MASS SPECTROMETRIC ISOMER CHARACTERIZATION OF PERFLUORINATED COMPOUNDS IN TECHNICAL MIXTURE, WATER AND HUMAN BLOOD

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TABLE OF CONTENTS

ABBREVIATIONS

3,7-DMPFOA	Perfluoro-3,7-dimethyloctanoic acid
APCI	Atmospheric pressure chemical ionization
BAF, BCF	Bioaccumulation, bioconcentration factor
CE	Collision energy
CID	Collision induced dissociation
ECF	Electrochemical fluorination
EI	Electron ionization
ESI	Electrospray ionization
FTOH	Fluorotelomer alcohol
GC	Gas chromatography
HPLC	High performance liquid chromatography
HR	High resolution
ISTD	Internal standard
IT	Ion trap
LD_{50}	Lethal dose 50%
LOD	Limit of detection
LOEL	Lowest observed effect level
LOQ	Limit of quantification
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS, MS^2	Tandem mass spectrometry
MS^n	Multiple stage mass spectrometry
m/z	Mass-charge-ratio
NOEL	No observable effect level
^{19}F-NMR	Fluorine nuclear magnetic resonance
PFAS	Polyfluoroalkylated substance
PFC	Perfluorinated compounds
PFCA	Perfluorocarboxylic acid
PFSA	Perfluorosulfonic acid
PFP	Perfluorophenyl
POP	Persistent organic pollutant
ppm	Part per million
SIM	Selected ion monitoring
SD	Standard deviation
SS	Standard solution
SQ	Sinple quadrupole
TOF	Time of flight
TQ	Triple quadrupole
ww	Wet weight

SUMMARY

Perfluorinated compounds (PFC) are special surfactants which have been used since the 1950s. Their detection in the environment started at the beginning of the 2000s. A lot has to be explored in different fields such as method development, understanding of their environmental distribution, human exposure and their transport to remote areas such as the Artic region. No degradation pathways are known for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), two predominant perfluorinated compounds in the biota and the environment. Their persistence in the environment emphasized the increasing interest of environmental scientists for this class of pollutant. In this work, besides method development, special attention was paid to the characterization of the isomers present in technical mixtures of PFC and in environmental samples.

Methodologies and quantification

Methodologies based on high performance liquid chromatography (HPLC) combined with mass spectrometry (MS) with electrospray in negative mode as ionization (ESI(-)) were developed for the analysis of perfluorinated compounds in biota. The extraction procedure consists of an ion pairing extraction using tetra-alkyl ammonium and methyl tert-butyl-ether. Triple quadrupole (TQ) mass spectrometry was better suited for quantification compared to ion trap MS. TQMS enabled the detection of perfluorinated compounds in lower limits of detection (pg range) required for ultra trace analysis. The possibility of systematic errors of the applied methods was investigated. Possible artefacts in the analysis of perfluorinated compounds were identified. One major drawback was the risk of

contamination originated by TEFLON®, which can contain perfluorocarboxylic acids. Matrix interference induced also ionization suppression/enhancement and emphasized the still need of method improvement. Moreover, the presence of by-products such isomers and homologues compounds in technical mixtures and in environmental samples make a proper quantification more difficult.

Determination of isomer patterns in technical mixture

Reversed phase chromatography was used to study the isomer composition of technical PFOS mixtures. Diperfluoromethyl, mono-perfluoromethyl substituted isomers and the linear isomer were identified. Seven isomers present in a technical PFOS mixture could be separated by HPLC. A new derivatization procedure was developed for PFOS and perfluorocarboxylic acids to allow high resolution gas chromatography (HRGC) separation by converting them into iso-propyl esters. An improvement of the separation of eleven PFOS isomers with a maximum of two coeluting isomers was achieved applying HRGC. It offers a promising alternative for the perfluorinated isomers separation in technical mixture.

Structural elucidation of PFOS, PFOA and PFOSA monosubstituted isomers was possible applying tandem MS. It allowed the differentiation of up to ten isomers in a technical PFOS mixture. Ion trap tandem MS was more suitable to elucidate the position of the CF_3 branching at the perfluorinated chain of monosubstituted PFOS isomers due to more structure-characteristic spectra. Tandem MS spectra of PFOA and perfluorooctane-sulfonamide (PFOSA) monosusbtituted isomers were more complex. Only slight differences were observed between their MS/MS spectra due to a different charge

stabilization. Currently, the lack of available pure isomer standards limits the isomer-specific analysis.

Determination of isomer patterns in technical mixture, water and human blood

Finally, the HPLC-MS method for isomer identification in technical mixtures was applied to water and human blood extracts. Isomer profile is indicative of the origin of PFAS contamination. Branched isomers are typical to an electrochemical fluorination (ECF) source, whereas mainly linear structures suggest a telomer source. PFOS detected in human blood collected in Sweden, Australia and United Kingdom was found to be produced by ECF process. However, the sources of PFOA in human were both an ECF and a telomerization processes.

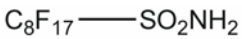
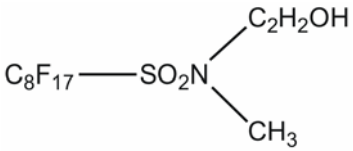
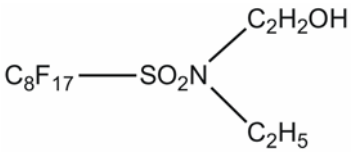
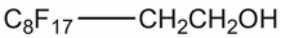
Moreover, the comparison of isomer pattern in human blood and water indicated different routes of exposure. The isomers present in technical mixtures were also present in environmental samples. These results showed the potential of isomer pattern for source characterization. This can be useful for risk assessment of humans and other living organisms. However, they could not be completely identified due to insufficient sensitivity. Improvement of the extraction procedure to lower matrix interferences is needed. The development of an alternative GC-MS method combined with a derivatization step could improve separation but not the detection limits due to low derivatization yield. Additional data about isomer patterns in different biota and human bloods from other geographic origins are required for comparison with present results.

1. Introduction

1.1. Identification

Polyfluoroalkylated substances (PFAS) is the surname of a compound class including PFOS (perfluorooctane sulfonate) and other partially fluorinated and perfluorinated compounds (PFC). These are perfluorosulfonic acids (PFSA), perfluorocarboxylic acids (PFCA), perfluorosulfonamides and fluorotelomer alcohols (FTOH). Their IUPAC names are long and impractical to use. Therefore, a simplified nomenclature and abbreviations were developed. Table 1.1 summarizes the current names for PFAS used in the literature and in this study.

Table 1.1: Chemical structures of the mostly analyzed polyfluorinated substances, their IUPAC and common names as well as their abbreviations used in literature.

Chemical structure	IUPAC name	Literature name (abbreviation)
	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonic acid	perfluorooctane sulfonate (PFOS)
	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid	perfluorooctanoic acid (PFOA)
	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonamide	perfluorooctane sulfonamide (PFOSA)
	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-(2-hydroxyethyl)-N-methyl-octane-1-sulfonamide	*N*-methyl perfluorooctane sulfonamidoethanol (*N*-Me-FOSE)
	*N*-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-*N*-(2-hydroxyethyl)octane-1-sulfonamide	*N*-ethyl perfluorooctane sulfonamidoethanol (*N*-Et-FOSE)
	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heptadecafluorodecan-1-ol	1H,1H,2H,2H-perfluorodecanol (8:2 FTOH)

The FTOH are assigned according to the number of fluorinated hydrogenated carbon atoms within the chain (see Table 1.1 for 8:2 FTOH given as example). Table 1.2 summarizes the monitored homologue series PFSA, PFCA and FTOH.

Table 1.2: Mainly monitored homologues perfluorosulfonic, perfluorocarboxylic acids and fluorotelomer alcohols.

Perfluorosulfonic acid **Name** **Elemental composition**	**Abbreviation**	**Perfluorocarboxylic acid** **Name** **Elemental composition**	**Abbreviation**
Perfluorobutane sulfonate $C_4F_{13}SO_3^-$	PFBS	Perfluorobutanoic acid $C_3F_7CO_2^-$	PFBA
Perfluorohexane sulfonate $C_6F_{15}SO_3^-$	PFHS	Perfluorohexanoic acid $C_5F_{11}CO_2^-$	PFHA
Perfluorooctane sulfonate $C_8F_{17}SO_3^-$	PFOS	Perfluoroheptanoic acid $C_6F_{13}CO_2^-$	PFHpA
Perfluorodecane sulfonate $C_{10}F_{19}SO_3^-$	PFDS	Perfluorooctanoic acid $C_7F_{15}CO_2^-$	PFOA
Fluorotelomer alcohol		Perfluorononanoic acid $C_8F_{17}CO_2^-$	PFNA
1H,1H,2H,2H-perfluorohexanol, $C_6H_5F_9O$	4:2 FTOH	Perfluorodecanoic acid $C_9F_{19}CO_2^-$	PFDA
1H,1H,2H,2H-perfluorooctanol, $C_8H_5F_{13}O$	6:2 FTOH	Perfluorundecanoic acid $C_{10}F_{21}CO_2^-$	PFUnA
1H,1H,2H,2H-perfluorodecanol, $C_{10}H_5F_{17}O$	8:2 FTOH	Perfluorododecanoic acid $C_{11}F_{23}CO_2^-$	PFDoA
		Perfluorotetradecanoic acid $C_{13}F_{27}CO_2^-$	PFTA

1.2. Production and application

PFAS are used in numerous applications due to their special surfactant properties (see Chapter 1.3.1). Table 1.3 lists their applications. The major applications areas are textiles, leather, carpet, paper and board impregnation. The exact use of each PFAS is not well documented. Moreover, mixtures of PFAS are commercially employed.

Table 1.3: Application and use of polyfluoroalkylated substances (Hekster *et al.*, 2002).

Application	Use (%)*
Carpet, leather and textile treatment	48.8
Paper and board treatment	15.0
Specialty surfactants (cleaning agents)	17.5
Fire-fighting foams	16.3
Chemical intermediate	2.5
Other: insecticide, polymerization aid, cosmetics, electronics, hydraulic oil	n.a

n.a: not available
*: in United Kingdom

In 2000, the global annual production of PFOS-based compounds was estimated to $3.7*10^6$ kg. 3M corporation is the major producer sharing 80-90 % of the market. FTOH production was approximately $5\text{-}6*10^6$ kg/year in 2000-2002 (OECD, 2002; US EPA, 2002). The worldwide global production of PFAS was about $5*10^7$ kg (AMAP working group, 2004). The C_8-based chemicals have been mostly produced.

1.3. Environmental fate

1.3.1. Properties

PFAS have surfactant properties. Presence of fluorine as the most electronegative element makes them different from hydrocarbonated surfactants. Whereas hydrocarbonated chains are lipophobic, the perfluorinated chains are both hydrophilic and lipophilic due to the strong polarity of the C-F bond. PFAS can repel both water and oil and are thus very useful for textile treatment.

Moreover, the perfluorinated chain is very rigid protecting it from biological attack (Key et *al*., 1997). Defluorination is hardly possible, and perfluorinated surfactants are thermally and chemically stable. The C-F bond of monofluoroacetate can withstand defluorination by boiling with 100 % sulfuric acid (Key *et al*., 1997). The terminal functionality is the only

possible part which can undergo further transformation. FTOH and perfluorosulfonamides show different degradation routes as shown in Figure 1.1. PFCA and/or PFSA are the final biodegradation products. PFOS and PFOA are very persistent in the environment and no further degradation is known. This should explain their predominance in biota. The degradation route to PFOS is better documented than the second pathway yielding PFOA.

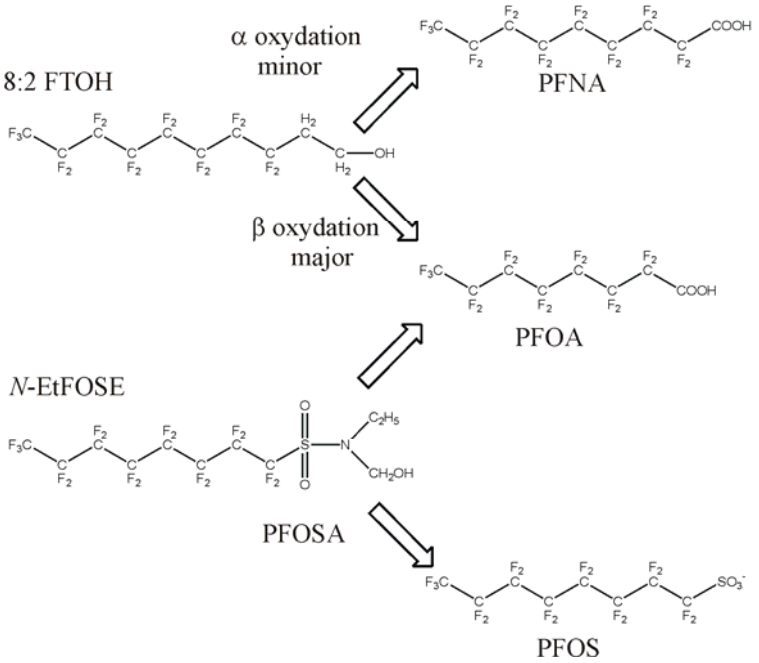


Figure 1.1: Degradation pathways of 8:2 FTOH and *N*-Et-FOSE to PFOS and PFOA (Dinglasan *et al.*, 2004, Lange, 2000).

Table 1.4 lists the physicochemical properties of important PFAS. The FTOH and the perfluorosulfonamides have higher vapor pressure. PFAS are very acid. A pKa value of 2.80 for PFOA was reported (Moody *et al*., 2000). A lower pKa value is expected for PFOS due to the presence of the sulfonate group but no exact value is available. Therefore, PFOS and PFOA are very water soluble. However, it is unclear if the determined water solubility is real due to possible micelle formation. Moreover, octanol-water partitioning coefficient (log K_{ow}), another important parameter for the characterization of a pollutant cannot be determined due to surfactant properties.

Table 1.4: Physicochemical properties of selected PFAS (Hekster *et al.*, 2002).

Compounds	Melting point (°C)	Boiling point (°C)	Vapor pressure (20 °C)	Water solubility (mg/l)
PFOS	>400	Not calculable	$3.3\ 10^{-4}$	570
PFOA	45-50	189-192	$9.3\ 10^{-3}$	3400
n-Et-FOSE	55-60	n.a	$5.0\ 10^{-1}$	0.15
8:2 FTOH	49-51	n.a	2.93	0.14

n.a: not available

1.3.2. Bioaccumulation

1.3.2.1. Target organs

PFAS have a high bioaccumulation potential due to their high stability. High levels of PFC were found mainly in liver and plasma of wildlife. Levels in adipose tissue are low due to the lipohobic behavior of PFAS. PFOS and PFOA bind to proteins such as albumin due to their ionic properties, which explains the primary accumulation of these compounds in blood (Jones e*t al.*, 2003). However, detection of PFOS in other organs such as spleen, brain, heart, lung, kidney and muscle questions the exact mechanism of bioaccumulation (Olivero-Verbel *et al*., 2006).

1.3.2.2. Bioaccumulation factors

Bioaccumulation factors increase by a factor of 8, when a CF_2 is added to the perfluorinated chain of PFCA. PFSA bioaccumulated to a greater extent than PFCA with the same perfluoroalkyl chain length indicating that the acid group has also influence (Martin *et al*., 2003).

The PFCA hepatic elimination is sex dependent under controlled laboratory conditions (Vanden Heuvel *et al*., 1991). Female rats eliminate faster PFCA. However, until now, no specific gender or age related elimination study was carried out.

1.4. Environmental levels

Giesy and co-workers first demonstrated the widespread occurrence of PFOS and related compounds in wildlife in 2001 (Giesy *et al*., 2001, Kannan *et al*. 2001). Many biota such as marine mammals and birds and to a less extent terrestrial mammals were contaminated by PFAS. PFAS were also detected in air, water and sediment. However, less information is available. Therefore, PFAS were considered as widespread environmental pollutant. Table 1.5 summarizes some examples of PFAS levels in different environmental compartments.

Table 1.5: Examples of PFAS concentrations in biota and abiotic samples (air, water and sediment).

Samples and location	Concentrations	References
Biota	**PFOS [ng/g]**	
Harbour porpoise		Van De Vijver *et al.*, 2004
Iceland	38 (tissue)	
Denmark	270 (tissue)	
German Baltic Sea	534 (tissue)	
Glaucous gull	100 (liver)	Bossi *et al.*, 2005
Norwegian Artic		
Other artic birds	1.3-20 (liver)	
Polar bear		
Alaska	180-680 (liver)	Kannan *et al.*, 2001b
Air	**[pg/m^3]**	
England	8:2 FTOH: 326 / 196 6:2 FTOH: 315 / 147	Berger *et al.*, 2005
North American troposphere	Perfluorosulfonamides: 22-403 FTOH: 11-165	Stock *et al.*, 2004
Air particulate matter-Manchester (England)	8:2 FTOH: 6 pg/m^3 *N*-Me-FOSE*:* 33 pg/m^3 PFOA: 828 pg/m^3 PFOS: 51 pg/m^3	Berger *et al.*, 2005
Indoor / outdoor air	*N*-Me-FOSE: 2590 / 23.8 *N*-Et-FOSE*:* 770 / 9.1	Shoeib *et al.*, 2004
Water	**[ng/l]**	
Elbe estuary / coast / open sea (Germany)	PFOS: 20 / 3 / 0.5 PFOA: 20 / 6 / 1.2	Caliebe *et al.*, 2005
Sediment & sewage sludge	**[ng/g](wet weight)**	
San Francisco		Higgins *et al*., 2005
Bay sediment	∑ PFAS: 0.141-16	
Bay sludge	∑ PFAS: 78-3390	

1.4.1. Concentrations in biota

PFOS is generally the most predominant PFAS in biota. PFOSA was only detected in lower concentrations, since it is expected to biodegrade to PFOS (see Chapter 1.3.1).

Samples from industrialized areas were less contaminated than those from remote regions far from any direct pollution source.

Unusually high PFOS concentrations in the range of μg/g (ppm) were determined after two pollution events. The highest PFOS concentration was reported for liver from wood mice with 179 μg/g wet weight (ww). The samples were collected in a natural reserve located 3 km downstream a 3M manufacturing site in Antwerp (Belgium) (Hoff e*t al.*, 2004). This high contamination also allowed the evaluation of PFOS effects under field conditions (see Chapter 1.6). A PFOS concentration of 72.9 μg/g in fish liver was found after an accidental release of 22000 l of fire fighting foam in the USA (Moody *et al.*, 2002). When no direct pollution was reported, the highest PFOS concentration was 3,68 μg/g ww in mink liver collected from Midwestern USA. (Giesy *et al*., 2001).

Species from higher trophic chain and non-polluted region such as glaugous gull or polar bear had higher PFOS concentrations (see Table 1.5). This indicated biomagnification of PFOS in the food chain.

Shorter and longer PFC were also recently detected in biota besides the mostly produced C_8-based compounds. Longer PFCA with C_9-C_{15} carbon chains were present in polar bear livers from the Canadian Artic (Van de Vijver *et al*., 2005, Moody *et al*., 2002). PFHS (C_6) was also found in biota however in lower concentration than PFOS and major PFCA (Giesy *et al.*, 2001, Bossi *et al*., 2005). In 2005, PFBS (C_4) was for the first time detected in harbor seal spleen from the Dutch Wadden Sea at concentrations of 1.7-3.3 ng/g (Hoff *et al.* 2004). So far, no PFBA (C_4) was reported in any samples.

1.4.2. Concentrations in water

Contamination of surface and coastal sea waters by PFAS has been reported. PFOS and PFOA were the main PFAS. Concentrations of a few ng/l to several hundreds of ng/l were found (see Table 1.5).

1.4.3. Concentrations in air

The highest volatile PFAS were also detected in air. These were the non-ionic perfluoro-sulfonamides and the FTOH (see Table 1.5). Moreover, some PFCA and PFSA are bounded to air particulate matter which is important for the understanding of the still largely unknown transport of PFAS.

Indoor air contained higher concentrations of *N*-Me-FOSE and *N*-Et-FOSE than outdoor air (see Table 1.5). These compounds are used in variety of consumer products such as carpet or upholstery protection. This established indoor air as an important source to the outside environment. Indoor air levels were even higher as those for polybrominated diphenyl ethers (Shoeib *et al*., 2004).

1.4.4. Concentration in sediment

Only few studies reported the distribution of PFAS in sediment. Very little is known about their sorption mechanism to solids. PFAS were detected in sediment at the low ng/g to sub-ng/g level (see Table 1.5). Higher concentrations were found in sewage sludge. Perfluorosulfonamides were the main PFAS in sediment and sludge. This is contrary to biota and water samples, where PFOS and PFOA tended to be the most predominant

PFAS. Perfluorosulfonamides are PFOS precursors (see Chapter 1.3.1). Thus, it was suggested that they may contribute significantly to the overall PFOS level in the environment.

1.5. Concentrations in human

The presence of organofluorine compounds in human blood was already published at the end of the 1960s (Taves, 1968). PFOA was thought to mostly account. Later, the use of more selective analytical techniques allowed to detect PFOS in blood. In 1976, the 3M corporation started the medical monitoring of employees involved with PFOA production. Mean concentrations of up to 10 μg/ml were found (3M, 1999). In 1997, the 3M company reported the presence of PFOS in commercial sera. (3M, 1999). In the beginning of 2000 the monitoring of PFOS was extended to blood from the not directly exposed population.

PFOS is the most dominant PFC in human blood and PFOA the next most abundant. Kärrman *et al*. reviewed PFOS and PFOA concentrations in human blood from different countries ranging from 60-10060 ng/ml (Kärrman *et al*. 2005). Only one study reported the monitoring of PFC in human milk. No measurable levels were found (Kuklenyik *et al.*, 2004).

The pathway leading to human exposure is still not well established. Surprisingly, similar concentrations as for adults were also detected in blood samples from children (OECD, 2002). Direct exposure via indoor air, through the use of clothes and carpet protection products may be important. Food intake may be a second route for human exposure (Tittlemier *et al.*, 2005).

1.6. Toxicity

The first toxicological studies were reported for PFOA (Griffith *et al.*, 1980). Until now, only few investigations have been carried out concerning the mechanism of toxicity of PFOS and its amide derivatives. FTOH have received little attention and nearly no literature about their toxicity is currently available. However, FTOH are not stable and biodegrade to perfluorocarboxylic acids in biota (see Chapter 1.3.1). Therefore, toxicological effects typical for perfluorocarboxylic acids should also be for FTOH (Kudo *et al.*, 2005).

Table 1.6 summarizes the so far known toxicological effects of PFOS and PFOA. Olsen *et al.* reviewed PFC toxicological effects in 2003. Perfluorinated acids are of special interest due to their structural similarity to endogenous fatty acids and their surface-acting physico-chemical properties, which can affect membrane properties.

The chain length and the functional group play both a key role in the toxicity mechanism. Table 1.6 shows the higher acute toxicity of PFOS compared to PFOA. Shorter PFAS were shown to be less toxic. PFDA (C_{10}) had a 4.6-fold lower lethal dose (LD_{50}) than PFOA (C_8) (Olson *et al.*, 1983). Therefore, C_4-based compounds were considered as not harmful (Hu *et al.*, 2003). Surprisingly, PFBS were only detectable in spleen tissue (Van de Vijver *et al.*, 2005). Only one study reported the effect of PFC to the spleen and the immune system leading to atrophy of thymus and spleen (Yang *et al.*, 2000).

Table 1.6: Toxicological effects of PFOS and PFOA (Purdy, 2000, Gilliland *et al.*, 1993, Olsen *et al.*, 2003).

Parameters	PFOS	PFOA
Acute toxicity LD_{50}	251 mg/kg (rat, oral)	>500 mg/kg (rat, male, oral), 250-500 mg /kg (rat, female, oral)
NOEL	15 mg/kg (liver), 5 mg/l (serum)	data not available
LOEL	48 mg/kg (liver), 19 mg/l (serum)	data not available
Mutagenicity	Non-mutagenic	
Carcino-genicity	Liver tumor response, prostate cancer	
Chronic/sub-chronic toxicity	Decreased body weight Increased liver weight Lowered serum total cholesterol, triglyceride and thyroid hormones Hepatic peroxisome proliferators Inhibition of gap junction in intercellular communication Increase of membrane fluidity	
Reproduction/ development	Post-natal death Developmental problems (reduction fetal weight, cleft palate, edema, delayed ossification of bones and cardiac anomaly)	

LD_{50}: lethal dose, NOEL: no observable effect level, LOEL: lowest observed effect level

Some effects presented in Table 1.6 were also observed in a field study (Hoff *et al.* 2004). Two studies also reported that workers employed in PFOS-based product plant with high exposure jobs had an increased number of deaths from bladder cancer. Those working in PFOA production plant exhibit a high prostate cancer mortality risk (Alexander *et al.* 2003, Olsen *et al.* 2003).

1.7. In conclusion: A new POP

1.7.1. POP criteria

The Stockholm Convention was signed in 2001 to finalize the negotiations on banning certain persistent organic pollutants (POP). The criteria persistence, bioaccumulation, long range transport and toxicity defined a POP. Table 1.7 summarizes the criteria as well as the corresponding properties of some initial selected POP (eg. polychlorinated biphenyls (PCB), toxaphenes and dichlorodiphenyltrichloro-ethane (DDT)) and of some other

organic halogenated chemicals which are now considered as “new POP” (chlorinated paraffins (CP), polybrominated diphenylethers (PBDE) and PFAS).

