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Liquid chromatography mass spectrometry determination of perfluoroalkyl acids in environmental solid extracts after phospholipid removal and on-line turbulent flow chromatography purification

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ABSTRACT

An on-line TFC (Turbulent Flow Chromatography) clean up procedures coupled with UHPLC–MS/MS (Ultra High Performance Liquid Chromatography Mass Spectrometry) multi-residue method was developed for the simultaneous determination of 8 perfluroalkyl carboxylic acids (PFCA, from 5 to 12 carbon atoms) and 3 perfluoroalkyl sulfonic acids (PFSA, from 4 to 8 carbon atoms) in environmental solid matrices. Fast sample preparation procedure was based on a sonication-assisted extraction with acetonitrile. Phospholipids in biological samples were fully removed by an off-line SPE purification before injection, using HybridSPE® Phospholipid Ultra cartridges.

The development of the on-line TFC clean-up procedure regarded the choice of the stationary phase, the optimization of the mobile phase composition, flow rate and injected volume.

The validation of the optimized method included the evaluation of matrix effects, accuracy and reproducibility. Signal suppression in the analysis of fortified extracts ranged from 1 to 60%, and this problem was overcome by using isotopic dilution. Since no certified reference materials were available for PFAS in these matrices, accuracy was evaluated by recoveries on spiked clam samples which were 98–133% for PFCAs and 40–60% for PFSAs. MLDs and MLQs ranged from 0.03 to 0.3 ng g^{-1} wet weight and from 0.1 to 0.9 ng g^{-1} wet weight respectively. Repeatability (intra-day precision) and reproducibility (inter-day precision) showed RSD from 3 to 13% and from 4 to 27% respectively. Validated on-line TFC/UHPLC–MS/MS method has been applied for the determination of perfluoroalkyl acids in different solid matrices (sediment, fish, bivalves and bird yolk).

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) constitute a class of man-made substances that have been produced and used in numerous industrial and commercial applications, mainly as fluorinated surfactants and fluoropolymer processing aids, since the 1950s [1]. PFASs include thousands of chemicals but the most prominent families are perfluoroalkyl carboxylic acids (PFCA), which include perfluorooctanoic acid (PFOA), and perfluoroalkyl sulfonic acids (PFSA), which include perfluorooctane sulfonic acid (PFOS) [2]. Perfluoroalkyl acids (PFAA) have a fully fluorinated carbon chain of variable length and a terminal carboxylate or sulfonate group. Because of their widespread distribution, high persistence in the environment and bioaccumulation capability [3], the European Commission included PFOS in the list of priority hazardous substances which must be monitored in biota living in the EU water bodies, setting an Environmental Quality Standard (EQS) in fish of 9.1 ng g^{-1} wet weight (ww) (Directive 2013/39/EU).

PFAA determination in biota by LC–MS is markedly affected by the influence of the biological matrix components on the analytes ionization in electrospray (ESI) [4], causing ion-suppression or –enhancement effects which were reviewed by Trufelli et al. [5]. Endogenous compounds and target analytes can compete for the available charge and space on the droplet surface causing an inhibition of ion ejection. Specific effects have been described for phospholipids [6] and fatty materials [7] which are thought to form a film on the droplet surface that inhibits ion evaporation.

Despite these effects could be less evident for PFAA which themselves act as surfactants, ion suppression or enhancement in ESI were reported by several authors [8]. In particular, phospholipids

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are a source of uncertainty in the quantification of PFAS in LC–MS in biological samples, because they are polar lipids and their highly ionic and surfactant nature influences the ionization and the desolvation of the droplets in ESI sources [9,10].

The biological matrix influence can be overcome by improving the preparative steps, e.g. by introducing clean-up after the extraction without adding excessive manipulation of the sample.

Turbulent Flow Chromatography (TFC) is a technique introduced in the late 1990s for the analysis of biological fluids that combines high-throughput, high reproducibility and reduced time-consuming sample clean-up [11]. TFC is effective in excluding molecules larger than 8000–10,000 Da, such as particulate and proteins. The sample is injected at high flow rate, higher than 1 mL min^{-1}, into 0.5–1.0 mm internal diameter columns packed with large particles (30–60 μm) whose pores are functionalized with different chemistries. Under the turbulent flow conditions the improved mass transfer across the bulk mobile phase allows all molecules to improve their radial distribution, but around the stationary phase particles a laminar zone persists, where diffusional forces still dominate the mass transfer process [12]. The smaller molecules, which diffuse faster than larger molecules, have time to interact with stationary phase and bind to pores, while the larger molecules are quickly flushed to waste. Because the resolution capability of the TFC column is low, the analytical separation is carried out on a coupled conventional analytical column under laminar flow.

