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DETERMINATION OF PERFLUORINATED ORGANIC ACIDS
IN SOIL BY GAS CHROMATOGRAPHY

Stanovení perfluorovaných organických kyselin v půdách
metodou plynové chromatografie

Diploma thesis

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ABSTRACT

A method employing solid-liquid extraction with methanol and solid-phase extraction (SPE) clean-up step using Supelco SupelcleanTM ENVITM-Carb 3 mL cartridges (0.25 g graphitized carbon adsorbent) followed by gas chromatography – mass spectrometry with negative chemical ionization (GC-NCI-MS) has been optimized and applied for determination of ultratrace concentrations of C₆ – C₁₂ perfluorinated carboxylic acids (PFCAs) in soil samples. A sophisticated multifactorial statistic method, response surface methodology, employing 1/16 fractional factorial design and the face centered central composite design as well has been applied to find the significant parameters which influence the extraction procedure of PFCAs and SPE clean-up step and to set the optimum extraction and clean-up levels of eight parameters evaluated yielding the maximum extraction recovery of all PFCAs. The analyte extraction recoveries and the limits of detection and quantification have been obtained. The recoveries of individual PFCAs were within a range from 85 to 100 % for analyte spiked concentration level of 1.1 ng g⁻¹ and within a range from 91 to 107 % for analyte spiked level of 2.1 ng g⁻¹. The values of limits of detection were 1.9 – 3.0 pg g⁻¹ and limits of quantification 6.4 – 10.1 pg g⁻¹. This analytical method has been tested on determination of C₆ – C₁₂ perfluorinated carboxylic acids in soil samples collected from nine different geographical locations in Prague and villages in the close neighbourhood. The concentrations determined were of the order of tens of pg g⁻¹ and C₈ – C₁₂ perfluorinated acids, except from C₉, occurred most often.

Subject words

Analytical chemistry, separation methods, gas chromatography

Keywords

Perfluorinated carboxylic acids, soil, gas chromatography

ABSTRAKT

Metoda využívající extrakce metanolem z pevné do kapalné fáze s přečištěním pomocí extrakce tuhou fází (SPE) za použití Supelco SupelcleanTM ENVITM-Carb 3 mL kolonek (0.25 g grafitizovaného uhlíkového adsorbentu) následovaná plynovou chromatografií – hmotnostní spektrometrií s negativní chemickou ionizací (GC-NCI-MS) byla použita pro stanovení ultrastopových koncentrací C₆ – C₁₂ perfluorovaných karboxylových kyselin (PFCAs) v půdních vzorcích. Vícefaktorová statistická metoda response surface methodology, využívající 1/16 frakční faktorový design a také face centered central composite design, byla využita k nalezení významných faktorů ovlivňujících extrakci a SPE přečištění perfluorovaných kyselin. Tato metoda také posloužila k určení optimálních hodnot osmi extrakčních a čistících parametrů poskytujících nejvyšší extrakční výtěžnost perfluorovaných kyselin. Následně byla stanovena účinnost extrakce a také limity detekce a kvantifikace. Účinnost extrakce se pohybovala v rozmezí od 85 do 100 % při přidaném množství analytů 1,1 ng g⁻¹ a v rozmezí od 91 do 107 % při přidaném množství 2,1 ng g⁻¹. Limity detekce se nacházely v intervalu 1,9 – 3,0 pg g⁻¹ a limity kvantifikace dosahovaly hodnot 6,4 až 10,1 pg g⁻¹. Tato analytická metoda byla testována stanovením perfluorovaných karboxylových kyselin v půdních vzorcích odebraných z devíti různých míst v Praze a okolí. Stanovené koncentrace perfluorovaných kyselin se pohybovali v desítkách pg g⁻¹ a nejčastěji se vyskytujícími byly C₈ – C₁₂ perfluorované kyseliny, kromě C₉ kyseliny.

Předmětová slova

Analytická chemie, separační metody, plynová chromatografie

Klíčová slova

Perfluorované organické kyseliny, půda, plynová chromatografie

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LIST OF SYMBOLS AND ABBREVIATIONS

APCI	atmospheric pressure chemical ionization	PLE	pressurized liquid extraction
c	analytical concentration [g L^{-1}]	R^2	correlation coefficient
CCD	central composite design	R^2	determination coefficient
CI	chemical ionization	RSD	relative standard deviation [%]
ECD	electron capture detector	RSM	response surface methodology
EI	electron impact	S/N	signal-to-noise ratio
EPA	Environmental Protection Agency	SIM	selected ion monitoring
ESI	electrospray ionization	SPE	solid-phase extraction
GC	gas chromatography	t	retention time [min]
HPLC	high-performance liquid chromatography	TIC	total ion current [a.u.]
ID	internal diameter [μm]	TOF	time-of-flight mass analyzer
K_{ow}	octanol-water partition constant		
LC	liquid chromatography		
LOD	limit of detection		
LOQ	limit of quantification		
m/z	mass-to-charge ratio		
MS	mass spectrometry		
MS/MS	tandem mass spectrometry		
NCI	negative chemical ionization		
OVAT	one-variable-at-a-time		
p	pressure of reagent gas [kPa]		
PFCAs	perfluorinated carboxylic acids		
PFHxA	perfluorohexanoic acid		
PFHpA	perfluoroheptanoic acid		
PFOA	perfluorooctanoic acid		
PFOS	perfluorooctanesulfonic acid		
PFNA	perfluorononanoic acid		
PFDA	perfluorodecanoic acid		
PFUnA	perfluoroundecanoic acid		
PFDoA	perfluorododecanoic acid		

1. INTRODUCTION

1.1 Aims of Diploma thesis

Despite the existence of studies reporting the presence of perfluorinated compounds in biological and abiotic matrices such as water and air, still very few studies have focused on their occurrence in solid matrices including soil, sludge and sediment. These compounds have been detected throughout the world in surface waters, in ground waters, in wastewaters, in air and in biological samples including species from remote polar region, but yet an explanation is needed of how they have been distributed in the environment and how much they have contaminated it.

Determination of perfluorinated compounds in solid matrices generally complicates the lack of reliable standards, impurities, complicated mixtures of isomers and congeners, their unique physical and chemical properties and mostly the contamination during all stages of the analytical procedure.

The aim of this Diploma thesis is to optimize a sensitive and accurate method employing solid-liquid extraction with methanol and SPE clean-up step followed by gas chromatography – mass spectrometry with negative chemical ionization for determination of C₆ – C₁₂ perfluorinated carboxylic acids and subsequently report the possible occurrence of these bioaccumulative and persistent compounds in the selected soil samples acquired from Prague and villages located in the close neighbourhood.

The occurrence of perfluorinated carboxylic acids has already been published in the Vltava and Elbe rivers [1] and afterwards in Central Wastewater Cleaning Plant in Prague [2]. Some of these acids were quantified in a concentration range from tens to hundreds pg mL⁻¹.

2. THEORETICAL PART

2.1 Perfluorinated carboxylic acids

Perfluorinated carboxylic acids (PFCAs) represent purely anthropogenic chemicals and have been classified as a new category of global environmental pollutants [3]. They are resistant to hydrolysis, photolysis, microbial degradation and metabolism by vertebrates, which makes them environmentally persistent [3, 4]. Moreover, these compounds bioaccumulate in food chains and have long half-lives in human organism [5].

Although these perfluorinated compounds have probably been present in the environment and biota since their industrial production, corresponding environmental and biological effects have emerged only recently [3]. A wide range of applications following from their unique chemical and physical properties complicates the understanding of how these compounds are distributed in the environment and how people become exposed to them [5].

2.1.1 Physical and chemical properties

Perfluorinated carboxylic acids are compounds consisting of a hydrophobic alkyl chain and a hydrophilic end group, with a typical structure of $F(CF_2)_n-COOH$, where $n = 1-13$ [1, 6]. They are analogues of fatty acids with linear chains in which all atoms of hydrogen have been replaced by fluorine [7]. It is mainly the high-energy bond between carbon and fluorine ($\approx 110 \text{ kcal mol}^{-1}$) as well as the three pairs of negatively charged unbinding electrons of fluorine that are responsible for an extreme chemical and thermal stability of these compounds [4]. In this Diploma thesis, the concern is focused on seven perfluorinated carboxylic acids, from C_6 to C_{12} chain length, which structural characteristics are summarized in Table 2.1.

Their unique properties, such as high surface activity, thermal stability, amphipathicity, density, weak intermolecular interactions and resistance to acidic and alkaline conditions have led to their extensive industrial application since 1940s [3]. These compounds function in conditions where other substances would rapidly degrade and therefore are irreplaceable in many industrial applications [8]. However, these properties are also responsible for their persistence in the environment and bioaccumulation in the food chains [3].

Based on their molecular properties they are called “supersurfactants” [1]. As mentioned above, their molecules contain polar and nonpolar domains that reduce water surface tension

more intensively than hydrocarbon-based surfactants, and therefore are more powerful wetting agents [4, 9]. Coating an exterior surface of a product leaves the hydrophobic tail of the molecule protecting away from the surface, repelling water, oil and fat [4]. These compounds have found numerous applications in the photographic, semi-conductor and aviation industry (hydraulic fluids), in upholstery, in metal plating and in the treatment of carpets, leather, paper, plastics, paints and packaging [4, 9, 10]. Moreover, they are widely used as leveling agents for lubricants, mist suppressions and fire fighting foams [5] to extinguish fires burning at high temperatures [11, 12]. A major use of perfluorinated carboxylic acids is as an emulsifier in the production of fluoropolymers, primarily the ammonium salt of perfluorooctanoate and perfluorononanoate, which serve as processing aids in the production of polytetrafluoroethylene, polyvinylidene fluoride, Teflon® or similar products [8, 13, 14].

Table 2.1 Structural characteristics of seven perfluorinated carboxylic acids (C₆ - C₁₂)

Compound	Abbreviation	Molecular weight	Structural formula
Perfluorohexanoic acid	PFH _x A	314.05	C ₆ HF ₁₁ O ₂
Perfluoroheptanoic acid	PFHpA	364.06	C ₇ HF ₁₃ O ₂
Perfluorooctanoic acid	PFOA	414.07	C ₈ HF ₁₅ O ₂
Perfluorononanoic acid	PFNA	464.08	C ₉ HF ₁₇ O ₂
Perfluorodecanoic acid	PFDA	514.08	C ₁₀ HF ₁₉ O ₂
Perfluoroundecanoic acid	PFUnA	564.09	C ₁₁ HF ₂₁ O ₂
Perfluorododecanoic acid	PFDoA	614.10	C ₁₂ HF ₂₃ O ₂

2.1.2 Sources in the environment

There are about 30 natural organofluorine molecules produced by geochemical and biological processes in the environment [15], e.g. by some higher plants and microorganisms. One of them is monofluoroacetic acid, which is produced by plants of the genus *Dichapetalum*, and certain antibiotics containing fluorine are produced by fungi [4]. Fluorinated organic compounds occurring in the environment usually contain one fluorine atom, whereas synthetic fluorinated compounds include many fluorine substitutes and moreover, perfluorinated compounds are fully fluorinated. All perfluorinated carboxylic acids are purely anthropogenic compounds. Although partially fluorinated compounds can undergo chemical breakdown at functional group bond in the environment, the perfluorinated compounds have no known natural decomposition processes [4]. They are also referred to as the “polychlorinated biphenyls of the 21st century” [16].

There are both direct and indirect sources of PFCAs emissions to the environment [10]. Direct sources of PFCAs emissions include PFCAs production, incorporation of PFCAs into products, distribution of the products to consumers and the use of these products [8]. On the other hand, indirect sources result from the degradation of primary substances that may form perfluorinated carboxylic acids or represent those sources, where PFCAs are present as chemical reaction impurities. The total global emissions of PFCAs from both direct and indirect sources over the period from 1951 to 2004 were estimated to be 3200 – 7300 tones [1, 10]. The majority ($\approx 80\%$) of PFCAs released to the environment came from fluoropolymer manufacture and use [10].

The number of sites producing fluorinated organic compounds is not clear but there is production in the USA, Italy, Switzerland, the United Kingdom and Japan [8]. In 2000, the major global producer 3M company announced the intention to phase out the manufacture of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). In October 2000, the US Environmental Protection Agency (EPA) proposed a significant new use rule for 88 PFOS-related compounds, in which companies have to file a notice with the US EPA 90 days before beginning any new manufacturing or importation of listed PFOS chemicals [8, 17]. During this time period, US EPA has the opportunity to evaluate any intended new use of PFOS-related substances and associated activities and, if necessary, to prohibit or limit the activity before it occurs. These substances have also been on the agenda of the Organisation for Economic Co-operation and Development since 2000 [8, 18]. As a result, competent governments should contact PFOS manufacturers to determine whether

the companies are planning to phase out PFOS production. In the EU, there is currently no legislation covering the use of PFOS-related compounds associated with their potential environmental and human health effects [8].

2.1.3 Behaviour in the environment

The transport and accumulation of organic chemicals in the environment is governed by their physical and chemical properties, i.e., their equilibrium partitioning between various media such as water, air and soil [19]. Partitioning coefficients, commonly required for environmental models describing the fate of particular organic pollutants in the environment, are the saturated subcooled liquid vapour pressure (p_L^*), the air – water partition constant (K_{aw}) and the octanol – water partition constant (K_{ow}) [20].

Due to high degree of fluorination, poly- and perfluorinated compounds have unique physical and chemical properties that differ from many other organic contaminants [20]. The partition behaviour of PFCAs strongly depends on their degree of dissociation [21]. The non-ionic form of PFOA has estimated a $\log K_{aw} = -2.4$ and a $\log K_{ow} = 4.3$, suggesting that both sorption in soils and sediments as well as volatilization and transport in air, play an important role in the environmental fate of PFOA [20, 21]. On the other hand, the anionic perfluorooctanoate is not expected to partition into the gas phase at all, and sorption by most soils and sediments is expected to be much smaller [21, 22], because electrostatic interactions with sediment and soil organic matter would likely be repulsive due to the presence of negative charges on both the sorbate and the organic matter [22].

Given the hydrophobicity and oleophobicity of the perfluorinated chain and the hydrophilicity of carboxylate head group, it is likely that both hydrophobic and electrostatic interactions will influence the sorption of PFCAs. Higgins and Luthy investigated the sorption of anionic poly- and perfluorinated alkyl substances to sediments [22]. According to their study, the sorption to sediment increases with decreasing solution pH and increasing Ca^{2+} concentration, suggesting that electrostatic interactions play a role. Moreover, sorption of these compounds was positively correlated with organic carbon content, indicating the importance of hydrophobic interactions.

2.1.4 Transport in the environment

Perfluorinated carboxylic acids can enter the environment in the same chemical form

as they were produced and applied in or as a precursor. Based on their molecular properties, such as low pK_a values ($pK_a = 2.80$ for PFOA), these compounds are considered to be strong acids [13, 14]. Therefore they should be mainly present in the environment in their anionic form, i.e. a non-volatile form [14]. After application, these perfluorinated surfactants reach the aquatic environment either through their release into rivers or via wastewater discharge into receiving waters [8]. They are predominantly adsorbed to sewage sludge, which is used for land treatment or disposed on dump sites [23]. It leads to a remobilization of these recalcitrant compounds, and due to their polarity and mobility in water and soil, they are able to reach the sea or ground water in unaffected or undegraded conditions [8, 23].

