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Development of an analytical strategy based on liquid chromatography–high resolution mass spectrometry for measuring perfluorinated compounds in human breast milk: Application to the generation of preliminary data regarding perinatal exposure in France

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ABSTRACT

Perfluorinated compounds (PFCs) are man-made chemicals for which endocrine disrupting properties and related possible side effects on human health have been reported, particularly in the case of an exposure during the early stages of development, (notably the perinatal period). Existing analytical methods dedicated to PFCs monitoring in food and/or human fluids are currently based on liquid chromatography coupled to tandem mass spectrometry, and were recently demonstrated to present some limitations in terms of sensitivity and/or specificity. An alternative strategy dedicated to the analysis of fourteen PFCs in human breast milk was proposed, based on an effective sample preparation followed by a liquid chromatography coupled to high resolution mass spectrometry measurement (LC–HRMS). This methodology confirmed the high interest for HRMS after negative ionization for such halogenated substances, and finally permitted to reach detection limits around the pg mL^{-1} range with an outstanding signal specificity compared to LC–MS/MS. The proposed method was applied to a first set of 30 breast milk samples from French women. The main PFCs detected in all these samples were PFOS and PFOA with respective median values of 74 (range from 24 to 171) and 57 (range from 18 to 102) pg mL^{-1}, respectively. These exposure data appeared in the same range as other reported values for European countries.

1. Introduction

Perfluorinated compounds (PFCs) are synthetic chemical substances produced and used for their amphiphilic properties (i.e. both lipophobic and hydrophobic) through anti-sticking material or surfactant related products (Kissa, 2001). PFCs are used in many applications, including oil and water repellent coatings for carpets, textiles, leather, paper, cardboard, and food packing materials; electronic and photographic devices; and surfactants in various cleaning agents, cosmetics, and fire-fighting foams (Prevedouros et al., 2005). They are also used as an essential processing aid in the manufacture of certain fluoropolymers such as polytetrafluoroethylene (PTFE) and to a lesser extent in industrial applications as antistatic additives, and in the electronics industry (Hansen et al., 2002). Consequently, consumers from industrialized countries are today in contact with these chemicals in their daily life, through a high number of manufactured products. In parallel, as many other chemicals of entropic origin, PFCs may be released into the environment at each step of their living cycle, and retrieved in various components of the food chain as well as in human biological matrices (Giesy and Kannan, 2001; Taniyasu et al., 2003; Simcik and Dorweiler, 2005; Fromme et al., 2007; Hölzer et al, 2009; Rylander et al., 2009).

Actually, PFCs are organic substances recognized to be persistent in the environment (Prevedouros et al., 2005), able to

Abbreviations: PFCs, perfluorinated compounds; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; EFSA, European Food Safety Agency.

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bio-accumulate and cause adverse effects to animals and human health. Thereby some of these substances (e.g. PFOS) fulfil the criteria to be classified as persistent organic pollutants (POPs) under the Stockholm convention (Wang et al., 2009). Hepatotoxicity (OCDE, 2002; 3M, 2003; US EPA, 2005), as well as impact on thyroid (US EPA, 2005) have been reported after exposure to PFOS and PFOA in laboratory animals. PFCs are also considered as endocrine disruptor chemicals (EDCs), with a particular debate related to their possible impact on reproductive functions in man (Liu et al., 2007; Fei et al., 2009; Liao et al., 2009). That's why, several studies looked into early exposure affecting prenatal and neonatal life, since it could explain the outbreak of diseases once fully grown. Thus, *in utero* exposure to PFCs in rodents was found to delay their development, reduce their postnatal survival and growth (Luebker et al., 2005; Abbott et al., 2009; Wang et al., 2010; Borg et al., 2010) and impairs their neurodevelopment (Apelberg et al., 2007). Regarding these observations, the question of a possible deleterious impact of an exposure to PFCs for human foetus and newborn is posed implying the need to generate accurate prenatal exposure data, for instance from cord blood samples (Inoue et al., 2004; Nakata et al., 2007), as well as newborn exposure data, notably from breast milk samples (Kuklenyik et al., 2004; Von Ehrenstein et al., 2009; Mosch et al., 2010). Such data were already provided from different countries but globally remain relatively scarce, and in particular never provided for France. The important analytical challenge associated to the measurement of these chemical pollutants (reduced sample volumes available for analysis, trace concentration levels, external contamination issues...) is one of the reasons explaining this situation.

