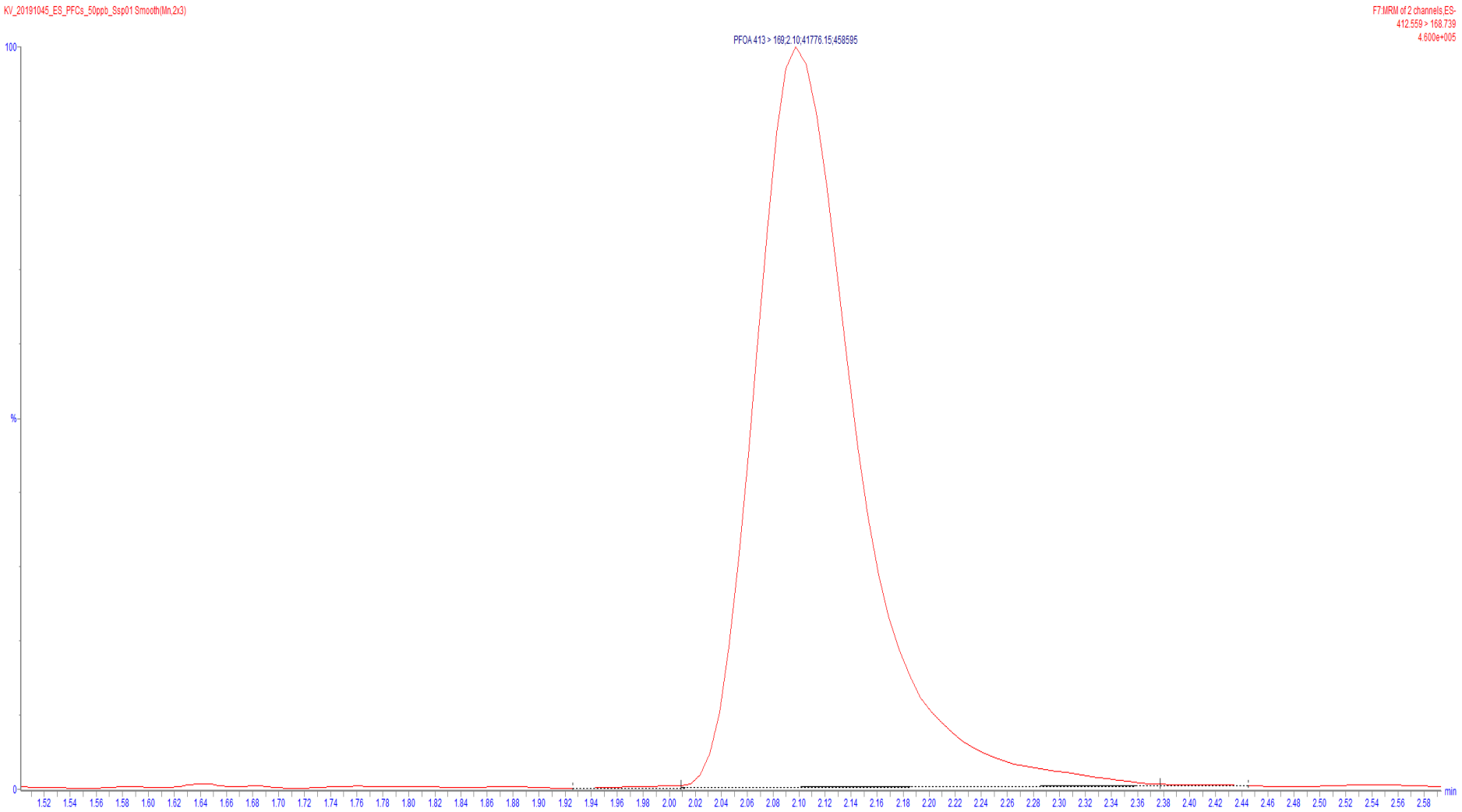


Figure D.17: Secondary transition for PFOA.

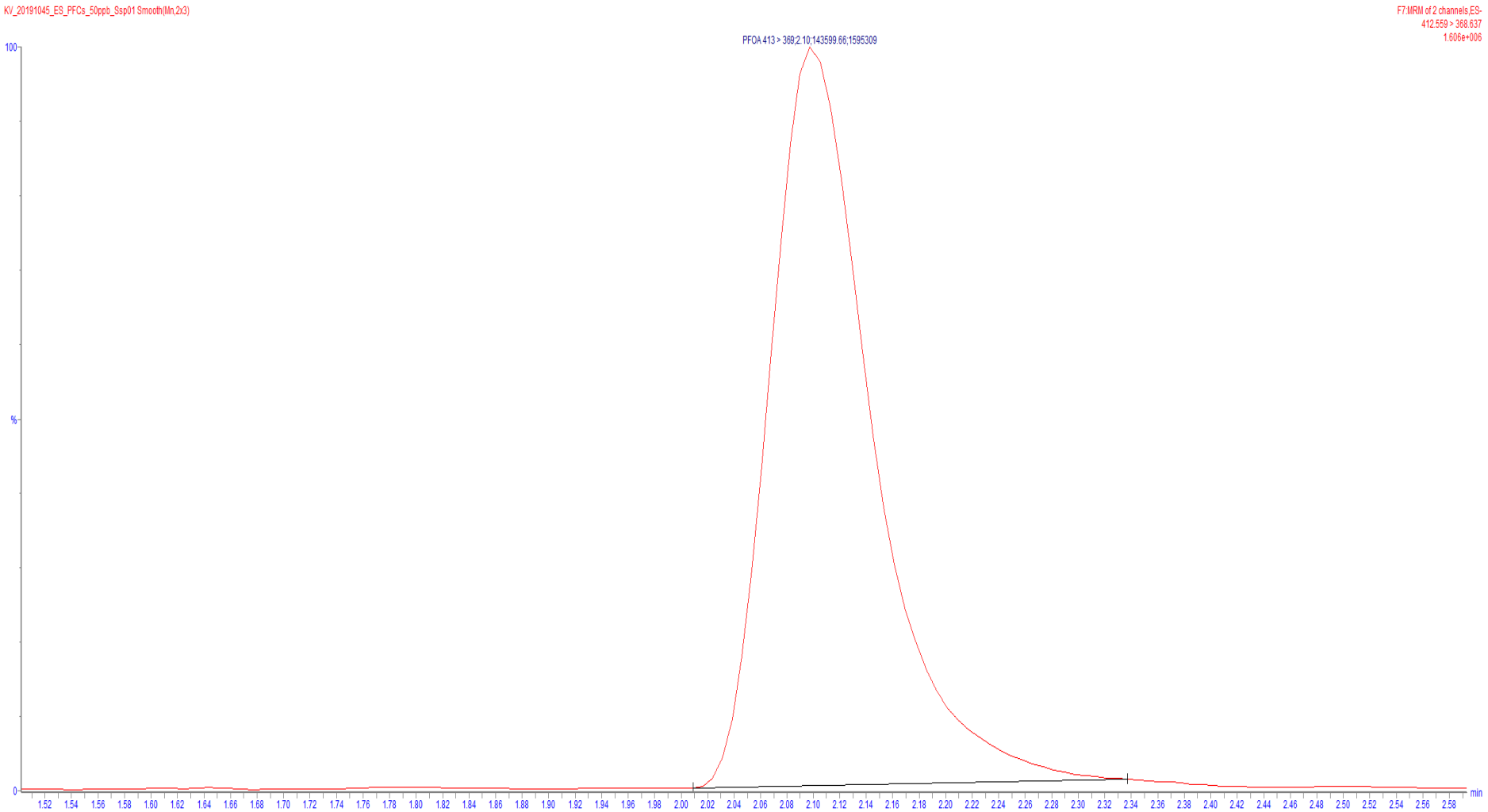


Figure D.18: Primary transition for PFOA.

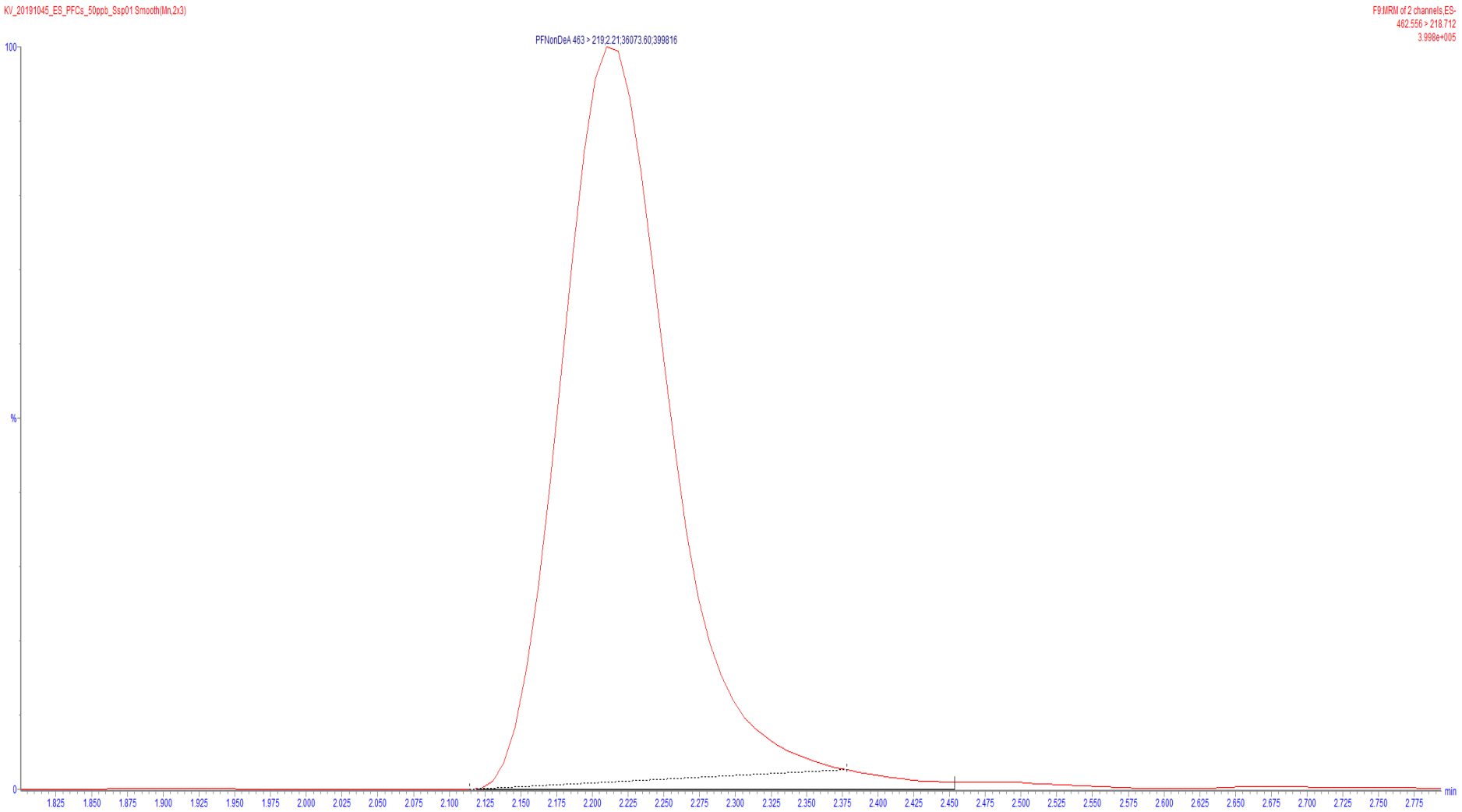


Figure D.19: Secondary transition for PFNA.