Thus, the global distribution of PFCAs does not result from their volatilization and transportation to remote regions [14]. However, the long carbon chain PFCAs were found to be present in animals from the Arctic and Northern Europe [24, 25]. Following the recent investigations, it has been suggested that the global distribution of PFCAs could be explained by the transport and environmental degradation of fluorotelomer alcohols [26, 27]. Fluorotelomer alcohols are neutral, volatile compounds with an estimated half-life of 20 days in the atmosphere, which is sufficient for their widespread distribution in the environment [28]. It is already known, that fluorotelomer alcohols are metabolically transformed to PFOA in rats [29] and are also metabolized through aerobic microbial systems [27] although the atmospheric degradation mechanism of fluorotelomer alcohols is unknown [26]. Nevertheless, it may be a possible explanation of why these long carbon chain PFCAs have been present in animals from such remote geographical regions [24, 25].

In addition to atmospheric transport and degradation of precursors, ocean water transport of PFCAs themselves could considerably contribute to their long-range transport [10]. It has been estimated that every year, 2 to 12 tonnes of PFOA in its anionic form are transported to the Arctic by oceanic transport, which is greater amount in comparison to the amount resulting from atmospheric transport and degradation of precursors [10].

A proposed fate of per- and polyfluorinated alkyl precursors in the environment is shown in Figure 2.1. The final breakdown products are perfluorinated carboxylic acids (PFCAs) and perfluoroalkane sulfonates (PFSAs) [30]. Precursor compounds which are suggested to be degraded or biotransformed to perfluorinated carboxylic acids are fluorotelomer alcohols (FTOHs), fluorotelomer aldehydes (FTALs), perfluorinated aldehydes (PFALs), fluorotelomer carboxylates (FTCAs), fluorotelomer unsaturated carboxylates (FTUCAs), fluorotelomer olefins (FTolefins) and fluorotelomer sulfonates (FTSs). The other poly- and perfluorinated precursors, i.e. fluorobutane sulfonamidoethanols (FBSEs), fluorooctane

sulfonamidoethanols (FOSEs), fluorobutane sulfonamides (FBSAs), fluoroctane sulfonamides (FOSAs), perfluorobutane sulfonamide (PFBSA), perfluoroctane sulfonamide (PFOSA) and perfluoroctane sulfinate (PFOSi) are degraded to both, perfluorinated carboxylic acids and perfluoroalkane sulfonates in the environment. Although many of these poly- and perfluorinated compounds have been detected throughout the world in surface waters at concentrations in the range of $\text{ng} - \text{mg L}^{-1}$, in ground waters ($\mu\text{g L}^{-1}$), in wastewaters, sediments and sewage sludge, in blood ($\text{ng} - \mu\text{g mL}^{-1}$), human liver (ng g^{-1}), and in the liver and fat of several wildlife species ($\text{ng} - \mu\text{g g}^{-1}$), including Arctic species, perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the two most widely detected compounds [8, 31].

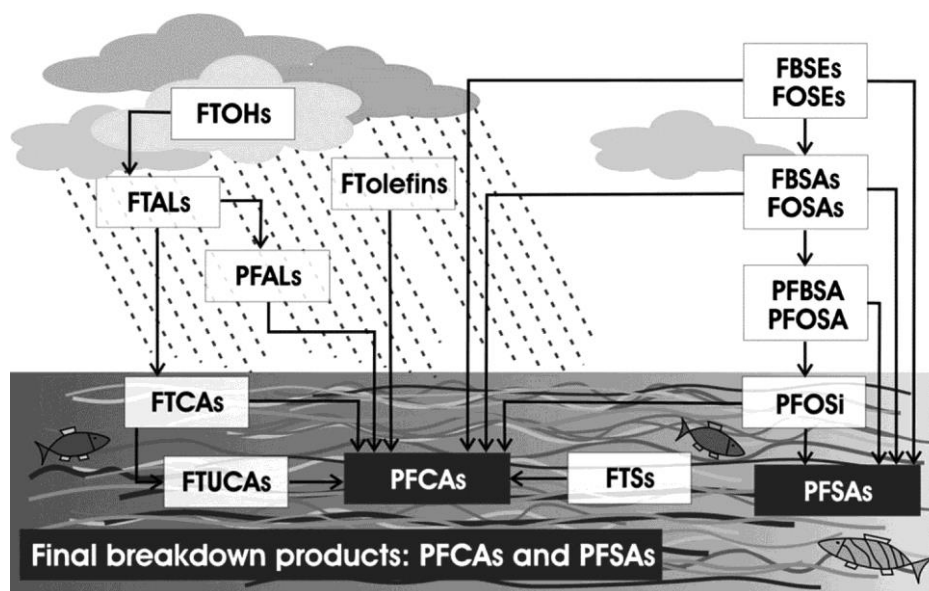


Figure 2.1 Environmental fate of per- and polyfluorinated alkyl precursors [30]

The bioaccumulation of PFCAs, as they move through food webs, has a tendency to increase with the carbon chain length of the perfluorinated acid [32, 33]. Compounds with a perfluoroalkyl chain length (number of carbons with fluorine bounds) ≥ 8 are generally more accumulative than those with ≤ 7 [32, 34]. Therefore, only perfluorinated acids with a longer carbon chain (typically $\text{C}_9 - \text{C}_{15}$) have been found in marine animals [32, 33] and in several species from various locations in the circumpolar region [24]. The functional group has also an effect on bioaccumulation, with a sulfonate being more likely to be retained than a carboxylate of the same size [34]. Generally, the odd numbered PFCAs, as opposed to the even numbered PFCAs, are the predominant environmental pollutants [24, 25].

2.1.5 Toxicity

The consequences of global distribution of PFCAs, involving long-term exposure to humans and living organisms, have still not been well clarified. Although these compounds had been considered to be metabolically inert and therefore non-toxic, indeed, they are biologically active and toxic [7].

Toxicokinetics of various per- and polyfluorinated compounds differ significantly between animal species and even between different genders within given species [5, 35]. It is mainly caused by different renal clearance and may possibly involve an organic ion transporter [14]. In comparison to mice, where the half-life of PFOA is measured in days, the half-lives of PFCAs in humans are much longer, with PFOA between 2 - 4 years depending on the study group and the way of exposure [36, 37]. No difference between human genders has been observed. The relatively long half-lives of PFCAs in humans increase concerns about the potential health effects [5].

Toxicological studies have shown significant metabolic changes in rodent organisms. An increased incidence of liver, pancreas and testicular tumours has been observed in rodents via dietary intake of PFOA [38]. An activation of a peroxisome proliferator-activated receptor α has been proposed as a mechanism for tumour induction and for the immune and hormonal changes seen in rodents [35]. Moreover, PFOA exposure causes liver enlargement as well as changes in lipid metabolism and liver enzymes [35, 39, 40]. The lowest doses at which these effects have been observed appear to be several orders of magnitude higher than human doses from drinking water contaminated at a level of 1 ng mL^{-1} [41].

In pregnant mice, a neonatal mortality and reduced growth for the surviving pups have been shown, when either PFOS or PFOA were dosed [35]. Furthermore, PFOA decreases the B-cell and T-cell immune responses in mice, while in rats an atrophy of the spleen and thymus, hepatomegaly and decrease of cholesterol level has been associated with the PFOA exposure [39, 40]. In rats, PFOA is easily absorbed via the gastrointestinal tract, binds to serum albumin and is excreted primarily from the kidney [35, 39, 41].

Most people tested in the USA have PFOA in their blood serum with a median of 4 ng mL^{-1} [41]. In comparison to the levels of PFOA found in the workers, the levels of PFOA in the general population are much lower [41, 42]. Perfluorooctanoic acid is not metabolized in the human body. High levels of PFOA are usually found in the liver and plasma, while levels in adipose tissue are low due to its lipophobic character [14]. It is extracted from human body in both, urine and excrement.

While the toxicity of PFOS and PFOA has been documented in animal studies, the potential health effects in humans show inconsistent results [5]. It is difficult to determine either positive or negative conclusive results due to small sample populations, uncertainty of historical exposure levels of PFCAs and the possibility that tested individuals had have simultaneous exposures to other compounds. All these factors complicate evaluations and therefore the data on the human health effects of PFCAs are sparse [41].

There is relatively consistent evidence of modest positive associations with cholesterol and uric acid, although the magnitude of the cholesterol effect is inconsistent across different exposure levels [41]. Many studies also showed positive relationship of PFOA with other lipids, such as low density lipoproteins and triglycerides [14, 41]. On the contrary, a significant negative correlation between PFOA and high density lipoproteins was noted.

Besides that, a correlation between PFOS and PFOA and decreased sperm count was observed [43]. Higher blood levels of PFOS and PFOA were related to current thyroid disease [44] and a negative association between PFOS and PFOA with birth size and weight was published [45, 46].

The toxicokinetics of PFCAs with longer carbon chains has not been well clarified in human body, while in the laboratory animals, the time of elimination has generally been observed to increase in proportion to compound chain length [47].

2.2 Analytical chemistry of poly- and perfluorinated compounds

2.2.1 Analytical standards

Some chemical standards are available by several manufacturers, although they can considerably vary in purity and isomeric profiles, which may lead to systematic errors [48]. Besides that, many standards do not exist or are difficult to obtain. The impurities are not well documented, but perfluoroalkyl standards commonly contain various short-chain analogues, which contribute to a negative bias when mixed standard solutions are used in quantification. The isomeric composition of these standards may also vary as a result of the production process used, either electrochemical fluorination or telomerization [13, 15]. Electrochemical fluorination yields many branched isomers, whereas telomerized products are predominantly linear. To avoid a false-positive detection or overestimated concentration, more than one product ion should be therefore monitored per analyte.

Moreover, perfluoroalkylated isomers may play an important role in toxicological and

environmental aspects [48]. Many environmentally relevant physical and chemical coefficients, including bioaccumulation potential, octanol-water partition constant (K_{ow}), vapour pressure and water solubility, are expected to be affected by perfluoroalkyl branching, which can lead to significantly different transport and partitioning behaviour of these isomers in the environment.

2.2.2 Internal standards

Isotopically labelled perfluoroalkyl internal standards such as ^{13}C -labelled or deuterium-labelled standards added before analysis may help improve the quality assurance of the analysis of poly- and perfluorinated alkyl substances [8], because they will elute from the chromatographic column at the same retention times as their non-labelled analogues [48, 49]. The mass enrichment should be more than 1 amu to minimize the overlap with non-labelled isotopes [48]. Besides that, isotopically labelled standards can be added early in the sampling or sample preparation process as surrogates to elucidate any problems caused by the extraction and purification procedures [50].

On the contrary, these standards are of limited availability, due to the cost of their synthesis [48] and even in these labelled standards traces of other PFCAs can be found [51]. Furthermore, some of ^{13}C -labelled standards may not be optimal for certain matrices, and therefore a careful selection of the internal standards for each type of matrix may be required [27]. For non-environmental samples, one perfluorinated acid may be used as an internal standard for the analysis of another perfluorinated acid [48]. Perfluoroheptanoic acid (PFHpA) is not a pre-dominant contaminant in the food web and therefore it may be a suitable internal standard for the analysis of other PFCAs in biological samples [24].

2.2.3 Contamination sources

Contamination sources of perfluoroalkyl substances in the laboratories have not yet been well characterized [8, 48]. Two distinct sources of contamination, instrumental and procedural, are expected to occur [8]. Fluoropolymers, such as polytetrafluoroethylene, are one known source of procedural contamination due to their presence in various common laboratory equipments [8, 48]. Wherever possible, contact with fluoropolymers during analysis should be avoided, because PFCAs are used as polymerization aids in their manufacture [48, 52]. Several experiments have been performed to evaluate the contamination

by fluorochemicals in procedural and instrumental blanks [52, 53]. Yamashita and co-workers have examined the source of blank contamination including sample collection, extraction and treatment of samples [52]. Two different solid-phase extraction (SPE) cartridges, Oasis HLB and Sep-Pak (C18), have been tested with Sep-Pak (C18) containing notable amounts of PFOA and PFOS. In the case of the Oasis HLB cartridges, PFOS, PFOA and two other fluorinated compounds have been detected, but at lower concentrations than those found in Sep-Pak (C18). Three different types of purified reagent water as well as nylon syringe filters have been tested. Traces of PFOA have been found in all three filters, but this contamination has been eliminated by washing the filters prior to filtration. Taniyasu and co-workers detected PFOS, PFOA, PFDA and PFUnA in the procedural blanks at the level of a few pg mL^{-1} in the final extracts [53].

Moreover, a post-injection contamination on HPLC systems has been consistently reported owing to internal fluoropolymer parts [48]. The potential sources of instrumental contamination from the different parts of the HPLC-MS/MS instrument have been investigated [52]. The HPLC tubing, internal fluoropolymer parts, solvent degassers and autosampler vial septum have been identified as the potential sources of fluorinated alkyl substances [50, 52]. The instrumental background can be reduced by replacing the fluoropolymer parts with alternate materials or by extensively flushing the LC system.

2.3 Processing of solid matrices

Despite the existence of studies reporting the occurrence of perfluorinated compounds in biological materials, only few reports have been published on the analysis of perfluorinated compounds in abiotic solid materials [6]. The molecular properties of PFCAs are a limiting factor for determination of these compounds in abiotic matrices. Therefore reliable techniques for sample pre-treatment, extraction and clean-up are required for the analysis of ionic and non-ionic PFCAs in environmental matrices. It is necessary to avoid contamination (e.g. from sample bottles, filters and laboratory equipment) and losses of perfluorinated compounds (e.g. adsorption, volatilization) during sampling, extraction and clean-up strategies [54].

2.3.1 Sampling

Sampling of sediments, soil and sewage sludge requires special precautions, including avoiding fluoropolymer materials [6]. Besides that, sediments may show vertical

heterogeneous distribution over distances of only a few cm. It is caused by the tendency of surfactants to preferentially sorb to interfaces. Moreover, a vertical distribution of sediments may also indicate historical exposure, as the layers have been deposited with settling particles over the years. Due to the heterogeneous distribution in sediments, a sampling of large areas as well as mixing of sediment samples, before an aliquot is taken, is necessary.

2.3.2 Storage of samples and conservation

During storage and conservation of samples for PFCAs analysis, losses and contamination can easily occur [48, 54]. It is necessary to check sample bottles for contamination, e.g. pre-cleaning them prior to sampling by rinsing with semi-polar or polar solvents such as deionized water, acetone, methanol or methyl *tert*-butyl ether [54, 55]. Adsorption of ionic perfluorinated compounds, mainly PFOS, to glass surfaces has been discussed [48, 55, 56], although it is not expected that this will happen in samples containing large amounts of matrix components [54]. Nevertheless most laboratories have switched to using polypropylene [48]. Although PFCAs will not degrade under any reasonable storage conditions, it is always preferred to analyze samples directly after sampling or alternatively stored them in refrigerators or freezers at -20°C until analysis to avoid possible degradation of precursors to perfluorinated acids [48, 54].

2.3.3 Sample pre-treatment

The aims of extraction and clean-up are firstly to transfer the analytes to the physical state that enables the analysis and final detection, secondly to enrich the analytes of interest and finally to purify the extract prior to instrumental detection [54].

