ANALYSIS OF PERFLUORINATED COMPOUNDS: COMPARISON BETWEEN LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY AND HIGH RESOLUTION MASS SPECTROMETRY

Veyrand B[1], Sticca P[2], Pagliuca G[2], Durand S[1], Duigou A[1], Monteau F[1], Cariou R[1], Marchand P[1], Le Bizec B[1]

[1]LABERCA, Route de Gachet, BP50707, 44307 Nantes Cedex 3, France; [2]University of Bologna, Department of Veterinary Public Health and Animal Pathology, Via Tolara di Sopra, 50 I 40064 Ozzano Emilia, BO, Italy

Abstract

Perfluorinated compounds, described by some scientist as the "PCBs of the twenty-first century", are a group of contaminants which present bioaccumulative and persistent properties. Significant amounts of these compounds were detected in human blood and in the liver of many species, and it appears that a potential risk could exist for developmental and other adverse effects associated with exposure in humans.

Due to the high affinity of these compounds with the proteins, milk appears to be a highly exposed matrix. Contamination could be present naturally (transfer from cow to milk) or during the processing of milk. The potential toxicity of this matrix is of high concern, considering the large amounts of milk consumed by children.

A sensitive and specific method has to be used in order to quantify accurately these compounds in cow milk. Extraction and purification processes present major problems due to the high complexity of the matrix, and they have been optimized using different protocols. A detection method by liquid chromatography coupled to tandem mass spectrometry has been developed, which enables the quantification of 15 perfluorinated compounds, especially PFOS and PFOA. The advantages of using a high resolution system are described in terms of sensitivity and specificity.

Introduction

Perfluorinated compounds (PFCs) comprise a family of manmade chemicals that have been used for decades to make products that resist heat, oil, stains, grease and water. The family of PFC includes the perfluoroalkyl sulfonates (of which perfluorooctane sulfonate, PFOS) and the perfluoroalkyl carboxylates (of which perfluorooctanoate, PFOA). Most of these compounds are present in the environment as a result of human manufacture and use. Release of PFCs into the environment can occur at each stage of the fluorochemical product's life cycle. Many of the degradation products of PFCs have been found in the environment throughout the world, but PFOS and PFOA are the two compounds most widely detected. Because of the strong carbon–fluorine (C–F) bond associated with PFCs, PFOS and PFOA are environmentally persistent substances that have been detected worldwide in human blood, water, soils, sediments, air, and biota samples. No clear association between human exposure to PFCs and adverse health effects has been established. However, on the basis of the following results from animal studies[1,2], a potential risk could exist for developmental and other adverse effects associated with exposures to PFCs in humans. Concerning metabolism and pharmacokinetics of PFOS and PFOA in humans, there are very limited data although these compounds have been fairly extensively studied in animals. Both compounds have been found to be persistent and bioaccumulative, with species differences in their elimination half-lives (via urine and faeces), which vary from days in rats and months in monkeys to years in humans[3,4]. In the year 2000, after 50 years of production, the company 3M announced that it was phasing out the manufacture of PFOS chemicals. Following this announcement, in October 2000, the US Environmental Protection Agency (EPA) proposed a significant new use rule (SNUR) for 88 PFOS-related substances. PFOS and related substances have also been on the agenda of the Organisation for Economic Co-operation and Development (OECD) since the year 2000. As a result, the OCDE has not recommended action for a ban but rather that the governments contact PFOS manufacturers in their countries to determine whether the companies have plans to phase out PFOS production. On the basis of a risk assessment, the European Commission has on December 2005 presented a proposal for a directive containing restriction measures for PFOS. With Directive 2006/122/EC, published in the Official Journal of the European Union, the placing on the market and use of PFOS as well as of PFOS containing preparations and articles is now prohibited. Exempted are only uses for which there are no suitable substitutes or alternative processes available. However, the Commission is obliged to review each of the derogations as soon as new information of uses and safer

alternative substances or technologies for the uses become available.
Perfluorinated compounds are generally detected using HPLC coupled to tandem mass spectrometry with electrospray ionisation in the negative ion mode (ESI-). Only trace levels of these contaminants are present in biological samples, therefore a sensitive and specific method had to be used in order to quantify them accurately, especially in high complex matrices such as milk. In this study, a detection method has been optimized and applied to the optimization of the milk purification steps. The advantages of using a high resolution system are described in terms of sensitivity and specificity

