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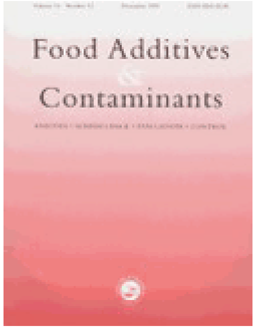

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	where encountered in the present case in fish samples but that may be present in other biological samples, were found particularly disturbing in low resolution MS/MS. Indeed, these interferences presented the same retention time and diagnostic signals as PFOS, leading to a possible overestimation of the PFOS quantification in LC-MS/MS. On the other hand, high resolution MS and MS/MS (LTQ-Orbitrap and Q-TOF) provided better results in terms of signal specificity and sensitivity. For instance, the estimated limits of detection (LOD) reached for PFOS on QqQ, Q-TOF and LTQ-Orbitrap instruments were 3.8, 0.7 and 0.5 pg injected, respectively.

Comparative study of low versus high resolution liquid chromatography - mass spectrometric strategies for measuring perfluorinated contaminants in fish

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Abbreviations: Multiple Reaction Monitoring (MRM), Quadrupole Time-of-flight (Q-TOF), High Resolution Mass spectrometry (HRMS), Low Resolution Mass Spectrometry (LRMS)

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Abstract

Perfluorinated compounds (PFCs) are usually monitored by high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) on triple quadrupole instruments. Although not yet widely implemented in the field, high resolution mass spectrometry (HRMS) today appears as a valuable alternative for these halogenated chemicals due to their significant mass defect. Indeed, this second approach offers a way to cope with particular matrix effects caused by co-eluting and isobaric interferences affecting the measurement of some PFCs in fish. The present study has compared three different LC-MS related instruments and various signal acquisition modes, from low resolution full scan and selected ion monitoring (SIM) mode on triple quadrupole (QqQ) instrument to high resolution full scan or product ion scan mode on orbital trap (LTQ-Orbitrap) or quadrupole-time-of-flight (Q-TOF) devices. Performances have been compared for 7 model compounds belonging to 7 PFCs sub-classes (perfluoralkylsulfonate, perfluoroalkylcarboxylate, perfluoroalkylsulfinate, perfluoroalkyl-sulfonamide, fluorotelomer saturated acid, fluorotelomer unsaturated acid and perfluoroalkylphosphonic acid). Low resolution MS/MS was found to be unsurprisingly reliable for extended multi-residue monitoring. However, the high stability of PFCs leads to a relatively poor and non-specific fragmentation pathway in MS/MS. In addition, biliary acid interfering compounds (e.g. taurochenodeoxycholic acid), that where encountered in the present case in fish samples but that may be present in other biological samples, were found particularly disturbing in low resolution MS/MS. Indeed, these interferences presented the same retention time and diagnostic signals as PFOS, leading to a possible overestimation of the PFOS quantification in LC-MS/MS. On the other hand, high resolution MS and MS/MS (LTQ-Orbitrap and Q-TOF) provided better results in terms of signal specificity and sensitivity. For instance, the estimated limits of detection (LOD) reached for PFOS on QqQ, Q-TOF and LTQ-Orbitrap instruments were 3.8, 0.7 and 0.5 pg injected, respectively.

Keywords: chemical food safety, mass spectrometry, perfluorinated contaminants, PFOS, PFOA.

INTRODUCTION

Perfluorinated compounds (PFCs) are synthetic chemical substances produced and used for their hydrophobic and lipophobic properties, as 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 some cleaning agents, cosmetics and fire-fighting foams. They are also used as an essential processing aid in the manufacture of some fluoropolymers such as polytetrafluoroethylene (PTFE) and to a lesser extent as antistatic additives in industrial applications and in the electronics industry (Hansen et al. 2002). Consequently, consumers from industrialised countries are daily in contact with these chemicals, through a high number of manufactured products. Furthermore, as many other chemicals of anthropogenic origin, PFCs may be released into the environment at each step of their life cycle and found in various food chain components. Food, especially through particular vectors of chemical exposure such as fish, represents a main source of consumers' exposure to PFCs (Fromme et al. 2007; Simcik and Dorweiler 2005; Taniyasu et al. 2003; Giesy and Kannan 2001; Rylander et al. 2009; Hölzer et al. 2009).