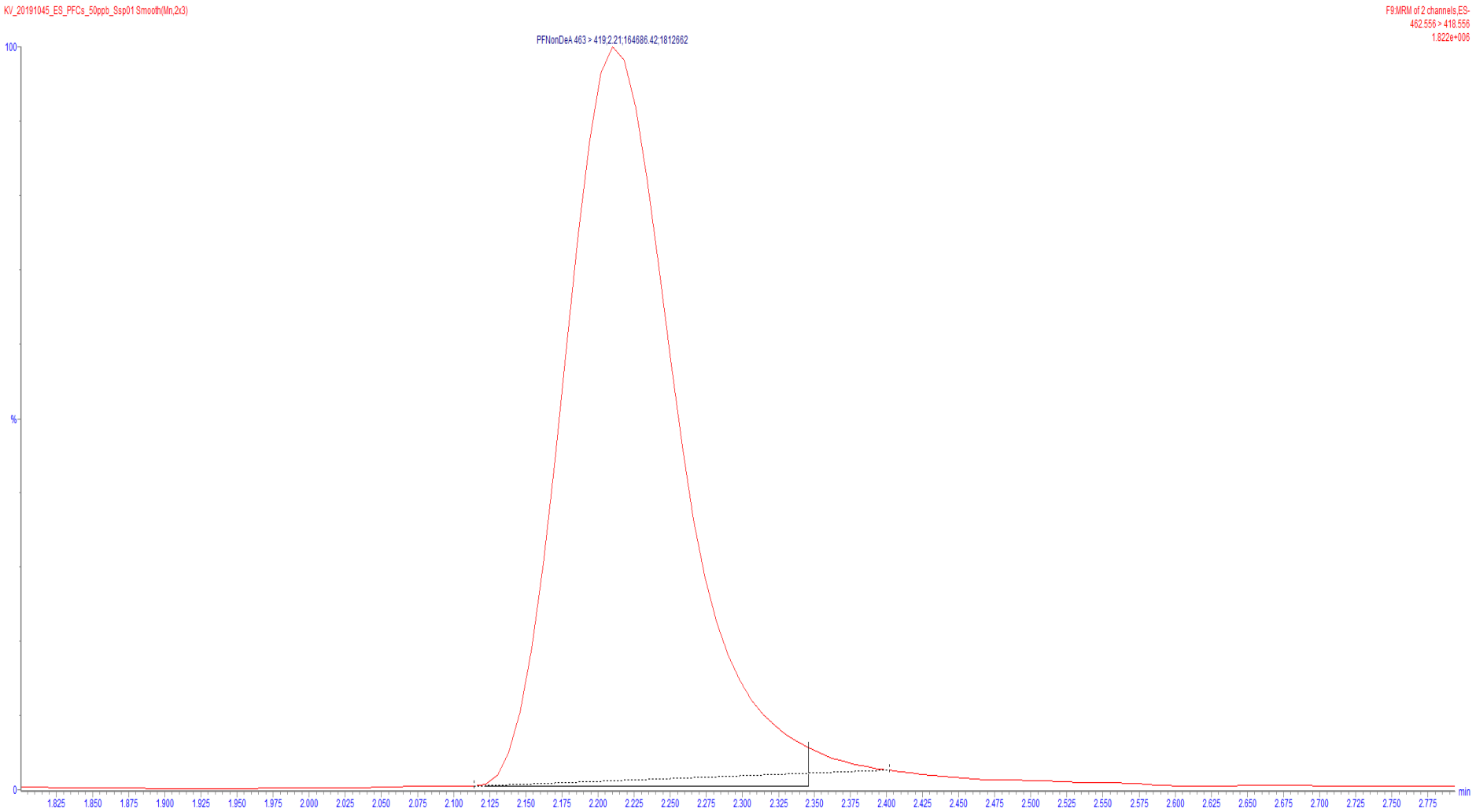


Figure D.20: Primary transition for PFNA.

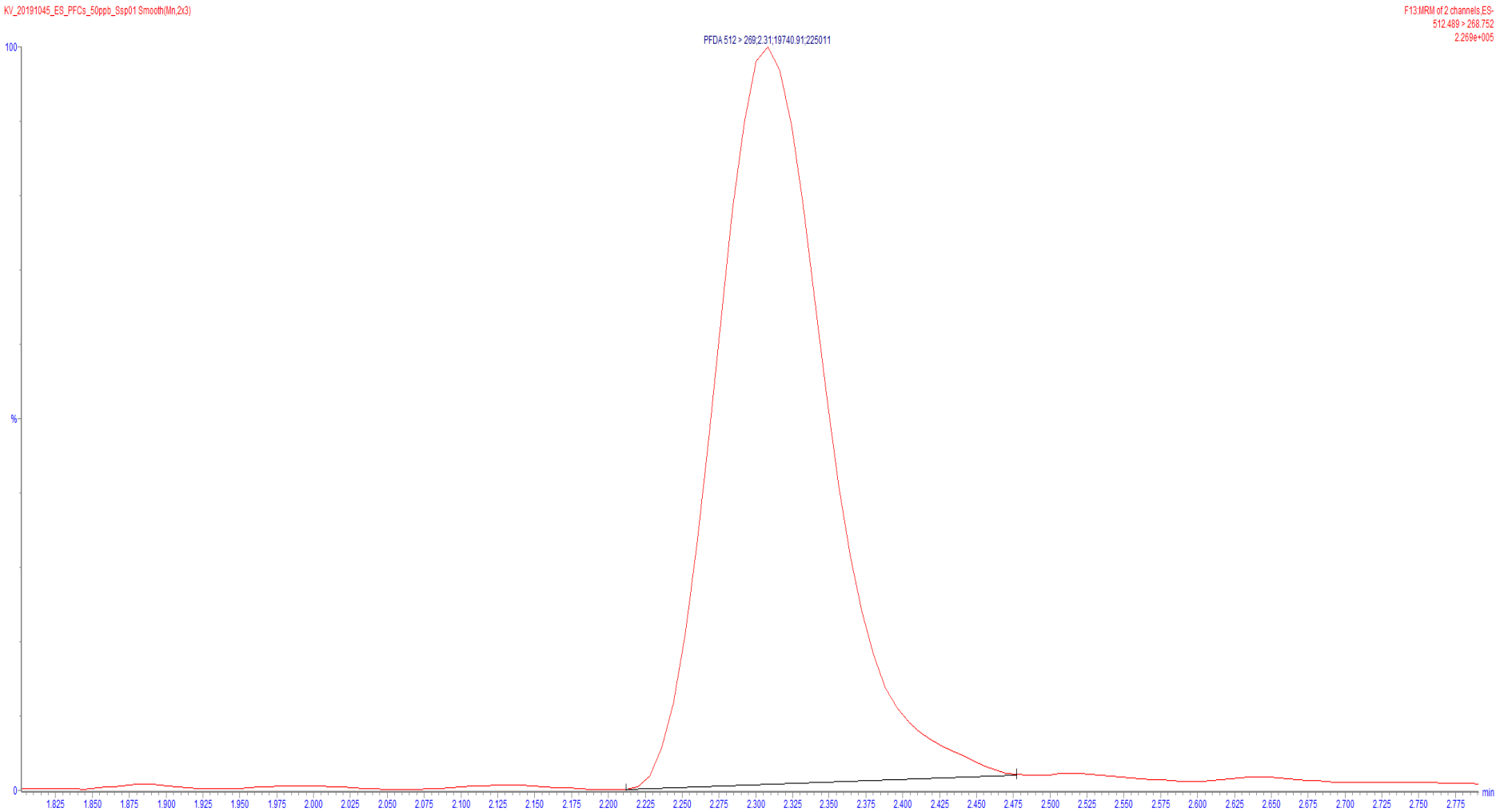


Figure D.21: Secondary transition for PFDA.

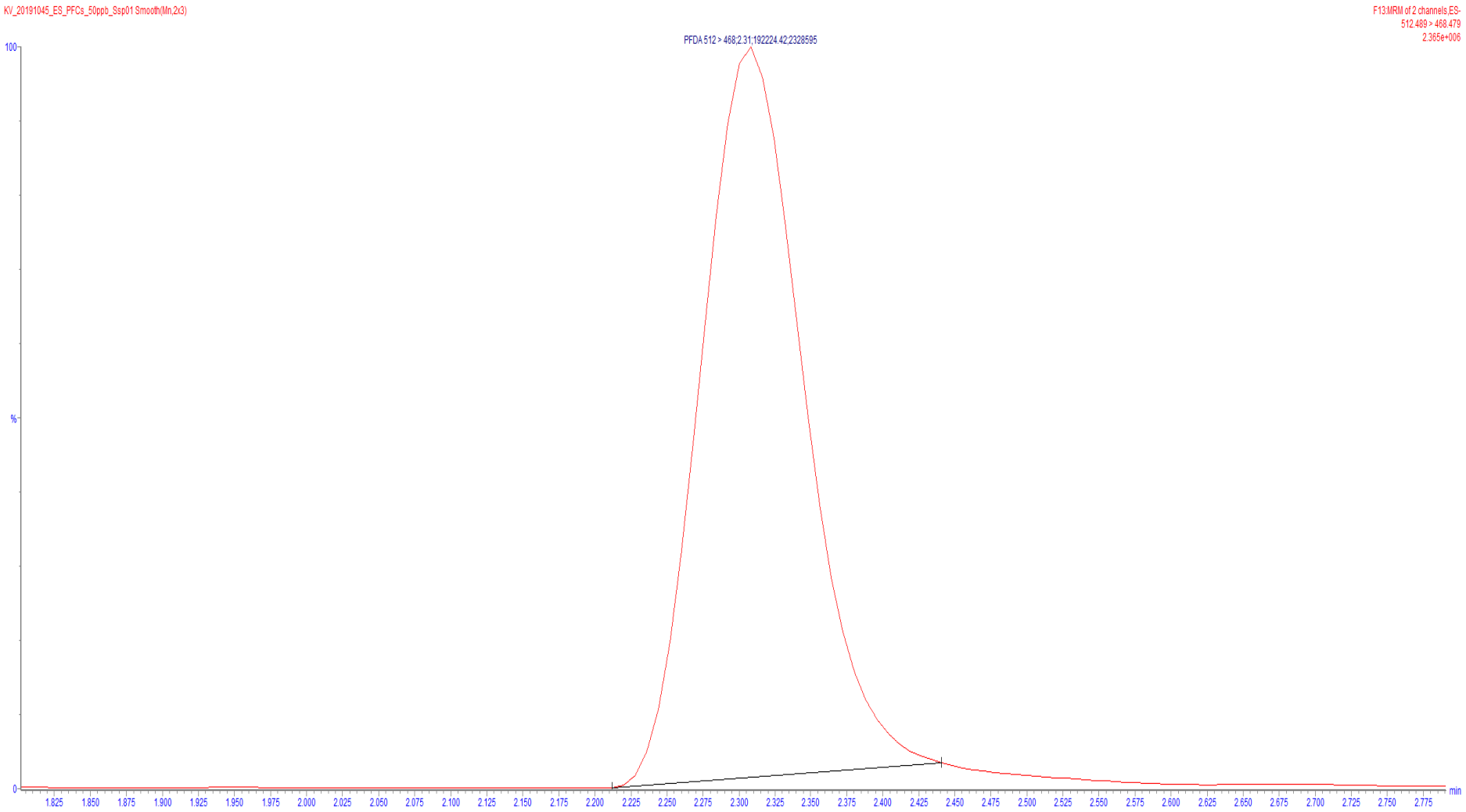


Figure D.22: Primary transition for PFDA.

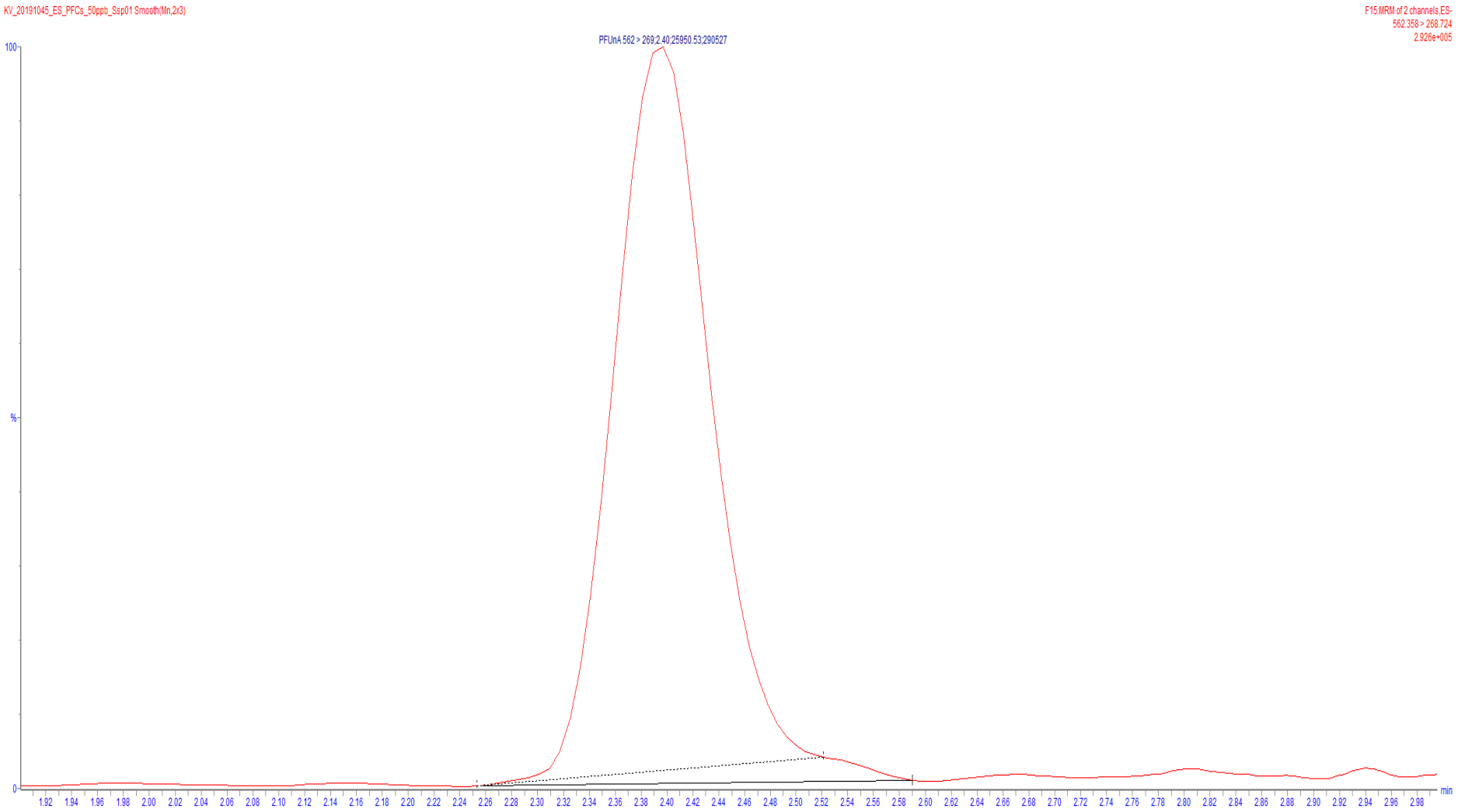


Figure D.23: Secondary transition for PFUnA.