PFOS is persistent, bioaccumulate, is detectable in remote areas and presents some adverse effects. Therefore, PFOS can be considered as a new POP. PFOS has a comparable vapor pressure as other POP (see Table 1.7). However, due to its very high water solubility (see Table 1.7), it is less likely that PFOS or PFOA are long-range dispersed via the “cold condensation” mechanisms as the POP in general can. Therefore, the detection of PFOS and PFOA in remote areas is still puzzling. It is hypothesized that volatile precursors of both such as fluorotelomers or some perfluorosulfonamides may be long-range transported. Then, they degrade to the stable PFOS and PFOA. Those hypotheses were partially confirmed by the PFAS distribution in the different environmental compartments. The volatile perfluorosulfonamides and FTOH were mainly detected in air. FTOH have a sufficient half life (20 days, see Table1.7) in air for long range transport.

Table 1.7: Persistent organic pollutant (POP) criteria of the Stockholm Convention (2001). Values of three "old" POP and three "new" POP are given.

		Total production (t)	$T_{1/2}$ in day (d), year (y) or month (m)	BAF or BCF	Log K_{ow} (25 °C)	Vapor pressure (Pa)	Water solubility (mmol/m^3 at 25 °C)	LD_{50} (mg/kg) in rat, oral dose
	Stockholm convention criteria[1]	-	Persistent air: >2 d	$>5*10^3$	>5	-	-	Adverse effects
"Old" POP	**DDT**	$2.6*10^6$ [1]	Persistent air: 7.1 d[1]	1000-$1*10^6$[2]	~6[1]	$87*10^{-5}$-$2.5*10^{-5}$[1]	0.1-0.02[1]	87[3]
	Toxaphenes	$1.3*10^6$ [1]	Persistent air: 7.1 d[1]	$2*10^4$-10^5 [4]	5.5[1]	$9*10^{-4}$ [1]	1.21[1]	13-1075[4]
	PCB	1.3 10^6 [1]	Persistent air: 2-34 d[1]	$6*10^4$-$8*10^5$ [5]	4.9-8.2 [1]	$2.0*10^{-5}$-0.28 [1]	$3.8*10^{-5}$-9.0 [1]	1010[6]
"New" POP	**Short chain CP**	$5\text{-}7*10^5$ [1]	Persistent air: 1.2-1.8 d[7]	>5000[8]	5.9-7.3 [1]	$2.8*10^{-7}$-0.5[1]	0.004-$6.3*10^{-7}$ [1]	0.3 (trout)[9]
	PBDE	1.3-$2.1*10^6$ [1]	Persistent human: BDE-183: 86 d [10]	BDE-47: $1.3*10^6$ [10]	6-10[1]	$<1*10^{-6}$-$13.2*10^{-3}$ [1]	$2*10^{-8}$ [1]	500-5000[10]
	PFAS	$5*10^4$ [1]	Persistent air (FTOH): 20 d[11] no PFOS route of biodegradation known	PFOS: 6300-$1.2*10^5$[12]	Not available	PFOS: $3.3*10^{-4}$ [1] 8:2 FTOH: 2.9[13]	PFOS: 1363 [1]	PFOS: 250[14]

$T_{1/2}$: half life, BAF: bioaccumulation factor, BCF: bioconcentration factor, LD_{50}: lethal dose

[1]: AMAP working group, 2004
[2]: US EPA, 1989
[3]: MSDS safety data for DDT, 2005
[4]: Saleh, 1991
[5]: International Council of Chemical Association, 1998
[6]: MSDS safety data for PCB, 2005
[7]: Muir *et al.*, 2000
[8]: WWF, 2005
[9]: European Commission, 2005
[10]: De Wit, 2002
[11]: Ellis *et al.*, 2003
[12]: Moody *et al.* 2002
[13]: Hekster *et al.*, 2002
[14]: OECD, 2002

PFAS levels were similar as for other POP in different environmental compartments. Muir et al. (2000) reported short chlorinated paraffin concentrations of 70 ng/l in water of industrialized area whereas PFOS concentration was 20 ng/l at the Elbe estuary (Caliebe *et al.*, 2005). Comparison with other POP is difficult since they bioaccumulate in different tissues. However, the concentration of total polychlorinated biphenyls (PCB) in polar bear fat was comparable (3330-7520 ng/g ww) to that of PFOS in blood of polar bears (up to

3100 ng/ml) (AMAP working group, 2004). Concentrations of PFAS (up to 3 ng/g) were lower in sediment compared to total PCB (138.5-329 ng/g), short CPs (7-290 ng/g) or PBDE (3.4-13.8 ng/g) (Wurl *et al*., 2005, Muir *et al.*, 2000). This is due to the higher water solubility of PFAS. Although PFAS concentrations are similar to other POP, the estimated global production for PFAS is lowest of all POP (see Table 1.7). This makes the monitoring of PFAS of special interest to better understand their environmental behavior.

1.7.2. Regulation

Currently, no specific regulation exists for PFAS. The organization for economic co-operation and development (OECD) has performed hazard assessments for PFOS and PFOA (OECD., 2002, Office of pollution prevention and toxic, 2003). Only one study reported reference values for avian species based on chronic and acute toxicity for PFOS (Newsted *et al*., 2005). Biological effects were not expected to occur for PFOS serum concentration below 1 μg/ml. The US environmental protection agency (US EPA) reported that a PFOS concentration of 1 μg/l in water does not present an absolute value above which health risk is imminent (Hansen *et al*., 2002).

3M corporation voluntary phased out the production of the C_8-based chemicals at the end of 2002 and replaced it by C_4-based chemicals (3M, 2000). Perfluorobutane sulfonate (PFBS) is the official successor of perfluorooctane sulfonate (PFOS). Shorter PFC are expected to offer improved environmental properties (Lau *et al.*, 2004, Hu *et al.*, 2003) (see Chapter 1.6). PFCA and fluorotelomers are still produced, since no replacement compounds have been found until now. Moreover, US EPA launched in January 2006 a global stewardship program inviting companies to reduce PFOA release as well.

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2. Aim of the work

The aim of this work was to develop methods for the analysis of perfluorinated compounds in environmental samples. Methodologies based on high performance liquid chromatography coupled to mass spectrometry with electrospray ionization were explored. Besides method development and validation, the followings problems and applications were studied:

First goal was to explore advantages and drawbacks of different mass spectrometers governing the detection of perfluorinated compounds. It included evaluation of an ion trap and a triple quadrupole mass spectrometer. Moreover, possible interferences encountered during the quantification procedure were investigated.

Another goal was the characterization of isomers present as by-products in technical mixtures by HPLC-MS. This work was focused on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonamide. This required optimization of the chromatographic separation and the use of tandem MS. Purified isomer obtained by fractionation of technical products were investigated. Additionally, a new gas chromatographic method was developed taking advantage of the high resolution of the isomer separation.

A further task concerned the identification of isomers in environmental samples based on the previous isomer characterization in purified mixtures. The isomer pattern in human blood and water samples should be determined and evaluated as a possible source indicator. Special attention should be focused on PFOS isomer pattern differences caused by origin, age and gender in human blood and to the comparison of PFOA isomer profile in human blood and water.

3. Experimental

3.1. Materials

3.1.1. Perfluorinated compounds

Table 3.1 shows the origin and purity of the perfluorinated compounds investigated in this work.

Table 3.1: Origin and purity of the perfluorinated compounds studied in this work.

Perfluorinated compound	Purity (%)	Company (City, Country)
PFOS ($C_8F_{17}SO_3^-$, K^+)	98	ABCR (Karlsruhe, Germany)
PFHA ($C_5F_{11}CO_2^-$)	98	
PFOA ($C_7F_{15}CO_2^-$)	98	
PFOSA ($C_8F_{17}SO_2NH_2$)	97	
Perfluoro-3,7-dimethyl octanoic acid ($C_9F_{19}CO_2^-$), (3,7-DMPFOA)	97	
PFHS ($C_6F_{13}SO_3^-$, K^+)	≥98	Sigma -Aldrich (Steinheim, Germany)
PFBA ($C_3F_7CO_2^-$)	99	
PFNA ($C_8F_{15}CO_2^-$)	≥95	
PFDA ($C_9F_{17}CO_2^-$)	≥97	
PFDS ($C_{10}F_{21}SO_3^-$, NH_4^+)	98	
PHpA ($C_6F_{13}CO_2^-$)	98	Fluka (Buchs, Switzerland)
PFUnA ($C_{10}F_{21}CO_2^-$)	95	
PFDoA ($C_{11}F_{23}CO_2^-$)	95	
PFTA ($C_{13}F_{27}CO_2^-$)	97	
PFBS ($C_4F_9SO_3^-$, K^+)	97	Dyneon (Zwijndrecht, Belgium)
PFHS ($C_6F_{13}SO_3^-$, K^+)*	98	Interchim (Montluçon, France)

*: used for the high resolution MS experiments

Purified isomer fractions (see Table 3.2) were gratefully provided by the laboratories Wellington Inc. (Guelph, Canada). Wellington Inc. also provided a PFOA solution with the typical isomer pattern of the electrochemical process.

Table 3.2: Composition of the isomer fractions obtained from a perfluorooctanesulfonyl fluoride technical product. Structure identification and approximate relative composition were carried out by ^{19}F-NMR spectroscopy.

Isomer fractions	Structure of isomers (relative abundance in %)
Fraction 1	Linear perfluorooctane sulfonate (L-PFOS) (97), 6-CF_3-PFOS (3)
Fraction 2	1-CF_3-PFOS (85), 6-CF_3-PFOS (15)
Fraction 3	3-CF_3-PFOS (93), 4-CF_3-PFOS (7)
Fraction 4	4-CF_3-PFOS (85), 3-CF_3-PFOS (5), 5-CF_3-PFOS (5), unknown (5)
Fraction 5	5-CF_3-PFOS (86), 2-CF_3-PFOS (7), 4-CF_3-PFOS (7)
Fraction 6	6-CF_3-PFOS (75), 5-CF_3-PFOS (12), 4-CF_3-PFOS (8), L-PFOS (5)
Iso-propyl fraction*	6-CF_3-PFOS (76), 5-CF_3-PFOS (12), 4-CF_3-PFOS (8), L-PFOS (4)

*: fraction used for GC analysis

Solutions containing linear, 6-CF_3, 4-CF_3 and 5-CF_3- PFOA and PFOSA were also donated by Wellington Inc..

3.1.2. Solvents and other materials

Methanol Pestipur (≥99.8 %) was delivered by SDS (Peypin, France) and by Labscan (Dublin, Ireland). Water was purified by an Elgastat Maxima HPLC water purification unit (Elga Ltd., Bucks, United Kingdom). Ammonium acetate of analytical grade (98 %) was obtained from Merck (Darmstadt, Germany). Formic acid (98-100 %) was obtained from Sigma-Aldrich (Seelze, Germany).

Methyl-tert-butyl-ether (MTBE, 99.9 %) was purchased from Merck (Darmstadt, Germany). Tetrabutyl ammonium acetate (TBA, >90 %) was obtained from Fluka (Buchs, Switzerland).

Cyclohexane, n-hexane, iso-octane and ethanol (HPLC grade) were delivered by Scharlau (Barcelona, Spain), sulfuric acid (95-98 %) by J.T Baker (Deventer, The Netherlands) and iso-propanol (HPLC grade) by Biosolve Ltd. (Berneveld, The Netherlands). Deuterium

labelled 1,1,1,3,3,3-D_6-iso-propanol (99.8 %) was purchased from CDN Isotopes Inc. (Pointe Claire, Canada). Trimethylchlorsilan (≥97 %) and N-O-bis(trimethylsilyl) acetamide (synthesis grade) were purchased from Sigma-Aldrich (Germany). Natrium sulfate (Na_2SO_4) (for organic trace analysis) was purchased from Merck (Darmstadt, Germany).

3.2. HPLC coupled to MS

3.2.1. HPLC instrumentation

A perfluorinated phenyl (PFP) phase (Thermo Electron, United Kingdom, 150 mm column length, 2.1 mm i.d., 5 µm particle size, 100 Å pore size), a X-Terra® C_{18} phase (Waters, USA, 100 mm length, 3.0 mm i.d., 3.5 µm particle size, 125 Å pore size) and a Discovery® HS C_{18} (Supelco, USA, 50 mm column length, 2.1 mm i.d, 3 µm particles size, 120 Å pore size) were used. Guard columns with PFP phase (10 mm) and Discovery® HS C_{18} (20 mm length) were employed for the analysis of environmental samples together with the corresponding HPLC columns.

A Rheos 2000 low-pressure mixing binary pump (Flux instruments, Basel, Switzerland) was employed for HPLC ion trap MS. Eluents were degassed with helium (99.999 %, Carbagas, Basel, Switzerland). A PAL autosampler (CTC Analytics, Zwingen, Switzerland) was used.

A Degassit degasser (Metachem Technologies, USA) as well as a two solvent delivery modules (Pro Star 210, Varian, USA) and a Triathlon autosampler (Varian, Walnut Creek, USA) were used together with the triple quadrupole MS.

The following gradient of aqueous 4 mM ammonium acetate and methanol was applied. Gradient 1 (slow) was: 30 % methanol for 1 min, to 65 % within 12 min, kept for 6 min, to 85 % within 6 min, isocratic for 6 min. Then, the column was rinsed with 100 % methanol for 1 min and returned to the starting conditions within 1 min. The following gradient of aqueous 2 mM ammonium acetate and methanol was applied. Gradient 2 (fast) was: 30% methanol for 1 min, to 95 % within 12 min, kept for 6 min. Then, the column was rinsed with 100 % methanol for 1 min and returned to the starting conditions within 1 min. The flow rate was in either case 200 µl/min.

The following gradient was applied when HPLC-SQMS was performed. The mobile phases consisted of 2 mM ammonium acetate in methanol and 2 mM ammonium acetate in water. The eluent gradient started at 35 % methanol followed by a 20 min ramp to 90 % methanol and finalized by a ten minute hold and a ten minute washing sequence with 100 % methanol. The flow rate was set to 300 µl/min.

3.2.2. Mass spectrometry coupled to HPLC

3.2.2.1. Ion trap mass spectrometer

An ion trap mass spectrometer (LCQ, Thermo Finnigan, San Jose, USA) was employed in the electrospray ionization mode detecting negative ions (ESI(-)). The following instrument parameters were applied: Nitrogen sheath gas flow, 60 arbitrary units, heated capillary temperature 200 °C, spray voltage 4.5 kV for PFSA and PFOSA and 3.5 kV for PFCA, capillary voltage -22 V, tube lens offset 10 V. The molecular ions of the PFC of interest were chosen for the selected ion monitoring mode (SIM) (see Table 3.3). MS^2 spectra of [M-H]- were recorded with the collision energy (CE) listed in Table 3.3 and an excitation time of 0.2 s. Helium was used as collision gas. The mass range of the ion trap

was set at the low mass cut-off of the analyte and at its molecular ions for the high mass range. The dwell time for each compound was optimized to obtain a minimum of 12 points per chromatographic peak.

Table 3.3: Ion trap and triple quadrupole MS/MS parameters applied to different perfluorinated compounds. The molecular ions were selected as precursor ions.

	Ion trap			Triple quadrupole		
	m/z→m/z	**CE (%)**	**Low mass cut-off**	***m/z→m/z***	**Capillary voltage (V)**	**CE (V)**
PFBS		n.a		299→80	-40	20.5
PFHS		n.a		399→80	-40	29
PFOSA	498→478	40	135	498	-40	21
PFOS	499→419	40	135	499→80	-40	35
	499→330	40		499→99		30
	499→280	40		499→130		35
PFDS	599→380	45	160	599→80	-40	45.5
	599→230					
	599→280					
PFBA		n.a		213→169		
PFHA	313→269	10	85	313→269		
PFHpA		n.a		363→319		
PFOA	413→369	10	110	413→369	-20	4
PFNA		n.a		463→419		
PFDA	513→469	10	140	513→469		
PFUnA		n.a		563→519		
PFDoA		n.a		613→569		

n.a: not applied during this study
CE: collision energy

3.2.2.2. Triple quadrupole mass spectrometer

The following instrument parameters were applied to the triple quadrupole 1200L mass spectrometer (Varian, Walnut Creek, USA): Nitrogen drying gas flow 140 kPa and 200 °C; spray voltage 4.0 kV. Nitrogen was used as nebulizing gas at a flow of 410 kPa. The heated capillary voltage was -45 V. Argon was used as collision gas at a collision cell pressure of 0.3 Pa. The optimized CE were obtained employing the work station software (Varian) and the option MS/MS breakdown. Collision energies applied to the molecular

ions are listed in Table 3.3. TQMS/MS spectra were recorded within a mass range of *m/z* 75-500 using a scan time of 0.2 s. Multiple reaction monitoring (MRM) mode was used for scan time optimization.

3.2.2.3. Single quadrupole

A single quadrupole mass spectrometer HP 1100 mass spectrometric detector (MSD, Waldbronn, Germany) was used. Electroyspray ionization in the negative ion mode was employed. The following parameters were applied: nitrogen nebulizer gas temperature 350 ºC, nebulizer gas pressure 138 kPa, nitrogen drying gas flow 13 ml/min, and capillary voltage 3500 V. Mass *m/z* 499 was measured in the selective ion monitoring mode at a fragmentation voltage of 150 V and a dwell time of 144 ms.

3.3. GC coupled to MS

3.3.1. Temperature program

The temperature program 1 was: 40 °C isothermal for 8 min, then 5 °C/min to 130 °C, 30 °C/min to 220 °C, and isothermal for 5 min. The temperature program 2 was: 50 °C isothermal for 3 min, then 5 °C/min to 150 °C, 30 °C/min to 180 °C.

3.3.2. GC-EI-LRMS

Separation was performed with a GC8060 gas chromatograph (Fisons Instruments, United Kingdom) equipped with a split/splitless injector and a A200S auto sampler (Fisons Instrument, United Kingdom). The separation was performed on a capillary column coated with HP-5MS (5 %-phenyl-95 %-methylpolysiloxane, 15 m length, 0.25 mm i.d, 0.25 μm

film thickness, J&W Scientific, USA). 1 µl of the sample was injected in the split mode (1:10). The injector temperature was set to 220 °C and the interface temperature to 250 °C. Helium (99.999 %, Carbagas, Switzerland) was used as carrier gas at a constant flow of 1 ml/min. Temperature program 1 was applied for the characterization of the homologues series of PFCA and PFSA.

A MD800 quadrupole mass spectrometer (Fisons instrument, United Kingdom) was employed in the EI mode (70 eV). Compounds were detected in the full scan mode (mass range *m/z* 60-600, scan time 0.8 s or *m/z* 60-800, scan time 0.6 s).

3.3.3. GC-NCI/EI-LRMS and MS/MS

Separation was performed with a CP 3800 gas chromatograph (Varian, USA) equipped with a split/splitless injector and a Combi PAL auto sampler (CTC Analytics, Switzerland). The same capillary column (see Chapter 3.2.2) was used. 2 µl of the sample were injected in the split mode (1:10). The injector temperature was set to 220 °C and the interface temperature to 220 °C. Helium (99.999 %, Carbagas, Switzerland) was selected as carrier gas at a constant flow of 1.5 ml/min. Temperature program 1 was used for NCI and the tandem MS experiments and program 2 for the PFOS isomer characterization.

A 1200 triple quadrupole mass spectrometer (Varian, USA) was employed in the NCI and EI mode. Methane at a pressure of 120 Pa (99.999 %, Carbagas, Switzerland) was used as reagent gas. Compounds were detected in the full scan mode applying a scan range of *m/z* 50-600 (scan time 0.2 s). MS/MS was performed at a collision energy of +15 V or -15 V (EI or NCI) with a scan time of 0.2 s.

3.3.4. GC-EI-HRMS

GC-EI-HRMS was used for accurate mass determination. Separations were carried out on an Agilent Technologies 6890N Series gas chromatograph (USA) using a DB5-MS capillary column (5 %-phenyl-95 %-methylpolysiloxane, 30 m length, 0.25 mm i.d., 0.1 µm film thickness, J&W Scientific, USA). Sample volumes of 1 µl were injected splitless (2 min splitless time) with an autosampler (7683B Series, ALS, USA). Helium was used as carrier gas at a constant flow of 1 ml/min. The injector temperature was set to 220 °C and the interface temperature to 250 °C. Temperature program 1 was used.

Exact mass determination was performed with a VG Autospec Ultima mass spectrometer (Micromass, England) at a resolution of 8000 (5 % valley). The MS was operated in the EI mode (70 eV, electron energy) with an ion source temperature of 200 °C and an acceleration voltage of 8000 V. Fragments of perfluorokerosene (PFK) were used as lock masses. Mass spectra were acquired in the full scan mode (mass range *m/z* 75-500) with a scan time of 1.5 s per decade. Mass accuracy was defined followed and was expressed in ppm: $(m_{measured}-m_{real})*10^6/m_{measured}$. The following restriction parameters were used for the calculation of elemental composition: 15 ppm tolerance for mass difference between experimental and theoretical mass, 0 to 4 double bond equivalents (DBE), monoisotopic, odd and even number of electrons possible, 0 to 12 C atoms, 0 to 10 H atoms, 0 to 3 O atoms, 0 to 1 S atoms and 0 to 13 F atoms.

3.4. Samples

3.4.1. Fish liver/tissue extraction procedure

Extraction is based on liquid-liquid extraction employing methyl-tert-butyl-ether. The different steps of the extraction procedure are described in Figure 3.1.

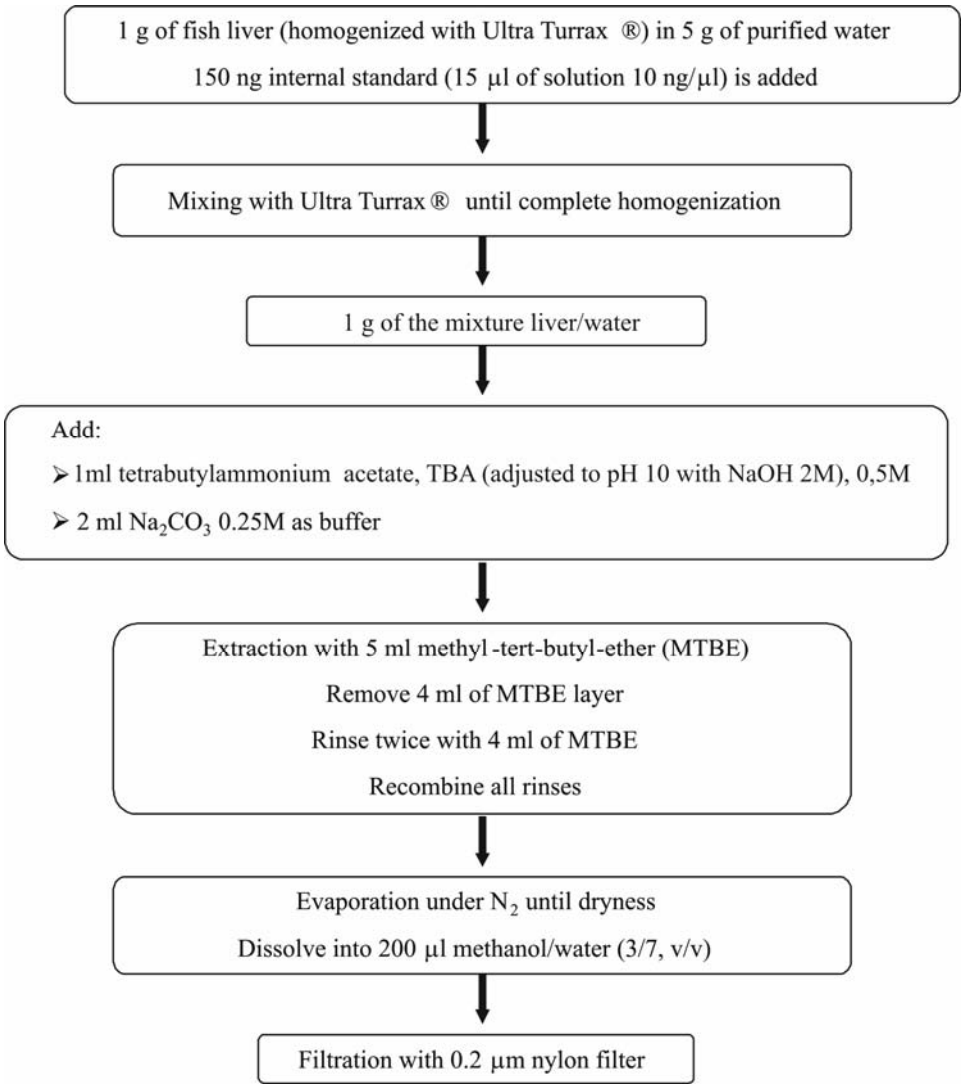

Figure 3.1: Extraction procedure applied on fish liver and tissue samples.

An Ultra-Turrax® T25 Basic (Janke and Kungel, Staufen, Germany) was used for homogenization and mixing of the sample. A centrifuge Minifuge T from Heraeus AG (Zürich, Switzerland) was used. The $NaCO_3$ buffer solution (pH 10) consisted of 1.05 g $NaHCO_3$ and 1.33 g of Na_2CO_3 in 50 ml water.