Materials and Methods
Standard compounds were obtained from Wellington Laboratories (Guelph, Canada). Solvents were Picograde® quality and provided by LGC Promochem (Wesel, Germany).
The LC separation was achieved on a Uptispher HSC stationary phase (50 x 2 mm, 5 µm, Interchim), coupled to an Agilent pump HP1200. The elution solvents were ammonium acetate in water (20 mM) (A) and methanol (B). The compounds were separated with the following gradient: A/B 75/25 for 1 min, from 75/25 to 50/50 in 0.55 min, from 50/50 to 30/70 in 1 min, from 30/70 to 0/100 in 14 min, 0/100 for 3 min. The gradient was linear and the flow rate was set at 0.2 mL/min. The injected volume was 10 µL. The detection was performed on a Agilent HP6410 triple quadrupole analyser (Palo Alto, CA, USA) operated in negative electrospray ionization (ESI-) and in selected reaction monitoring acquisition mode (SRM). Nitrogen was used as nebulisation and desolvatation gas, at 40 psi and 6 L/min, respectively. Potential applied onto the capillary was 2.0 kV. Cone potential and collision energy were optimized for each molecule. In the collision cell, nitrogen was used as a collision gas.
LTQ-Orbitrap was performed on a Thermo Electron model LTQ-Orbitrap hybrid MS system equipped with a model Surveyor LC. The linear ion trap (LTQ) part of the hybrid MS system was equipped with an electrospray ionization probe and operated in the negative ion mode. Product ions were transferred to the Orbitrap part of the instrument for accurate mass measurement at high resolution (15 000 FHWM on m/z 400). Datas were acquired from 55 to 500 Da and processed using XCalibur v 2.0 software.

Results and Discussion

Detection method
A detection method has been optimized using liquid chromatography coupled to tandem mass spectrometry with negative electrospray ionization. MS/MS detection of perfluorocarboxylic acids does not present major problems due to the relatively good fragmentation of the compounds leading numerous diagnostics ions either in MS^2 or MS^3. Typically, the ion resulting from the CO_2 loss ($[M\text{-}H\text{-}CO_2]$) is characterized by a good sensitivity, thus permitting to achieve low LODs. Detection of perfluorosulfonates is more complex, due to the high stability of the molecular ion ($[M\text{-}H]^-$). Even under drastic conditions (high energy voltage applied to the fragmentor and the collision cell), only few fragments ions are generated; when observed, they mainly correspond to low m/z ions such as $[SO_3]^-$ or $[FSO_3]^-$ whatever the conditions used, $[M\text{-}H]^-$ remains the base peak of the MS. In order to increase the detection level, a pseudo-MRM (following the transition $[M\text{-}H]^- > [M\text{-}H]^-$) was tested and showed a high sensitivity: 20V applied in the collision cell enabled a high fragmentation of interferences a kind of mass clean-up), and finally an improved signal-to-noise in biological matrices. This sensitive transition, coupled to an additional one consisting in $[M\text{-}H]^- > [SO_3]^-$ or $[M\text{-}H]^- > [FSO_3]^-$, allows for a very sensitive and specific detection.

Performances of the analytical method were evaluated on standards samples, and very good results were obtained: as illustrated in Figure 1, perfluorosulfonates were detected with a good sensitivity for an injection of 5 picogrammes of each compound. Perfluorocarboxilic acids were all detected at 10 pg injected with excellent signal-to-noise ratios. This very promising detection would be in accordance with the relatively low concentrations expected in cow milk.