A solid-liquid extraction is the usually applied extraction technique for solid matrices such as soil, sediment and sludge [54]. Furthermore, SPE of fluid samples as well as pressurized liquid extraction (PLE) has been applied for these matrices. The ionic PFCAs are usually extracted by moderate polar media (Oasis Wax SPE or methanol and acetonitrile) for efficiently trapping of water soluble short chain PFCAs (C_4 - C_6). For extraction of PFCAs with longer chain, less polar or non-polar SPE phases (C_{18} and Oasis HLB) can be used. The ion-pairing extraction of PFCAs can be applied as well. An ion-pairing agent decreases the polarity of the ion pair complex and therefore a non-polar solvent such as methyl *tert*-butyl ether may be used for extraction. The non-ionic PFCAs may be extracted from matrix

either by non-polar media (C_{18} SPE or hexane) or by moderately polar media (Oasis HLB, Oasis WAX SPE, hexane-acetone mixture or acetonitrile).

Further clean-up of crude extracts is essential for destruction and removal of co-extractive compounds that can either suppress or enhance ionization, resulting in considerable inaccuracies [48]. Clean-up methods are relatively simple and straightforward, involving normal phase adsorption chromatography with fluoros silica adsorbent or C_{18} materials in SPE set-up. Alternatively dispersive graphitized carbon adsorbent can be applied or destructive methods such as sulphuric acid treatment [6, 48, 54].

Finally, samples may be filtrated to remove solids from the final analysing extract, although filtration can result in losses by adsorption of perfluorinated surfactants on the filters [54]. On the contrary, Schultz and co-workers observed contamination of samples originating from four types of filters (namely glass fiber, nylon, cellulose acetate and polyethersulfone filters) [57]. A centrifugation as alternative for separation the liquid from solids can be applied. A summary of various sample pre-treatment techniques, extraction procedures and clean-up strategies for the determination of PFCAs in solid matrices is shown in Figure 2.2.

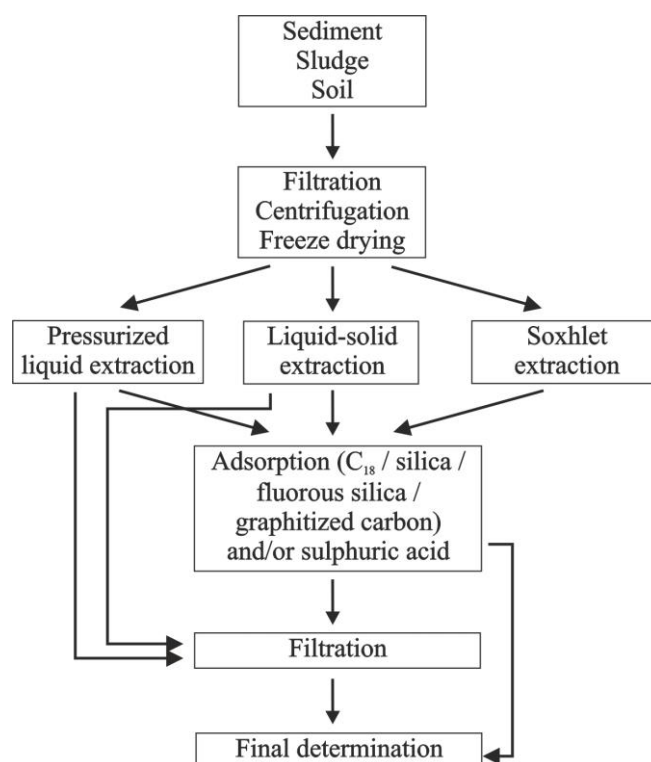


Figure 2.2 Extraction and clean-up techniques for the analysis of PFCAs in solid matrices

2.4. Instrumental analysis

In the following subchapters two analytical techniques are generally discussed, liquid chromatography (LC) and gas chromatography (GC) coupled to various detectors and mass analyzers, commonly used for determination of poly- and perfluorinated alkyl substances in environmental or non-environmental matrices. The other analytical techniques, such as ^{19}F nuclear magnetic resonance spectroscopy, which determines the presence of CF_2 and CF_3 moieties in the sample, combustion ion chromatography, neutron activation, X-ray fluorescence or radiochemical methods, have also been applied for the analysis of poly- and perfluorinated compounds, although these techniques are nonspecific and may yield erroneous quantification results [58] and thus are not discussed in this Diploma thesis.

2.4.1 Liquid chromatography – mass spectrometry

A preferred and most frequently used instrumental method for the determination of anionic perfluorinated compounds such as perfluoroalkane sulfonates and PFCAs is high-performance liquid chromatography coupled to triple-quadrupole tandem mass spectrometry applying negative electrospray ionization interface (HPLC/(-)ESI-MS/MS) [8, 30, 48]. It is due to its sensitivity, selectivity and applicability to the analysis of all classes of fluorinated alkyl substances without necessary derivatization [8]. Liquid chromatography with single-quadrupole MS is also often used, although requires more thorough clean-up of the sample in order to remove interferences [6, 48] and shows lower selectivity, particularly for PFOS analysis where known mass interferences exist [48]. According to literature, liquid chromatography separations are mainly carried out on C_{18} and C_8 columns [8].

The pseudo-molecular ion $[\text{M}-\text{H}]^-$ is commonly used for quantification in LC-single-quadrupole MS systems, whereas in LC-MS/MS can serve as the parent ion for multiple ion reactions [6]. Under collision-induced dissociation conditions, the CO_2 is readily lost from the molecular anion, forming the perfluorinated anion $\text{CF}_3(\text{CF}_2)_n^-$, which can subsequently lose CF_2 groups [50].

For the determination of poly- and perfluorinated alkyl substances, liquid chromatography does not need to be necessarily coupled to quadrupole mass analyzers, but also to time-of-flight mass analyzers (TOF) or ion-trap mass analyzers. In comparison to TOF mass analyzers, triple-quadrupole MS/MS analyzers have higher sensitivity and linear range, while TOF systems prove to be instruments of the choice for the identification of poly- and

perfluorinated alkyl substances in the environmental matrices [24, 56]. Ion trap mass-analyzers, which perform MS^n experiments, can be useful for environmental monitoring of PFCAs because collision-induced dissociation results in decarboxylation [48]. However, due to large mass difference between the parent ion (m/z 499) and the product ion (m/z 99) of PFOS, these analyzers cannot be used for MS/MS analysis of this compound [48].

Recently, a liquid chromatography coupled to three different MS techniques, specifically ion-trap MS, triple-quadrupole MS and high-resolution TOF-MS, has been tested and compared by Berger and co-workers [59]. The electrospray ionization (ESI) has been evaluated as the most suitable interface to the analysis of poly- and perfluorinated substances for all three LC-MS techniques. The best results for qualitative interpretation and identification of branched isomers have been obtained by ion-trap MS, whereas triple-quadrupole MS has been best suited for quantitative analysis of fluorotelomer alcohols with limits of detection (LOD) in the low pg range and well suited for analysis of other poly- and perfluorinated compounds (LODs of 10 - 100 pg). According to Berger's study, LC-TOF-MS has been selected to be the optimal quantitative method for these compounds, combining high selectivity with high sensitivity (LODs of 2 - 10 pg). However, due to the low distribution of this type of instrument in analytical laboratories, quadrupole MS/MS is still most frequently used [30, 48].

2.4.2 Liquid chromatography coupled to conventional detectors

Except from MS analyzers, several conventional detectors have also been applied in combination with LC, e.g. conductimetric detector [60, 61] or fluorescence detector, which can be employed only after derivatization of poly- and perfluorinated samples [6, 7], because of the general absence of fluorophores in these compounds. Due to the fact that most perfluorinated surfactants do not contain chromophores, UV detection also does not provide sufficient selectivity or sensitivity [8].

2.4.3 Matrix suppression and enhancement

Although HPLC/(-)ESI-MS/MS demonstrates highly sensitive and specific method for the determination of PFCAs in a wide variety of matrixes without the need of chemical derivatization, the quantitative results can be often adversely affected by a matrix suppression or a matrix enhancement, if unpurified samples of soil, sludge and sediment are

analyzed [43]. The ionization efficiency of the analyte can be affected by co-eluting peaks either positively or negatively, depending on the nature and concentration of the co-elutants [50]. When co-eluting matrix components compete with the analyte for charge, thereby reducing the number of gas-phase ions available for detection, the matrix suppression occurs in the electrospray interface [62]. Conversely, if a matrix component facilitates the ionization process (e.g. by reducing surface tension) an enhancement is obtained [62]. Using isotopically labelled internal standards and matrix-matched standards is one possible alternative or when matrix effects are unavoidable, standard addition quantification, involving spiking successive known quantities of a standard and reanalyzing, is another available option [48]. Atmospheric pressure chemical ionization (APCI) is less prone to matrix effects than ESI because ionization takes place in the gas phase [8]. However, the range of compounds that can be analyzed by APCI is shorter than in ESI, thus this interface has found less application in environmental analysis [8, 23]. Takino and co-workers developed a method based on the use of atmospheric pressure photoionization [63]. The best advantage of this technique was the absence of matrix effects, however, the detection limits achieved were considerably higher than those obtained by HPLC/(-)ESI-MS/MS.

2.4.4 Gas chromatography coupled to conventional detectors

Neutral and volatile per- and polyfluorinated alkyl compounds such as sulfonamides, fluorotelomer alcohols and olefins are usually analysed directly by gas chromatography techniques, because of their high vapour pressures, typically up to several hundred Pa [6]. In contrast, perfluorinated carboxylic acids need to be firstly derivatized in order to be amenable to GC analysis.

Gas chromatography coupled to electron capture detector (ECD) has been published for determination of selected PFCAs in biological samples after derivatization with diazomethane [64, 65]. Due to the high amount of fluorine atoms in these compounds, the electron capture detector seems to be an obvious detector for GC analysis. However, in the case of complex matrices, the specificity of this detector may be insufficient. Co-eluting analytes may disproportionately impact the amplitude of the detector response [50]. Alternatively to ECD detector, a helium-specific microwave plasma detector has been tested [29] or a combination of GC with a flame ionization detector has also been applied to analysis of polyfluorinated alkyl substances after conversion to their benzyl esters [66].

2.4.5 Gas chromatography – mass spectrometry

Gas chromatography coupled to mass spectrometry, either in combination with electron impact (EI) or chemical ionization (CI), represents a predominant GC technique for determination of poly- and perfluorinated substances. The advantage of electron impact is the applicability of mass spectral libraries, whereas chemical ionization is a softer and more sensitive ionization technique [30, 48]. According to the positive or negative ion mode, the chemical ionization shows the pseudo-molecular ion $[M+H]^+$ or $[M-H]^-$. Moody and Field determined methylated PFCAs in groundwater after derivatization with methyl iodide using GC-EI-MS [11], whereas Alzaga analyzed butyl esters of PFCAs in effluents from wastewater treatment plants by GC-NCI-MS [67]. Scott and co-workers determined PFCAs after their conversion to 2,4-difluoroanilides in surface waters and precipitation by GC-MS and compared results with LC-MS/MS analysis [68]. The obtained results were nearly identical with low standard deviation.

The determination of PFCAs by gas chromatography coupled to quadrupole mass spectrometry (GC-MS) after a derivatization step offers the advantage of absence of instrumental blank contamination and better separation power compared to HPLC instrumentation [30]. On the contrary, GC methods require multiple, not easily automated steps and lengthy sample preparation, which makes these methods subject to error [50]. In principle, limits of detection of LC-MS, LC-MS/MS and GC-MS analytical methods are sufficiently low to allow determination of environmental levels of poly- and perfluorinated alkyl substances in abiotic and biotic samples [6]. Generally, detection limits vary between methods but they are in the pg or low ng L⁻¹ range in the case of water samples, in the pg – ng mL⁻¹ range in the case of biological samples, and in pg – ng g⁻¹ in the case of solid environmental samples [8].

2.5 Determination in solid matrices

Selected publications of analytical methods determining PFCAs in abiotic matrices such as soil, sludge and sediment are reported in the following chapter. This short overview focuses on the various pre-treatment techniques used and differences between them, whereas the determination is usually carried out by liquid chromatography coupled to mass spectrometry.

2.5.1 Soil

A 'matrix effect-free' analytical method for determination of C₆-C₁₄ PFCAs in soil, sediment and sludge with limits of quantification (LOQ) of 1 ng g⁻¹ has been published by Powley and co-workers [24]. Method consisting of dissolving samples in 200 mmol L⁻¹ NaOH, followed by solid-liquid extraction with methanol, dispersive clean-up using graphitized carbon adsorbent (Envi-Carb) and LC-MS/MS analysis have negligible matrix enhancement or suppression due to effective removal of matrix constituents by the graphitized carbon, which does not retain perfluorinated compounds. The method recoveries for all PFCAs are in range from 75 to 120 % [49].

Washington and co-workers reported on extensive experiments with alkaline soil pre-treatment (sodium hydroxide vs. sodium carbonate) and extraction (sonication in acetonitrile-water vs. ion pair extraction with methyl *tert*-butyl ether). Their final method included NaOH pre-treatment, extraction with acetonitrile-water (3:2) by sonication and an ion-pair extraction clean-up step prior to LC-MS/MS analysis [69, 70].

2.5.2 Sediment and sludge

A method based on the acidic extraction of ionic and non-ionic perfluorinated compounds from sediment and sludge has been applied by Higgins and co-workers. The method included a washing step with acetic acid followed by repeated extractions of the acidified sediments using a solvent mixture of methanol-water (9:1) and 0.1 % acetic acid. Thereafter, a clean-up step was performed using C₁₈-SPE cartridge to concentrate the extracts and remove the acetic acid, salts and potential matrix interferences [71]. Finally, the extracts were analyzed by LC-MS/MS. For the perfluorinated carboxylic acids, [¹³C₂]-PFOA was selected as an internal standard. The obtained LODs were from 0.011 to 2.2 ng g⁻¹ (dry weight) and the method recoveries ranged from 73 - 98 % (dry sediment) and 41 - 91 % (digested sludge).

Schröder compared three different techniques, i.e. Soxhlet extraction, hot vapour extraction and pressurised liquid extraction, for the extraction of per- and polyfluorinated alkyl substances from sewage sludge. The sequential pressurized liquid extraction using ethyl acetate-dimethylformamide (8:2) and methanol-phosphoric acid (99:1) followed by LC-MS detection provided the most efficient analyte extraction, whereas Soxhlet and hot vapour extraction were found to be relatively inefficient [23]. The sewage sludge extracts were volume-reduced and analyzed by LC-MS without prior clean-up. Moreover, Schröder also

investigated the possibility of using flow injection analysis with mass spectrometry (FIA-MS) instead of LC-MS. However, the application of FIA-MS failed because of the high load of co-extracted matrix components. As a result, the whole method was not sensitive enough to find any of the fluorinated surfactants investigated at concentrations higher than the corresponding limits of detection ($6 \mu\text{g g}^{-1}$ dry residue for PFOA) in any of the 80 real sludge samples analyzed.