3.4.2. Human blood

3.4.2.1. Samples

The human blood were extracted and provided by Anna Kärrman, Prof. Bert van Bavel and Prof. Günilla Lindström from the Man Technology Environment Center Research Centre at the University of Orebrö (Sweden). Human blood samples from Sweden were collected between 1997 and 2000 and consisted of plasma from men at the age of 19-46 years (n=10) and women at the age of 46-75 years (n=8). The human plasma samples from the United Kingdom were from 2003 and consisted of plasma from men at the age of 28-40 years (n=6) and women at the age of 26-45 years (n=7). Plasma samples were obtained by centrifugation of the whole blood collected in heparin tubes. Forty pooled human serum samples were obtained from different regions of Australia. Sampling was carried out between November 2002 and April 2003. They represented the following age groups of men and women: <16, 16-30, 31-45, 46-60 and >60 years. Samples representing an urban population were obtained from the Southeast of Australia (Sydney, Canberra, Wollongong, Newcastle and other major urban centers of New South Wales). Rural samples were obtained from the Northern Territory and areas in all states with postcodes outside metropolitan or major regional centers. Two pools containing 100 individual samples were prepared for each age, gender and region group. In addition, samples from five individuals from Sweden donating whole blood, plasma and serum in 2004 were included to evaluate matrix-dependent differences.

3.4.2.2. Extraction procedure

The extraction procedure is described in details elsewhere (Kärrman *et al.*, 2005). Briefly, to 0.75 ml serum or plasma 3 ml of a formic acid (98-100 %)/water (ultra pure) mixture

(50/50, v/v) was added. The solution was sonicated for 15 min and centrifuged at 10000 x g for 30 min. An aliquot of 2.5 ml corresponding to 0.5 ml sample was extracted with a solid phase extraction (SPE) column. Elution was performed with 0.5 ml methanol. In addition, the United Kingdom samples were extracted in duplicate. The duplicate was extracted with Waters Oasis® WAX SPE cartridges (60 mg, 30 μm) and with 1 ml 2 % ammonium hydroxide in methanol as elution solvent.

3.4.3. Water sample

The water extracts were provided by the German Federal Maritime Hydrographic Agency. Surface water samples (5 m depth) were collected in May 2004. Sample sites were located at the estuary of the river Elbe and the German Bight in the North Sea (53°52, 4'N; 8°43, 8'E and 53°36, 8'N; 9°33, 5'E). The extraction procedure is described into details elsewhere (Caliebe *et al*., 2005). Briefly, sea water (10 l) was acidified with 10 ml of hydrochloric acid (25 %) to pH 2-3. The sample was extracted with a SPE cartridge (4 g of Chromabond HR-P resin, Macherey-Nagel, Düren, Germany). After washing with water and drying in a stream of nitrogen, the cartridges were eluted with 100 ml of methanol in the reversed direction. Finally, the sample was concentrated to 200 μl with a nitrogen stream.

3.5. Derivatization

Derivatization was qualitatively performed by adding ca. 1-2 mg of the analyte to 1 ml of the derivatization reagent and to concentrated sulfuric acid (H_2SO_4). Four reagents were tested: ethanol, iso-propanol, trimethylchlorsilan (C_3H_9ClSi) and N,O-Bis (tri-methylsilyl) acetamide ($(CH_3)_3SiO(CH_3)CNSi(CH_3)_3$). The derivatization procedure was tested with 0, 2 and 5 % of concentrated H_2SO_4 (v/v with derivatization reagent). The mixture was shaken for 1 min, 3 hours or overnight. 800 µl of extraction solvent and 200 µl of purified water were added to extract the derivative. An additional clean-up with 300 µl of water was performed to remove the remaining acid, not compatible with stationary phases employed in GC. Cyclohexane, n-hexane and iso-octane were tested as extraction solvents. Cyclohexane was used as extraction solvent for LRMS experiments and n-hexane for HRMS experiments. Na_2SO_4 was added. Alternatively, deuterium labelled 1,1,1,3,3,3-D_6-iso-propanol was applied as derivatization reagent.

4. Methodology and quantification

4.1. Environmental analysis and PFAS analysis

The interest in environmental analysis started s with the discovery of the first pollutants in the environment during the late 1960s. The early success of gas chromatography (GC) coupled to mass spectrometry (MS) demonstrated the benefits of this technique to this detection mode. MS could be successfully combined with high performance liquid chromatography (HPLC) first in the 1980s. It widened considerably the range of detectable compounds. Today, HPLC-MS is a well established technique in the environmental analysis.

Quantitative analysis of trace contaminants in complex matrices requires selectivity and sensitivity. MS detection offers both. The high selectivity can be improved further by the use of tandem MS (MS^2 or MS/MS). Triple quadrupole mass spectrometer (TQ) combined with multiple reaction monitoring (MRM) is very best suited for quantification of contaminants. However, the selection of the type of mass spectrometer is often driven by availability. Therefore, the performance of ion trap (IT) and time of flight (TOF) was also considered for trace quantification. Though TOF has no ability for MS^2, it shows improved selectivity due to its higher mass resolution.

Analysis of PFAS followed the development of analytical instrumentation. The first reports on fluorine compounds in human blood were based on fluorine nuclear magnetic resonance (NMR) (Taves, 1968). However, this method suffered from the lack of selectivity since it detected only the presence of CF_3 and CF_2 moieties as well as of sensitivity with a reported limit of detection of 10 µg/l for water samples (Moody *et al.*, 2001). In the 1980s, the total

organofluorine levels in plasma and urine were determined by GC coupled to flame ionization or electron capture detection and to mass spectrometry (MS) (Belisle *et al.*, 1980; Ylinen *et al.*, 1985; Yamamoto *et al.*, 1989)

The successful development of interfaces for HPLC-MS allowed to improve the analysis of PFAS in environmental samples. Moreover, it is the only tool enabling the analysis of ionic and non-ionic PFAS. Therefore, became the mostly current technique for their analysis

This chapter will discuss some methodologies used for the analysis of PFAS focusing mainly on PFOS, PFOA and PFOSA. Differences between IT and TQ will also be presented concerning interferences during quantification.

4.2. Ion trap vs. triple quadrupole MS

4.2.1. Ionization and fragmentation

PFAS contain carboxylic, sulfonic, hydroxy or sulfonamide group. They have acidic properties and can therefore dissociate. Therefore, electrospray ionization in the negative mode (ESI(-)) is best suited.

Pseudomolecular ions are formed such as $[M-K]^-$ for PFOS (m/z 499), $[M-H]^-$ for PFOA (*m/z* 413) and PFOSA (*m/z* 498). No other product ions are generated reflecting the high stability of these compounds. The molecular ions were selected as precursor ions for MS2 experiments using ion trap and a triple quadrupole instruments. Both mass spectrometers will be discussed concerning their ability to analyze PFAS. A comparison of both instruments with a TOF mass analyzer were described elsewhere (Berger *et al.*, 2004).

Typical ESI(-)ITMS^2 and ESI(-)TQMS^2 spectra of PFOS, PFOA and PFOSA are shown in Figure 4.1.

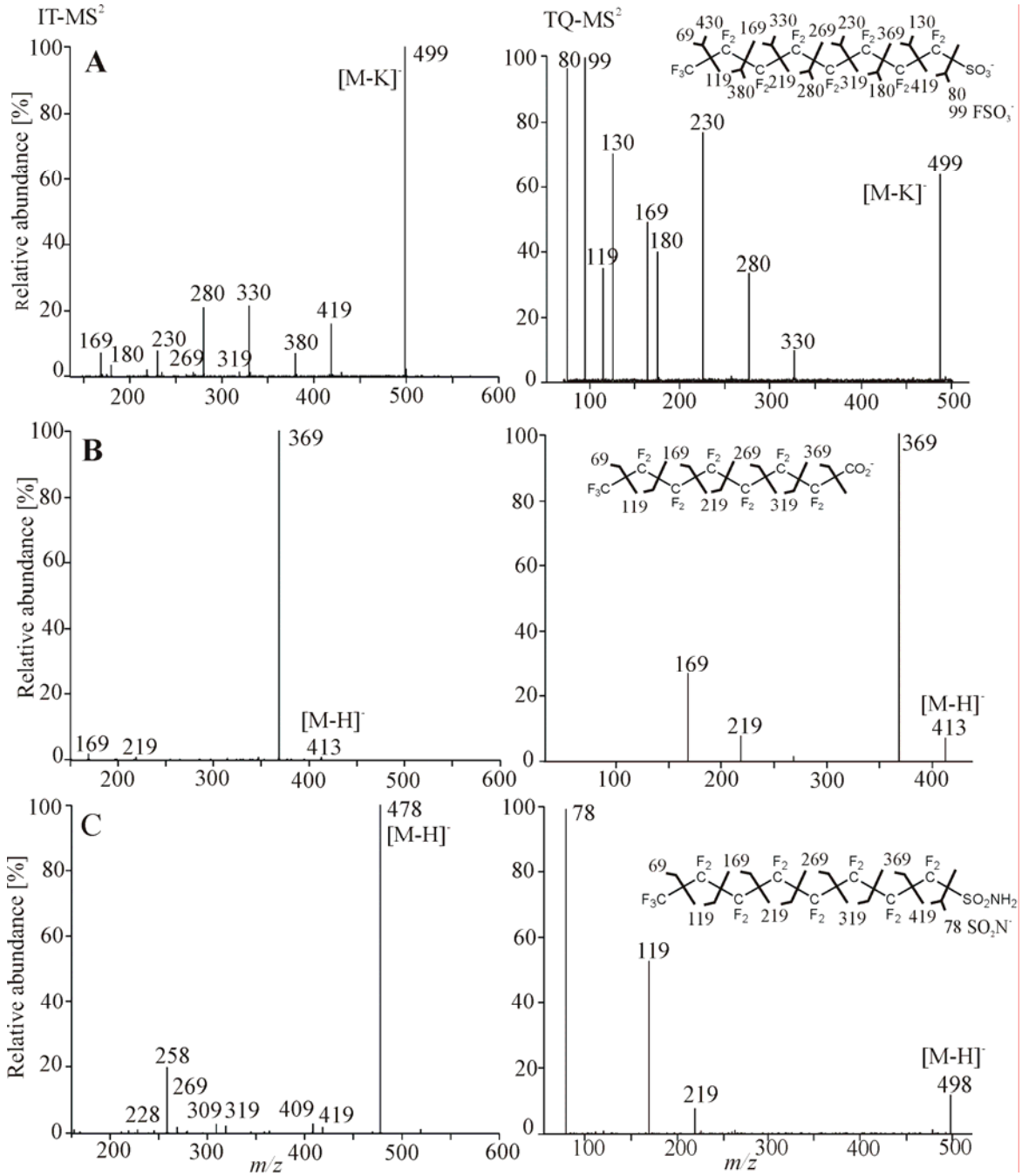

Figure 4.1: ESI(-)ITMS^2 (left) and TQMS^2 (right) spectra of PFOS (A), PFOA (B) and PFOSA (C). The molecular ions were fragmented at 40 %, 25 % and 40 % collision energy with IT at -35 V, -20 V and -40 V collision energy with TQ.

The molecular ions provide main series of fragments typical for each family of compounds under MS^2 conditions. Table 4.1 summarizes the obtained product ions and their proposed

structures. They can be divided into three main series called “9-series” (ending with a “9”) and “0-series” (ending with a “0”) and “other”. The series contained fragments with a mass difference of 50 u corresponding to -CF_2-. The first 9-series is common for PFOS, PFOA and PFOSA. The 0-series is typical for PFOS and other perfluorosulfonic acids. PFOSA provided a second 9-series as shown in Table 4.1 and Figure 4.2.

Table 4.1: Fragment series and other product ions produced by tandem mass spectrometry. Molecular anions were dissociated (PFOS (*m/z* 499), PFOA (*m/z* 413) and PFOSA (*m/z* 498)).

Assignment	Proposed structure	Product ions (*m/z*)
9-series	$[C_nF_{2n+1}]^-$ $1\leq n\leq 8$	69*, 119*, 169, 219, 269, 319, 369, 419 (PFOS, PFOA, PFOSA)
0-series	$[C_mF_{2m}SO_3]^-$ $1\leq m\leq 7$	130*, 180, 230, 280, 330, 380, 430 (PFOS)
9-series	$[M\text{-}HF\text{-}C_XF_{2X+1}\text{-}H]^-$	209, 259, 309, 359, 409 (PFOSA)
other	$[FSO_3]^-$	99* (PFOS)
	$[SO_3]^-$	80* (PFOS)
	$[SO_2N]^-$	78 (PFOSA)
	$[M\text{-}HF\text{-}C_5F_{10}\text{-}H]^-$	228 (PFOSA)

* Product ions not detectable by ion trap MS/MS due to the operational mass cut-off at *m/z* 135

Ion yields of PFSA, PFCA with C_6, C_8 and C_{10} chain length as well as of PFOSA were calculated and were defined as followed. The abundance of the precursor ions was defined as 100 %. The signal intensity of the product ions in the MS/MS spectrum was calculated as relative percentage of the precursor ion. The abundances obtained by IT and TQ MS/MS are listed in Table 4.2.

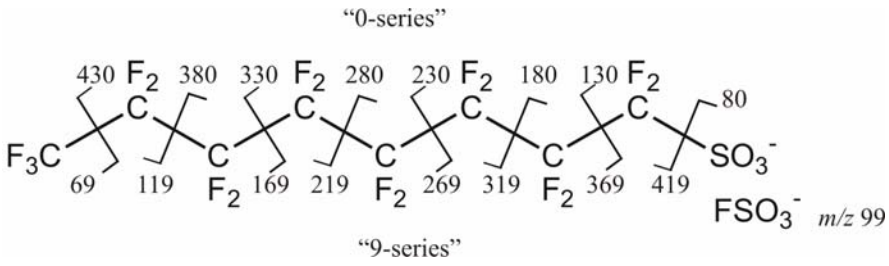

Figure 4.2: Fragmentation series and product ions obtained when applying MS^2 on PFOS molecular ions $[M\text{-}K]^-$ (*m/z* 499).

Table 4.2: Ion yields of MS^2 fragmentation (in %) of seven PFC by triple quadrupole (TQ) MS^2 and ion trap (IT) MS^2.

	$TQMS^2$			$ITMS^2$		
	m/z→ m/z	**CE (V)**	**Ion yield (%)**	***m/z→ m/z***	**CE (%)**	**Ion yield (%)**
PFHA	313→269	10	23	313→269	20	0.3
				269→119	30	0.2
PFOA	413→369	10	19.7	413→369	20	0.3
				369→169	35	0.6
PFDA	513→469	10	25.3	513→469	20	0.3
				469→269	30	0.3
PFHS	399→80	25	0.7	399→319	35	0.04
	399→99	30	0.6	399→230	35	0.13
				399→280	35	0.15
PFOS	499→80	35	0.4	499→419	42	0.3
	499→99	40	0.6	499→330	42	0.4
	499→130	35	0.4	499→280	42	0.3
PFDS	599→80	40	0.6	599→280	45	0.4
	599→99	45	0.3	599→330	45	0.1
	599→130	45	0.4	599→380	45	0.3
PFOSA	498→78	35	3.8	498→478	30	7.7

CE: collision energy

In general, HPLC-MS^2 of PFAS suffered from the very low ion yields ranging from 0.04 to 25.3 %, which reflected their very high stability. PFCA are less stable than PFSA. The loss of CO_2 led to most abundant product ions obtained by $TQMS^2$ (ca 20 %). Higher collision energies were necessary to induce optimal PFSA dissociation on both instruments. Decarboxylation of the PFCA molecular ion is also possible by in-source fragmentation after increasing the capillary voltage. The collision energy has to be increased with the chain length of the PFSA to induce optimal fragmentation. This reflects the elevated stability of PFHS, PFOS and PFDS as shown in Table 4.2.

Moreover, the overall PFSA ion yields were lower for $ITMS^2$ (0.04-0.4 %) than for $TQMS^2$ (0.3-0.6 %) (see Table 4.2). This was due to the low mass cut-off limitations of the ion trap instrument, which reduces the mass range at each fragmentation step. Therefore,

the abundant low mass fragments *m/z* 80, 99, 130 could not be detected by $ITMS^2$. This was also valid for PFOSA (see Table 4.2).

4.2.2. Linear ranges and limits of detection

Linear ranges and detection limits for PFHA and PFHS (C_6), PFOA and PFOS (C_8), PFDA and PFDS (C_{10}) and PFOSA obtained by ITMS and TQMS are given in Table 4.3.

Table 4.3: Limits of detection (LOD, signal-to-noise ratio 3:1)) and linear range of selected PFAS obtained by ion trap MS in the full scan mode and triple quadrupole employing ion monitoring mode (SIM). Multiple reaction monitoring (MRM) was used with both instruments.

	Ion trap		**Triple quadrupole**	
	Full scan	**MRM**	**SIM**	**MRM**
PFHA	313	313→269	313	313→269
LOD (pg)	50	2000	15	50
Linear range (ng)	0.15-2.5	8-100	0.05-3	0.2-25
PFHS	399	399→99	399	399→99
LOD (pg)	20	2500	2.5	50
Linear range (ng)	0.08-2.5	10-100	0.03-2	0.5-25
PFOA	413	413→369	413	413→369
LOD (pg)	100	4000	15	100
Linear range (ng)	0.3-2.5	15-150	0.05-2.5	0.3-20
PFOS	499	499→99	499	499→99
LOD (pg)	25	5000	5	150
Linear range (ng)	0.08-2.5	15-100	0.02-3	0.5-20
PFDA	513	513→469	513	513→469
LOD (pg)	150	5000	20	100
Linear range (ng)	0.3-3	15-500	0.08-4	0.5-20
PFDS	599	599→99	599	599→99
LOD (pg)	50	6000	5	100
Linear range (ng)	0.1-2	10-500	n.c	n.c
PFOSA	498	498→98	498	498→98
LOD (pg)	50	5000	10	100
Linear range (ng)	0.15-2.5	15-100	0.1-4	0.3-25

n.c: not calculated

Limits of detection at a signal-to-noise ratio of 3:1 were 2.5 to 20 pg and 50-150 pg for TQMS (SIM mode) and TQMS/MS respectively (see Table 4.3). These values are one magnitude higher than those reported by Berger *et al.* (2004) for the TQMS QuattroLC (Micromass). Ion trap MS provided higher LOD in both the full scan mode and by MS/MS compared with TQ (see Table 4.2). Therefore, a TQ mass spectrometer is better suited for quantification of PFAS in the MS/MS mode.

Linear ranges reported in Table 4.3 started with the limit of quantification (LOQ) at a signal-to-noise ratio of 10:1. The IT mass analyzer provided the most limited linear ranges covering only two orders of magnitude. Berger *et al*. (2004) reported a similar linear range for a TOF instrument.

4.3. HPLC separation

PFAS were separated by reversed phase HPLC. The use of ammonium acetate (NH_4OAc) as buffer in the mobile phase was necessary to enhance the peak shape, when a C_{18} was applied. The absence of buffer led to broad peak (see Figure 4.3). NH_4OAc acts as a kind of ion-pairing reagent, since all the compounds under investigation are ionic except PFOSA. However, longer retention times and a reduced signal intensity were the consequence. Typically, a concentration of 2 mM of NH_4OAc was used for the separation of the PFAS. Figure 4.3 shows an example of the separation of ten PFAS.

Separation was driven by PFAS chain length. PFAS with the longest chain eluted last followed by the more lipophobic PFOSA. The chromatographic signals of PFOS, PFDS and PFOSA were not symmetrical due to some minor isomers present in the technical

solution. Separation of these isomers and their characterization will be discussed in the next chapter (see Chapters 5.3, 5.4 and 5.5). A compromise had to be found between separation of PFAS and separation of the isomers for their quantification.

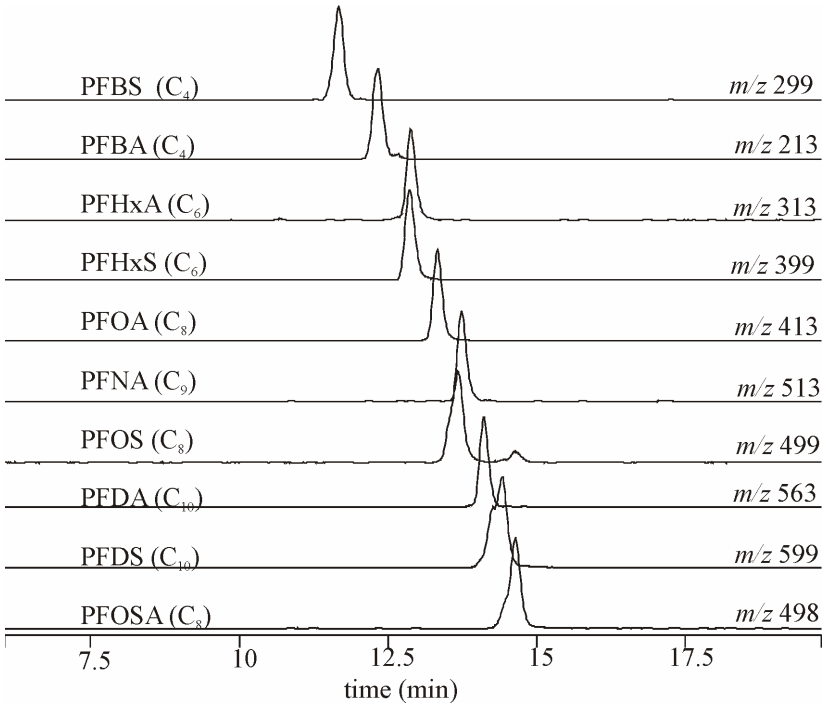

Figure 4.3: Separation of selected PFAS on a C_{18} phase recorded with ESI(-)-TQMS in the full scan mode (*m/z* 250-650) using 2 mM NH_4OAc in the mobile phase with the gradient 2.

4.4. Extraction procedure

The extraction procedure described in chapter 3.4 is based on an ion-pair formation between PFAS and a tetra-alkyl ammonium reagent, which is then extracted from the liver sample with methyl-tert-butyl-ether. 1H,1H,2H,2H-tetrahydroperfluorooctane (THPFOS, see chemical structure in Figure 4.4) was mainly used as internal standard for the quantification of PFAS until its detection in groundwater collected from military bases (Schultz *et al.*, 2004). Therefore, a new internal standard had to be found. In this study, the

use of perfluoro-3,7-dimethyloctanoic acid (3,7-DMPFOA, see chemical structure in Figure 4.4) was proposed as a new internal standard.

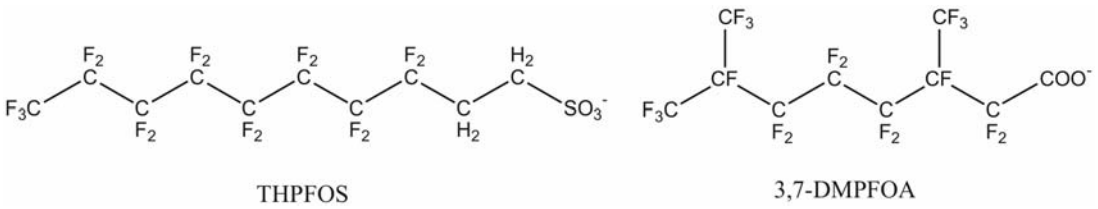

Figure 4.4: Chemical structures of 1H, 1H, 2H, 2H-tetrahydroperfluorooctane sulfonate (THPFOS) and perfluoro-3,7-dimethyloctanic acid (3,7-DMPFOA).

The different chemical properties of the carboxylic and sulfonic acids as well as the chain length influenced their recoveries relative to 3,7-DMPFOA as shown in Figure 4.4. However, this also occurred with THPFOS. PFCA with the same similar chain length as PFSA provided higher recoveries relative to 3,7-DMPFOA. Recoveries decreased with increasing chain length to ≤1 % for C_{10} (see Figure 4.5).

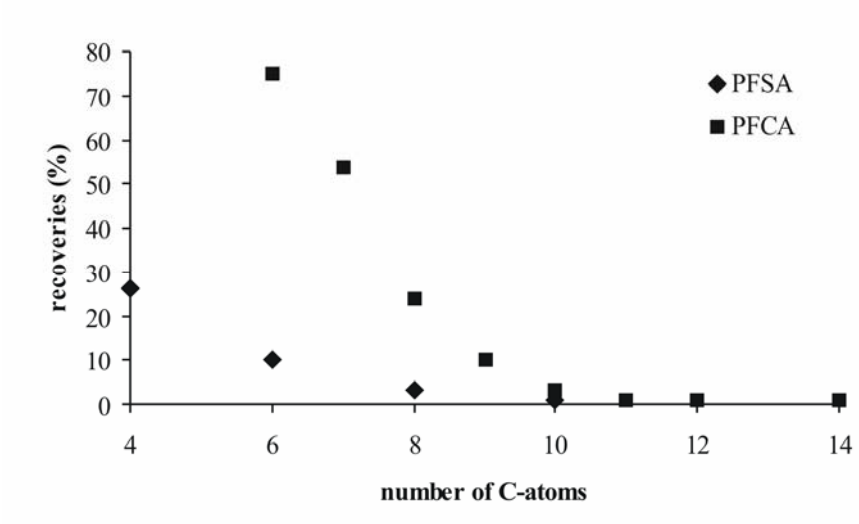

Figure 4.5: Recoveries relative to the internal standard 3,7-DMPFOA (in %) of perfluorosulfonic acids (PFSA) and perfluorocarboxylic acids (PFCA) in spiked fish liver samples.