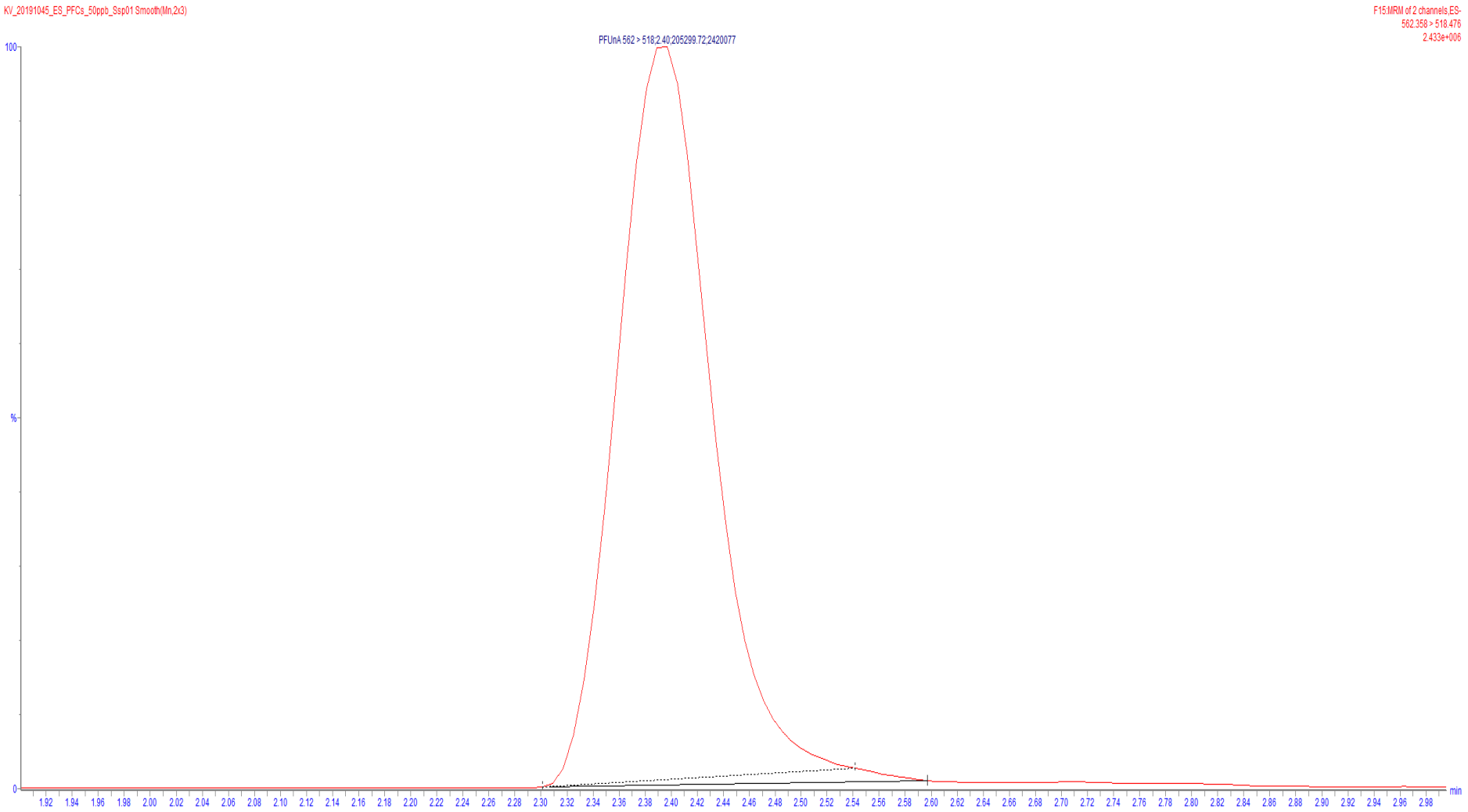


Figure D.24: Primary transition for PFUnA.

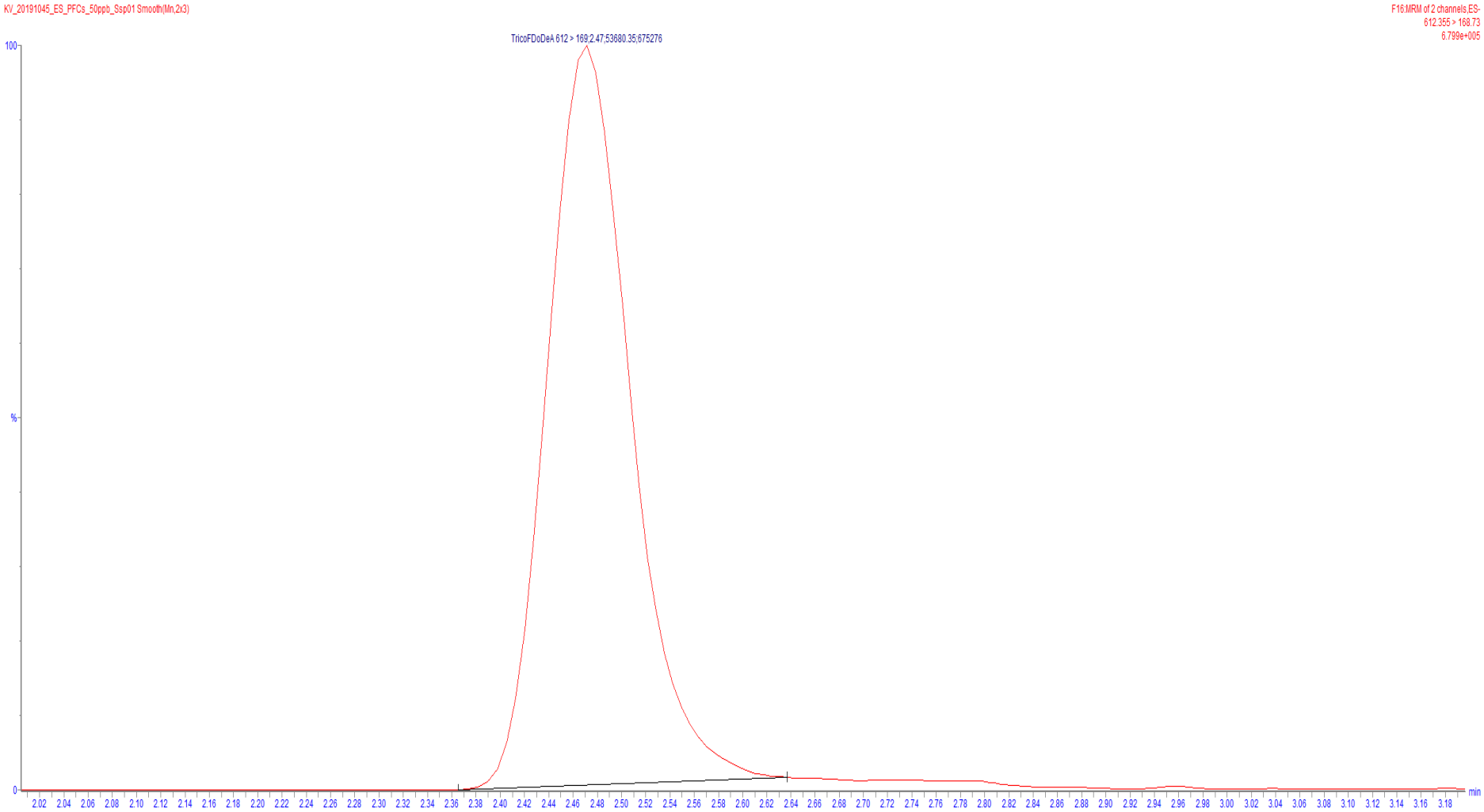


Figure D.25: Secondary transition for PFDoDeA.

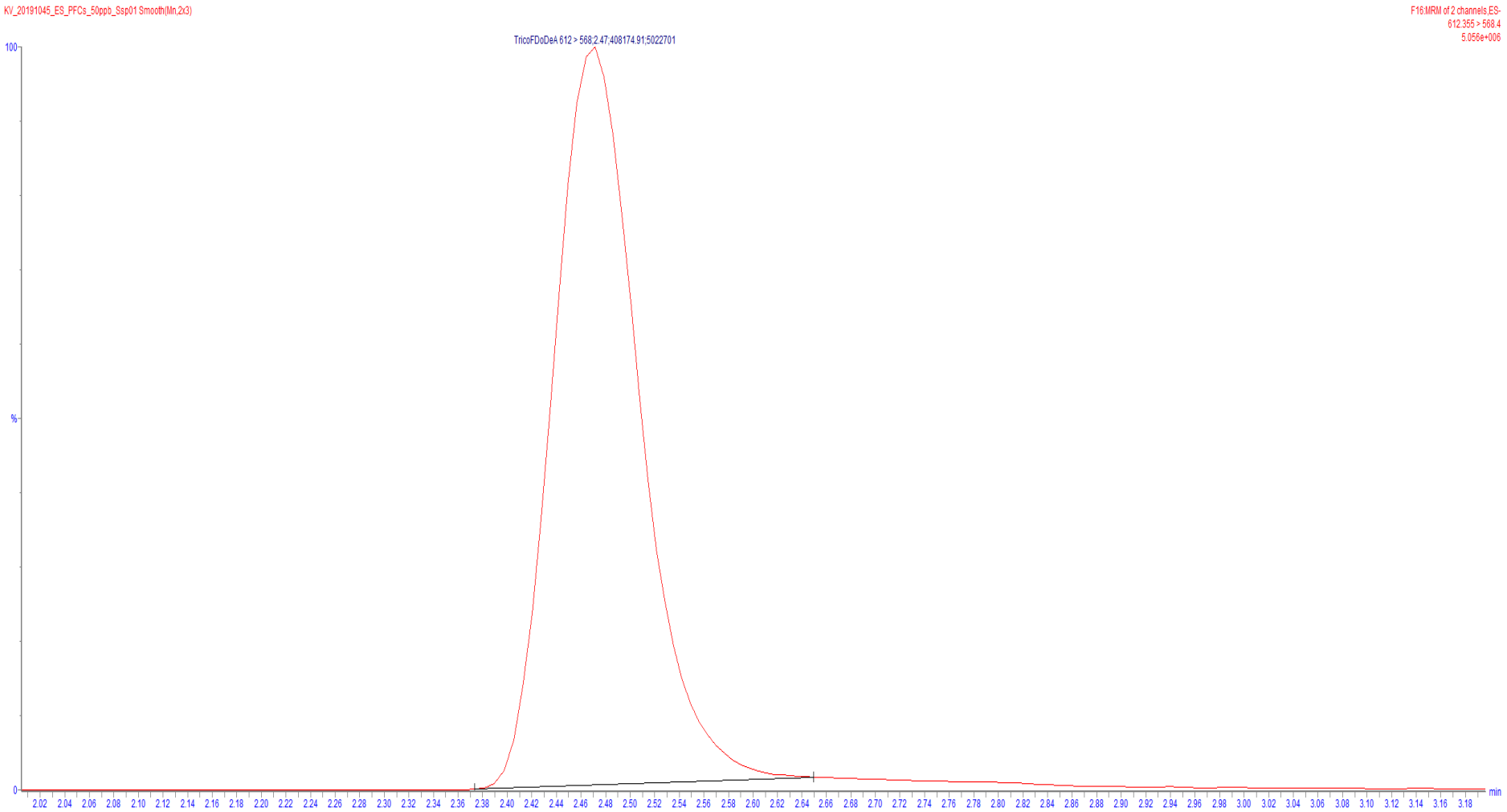


Figure D.26: Primary transition for PFDoDeA.