Perfluorinated compounds include a large group of chemicals which are characterized by a fully fluorinated hydrophobic linear or branched carbon chain attached to various hydrophilic moieties. This chemical structure explains their physico-chemical characteristics such as chemical and thermal stability, low surface free energy and surface active properties. The C–F bond is particularly strong, and is resistant to various degradation modes, including reaction with acids and bases, oxidation, and reduction. While some PFCs undergo chemical transformations, these reactions occur mainly on the hydrophilic group of the molecule rather than on the perfluorinated alkyl chains moiety (3M 1999). The most commonly studied PFC substances are perfluoalkylsulfonates and carboxylates. Among these, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are two main representative of the PFCs family (Figure 1).

PFOS is classified as a persistent, bioaccumulative and toxic chemical substance, thus fulfilling the criteria for being considered as a persistent organic pollutant (POP) under the Stockholm Convention (Wang et al. 2009). Moreover, recommendation 2010/161/EU was adopted to encourage member states to develop methods for the monitoring of perfluorinated compounds in food. The toxicity of PFOS and PFOA has been studied mainly in rodents. On the whole, hepatotoxicity, developmental toxicity, immunotoxicity, hormonal and neuroendocrine effects, as well as carcinogenic potency are the observed effects of main concern (Fei et al. 2009, Olsen et al. 2009, Wolf et al. 2007; Austin et al. 2003). More recently, some *in vitro* genotoxicity studies indicated that PFOS and/or PFOA may induce oxidative stress, apoptosis or increase the potential genotoxicity of other chemicals in a multi-exposure context (Liu et al. 2007a, 2007b, Yao and Zong 2005, Jernbro et al. 2007). Moreover, PFOS and PFOA have been suspected to have an effect on human reproduction and development during pregnancy (Trudel et al. 2008, Fromme et al. 2009). Recent studies on fluorotelomer acids – another class of fluorinated compounds – have revealed that their toxicity was higher than that of carboxylic acids on crustacean (Philipps et al. 2007).

Numerous PFCs have been detected in nearly all environmental media and biota, reflecting the widespread global pollution in all parts of the ecosystem (Pistocchi et al. 2009). Two recent surveys of PFCs in food samples, carried out in the UK and Sweden (EFSA 2008), provided some European country-related data. According to the EFSA (European Food Safety Agency) expert panel, "*Data on PFAS in food from monitoring activities in the EU countries are on the whole insufficient and the contamination of most foodstuffs cannot be characterised at present*" (EFSA 2008). However, fish and fisheries products have been identified as particularly contaminated products. Several studies report PFCs' presence in fish at concentrations varying from some ppb to hundreds of ppb (Taniyasu et al. 2005, Furdui et al. 2007, Nania et al. 2009). In this matrix, PFOS concentrations are almost invariably higher than PFOA concentrations and the PFOS concentrations in fish liver are consistently higher than those in fillet. PFOS has been shown to bioaccumulate in fish, with an estimated time clearance of 50% in fish around 100 days (EFSA 2008). Thus, fish seems to be an important source of human exposure to PFOS, as confirmed by EFSA in 2008: "*Based on the limited information available, fish and fishery products seem to be one important source of human exposure to PFOS and PFOA*". A specific population characterised by high fish consumption such as the Inuit community was also confirmed as particularly exposed to PFOS (Dallaire et al. 2009).

The main measurement technique for perfluorinated compounds remains liquid chromatography coupled to tandem mass spectrometry on triple quadrupole instruments after negative electrospray ionization. However, the need for an unambiguous quantification of an extended range of monitored compounds justified the evaluation of alternative approaches, especially those based on high resolution instruments. Indeed, Guo et al. 2008 reported the benefits of using other instruments such as a Quadrupole –time of flight (Q-TOF). Besides, Llorca et al. 2009 suggested a method based on an hybrid mass analyzer QqLIT (Quadrupole-Linear Ion trap) system to achieve unequivocal identification and quantification of PFCs compounds. So far, no comparison of the performances for different systems has yet been performed. For that purpose, the present study has compared three different LC-MS related instruments and various signal acquisition modes, from low resolution full scan and selected ion monitoring acquisition on triple quadrupole (QqQ) to high resolution full scan or product ion scan mode on an orbital trap (LTQ-Orbitrap) or a quadrupole-time-of-flight (Q-TOF). After having characterized the global spectrometric behavior of 7 PFCs in MS and MS/MS modes, the different tested technologies were discussed in terms of performances (specificity, sensitivity, dynamic range, linearity…), advantages and limitations, and possible drawbacks in the scope of a multi-residue monitoring.