Berger *et al.* (2005) reported the successful use of 3,7-DMPFOA as internal standard for the quantification of PFCA and PFSA in air samples. Occurrence of 3,7-DMPFOA in the environment is unlikely due to its very specific structure with double branching on two different C-atoms.

4.5. Interferences

A first interlaboratory study was conducted for the analysis of selected PFAS in 2005 (Van Leeuwen *et al.*, 2005). These were perfluorocarboxylic and perfluorosulfonic acids as well as perfluorooctanesulfonamide. In general, little agreement was found for the complete participating laboratories. Moreover, the level of agreement between the laboratories decreased with the complexity of the matrix (standard solution, fish liver extract and fish tissue). For example high relative deviations from means of 25-256 % in standard solution, 37-202 % in fish liver extract and 88-236 % in fish tissue were found. This study demonstrated that the current methods still required improvement. Therefore, analytical problems encountered during the analysis of PFAS will be listed and discussed in this chapter. These can lead to systematic errors and might explain the unsatisfactory data comparability of the first interlaboratory study (Van Leeuwen *et al.*, 2005).

4.5.1. Contamination and carry-over

Trace analysis in the ng/g range requires sufficiently low blank values. PFOA is used as polymerization aid in the manufacture of fluoropolymer resins and dispersion. PFOA is also a by-product of the TEFLON® manufacturing process (Dupont, 2006). TEFLON® and fluoropolymers are very common materials used in analytical laboratories such as tubings for HPLC and seals. Therefore, TEFLON® is a potential source of contamination for

PFOA and should be avoided in analytical laboratories dealing with PFAS analysis. Longer and shorter chained PFCA are also expected to be potential contaminants. Some solvent filters as well as one tested degasser system (Hewlett Packard, HP series 1050) released PFOA. The limit of detection obtained for PFOA (15 pg, see Table 4.3) by TQMS in the full scan mode was thus increased by a factor around ten. This disenabled quantification of environmental with typical level ranged around 100-200 pg. Results obtained during the first interlaboratory revealed as well PFNA and PFDA contaminations. For example, twelve values were submitted for PFNA in fish liver extract and in fish tissue although this chemical was not spiked (Van Leeuwen *et al.* 2005). PFBA was also found in the extraction blank. However, this C_4-based compound is not extensively quantified. Water sampling equipment can also be contaminated (Taniyasu *et al.*, 2005). It was also found out during the participation at the interlaboratory study that some carry over effects was possible with PFAS employing one autosampler (Varian, Triathlon, USA). Limits of detection were increased by a factor around eight.

4.5.2. Reference solutions and instrument response

Quantification based on similar technical mixtures is necessary for providing comparable results. However, batch to batch variations were reported by 3M company, the main PFC producer. Both the level of impurities and the isomer pattern may vary (3M, 1999).

Moreover, two production processes exist for PFOA yielding completely different isomer compositions. Figure 4.6 shows the isomer profiles of two PFOA technical products, originating from different production processes. The PFOA solution produced by electrochemical fluorination contained up to 22 % branched isomers, whereas the PFOA

solution manufactured by telomerization was pure. The isomer patterns of technical PFAS will be discussed into more detail in the next chapter.

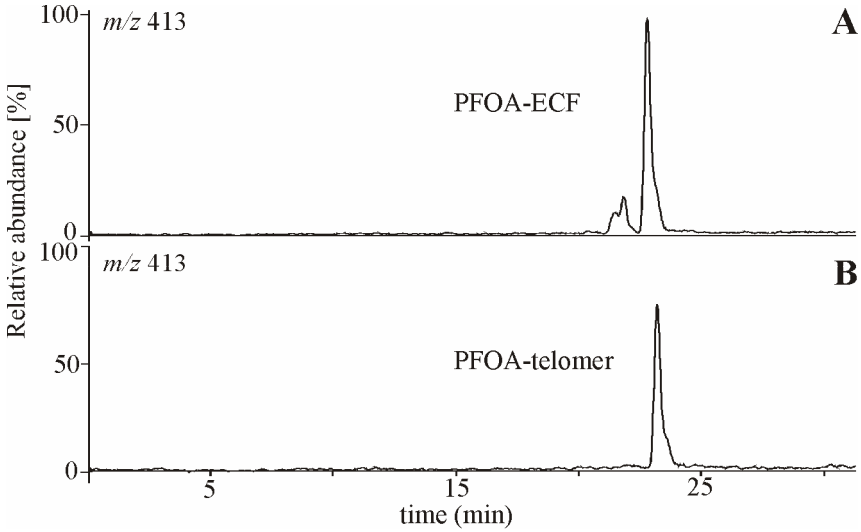

Figure 4.6: ESI(-)-TQMS mass chromatograms (*m/z* 413) of two PFOA technical products produced by electrochemical fluorination (ECF) (A) and by telomerization (B).

PFOS mixtures contained some homologues as main impurities with chain between C_5 to C_{10}. Table 4.6 shows their content in three different technical PFOS mixtures. Their composition varied both between companies (Fluka vs. ABCR) and from batch to batch (Fluka 1 vs. Fluka 2). The solution Fluka 2 did not contain any homologues. On the opposite, the solution Fluka 1 contained perfluoroheptane sulfonate (homologue C_5) as the main impurity besides homologues with C_6 to C_{10} chains. The solution obtained from ABCR contained only the homologue C_7. Thus, use of these solutions for quantification will cause some systematic result deviations.

Table 4.6: Variability the homologue composition relative to the C_8 compounds of technical PFOS product from two companies and in different batches.

	Composition (%)				
PFSA homologues	**C_{10}**	**C_9**	**C_7**	**C_6**	**C_5**
Fluka 1	2.3	4.7	12.4	32.8	42.6
Fluka 2	n.d	n.d	n.d	n.d	n.d
ABCR	n.d	n.d	100	n.d	n.d

n.d: not detected

The employed analytical technique can also induce errors concerning the relative composition of the technical products. The response factors of the isomers present varied between different methodologies. Table 4.7 shows the relative isomer composition of a technical PFOS mixture obtained in three different ways using ^{19}F-NMR and mass spectrometry. The results obtained by IT and TQ analyzers varied by ca. a factor of two. The ionization process overestimated the presence of branched isomers and formed only 40 % of the linear isomer composed to 69 to 79 % found with the other techniques.

Table 4.7: Relative isomer composition (in %) of a technical PFOS mixture obtained with different analytical methods.

	Minor isomers	**Major isomer**	**L-PFOS**
F^{19}-NMR			
3M company (Kestner, 1997)	0.38	28.9	70
Wellington Inc. (Arsenault *et al.*, 2005)	0.5	30.6	68.9
Mass spectrometry			
Single quadrupole*	0.6	20.8	79
Ion-trap (n= 5)	7.3 (± 1.2)	55.9 (± 2.7)	36.8 (± 3.4)
Triple quadrupole (n= 5)	2.7 (± 0.6)	28.4 (± 3.1)	68.9 (± 3.3)

*: Results provided by Anna Kärrman (University of Orebrö)

4.5.3. Matrix interference and mass monitored

Matrix interference was studied by HPLC-ESI(-)MS^2 with three different matrices on PFOS quantification. These were human plasma and serum extracts as well as water samples. Moreover, extracted PFOS-free fish liver was spiked with a technical PFOS mixture. Two different HPLC stationary phases, a C_{18} (Discovery® HS) and a PFP phases (see Chapter 3.2.1) were employed. Three mass transitions were monitored. The matrix effect was expressed as the deviation of the signal intensity compared to a technical PFOS mixture diluted in a solvent. Values higher than 1 in Table 4.8 correspond to signal enhancement and those lower than 1 to signal suppression.

Table 4.8: Matrix effect affecting PFOS quantification. The stationary phase used for separation, the kind of matrix and mass transition monitored were investigated.

Matrix	**Human plasma**		**Human serum**		**Water**	**Spiked fish liver**
Stationary phase	**C_{18}**	**PFP**	**C_{18}**	**PFP**	**C_{18}**	**C_{18}**
***m/z* 499→80**	0.99	0.69	1.13	0.82	0.97	0.97
***m/z* 499→99**	1.04	0.61	0.92	0.55	1.12	1.09
***m/z* 499→130**	0.87	0.74	1.01	0.71	0.60	0.12

Deviations compared to technical mixture were observed in mostly all cases. The use of a PFP stationary phase led to higher signal suppression in human plasma and serum. The relative matrix effect varied between 0.87 and 1.13 for the C_{18} phase and between 0.55 and 0.82 for the PFP phase. This is probably caused by ionization suppression/enhancement of matrix compounds coeluting differently on both phases. Coeluting impurities present in fish liver samples led a high signal suppression when monitored *m/z* 499→130. The matrix effect can occur in the electrospray interface when coeluting matrix components compete with the analyte for charge.

4.6. Conclusions

Triple quadrupole MS in the MRM mode is the best suitable technique for the determination of PFAS traces in environmental samples. It combines both high sensitivity and selectivity. Problems associated with the quantification of PFAS were identified such as lack of defined standards, technical mixtures consisting of complex mixtures of isomers and homologues, matrix effects affecting ionization and finally contamination by all stages of the analytical procedure. All might generate systematic errors. Participation at the first interlabory study on PFC revealed blank and carry-over problems. Contamination problems by TEFLON® containing PFOA and PFCA were also encountered. Finally, the isomer pattern of technical PFOS mixture might be different from environmental samples, which will also lead to faulty results.

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5. Isomer identification in standard solution

5.1. State of the art on PFAS isomers

5.1.1. Production process

Technical PFAS products are not pure. They contain as main impurities some isomers besides the linear product. So far, no study reported the influence of the isomer composition on the properties and qualities of the commercial product. The isomers are formed during the production and are process specific. There are two major commercial processes: electrochemical fluorination (ECF) and telomerization. Additional fluorination processes exist. However, they are not important for the commercial market and will not be discussed (Hekster *et al.*, 2002; De Silva and Mabury, 2004).

ECF is the major production route for PFAS. It is used by the 3M company since the 1950s. ECF replaces the C-H by C-F bonds. An electric current is passed through a solution of anhydrous hydrogen fluoride (HF) and the educt, n-1-octanesulfonyl fluoride ($C_8H_{17}SO_2F$). The obtained perfluorooctanesulfonyl fluoride ($C_8F_{17}SO_2F$) is the starting material for PFOS-related chemicals. During the fluorination step (see Figure 5.1 A), C-C bond rupture and molecular rearrangements accompany conversion of C-H to C-F, which is not always complete. Therefore, many by-products with even and odd number of C-atoms are formed besides the n- product. Therefore, the technical product contain between 10 and 30 % of homologues and branched isomers (Moldavsky *et al.*, 1998, OECD., 2002; De Silva and Mabury, 2004).

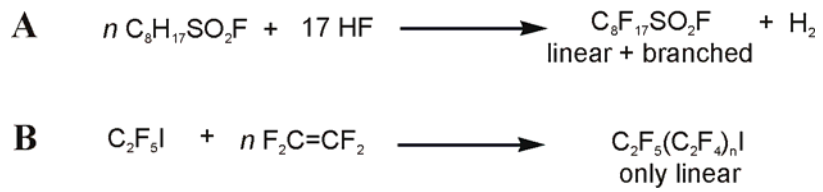

Figure 5.1: Main reactions of the two production processes of PFAS: electrochemical fluorination (A) and telomerization (B).

Telomerization is applied by for example Dupont, Clariant, Atofina and Asahi Glass. It is a polymerization reaction between tetrafluoroethylene (CF_2=CF_2) with a perfluoroalkyl iodide (CF_3CF_2I) (see Figure 5.1 B). In a first stage, the chain length is increased by n= 2, 4, 6…C atoms. Then, the iodide is substituted by a functional group depending on the application. Telomerization yields mainly n-alkyl products with an even number of C atoms.

The products obtained by telomerization are purer than those obtained by ECF. However, the ECF process is less expensive and therefore mostly used. The isomer profile of the technical product reflects the production process and allows also to trace the origin of pollution in environmental samples. PFOS, PFOSA as well as the perfluorosulfonamides are only produced by ECF. PFOA can be produced by both processes (Hekster *et al.*, 2002).

5.1.2. Previous ^{19}F-NMR results

Hundreds of PFOS isomers with the elemental composition $C_8F_{17}SO_3^-$ are theoretically possible. However, ^{19}F-NMR analysis of technical PFOS product allowed the identification of three groups of isomers (Arsenault *et al.*, 2005, Kestner, 1997). These were: the linear isomer, monoperfluoromethyl substituted and diperfluoromethyl geminal substituted isomers. The chemical structures of these PFOS isomers are shown in Figure 5.2 together

with the abbreviations used. ^{19}F-NMR analysis enabled the differentiation of the isomers 1-, 2-, 3-, 4-, 5- and 6-CF_3-PFOS. However, only the tert-butyl-PFOS was identified among the geminal diperfluoromethyl substituted isomers. Table 5.1 shows the isomer composition of commercial PFOS product obtained by ^{19}F-NMR spectroscopy.

Table 5.1: Relative isomer composition of technical PFOS obtained by ^{19}F-NMR spectroscopy

References	Gem-substituted PFOS	Tert-butyl-PFOS	1-CF_3-PFOS	2-CF_3-PFOS	3-CF_3-PFOS	4-CF_3-PFOS	5-CF_3-PFOS	6-CF_3-PFOS	L-PFOS
1	0.3	0.2	1.9	1.9	5.0	4.8	6.2	10.8	68.9
2	0.15	0.23	1.6			17		10.3	70

1: Arsenault *et al.*, 2005, 2: Kestner, 1997.

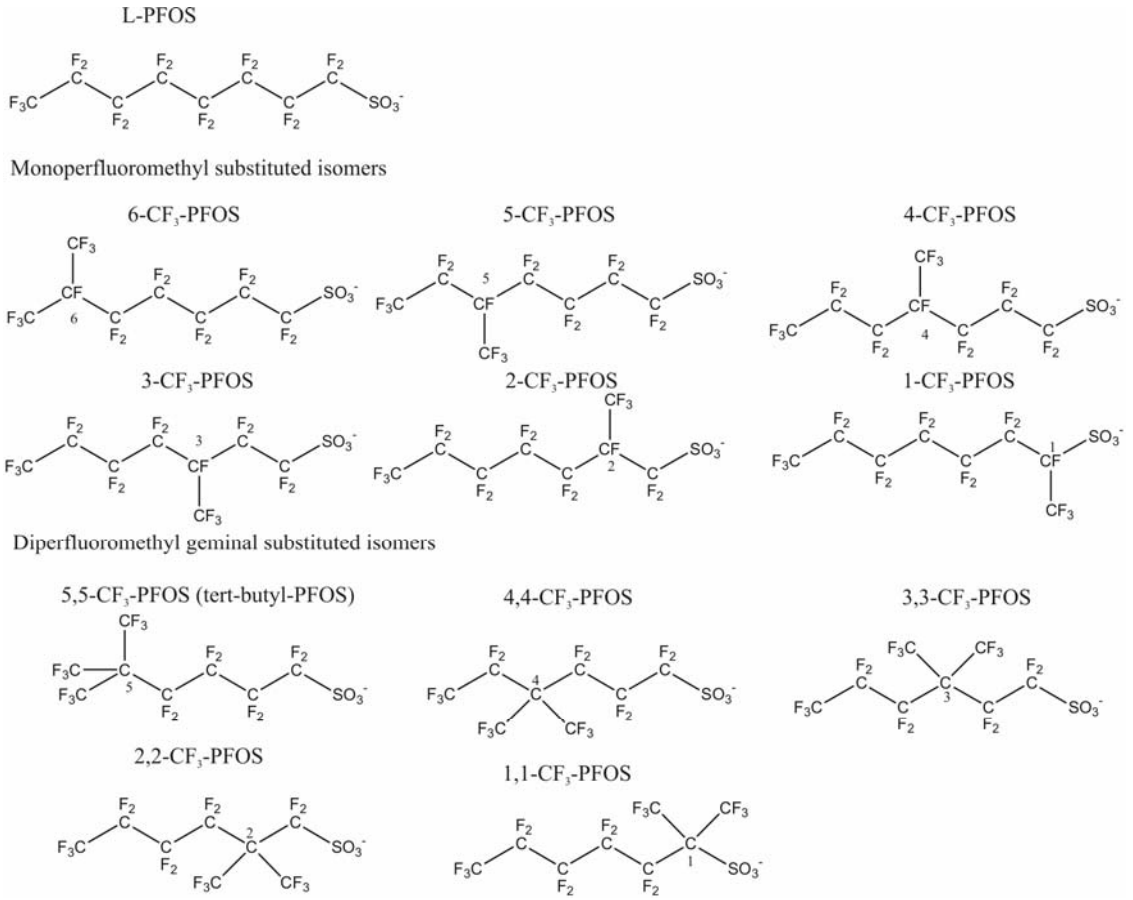

Figure 5.2: Structures of linear-PFOS (L-PFOS), perfluoroisopropyl (6-CF_3-PFOS), 5-perfluoromethyl- (5-CF_3-PFOS), 4-perfluoromethyl- (4-CF_3-PFOS), 3-perfluoromethyl- (3-CF_3-PFOS), 2-perfluoromethyl- (2-CF_3-PFOS), 1-perfluoromethyl- (1-CF_3-PFOS), tert-perfluorobutyl-PFOS and of the diperfluoro-methyl geminal substituted PFOS isomers.

5.2. Tandem MS: A tool for structural elucidation

During the last decade the development of soft ionization techniques such as atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) was decisive for the establishment of HPLC-MS as a standard method in pharmaceutical, biochemical and environmental analysis. ESI and APCI provide mainly protonated or deprotonated molecules. However, very few additional fragments are formed. Therefore, structural information can be gained. Moreover, isomers cannot be differentiated.

A precursor ion is selected in tandem mass spectrometry (MS/MS or MS^2) and then fragmented by collision induced dissociation (CID) resulting in product ions. Typically, helium or argon is used as collision gas. The product ions allow in many cases to determine the structure of the precursor ion. Moreover, the MS^2 spectrum gives a more specific fingerprint.

Tandem MS can be performed in time with an ion trap (IT) instrument or in space with the combination of mass spectrometers such as triple quadrupoles (TQ). Due to the methodological differences, the $ITMS^2$ and $TQMS^2$ spectra of a compound are not completely similar. With an ion trap, CID is only applied to the selected precursor ions in an ion trap. In $TQMS^2$ the precursor ions selected by the first quadrupole are fragmented by a cascade of collision induced processes leading to more ions.

Ion trap MS is the method of choice for structure elucidation. First, it provides high sensitivity in the product ion scan mode. Moreover, its ability for multiple stage fragmentation (MS^n) makes the ion trap a powerful tool for structural elucidation when

tandem MS is not sufficient. The main drawback of the ion trap spectrometer is the restricted mass registration range of the product ions.

The ion trap consists of a ring electrode and two end-cap electrodes. A direct current voltage is connected to both end caps. A radio frequency (rf) voltage with variable amplitude and constant frequency is applied to the ring electrode generating a three dimension potential field. This will trap ions on stable oscillating trajectories within the center of the trap. They are described by the Mathieu equations. According to them, the rf applied to the ring electrode influences the orbits of the ions in relation to their m/z ratios. A low mass cut-off of stable trajectories is defined at each multiple MS stage for any given value of a rf voltage. Therefore, ions with lower *m/z* values will not be trapped. The low mass cut-off value limits the mass range to approximately 1/3 of the mass of the precursor ions. This effect can prevent the detection of lower fragments.

5.3. Identification of PFOS isomers by HPLC-MS/MS

5.3.1. HPLC separation

The ESI(-)MS spectrum of all PFOS isomers contained only the molecular anion $[M-K]^-$ (*m/z* 499). Therefore, the optimization of the HPLC separation was carried out in the MS mode. ITMS was selected for detection. The perfluorinated phenyl (PFP) phase and the X-Terra® C_{18} phase were best suited and gave complementary information as the mass chromatograms of the molecular anion *m/z* 499 shows in Figure 5.3.

The conventional C_{18} phase allowed to separate PFOS isomers according to their degree of lipophilicy, if a silica support with a small pore size (120 Å) was used. Lipophilicy is

influenced by the number of branching points and their positions as well as the length of the side chains. Since all these parameters have a simultaneous influence, different isomers can have similar lipophilic properties and hence retention times. Three main PFOS isomer classes could be distinguished according to their elution order. A minor group of isomers eluted first followed by a medium one and then the most abundant signal. Linear chains are most lipophilic and will elute last, while an increase of branching points will shorten retention. Therefore, disubstituted and monosubstituted isomers were tentatively assigned as the first minor group and the second major group in Figure 5.3. The identification of these three isomer groups in technical PFOS mixture is in agreement with ^{19}F-NMR analysis.

The PFP phase allowed a further separation of the three groups into totally six signals due to the shape/size selectivity of the phase (Przybyciel, 2003). At least two isomers were present in the first eluting group on the PFP phase and three in the second one (see Figure 5.3 B). No further signal was detected in the last group tentatively assigned as the linear isomer.

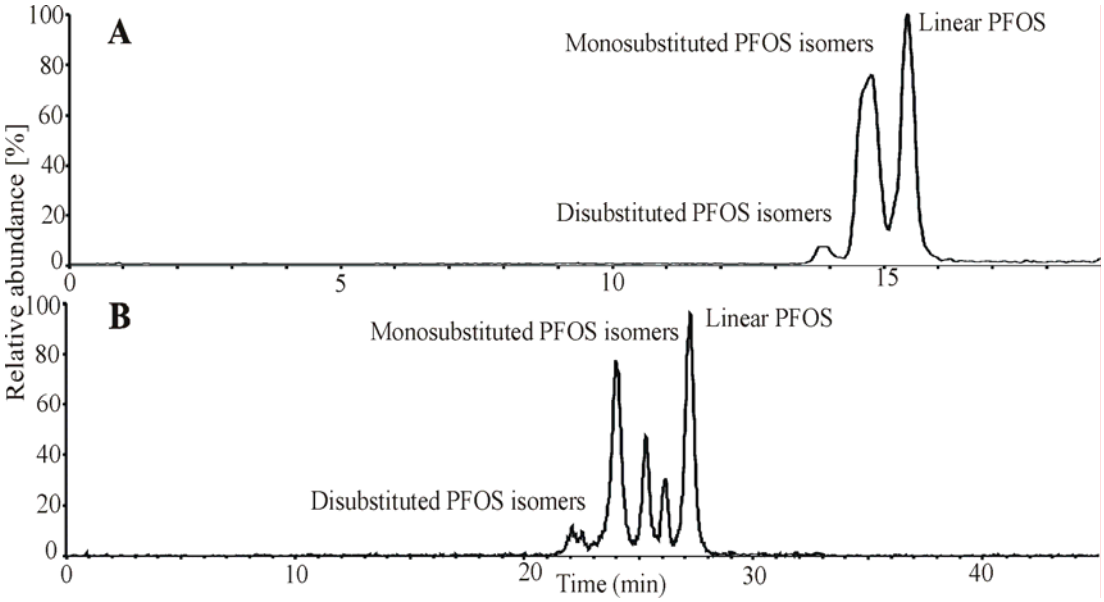

Figure 5.3: Separation of PFOS isomers (*m/z* 499) present in a technical mixture on (A) a C_{18} (X-Terra®) and (B) perfluorophenyl phase.

However, the main restriction concerning the use of such phase is the lack of reproducibility of the separation between columns.

5.3.2. Tandem MS

ESI(-)MS/MS spectra were recorded for each signal of the HPLC chromatogram of an technical PFOS product as well as for the purified isomer fractions listed in Table 3.2. Both series of fragmentation typical for PFOS were formed ("0-series"and "9-series", see Table 4.1) as well as $[SO_3]^-$ (m/z 80) and $[FSO_3]^-$ (*m/z* 99). A third minor series not listed in Table 4.1 with elemental composition $[C_pF_{2p-1}SO_3]^-$ was also observed and called "1-series".

5.3.2.1. Ion trap MS/MS

5.3.2.1.1. Isomer differentiation

Ion trap MS/MS spectra allowed to identify ten isomers. They were not completely chromatographically resolved on the PFP phase, but could be distinguished in the mass chromatograms (see Figure 5.4). Isomers belonging to the first eluting group were assigned with an "F" (first). At least four compounds/classes were identified and marked as F1 to F4 in the mass chromatograms shown in Figure 5.4. F1 and F2 eluted first on the PFP phase and contained the fragment ion *m/z* 280. F3 had an intense *m/z* 419 ion and F4 one at *m/z* 380. Five isomers were identified in the middle of the elution range and assigned as M5 to M9. M5 had an abundant fragment at *m/z* 280, M6/M9 at *m/z* 330, M7 at *m/z* 419 and M8 at *m/z* 380 (see Figure 5.4). M7 showed a better separation in the mass chromatograms of the C_{18} phase, and these mass abundances are presented instead of those of PFP phase. The last eluting signal (L10) contained the linear PFOS. Table 5.2 summarizes the relative abundances of the product ions of each isomer.