In recent years TFC was applied to environmental waters and sediment samples as an automated clean-up step in the determination of emerging pollutants such as perfluoroalkyl compound [13], pharmaceuticals [14] and endocrine disrupters [15]. It has some advantages like minimum sample manipulation, low error introduction, very efficient extraction and good reliability, but exhibits also some limitations [16]. Matrix molecules are difficult to remove because physical mechanism of exclusion probably is not very efficient. In fact, in some cases target compounds and matrix molecules have the same dimensions and, consequently, same diffusion capability in the stationary phase and are not washed out by flow. Besides, if the analytes molecules have a wide spectrum of polarities and acidities, it is necessary to use different serially connected TFC columns to cover the whole range of analyte properties, but losing in selectivity [14].

The aim of our work is to optimize an on-line TFC purification and phospholipid removal procedure for the detection of PFAA in animal tissues and sediments. The optimized and validated method has then been applied to soft tissue of bivalve, egg, fish fillets and sediment samples collected during field research in Northern Italian and Swiss water bodies.

2. Materials and methods

2.1. Chemicals and reagents

Perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), tetrabutylammonium perfluorobutane sulfonate (PFBS), potassium perfluorohexane sulfonate (PFHxS), and tetrabutylammonium perfluorooctane sulfonate (PFOS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stable isotope labelled PFCA and PFSA used as internal standards (SIL-IS) (^{13}C2-PFHxA, ^{13}C4-PFOA, ^{13}C5-PFNA, ^{13}C2-PFDA, ^{13}C2-PFUnDA, ^{13}C2-PFDoDA, ^{18}O2-PFHxS, and ^{13}C4-PFOS) were purchased from Wellington Laboratories (Guelph, ON, Canada) as 2 μg mL^{-1} solution mixtures.

HybridSPE® Phospholipid Ultra cartridges (30 mg, 1 mL SPE Tubes) were obtained by Sigma Aldrich (St. Louis, Missouri, USA). Phosphatidylcholine solution (10 mg L^{-1}) was prepared by dissolution in acetonitrile of purified phosphatidylcholine of soybean origin (Epikuron 200 purchased from Cargill Inc. Minneapolis, MN, USA).

All reagents were analytical reagent grade. LC–MS grade Chromasolv methanol, LC–MS grade Chromasolv acetonitrile, ammonium acetate (99%), and concentrated formic acid were purchased from Sigma-Aldrich. Water (<18 MΩ cm resistivity) was produced by a Millipore Direct-QUV water purification system (Millipore, Bedford, MA, USA).

2.2. Standard preparation

1 g L^{-1} stock solutions of each analyte were prepared dissolving pure compounds in methanol. An intermediate mixed solution in methanol containing the 12 selected analytes at 5 or 10 mg L^{-1} was prepared by diluting the stock solutions. Acetonitrile standard solutions for calibration (0–100 μg L^{-1}), containing all analytes, were weekly prepared by serial dilution of the intermediate mixed solution. The SIL-IS solution was diluted to 40 μg L^{-1} with methanol. All standard solutions were stored at 4 °C.

2.3. Sample preparation

Few grams of environmental solid samples were just homogenized and weighed (soft tissue of bivalves: 5–10 g; yolk: 1 g; sediment: 5 g; fish fillet: 2 g) before extraction. The extraction was carried out using a slightly modified method after Lacina et al. [17]. 1.5 mL of water and acetonitrile solution (10:90 v/v) per gram of fresh sample were added to the solid sample into a 50 mL polypropylene (PP) centrifuge tube. Extraction mixture was spiked with 100 μL SIL-IS (40 μg L^{-1}) and 40 μL of formic acid and vigorously shaken.

Subsequently the tube was immersed in an ultrasonic bath for 15 min and then centrifuged for 10 min at 11,000 rpm at 10 °C. The extraction was repeated twice and the combined supernatants were transferred in a new 50 mL PP tube where 0.6 g $MgSO_4$ and 0.2 g NaCl per gram of fresh sample were added. The tube was immediately shaken to prevent coagulation of $MgSO_4$, centrifuged and stored at −4 °C for one night. The extract volume was reduced to 1 mL under a gentle nitrogen stream and, if needed, filtered through HybridSPE® Phospholipid Ultra cartridge to remove phospholipids which can interfere with the analysis of PFAAs [6,18]. Finally the extract was acidified with 50 μL of formic acid and transferred into the autosampler vial.

2.4. On-line-turbulent flow chromatography/UHPLC–MS/MS

The on-line TFC-UHPLC analysis was performed using a modified Thermo EQuan system which consists of a CTC PAL autosampler equipped with four 6-way VICI valves, two Thermo Scientific Accela LC pumps (600 and 1200) equipped with serially connected TFC (Thermo Fluoro XL, 50 × 0.5 mm and Thermo Cyclone™, 50 × 0.5 mm) and analytical columns (Thermo Hypersil GOLD PFP 1.9 μm, 50 × 2.1 mm). By this configuration (isocratic focusing mode) the organic solvent used to elute sample from the cleanup column is connected via a tee (T) junction into the aqueous flow from the analytical pump. The analytes are focused at the head of the analytical column by the isocratic aqueous makeup flow from the analytical pump, which makes hydrophilic compounds easier to capture [19].

Table SM1 reports the settings of the loading and analytical pumps at different times, while Fig. SM1 shows the operative schemes of the preparative steps. 50 μL of sample are injected into