Alzaga and co-workers tested pressurized liquid extraction with different solvents, reporting the best results with acetone-methanol (1:3) for extraction of PFCAs from harbour sediments [72]. Extraction efficiencies increased with increasing chain length (C_7 – C_{10} from 70 to 100 %). A derivatization step followed by headspace solid-phase micro-extraction combined with GC-NCI-MS was employed obtaining LODs from 0.5 ng g^{-1} to 0.8 ng g^{-1} . The NCI mode was chosen due to its selectivity and sensitivity for the PFCAs alkyl ester derivatives. Quantification was based on the sum of the following m/z : $[\text{M}]^-$, $[\text{M-HF}]^-$, $[\text{M-OC}_4\text{H}_9\text{F}]^-$, $[\text{M-O}_2\text{C}_5\text{H}_9\text{F}]^-$ and $[\text{M-O}_2\text{C}_5\text{H}_9\text{F}_3]^-$. The main disadvantages of this analytical method were the necessity of having a derivatization step and the low recoveries observed for a short carbon chain PFCAs ($\text{C} < 8$). It is, however, not likely that these polar short chains sorb to sediments to a large degree.

3. EXPERIMENTAL PART

3.1 Chemicals and reagents

The following substances were used as the analytical standards: perfluorohexanoic acid (>97 %, Fluka, Germany), perfluoroheptanoic acid (99 %, Sigma-Aldrich, Germany), perfluorooctanoic acid (96 %, Sigma-Aldrich, Germany), perfluorononanoic acid (97 %, Sigma-Aldrich, Germany), perfluorodecanoic acid (98 %, Sigma-Aldrich, Germany), perfluoroundecanoic acid (95 % Sigma-Aldrich, Germany) and perfluorododecanoic acid (95 % Sigma-Aldrich, Germany). As an internal standard a solution of perfluoro-*n*-[1,2-¹³C₂]-octanoic acid (>98 %, ≥99 % ¹³C, >99 % linear, 1.2 mL, Wellington Laboratories, Ontario, Canada) in methanol at a concentration of 50 ± 2.5 µg mL⁻¹ was used.

All the other chemicals, acetonitrile (≥99.5 % Sigma-Aldrich, Germany), *n*-hexane (≥97 %, Sigma-Aldrich, Germany), methanol (≥99.9 %, Sigma-Aldrich, Germany), isobutyl chloroformate (98 %, Sigma-Aldrich, Germany), methyl chloroformate (99.8 %, Sigma-Aldrich, Germany), ethyl chloroformate (97 %, Sigma-Aldrich, Germany), pyridine (99+ %, Sigma-Aldrich, Germany), 2-methyl-1-propanol (isobutyl alcohol, p.a., Penta, Chrudim, Czech Republic), sodium hydroxide (p.a., Penta, Chrudim, Czech Republic), hydrochloric acid (35 %, p.a., Lach-Ner, Neratovice, Czech Republic) and deionized water (18 MΩ, Millipore, USA) were used as received.

3.2 Instruments

To determinate a homological scale of seven perfluorinated carboxylic acids, the gas chromatography – mass spectrometry analyses with negative chemical ionization (GC-NCI-MS) were performed using a GCMS-QP2010 Plus Shimadzu instrument (Analytical and Measuring Instruments Division, Kyoto, Japan), equipped with 20 m length, 0.15 mm internal diameter and 0.15 µm film thickness Rtx-®-200 MS column (trifluoropropylmethyl polysiloxane, Restek, USA). As a carrier gas was used helium (99.999 %, Linde, Czech Republic) and methane was used as a reagent gas (99.9995 %, Linde, Czech Republic).

The splitless-mode injection was employed. The injection temperature was 250 °C and the volume of samples injected onto the chromatographic column was 1 µL. The sampling time was 0.60 min.

3.2.1 Temperature program

In the Bachelor thesis the chromatographic column Rtx-®-200 MS (trifluoropropylmethyl polysiloxane, Restek, USA), with the following parameters: 30 m length, 0.25 mm internal diameter and 0.25 μm film thickness, was used [2]. The temperature program, applied for determination of perfluorinated carboxylic acids in the Bachelor thesis, has been recalculated according to the current chromatographic column parameters. For recalculation of the temperature gradients the following theoretical formula has been employed:

$$(\text{temperature ramp})_{\text{new}} = (\text{temperature ramp})_{\text{old}} \frac{L_{\text{old}} \bar{u}_{\text{new}} \beta_{\text{new}}}{L_{\text{new}} \bar{u}_{\text{old}} \beta_{\text{old}}} \quad (3.1)$$

where L_o and L_n is a length of the chromatographic column used [m], \bar{u}_o and \bar{u}_n express a linear flow velocity of the carrier gas in the chromatographic column [cm s^{-1}], whereas β_o and β_n is a phase ratio of the chromatographic column.

The following temperature program for separation of perfluorinated carboxylic acids has been obtained. The initial column oven temperature, 40 $^{\circ}\text{C}$, was maintained for 3 min then the first temperature gradient 21 $^{\circ}\text{C min}^{-1}$ to 150 $^{\circ}\text{C}$ was employed. After reaching 150 $^{\circ}\text{C}$, the second temperature gradient 62 $^{\circ}\text{C min}^{-1}$ to 200 $^{\circ}\text{C}$ was employed. The final temperature, 200 $^{\circ}\text{C}$, was hold for 2 min. The total analysis time was 11.04 min. The linear flow velocity of the carrier gas has been set constantly at 50 cm s^{-1} and the other instrumental parameters have been recalculated according to the linear flow velocity value.

3.2.2 Detection conditions

Since the sample injection the mass spectrometer was out of detection for 4 min due to the elution of the dissolving agent (hexane). The MS transfer line was maintained at 210 $^{\circ}\text{C}$. The temperature of ion source was 200 $^{\circ}\text{C}$. The detector voltage was relative to the tuning result. The mass spectrometer was operated in selected ion monitoring (SIM) at specific mass-to-charge values (m/z) for each perfluorinated acid. All monitored m/z values were applied for quantification of individual perfluorinated carboxylic acids and are listed in Table 3.1. The predominant m/z values for each perfluorinated carboxylic acid are: 278 (PFHxA), 328 (PFHpA), 378 (PFOA), 380 (1,2- $^{13}\text{C}_2$ PFOA), 428 (PFNA), 478 (PFDA), 528 (PFUnA) and 578 (PFDoA).

Table 3.1 The specific m/z values monitored for each PFCA including the retention time (*t*) of PFCAs

PFCA	Time window monitored min	<i>t</i> min	m/z monitored
PFHxA	4.00 – 5.10	4.781	278, 350, 250, 307, 297, 294
PFHpA	5.10 – 5.75	5.364	328, 300, 400, 357, 262, 344
1,2- ¹³ C ₂ PFOA	5.75 – 6.25	5.906	380, 351, 313, 452, 409, 211
PFOA	5.75 – 6.25	5.908	378, 350, 312, 450, 407, 209
PFNA	6.25 – 6.75	6.403	428, 400, 281, 362, 209, 500
PFDA	6.75 – 7.10	6.861	478, 450, 412, 281, 209
PFUnA	7.10 – 7.50	7.287	528, 462, 500, 331, 209
PFDoA	7.50 – 11.04	7.687	578, 512, 550, 209, 331

3.2.3 Other laboratory equipment

In solid-liquid extraction procedure an orbital shaker (Vibramax 100, Heidolph Instruments) was applied. To remove soils from the liquid extracts a centrifuge AllegraTM X-22R (Beckman CoulterTM) was used in the co-operation with the Department of Biochemistry, Charles University in Prague.

A clean-up of extracted samples was performed in the solid-phase extraction (SPE) vacuum manifold (Visiprep, Supelco) using Supelco SupelcleanTM ENVITM-Carb 3 mL SPE tubes with 0.25 g graphitized carbon adsorbent as a stationary phase, purchased from Supelco, Sigma-Aldrich, Germany. To evaporate extracts to dryness a thermostat (Start, SBH 130 DC) was used. In the derivatization step a shaker (Vortexgenie 2, Scientific Industries) was applied.

3.3 Operation procedure

3.1.1 Preparation of stock standard solutions

The stock standard solutions of seven perfluorinated carboxylic acids were prepared with a concentration of 1 mg mL⁻¹ into 10 mL glass measuring flasks. Each stock solution was prepared by dissolving weighed 10.0 mg of particular perfluorinated carboxylic acid in acetonitrile. Subsequently the standard solutions were quantitatively transferred into 15 mL polypropylene screw-up tubes and stored until analysis in refrigerator at 4 °C.

3.3.2 Collection of soil samples

The reference soil material was collected from the Botanical Garden of the Charles University in Prague. This soil was set to be an appropriate solid material for the optimization of the whole solid-liquid extraction procedure followed by SPE clean-up step since it should not be contaminated by the perfluorinated carboxylic acids. In March 2013, fresh samples were acquired from A-horizon surface soil using a polypropylene paddle and collected into three polypropylene self-locked packages. Each polypropylene package contained approximately 2 kg moist solid material. After collection, samples were delivered to the laboratory and stored in a freezer until processing.

In April 2014, samples from different geographical locations were acquired. The first location was nature reserve Černínovsko, in the close distance of Spolana Neratovice, one of the biggest chemical companies within Czech industrial landscape. Soil samples from Kralupy nad Vltavou with the location of petrochemical industrial company Česká rafinérská a.s. and sample from the bank of Vltava river downstream from Trója, the Central Wastewater Cleaning Plant in Prague, were acquired as well. After floods of the Elbe and Vltava rivers in 2013, it has been expected that PFCAs could be present in these locations. To demonstrate a potential distribution of PFCAs in soils, samples at two sites within 0.5 km air distance were acquired from nature reserve Černínovsko and within 3 km air distance from Kralupy nad Vltavou. Samples from all locations were collected from A-horizon surface soil into polypropylene self-locked packages, transferred to the laboratory and immediately processed.

Other soil samples have been obtained after sampling, specifically samples from Zadní Kopanina and Zbraslav (both former military areas), Evropská street in Prague (the building of tunnel Blanka in progress) and from Radotín (a cement factory). As soon as it was possible, these samples were transferred to the laboratory and processed.

3.3.3 Processing of soil samples

Soil samples in corresponding polypropylene packages were defrosted at the laboratory temperature. Afterwards, they were placed in porcelain and metal bowls and dried at 50 °C in drier for 24 hours. Soil in the bowls was continuously stirred with a stainless steel spatula to get the moist content on the top and accelerate the drying.

All dried samples were passed through a system of cleaned #5.0 mesh, #2.8 mesh and #1.0

mesh steel sieves by mechanical shaking. Material not passing through the sieves was discarded. Soil passing through the last 1 mesh sieve was stored in 500 mL glass bottles at the laboratory temperature. After sieving no more homogenization of the soil samples was processed. Fluoropolymer materials were avoided throughout the pre-treatment and analysis as interferences may be introduced to the samples. Thus processed soil samples were extracted with methanol, cleaned-up using SPE, derivatized and analyzed by GC-NCI-MS.

3.3.4 Composition of the reference soil material

The reference soil material was analyzed by roentgen diffraction in collaboration with the Geological Institute of the Faculty of Science, Charles University in Prague. The most occurring crystalline phase was quartz. Secondary, there were present an albite, a microcline, a muscovite and a hornblende. They are remnants of erosion, specifically minerals descending from weathered parent rock. Semi-quantitative contents of these crystalline phases, expressed in percentage, are listed in Table 3.2. The high content of microcline and muscovite is based on the coincident signals during analysis, in fact these two crystalline phases quantitatively correspond to the content of albite.

Apart from crystalline phases, phases emerging in soil occurred as well, namely a calcite, a kaolinite and a dolomite. A complete laboratory report of the roentgen diffraction analysis of reference soil material, including the X-ray spectrum, is available in Appendix of this Diploma thesis.

Table 3.2 A semi-quantitative content of particular minerals found by roentgen diffraction in soil collected from the Botanical Garden of the Charles University in Prague

Minerals	Score	Total Lines	Scale Factor	Semi-quantitative content %
Quartz	62	12	0.988	32
Albite	36	52	0.032	3
Dolomite, ferroan	23	13	0.004	< 1
Calcite	23	12	0.015	< 1
Muscovite	24	95	0.156	35
Microcline	17	120	0.145	27
Hornblende	7	97	0.009	1
Kaolinite	10	21	0.021	1

3.3.5 Solid-liquid extraction and clean-up procedure of soil samples

An aliquot of 5.0 g dry soil was weighed in a clean 50 mL polypropylene Falcon tube and 20 mL of methanol as an extraction agent were added. The extraction mixture was shaken in an orbital shaker (Vibramax 100, Heidolph Instruments) for 20 min at 300 rpm and then sonicated in a water bath for 40 min (37 Hz, 100 % frequency). To separate soil from the liquid extract, sample was centrifuged for 15 min at 4500 rpm corresponding to 3901 g (Allegra™ X-22R, Beckman Coulter™). The methanolic supernatant was subjected to SPE clean-up using Supelco Supelclean™ ENVI™-Carb 3 mL cartridges (0.25g graphitized carbon adsorbent). The SPE cartridge was placed in a vacuum manifold (Visiprep, Supelco) and pre-conditioned by passage of 13 mL of methanol at a flow rate of 1 to 2 drops per second and vacuum of -40 kPa. Afterwards, the entire methanolic extract was passed through the SPE cartridge using polypropylene tubing, at a rate of 1 to 2 drops per second and vacuum of -30 kPa, and was collected in a clean 50 mL polypropylene Falcon tube. Subsequently, the methanolic extract was transferred into a clean 15 mL polypropylene screw-up tube and concentrated under nitrogen or air stream at 64 °C to dryness. Finally, the sample was reconstituted in 625 µL of acetonitrile containing 1,2-¹³C₂-PFOA as an internal standard (140 µL of 1,2-¹³C₂-PFOA in acetonitrile at a concentration of 5 µg mL⁻¹ were added to 485 µL of acetonitrile, thus obtaining the volume of 625 µL) and shaken for 1 min (Vortexgenie 2, Scientific Industries). The concentration of 1,2-¹³C₂-PFOA after derivatization was 1 µg mL⁻¹, corresponding to the concentration of internal standard in the calibration dependencies of individual PFCAs. This solid-liquid extraction with methanol followed by SPE clean-up step corresponds to the pre-concentration factor 32 (the original sample volume was 20 mL). The reconstituted sample was divided into three sub-samples of 178 µL, which were subjected to derivatization and determination by gas chromatography – mass spectrometry with negative chemical ionization. Exactly the same solid-liquid extraction procedure followed by SPE clean-up step has been applied to blank samples.

3.3.6 Derivatization procedure

The derivatization process is based on the reaction between particular perfluorinated carboxylic acid and alkyl chloroformate [2, 73]. In the first place, the particular alkyl chloroformate is bound to the acid with the liberation of hydrochloric acid and simultaneous formation of the labile acid anhydride. The acid anhydride decarboxylates rapidly and

provides the ester and carbon dioxide.

Apart from particular PFCA and alkyl chloroformate, pyridine and isobutyl alcohol are present in the reaction mixture as well [73]. Pyridine is used as the catalyst and the isobutyl alcohol sometimes serves as an auxiliary agent. The reaction scheme is shown in Figure 3.1, where R_1 represents the perfluorinated acid carbon chain and R_2 the alkyl chain of chloroformate. Besides the alkyl esters of PFCAs, small amount of several volatile compounds is formed. However, they do not interfere with GC-NCI-MS determination of the alkyl esters of PFCAs.

The possibility of combination this derivatization step with an efficient extraction technique makes it suitable for the determination of PFCAs in abiotic matrices such as water and soil.

