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Determination of perfluorinated carboxylic acids in fish fillet by micro-solid phase extraction, followed by liquid chromatography–triple quadrupole mass spectrometry

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ABSTRACT

In the current study, a simple, fast and efficient combination of protein precipitation and micro-solid phase extraction (μ-SPE) followed by liquid chromatography–triple quadrupole tandem mass spectrometry (LC–MS/MS) was developed for the determination of perfluorinated carboxylic acids (PFCAs) in fish fillet. Ten PFCAs with different hydrocarbon chain lengths (C5–C14) were analysed simultaneously using this method. Protein precipitation by acetonitrile and μ-SPE by surfactant-incorporated ordered mesoporous silica were applied to the extraction and concentration of the PFCAs as well as for removal of interferences. Determination of the PFCAs was carried out by LC–MS/MS in negative electrospray ionization mode. MS/MS parameters were optimized for multiple reaction monitoring of the analytes. ^{13}C mass labelled PFOA as a stable-isotopic internal standard, was used for calibration. The detection limits of the method ranged from 0.97 ng/g to 2.7 ng/g, with a relative standard deviation of between 5.4 and 13.5. The recoveries were evaluated for each analyte and were ranged from 77% to 120%. The *t*-test at 95% confidence level showed that for all the analytes, the relative recoveries did not depend on their concentrations in the explored concentration range. The effect of the matrix on MS signals (suppression or enhancement) was also evaluated. Contamination at low levels was detected for some analytes in the fish samples. The protective role of the polypropylene membrane used in μ-SPE in the elimination of matrix effects was evaluated by parallel experiments in classical dispersive solid phase extraction. The results evidently showed that the polypropylene membrane was significantly effective in reducing matrix effects.

1. Introduction

Perfluorinated compounds (PFCs) are anthropogenic materials, widely used in numerous industrial and domestic applications, because of their unique physicochemical characteristics [1–3]. Some members of these ubiquitous compounds have been identified as toxic and extremely persistent with bioaccumulative potential. Consequently, global concern regarding PFCs has been increasing rapidly among the environmental scientific community [4,5]. The relative importance of different routes of human exposure to PFCs is not yet well established; however, it has been suggested that water, food and dietary intake are potentially significant routes. In 2008, the European Food Safety Authority professed that considering human exposure assessment, exclusive data related to the PFCs levels in food and human tissues is needed [6]. From the analytical point of view, the analysis of PFCs in such complex matrices is challenging and associated with uncertainties. These challenges and uncertainties have been identified in detail and must be considered by researchers in analytical works related to PFCs [7,8]. Matrix effects have been considered as one of the most important sources of uncertainties in quantitative analysis of PFCs. These can have suppressive and/or enhancing effect on the results. In a comprehensive review paper, Powley and coworkers [9] have given a detailed description of this phenomenon in liquid chromatography–mass spectrometry (LC–MS).

Overall, there is no unique or universal strategy or solution concerning matrix effects, even though several practical suggestion have been made, and evaluated to overcome this phenomenon [7]. However, it has been confirmed that sample preparation is the key step for minimizing the presence of interfering compounds in

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complex matrices before analysis. The use of an internal standard (IS) has been also suggested to be taken into consideration during sample preparation. All these strategies lead to adequate and reliable quantitative results [8].

Recently much effort has been devoted to the monitoring and determination of PFCs from biota. However it is difficult to identify a robust extraction and sample preparation strategy which can cover a wide range of PFCs in complex matrices. Since 2000, three major extraction procedures followed by different clean-up strategies for the analysis of these compounds in biota have been suggested: ion pair extraction (IPE), solid–liquid extraction (SLE), and alkaline digestion. Despite all the advantages of the above mentioned methods, they still suffer from shortcomings. In IPE, tetrabutylammonium hydrogen sulphate is used for the ion pairing of the target compounds and subsequently extracted with methyl tert-butyl ether (MTBE) [10,11]. This method has been widely used for biological matrices like fish, molluscs, and tissues such as those of liver, kidney, gall bladder and blood. However, this method is relatively laborious and the co-extraction of lipid and the interference of matrix have been also reported. The method based on alkaline digestion, improves the extraction of targets bound to biological tissue, and reduces interferences from the matrix [12–14]. However, it is time consuming and involves many steps. Hence it runs the risk of increased contamination during processing. Methods having as few steps as possible are preferred in order to avoid analyte losses, which is likely during practical processes such as solvent change, solvent evaporation or the transfer of extract between containers. In comparison to the previous extraction methods, SLE has the benefit of simplicity; however, it is not without its own problems. For example, tetrahydrofuran/water has been reported as a successful solvent mixture, with good recovery and rapid extraction rate [13,15,16]. Nevertheless, it is necessary to control the amount of water in the sample. MTBE is another solvent which has been used for the SLE process [17]. Nonetheless, in this case the process needs solvent reduction and solvent exchange which could lead to the loss of the analytes. A mixture of mobile phase (methanol/ammonium acetate) has also been used as a quick and cost effective screening extraction method [18], but a high matrix effect has been observed. Moreover, due to the low solubility of the long-chain compounds in this mixture, it is not possible to extract these particular compounds.