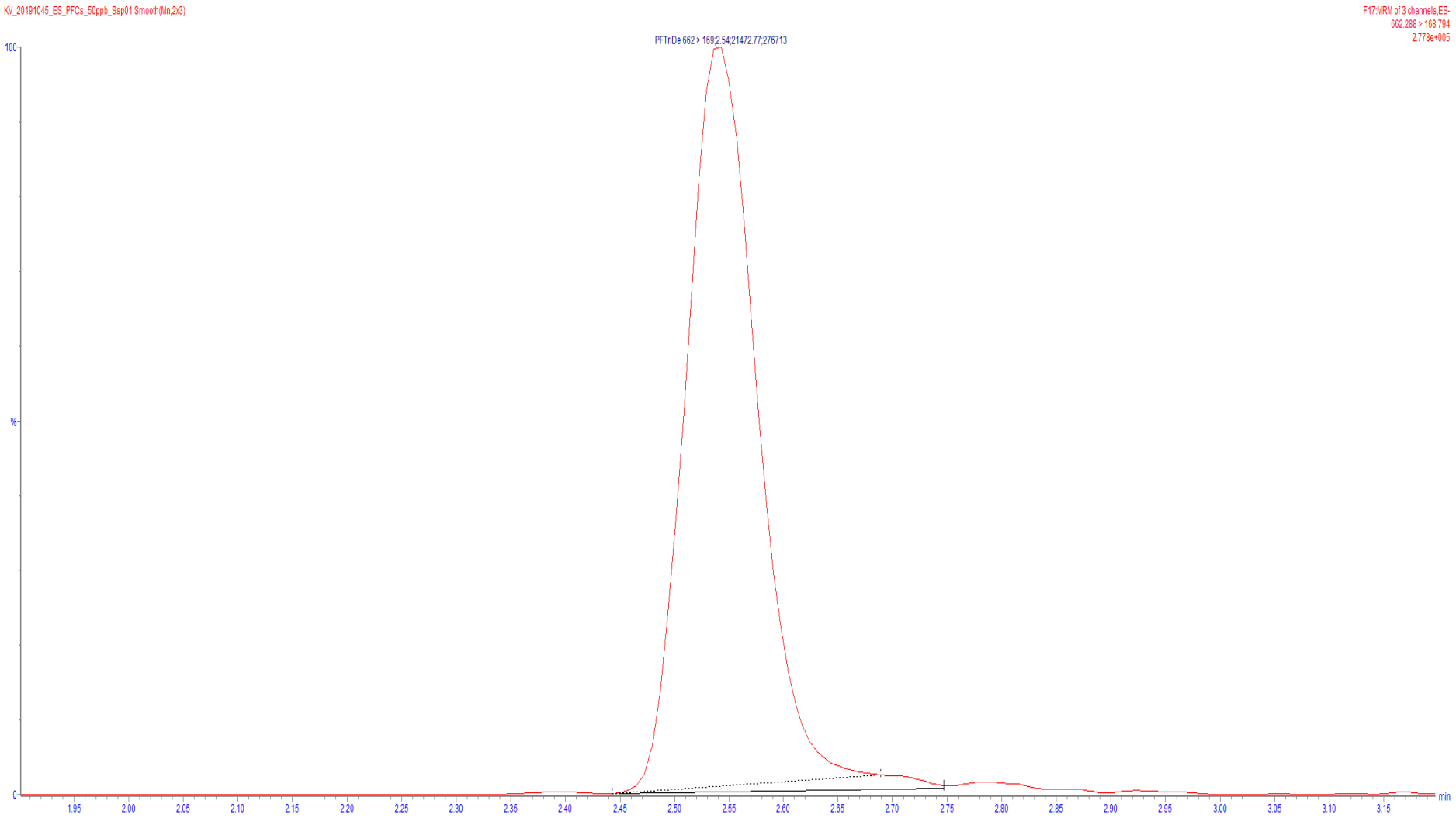


Figure D.27: Secondary transition for PFTriDe.

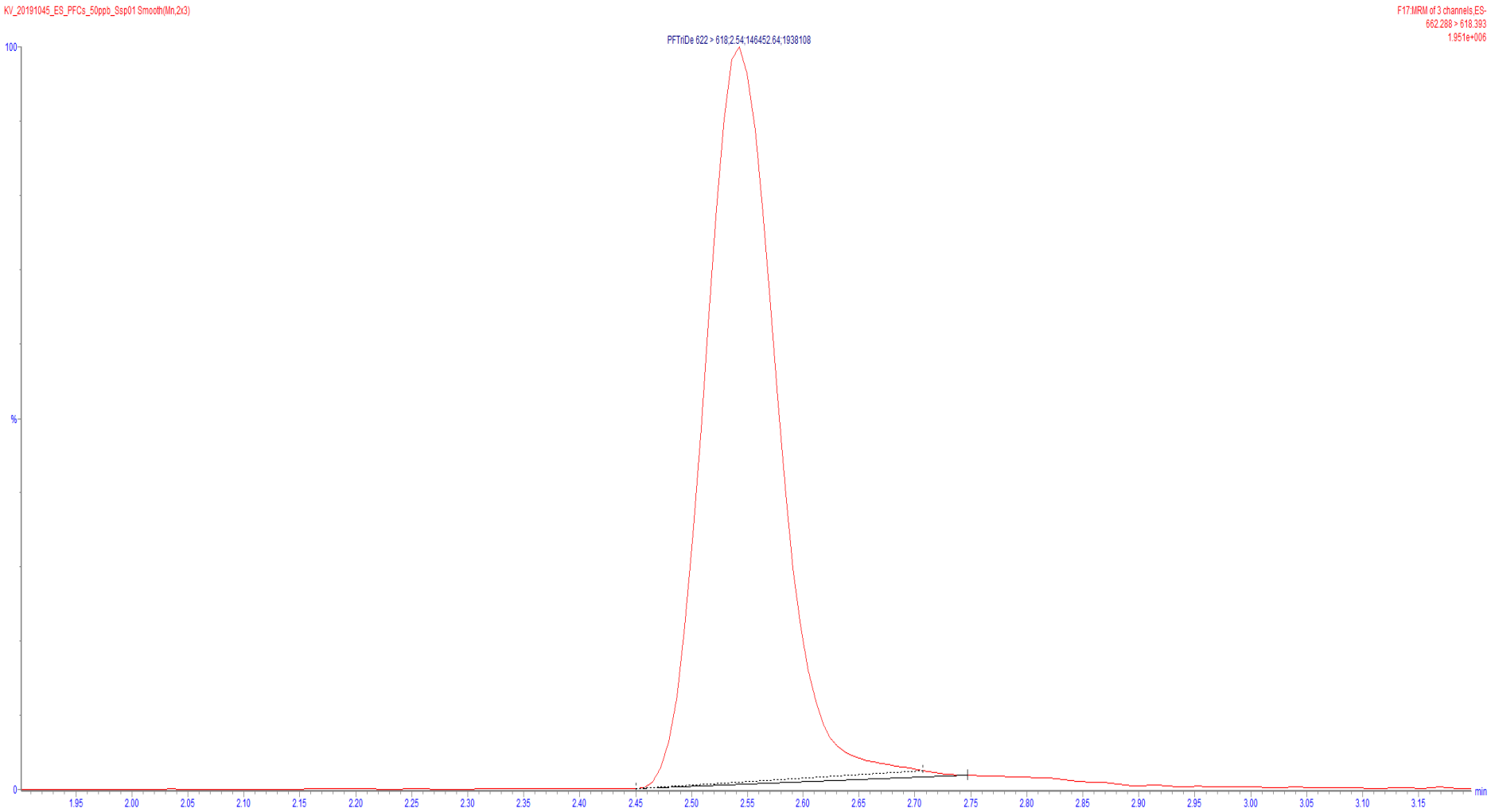


Figure D.28: Primary transition for PFTriDe.

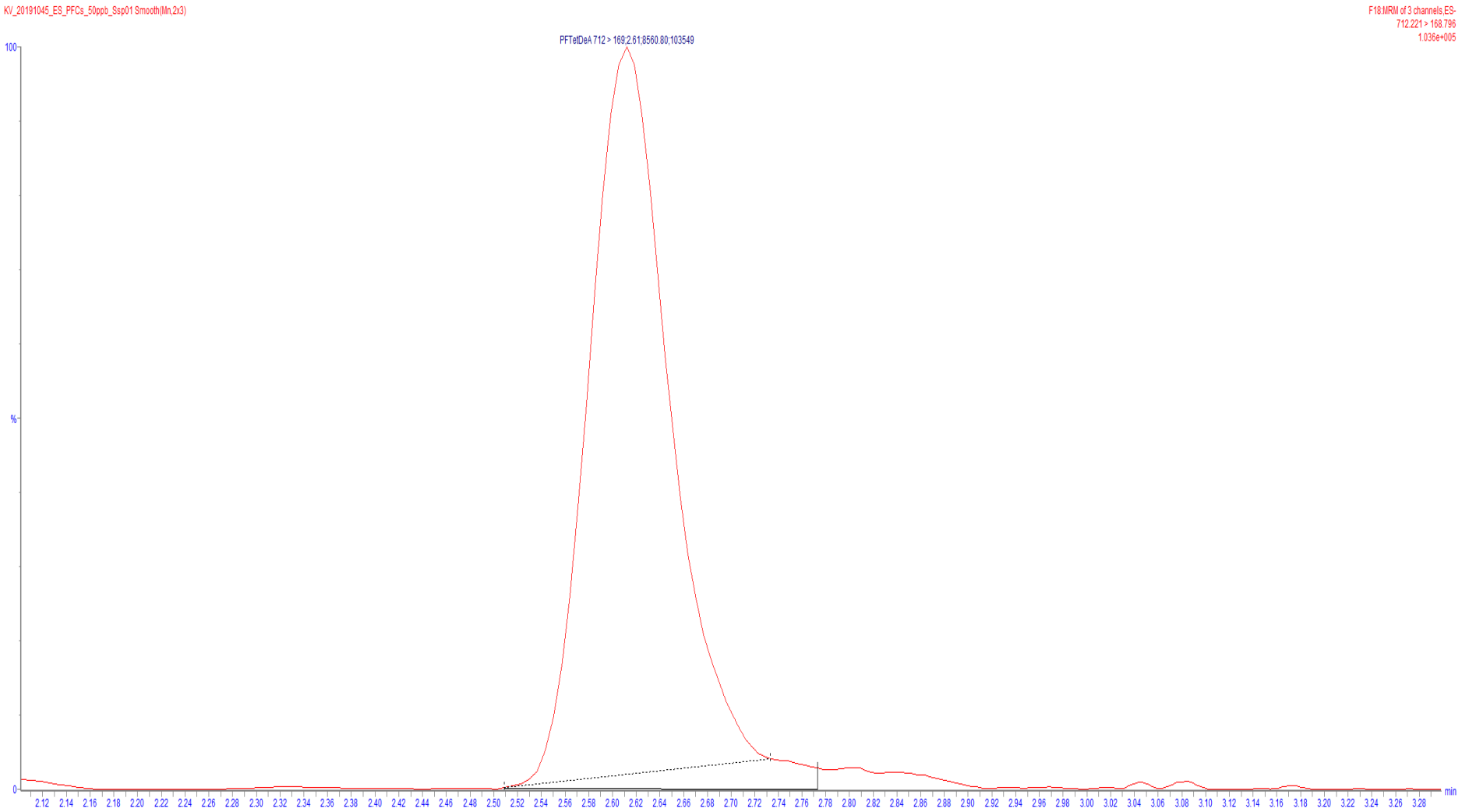


Figure D.29: Secondary transition for PFTetDeA.

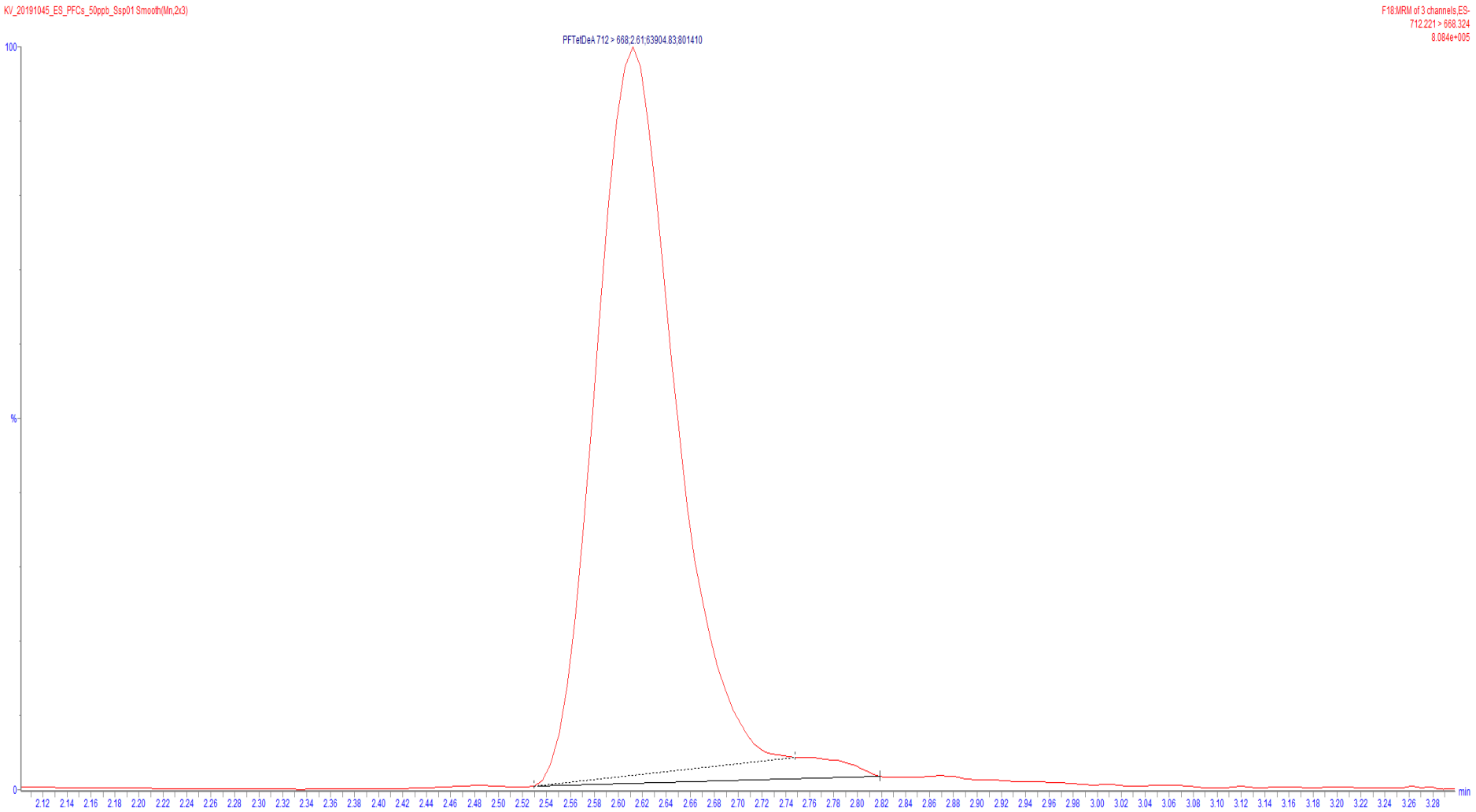


Figure D.30: Primary transition for PFTetDeA.

