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Simple, high throughput ultra-high performance liquid chromatography/tandem mass spectrometry trace analysis of perfluorinated alkylated substances in food of animal origin: Milk and fish

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ABSTRACT

The present study documents development and validation of a novel approach for determination of 23 perfluorinated alkylated substances (PFASs) in food of animal origin represented by milk and fish. The list of target analytes comprises four classes of PFASs, both ionic and non-ionic: 11 perfluorocarboxylic acids (PFCAs), 4 perfluorosulphonic acids (PFSAs), 5 perfluorosulphonamides (FOSAs) and 3 perfluorophosphonic acids (PFPAs). Fast sample preparation procedure is based on an extraction of target analytes with acetonitrile (MeCN) and their transfer (supported by inorganic salts and acidification) into the organic phase. Removing of matrix co-extracts by a simple dispersive solid phase extraction (SPE) employing ENVI-Carb and C18 sorbents is followed by an efficient sample pre-concentration performed by acetonitrile evaporation and subsequent dilution of residue in a small volume of methanol (matrix equivalent in the final extracts was 16 and 8 g mL^{-1}, for milk and fish respectively). Using modern instrumentation consisting of ultra-high performance liquid chromatography (UHPLC) hyphenated with a tandem mass spectrometer (MS/MS), limits of quantification (LOQs) as low as 0.001–0.006 μg kg^{-1} for milk and 0.002–0.013 μg kg^{-1} for fish can be achieved. Under these conditions, a wide spectrum of PFASs, including minor representatives, can be determined which enables collecting data required for human exposure studies. The pilot study employing the new method for examination of milk and canned fish samples was realized. Whereas in majority of canned fish products a wide spectrum of PFCAs, perfluorooctanesulphonic acid (PFOS) and perfluoro-1-octanesulphonamide (PFOSA) was detected, only in a few milk samples very low concentrations (LOQ levels) of PFOS and perfluorooctansulphonic acid (PFDS) were found.

1. Introduction

Perfluorinated alkylated substances (PFASs), a broad group of anthropogenic chemicals, are widely used in various industrial and consumer applications, mainly thanks to their unique ability to repel both water and oil [1]. Within the last decade, PFASs have been identified as "emerging" food and environmental contaminants, due to their presence in various types of abiotic and biotic matrices, including human tissues and fluids [2,3]. In order to enable a risk assessment associated with dietary exposure to PFASs, EFSA (the European Food Safety Authority) recommended that further data on their levels in foods and in humans would be desirable, particularly with respect to the human exposure assessment [4]. Therefore, an additional monitoring focused not only on perfluorooctanesulphonic acid (PFOS) and perfluorooctanoic acid (PFOA), which are the most known representatives of this group, but also on other PFASs is needed. On this account, in March 2010, Commission Recommendation 2010/161/EU invited the Member States to monitor the presence of PFOS and PFOA, compounds similar to PFOS and PFOA but with different chain length (C4–C15) and their precursors (perfluorooctane sulphonamide (PFOSA), N-ethyl perfluorooctane sulfon-amidoethanol (NEtFOSE) and 8:2 fluorotelomer alcohol), in order to estimate the relevance of their presence in food [5]. For this purpose, it is required to use a method of analysis that has been proven to generate reliable results. Ideally, the recovery rates should be in the range 70–120%, with limits of quantification (LOQs) of 1 μg kg^{-1}.

Currently, due to its high sensitivity and selectivity, liquid chromatography hyphenated with tandem mass spectrometry (LC–MS/MS) operated in the multiple reaction monitoring mode (MRM) is the preferred technique for a quantitation of PFASs trace levels. While more or less general agreement exists on an optimal determinative step, it is rather difficult to find an extraction strategy that would enable a rapid analysis of a wide range of both ionic and

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non-ionic PFASs in complex matrices such as food. One of the first sample processing procedures, developed by Hansen et al. [6] for trace analysis of PFASs in biotic matrices, was an ion-pair extraction into methyl *tert*-butyl ether (MTBE) employing tetrabutylammonium (TBA) as an ion-pairing agent. This approach was then used in various environmental studies; nevertheless, the robustness of such extraction strategy is questionable, since very variable recoveries of PFASs, ranging from <50 to >200%, were reported [7]. For instance, PFOS recoveries reported by Kannan et al. [8] for tuna, swordfish and dolphin livers were in the range 66–140%, for tuna blood 37–47%. In any case, ion-pair method is relatively laborious; and suffers from drawbacks such as co-extraction of lipids and other lipophilic matrix components, which significantly complicates PFASs analysis in fatty matrices. As regards the reported LOQs, when employing the ion-pair extraction method, these were typically in the range 0.1–1 $\mu g\,kg^{-1}$.

As a less laborious, faster alternative, solid phase extraction (SPE) represents the option for isolation and/or pre-concentration of PFASs from different biotic and abiotic samples. In the study by Taniyasu et al. [9], who tested HLB (hydrophilic–lipophilic balanced sorbents) and WAX (weak anion exchanger) cartridges in water analysis, it was observed that the latter ones were found more effective, because almost all tested PFASs were retained from water and recoveries ranged from 50 to 90%. The exceptions were neutral perfluorosulphonamides (PFOSAs) and perfluorotelomer alcohols (FTOHs), recoveries of which were lower, varying between 35 and 55%. Contrary to these results, Fromme et al. [10] reported mean recoveries on WAX cartridge only 12% for PFOSA and 63% for PFOA. Kärrman et al. [11], who also used Taniyasu's WAX SPE method for milk and serum obtained relatively low recoveries (not exceeding 50%) for all long chain perfluorocarboxylic acids (PFCAs) (>C11), perfluorosulphonic acids (PFSAs) (C10) and PFOSA.