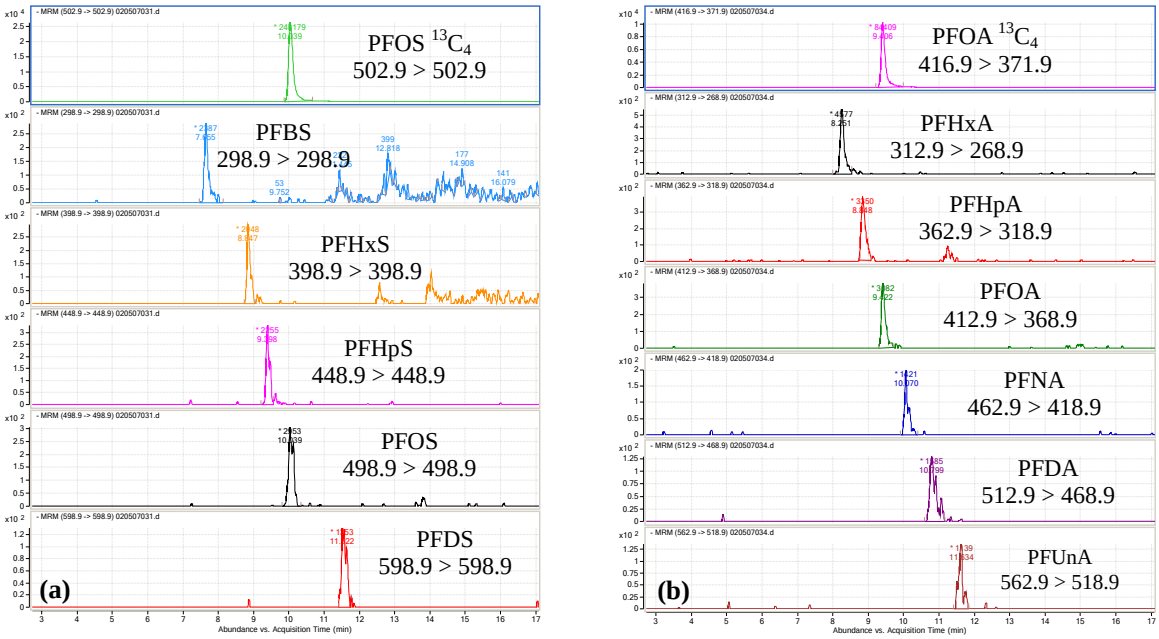

Figure 1 : Chromatograms of : (a) standard mixture of perfluorosulfonates (5 pg injected) and (b) perfluorocarboxylic acids (10 pg injected)

The linearity was assessed on eight calibration levels for each analyte, based on the analysis of different standards solutions, over the respective range of 0 to 5 ng injected. PFOA and PFOS, correlation coefficients (R^2) were better than 0.99 for all analytes, proving the adequacy between the measured signal and the concentration, and the adequacy of the internal standards.

• High resolution mass spectrometry

Perfluorinated compounds were analysed using LTQ-Orbitrap® in order to compare sensitivity and specificity of both instruments. Mix solutions were analysed under MS^3 conditions (linear trap) and accurate mass acquisition (orbitrap).

Perfluorocarboxylic acids, which are characterised by a propensy to fragment at low energy potentials, can be analysed under MS^3 conditions, as described in Figure 2 for PFOA. Use of transition 413>369>219 or 413>369>169 have been found very specific and the resulting chromatograms are characterised by a quasi-absence of interferences.

Perflorosulfonates, which are very stable even under drastic conditions (high collision energy) could not be efficiently analysed under MS^n conditions. The approach consisting in accurate mass measurement (R>15000) is obviously a better strategy for this class of compounds. As shown in Figure 2, chromatograms of a fortified milk sample (0.1 ng/mL) are characterized by an absence of noise, guaranteeing high sensitivity and specificity.

