DETERMINATION OF 16 PERFLUORINATED COMPOUNDS IN MILK AND BREAST MILK BY UPLC - MS/MS

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Introduction

Per and polyfluorinated compounds (PFASs) show at once very high chemical, thermal, biological stability due to the strength of the C-F bound and amphiphilic properties. These particular chemical properties make these synthetic substances widely used through numerous industrial and commercial applications. Indeed, these applications cover a large spectrum from oils and water repellent coatings for carpets, textiles, leather, paper, cardboard and food packing materials to electronic photographic devices or surfactant in various cleaning agent, cosmetics and fire-fighting foams[1].

Due to the extensive human exposure, hence the European Union encourages member states to develop methods for the monitoring of PFAS in food[2]. In the same time several publications revealed the presence of PFAS in human breast milk[3-5]. Therefore we developed a method for PFAS analysis in milk and breast milk.

The selection of PFAS was based upon the last scientific report of EFSA[6]. We focused our development on the most frequently discovered PFAS: 11 perfluoroalkyl carboxylic acids (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoA, PFTrDA, PFTeDA), 4 perfluoroalkyl sulfonates (PFBS, PBHxS, PFOS, PFDS) and 1 perfluoroalkyl sulfonamide (PFOSA).

Our methods for PFAS determination were developed and validated with the aim to meet the EU recommendations that suggest both limits of quantification of 1µg/kg and recoveries between 70 and 120%[2]. As we are dealing with population exposure studies, the optimization of the method has been designed to lower the limit of quantification to 0.1 µg/kg, well below the limits currently requested by the EU.
Finally the method has been used to quantify PFASs in several Belgian breast milk samples.

Materials and methods

Sample preparation

The sample extraction procedure for the milk matrix we use, has been derived from a protocol described elsewhere [3, 7]. First of all, labeled standards for 10 PFASs were added just before extraction as internal standards. The selected method begins with a liquid/liquid extraction with acetone. This extraction was followed by a first clean-up on a SPE-WAX (OASIS) column and a second clean up on an ENVI-Carb (SUPELCO) column. M8PFOS was added as external standard during the reconstitution of final extract.

UPLC-MS/MS measurement

The determination was achieved with an effective run of 7 min thanks to an UHPLC-ESI$^-$-MS/MS (Acquity System coupled with a XEVO tandem mass spectrometer, Waters) equipped with an Acquity BEH C18 column (2.1*100mm, 1.7µm). Mobile phase A contained H_2O ammonium acetate 20 mM and mobile phase B contained methanol. The percentage of mobile phase A was changed as follows: 0 min: 70%; 7 min: 0%; 9 min: 0%, 9.1 min: 70%; 12 min: 70%. The injection volume was 7.5 µl in partial loop mode with needle overfill.
Background interferences from apparatus, especially PFOS, were eliminated by PFAS isolator column (Waters) placed between the solvent mixer and the injector.
In order to confirm the compounds presence or absence, a secondary transition was also followed by MS/MS. While the secondary transition did not exist or was too weak, other columns with different selectivity were used

to confirm the presence or the absence of compounds. These columns were Supelcosil LC-PAH 150*4.6mm, 5µm, SUPELCO and Hypercarb 100*2.1mm,5µm, THERMO.

Results and discussion

Preliminary studies for method development

The method development revealed that the solvent composition of the final extract is a critical point. To illustrate that, PFAS solvent mixture at 10 ng/ml was prepared with 2 different proportions of methanol/water : 50/50 (Fig.1 a, b) and 90/10 (Fig.1c, d). The signal increased by a factor 10 for the PFTrDA when the proportion of methanol in the vial increased from 50% (Fig.1a) to 90% (Fig.1c). A high proportion of methanol in the vial enables to avoid losses of the long chain PFAS. However when the proportion of methanol is higher than the initial condition, the peak shape of the short chain becomes wider (fig. 1d) nevertheless without consequences on the quantification of these compounds.