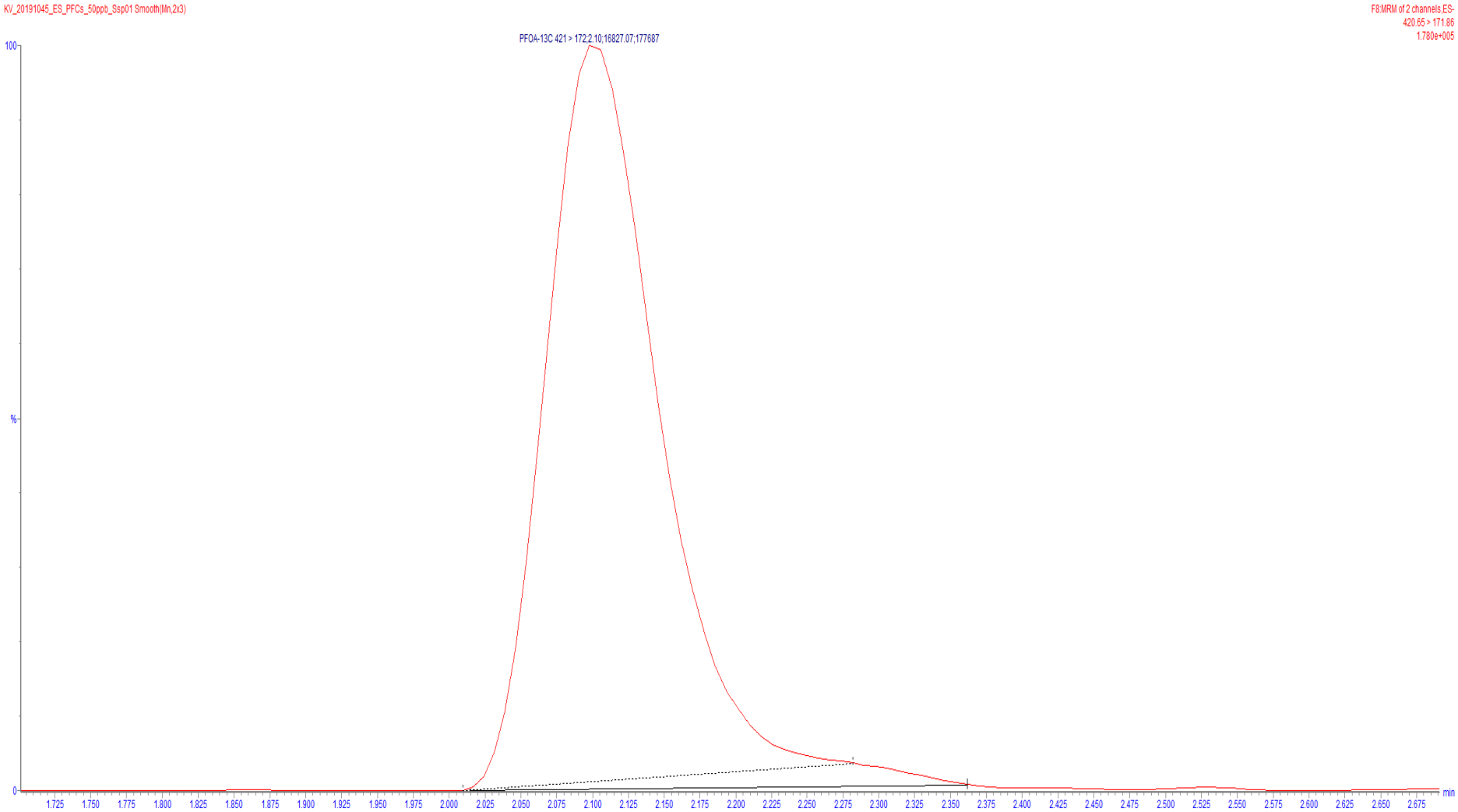


Figure D.31: Secondary transition for ISTD PFOA-13C.

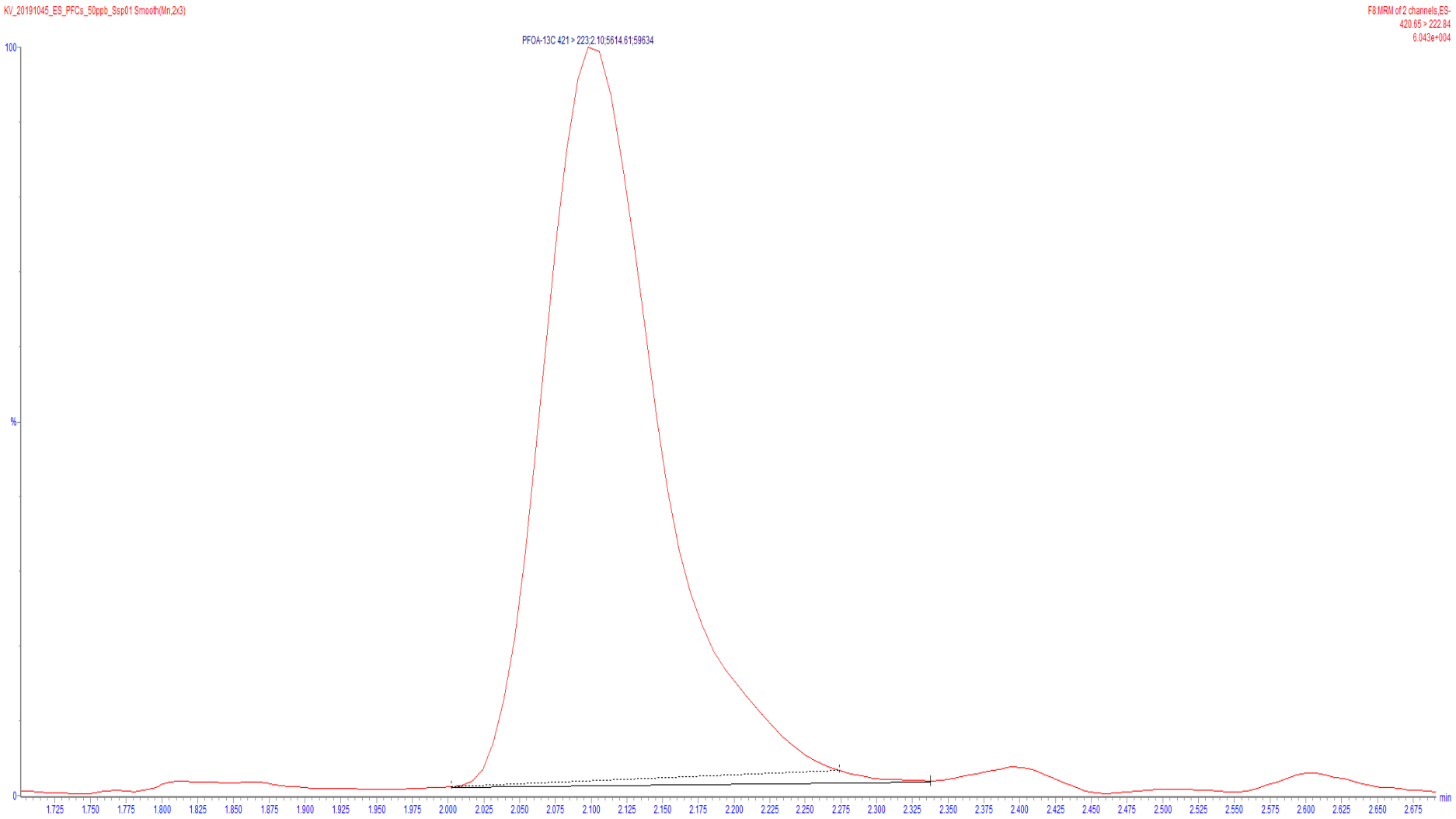


Figure D.32: Primary transition for ISTD PFOA-13C.

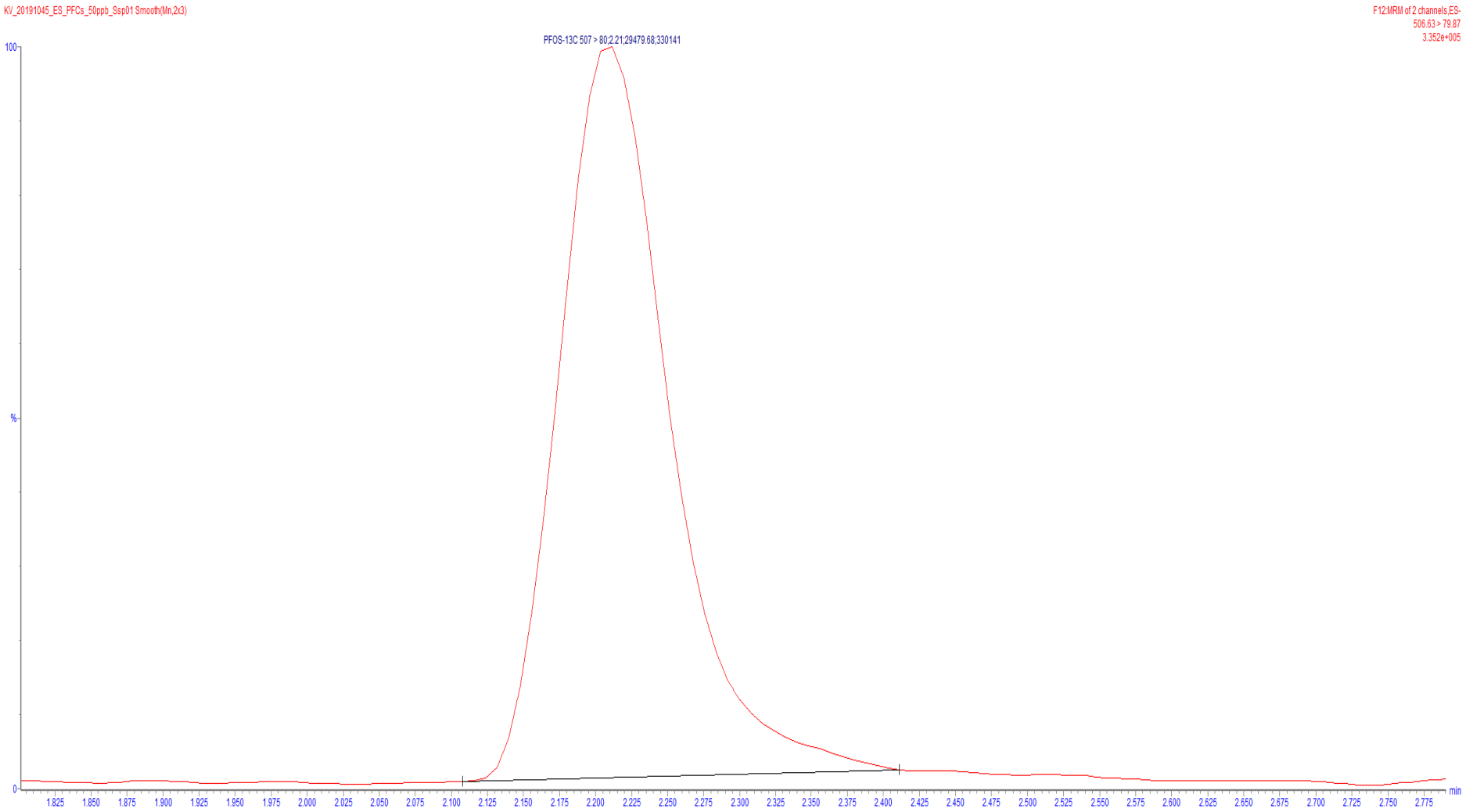


Figure D.33: Secondary transition for PFOS-13C.