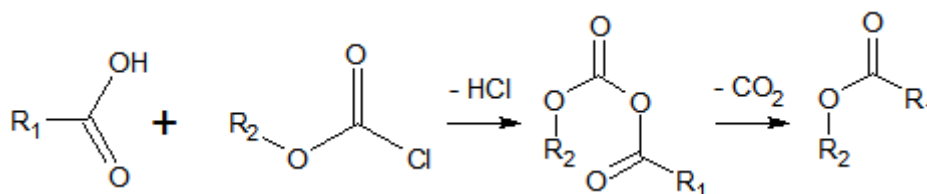


Figure 3.1 The scheme of PFCAs derivatization procedure [2, 73]

After solid-liquid extraction with methanol followed by SPE clean-up, it is necessary to immediately derivatize samples, because eventually degradation and loss of PFCAs may occur. To 178 μL of extracted PFCAs in acetonitrile in 500 μL polypropylene microvial were gradually added 8 μL of isobutyl alcohol, 4 μL of pyridine and 10 μL of alkyl chloroformate. The reaction mixture was stirred for 20 s in an ultrasonic bath, maintained quiet for 8 min and finally 200 μL of hexane were added. The alkyl esters formed were extracted into hexane for 1 min in a shaker (Vortexgenie 2, Scientific Industries) at a shaking velocity between 4 and 5. Subsequently the upper hexane phase was separated into a clean 200 μL polypropylene vial and injected onto the gas chromatographic column. It is supposed, that 100 % of perfluorinated carboxylic acids converted into their alkyl esters is extracted to hexane.

The same procedure was used for the derivatization of the standard stock solutions of PFCAs to obtain calibration dependencies of individual PFCAs.

3.3.7 Calibration dependencies of individual PFCAs

For GC-NCI-MS determination of alkyl esters of PFCAs in soil samples, the calibration curves for individual perfluorinated acids have been obtained in a concentration range from 0.005 to 10 $\mu\text{g mL}^{-1}$. To obtain a calibration dependency for each PFCA, standard stock solutions of PFCAs in acetonitrile at a concentration of 1 mg mL^{-1} were used. Eight calibration points for each PFCA at a concentration of: 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5 and 10 $\mu\text{g mL}^{-1}$ have been prepared by diluting the standard stock solutions of individual PFCAs. A particular volume of each standard stock solution, calculated for each PFCA separately, as well as 224 μL of 1,2- $^{13}\text{C}_2$ -PFOA in acetonitrile was pipetted to 1.5 mL Eppendorf tube and acetonitrile was added to the mixture to obtain final volume of 1 mL. The 178 μL from as follows prepared solutions for each calibration point were subjected to derivatization and GC-NCI-MS analysis. Each calibration point has been measured in triplicates. Calibration curves were constructed to perform linear regression of plots of peak area – internal standard area ratio versus standard concentration of PFCAs.

The internal standard (1,2- $^{13}\text{C}_2$ -PFOA) was purchased from Wellington Laboratories (Ontario, Canada) in methanol at $50 \pm 2.5 \mu\text{g mL}^{-1}$. Therefore, 100 μL of 1,2- $^{13}\text{C}_2$ -PFOA in methanol were pipetted to a glass vial with 900 μL of methanol as well. This solution (1 mL) at a concentration of 5 $\mu\text{g mL}^{-1}$ was evaporated to dryness under nitrogen, reconstituted in 1 mL of acetonitrile and stored until usage in refrigerator at 4 °C. The 224 μL of by this means prepared solution were pipetted to 1.5 mL Eppendorf tube as described above.

3.3.8 Evaluation of experimental data

For evaluation of the chromatographic reports and attainment of chromatographic parameters (e.g. absolute and relative peak areas, S/N ratios) a commercial program GCMS LabSolution 2.70 (Shimadzu Corporation, Japan) was applied. Obtained results were evaluated using programs Origin Pro 8.0 software (OriginLab Corporation, USA) and Microsoft Office Excel 2007 (Microsoft Corporation, USA). The construction and analyses of the experimental design and the response surfaces were carried out using the Minitab 16 statistical package (Minitab Inc., USA).

4. RESULTS AND DISCUSSION

4.1. Optimization of derivatization procedure

According to the Bachelor thesis, the most efficient separation of PFCAs on chromatographic column Rtx-®-200 MS (30 m × 0.25 mm ID, 0.25 μm) was obtained using isobutyl chloroformate in the derivatization procedure. However, isobutyl esters of PFHpA, PFOA and PFNA did not yield symmetrical peaks after separation on the new chromatographic column Rtx-®-200 MS (20 m × 0.15 mm ID, 0.15 μm). A chromatogram of isobutyl esters of PFCAs is shown in Figure 4.1.

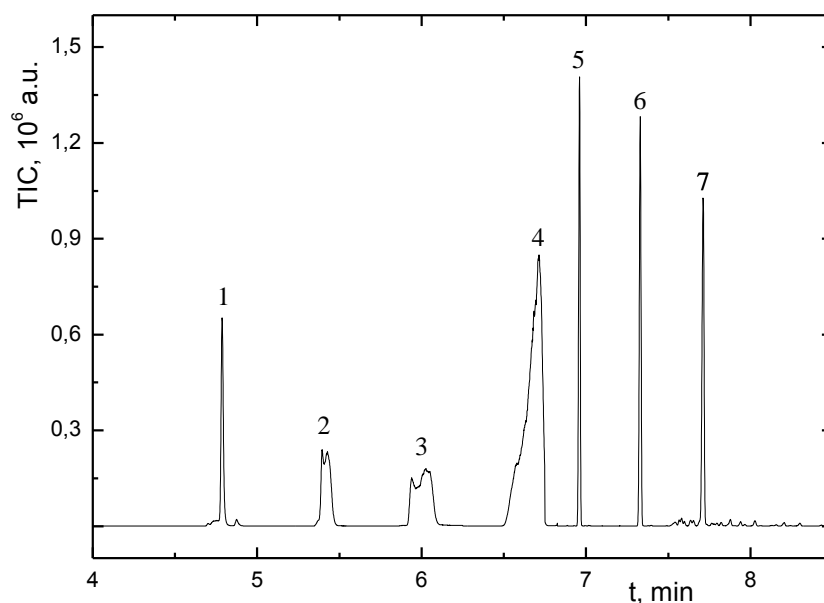


Figure 4.1 A GC-NCI-MS chromatogram of isobutyl esters of PFCAs (5 μg mL⁻¹): (1) PFHxA, (2) PFHpA, (3) PFOA, (4) PFNA, (5) PFDA, (6) PFUnA and (7) PFDoA; Rtx-®-200 MS column (20 m × 0.15 mm ID, 0.15 μm); 1 μL sample injected (splitless, 0.60 min); ion source temperature 200°C; injector temperature 250°C; $p(\text{CH}_4)$ 250 kPa; constant He flow rate 50 cm s⁻¹; temperature program: 40 °C (3 min), 21 °C min⁻¹ to 150°C, 62 °C min⁻¹ to 250°C (2 min)

Therefore, the derivatization procedure has been optimized with ethyl and methyl chloroformate instead of isobutyl chloroformate. A chromatogram of ethyl esters of PFCAs is shown in Figure 4.2. The shape symmetry of problematic peaks (PFHpA, PFOA and PFNA) is considerably better in comparison to isobutyl esters of PFCAs. Nevertheless, there are

significant differences in intensities and corresponding signal-to-noise ratios (S/N) among particular PFCAs. While the chromatographic peak of PFHpA is extremely high, the intensity of PFHxA is considerably lower mainly in comparison to PFHpA and to others PFCAs as well. An extremely high intensity of ethyl ester of PFHpA may be caused by contemporary elution of another volatile compound formed during the derivatization procedure probably enhancing the ionization.

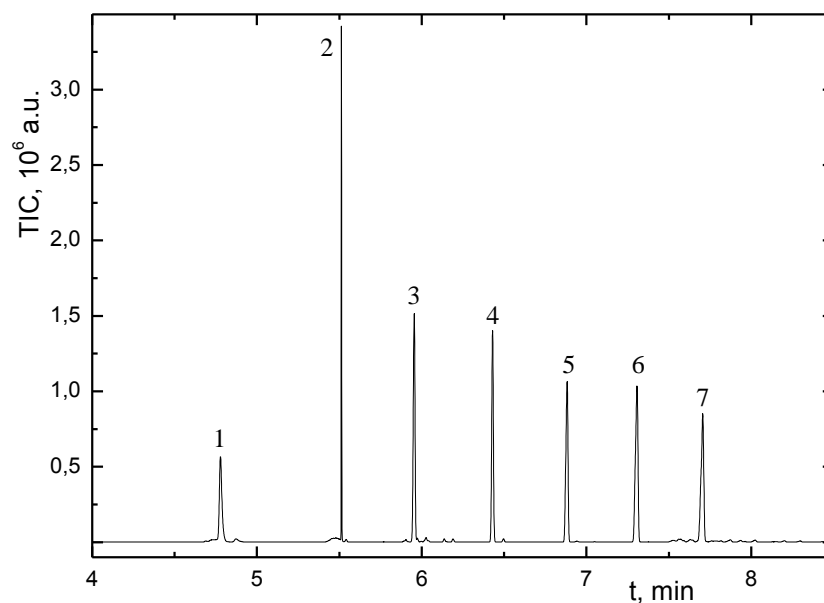


Figure 4.2 A GC-NCI-MS chromatogram of ethyl esters of PFCAs ($5 \mu\text{g mL}^{-1}$) PFCAs: (1) PFHxA, (2) PFHpA, (3) PFOA, (4) PFNA, (5) PFDA, (6) PFUnA and (7) PFDoA; experimental conditions same as in Fig. 4.1

The last tested alkyl chloroformate in the derivatization procedure was methyl chloroformate. A particular chromatogram of methyl esters of PFCAs is shown in Figure 4.3. Comparing it to the chromatogram of ethyl esters of PFCAs, chromatographic peaks of methyl esters of PFCAs provide similar intensities to ethyl esters of PFCAs, except from PFHxA and PFHpA. The intensity of methyl ester of PFHxA is much higher than the intensity of ethyl ester. On the other hand, the intensity of methyl ester of PFHpA is significantly smaller in comparison to the intensity of ethyl ester and corresponds more to the intensities obtained for other methyl esters of PFCAs. Therefore the methyl esters of PFCAs have been chosen for the further experiments.

Besides alkyl chloroformates, methanol and ethanol instead of isobutyl alcohol as an auxiliary agent have been tested. However, it did not lead to any further improvement.

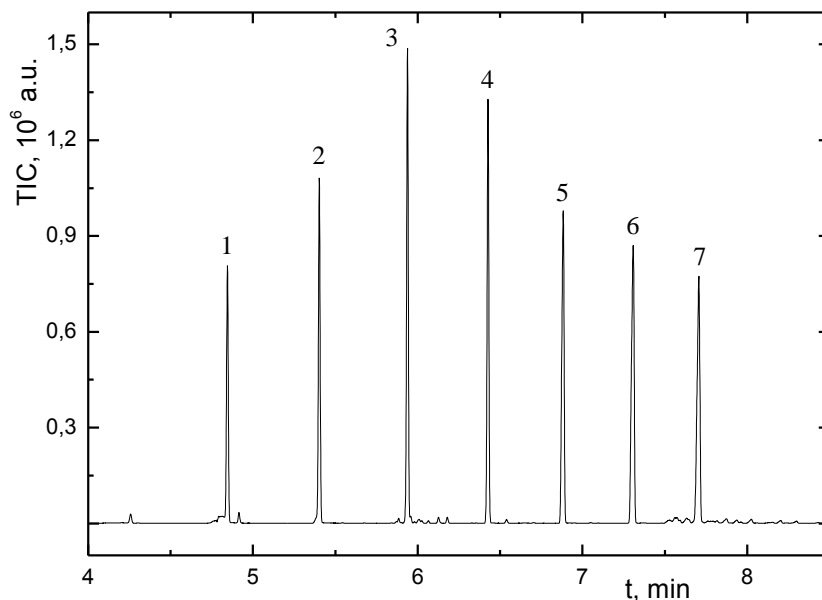


Figure 4.3 A GC-NCI-MS chromatogram of methyl esters of PFCAs ($5 \mu\text{g mL}^{-1}$): (1) PFHxA, (2) PFHpA, (3) PFOA, (4) PFNA, (5) PFDA, (6) PFUnA and (7) PFDoA; experimental conditions same as in Fig. 4.1

4.2 Optimization of reagent gas pressure

Methane (99.9995 %, Linde, Czech Republic) was used as the reagent gas in the gas chromatography – mass spectrometry analysis with negative chemical ionization. The pressure of methane has been optimized with three different values: 200 kPa, 250 kPa and 300 kPa. After derivatization, a sample of $1 \mu\text{L}$ of methyl esters of PFCAs in hexane at a concentration of $5 \mu\text{g mL}^{-1}$ was injected in splitless-mode and analyzed by GC-NCI-MS in triplicate, always with a different reagent gas pressure.

A signal-to-noise ratio (S/N) has been calculated for the predominant m/z value of each perfluorinated acid, specifically 278 (PFHxA), 328 (PFHpA), 378 (PFOA), 428 (PFNA), 478 (PFDA), 528 (PFUnA) and 578 (PFDoA). According to Figure 4.4, the lowest S/N values for all PFCAs have been obtained by 200 kPa. On the contrary, the highest S/N values of methyl esters of PFCAs have been achieved by 300 kPa, although some S/N values did not considerably differ from S/N values obtained by 250 kPa. The higher values ($> 300 \text{ kPa}$) of

the reagent gas pressure have not been tested due to decreasing vacuum below optimal conditions with increasing pressure of methane flowing into ion source cell. For further experiments, the value of 300 kPa for methane was used.

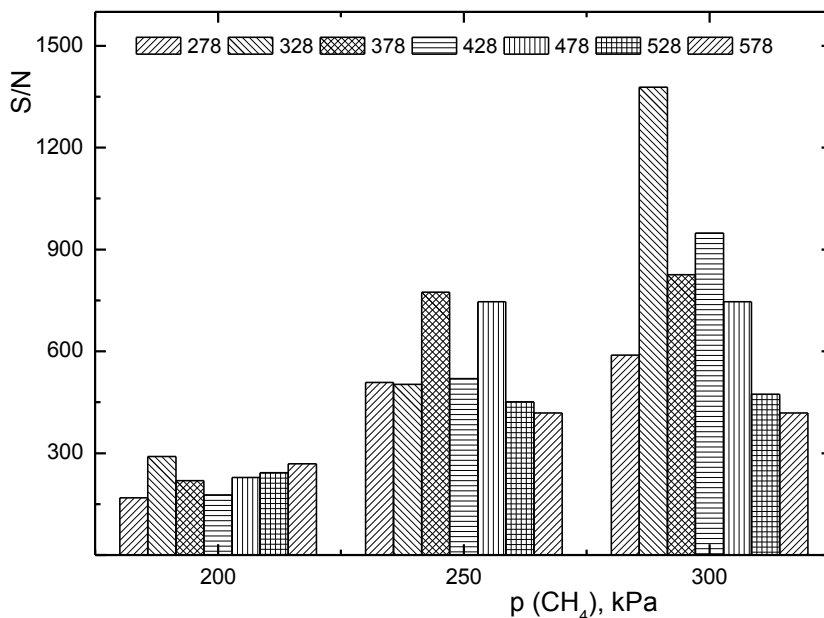


Figure 4.4 Dependency of the S/N values obtained for predominant m/z values of PFCAs on the pressure of CH₄ (kPa)

4.3 Evaluation of calibration dependencies

For each perfluorinated carboxylic acid, a calibration curve has been obtained. The concentration range of 8 calibration standards prepared was from 0.005 to 10 $\mu\text{g mL}^{-1}$. Each calibration standard also contained internal standard (1,2-¹³C₂-PFOA) at a concentration of 1 $\mu\text{g mL}^{-1}$. Samples of 178 μL containing PFCAs and 1,2-¹³C₂-PFOA in acetonitrile at particular concentrations were derivatized and analyzed by GC-NCI-MS. Calibration curves were constructed to perform linear regression of plots of peak area - internal standard area ratio (A/A_i) versus standard concentration of PFCAs. Because of the transparency, the plots of linear regression of PFHxA, PFHpA and PFOA are shown in Figure 4.5, whereas the plots of linear regression of PFNA, PFDA, PFUnA and PFDoA are shown in Figure 4.6.