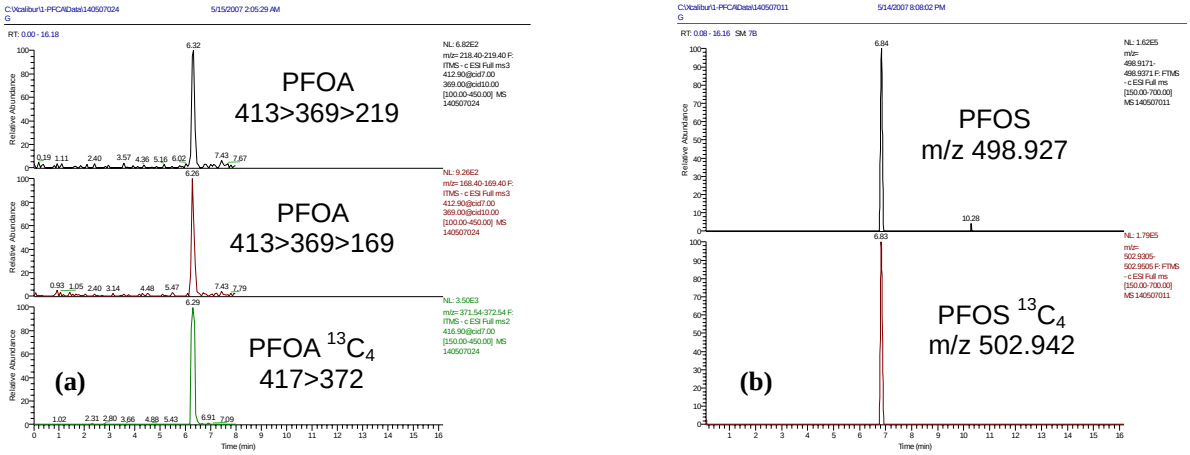

Figure 2 : Milk sample spiked to 0.1 ng/ml – Ion chromatograms observed on a LTQ-Orbitrap after negative electrospray ionisation using (a) LC-MS^3, SRM acquisition (linear trap) characteristic of PFOA (413>369>219 ; 413>369>169) and PFOA $^{13}C_4$ (417>372) (b) LC-HRMS (Orbitrap) characteristic of PFOS (m/z = 498.927) and PFOS $^{13}C_4$ (502.942).

Method development

In this study, different techniques of extraction (Ion Pairing Extraction) and purification (SPE Oasis HLB, SPE Oasis WAS, SPE Envi-Carb…) of PFCs in milk were tested.

Protocols have been already described[5], but only for human milk. Extraction-purification in cow milk is more complex due to higher protein content and lower carbohydrate content, and the transposition from human to bovine is not directly feasible.

Conclusions

Analysis of perfluorinated compounds in biological matrices is a real challenge. Three approaches were compared to analyse PFCs in biological matrices: MS^2 using a triple quadrupole, MS^3 using a linear trap and HRMS using Orbitrap. All approaches demonstrated linearity in the response. For PFOS, the MS^n approach was not found as the ideal strategy due to the high stability of this class of PFCs. A HRMS approach (R>15000) appeared as an interesting alternative when a multiresidue survey is requested.

Acknowledgements

We would like to thank Dr. Antonia Calafat, Dr. Charles Powley and Dr. Stefan Van Leeuwen for their kind response to our requests and Florence Ramdin for the help of the redaction of this proceeding.

References

1 - EPA, Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic acid and its salts (PFOA). U.S. Environmental Protection Agency: Washington, DDDDC, 2005

2 - OECD. Hazard Assessmen of Perfluorooctane Sulfonate (PFOS) and its salts. http://www.oecd.org/dataoecd/23/18/2382880.pdf, pp 1-362. Organisation for Economic Cooperation and Dvelopment (OECD).2002

3 - Footitt A, Nwaogu T (2004) Proposal for regulations on PFOS related substances. Risk & Policy Analysts Ltd./BRE Environment, London

4 -Burris JM, Lundberg JK, Olsen GW, Simpson C, Mandel JH (2002) Determination of serum half-lives of several fluorochemicals: Interim Report 2 (US EPA Doc: AR226-1086). 3M/Corporate Occupational Medicine Department, US EPA, Washington, DC

5 - Kuklenyik Z., Reich J.A., Tully J.S., Needham L.L., Calafat A., *Environ. Sci. Technol*., 38 (2004) 3698-3704