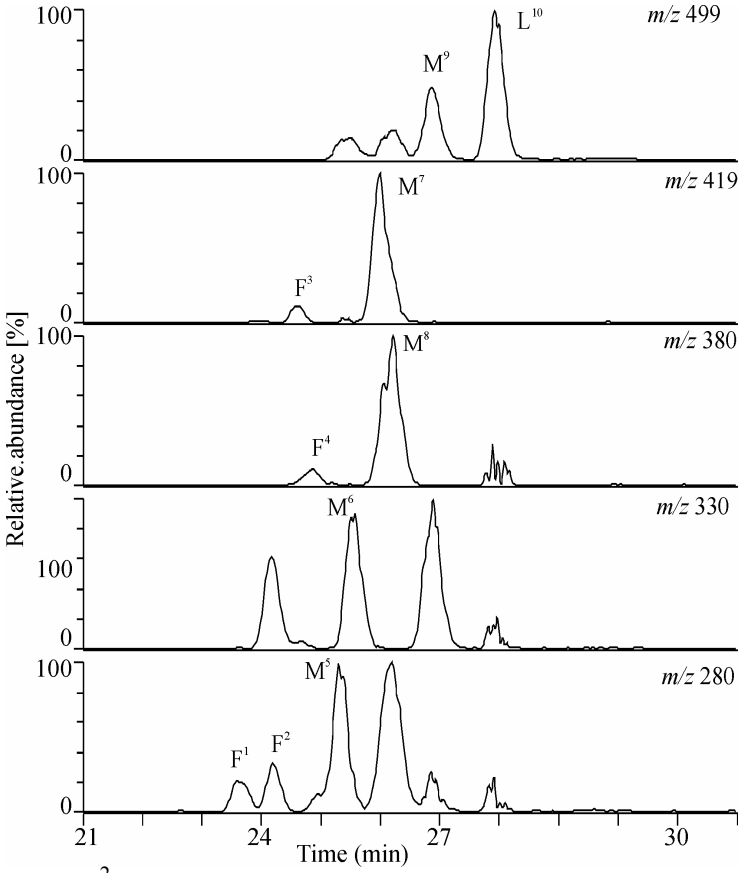

Figure 5.4: ESI(-)ITMS^2 product ions mass chromatograms of the HPLC separation of a technical PFOS product on a PFP phase. Molecular ions were fragmented with 40 % collision energy. The structure assignment is given in Table 5.2.

A correlation between the relative abundance of the molecular anions and retention time could be deducted from Table 5.2. The lower the retention the less was the abundance of the molecular anion. This indicates that early eluting isomer groups are more branched, since charge stabilization at branching points promotes fragmentation. The isomers F1 to F3 showed no molecular anion at all, while that of F4 had at a relative abundance of 32 %. The abundance of the molecular anion of M5 to M8 was 30-53 %, while it was the base ion for M9 and L10. L10 formed only one product ion formed at relative abundance of 5 %, which confirms the stability of the linear structure (see Table 5.2).

Table 5.2: Relative abundance (≥3%) of product ions of PFOS isomers in a technical product obtained by fragmentation of the molecular ions *m/z* 499 by ion trap (IT) MS/MS (40% collision energy) and triple quadrupole (TQ) MS/MS (-40 V collision energy). the typical relative uncertainty of the abundances is ca. 1-2 % .

Isomer	**F[1]**		**F[2]**		**F[3]**		**F[4]**		**M[5]**	**M[6]**	**M[5-6]**	**M[7]**		**M[7-8]**		**M[9]**		**L[10]**	
Type	**IT**	**TQ**	**IT**	**TQ**	**IT**	**TQ**	**IT**	**TQ**	**IT**	**IT**	**TQ**	**IT**	**TQ**	**IT**	**TQ**	**IT**	**TQ**	**IT**	**TQ**
m/z																			
499	-	11	-	-	-	-	32	9	53	55	5	30	-	53	-	100	22	100	26
430	-	-	-	-	-	-	-	-	3	3	-	-	-	3	-	3	-	-	-
419	8	-	-	-	100	-	-	-	-	-	-	100	-	100	-	-	-	-	-
380	-	-	-	-	14	-	45	-	-	-	-	-	-	36	-	-	-	-	-
369	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
330	11	12	-	-	29	-	5	-	7	100	5	-	-	-	-	48	28	-	-
319	59	-	-	22	-	-	-	23	5	-	-	-	-	-	-	-	-	-	-
280	100	-	44	-	7	20	100	-	100	16	28	-	-	57	-	10	29	-	5
269	19	20	7	18	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-
261	-	-	-	-	8	6	-	10	3	-	-	-	-	-	-	-	-	-	-
230	-	-	100	32	-	-	26	22	-	39	42	-	-	19	33	3	89	-	25
219	-	24	-	-	7	18	75	34	-	5	6	-	21	3	5	-	6	-	-
180	-	-	11	100	-	18	10	42	7	19	68	-	-	-	12	3	34	-	9
169	-	-	-	-	6	-	-	25	-	-	15	-	26	-	-	9	100	5	14
130	*	100	*	40	*	100	*	100	*	*	100	*	-	*	100	*	60	*	5
119	*	-	*	14	*	-	*	58	*	*	7	*	14	*	-	*	-	*	21
99	*	-	*	-	*	-	*	45	*	*	15	*	100	*	13	*	15	*	100
80	*	12	*	36	*	10	*	-	*	*	90	*	-	*	16	*	48	*	77

-: below 3 %

*: not detected due to the low mass cut-of of the ion trap instrument

5.3.2.1.2. Isomer identification

^{19}F-NMR analysis of the purified isomer fractions of PFOS was also carried out and described in detail elsewhere (Arsenault *et al*., 2005). It allowed the calculation of the exact composition of the technical PFOS mixture. The same fractions were also analyzed with HPLC-ITMS/MS and enabled together with the ^{19}F-NMR results the identification of the following isomers. The perfluoromonomethyl isomers in the middle signal of the HPLC chromatogram shown in Figure 5.5 were assigned in the order of their retention time as 3-CF_3-PFOS (M5), 4-CF_3-PFOS (M6), 1-CF_3-PFOS (M7), 5-CF_3-PFOS (M8) and 6-CF_3-PFOS (M9). Their ITMS^2 spectra are presented in Figure 5.6. In addition, L10 was identified as the linear PFOS. The presence of 2-CF_3-PFOS in the technical product could not be confirmed, since no signal contained a well detectable fragment *m/z* 219, which is characteristic for this isomer (Figure 5.6 F). ^{19}F-NMR identified 2-CF_3-PFOS as a minor isomer (1.9 %) in the technical mixture (see Table 5.1).

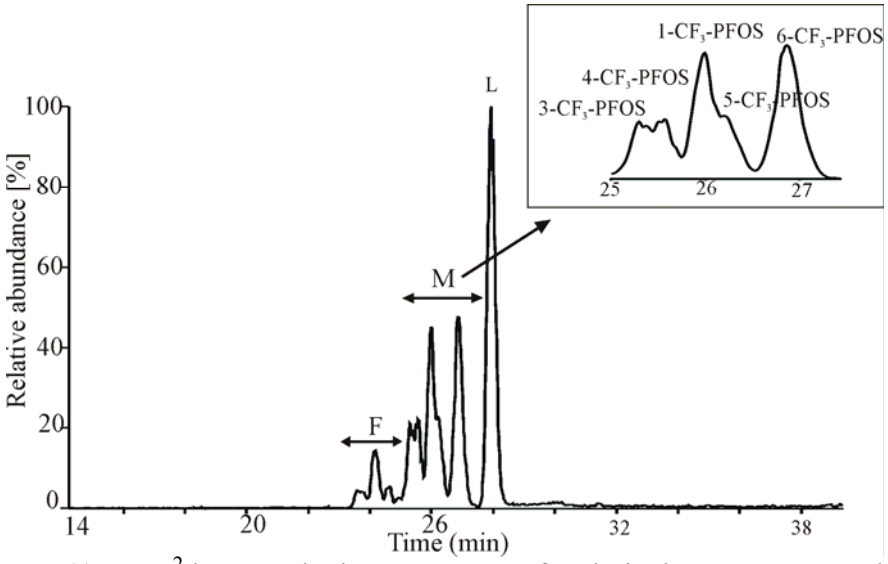

Figure 5.5: ESI(-)ITMS^2 base peak chromatogram of technical PFOS separated on a PFP phase and assignment of identified isomers. Molecular anions were fragmented with 40 % collision energy.

The structure elucidation of the M-isomers by ^{19}F-NMR allowed the further interpretation of their ITMS/MS spectra. The relative abundance of the molecular anion *m/z* 499 decreased with the CF_3 substitution closer to the sulfonate group (see Figure 5.6). No molecular ion was visible for 1-CF_3-PFOS, but it was the base ion for 6-CF_3-PFOS (Figure 5.6 G and B). Moreover, the position of the CF_3-substitution could be clearly elucidated from the mass spectra as Figure 5.6 demonstrates. The corresponding fragment ion of the "0-series" was missing, if a CF_3-group was present at the C-atom adjacent to the originally breaking bond. No fragment *m/z* 380 was visible for 6-CF_3-PFOS and correspondingly *m/z* 330, 280, 230 and 180 for 5-CF_3-PFOS, 4-CF_3-PFOS, 3-CF_3-PFOS and 2-CF_3-PFOS (see arrows in Figure 5.6). Figure 5.7 shows the fragmentation pattern of 5-CF_3-PFOS as an example.

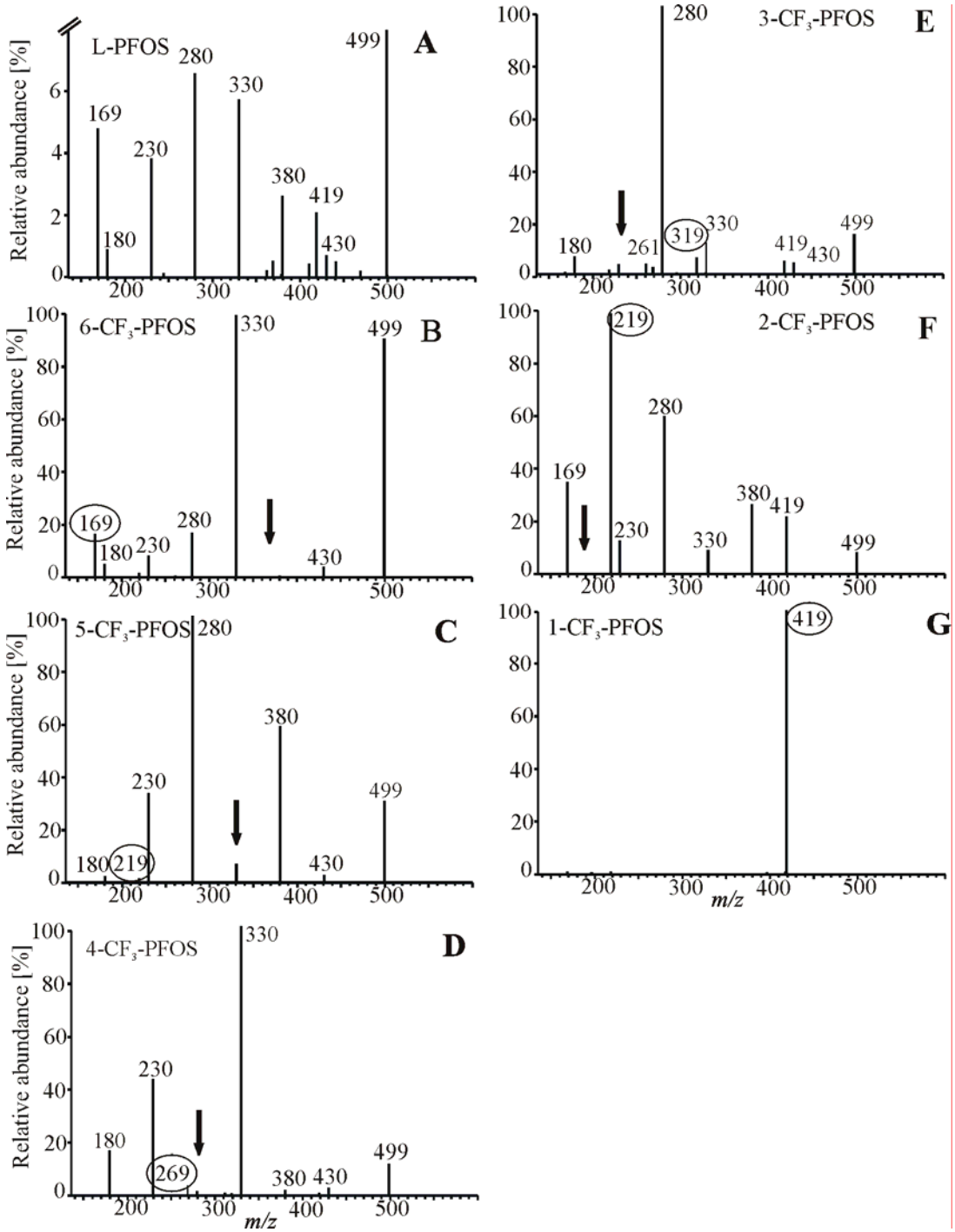

Figure 5.6: ESI(-)ITMS/MS spectra of L-PFOS, 6-CF_3-PFOS, 5-CF_3-PFOS, 4-CF_3-PFOS, 3-CF_3-PFOS, 2-CF_3-PFOS and 1-CF_3-PFOS. An arrow marks the missing product ion in the "0 series" due to branching and a circle the typical fragment in the "9-series". Molecular ions (*m/z* 499) were fragmented with 40 % collision energy.

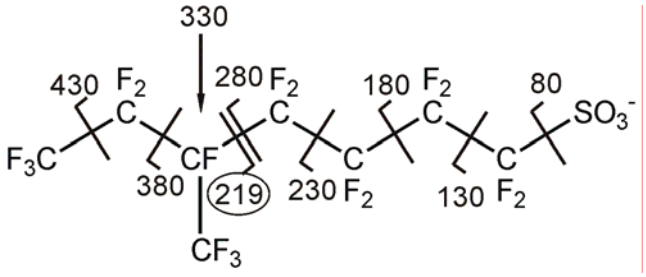

Figure 5.7: Fragmentation pattern of the molecular ion of 5-CF_3-PFOS. The arrow marks the missing fragment in the "0-series" and the encircled product ions correspond to the preferential cleavage in the "9-series".

The ion *m/z* 130 should also be absent in the MS/MS spectrum of 1-CF_3-PFOS. However, this could not be checked due to the operational mass range limitation of the ion trap to *m/z* 135. Nevertheless, 1-CF_3-PFOS could be unequivocally identified due to the presence of the unique fragment ion *m/z* 419 $[M\text{-}K\text{-}SO_3]^-$ formed by the inductive cleavage of the SO_3 group and the charge stabilization by the secondary carbonium ion (see Figure 5.6 G).

The fragment *m/z* 419 was also present in the ITMS/MS spectra of the other perfluoromonomethylated isomers. However, its abundance decreased with increasing distance of the substituted C-atom from the sulfonate group (see Figure 5.6). The ions from the "9-series" were visible at *m/z* 169 ($[(CF_3)_2CF]^-$), *m/z* 219 ($[CF_3CF_2(CF_3)CF]^-$), *m/z* 269 ($[CF_3(CF_2)_2(CF_3)CF]^-$) and *m/z* 319 $[CF_3(CF_2)_3(CF_3)CF]^-$) for 6-CF_3-PFOS , 5-CF_3-PFOS, 4-CF_3-PFOS and 3-CF_3-PFOS (see Figure 5.6). The stabilization effect of secondary carbonium supported the formation of these product ions as illustrated in Figure 5.7 for 5-CF_3-PFOS. Table 5.3 summarizes the typically fragments of the PFOS isomers.

Charge stabilization by the ternary carbonium ion formed from the disubstituted C-atom will lead to an abundance increase of the fragments of the "9-series" such as *m/z* 219, 269, 319, 369 and 419 for tert-butyl, 4,4-, 3,3-, 2,2- and 1,1- diperfluoromethyl-PFOS, respectively. All these masses were found in the signals of the first eluting F-group (see

Table 5.3). However, the isomers coeluted partly as Figure 5.4 shows. Therefore, only a tentative assignment of the retention time was possible for the PFP phase.

Table 5.3: Overview of the missing fragments of the "0-series" due to the CF_3 substitution and enhanced fragments of the "9-series" for perfluoromethyl substituted isomers.

	Missing fragment "0-series"	**Enhanced fragment "9-series"**
1-CF_3-PFOS	130	419
2-CF_3-PFOS	180	369
3-CF_3-PFOS	230	319
4-CF_3-PFOS	280	269
5-CF_3-PFOS	330	219
6-CF_3-PFOS	380	169

Only 3-CF3-PFOS showed the "1-series" fragment *m/z* 261. According to Lyon *et al*. (1985), it is generated by a 1,4-elimination of F_2 from the longer perfluoroalkyl chain resulting in the formation of a neutral alkene (C_4F_8) and a charged alkenesulfonate ($[CF_2{=}CF(CF_2)_2SO_3]^-$, *m/z* 261).

MS^3 experiments were performed on the main product ions produced by MS^2 but did not bring additional information concerning structural elucidation of the isomers.

No isomer enriched fractions were available for the first eluting isomers. However, ^{19}F-NMR studies of technical PFOS by Kestner (1997), which were confirmed by Arsenault et *al*. (2005), revealed the presence of perfluorodimethyl substituted compounds having both CF_3-groups in geminal position. Possible structures are then tert-perfluorobutyl-PFOS and the four isomers 1,1-, 2,2-, 3,3- and 4,4-diperfluoromethyl-PFOS (see Figure 5.2). They should elute first in the HPLC chromatogram and were therefore tentatively linked to the F-group.

5.3.2.2. Triple quadrupole MS/MS

The principal fragmentation pattern obtained with ion trap MS/MS was confirmed by triple quadrupole MS/MS. Separations were carried out on the PFP phase with exception of M7, which showed a better mass chromatographic separation on the C_{18} phase.

The main difference in the TQMS/MS spectra was the missing instrumental mass cut-off of the ion trap at *m/z* 135. Therefore, also low mass fragments were detectable such as *m/z* 80, 99, 119 and 130. Moreover, the dissociation processes in the collision cell led to a more pronounced fragmentation. Masses above *m/z* 269 had decreased relative abundances of <30 %, and base ions were only observed below 180 u. Consequently, the isomers M5 and M6 could not be differentiated. Most isomers formed *m/z* 130 as base ion such as F1, F3, F4, M5/6 and M8. The relative abundance was still 60 % for M9. Table 5.2 summarizes and compares the relative abundances of the product ions of the isomer groups.

Figure 5.8 shows the $TQMS^2$ spectra obtained with purified isomer fractions. Differences between the perfluoromethyl isomers were less pronounced due to reduced abundances above *m/z* 269. Nevertheless, the same masses were missing in the "0 series" as for $ITMS^2$ and allowed the identification of the single isomers (see arrows in Figure 5.8).

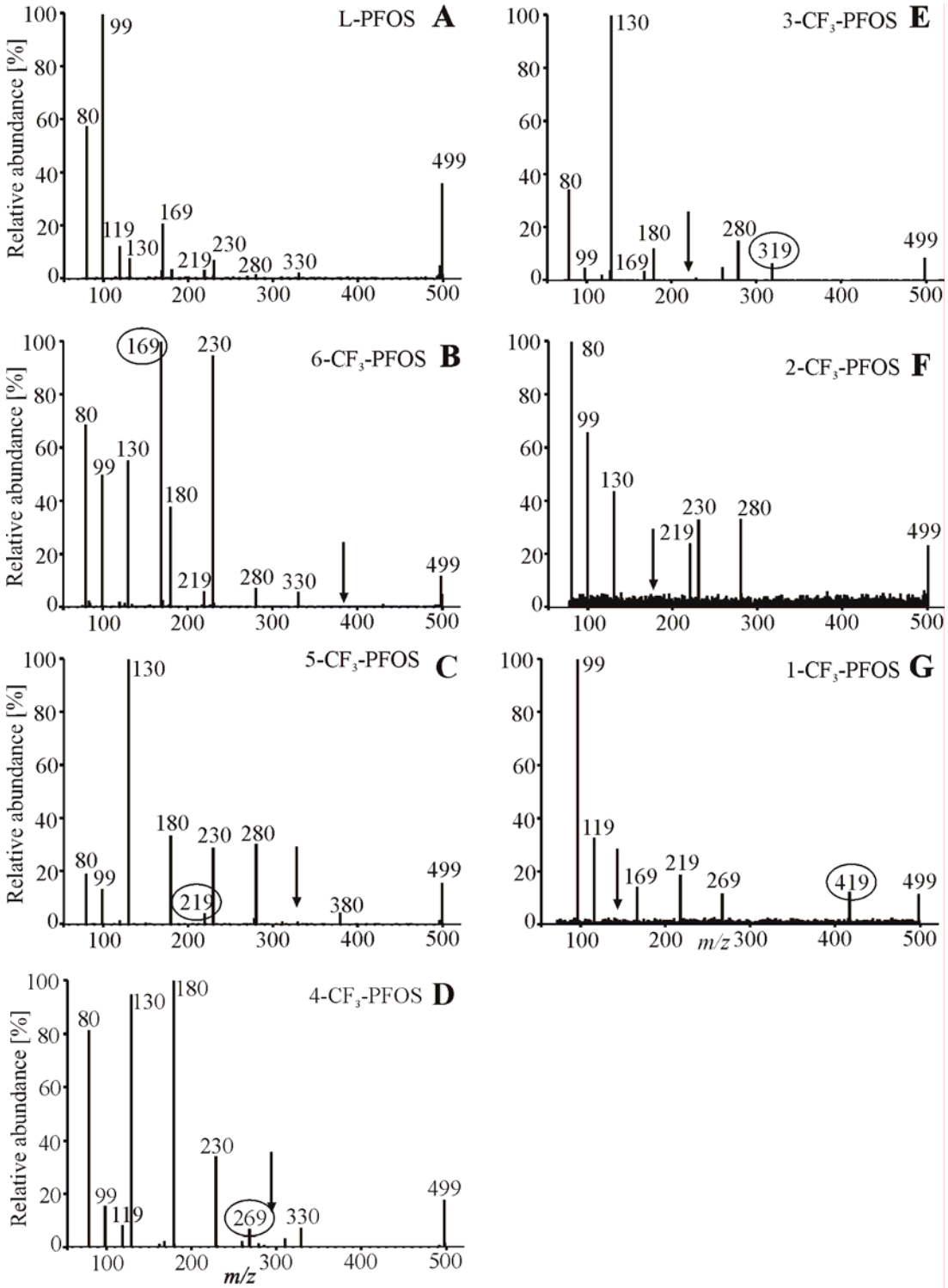

Figure 5.8: ESI(-)-TQMS^2 spectra of L-PFOS, 6-, 5-, 4-, 3-, 2- and 1-CF_3-PFOS. An arrow marks the missing product ion in the "0 series" and the circle the typical fragment of the "9-series". Molecular ions (*m/z* 499) were fragmented with -40V collision energy.

The $TQMS^2$ spectrum of 1-CF_3-PFOS contained more fragments compared to $ITMS^2$. The base ion was fluorosulfonate (*m/z* 99). Fluorine transfer from the perfluoro group at the α-C-atom may explain this high abundance. Moreover, *m/z* 130 from the "0-series" was missing together with the sulfonate ion *m/z* 80. This might be due to a better stabilization charge at the α-C-atom caused by the branching at this site.

The "9-series" was most abundant in the $TQMS^2$ spectrum of 1-CF_3-PFOS due to a general suppression of all ions of the "0-series". This indicates that the "9-" and "0-series" are formed by two different mechanisms. Lyon *et al.* (1985) proposed that the formation of the "0-series" occurs via a cyclic transition state. This is supported by the missing fragment *m/z* 130, since the formation of a cycle is sterically hindered at a branching point.

5.3.3. Implication for PFOS quantification

The first interlaboratory study on PFAS quantification showed a low agreement between the participants (Van Leeuwen *et al.*, 2005). So far, PFOS was mainly quantified in environmental samples by TQMS/MS using the mass transition *m/z* 499→99. However, *m/z* 99 is only base ion in the MS/MS spectra of linear and 1-CF_3-PFOS. Its relative low relative abundance is below <20 % for 3-CF_3-PFOS, 4-CF_3-PFOS and 5-CF_3-PFOS (see Figure 5.8). This means both a higher detection limit for these isomers and response factors different to L-PFOS. The latter will cause systematic quantification errors. This can only be avoided by specific reference standards for the corresponding isomers.

Moreover, only the isomer F^4 formed *m/z* 99 and the remaining diperfluoromethyl isomers can only be detected, if their abundant fragments *m/z* 130 and 180 are included into the

quantification procedure. The geminal diperfluoromethyl isomers represented up to 7.3% of the total isomer signal. Only then, a complete analysis of PFOS and its by-products can be carried out in environmental samples. According to ^{19}F-NMR data, technical PFOS contains about 20-30 % of perfluoromonomethyl isomers and less then 1 % of diperfluoromethyl substituted compounds.