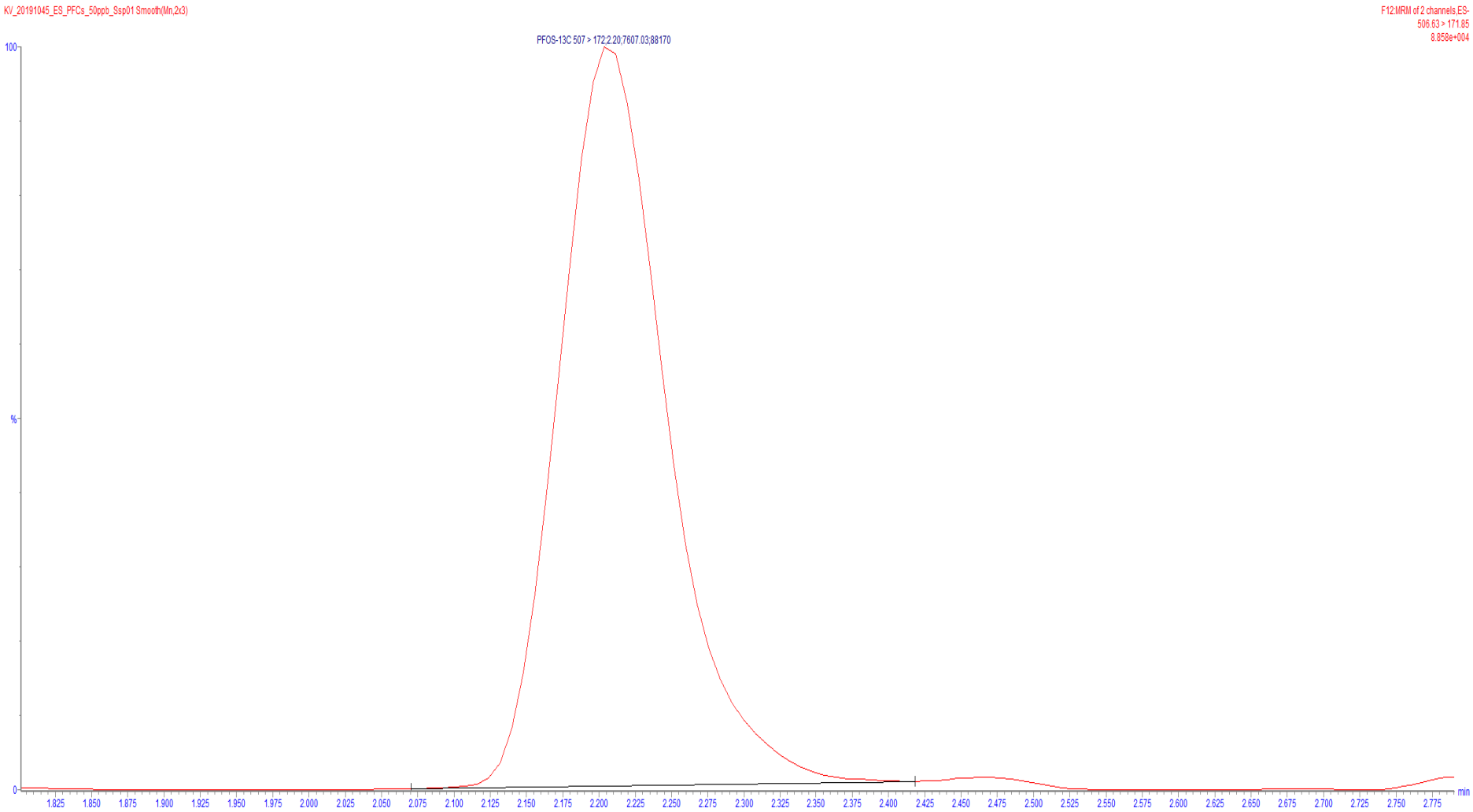


Figure D.34: Primary transition for ISTD PFOS-13C.

Appendix E Results

E.1 Group 1 feather samples – trace element analysis

Table E.1: Quantification limits (QL) in µg/g, mean detected concentration (µg/g) and RSD% values for group 1 feather samples – trace element analysis.

Element	QL (µg/g)	Mean (µg/g)	RSD%	Element	QL (µg/g)	Mean (µg/g)	RSD%
Ag	0.05	0.02	37.7	**Mn**	0.01	0.16	7.70
Al	0.49	17.9	3.80	**Mo**	0.05	0.30	13.2
As	0.06	0.09	14.8	**Na**	24.3	4.72	8.30
Au	0.0005	0.003	36.9	**Ni**	0.04	0.10	15.1
B	0.12	1.00	2.50	**P**	0.97	43.7	2.70
Ba	0.03	0.10	27.3	**Pb**	0.005	0.08	6.20
Bi	0.002	0.003	21.3	**Rb**	0.03	0.02	20.6
Ca	4.9	84.8	3.30	**S**	49.0	27 991	2.00
Cd	0.005	0.004	34.2	**Sb**	0.005	0.01	37.4
Ce	0.0005	0.02	7.60	**Se**	0.12	9.10	3.00
Co	0.01	0.02	22.4	**Sn**	0.002	0.03	12.9
Cr	0.05	0.23	7.50	**Sr**	0.06	1.33	3.1
Cs	0.001	0.001	24.3	**Th**	0.001	0.002	19.9
Cu	0.07	3.07	3.60	**Ti**	0.05	1.33	7.10
Fe	0.05	15.4	1.90	**Tl**	0.0006	0.0006	64.1
Hg	0.005	10.5	1.40	**U**	0.0006	0.006	15.7
K	2.40	4.79	3.80	**V**	0.007	0.13	9.00
La	0.001	0.01	11.3	**W**	0.002	0.0001	31.1
Li	0.01	0.01	10.0	**Zn**	0.06	10.4	5.30
Mg	0.24	98.8	5.40				

E.2 Group 3 feather samples – trace element analysis

Table E.2: Quantification limits (QL) in µg/g, mean detected concentration (µg/g) and RSD% values for group 3 feather samples – trace element analysis.

Element	QL (µg/g)	Mean (µg/g)	RSD%	Element	QL (µg/g)	Mean (µg/g)	RSD%
Ag	0.007	0.02	15.5	**Mn**	0.002	0.11	5.30
Al	0.07	2.10	5.70	**Mo**	0.007	0.006	29.6
As	0.009	0.04	7.60	**Na**	3.60	0.57	8.70
Au	0.0001	0.003	26.2	**Ni**	0.005	0.02	15.7
B	0.02	0.06	2.80	**P**	0.14	75.7	2.60
Ba	0.005	0.05	18.3	**Pb**	0.0007	0.06	6.10
Bi	0.0004	0.16	2.20	**Rb**	0.004	0.002	34.2
Ca	0.70	12.8	2.70	**S**	7.00	26 387	2.20
Cd	0.0007	0.0009	60.0	**Sb**	0.0007	0.006	27.6
Ce	0.0001	0.003	27.1	**Se**	0.02	1.34	3.20
Co	0.001	0.004	21.8	**Sn**	0.0004	0.02	8.20
Cr	0.007	0.05	8.00	**Sr**	0.009	0.08	4.1
Cs	0.0002	0.0002	27.1	**Th**	0.0002	0.0003	28.0
Cu	0.01	1.79	2.20	**Ti**	0.007	0.69	6.10
Fe	0.007	2.93	1.80	**Tl**	0.0001	0.0001	74.5
Hg	0.0007	3.30	1.20	**U**	0.0001	0.0003	26.5
K	0.40	0.35	4.30	**V**	0.001	0.005	15.8
La	0.0002	0.20	9.20	**W**	0.0004	0.0005	25.7
Li	0.002	0.001	13.0	**Zn**	0.009	8.60	2.20
Mg	0.04	6.10	7.20				

E.3 Group 2 feather samples – trace element analysis

Table E.3: Quantification limits (QL) in µg/g, mean detected concentration (µg/g) and RSD% values for group 2 feather samples – trace element analysis.

Element	QL (µg/g)	Mean (µg/g)	RSD%	Element	QL (µg/g)	Mean (µg/g)	RSD%
Ag	0.03	0.02	38.1	**Mo**	0.03	0.08	20.3
Al	0.28	573	1.50	**Na**	14.2	263	3.40
As	0.04	0.09	9.40	**Ni**	0.02	0.44	11.0
Au	0.0003	0.004	27.7	**P**	0.57	133	2.20
B	0.07	0.74	2.70	**Pb**	0.003	0.21	3.30
Ba	0.02	2.06	7.60	**Rb**	0.02	0.74	5.10
Bi	0.001	0.007	14.7	**S**	28.0	25 756	1.80
Ca	2.80	271	2.00	**Sb**	0.003	0.02	37.3
Cd	0.003	0.003	32.3	**Sc**	0.006	0.05	11.6
Ce	0.0003	0.47	2.30	**Se**	0.07	0.95	16.9
Co	0.006	0.19	8.90	**Sn**	0.001	0.06	9.70
Cr	0.03	0.85	6.10	**Sr**	0.04	1.18	3.50
Cs	0.0007	0.03	6.10	**Th**	0.0007	0.06	5.10
Cu	0.04	5.63	2.70	**Ti**	0.03	32.5	2.70
Fe	0.03	333	1.80	**Tl**	0.0004	0.004	22.7
Hg	0.003	0.48	4.60	**U**	0.0004	0.02	9.10
K	1.40	227	3.20	**V**	0.004	0.78	4.50
La	0.0007	0.26	3.00	**W**	0.001	0.009	16.0
Li	0.007	0.30	3.30	**Y**	0.0006	0.10	4.00
Mg	0.14	195	1.70	**Yb**	0.0006	0.01	16.3
Mn	0.009	3.96	2.60	**Zn**	0.04	61.6	2.50

E.4 Group 1 blood samples – trace element analysis

Table E.4: Quantification limits (QL) in µg/g, mean detected concentration (µg/g) and RSD% values for group 1 blood samples – trace element analysis.

Element	QL (µg/g)	Mean (µg/g)	RSD%	Element	QL (µg/g)	Mean (µg/g)	RSD%
Ag	0.001	0.001	78.9	**Mo**	0.001	0.01	25.7
Al	0.01	0.16	3.00	**Na**	0.67	1239	2.10
As	0.0007	0.50	2.5	**Ni**	0.001	0.003	25.5
Au	0.00001	0.00008	34.0	**P**	0.03	825	1.70
B	0.003	0.06	2.50	**Pb**	0.0001	0.002	8.70
Ba	0.0009	0.003	33.8	**Rb**	0.0008	0.31	3.20
Bi	0.00007	0.0007	22.9	**S**	0.001	1056	1.70
Ca	0.001	28.0	2.20	**Sb**	0.0001	0.005	30.8
Cd	0.0001	0.002	19.6	**Sc**	0.0003	0.0006	49.2
Ce	0.00001	0.0001	28.4	**Se**	0.003	10.4	1.90
Co	0.0003	0.009	15.8	**Sn**	0.00007	0.0006	17.4
Cr	0.001	0.002	12.6	**Sr**	0.002	0.02	7.90
Cs	0.00003	0.002	9.00	**Th**	0.00003	0.000008	53.1
Cu	0.002	0.13	6.0	**Ti**	0.001	0.005	24.1
Fe	0.001	241	1.8	**Tl**	0.00002	0.00005	47.5
Hg	0.0001	0.53	2.00	**U**	0.00002	0.00009	32.2
K	0.07	1060	3.40	**V**	0.0002	0.002	19.4
La	0.00003	0.00008	30.4	**W**	0.00007	0.00007	26.3
Li	0.0003	0.01	3.10	**Y**	0.00003	0.00004	22.8
Mg	0.007	41.0	1.80	**Yb**	0.00003	0.000008	87.6
Mn	0.0004	0.02	6.70	**Zn**	0.001	2.95	4.5

E.5 Group 2 feather samples – organic analysis

Table E.5: Calculated concentrations (ng/g) of 8/16 chosen perfluorinated compounds in 38 feathers from birds. Concentrations detected below LOD are not included in the table. The table continues over the next 3 pages. Results from external contamination is represented as "hexane" from the washing process.