As regards HLB SPE cartridge-based sample preparation, the limitation is low recoveries of the most polar, short chain (C4–C6) ionic PFCAs (typically less than 30%). It should be noted, that LOQs in published methods employing SPE for the analysis of biotic matrices, were significantly lower (varied from 0.01 to 0.2 $\mu g\,kg^{-1}$) compared to those achieved by ion pair methods. Unfortunately, none of the cartridges available at the market allows simultaneous retaining of all commonly monitored representatives of PFASs with acceptable recoveries.

To avoid the above limitations, sample preparation strategies consisting of a simple homogenization of a sample with respective extraction solvent, (optionally) followed by a simple clean-up and centrifugation/filtration and direct LC–MS/MS analysis were developed. Powley et al. [12] used methanol (MeOH) for extraction of environmental matrices and dispersive SPE with ENVI-Carb graphitized carbon for treatment of crude extract to remove matrix interferences. Hradkova et al. [13] used a similar approach to analyse PFASs in canned fish and seafood; MeOH was employed as an extraction solvent and activated charcoal for clean-up, thus replacing more expensive ENVI-Carb. Berger and Haukås [14], who analysed PFASs in animal livers, used a MeOH/2 mM aqueous ammonium acetate mixture (50:50, *v*/*v*), but this method provided lower recoveries (<50%) of long chain carboxylic acids (>C10) and non-ionic PFOSA. Recently, a micro-extraction method was developed by Luque et al. [15] for analysis of PFASs in biota; it employs a mixture consisting of tetrahydrofuran (THF) water (75:25, *v*/*v*). The main advantages of this approach were not only low sample amount required for analysis, but also rapid extraction and good recoveries of tested analytes. It should be emphasized, that although the methods mentioned in this paragraph are simple, fast and provide acceptable recoveries for a wide range of analyte/matrix combinations, due to the absence of any enrichment step, the achievable LOQs are similar to those of the ion-pair method.

The aim of the present study was to implement an innovative solution that would enable not only high throughput sample handling, but also accurate determination of entire set of analytes of concern at the ultra-trace level. For this purpose, QuEChERS (Quick, Easy, Cheap, Rugged and Safe) approach, originally developed by Anastassiades et al. [16] for determination of a wide range of pesticide residues in fruits and vegetables and then modified by Lehotay et al. for analysis of fatty matrices [17], was selected for a feasibility testing and follow-up validation. The benefits resulting from integrating of a new rapid sample processing QuEChERS strategy with a well-established LC–MS/MS determinative step were demonstrated. The key requirement for performance characteristics of this new analytical procedure was to achieve LOQs $\approx 0.01\ \mu g\,kg^{-1}$, which would enable generation of data needed for the dietary exposure assessment.

2. Experimental

2.1. Chemicals and reagents

Certified standards of PFAS in methanol and their isotopically labelled internal standards (see Table 1) were purchased from Wellington Laboratories (Canada). The purity of each standard was >98%. Anhydrous magnesium sulphate ($MgSO_4$) was obtained from Fluka (Germany), formic acid (95%), ammonium acetate for LC–MS and HPLC grade acetonitrile and methanol were purchased from Sigma–Aldrich (Germany). Sodium chloride, sodium hydroxide, hydrochloric acid and sulphuric acid were bought from Penta (Czech Republic), Bondesil C18 sorbent (40 μm) from Varian (USA) and Supelclean ENVI-Carb (particle size: 120–400 mesh) was obtained from Sigma–Aldrich (Germany).

2.2. Samples

The analytical method was developed and validated using samples of fresh fish (salmon, trout) and milk purchased in a local store. The method was also used for preliminary PFASs screening in 12 samples of milk (pasteurized and UHT, fat content varied from 0.5 to 3.5%) from different Czech producers and 16 canned fish products (7 mackerels, 3 sardines, and 6 cod livers). The canned fish were from Poland, Latvia and Sweden, thus most likely all fish were from Baltic Sea.

From the fresh fish (salmon, trout) only edible parts were homogenized and stored in freezer until its use. All canned fish products were in the vegetable or olive oil. Whole content of cans was homogenized and stored in freezer.

2.3. Sample preparation

7.5 g of homogenized fish muscle tissue and 10 g water were weighted into a 50 mL polypropylene (PP) centrifuge tube and mixed by shaking for 1 min. It should be noted that added water was 18 MΩ MiliQ water from apparatus without parts made from PFASs containing polymers and water was tested for contamination by target analytes. In the case of milk samples, 15 g was weighted and no water was added.

The following steps of sample preparation were the same for both matrices, fish and milk. Isotopically labelled internal standards were added to the sample and sample was mixed. 0.2 mL formic acid and 15 mL acetonitrile (MeCN) were added and the tube was vigorously shaken for 1 min by hand. In the next step, 6 g of $MgSO_4$ and 1.5 g NaCl were added and the tube was immediately shaken to prevent coagulation of $MgSO_4$. The tube was then centrifuged (Hettich, Germany) for 5 min at 11 000 rpm and 12 mL aliquot of the upper acetonitrile phase was transferred to a new 50 mL PP