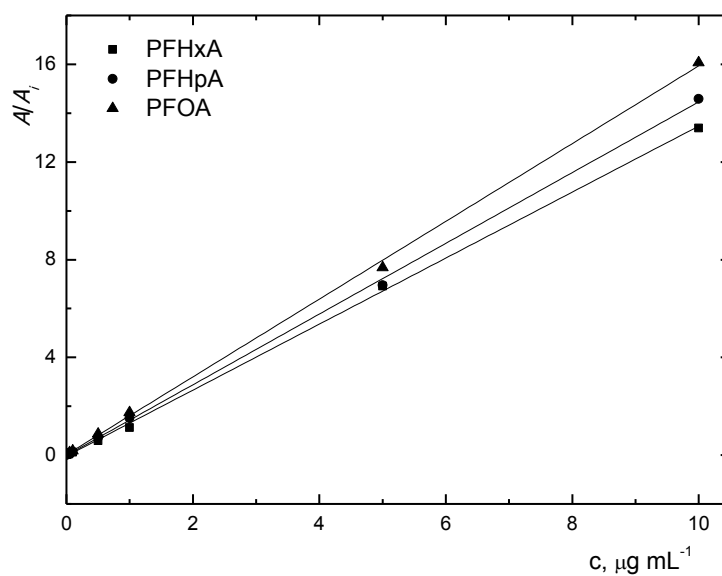


Figure 4.5 Calibration curves of methyl esters of PFHxA, PFHpA and PFOA ($0.005 - 10 \mu\text{g mL}^{-1}$); experimental conditions same as in Fig. 4.1 except from $p(\text{CH}_4)$ 300 kPa

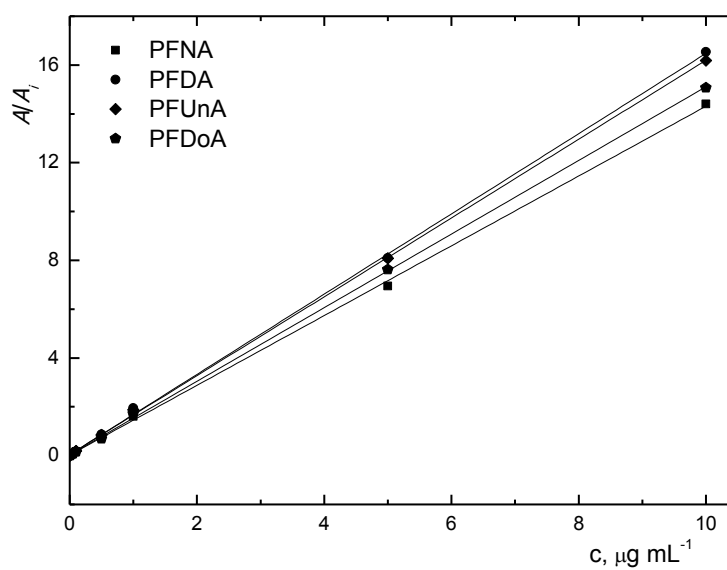


Figure 4.6 Calibration curves of methyl esters of PFNA, PFDA, PFUnA and PFDoA ($0.005 - 10 \mu\text{g mL}^{-1}$); experimental conditions same as in Fig. 4.5

Obtained calibration curves were generally linear with correlation coefficients (R^2) from 0.9994 to 0.9998. The correlation coefficients and values of linear regression ($y = A \cdot x + B$) for each perfluorinated carboxylic acid are listed in Table 4.1.

Table 4.1 Values of linear regression and correlation coefficients for particular PFCAs

PFCA	A	B	R^2
PFHxA	1.3505	-0.0353	0.9995
PFHpA	1.4475	-0.0130	0.9995
PFOA	1.5921	0.0175	0.9994
PFNA	1.4289	0.0223	0.9996
PFDA	1.6433	0.0421	0.9996
PFUnA	1.6143	0.0477	0.9998
PFDoA	1.5080	0.0318	0.9998

4.4 Multifactorial statistic optimization of extraction conditions

Since the determination of PFCAs in solid materials requires multiple, not easily automated steps and lengthy sample preparation, the response of the measurement system may depend on a variety of experimental factors, i.e. aspects of the experimental conditions which affect the result obtained from an experiment. Therefore a sophisticated multifactorial statistic method, response surface methodology (RSM), has been applied to find particular extraction and clean-up parameters yielding the optimum system response. The multifactorial statistical methods are recently gradually replacing the common one-variable-at-a-time (OVAT) procedures, because they are much more effective and make it also possible to find interactions among individual factors influencing the system response [74].

The RSM procedure can be divided into three principal steps: (a) screening, to find the parameters which exert statistically significant influence on the response studied, (b) modelling, to find a mathematical description of the system response as a function (mostly polynomial one) of the statistically significant parameters and (c) optimization, to specify the combination of the parameters yielding the optimum response [75].

4.4.1 Selection of factors

On the basis of the literature, a solid-liquid extraction followed by SPE clean-up employing dispersive graphitized carbon adsorbent has been chosen as an extraction technique prior GC-NC-MS determination of PFCAs in soils since it does not involve so many procedural steps in comparison to other extraction techniques published. The eight

factors evaluated have been selected on the basis of the literature and the extraction process proposed. They involve the amount of soil (m_{soil}), the extraction solvent volume (V_{MeOH}), the shaking time ($t_{shaking}$) and velocity ($v_{shaking}$) of the extraction mixture, the sonication time ($t_{sonication}$), the volume of methanol ($V_{condit. MeOH}$) used for pre-condition of the SPE cartridge, the vacuum of pre-condition ($p_{condition}$) and the vacuum of extraction procedure ($p_{extraction}$). Two more factors, an addition of NaOH and neutralization with HCl have been tested as well, however obtained recovery results of particular PFCAs were so poor, that these two factors were removed.

4.4.2 Screening and modelling

To reduce time and number of the experiments, screening and modelling of the eight factors selected were performed in one step employing 1/16 fractional factorial design and face centered central composite design as well. Three values have been attributed to each factor, the low and the high one and one corresponding to the central point, which has been calculated by Minitab 16 program according to those two values. The different values that a factor takes are known as different levels [75]. The selected factors with their high and low levels are listed in Table 4.2.

Table 4.2 Experimental factors with their high and low levels used for optimization of solid-liquid extraction and SPE clean-up step to determine PFCAs in soil samples

Factor (unit)	Low level	High level
m_{soil} (g)	5	15
V_{MeOH} (mL)	20	40
$v_{shaking}$ (rpm)	20	40
$t_{shaking}$ (min)	300	600
$t_{sonication}$ (min)	20	40
$V_{condit. MeOH}$ (mL)	5	15
$p_{condition}$ (kPa)	-40	0
$p_{extraction}$ (kPa)	-50	-30

A set of 35 experiments shown in Table 4.3 with various combinations of the factor levels has been created in Minitab 16 program.

Table 4.3 Modelling experimental plan of 35 measurements created in Minitab 16

-	m_{soil}	V_{MeOH}	$v_{shaking}$	$t_{shaking}$	$t_{sonication}$	$V_{condit. MeOH}$	$p_{condition}$	$p_{extraction}$
1	10	30	450	30	30	10	-20	-40
2	15	20	300	40	40	5	0	-50
3	5	20	600	20	40	15	0	-50
4	5	40	600	40	40	5	-40	-30
5	5	40	600	20	20	15	-40	-30
6	15	40	600	40	40	15	0	-50
7	15	20	600	40	20	15	-40	-30
8	5	40	300	20	40	5	0	-30
9	5	20	300	40	40	15	-40	-30
10	5	20	300	20	20	5	-40	-50
11	5	20	600	40	20	5	0	-30
12	10	30	450	30	30	10	-20	-40
13	15	20	600	20	40	5	-40	-30
14	5	40	300	40	20	15	0	-50
15	15	40	600	20	20	5	0	-50
16	10	30	450	30	30	10	-20	-40
17	15	20	300	20	20	15	0	-30
18	15	40	300	40	20	5	-40	-30
19	15	40	300	20	40	15	-40	-50
20	5	30	450	30	30	10	-20	-40
21	15	30	450	30	30	10	-20	-40
22	10	20	450	30	30	10	-20	-40
23	10	40	450	30	30	10	-20	-40
24	10	30	300	30	30	10	-20	-40
25	10	30	600	30	30	10	-20	-40
26	10	30	450	20	30	10	-20	-40
27	10	30	450	40	30	10	-20	-40
28	10	30	450	30	20	10	-20	-40
29	10	30	450	30	40	10	-20	-40
30	10	30	450	30	30	5	-20	-40
31	10	30	450	30	30	15	-20	-40
32	10	30	450	30	30	10	-40	-40
33	10	30	450	30	30	10	0	-40
34	10	30	450	30	30	10	-20	-50
35	10	30	450	30	30	10	-20	-30

These 35 experiments have been performed using soil collected from the Botanical Garden of the Charles University in Prague. Soil samples were spiked with 140 μL of PFCAs mixture at a concentration of 5 $\mu\text{g mL}^{-1}$ in acetonitrile to obtain final concentration of 1 $\mu\text{g mL}^{-1}$ of each PFCA after derivatization. Samples were allowed to soak for 30 min. Subsequently, the extraction procedure followed by SPE clean-up step was performed according to the experimental plan with levels of factors changing.

The recoveries of individual PFCAs obtained were used as the system response. They were calculated as the ratio of experimentally found concentration of particular PFCA to the theoretical concentration, using the calibration curves for each PFCA with a concentration range from 0.005 to 10 $\mu\text{g mL}^{-1}$. The calibration curves were constructed to perform linear regression of plots of absolute peak areas of particular PFCAs versus standard concentration of PFCAs. Obtained calibration curves were generally linear with correlation coefficients (R^2) from 0.9998 to 1 for all PFCAs except from PFHxA, where R^2 was 0.9991.

The results obtained from the first 19 experiments have been evaluated using the ANOVA test, determining the factors and their interactions with main effects on sum of the PFCAs recoveries as well as on the recovery of each perfluorinated acid separately at a significance level of 95 %. To show the main effects of particular factors and their interactions, the Pareto chart, which is shown in Figure 4.7, has been applied for sum of the PFCAs recoveries with standardized effects shown on X axis and particular factors or possible interactions on Y axis.

This test of the factor significance has indicated that the amount of soil has the greatest and only significant influence on the system response at a significance level of 95 %. Two more factors, the vacuum of pre-condition and the volume of methanol used as an extraction agent, may play a role in recovery of PFCAs from soil samples. The other factors exert a small effect on the overall analyte extractions. No interactions between factors have been observed at a significance level of 95 %.

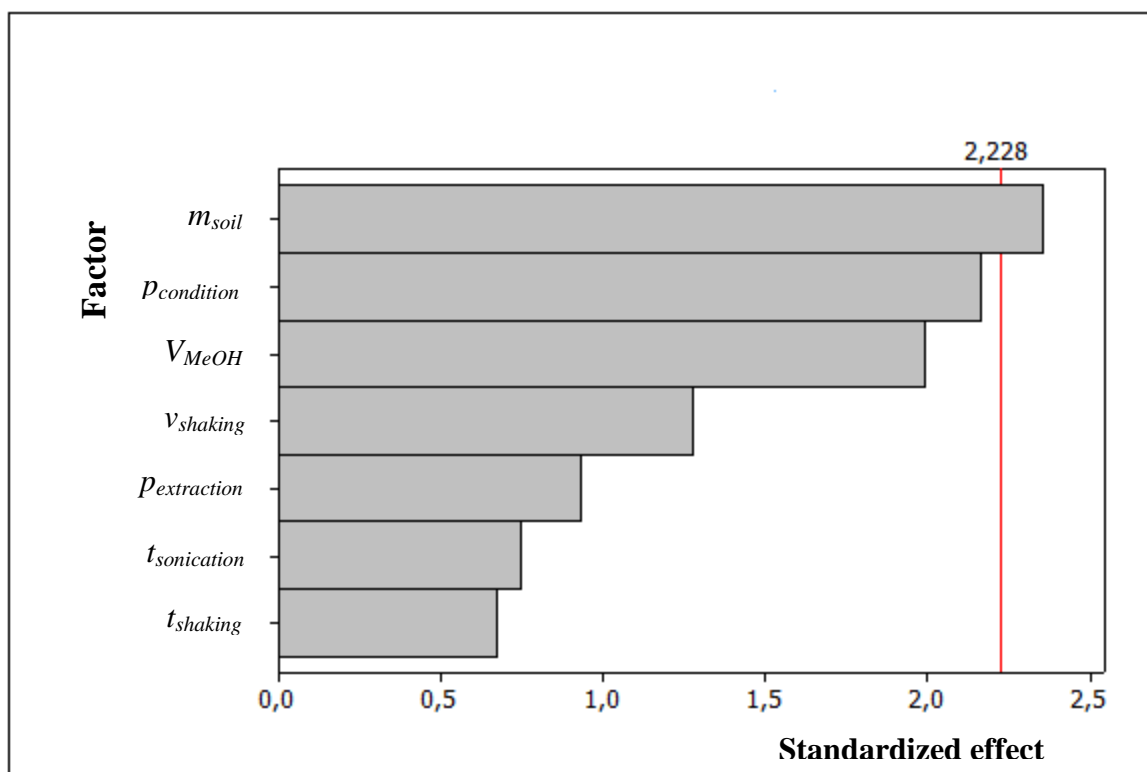


Figure 4.7 Standardized main effect Pareto chart for sum of the PFCAs recoveries with a vertical line defining 95 % confidence level

To show main effects of particular factors and their possible interactions on the recovery of individual PFCAs, the Pareto charts have been constructed for each PFCA. Comparing these results with the Pareto chart obtained for sum of the PFCAs recoveries indicates that each PFCA is influenced by different factors and that only on the recovery of PFDoA no factor evaluated plays a significant role. No interactions between factors have been observed at a significance level of 95 % when evaluating the recovery of individual PFCAs.