5.3.4. Conclusions

HPLC-MS^2 enabled for the first time the identification of the PFOS isomers present in technical product. Ten isomers could be differentiated. Geminal diperfluoromethyl substituted isomers eluted first from the best suited HPLC-phase (PFP) followed by perfluoromonomethyl isomers and finally the n-alkyl chain. Such an elution order was also observed for the C_{18} phase. Moreover, the position of the CF_3 groups could be clearly elucidated due to some differences in the MS^2 spectra of the monosubstituted isomers. Structural elucidation of the geminal diperfluoromethyl isomers could not be completely carried out due to their very low abundance and coelution. Similar isomer pattern can be expected for the other PFAS produced by the ECF process such as PFOSA and further perfluorosulfonamides.

5.4. Separation of PFOS isomers by GC

5.4.1. GC analysis of PFAS

HPLC coupled to MS is the only technique enabling detection of all PFAS including ionic and non ionic compounds as shown in Table 1.1 (Berger *et al.*, 2004). Alternatively, non ionic PFAS such as fluorotelomers and perfluorosulfonamides can be analyzed by high resolution gas chromatography (HRGC) combined with MS due to their higher volatility (Shoeib *et al.*, 2005, Stock *et al.*, 2004). Analysis of ionic PFAS by GC requires a previous derivatization step. Esterifications with methyl iodide as well as with butanol and thionyl chloride or derivatization with 2, 4-difluoroaniline were described for PFCA (Moody *et al.*, 1999, Alzaga and Bayona, 2004, Ellis *et al.*, 2003).

Several derivatization procedures exist for sulfonate moieties as for example described for the analysis of linear alkyl benzene sulfonates by GC (Trehy *et al.*, 1990, Reiser *et al.*, 1997). However, the ionic properties of PFSA due to the sulfonate group as well as by their amphibilic character due to the perfluorated alkyl rest make derivatization very demanding. Therefore, so far, no successful methodology is available.

5.4.2. Method optimization

5.4.2.1. Derivatization conditions

Silylation and alkylation reactions are commonly used for GC-derivatization. Silylation derivatization of PFOS was evaluated using trimethylchlorsilan (C_3H_9ClSi) and *N,O*-Bis(tri-methylsilyl) acetamide ($((CH_3)_3SiO(CH_3)CNSi(CH_3)_3$), however without success. The targeted derivatives were may be not formed, or they were unstable under the applied GC conditions.. Alkylation derivatizations using ethanol and iso-propanol under acidic condition were also tested. The derivatization reaction did not occur with ethanol.

However, surprisingly iso-propanol was a possible derivatization reagent for PFOS and PFOA. Figure 5.9 shows the derivatization reaction.

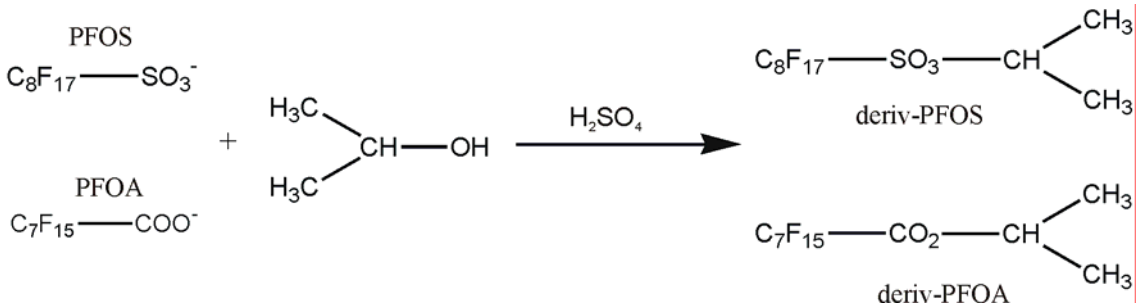

Figure 5.9: Derivatization reaction with iso-propanol applied to PFOS and PFOA.

The derivatization procedure with iso-propanol was optimized by testing different extraction solvents (n-hexane, iso-octane, and cyclohexane), acid amounts (0, 2 and 5 %) and reaction times under shaking (1 min, 3 hours and over night). The yield of derivatization for PFOA increased by a factor seven from 1 min to over night. Increasing the amount of acid from 0 to 5 % resulted in two fold higher yield. The PFOS derivatized (deriv-PFOS) was most stable in n-hexane.

5.4.2.2. GC separation

Mainly non-polar phases (DB5) and to less extent medium polar phases (DB 1701) are used for the separation of PFCA derivatives, perfluorosulfonamides and fluorotelomers (Moody *et al.*, 1999, Alzaga and Bayona., 2004, Ellis *et al.*, 2003). The isopropyl PFCA derivatives showed extremely low retention on the stationary phase with starting temperature of 40 or 50 °C (see Chapter 3.3.1). This was confirmed by Moody *et al.* (1999) reporting the necessity of a film thickness of 4 µm to obtain an acceptable retention time for the methyl PFCA derivative. The iso-propylated PFOS and PFOA eluted at

acceptable retention times of 14 and 7 minutes respectively on a DB5 MS capillary column (15 m, 0.25 mm i.d, 25 µm film thickness) with temperature program 1 (see Chapter 3.3.1).

5.4.3. PFOS derivatized spectra

Figure 5.10 (A) shows the EI-LRMS spectra of deriv-PFOS. A high degree of fragmentation was observed and no molecular ions (*m/z* 542) were detected.

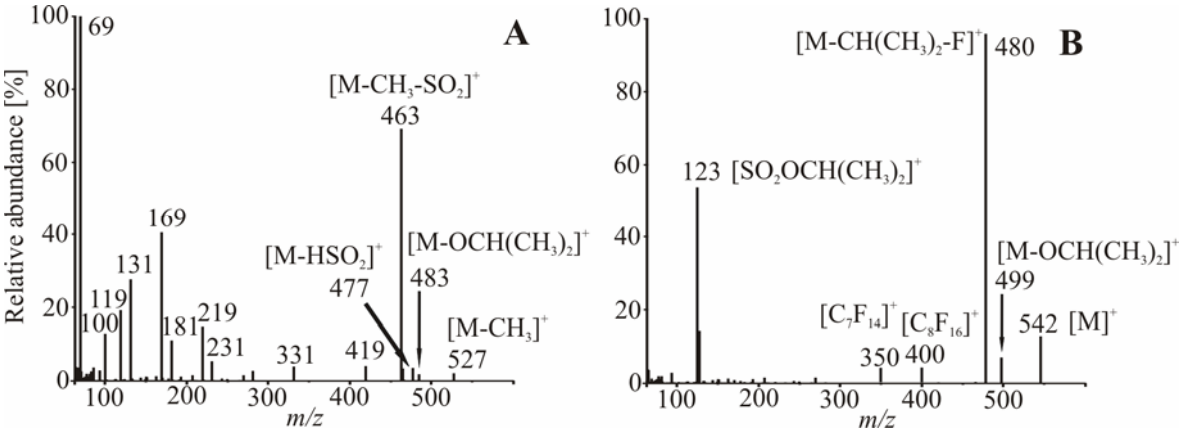

Figure 5.10: EI-LRMS (A) and NCI-LRMS (B) spectra of derivatized PFOS obtained with 70 eV ionization energy and methane as reagent gas.

Two series of fragmentation were observed which are typical for PFC (Kuehl *et al.*, 2003). One series started at *m/z* 69 $[CF_3]^+$ ("9-series") and a second series at *m/z* 131 $[C_3F_5]^+$ ("1-series"). Both contained fragments with a mass difference of 50 u corresponding to $-CF_2-$. Furthermore, *m/z* 93 $[C_3F_3]^+$ and *m/z* 100 $[C_2F_4]^+$ were observed. Loss of the methyl group from the molecular ions gave the fragment *m/z* 527 followed by a loss of SO_2 forming the fragment *m/z* 463 $[M\text{-}CH_3\text{-}SO_2]^+$. A similar loss of SO_2 was also reported for some perfluorosulfonamides in the EI mode (Kuehl *et al.*, 2003). Moreover, $-O\text{-}CH(CH_3)_2$ was cleaved off from the molecular ion leading to *m/z* 483. A third cleavage with the loss of HSO_2 yielded *m/z* 477. Figure 5.11 summarizes the EI fragmentation pathways observed for deriv-PFOS.

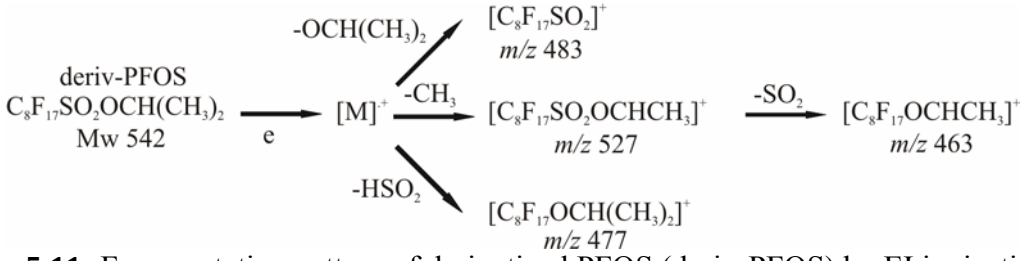

Figure 5.11: Fragmentation pattern of derivatized PFOS (deriv-PFOS) by EI ionization.

The molecular ion $[C_8F_{17}SO_3CH(CH_3)_2]^-$ of deriv-PFOS (*m/z* 542) was present in the NCI-MS spectra of deriv-PFOS (see Figure 5.10 B). Fragment ions of deriv-PFOS were *m/z* 123 $[SO_2OCH(CH_3)_2]^-$, *m/z* 350 $[C_7F_{14}]^-$, *m/z* 400 $[C_8F_{16}]^-$, *m/z* 480 $[C_8F_{16}SO_3]^-$ and *m/z* 499 $[C_8F_{17}SO_3]^-$.

The elemental composition of the fragments formed by EI and NCI was confirmed with 1,1,1,3,3,3-D_6-iso-propyl derivatives. Figure 5.12 shows the corresponding EI-LRMS and NCI-LRMS spectra of deriv-PFOS. Fragment *m/z* 483 $[M\text{-}OCH(CH_3)_2]^+$ had a low abundance for the native iso-propylated PFOS (0.2 %, see Figure 5.10 A), but was more dominant for the deuterium labelled derivative due to the isotope effect (4.8 %, see Figure 5.12 A). Fragment *m/z* 483 originated from $[M\text{-}OCH(CD_3)_2]^+$ and $[M\text{-}HSO_2]^+$. Moreover, 1,1,1,3,3,3-D_6-iso-propyl PFOS revealed that the H atom from the loss of HSO_2 originated from the not deuterium-labelled tertiary C-atom of the iso-propyl rest, since the rest still contained all six D (*m/z* 483).

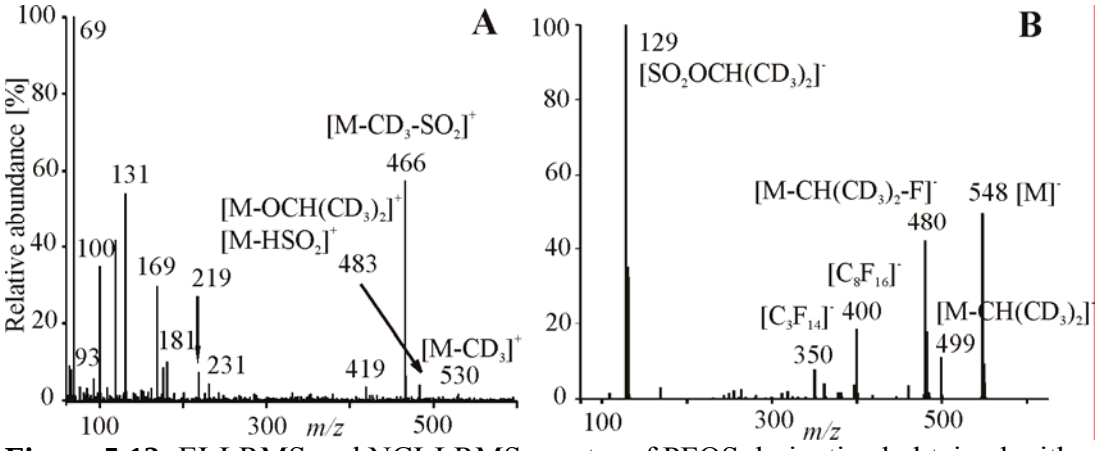

Figure 5.12: EI-LRMS and NCI-LRMS spectra of PFOS derivatized obtained with 1,1,1,3,3,3-D_6-iso-propyl PFOS.

Moreover, high resolution MS (resolution 8000) was applied to the EI product ions of PFHS derivatized to confirm the elemental composition by exact mass determination. All elemental compositions as shown in Table 5.4 were confirmed.

Table 5.4: Masses determined by HRMS (resolution 8000), theoretical masses and proposed formulas of the relevant EI product ions of deriv-PFHS and deriv-PFHA. The mass deviation and double bond equivalent (DBE) are also given.

Measured mass	Theoretical mass	Tolerance (ppm)	DBE	Formula
deriv-PFHS				
440.9782	440.9830	-10.9	0.5	$C_9H_6O_3F_{13}S$
426.9653	426.9674	-4.8	0.5	$C_8H_4O_3F_{13}S$
382.9374	382.9411	-9.8	0.5	$C_6O_2F_{13}S$
377.0218	**377.0211**	**1.8**	**0.5**	**$C_9H_6OF_{13}$**
	377.0258	-10.6	2.5	$C_{11}H_{10}O_2F_9S$
363.0050	**363.0055**	**-1.3**	**0.5**	**$C_8H_4OF_{13}$**
	363.0101	-14.1	2.5	$C_{10}H_8O_2F_9S$
deriv-PFHA				
355.0251	355.0228	6.5	0.5	$C_8H_8O_5F_9$
	355.0214	10.3	0.5	$C_9H_9OF_{10}S$
	355.0192	16.6	1.5	**$C_9H_6O_2F_{11}$**
340.9993	341.0036	-12.5	1.5	$C_8H_4O_2F_{11}$
327.0237	327.0243	-1.8	0.5	$C_8H_6OF_{11}$
313.0093	313.0087	2.1	0.5	**$C_7H_4OF_{11}$**
	313.0133	-12.9	2.5	$C_9H_8O_2F_7S$
296.9732	296.9774	-14	1.5	C_6OF_{11}

The most probable formula is marked in bold when different possibilities were proposed

5.4.4. PFOS isomer pattern obtained by HRGC

Figure 5.13 shows the EI-LRMS mass chromatogram (*m/z* 463 $[M\text{-}CH_3\text{-}SO_2]^+$) of the GC separation of a technical PFOS mixture after derivatization using temperature program 2 (see Chapter 3.3.1). Only seven isomer groups were completely resolved by HPLC-MS separation (see Figure 5.3) and up to ten by HPLC-MS^2 (see Figure 5.4, Langlois and Oehme. 2006). However, the high resolution of the GC capillary column enabled the separation of eleven PFOS isomers (see Figure 5.13). Five minor isomers (isomers #1, 2, 3, 4, 5) eluted first followed by two medium isomer groups (isomers #6 and 7). The main isomer, L-PFOS was followed by two major branched isomers. A minor isomer (isomer #11) completed the elution. This is different to HPLC where L-PFOS eluted last (see Figure 5.4). Isomers #6, 7, 9 and 10 were expected to be the mono-substituted isomers due to their higher relative abundances. Separation according to both polarity and boiling point led to different elution order of HRGC compared with HPLC.

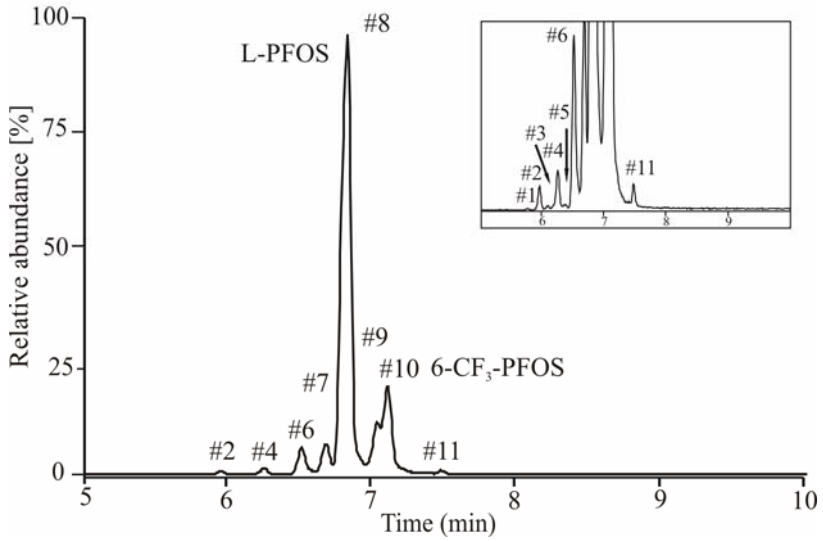

Figure 5.13: HRGC- EI-LRMS (70 eV) mass chromatogram (*m/z* 463) of a PFOS technical mixture and numbered isomers.

Theoretically, twelve isomers are possible (see Chapter 5.1.2). HRGC separation showed eleven signals. Therefore, maximum two signals contain coeluting diperfluoromethyl substituted isomer.

Table 5.5 summarizes the relative abundances of the EI fragments for the eleven PFOS isomers. The availability of a purified 6-CF_3-PFOS standard enabled its identification as isomer #10, major substituted isomer. This is in agreement with ^{19}F-NMR results (Arseneault *et al.* 2005). However, the characterization of further isomers was not possible. Some differences were observed between their EI-MS spectra. For example, isomer #2 and 3 contained fragments *m/z* 527 $[M\text{-}CH_3]^+$ at relative abundances of 60 % and 48 % respectively (see Table 5.5). Isomer #2 and 3 showed the ions *m/z* 131 $[C_3F_5]^+$ as base peak. The mass spectrum of isomer #5 showed the ions *m/z* 269 $[C_3F_7]^+$ and 331 $[C_7F_{13}]^+$ with high abundance. However, those fragments were not sufficient to clearly elucidate the position of the CF_3 groups as ESI-MS^2 spectra (see Chapter 5.3.2). Identification of all PFOS isomers requires pure standards. However, so far, they are not commercially available.

Table 5.5: EI-LRMS relative abundances (≥0.1 %) of the fragments of derivatized PFOS isomers separable in a technical product. Linear PFOS (L-PFOS) and 6-CF_3-PFOS could be identified.

Isomer/ *m/z*	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8 L-PFOS**	**#9**	**#10 6-CF_3-PFOS**	**#11**
100	-	11	-	10	1	12	5	8	6	5	2
119	3.2	56	5	41	-	51	8	33	37	12	0.2
131	-	100	100	69	36	49	23	50	32	32	0.3
169	-	92	5	54	-	13	100	69	13	21	25
181	-	59	98	79	100	35	16	19	18	20	1
219	-	38	6	13	-	55	2	32	26	8	0.7
231	-	18	19	11	3	8	9	11	6	5	0.2
269	-	10	14	16	45	0.1	-	2	1	5	0.1
281	-	17	49	8	29	9	1.1	3	5	2	0.1
319	-	0.3	3	-	4	-	0.2	32	0.1	-	-
331	-	13	24	52	75	22	5	5	7	12	0.1
369	-	-	-	0.6	-	0.1	-	-	-	-	-
381	-	3	-	2	9	0.1	0.1	0.1	0.1	-	-
419	-	3	18	14	1	7	4	7	5	6	0.1
463	0.1	72	84	100	84	100	77	100	100	100	1
477	0.1	4	2	4	3	5	3	6.3	4	4	0.1
527	-	60	48	0.8	4	2	1	4	2	2	-

-: below 0.1 %.

5.4.5. Results obtained for PFCA and other PFSA

The derivatization procedure could be also applied to PFCA. Similar fragmentation patterns were observed as for deriv-PFOS (see Figure 5.14).

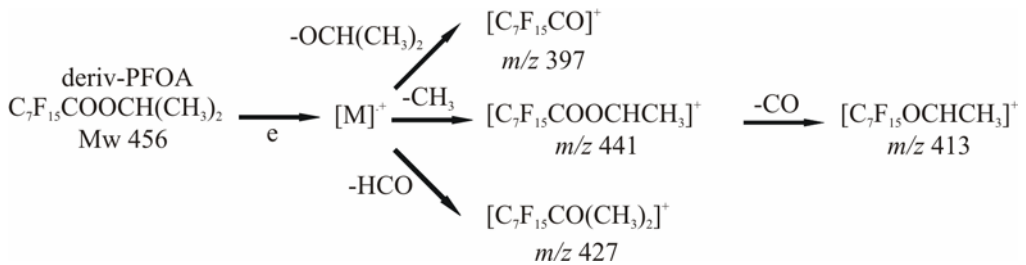

Figure 5.14: EI fragmentation pathways observed for derivatized PFOA (deriv-PFOA).

Figure 5.15 shows the EI-LRMS spectra for a series of PFCA derivatives. The proposed elemental composition of the fragments were confirmed by the respective deuterium labelled compound using 1,1,1,3,3,3-D_6-iso-propanol (results not shown). Moreover, it was also checked by high resolution MS applied to derivatized PFHA (see Table 5.4).

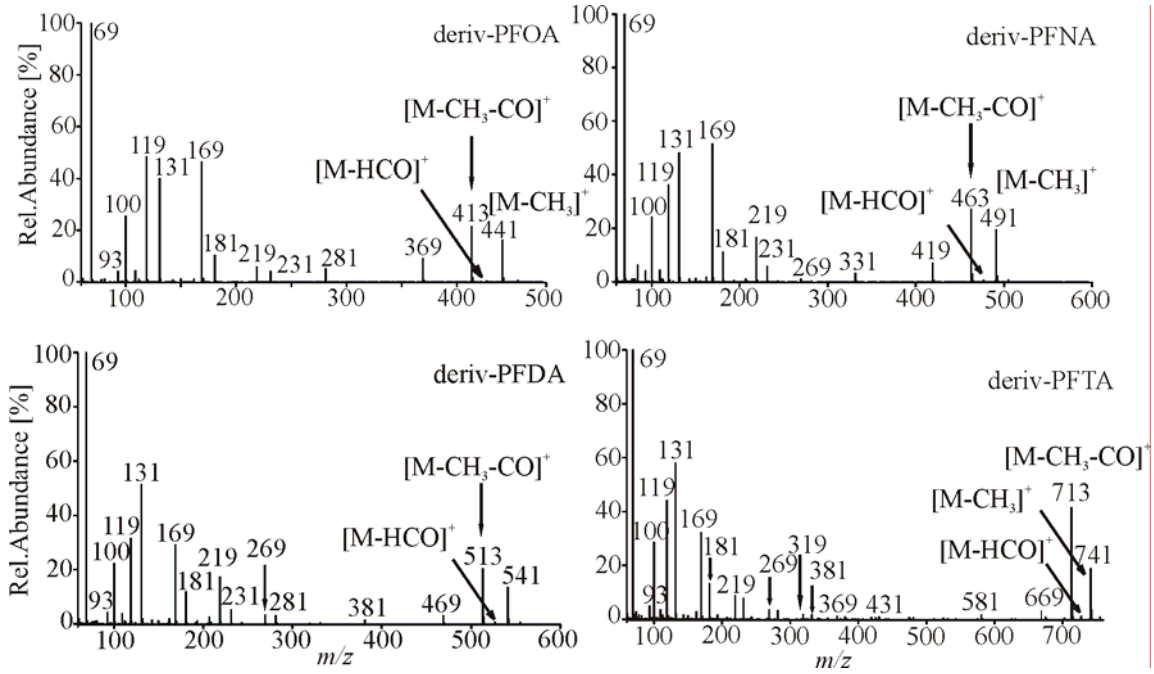

Figure 5.15: EI-LRMS spectra of perfluorocarbobylic acid derivatives: deriv-PFOA (C_8), deriv-PFNA (C_9), deriv-PFDA (C_{10}) and deriv-PFTA (C_{14}).

5.4.6. Conclusions

For the first time, a GC derivatization procedure was described for the characterization of PFOS mixtures by HRGC-MS. The derivatization step is easy to carry out and allowed the conversion of PFSA in their iso-propyl esters. HRGC enabled the separation of eleven isomers. No other technique allowed the differentiation of so many isomers in technical mixtures. Four additional isomers were identified compared with HPLC-MS and one compared to HPLC-MS^2. The derivatization reaction could be also applied to PFCA and

offers an alternative to the other derivatization procedure described in the literature. However, the yield of the presented technique is still currently low and therefore only suitable for qualitative work.

5.5. Identification of PFOA and PFOSA isomers by HPLC-MS

The abbreviations of the discussed isomers are listed in Table 5.6. Similar abbreviations as thoses used for PFOS isomers were employed. Both PFOA and PFOSA can be produced both by ECF and may therefore contain similar isomer patterns as PFOS.

Table 5.6: PFOA and PFOSA isomer abbreviations.

Isomer	PFOA	PFOSA
Linear	L-PFOA	L-PFOSA
Perfluoroisopropyl-	6-CF_3-PFOA	6-CF_3-PFOSA
5-perfluoromethyl-	5-CF_3-PFOA	5-CF_3- PFOSA
4-perfluoromethyl-	4-CF_3-PFOA	4-CF_3-PFOSA
3-perfluoromethyl-	not available	3-CF_3-PFOSA

5.5.1. PFOA isomers

Molecular anions $[M-H]^-$ of PFCA were formed by ESI(-) in full scan mode. They tend to loss the carboxylic group and the fragment $[M-H-COO]^-$ was also observed. An increase of the capillary voltage from -20 V to -40 V (TQ instrument) induced decarboxylation.