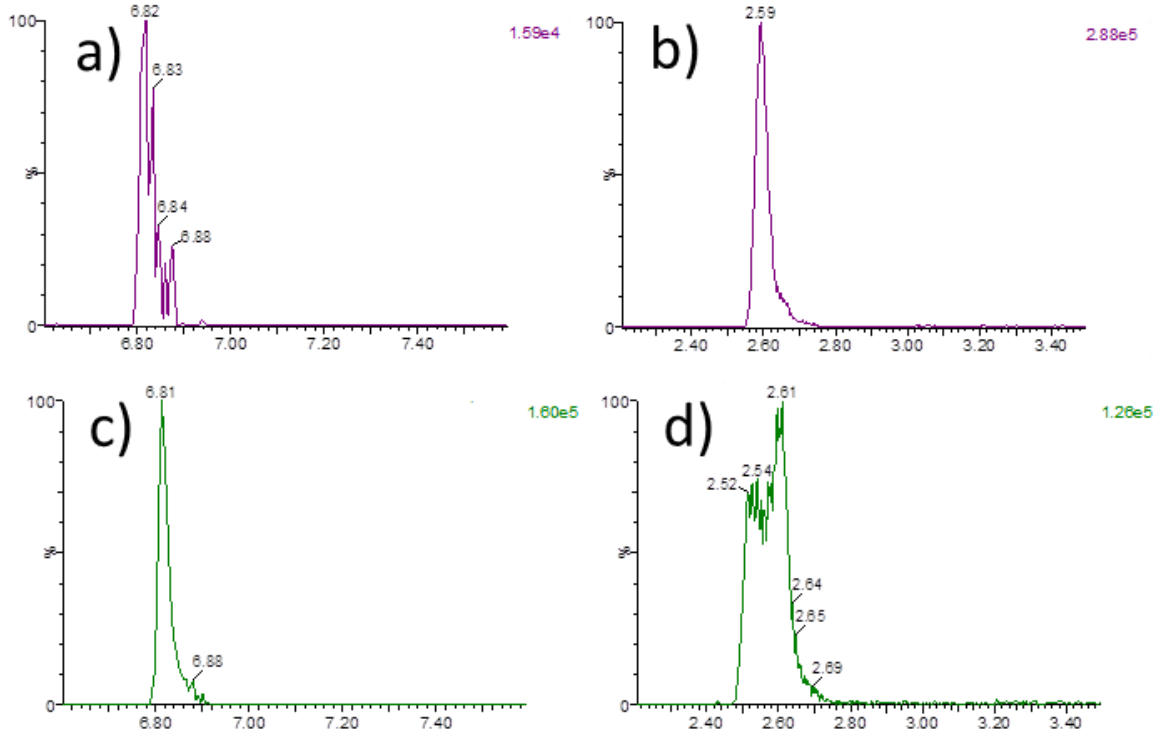

Figure1: Chromatograms obtained at 10ng/ml for PFPeA (b, d) and PFTrDA (a, c) in different proportion of methanol/water: 50/50 (a,b) and 90/10 (c,d).

Validation study

The performance of the method was evaluated through the limits of detection and quantification, the linearity, the matrix effect, the recovery, the within-day repeatability and day-to-day reproducibility.

Practical limits of quantification of 0.1 µg/kg (even 0.05 µg/kg for few compounds) were achieved for milk matrix which is much lower than the EU recommendation. Thank to labeled surrogates no matrix effect were observed.

The precision and the recovery of the analytical method were evaluated on milk samples spiked at 3 concentration levels (0.5, 1 and 2 µg/kg). 3 samples per concentration level were analyzed at 3 different days. The procedure was also evaluated on breast milk samples from 6 samples spiked at 1 µg/kg. All PFASs had apparent recoveries close to 100% using labelled surrogates. The extraction yield was around 50-70% for the majority of compound except for PFOSA, PFTrDA, PFTeDA (20-30%) however isotope dilution methodology corrects this drawback. Similar recoveries were obtained for milk and breast milk samples.
For the majority of PFASs, the relative standard deviations (RSDs) were much lower than 20% for within-day repeatability and day-to-day within-reproducibility.

Analysis of real breast milk sample
Several Belgian breast milk sample were collected and analysed following protocol described hereinabove. A previous study[8] on these samples was focused on the analyses of individual milk samples for nine ‘‘basic POPs” (chlorinated pesticides and indicator PCBs) and of pooled milk samples for ‘‘basic POPs”, ‘‘advanced POPs” (dioxins and dioxin-like PCBs) and ‘‘optional POPs” (polybrominated diphenylethers [PBDEs], polybrominated dioxins and dibenzofurans [PBrDD/F], mixed halogenated dioxins and dibenzofurans [PXDD/F] and hexabromocyclododecane [HBCD]).

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