The comparison of various factors with main effects on the recovery of individual PFCAs at a significance level of 95 % is shown in Figure 4.8. The recovery of PFHxA and PFHpA is affected by three factors, in case of PFHxA by the shaking velocity of the extraction mixture, the vacuum of pre-condition and the extraction solvent volume and in case of PFHpA by the vacuum of pre-condition, the amount of soil and the extraction solvent volume. The vacuum of pre-condition and the amount of soil have the greatest influence on the recovery of PFOA, whereas for PFNA, PFDA and PFUnA only the amount of soil is statistically significant. Surprisingly two perfluorinated acids, PFHxA and PFDoA, are not statistically affected by the amount of soil.

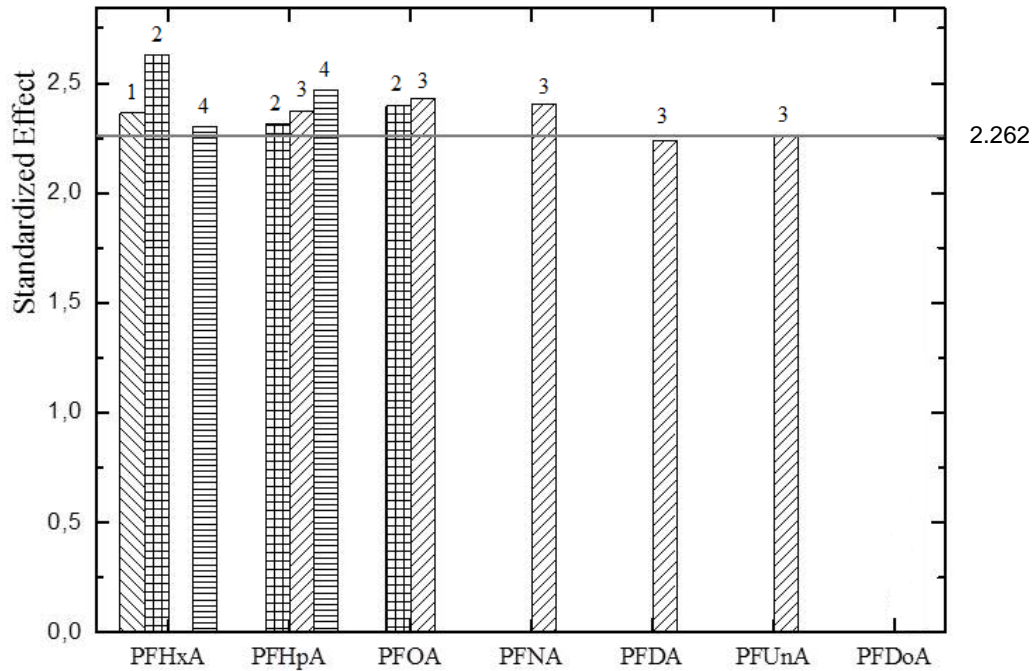


Figure 4.8 Standardized main effects of four factors: (1) the shaking velocity of the extraction mixture, (2) the vacuum of pre-condition, (3) the amount of soil and (4) the extraction solvent volume affecting the recovery of individual PFCAs with a horizontal line defining 95 % confidence level

To find a mathematical description of the system response as a function of the statistically significant factors, the results obtained from all 35 experiments have been evaluated by the ANOVA method. The following functional polynomial dependence has been employed:

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i < j} \beta_{ij} x_i x_j \quad (4.1)$$

where y is the response, β_0 is the constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient and x_i is the level of the individual factor [74].

The quadratic equations have been obtained for the recovery of individual perfluorinated acids as well as for sum of the PFCAs recoveries. On treating the results for sum of the PFCAs recoveries, the determination coefficient 0.7427 has been obtained, which indicates that there is an acceptable agreement between the experimental data and the model created. The determination coefficients for individual PFCAs, describing the agreement between experimental data and the model created for each PFCA separately, are summarized

in Table 4.4. The highest agreement with the statistical model was obtained by PFHxA, whereas the lowest agreement was obtained by PFNA.

Table 4.4 Determination coefficients (R^2) obtained for recovery of individual PFCAs showing the agreement between experimental data and the model created

PFCA	Determination coefficient (R^2)
PFHxA	0.8095
PFHpA	0.7734
PFOA	0.7532
PFNA	0.6979
PFDA	0.7235
PFUnA	0.7007
PFDoA	0.7691

The response surface plots have been created in Minitab 16 program. These plots demonstrate the interrelationships of two selected factors with the system response, whereas other factors are set at their optimum levels. Two response surface plots are shown in Figures 4.9 and 4.10 demonstrating the dependency of sum of the PFCAs recoveries on different factors.

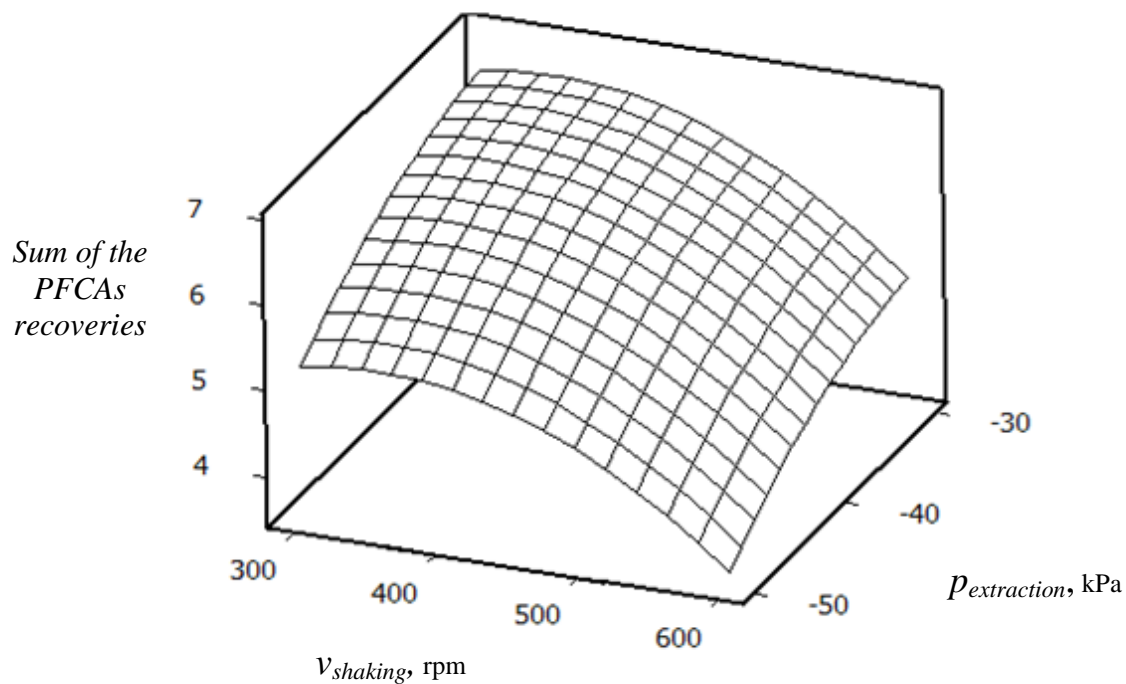


Figure 4.9 Response surface plot showing the dependency of sum of the PFCAs recoveries on the shaking velocity and on the vacuum of extraction procedure

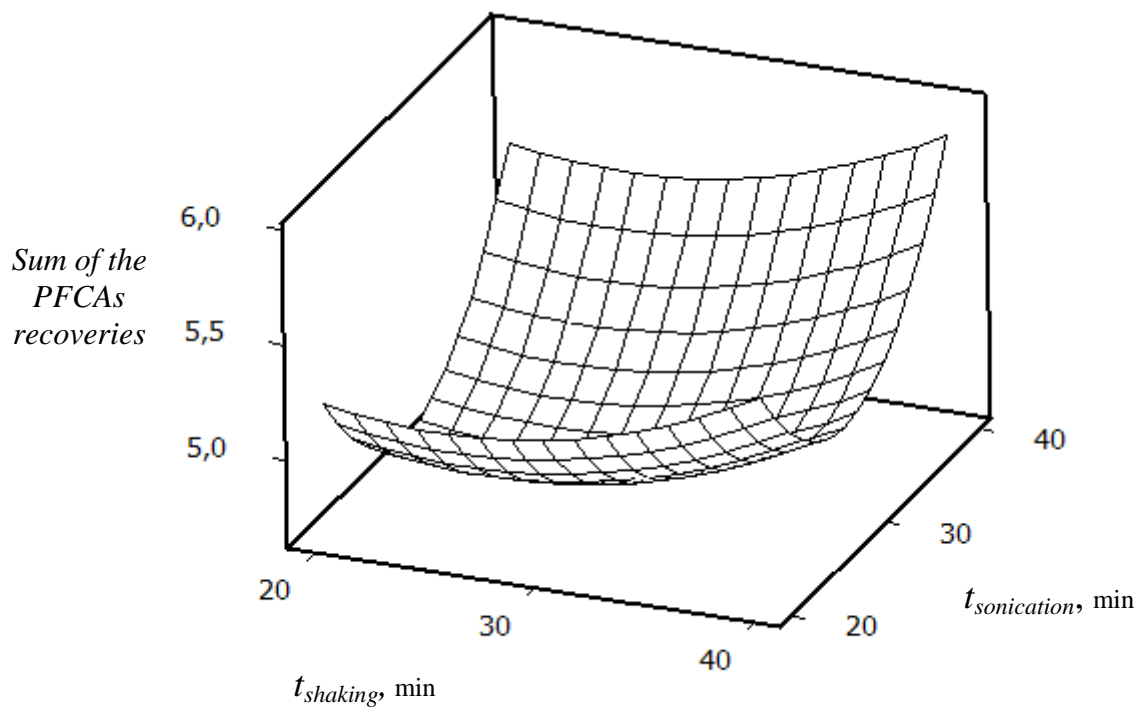


Figure 4.10 Response surface plot showing the dependency of sum of the PFCAs recoveries on the shaking time and on the sonication time

4.4.4 Optimization

The system has been optimized in Minitab 16 program (Minitab Inc.), using the desirability function, to find the optimal combination of the factor levels which yields the response value (the sum of PFCAs recoveries) most corresponding to the theoretical expected value. The following optimum levels of particular factors are shown in Table 4.5.

Table 4.5 Optimum levels of eight factors found for the solid-liquid extraction of PFCAs from soil samples followed by SPE clean-up step

Factor (unit)	Optimum level
m_{soil} (g)	5
V_{MeOH} (mL)	20
$v_{shaking}$ (rpm)	300
$t_{shaking}$ (min)	20
$t_{sonication}$ (min)	40
$V_{condit. MeOH}$ (mL)	13
$p_{condition}$ (kPa)	-40
$p_{extraction}$ (kPa)	-30

The extraction procedure of PFCAs from soil samples followed by SPE clean-up step has been repeated three times with the optimum levels of particular factors used to confirm the found optimum. The recoveries of individual PFCAs were within a range from 0.7 to 1.1 (70 to 110 %). Nevertheless, these recovery values have not been used as the final recovery results of individual PFCAs because they were evaluated from the absolute peak areas corresponding to particular concentrations and moreover they were measured at high concentration ($1 \mu\text{g mL}^{-1}$) in comparison to concentrations of PFCAs commonly found in soil samples. Therefore these optimum levels of eight factors have been applied for further recovery experiments of PFCAs from soil samples and for extraction and subsequently determination of PFCAs in soil samples acquired from Prague and villages located in the close neighbourhood.

4.5 Recovery of the extraction procedure

The recovery experiments were performed using soil collected from the Botanical Garden of the Charles University in Prague. The samples were spiked to two different levels and the concentrations of PFCAs experimentally found were compared with the theoretical ones, thus obtaining the real recoveries.

An aliquot of 5.0 g dry soil was weighed in a clean 50 mL polypropylene Falcon tube and spiked with 10.5 μL of PFCAs mixture at a concentration of $0.5 \mu\text{g mL}^{-1}$ in acetonitrile to obtain final concentration of 7.5 ng mL^{-1} of each PFCA after derivatization, approximately corresponding to $5 \times \text{LOQ}$ values of PFCAs. Samples were allowed to soak for 30 min. Afterwards 20 mL of methanol were added to samples and the extraction was further performed as described in 3.3.5. To obtain final concentration of 15 ng mL^{-1} of each PFCA after derivatization, approximately corresponding to $10 \times \text{LOQ}$ values of PFCAs, an aliquot of 5.0 g dry soil was spiked with 21 μL of PFCAs mixture at a concentration of $0.5 \mu\text{g mL}^{-1}$ in acetonitrile. After solid-liquid extraction followed by SPE clean-up step, sample was divided into three sub-samples of 178 μL , which were subjected to derivatization and determination by gas chromatography – mass spectrometry with negative chemical ionization.

The peak areas of individual perfluorinated carboxylic acids were recalculated to corresponding concentrations of PFCAs using calibration curves constructed for each PFCA within a concentration range from 0.005 to $1 \mu\text{g mL}^{-1}$. Calibration curves were constructed to perform linear regression of plots of peak area - internal standard area ratio (A/A_i) versus standard concentration of PFCAs as described in 4.3 only with a slight difference, that the two last calibration points (5 and $10 \mu\text{g mL}^{-1}$) were removed because they yield inaccuracy in quantification of individual PFCAs. Obtained calibration curves were generally linear with correlation coefficients (R^2) from 0.9995 to 1 for all PFCAs.

The recovery of individual PFCAs was calculated as the ratio of experimentally found concentration of particular PFCA to the theoretical concentration. To obtain the recovery results in percentage, the ratio was multiplied by 100 and finally a median for each PFCA was calculated from the three recovery values obtained. The recovery results obtained after spiking 1.1 ng g^{-1} and 2.1 ng g^{-1} of PFCAs are summarized in Table 4.6.

Table 4.6 Recovery of PFCAs in percentage after spiking 1.1 ng g^{-1} and 2.1 ng g^{-1} of PFCAs mixture in acetonitrile to soil samples

PFCA	1.1 ng g^{-1}	2.1 ng g^{-1}
	%	%
PFHxA	85	91
PFHpA	96	96
PFOA	94	106
PFNA	100	97
PFDA	94	100
PFUnA	97	107
PFDoA	100	105

The ability of graphitized carbon is that absorbs compounds via dispersive interaction with π electrons. In PFCAs, π electrons are strongly associated with the highly electronegative fluorine atoms. Therefore no effective interaction of PFCAs with the sorbent will be observed, not even in the presence of a weak eluting solvent such as methanol. However, most nonperfluorinated compounds with any degree of aromaticity will be strongly associated with the graphitized carbon sorbent.

The results demonstrate that the recovery of particular PFCAs is within a range from 85 to 107 %. No correlation between extraction recovery and the length of the carbon chain of PFCAs has been observed. The lowest recovery was obtained for PFHxA when spiking 1.1 ng g^{-1} of PFCAs, whereas the recovery results of PFOA, PFUnA and PFDoA exceeded 100 % when spiking 2.1 ng g^{-1} of PFCAs. The t-test ($\alpha = 0.05$, $n = 3$) indicates that the recovery values do not differ statistically for the two PFCAs concentrations spiked. According to literature, it is a frequent problem with the extraction efficiency values exceeding 100 % at low concentrations.