MS/MS fragmentation with TQ and IT instruments will be discussed for the PFOA anion ($C_7F_{15}CO_2^-$) in detail. However similar results can be expected for other PFCA homologues with longer or shorter chain. MS/MS spectra of PFOA isomers present in a ECF-product were not sufficiently specific with base ion *m/z* 369. Therefore, structural

elucidation of PFOA isomers was performed by further fragmentation of *m/z* 369 [M-CO_2]$^-$. Figure 5.16 shows the ESI(-)ITMS^2 and ESI(-)TQMS^2 spectra of L-PFOA, 6-CF_3-PFOA and 5-CF_3-/ 4-CF_3-PFOA from purified isomer fractions (see Table 2.1). The isomers 3-, 2- and 1-CF_3-PFOA were not available as purified fractions. No individual spectrum of 4-CF_3-PFOA and 5-CF_3–PFOA could be obtained due to coelution and too similar MS spectra. Therefore, an averaged spectrum of these two isomers is given in Figure 5.16.

ITMS^2 and TQMS^2 spectra of PFOA isomers were very similar. Here, the low mass cut-off of the IT instrument did not cause differences. Mainly product ions from the "9-series" $[C_nF_{2n+1}]^-$ as already summarized in Table 4.1 were formed from *m/z* 369 (see Figure 5.16). The fragments *m/z* 119 $[CF_3CF_2]^-$ or *m/z* 169 $[C_3F_7]^-$ were base ions. Fragments *m/z* 119 was most abundant for 4-CF_3-/5-CF_3-PFOA and *m/z* 169 for L-PFOA and 6-CF_3-PFOA with both instruments. The structure of *m/z* 169 in the MS^2 spectra of 6-CF_3-PFOA is perfluoroisopropyl group $[(CF_3)_2CF]^-$. The ions *m/z* 219 $[C_4F_9]^-$ and 269 $[C_5F_9]^-$ were also present but at too low abundance to enable structural elucidation.

ITMS^2 formed an additional series containing *m/z* 197, 297 and 347 as shown in Figure 5.16. The respective elemental composition were $[C_3F_7CO]^-$, $[C_5F_{11}CO]^-$ and $[C_6F_{13}CO]^-$. Their formation required a reaction with oxygen within the trap expected to originate from air since the precursor ions $[M\text{-}H\text{-}CO_2]^-$ did not contain anymore oxygen atoms.

The position of the CF_3 groups in the chain could not be clearly elucidated for PFOA isomers. Coelution between 4-CF_3-PFOA and 5-CF_3-PFOA and lack of pure standard were

the main reasons. However, 6-CF_3-PFOA could be differentiated from 4-CF_3-/5-CF_3-PFOA due to enhancement of the product ions *m/z* 169 and the absence of *m/z* 119.

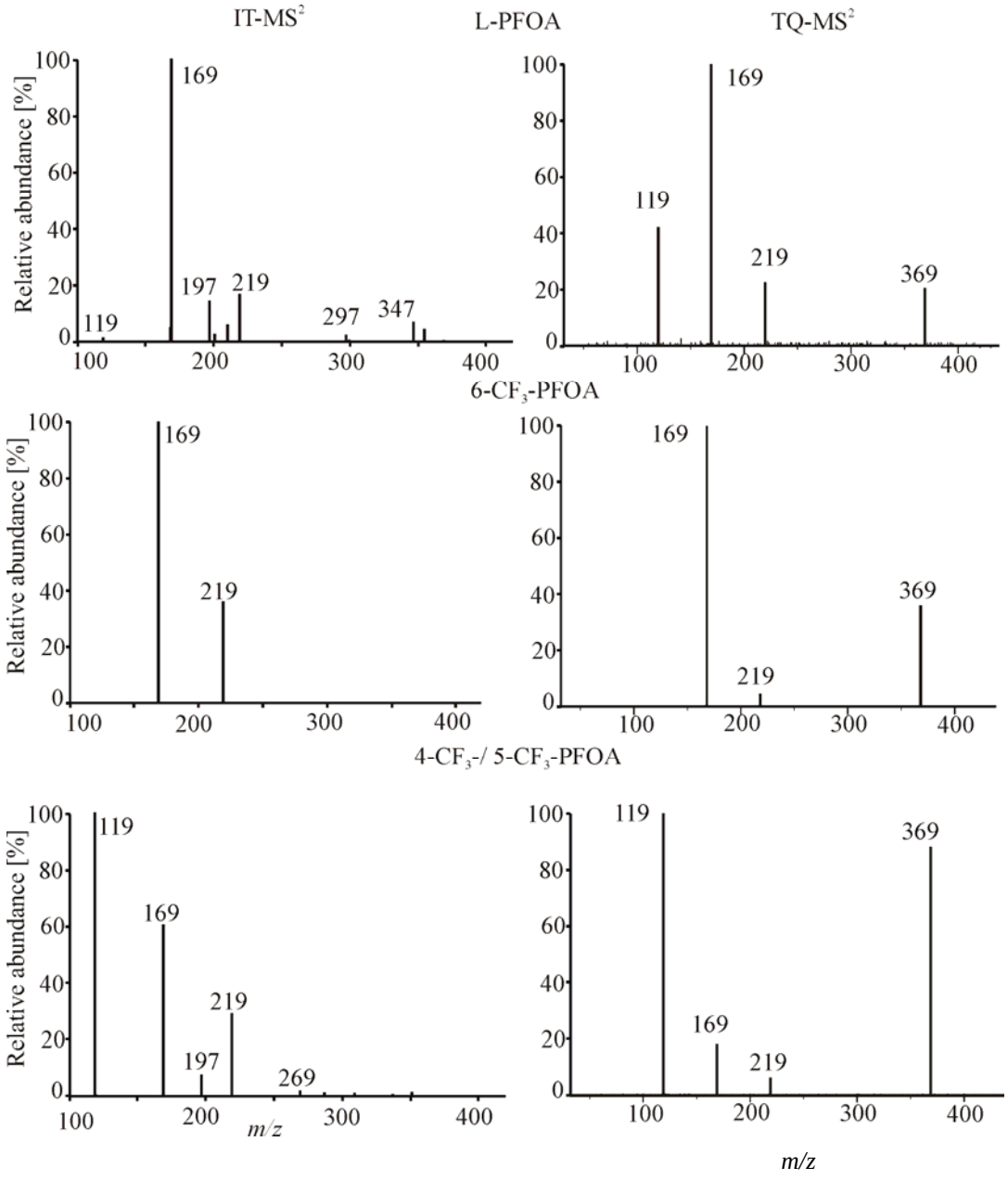

Figure 5.16: ESI(-)ITMS^2 and ESI(-)TQMS^2 spectra of L-PFOA, 6-CF_3-PFOA and 4-CF_3-PFOA/5-CF_3-PFOA. The anions *m/z* 369 were fragmented with 35% (IT) and -20 V (TQ) collision energy.

5.5.2. PFOSA isomers

A typical isomer pattern of ECF-based technical PFOSA product is shown in Figure 5.17. Similarly to PFOS, the disubstituted isomers eluted first, followed by the monosubstituted isomers. L-PFOSA eluted last. No further separation between the isomers was obtained.

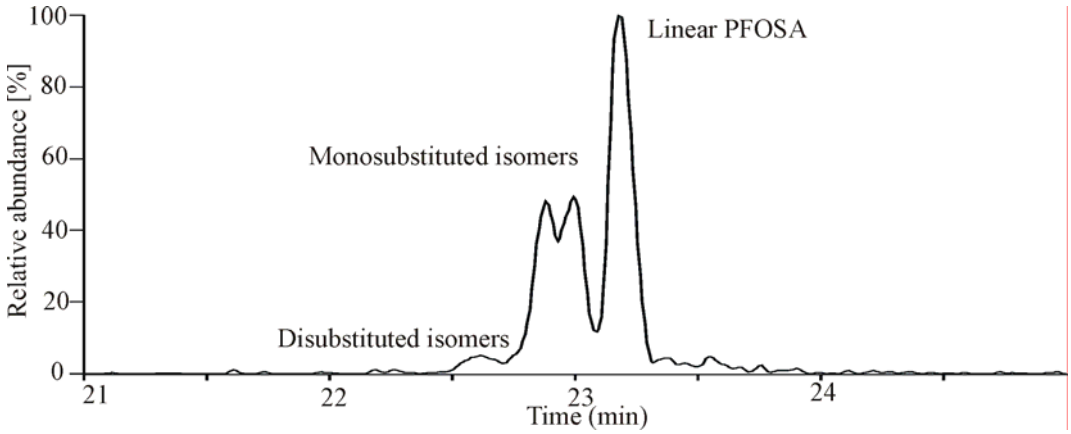

Figure 5.17: ESI(-)ITMS mass chromatogram (molecular anions, *m/z* 498) of the HPLC separation of a technical PFOSA mixture on a PFP phase.

Only molecular anions $[M\text{-}H]^-$ (*m/z* 498) were formed by ESI(-) and were selected for tandem MS. $ITMS^2$ and $TQMS^2$ were applied to purified isomer fractions containing L-PFOSA, 6-, 5-, 4-, and 3- CF_3-PFOSA. The $ITMS^2$ spectrum of 1-CF_3-PFOSA was obtained from a HPLC separation of a technical PFOSA mixture. Figure 5.18 and 5.19 present all registered mass spectra.

$ITMS^2$ fragmentation produced more ions than $TQMS^2$. Two "9-series" $[C_nF_{2n+1}]^-$ and $[M\text{-}H\text{-}C_xF_{2x+1}\text{-}H]^-$ were formed by $ITMS^2$ whereas $TQMS^2$ generated only the first one with the product ions *m/z* 119, 169, 219… (see Figure 5.18, 5.19 and Table 4.1). One additional product ion *m/z* 345 was present in the $ITMS^2$ of 3-CF_3-PFOSA with a tentative elemental composition $[M\text{-}H\text{-}SO_2N\text{-}C_3F_2\text{-}H]^-$ (see Figure 5.18).

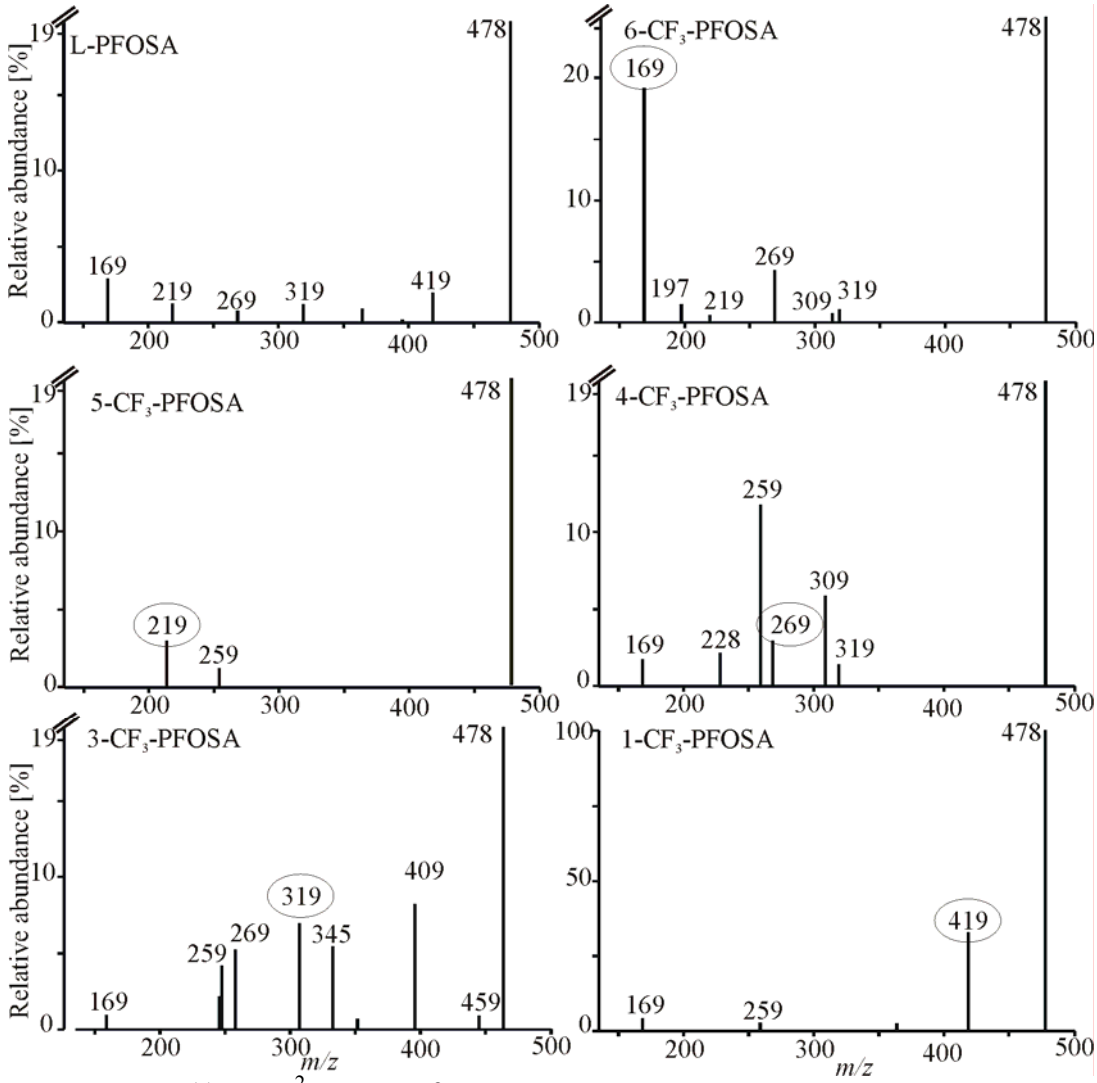

Figure 5.18: ESI(-)ITMS^2 spectra of L-PFOSA, 6-CF_3-PFOSA, 5-CF_3-PFOA, 4-CF_3-PFOSA, 3-CF_3-PFOSA and 1-CF_3-PFOSA. A circle marks the typical fragments from the "9-series". The molecular anions *m/z* 498 were fragmented with 35 % collision energy.

The MS^2 spectra for both instruments contained one dominant product ion. This is *m/z* 478 $[M\text{-}HF]^-$ for ITMS^2 and *m/z* 78 $[SO_2N]^-$ for TQMS^2. The fragment *m/z* 78 was not detectable by ITMS^2 due to the low mass cut off of the instrument at *m/z* 135.

For PFOS, charge stabilization on secondary carbonium ions led to the formation of *m/z* 169, 219, 269, 319 and 419 in the TQMS^2 spectra of 6-, 5-, 4-, 3- and 1-CF_3-PFOS (see Table 5.3). Similar formation was observed for PFOSA isomers as shown in Figure 5.18. 1-CF_3-PFOSA was the only isomer containing the product ions *m/z* 419 at a relative abundance >20 %. This allowed its identification in technical mixture. On the contrary, the

product ions *m/z* 169, 219 and 269 were only present in the MS^2 spectra of 6-CF_3-PFOSA, 5-CF_3-PFOSA and 4-CF_3-PFOSA respectively (see Figure 5.18 and 5.19). TQMS led to a more extensive fragmentation. Therefore, the fragment *m/z* 369 was not present in the $TQMS^2$ spectrum of 3-CF_3-PFOSA (see Figure 5.19).

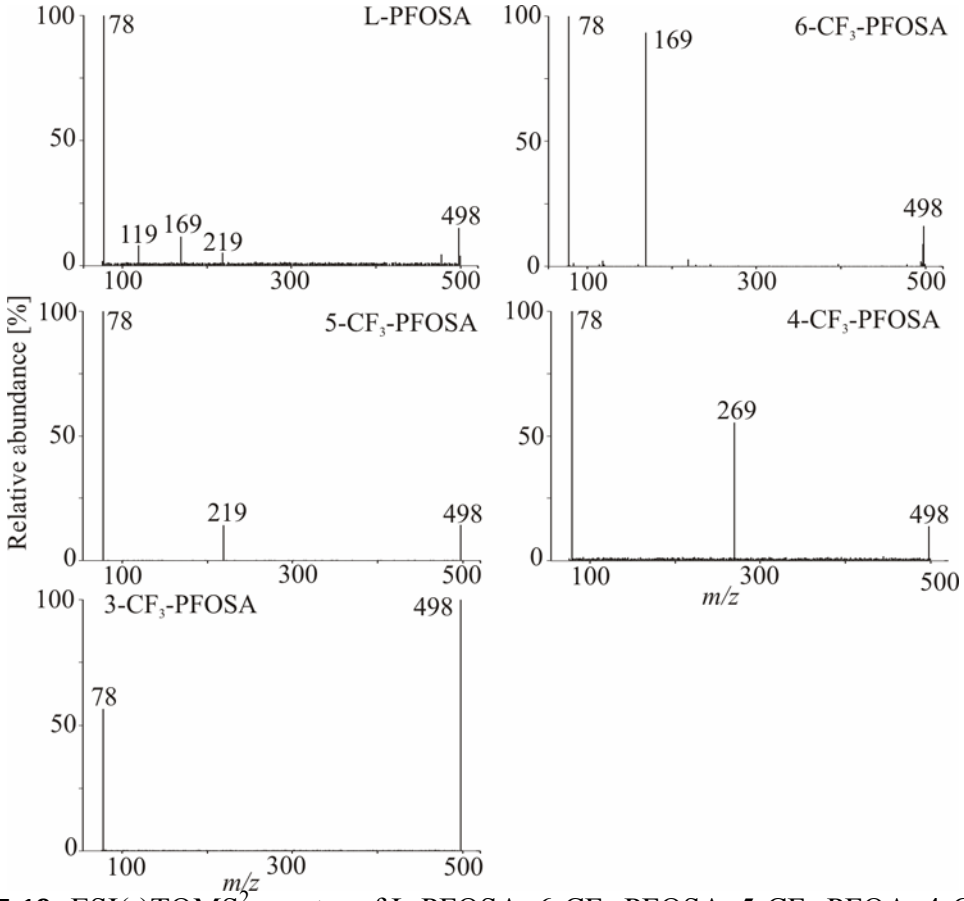

Figure 5.19: ESI(-)$TQMS^2$ spectra of L-PFOSA, 6-CF_3-PFOSA, 5-CF_3-PFOA, 4-CF_3-PFOSA, and 3-CF_3-PFOSA. A circle marks the typical fragment of the "9-series". The molecular anions *m/z* 498 were fragmented with -40 V and -30 Vcollision energy (3-CF_3-PFOSA).

5.6. Conclusions

Isomers present in technical PFC mixtures were characterized by HPLC combined with triple quadrupole and ion trap MS/MS. PFOS, PFOA and PFOSA were selected as representatives of the compound classes of PFSA, PFCA and perfluorosulfonamide since until now the C_8-based compounds were mostly produced. These were also the mostly monitored in the environment.

HPLC separation allowed to differentiate three main groups of isomers in technical ECF mixtures. Those were the diperfluoromethyl geminal substituted isomers (minor group), the perfluoromonomethyl isomers (major group) and the linear isomer (the main isomer). Separation of individual isomers was difficult. Up to ten isomers were separated. Moreover, tandem MS allowed to elucidate for PFOS isomers the location of the CF_3 position. This was hardly possible for PFOA and PFOSA due to a charge different stabilization. Only slight differences were observed between their MS/MS spectra.

Identification of these isomers in environmental samples should be now possible. However, differentiation is based on product ions of low abundance leading to rather high detection limits of MS^2. Therefore, a good isomer separation and detection by MS only is preferred. Here, the development of a new GC method with a derivatization offers a promising alternative technique to reach this aim. It allowed the separation of eleven PFOS isomers. Currently, lack of pure isomer standards limits the isomer-specific analysis of PFOS, PFOA and PFOSA.

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6. Determination of isomer pattern in environmental samples

6.1. Current PFAS isomer identification in environmental samples

Identification of PFAS isomers in environmental samples was until now hardly reported in the literature. Chromatographic conditions were optimized to obtain one signal containing all isomers. Isomers were only reported as additional signal “shoulder” and as “split chromatographic peaks” in human blood and water samples (Hansen *et al.*, 2001; Schultz *et al.*, 2004).

Only three references describe the detection of some isomers in polar bear liver and in human blood. The analysis was restricted to perfluorocarboxylate substances applying GC. (De Silva and Mabury, 2004, 2005, 2006). Kärrman *et al.* (2005a) reported the HPLC separation of several PFOS isomers in human blood samples for the first time. All these studies differentiated only the linear structure from branched ones. Their further characterization is difficult, since they are less abundant in environmental samples. Moreover, no further identification was possible due to the lack of pure isomer standards and of specific MS spectra. Furthermore, the HPLC separation of PFOS isomers is not sufficient in environmental samples and a suitable GC method is lacking.

The different isomer composition of product produced by ECF or telomerization should also allow to give an indication of the origin of PFAS contamination. The presence of branched isomers suggests an ECF process as source, whereas the detection of a high content of the linear isomer indicates a telomerization process (see Chapter 5.1.1.). Moreover, the isomer-specific determination of samples for different environmental

compartments adds new information about environmental behavior and bioaccumulation of PFAS isomers.

6.2. Identification of PFOS isomers in human blood extracts

6.2.1. Identification of PFOS isomers

Identification of PFOS isomers in human blood extracts from Australia, Sweden and the United Kingdom (UK) was first performed by Kärrman *et al.* (2005a). HPLC with the PFP phase coupled to a TQ instrument in SIM mode (*m/z* 499) was used for the analysis of PFOS isomer fractions (see Table 2.2) and PFOS technical mixture which enabled the identification of several isomers.

Figure 6.1 illustrates the PFOS isomer pattern present in human blood samples from Sweden and Australia. They are quite similar to the isomer profile of a technical PFOS mixture. The three main isomer groups (diperfluoromethyl substituted, monoperfluoromethyl substituted and linear isomers) described in the chapter 5.3 were also detected in human blood (see Figure 6.1 C and D).

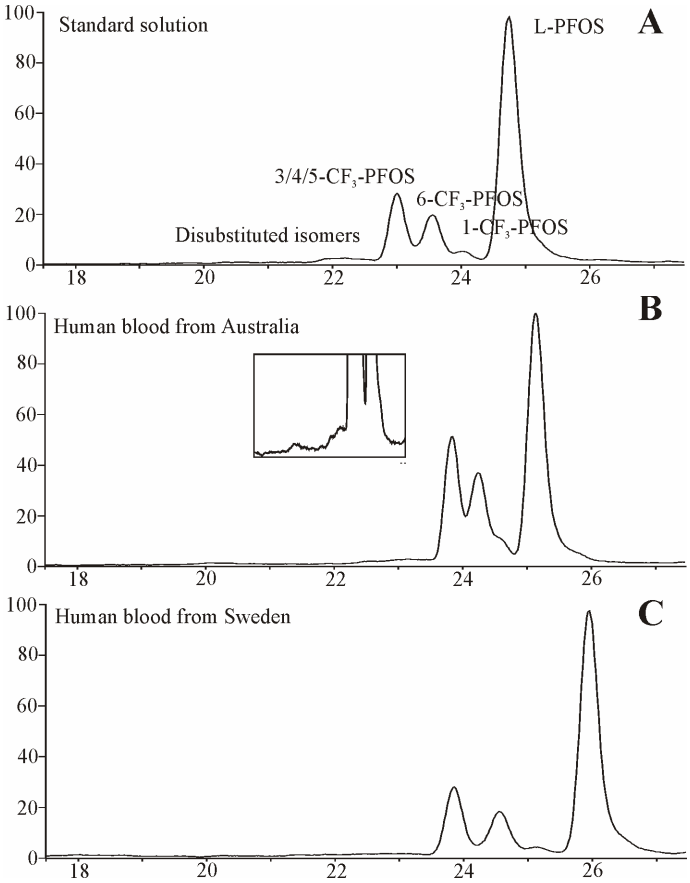

Figure 6.1: PFOS isomer pattern of a technical mixture (A) and in human blood samples from Australia (B) and Sweden (C) recorded by HPLC-ESI(-)TQMS (*m/z* 499).

The first eluting group of human blood contained up to four peaks when a C_{18} Discovery® phase was employed (Kärrman *et al,* 2005a). Two of them were identified as diperfluoromethyl substituted isomers (see chemical structures in Figure 5.2) due to retention times identical with a PFOS technical solution. Pure standards of diperfluoromethyl substituted isomers were not available for further identification.