Perfluorooctanesulfonic acid (PFOS) and perfluorinated carboxylic acids (PFCAs) are persistent against the typical environmental degradation processes (hydrolysis, photolysis, microbial degradation, and metabolism) compared to other members of PFCs, moreover, they are known to bioaccumulate [19,20]. A comprehensive survey, conducted in a wide range of geographical locations (e.g., South America, Russia, Antarctica) on monitoring of PFCs in aquatic ecosystems, has revealed a decrease in PFOS levels over time. In contrast, PFCAs have tended to increase in biota at many of the locations under survey [21]. Moreover, most of the studies hitherto on monitoring of PFCs have only been focused on PFOS and perfluorooctanoic acid (PFOA) – the most well-defined toxic PFCs. In 2010, however, the commission recommendation 2010/161/EU document invited member states to monitor and study other similar PFOA compounds with different carbon chain lengths in food matrices [22].

Hence, in this study, PFCAs with different carbon chain lengths have been chosen as an important group of PFCs. The aim of the present study was to develop a sorption-based method as an innovative strategy in the analysis of these PFCAs in fish fillet. There are two basic potential advantages related to sorption-based microextraction and concentration. The first operational advantage is focused on choosing the proper sorbent which might have specific or (and) particular compatibility with the analytes. The second operational part of a sorption based extraction method is based on the targeted selection of the washing and eluting solvent. These advantages could lead to the selective purification of the target analytes from interferences.

In 2006, micro-solid-phase extraction (μ-SPE), as a mode of sorption based extraction, based on the packing of sorbent material in a sealed porous polypropylene membrane envelope (bag), was reported by our group [23]. Extraction and concentration of the analyte in one single step, and easy manipulation, are some of the significant benefits of μ-SPE. In μ-SPE, the bag can be quickly isolated from the matrix solution using a pair of tweezers and it does not suffer from the usual difficulties related to other sorption based methods such as the high back-pressure or blockage of the column associated with the use of cartridge based SPE, collection of the sorbent in dispersive solid phase extraction (dSPE), or difficulties related to the fragility of the fiber in solid phase microextraction. μ-SPE is relatively inexpensive and consumes only small amounts (a few mg) of sorbent. Moreover, the protective role of the membrane in preventing interferences in the extraction leads to successful removal of matrix effects. The latter benefit makes μ-SPE a useful technique in the extraction and analysis of various target analytes from complex matrices like aqueous samples, food products, and biological tissues, without additional sample clean up [24–29]. The kind of sorbent used in μ-SPE is obviously an important factor, which influences greatly the extraction of the analytes from the matrix. In the current study, we evaluated the adoptive properties of uncalcined MCM-41 (cetyltrimethylammonium bromide contained MCM-41, noted as CTAB-MCM-41) for the extraction and pre-concentration of perfluorinated carboxylic acids (PFCAs) by μ-SPE. MCM-41 is a well-known member of the mesoporous molecular sieve which is largely used in selective adsorption and separation processes, shape selective catalysis, and chemical sensors. A narrow pore size distribution, high surface area and pore volume are some of the properties of this material, which make it a desirable sorbent. It is synthesized via a liquid-crystal templating mechanism [30]. During synthesis, the silicate material forms inorganic walls between the ordered surfactant micelles. After polymerization of the silica, the surfactant template is removed through either chemical or thermal treatment (calcination process). The hydrophobic and positively charged surface of CTAB on the CTAB-MCM-41 may conceivably trap PFCAs, with their anionic properties [31]. The ordered structure of the CTAB-MCM-41 conceivably enforces this interaction. Thus, CTAB-MCM-41 may result in high extraction efficiency, resulting in the efficient preconcentration and extraction of PFCAs.

In this study, we report a simple protein precipitation with consequent extraction and concentration by μ-SPE followed by LC–MS/MS analysis for the determination of 10 PFCAs including perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), and perfluorotetradecanoic acid (PFTeDA), in fish fillet. This protein precipitation sorption based assisted extraction method was used for the extraction of analytes and reducing interferences and matrix effects. A stable-isotopic internal standard (IS) was used for calibration. The method was then applied to the analysis of PFCAs in different fish fillet samples.

2. Experimental

2.1. Chemicals and materials

PFPA (97% purity), PFHxA (97%), PFHpA (99%), PFOA (96%), PFNA (97%), PFDA (96%), PFUdA (95%), PFTrDA (97%), and PFTeDA