To evaluate the possible losses of PFCAs, the whole extraction procedure was performed as described above with a difference of the addition of $140 \mu\text{L}$ of internal standard ($1,2\text{-}^{13}\text{C}_2\text{-PFOA}$) to 20 mL of methanol instead of to $485 \mu\text{L}$ of acetonitrile after sample evaporation to dryness. The loss of $1,2\text{-}^{13}\text{C}_2\text{-PFOA}$ has been observed as well, which indicates that the losses of PFCAs will not be caused by insufficient extraction from soil, though may occur during the extraction or SPE clean-up procedure.

To determine the PFCAs concentrations in soil samples, the recovery results of the solid-

liquid extraction followed by SPE clean-up step obtained for the lower spike (1.1 ng g^{-1}) were used because the concentration spiked is closer to concentrations of PFCAs commonly found in soil samples and the recovery of all PFCAs did not exceed 100 %.

4.6 Determination of instrumental LOD and LOQ

To determine instrumental limit of detection (LOD) and limit of quantification (LOQ) values for each perfluorinated carboxylic acid, the signal-to-noise (S/N) values were used for the peak areas corresponding to the lowest concentrations of the acid methyl esters in calibration curves (5 ng mL^{-1}). These values were recalculated to the acid methyl esters concentrations corresponding to the S/N ratios of 3 for LOD and to the S/N ratios of 10 for LOQ. The instrumental LOD and LOQ values of particular perfluorinated acids with the relative standard deviations of S/N values in percentage are listed in Table 4.7.

Table 4.7 Instrumental LOD and LOQ values of PFCAs for GC-NCI-MS analysis with the relative standard deviations of S/N values in percentage (n = 3)

PFCA	LOD	LOQ	S/N RSD
	ng mL^{-1}	ng mL^{-1}	%
PFHxA	0.4	1.5	9.2
PFHpA	0.5	1.7	4.1
PFOA	0.5	1.6	7.2
PFNA	0.3	1.0	4.7
PFDA	0.4	1.2	10.0
PFUnA	0.5	1.5	12.5
PFDoA	0.5	1.6	9.4

The LOD and LOQ values of the whole analytical method including solid-liquid extraction followed by SPE clean-up step and GC-NCI-MS analysis were then obtained for individual analytes by including the pre-concentration factor $f = 32$, the recovery of PFCAs after extraction procedure and the amount of soil (5 g). These values are listed in Table 4.8.

Table 4.8 The LOD and LOQ values of PFCAs for the whole analytical method with the relative standard deviations of S/N values in percentage (n = 3)

PFCA	LOD pg g ⁻¹	LOQ pg g ⁻¹	S/N RSD %
PFHxA	2.3	7.8	9.2
PFHpA	3.0	10.1	4.1
PFOA	2.7	8.8	7.2
PFNA	1.9	6.4	4.7
PFDA	2.1	7.1	10.0
PFUnA	2.7	9.0	12.5
PFDoA	2.9	9.7	9.4

According to LOD and LOQ values obtained for each perfluorinated carboxylic acid, this analytical method is sufficiently sensitive for PFCAs determination in soil samples. There is no significant difference among LOD and LOQ values obtained for individual perfluorinated carboxylic acids therefore this analytical method is equally sensitive to each PFCA.

4.7 Evaluation of soil samples

The soil samples collected from Prague and villages located in the close neighbourhood were analyzed by solid-liquid extraction with methanol and SPE clean-up step followed by gas chromatography – mass spectrometry with negative chemical ionization. The peak areas of individual perfluorinated carboxylic acids were recalculated to corresponding concentrations of PFCAs using the same calibration curves which were applied for evaluation of recovery of individual PFCAs. Finally a median for each PFCA was calculated from three values obtained. All concentrations of PFCAs determined in nine soil samples with corresponding relative standard deviations in parentheses (expressed in percentage) are listed in Table 4.9.

Samples (A and B) acquired at two sites from Kralupy nad Vltavou with the location of petrochemical industrial company Česká rafinérská a.s. were the most contaminated, determining five from seven PFCAs measured. A concentration range of these PFCAs was 11 - 45 pg g⁻¹. The occurrence of PFCAs, specifically PFHxA, PFOA, PFDA, PFUnA and

PFD_oA, was in both samples similar and did not show any significant differences in PFCAs distribution in soil within 3 km air distance.

In three soil samples, the Evropská street in Prague (building of tunnel Blanka in progress), Radotín (a cement factory) and Zbraslav (a former military area), have not been detected any PFCAs. In sample from the bank of the Vltava river downstream from Central Wastewater Cleaning Plant only PFOA and PFUnA were detected.

On the other hand, an interesting phenomenon has been observed in samples collected within 0.5 km air distance at the beginning of nature reserve Černínovsko in the close distance of Spolana Neratovice, one of the biggest chemical companies within Czech industrial landscape. The occurrence of PFCAs in both samples significantly differed. While Spolana A sample contained only PFOA below LOQ value, in Spolana B sample (collected in closer distance to the factory) PFOA, PFDA, PFUnA and PFD_oA were quantified. Moreover, an extremely high concentration of PFUnA (159 pg g⁻¹) was determined in this soil sample. The 159 pg g⁻¹ value was the overall highest concentration determined in all nine soil samples and has demonstrated an important role of different distribution of PFCAs in soils, which generally complicates determination of PFCAs in solid matrices and makes it necessary to collect more samples from one geographical location to properly determine the potential occurrence of PFCAs.

The second highest concentration (69 pg g⁻¹) was obtained for PFUnA in Zadní Kopanina sample (a former military area) with PFOA and PFDA determined as well. Two perfluorinated carboxylic acids, PFHpA and PFNA, have not been detected in any sample analyzed. On the other hand, the most frequently occurred PFCAs were PFOA, PFDA, PFD_oA and PFUnA with two overall highest concentrations determined. Selected chromatograms of Kralupy nad Vltavou A and B sample are shown in Figures 4.11 and 4.12, whereas chromatographic report of Spolana Neratovice B sample is shown in Figure 4.13.

Table 4.9 Nine soil samples containing particular concentrations of PFCAs determined with relative standard deviations expressed in percentage in parentheses (n = 3)

Geographical location	PFHxA pg g ⁻¹	PFHpA pg g ⁻¹	PFOA pg g ⁻¹	PFNA pg g ⁻¹	PFDA pg g ⁻¹	PFUnA pg g ⁻¹	PFDoA pg g ⁻¹
Kralupy nad Vltavou A	43 (13)	< LOD	19 (13)	< LOD	11 (19)	19 (12)	45 (15)
Kralupy nad Vltavou B	19 (14)	< LOD	12 (5)	< LOD	< LOQ	19 (4)	26 (8)
Evropská street	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Radotín	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Zbraslav	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOQ
Zadní Kopanina	< LOD	< LOD	16 (20)	< LOD	29 (10)	69 (14)	< LOD
Spolana Neratovice A	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOD
Spolana Neratovice B	< LOD	< LOD	20 (7)	< LOD	14 (13)	159 (12)	39 (11)
Trója	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOD	33 (8)

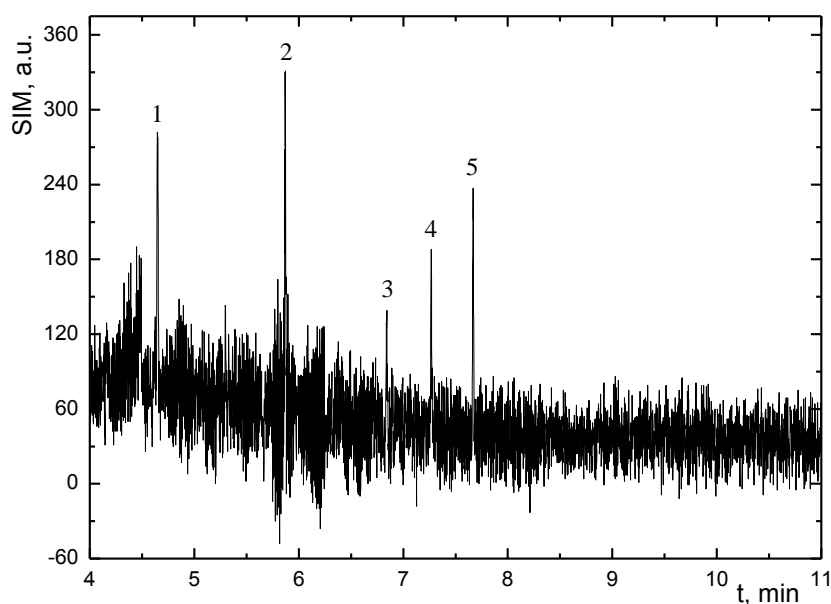


Figure 4.11 A GC-NCI-MS chromatogram of methyl esters of PFCAs from Kralupy nad Vltavou A sample: (1) PFHxA, (2) PFOA, (3) PFDA, (4) PFUnA and (5) PFDoA; experimental conditions same as in Fig. 4.5; SIM (pre-dominant m/z monitored for each PFCA; 1,2-¹³C₂-PFOA not shown)

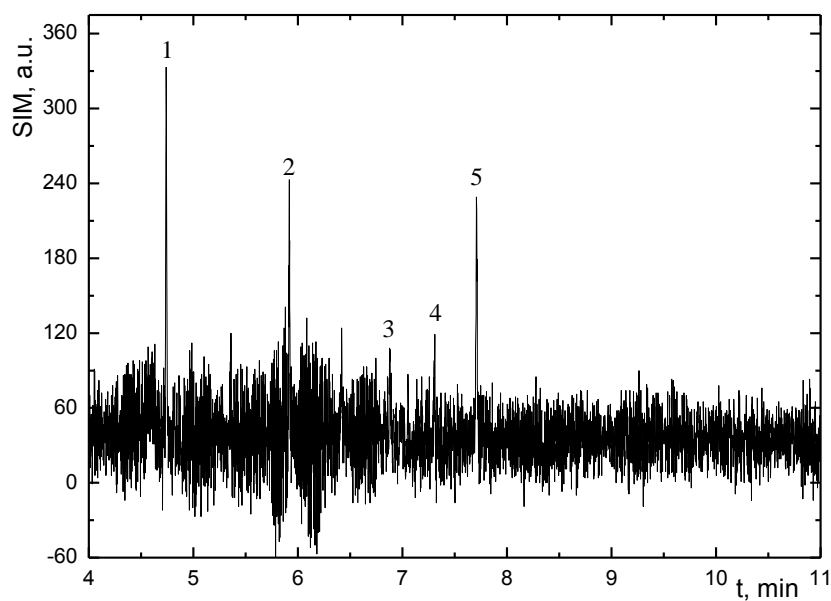


Figure 4.12 A GC-NCI-MS chromatogram of methyl esters of PFCAs from Kralupy nad Vltavou B sample: (1) PFHxA, (2) PFOA, (3) PFDA, (4) PFUnA and (5) PFDoA; experimental conditions same as in Fig. 4.5; SIM (pre-dominant m/z monitored for each PFCA; 1,2- $^{13}\text{C}_2$ -PFOA not shown)

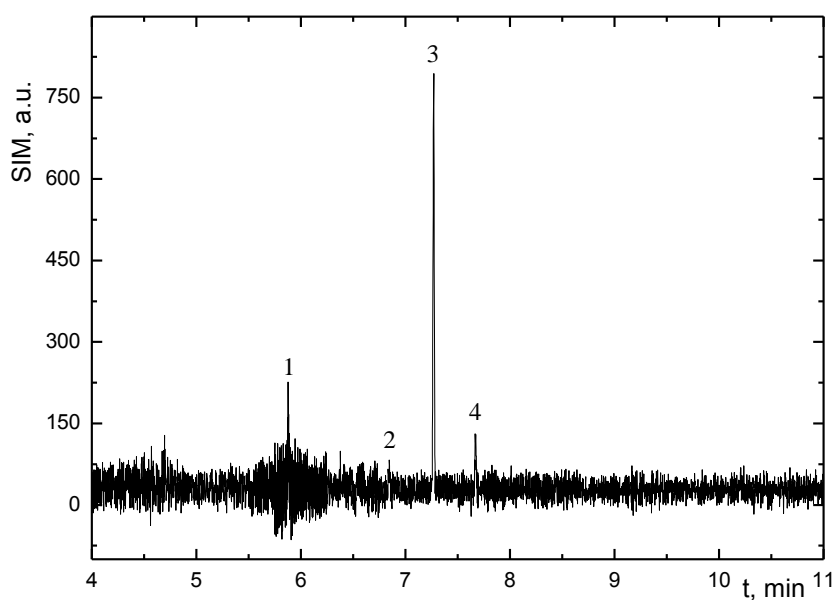


Figure 4.13 A GC-NCI-MS chromatogram of methyl esters of PFCAs from Spolana Neratovice B sample: (1) PFOA, (2) PFDA, (3) PFUnA and (4) PFDoA; experimental conditions same as in Fig. 4.5; SIM (pre-dominant m/z monitored for each PFCA; 1,2- $^{13}\text{C}_2$ -PFOA not shown)

5. CONCLUSION

The aim of this Diploma thesis was to optimize a sensitive and accurate method employing solid-liquid extraction with methanol and SPE clean-up step followed by gas chromatography – mass spectrometry with negative chemical ionization for determination of C₆ – C₁₂ perfluorinated carboxylic acids in selected soil samples acquired from Prague and villages located in the close neighbourhood. The occurrence of perfluorinated carboxylic acids has already been published in the Vltava and Elbe rivers [1] and afterwards in Central Wastewater Cleaning Plant in Prague [2]. Some of these acids were quantified in a concentration range from tens to hundreds pg mL⁻¹.

Firstly, the derivatization step prior to GC-NCI-MS analysis has been optimized according to the dimensions of the chromatographic column (Rtx-®-200 MS; 20 m × 0.15 mm ID, 0.15 µm) used with best results obtaining using methyl chloroformate. Therefore, PFCAs converted to their methyl esters have been detected in soil samples. Furthermore, calibration dependencies for each PFCA have been obtained in a concentration range from 0.005 – 10 µg mL⁻¹. Calibration curves were constructed to perform linear regression of plots of peak area - internal standard area ratio versus standard concentration of PFCAs with correlation coefficients from 0.9995 to 0.9998.

A sophisticated multifactorial statistic method, response surface methodology, has been applied to find the significant factors which influence the extraction procedure of PFCAs and SPE clean-up step as well and to set the optimum extraction and clean-up levels of eight factors evaluated yielding the maximum extraction recovery of all PFCAs.

After finding optimum conditions, soil samples were spiked to two different analyte concentration levels of 1.1 ng g⁻¹ and 2.1 ng g⁻¹ with recoveries obtained for individual PFCAs within a range from 85 to 100 % and from 91 to 107 %, respectively. Moreover, the LOD and LOQ values for each PFCA have been calculated. The limit of detection values were 1.9 – 3.0 pg g⁻¹ and limit of quantification values were 6.4 – 10.1 pg g⁻¹.

Finally, the method has been tested on determinations of C₆ – C₁₂ perfluorinated carboxylic acids in soil samples. The concentrations of PFCAs determined were within a range from 11 pg g⁻¹ (PFDA) to 159 pg g⁻¹ (PFUnA). Two perfluorinated carboxylic acids, PFHpA and PFNA, have not been detected in any sample analyzed. On the other hand, the most frequently occurred PFCAs were PFOA, PFDA, PFUnA and PFDoA. A different distribution of PFCAs in soil samples has been observed.

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7. APPENDIX