The current highest state-of-the-art analysis in the field of PFCs is based on solid phase extraction (SPE) followed by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS) on triple quadrupole instruments (Berger et al., 2004; Kärrman et al., 2006; So et al., 2006; Bernsmann and Fürst, 2008; Liu et al., 2010; Roosens et al., 2010). These approaches were most often developed for perfluoroalkylcarboxylic and perfluoroalkylsulfonic acids, in most cases with a focus on PFOS and PFOA only, but sometimes on a larger range of compounds. Although these approaches are globally of high efficiency both from a qualitative and quantitative point of view, some limitations may be underlined in terms of sensitivity (with regard to the usually extremely reduced sample volumes available in the frame of such human studies) and/or specificity (with regard to the high stability of PFCs leading to limited fragmentations in MS/MS) (Benskin et al., 2007). In addition, some quantification problems due to coeluting interferents presenting the same diagnostic signals as certain PFCs were also reported (Inoue et al., 2004; Berger and Haukås, 2005; Chan et al., 2009). Chan et al. (2009) reported a possible overestimation of PFOS and PFHxS concentrations in serum samples using LC–MS/MS because of matrix interferences contributing to the monitored signal. In order to analyze these compounds with better specificity in biota, Berger and Haukås (2005) previously mentioned the use of a new analytical strategy based on high resolution mass spectrometry. However, this analytical strategy proved better sensitivity and specificity than tandem mass spectrometry; it was only used as a screening method.

In this context, the present study aimed at presenting an alternative confirmatory and quantitative method for the determination of PFCs in human breast milk based on liquid chromatography coupled to high resolution mass spectrometry (LC–HRMS). This methodology covered 14 target compounds belonging from the perfluoroalkylcarboxylic acids and perfluoroalkylsulfonic acids families, which are both the most commonly studied and effectively detected PFC representatives (Liu et al., 2010; Roosens et al., 2010). The method was validated according to current European criteria. Then it was applied to a set of 30 breast milk samples collected at a regional scale, providing the first set of preliminary exposure assessment data in France, which was compared to other similar data collected in other countries (Bets, 2007; Tao et al., 2008a,b; Völkel et al., 2008; Llorca et al., 2010).

2. Material and methods

2.1. Reagents and chemicals

Targeted compounds including 14 native and 10 ^{13}C-labeled internal standards are listed in Table 1. These standards (purity higher than 98%) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Methanol and acetone (picograde quality) were from UGC Promochem (Wesel, Germany). Mix working solutions containing the 14 target native PFCs at 0.1, 0.01, and 0.001 ng μL^{-1} as well as a mix containing 10 ^{13}C-labeled internal standards at 0.01 ng μL^{-1} were prepared by appropriate dilutions of commercial standards in methanol. All these solutions were stored in the dark at −20 °C. The concentration levels covered by the prepared calibration solutions ranged from 25 to 2000 pg mL^{-1}. Ammonium acetate, glacial acetic acid and ammoniac (32%) were purchased from Merck (Darmstadt, Germany). Deionised water (>18 m Ω cm) was obtained from nanopure system (Barnstead, Germany). Formic acid and 500 mg single use Envicarb® cartridges were acquired from Supelco (Sigma-Aldrich, Saint-Quentin Fallavier, France). Oasis® HLB 500 mg cartridges were from Waters (Guyancourt, France).

2.2. Sample collection

The 30 breast milk samples analyzed in the present study were collected in the frame of a French regional research project globally aiming to investigate the link between perinatal nutrition and metabolic fingerprint/programming. Thus, one particular aspect of this project was dedicated to the characterization of the chemical pollutant content of breast milk as a possible contributor to the studied biological endpoints (metabolic syndrome, prematurity...) in addition to the genetic or nutritional factors. Breast milk samples were randomly collected from several nurseries in Human Milk Biobank (LACTATHEQUE DC-2009-0982), declared and approved by local ethic committee on 24th June 2010. For each donation, mothers were asked to complete a medical questionnaire integrating biobank consent. Socio-demographic information including gestity, parity, age, number of infants, number of infants breastfed, newborn weight, and mother's profession were collected. All samples were stored at −20 °C until analysis.

2.3. Sample preparation

The analytical strategy used for measuring PFCs in breast milk included a liquid/liquid extraction (LLE) followed by a purification on two successive Solid Phase Extraction (SPE) systems, i.e. Oasis® HLB and carbon graphitized (Envicarb®) cartridges. First of all in a polypropylene (PP) tube, 9 mL of acetone were added to 3 mL of milk previously spiked with 0.5 ng of internal standards. The PP tube was then capped and thoroughly mixed using a vortex chemical mixer for 30 s and then allowed to extract during 10 min in an ultrasonic bath at room temperature. Afterwards the sample was centrifuged and the supernatant was transferred to another PP tube and evaporated to 3 mL at 45 °C under a gentle nitrogen stream. Then, 8 mL of 0.1 M formic acid were added to adjust the pH, and the extract was loaded on the Oasis® HLB cartridge which was previously conditioned with 10 mL of MeOH, and 10 mL of 0.1 M formic acid. The cartridge was then washed twice with first 5 mL of 0.1 M formic acid and then 5 mL of MeOH/0.1 M formic acid (50:50, *v*/*v*), before eluting the target compounds with 6 mL of MeOH/NH_4OH (99:1, *v*/*v*). The extract (evaporated to around