Sample number	Tissue	DecaS	NonaFBS	TriDeFHxSA	Sulf	PFOS	PFOSA	PFPA	PFHxA
1	Feather	38.9	3.56	-	0.16	61.7	4.20	21.6	8.39
2	Feather	39.6	1.52	-	-	14.3	1.27	24.5	6.94
3	Feather	43.4	1.94	-	0.11	11.8	0.34	13.5	8.93
4	Feather	49.3	2.61	5.38	-	11.0	0.29	5.48	8.56
5	Feather	28.2	1.03	-	0.08	7.10	0.07	11.4	5.89
6	Feather	21.2	1.89	-	0.17	14.5	0.25	9.60	7.35
7	Feather	40.7	2.51	-	-	31.1	0.54	13.7	-
8	Feather	36.8	1.50	2.01	-	21.0	0.43	26.6	15.3
9	Feather	15.8	2.77	1.58	-	12.3	0.05	23.7	12.7
10	Feather	27.6	3.39	27.5	-	16.4	0.02	15.3	22.0
11A	Feather	38.5	3.09	4.85	-	25.5	0.20	32.4	29.4
11B	Feather	21.4	7.14	5.61	0.14	18.2	-	33.7	19.8
11C	Feather	24.4	4.84	5.27	-	20.6	0.05	29.6	24.9
12	Feather	28.2	5.53	2.12	-	17.9	0.03	22.3	-
13	Feather	27.0	4.45	-	-	20.2	0.02	597	14.6
14	Feather	41.2	9.58	-	-	66.5	0.002	-	19.1
15	Feather	31.4	4.94	-	-	10.5	0.02	18.3	20.7
16	Feather	20.6	3.84	1.90	-	42.3	0.003	11.1	10.2
17	Feather	15.9	5.24	-	-	8.10	0.04	11.8	6.51
18	Feather	30.6	2.86	-	-	15.4	0.05	16.3	6.27
19	Feather	29.6	2.08	-	0.10	11.6	0.004	16.3	14.3

20	Feather	17.5	1.29	-	-	7.52	0.006	9.48	6.67
21	Feather	23.7	0.90	-	0.05	11.2	0.07	-	8.61
22	Feather	32.7	6.00	-	-	8.10	0.08	37.9	20.3
23	Feather	12.9	2.92	-	0.28	19.1	0.11	12.4	10.1
24	Feather	15.2	-	-	-	24.9	0.05	9.38	-
25	Feather	15.5	2.38	2.86	-	11.9	0.01	12.1	11.0
26	Feather	13.6	1.07	-	0.07	9.47	0.04	-	5.94
27	Feather	13.9	1.27	1.67	0.19	5.97	0.05	13.3	-
28	Feather	10.7	3.18	-	-	8.96	0.03	15.1	15.6
29	Feather	17.1	-	-	0.07	27.8	-	339	11.3
30	Feather	22.0	5.07	-	-	48.6	0.04	21.8	9.38
31	Feather	15.5	1.96	-	-	8.34	0.005	13.1	4.60
32	Feather	14.7	3.32	-	-	12.0	0.09	-	12.6
33	Feather	18.1	3.54	2.86	0.14	5.69	0.006	21.3	-
34	Feather	32.2	5.17	-	-	20.6	0.05	11.4	13.0
35	Feather	14.9	2.41	-	-	14.9	0.002	14.0	10.0
36	Feather	-	2.87	-	-	16.2	0.07	18.1	10.8
37	Feather	3.91	0.74	-	0.05	2.67	0.002	2.30	2.41
38	Feather	-	0.86	0.80	-	2.75	0.002	3.81	2.65
		24.8	3.19	4.96	0.12	18.1	0.23	41.9	11.9
1	Hexane	800	17.1	31.6	22.8	351	139	138	-
2	Hexane	7878	53.3	283	-	-	-	2675	-
3	Hexane	2196	-	69.6	-	-	-	-	540
4	Hexane	4676	39.1	-	-	-	-	-	909
5	Hexane	-	-	-	-	-	-	-	-
6	Hexane	-	-	-	17.3	-	44.2	-	-

7	Hexane	-	-	-	2.41	148	3.67	-	-
8	Hexane	-	-	-	22.1	365	11.6	-	-
9	Hexane	-	-	-	14.1	-	0.25	-	-
10	Hexane	-	-	-	-	861	29.6	-	-
11	Hexane	4142	-	-	127	6756	157	-	-
12	Hexane	-	-	-	-	772	57.4	-	-
13	Hexane	-	-	-	37.6	770	7.27	-	-
14	Hexane	-	-	-	-	-	-	-	-
15	Hexane	28329	-	-	2.52	883	7.51	5893	-
16	Hexane	-	-	-	-	-	-	-	-
17	Hexane	17433	-	-	2.58	319	9.62	5243	-
18	Hexane	-	-	-	16.3	1048	1.55	-	-
19	Hexane	-	-	-	4.03	435	-	-	-
20	Hexane	1850	-	-	-	-	-	509	-
21	Hexane	-	-	-	71.6	2716	-	-	-
22	Hexane	-	-	-	8.09	234	2.94	-	-
23	Hexane	1624	22.1	41.4	19.1	587	-	-	-
24	Hexane	2500	-	-	7.57	539	2.93	-	409
25	Hexane	-	-	-	-	-	-	-	-
26	Hexane	-	-	-	2.47	101	2.70	-	-
27	Hexane	-	-	-	22.2	988	-	-	-
28	Hexane	-	-	-	3.92	412	-	-	-
29	Hexane	7388	-	-	5.62	101	-	948	-
30	Hexane	-	-	-	2.11	1564	3.60	-	-
31	Hexane	14207	-	-	-	-	-	5823	-
32	Hexane	-	-	-	2.95	717	-	-	-

33	Hexane	-	-	-	303	8657	-	-	-
34	Hexane	-	-	-	6.38	403	-	-	-
35	Hexane	-	-	-	-	807	6.41	-	-
36	Hexane	-	-	-	-	-	-	-	-
37	Hexane	3269	11.7	-	7.28	519	-	-	212
38	Hexane	-	-	-	-	321	2.88	-	-
		7407	28.7	106	30.5	1207	27.2	3033	517

Table E.6: Calculated concentrations (ng/g) of the next 8/16 chosen perfluorinated compounds in 38 feather samples from birds. The table also includes results from external contamination analysis, marked as "hexane" samples in the tables. Concentrations detected below LOD are not included in the table. The table continues over the next 3 pages. Results from external contamination is represented as "hexane" from the washing process.

Sample number	Tissue	PFHeA	PFOA	PFNA	PFDA	PFUnA	PFDoDeA	PFTriDe	PFTetDeA
1	Feather	-	7.19	5.58	7.31	5.66	6.45	3.99	4.70
2	Feather	-	8.36	2.55	2.96	-	3.29	1.99	-
3	Feather	2.75	3.50	2.06	1.63	-	1.39	1.05	-
4	Feather	2.53	2.10	0.67	0.99	-	0.61	-	-
5	Feather	8.37	-	-	-	-	0.49	-	-
6	Feather	2.92	3.84	0.76	0.95	-	1.00	-	-
7	Feather	-	5.38	2.25	1.15	-	1.88	1.48	-
8	Feather	-	4.61	1.55	-	-	1.61	-	-
9	Feather	-	3.78	1.76	1.45	-	0.76	-	-
10	Feather	-	3.95	1.94	1.09	-	0.72	-	-
11A	Feather	-	7.93	7.30	4.25	-	2.72	2.03	-
11B	Feather	-	5.33	5.37	3.14	-	2.51	-	-
11C	Feather	-	4.81	8.21	3.58	-	2.56	2.77	-
12	Feather	-	-	1.01	-	-	0.72	-	-
13	Feather	-	-	2.30	1.21	-	0.83	-	-
14	Feather	-	-	-	-	-	-	-	-
15	Feather	-	4.87	-	-	-	0.44	-	-
16	Feather	-	-	-	-	-	1.45	-	-
17	Feather	4.44	-	-	-	-	0.51	-	-
18	Feather	-	-	-	-	-	0.87	-	-
19	Feather	-	-	2.19	1.06	-	0.96	-	-

20	Feather	4.49	3.11	0.62	-	-	0.44	-	-
21	Feather	1.81	-	0.48	-	-	0.46	-	-
22	Feather	-	-	1.33	-	-	-	-	-
23	Feather	-	-	0.44	-	-	0.73	-	-
24	Feather	1.27	1.50	0.80	0.46	-	0.64	-	-
25	Feather	2.06	-	0.53	-	-	1.48	-	-
26	Feather	-	-	1.78	1.22	-	0.39	-	-
27	Feather	-	-	0.92	-	-	0.43	-	-
28	Feather	2.35	3.14	1.34	1.52	-	0.82	-	-
29	Feather	-	-	2.21	1.02	-	0.78	-	-
30	Feather	-	-	1.06	1.15	-	1.72	-	-
31	Feather	-	-		-	-	-	-	-
32	Feather	-	-	1.78	-	-	1.23	-	-
33	Feather	-	5.23	1.13	-	-	0.90	-	-
34	Feather	-	5.66	2.02	-	-	0.96	-	-
35	Feather	-	6.47	4.44	1.64	-	-	-	-
36	Feather	-	-	2.46	1.58	-	0.89	-	-
37	Feather	1.15	-	0.53	0.27	-	-	-	-
38	Feather	1.78	-	0.22	-	-	-	-	-
1	Hexane	77.8	279	426	341	349	30.8	318	-
2	Hexane	795	1700	-	-	-	-	-	-
3	Hexane	-	207	-	-	-	-	-	-
4	Hexane	-	533	-	-	-	-	-	-
5	Hexane	-	-	-	-	-	-	-	-
6	Hexane	-	-	152	-	-	142	-	-
7	Hexane	-	-	91.9	-	30.5	39.9	74.2	-

8	Hexane	-	-	114	-	196	129	135	-
9	Hexane	-	-	12.2	-	-	104	167	-
10	Hexane	-	-	177	-	254	164	176	-
11	Hexane	56.0	894	380	-	-	1140	2230	-
12	Hexane	-	-	8.26	-	152	-	473	-
13	Hexane	-	-	9.81	-	-	186	155	-
14	Hexane	-	-	-	-	-	-	-	-
15	Hexane	-	-	196	-	-	-	-	-
16	Hexane	-	-	-	-	-	-	-	-
17	Hexane	-	-	66.2	-	118	-	131	-
18	Hexane	-	-	231	-	-	-	-	-
19	Hexane	-	-	213	70.5	-	-	-	-
20	Hexane	-	280	-	-	-	-	-	-
21	Hexane	-	-	274	-	787	174	-	-
22	Hexane	-	-	-	-	-	47.5	24.7	-
23	Hexane	-	357	166	-	-	48.2	-	-
24	Hexane	-	181	90.1	-	30.1	63.4	-	-
25	Hexane	-	-	-	-	-	-	-	-
26	Hexane	-	-	-	11.2	33.9	24.8	-	-
27	Hexane	-	-	16.6	-	148	-	-	-
28	Hexane	-	-	-	-	131	-	201	-
29	Hexane	-	-	-	-	-	-	65.2	-
30	Hexane	-	-	-	-	-	216	552	-
31	Hexane	-	-	-	-	-	-	-	-
32	Hexane	-	-	-	112	-	-	186	-
33	Hexane	-	-	1120	-	2472	-	-	-

34	Hexane	-	-	7.87	25.3	98.6	43.5	217	-
35	Hexane	-	-	185	-	-	-	-	-
36	Hexane	-	-	-	-	-	-	-	-
37	Hexane	-	535	44.4	-	-	135	-	-
38	Hexane	-	-	22.9	44.6	-	-	47.2	-

Appendix F

Correlation

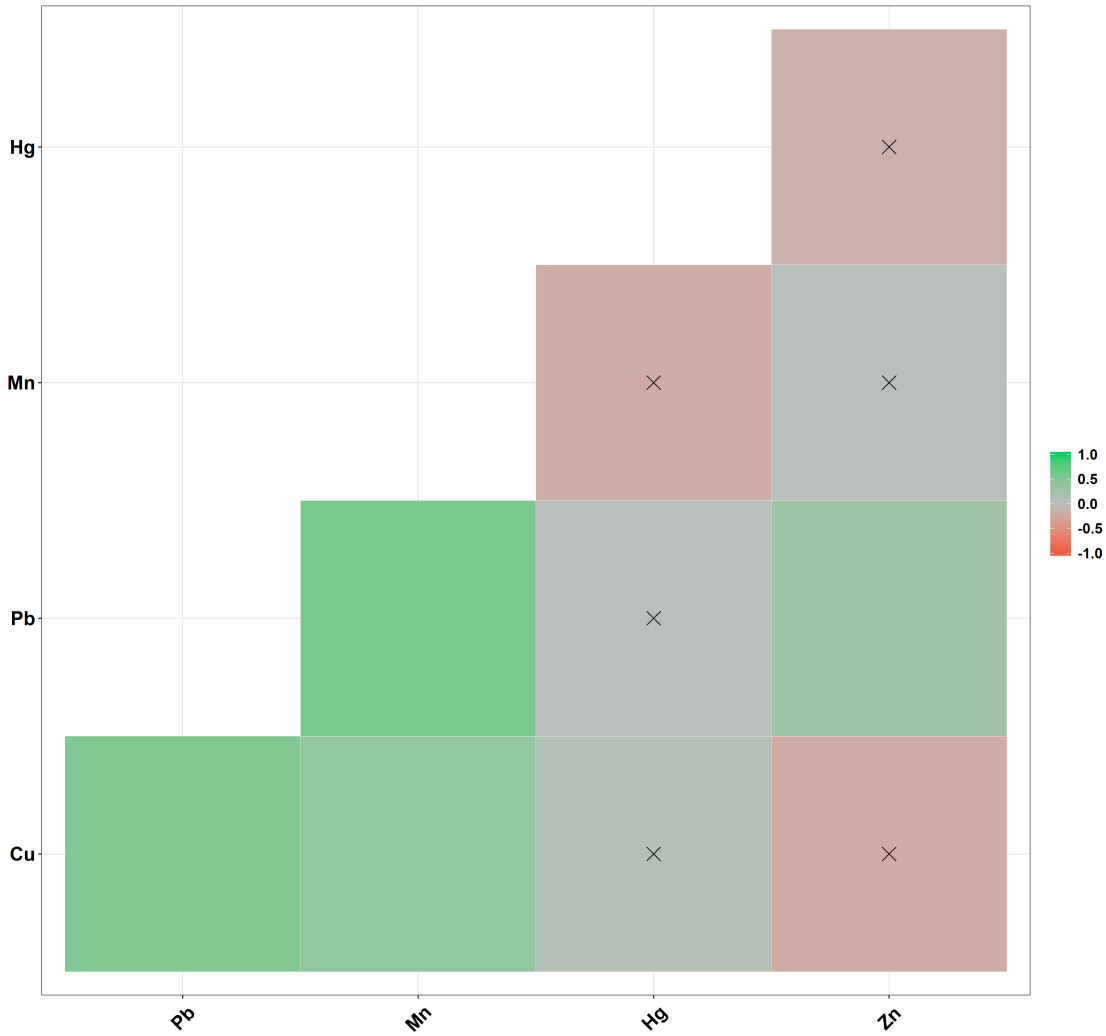


Figure F.1: Correlation for group 1 feathers P1 and P10.