Monoperfluoromethyl substituted isomers (see chemical structures in Figure 5.2) were identified in a technical PFOS mixture and in human blood by HPLC-TQMS using the purified isomer mixtures (see Figure 6.1). HPLC separation was not sufficient to distinguish 3-CF_3-PFOS, 4-CF_3-PFOS and 5-CF_3-PFOS. A slight retention time difference was observed between 3-CF_3-PFOS and 4/5-CF_3-PFOS for the purified fractions but not

for human blood. Further differentiation between 3-, 4- and 5-CF_3-PFOS was tried for human blood samples applying tandem MS. The $TQMS^2$ of 3-, 4- and 5-CF_3-PFOS were very similar with the product ion *m/z* 130 as base peak (see Figure 5.8 C, D and E). Differences were observed for the higher mass range. The $TQMS^2$ spectra of 4-CF_3-PFOS showed higher relative abundances of the product ions *m/z* 80 and 180 compared to the spectra of 3-CF_3-PFOS and 5-CF_3-PFOS. The abundance of the product ions *m/z* 280 was also different. It was not present in the $TQMS^2$ spectra of 4-CF_3-PFOS. However, the abundance was too low in the other isomer mass spectra to enable identification in human blood. Multiple reaction monitoring was applied to the mass transitions *m/z* 499→80, 99 and 180 to differentiate 4-CF_3-PFOS from 3/5-CF_3-PFOS. However, the HPLC separation was not sufficient for an identification.

The branched monoperfluoromethyl 1-CF_3-PFOS to 6-CF_3-PFOS could be clearly differentiated from linear PFOS (L-PFOS) and were detectable in human blood samples after separation on a PFP phase. However, the isomer 2-CF_3-PFOS was not identified due to too similar MS/MS spectra.

Furthermore, a signal with a higher retention time than L-PFOS (*m/z* 499) was observed in the human blood extracts. It was assigned as an additional isomer by Kärrman *et al.* (2005a). This compound formed the product ions *m/z* 80 from the precursor ions *m/z* 499 and had an abundance of up to 9 % relative to L-PFOS. However, no additional fragments (*m/z* 130 and 99) typical for PFOS were present. Furthermore, this compound was not present in the technical mixture and ^{19}F-NMR analysis gave no indication about the presence of further PFOS related isomers. Its elution after L-PFOS indicated an even more lipophilic structure. No further structural information could be obtained.

6.2.2. PFOS isomer pattern and route of exposure

The PFOS isomer profiles in all human blood samples were similar to those of technical PFOS. The presence of several branched isomers at a relative abundance around 30 % indicated an ECF contamination source for humans from Australia, Sweden and UK. Table 6.1 summarizes the isomer composition of all human blood samples obtained by HPLC-SQMS.

Table 6.1: PFOS isomer composition (area %) of standard solutions and human blood samples from Sweden, Australia and the UK. HPLC-SQMS was applied.

Identity	**	**	**Diperfluoro-methyl substituted**		**3/4/5CF_3-PFOS**	**1/6-CF_3-PFOS**	**L-PFOS**	**
Relative retention time	0.86	0.90	0.93	0.94	0.96	0.97	1	1.03
Standard (Fluka)								
mean (n= 5)			0.4	0.2	8.0	14.4	78	
SD					1.1	2.4	1.1	
Sweden, serum								
mean (n= 17)	0.69	0.18	0.22	0.20	12.6	18.0	68.1	0.43
SD	0.57	0.16	0.08	0.18	2.3	3.6	3.8	0.75
Australia, serum								
mean (n= 40)	2.5		0.33	0.44	17.1	21.3	58.7	0.39
SD	0.83		0.11	0.14	2.1	2.1	2.3	0.23
UK, plasma								
mean (n= 13)			0.26	0.21	17.7	20.4	59.6	2.5
SD			0.13	0.10	3.3	3.3	5.2	3.1

**: unidentified signals
SD: standard deviation

The relative abundance of L-PFOS in blood was significantly lower ($p<0.001$) than in technical PFOS as can be seen in Table 6.1. The relative amount of L-PFOS was 58-70 % in blood and 76-79 % in the technical mixture. The possibility of isomer discrimination was checked for extraction and sample clean-up. No indication for losses of isomers was found.

The most abundant branched isomers in the technical product were 6/1-CF_3-PFOS (18-20 % in total) followed by 3/4/5-CF_3-PFOS (13-18 %). These results are in agreement with previously reported ^{19}F-NMR data for technical mixtures (6/1-CF_3-PFOS, 16 %; 3/4/5-CF_3-PFOS, 13 %). Moreover, HPLC on the PFP phase and TQMS revealed that plasma samples from Sweden contained less 1-CF_3-PFOS (0-2.7 %) compared to technical PFOS (2.9 %). Samples from Australia had a higher content of 6-CF_3-PFOS (17.3-17.5 %) compared to the technical mixture (11.3 %) and samples from Sweden (12.5-16.4 %).

PFOS is the final degradation product of perfluorooctanesulfonyl fluoride based compounds mainly produced by the process (Tomy *et al.*, 2003, Xu *et al.*, 2003). The observed isomer profiles in human blood from Australia, Sweden and the United Kingdom indicated ECF as synthesis process, which is in agreement with the main production process for PFOS.

The observed differences in the isomer profiles of human blood and technical mixture suggested a preferential bioaccumulation of the branched PFOS isomers and/or a preferential elimination of linear PFOS. A direct up-take of PFOS is less likely, since PFOS itself represents only 2-3 % of the total ECF production (OECD., 2002).

Additionally, differences in the kinetics of up-take and elimination of between branched and linear PFOS have also to be taken into account. Recently Loveless *et al* reported that oral administration of PFOA isomers to mice and rats resulted in lower serum concentrations of branched PFOA isomers compared to the linear one (Loveless *et al.*,

2006). Possible explanations were that branched PFOA were excreted or not absorbed to the same extent as linear PFOA.

6.2.3. Country, age and gender related comparison

The mean PFOS concentration was 33.4 ng/ml (range 10.1-90.9 ng/ml) for the Swedish samples, 14.4 ng/ml (range of 6.7-28.1 ng/ml) for those from UK and 23.4 ng/ml (range 12.7-29.5 ng/ml) for the Australian ones (see Table 6.2). The samples from Sweden contained in average 20-50% more PFOS compared to Australia and the UK. PFOS isomer patterns of the different countries are compared in Figure 6.1. The mean relative abundance of L-PFOS was 68 % for the Swedish samples and 59 % for the Australian/UK samples.

Table 6.2: PFOS concentrations (ng/ml) in human blood from different countries.

	PFOS [ng/ml]
Sweden, plasma n=17	
mean (SD)	33.4 (19.6)
median	29.0
Australia, serum n=40	
mean (SD)	23.4 (13.6)
median	21.1
UK, plasma n=13	
mean (SD)	14.4 (6.0)
median	12.9

SD: standard deviation

Possible correlations between age, gender and isomer pattern were studied for the Australian samples (see Figure 6.2). No significant abundance difference was observed for L-PFOS. However, gender and age influenced the relative abundances of 3/4/5-CF_3-PFOS and 1/6-CF_3-PFOS. 3/4/5-CF_3-PFOS were less abundant in women. The age groups <16

and 16-30 years also contained less of this isomer group compared to the medium age group (30-60 years). However, no age trend was observed, since the age group >60 years had a decreased relative level again. Moreover, the group 30-60 years showed a lower relative burden of the isomer group 1/6-CF_3-PFOS.

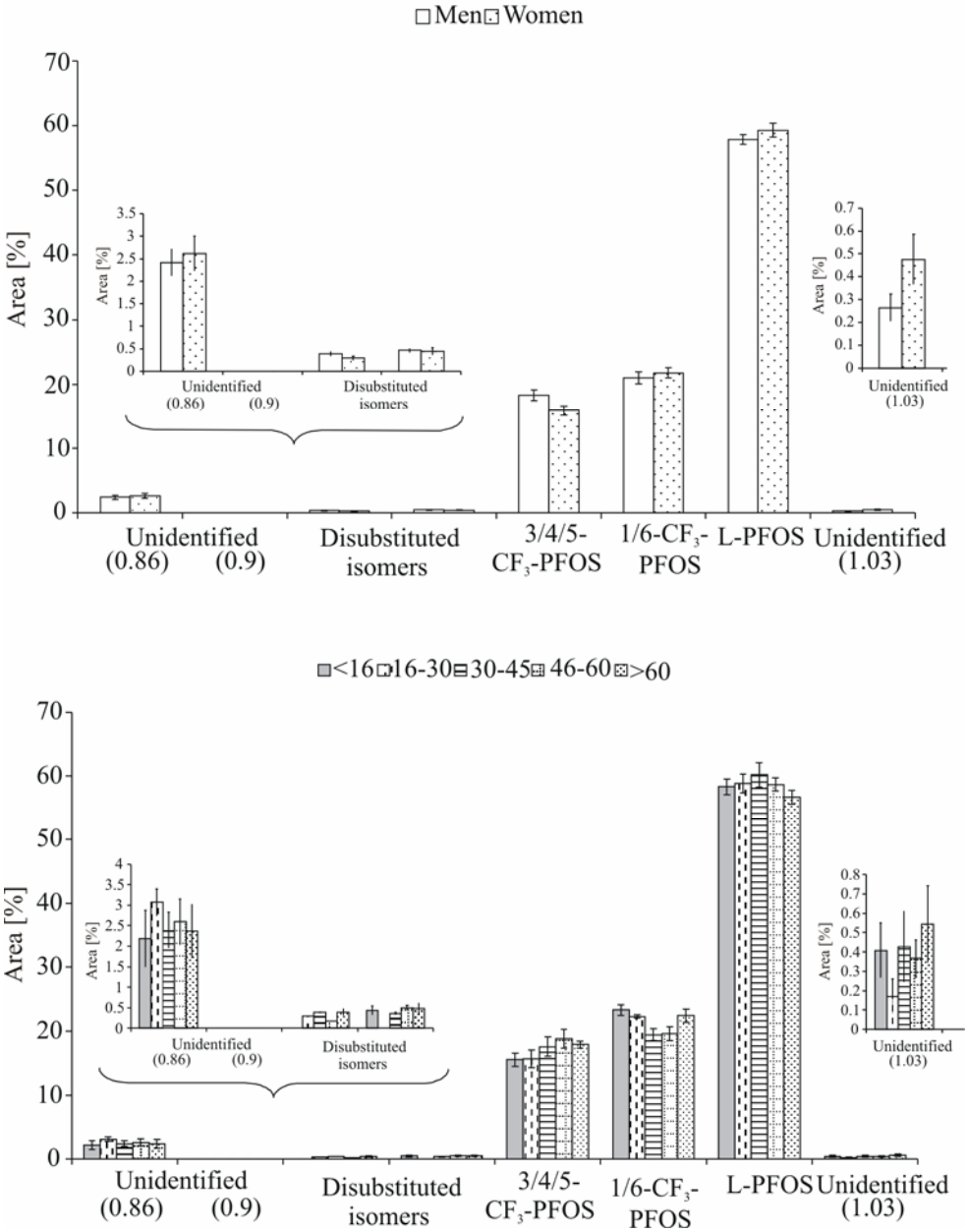

Figure 6.2: Isomer pattern of PFOS in human blood (n=40) from Australia related to gender and age. Samples were divided into five age groups. The bars represent the mean abundance and the whiskers the 95 % confidence interval. The retention time relative to L-PFOS is given for the unidentified peaks.

Furthermore, no significant differences were observed between the isomer patterns in the samples from Australia presenting the Southern Hemisphere and from the UK (Northern Hemisphere). The observed differences between the isomer pattern in Sweden and the UK/Australia have to be compared with more data from other regions to conclude whether this is consumer product derived or caused by other processes.

6.2.4. Observed problems

Problems of retention time reproducibility occurred with the PFP phase. The HPLC separation of monoperfluoromethyl substituted PFOS isomers shown in Figure 5.3 B could not be repeated with another PFP column (see Figure 6.1). The resolution between the isomers was lower. Moreover, a change in the elution order was also observed between 6-CF_3-PFOS and 1-CF_3-PFOS limits the application of this phase.

Moreover, the isomer resolution was reduced after around twenty injections (loop of 20 µl). Matrix components such as rest of lipids and/or proteins may be irreversibility adsorbed on some active sites of the phase (Oehme *et al.*, 2002). Regeneration was tried by running repeated gradients with aqueous acetic acid. Moreover, injection of acetonitrile with 1 % acetic acid or trifluoroacetic acid (100 µl injected) did not lead to the initial separation as proposed by Major (2003).

Release of phase impurities was observed for two PFP columns from the same company. This led to a very high background in the mass range of PFOS compounds and caused detection problems. These impurities were assumed to be by-products of the phase production.

Response factors for isomers varied between different mass spectrometers as shown in Table 4.7 for a PFOS standard and human blood extracts. The relative amount of L-PFOS applying the SIM mode (*m/z* 499) was 68.9 % employing the Varian TQMS and 79 % with the Agilent SQMS. The relative quantity of 3/4/5-CF_3-PFOS was 6 % for the Varian TQMS and 9 % for the Agilent SQMS. These instruments related differences in will lead to systematic deviations of isomer composition, when data are reported from different laboratories.

6.3. PFOA isomer pattern in water and human blood extracts

6.3.1. PFOA isomer patterns

The PFOA isomer patterns were determined in two water samples and four human blood samples from different locations. Table 6.3 summarizes the samples, their geographical origin and the results of the quantitative analysis according to Kärrman *et al.* (2005b) for human blood samples and Caliebe *et al.* (2005) for water samples. The concentration range in blood was in agreement with published PFOA levels in a non-exposed population (see Chapter 1.5). Sample Sw from Sweden had a high PFOA concentration of 49.8 ng/ml. The water samples (94.8 and 170.5 ng/ml) were collected in the industrialized area of the Elbe estuary in Germany.

Table 6.3: PFOA concentrations in plasma samples from Sweden, in serum samples from Australia, in water samples and in a technical mixture (TM). The relative isomer compositions (linear: L, branched: B, in %) are also given. TQMS was applied.

Sample				**Human blood**			**Water**	
Name		**TM**	**Sw**	**UK**	**AS1**	**AS2**	**W1**	**W2**
Location		-	Sweden	United Kingdom	Australia		North Sea	
PFOA conc [ng/ml]		-	49.8	12.6	8.0	9.6	94.8	170.5
Isomer composition [%]	**L:**	78	100	100	100	L+B isomers	92.3	92.3
	B:	22					7.7	7.7

Two technical mixtures obtained by ECF and telomerization were also studied. HPLC on a PFP phase and TQMS were applied. Figure 6.3 shows the PFOA isomer pattern of both processes. The telomer solution did not contain any branched isomers.

The water samples were analyzed in the SIM mode monitoring $[M\text{-}H]^-$ (*m/z* 413) and $[M\text{-}CO_2]^-$ (*m/z* 369). Tandem MS was necessary to increase the selectivity for human blood samples due to matrix residues. The mass transition *m/*z 413$\rightarrow$369 and *m/*z 369$\rightarrow$169 were selected for the MRM mode.

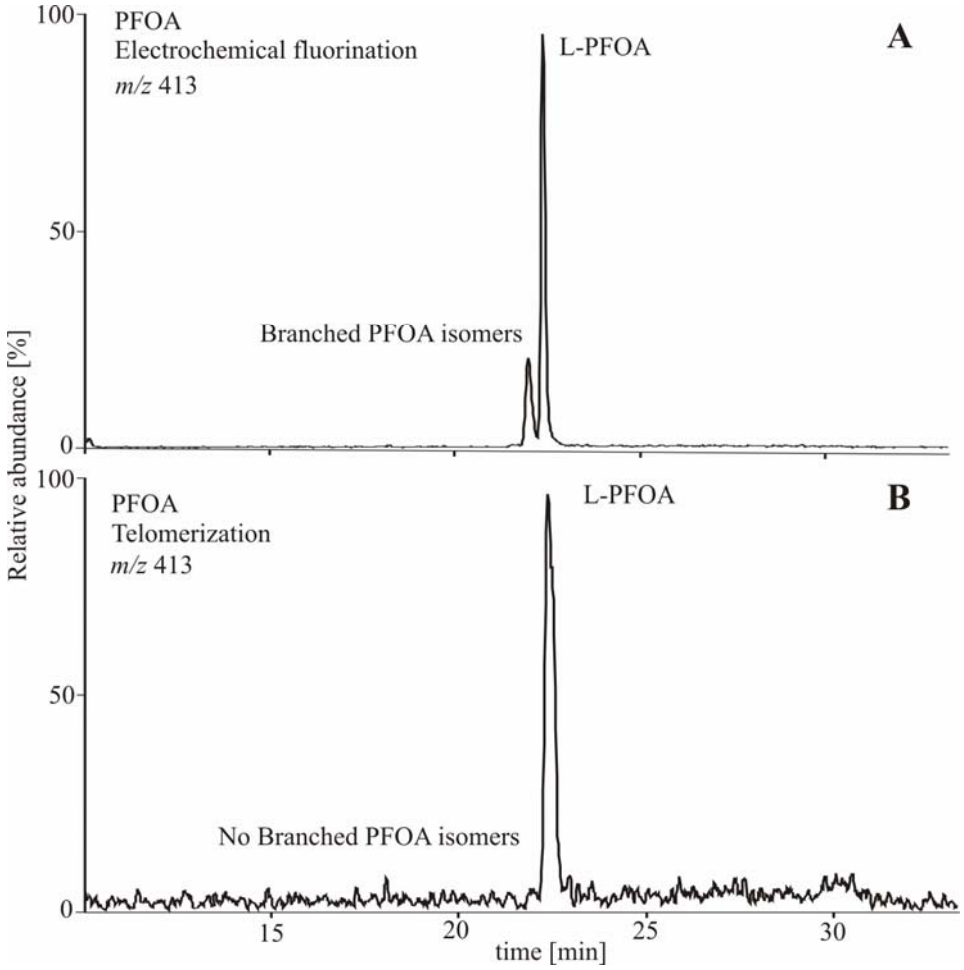

Figure 6.3: PFOA isomer distribution in technical mixtures produced by ECF (A) and by telomerization (B). The selected ion monitoring mode was applied.

The PFOA isomer patterns of water and human blood are shown in Figure 6.4. The isomer profile was dominated by the linear isomer.

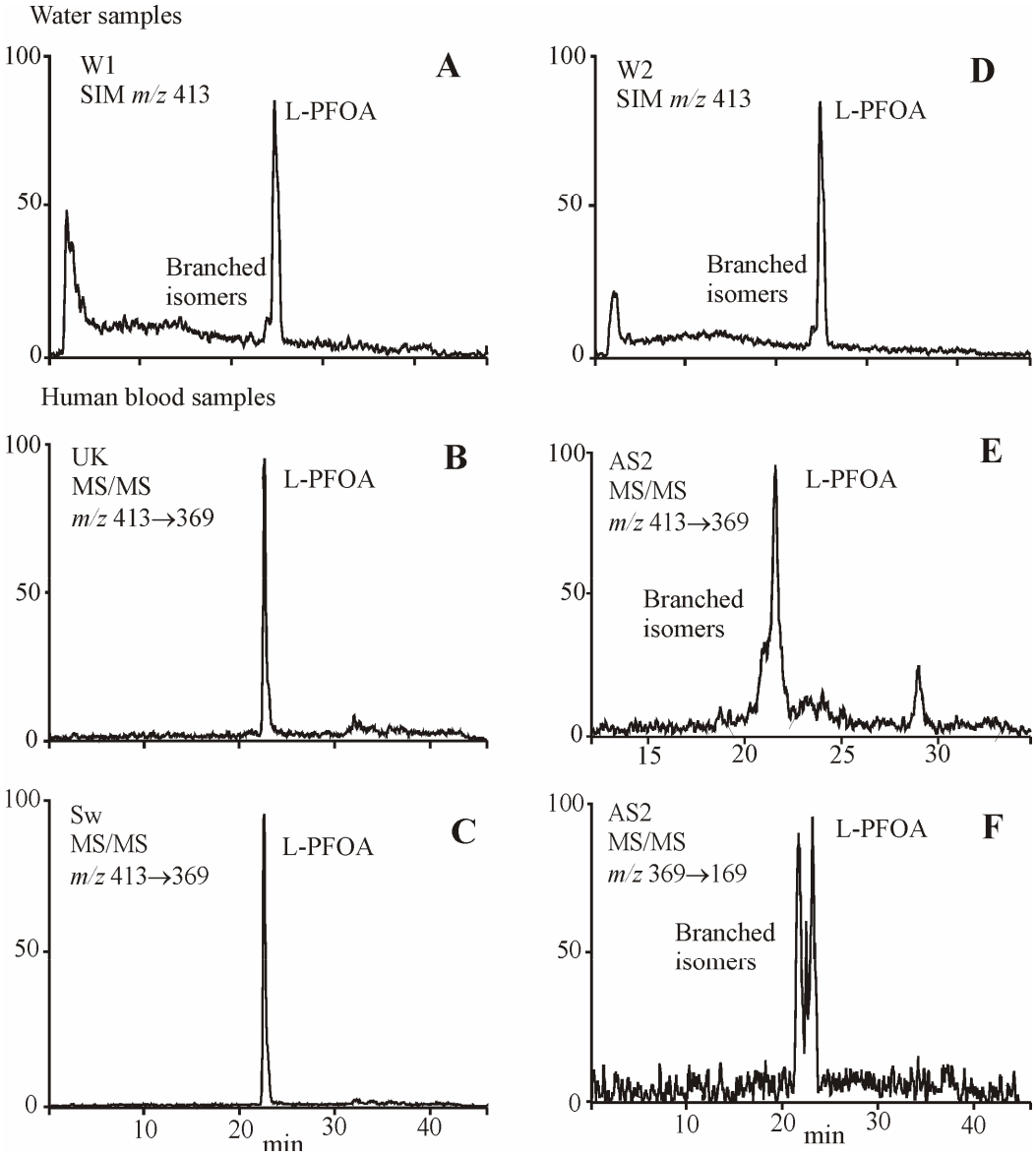

Figure 6.4: PFOA isomer patterns in water (A and D) and human blood from United Kingdom (B), Sweden (C) and Australia (E and F) obtained by HPLC-MS in the selected ion monitoring mode (*m/z* 413) and by multiple reaction monitoring of the mass transition *m/z* 413→369 and *m/z* 413→169.

The content of branched isomers was the main difference between both matrices. The relative isomer composition of branched PFOA isomers is given in Table 6.3. Both water samples had a relative abundance of 7.7 % (see Table 6.3 and Figure 6.4 A, D), which is between telomer-PFOA (0 %) and ECF-PFOA (22 %) This indicates that the PFOA origin in the Elbe estuary is both from telomerization and ECF. This is the first determination of PFOA isomer patterns in water. Therefore, a further comparison is not possible. Surface water and especially the oceans contain the majority of PFOA. Thus, determination of the

PFOA isomer pattern is important for source identification of PFOA in water (Prevedouros *et al.*, 2006).

L-PFOA was the only isomer detected in human blood from the United Kingdom and Sweden (see Figure 6.4 C, E). This is in agreement with previous studies of De Silva and Mabury (2006, 2005) reporting a relative L-PFOA abundance of 98 %. The Australian extracts contained more impurities which limited the identification of branched isomers. Signal suppression was observed by tandem MS. However, the Australian samples show a small first eluting peak of presumably branched isomers. The blank was free of this signal.

6.3.2. PFOA route of exposure

The characterization of PFOA isomers in environmental samples is more problematic compared to PFOS. First, the PFOA isomer $TQMS^2$ spectra are less structure specific compared to PFOS isomers (see Chapters 5.3 and 5.5). Moreover, there are two PFOA release patterns. The first one contains only linear isomers (telomerization process and degradation of fluorotelomer alcohols to PFOA). The second one releases a mixing of branched and linear isomer (ECF process and biodegradation of ECF-based compounds) (Hekster *et al.*, 2002, Hagen *et al.*, 1981, Dinglasan *et al.*, 2004, Gauthier *et al.*, 2005, Wang *et al.*, 2005, Lange, 2000). Consequently, the observed environmental patterns are a mixture of both, which are not interpretable, since no estimation quantities for both processes exist.

Different PFOA isomer patterns were observed for water and human blood. Water samples contained more branched PFOA isomer reflecting the origin of the release. The profiles in human blood are influenced by the up-take process. Recently Loveless *et al.* reported that

the up-take and the elimination of the branched PFOA isomers are different to L-PFOA resulting in a lower amount of branched PFOA in serum of mice and rats (Loveless *et al.*, 2006).

The sample Sw had a rather high PFOA concentration of ca. 50 ng/ml (see Table 6.2). It contained only L-PFOA (see Figure 6.4). Special exposure of this individual was from a telomer source is likely. This person was a regular ski wax user and such products were therefore considered as a potential source of telomer compounds.

6.4. Conclusions

Isomer patterns of PFOS and PFOA in environmental samples allowed to draw conclusions about their origin. PFOS isomers were determined in environmental samples for the first time as well as PFOA isomer patterns determined in water and in human blood. The linear structure (PFOS and PFOA) is dominant in the environment. However, some differences were observed between PFOA and PFOS depending on the matrix.

L-PFOS bioaccumulates less than the branched isomers in human blood. However, L-PFOA is more enriched than branched isomers in human blood. Moreover, the first PFOA isomer profile in water suggested a both telomerization and ECF as source of PFOA release.

The separation of PFOS or PFOA isomers is a demanding task. Besides the requirement of purified isomer standards, identification of PFOS and PFOA isomers in the environment was also limited by their still too poor HPLC separation. As a consequence, most of the

studies reporting isomer patterns in environmental samples used GC which allows a higher resolution. HPLC coupled to tandem MS suffered from high limits of detection and did not always enabled identification of all the isomers. Challenges were additionally brought by the detection of an unidentified peak after L-PFOS.

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