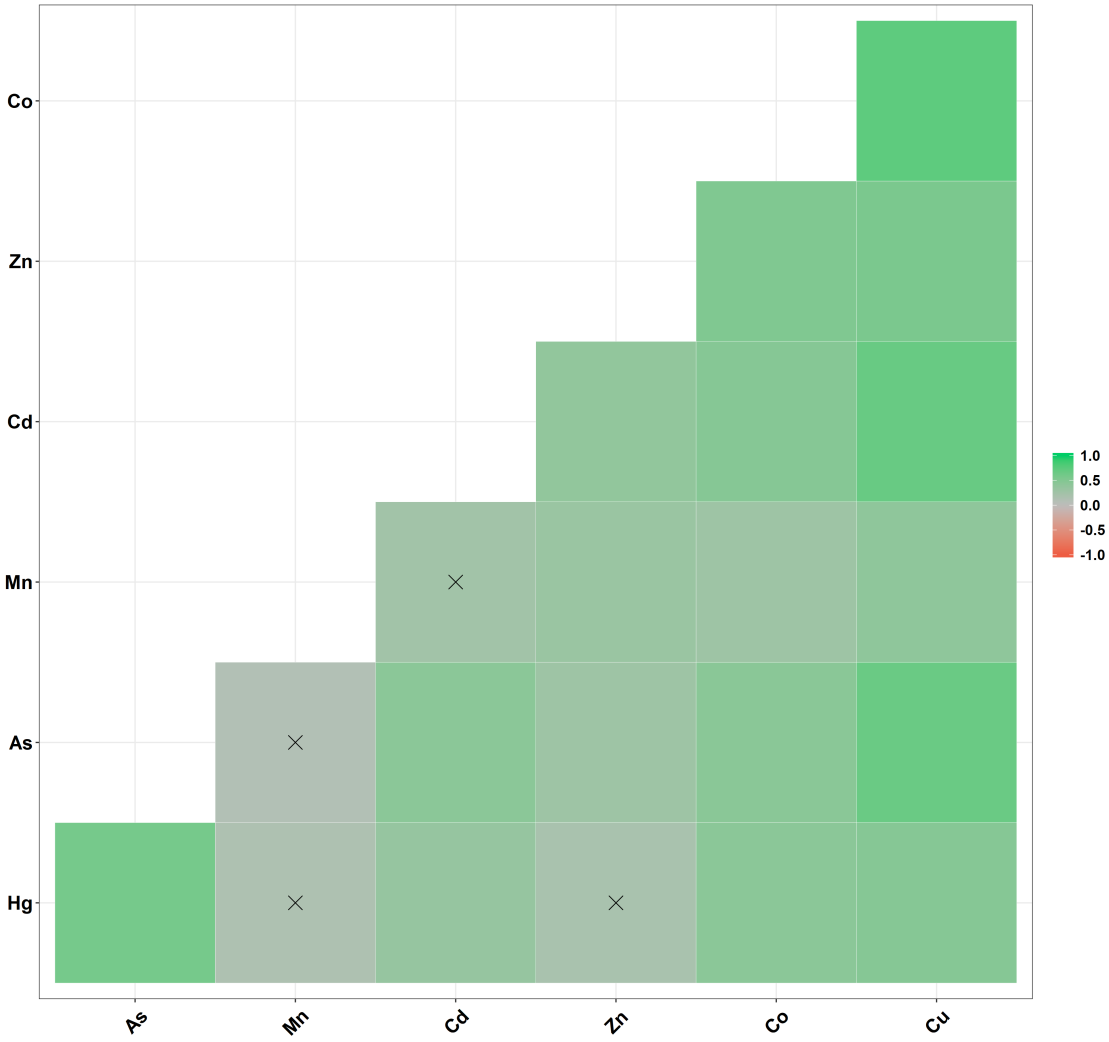


Figure F.2: Correlations for group 1 blood samples.

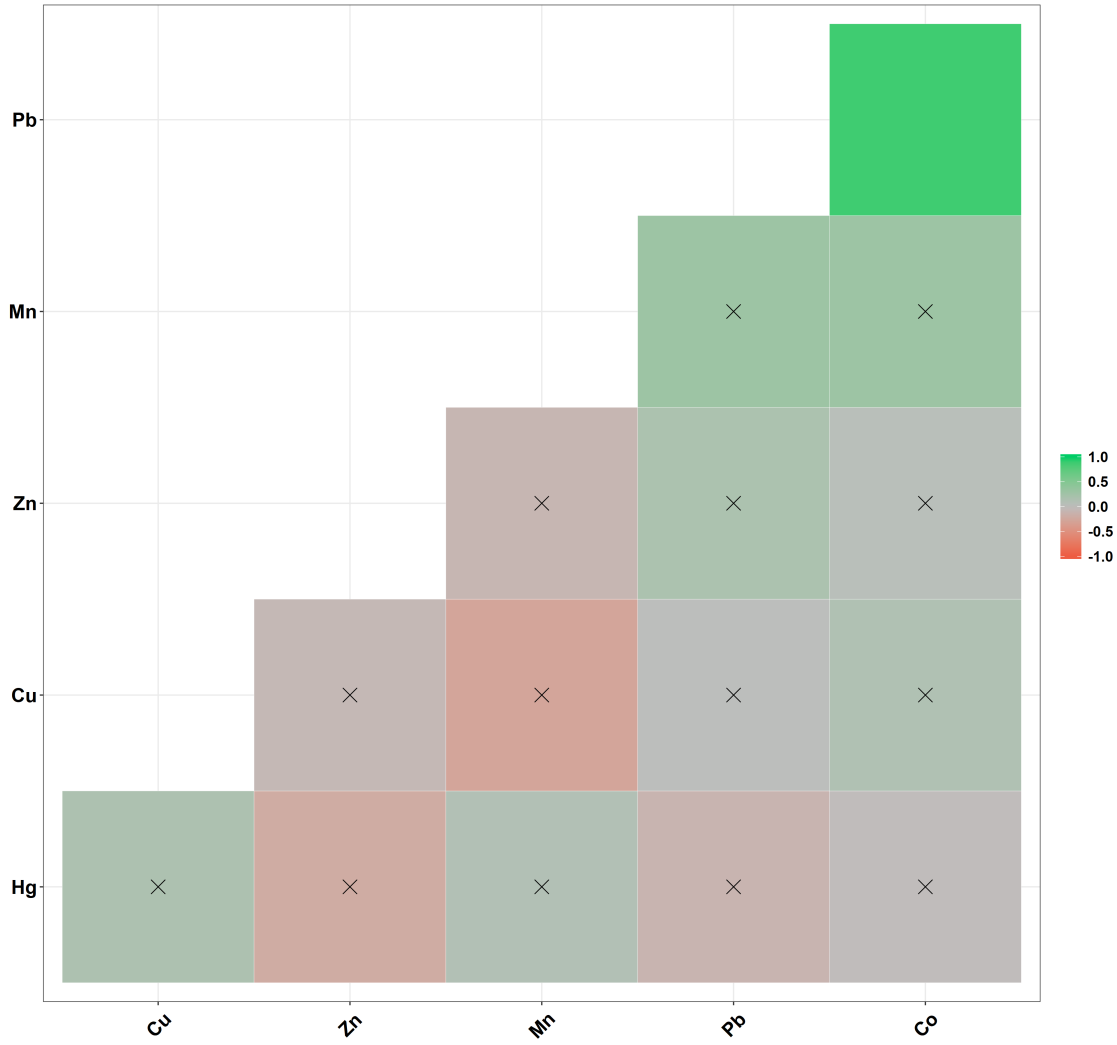


Figure F.3: Correlation for group 2 P10 feather samples.

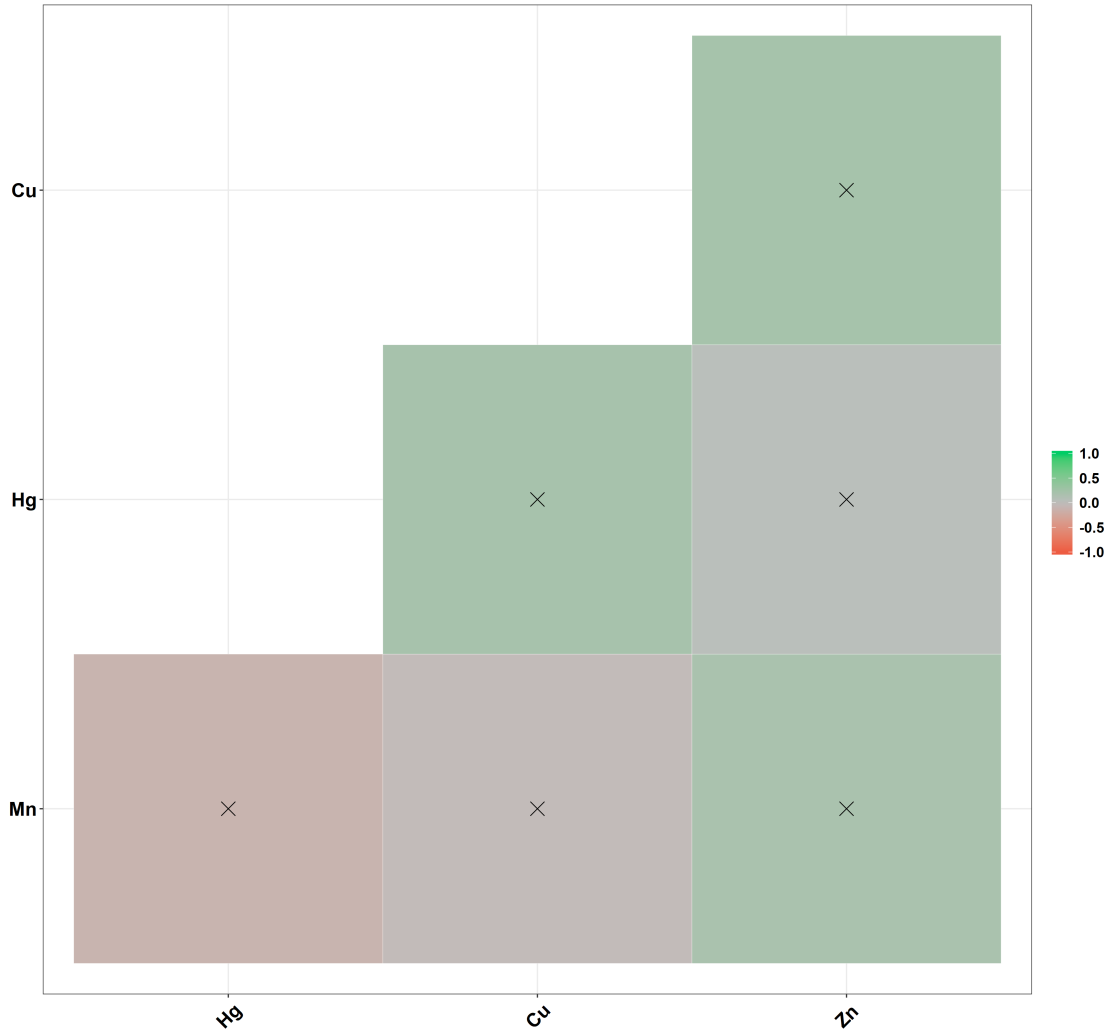


Figure F.4: Correlation for group 3 feather samples.