Materials and methods

Reagents and Chemicals

All the following reference substances were purchased from BCP Instruments (Wellington Laboratories, Guelph, Ontario, Canada): PFOA (perfluoro-n-octanoic acid) ; PFDA (perfluoro-n-decanoic acid); PFOS (potassium perfluorooctanesulfonate) ; FOEA (2-perfluorooctylethanoic acid) ; FOUEA (2H-perfluoro-2-decenoic acid); PFOSi (sodium perfluoro-1-octanesulfinate acid); PFDPA (perfluorodecylphosphonic Acid) ; FOSA (perfluoro-1-octanesulfonamide) and mass labeled standards PFOA $^{13}C_4$ (perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid); PFOS $^{13}C_4$ (sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate); FOUEA $^{13}C_2$ 2H-perfluoro-[1,2-$^{13}C_4$]-2-decenoic acid; PFOSi $^{13}C_4$ sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]-octanesulfinate; FOSA $^{13}C_8$ perfluoro-1-[$^{13}C_8$]octanesulfonamide. Taurochenodeoxycholic acid, tauroursodeoxycholic acid, taurodeoxycholic acid, taurohyodeoxycholate acids were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Methanol (picograde quality) was provided by UGC Promochem

(Wesel, Germany). Ammonium acetate and glacial acetic acid were from Merck (Darmstadt, Germany). Deionised water (<18 MΩ.cm) was obtained from nanopure system (Barnstead, Germany). Supelclean envicarb® was acquired from Supelco (Sigma-Aldrich, Saint-Quentin Fallavier, France).

Sampling and sample preparation

As a prerequisite, all materials used for sampling and sample preparation were washed with acetone in order to avoid cross-contamination between samples. Fish samples were freeze-dried to obtain a minimum of 2 g for each sample, further ground and homogenized. Then, 1 g of dried sample was transferred in a 50 mL polypropylene tube, in which 2 ng of each internal standard were added before vortexing. A liquid solid extraction (LSE) using 15 mL of MeOH was performed. The sample was then mechanically agitated for 15 minutes. After centrifugation, 3 mL of supernatant were purified using dispersive solid phase extraction with Envicarb stationary phase, according to a method described by Powley et al. (2005). An adaptation of this procedure included the addition of 200 µL of glacial acetic acid to 150 mg of the graphitized carbon stationary phase (Envicarb®). Finally, 2 mL of the resulting extracts were evaporated to dryness under nitrogen, and reconstituted in 200 µL MeOH/H_2O 0.02M ammonium acetate, (50/50, *v/v*). A final centrifugation step was performed to discard most of the lipid fraction present in fish, and the supernatant was transferred into a polypropylene vial vessel.

HPLC separation

An identical liquid chromatographic system was used for the three different tested MS systems, based on a 1200 series binary pump from Agilent (Palo Alto, CA, USA). The separation was performed on a Gemini C_{18} reverse phase column (3 µm, 50 x 2.0 mm) fitted with a guard column (3 µm, 10 x 2.0 mm) (Phenomenex, Torrance CA, USA). The mobile phase consisted of MeOH (Solvent A) and ammonium acetate 20 mM (Solvent B). The elution gradient started with 30% A for 2 min, followed by a 7 min linear gradient to 100%, then 5 min hold at 100%, and returned back to 30% in 3 min. The column, kept at 40°C was equilibrated during 3 min prior to the next injection. Injection volume was set at 20 µL for both LTQ-Orbitrap and Q-TOF systems and 50 µL on the triple quadrupole system.

Low resolution MS and MS/MS on triple quadrupole

A triple quadrupole instrument (Agilent 6410, Palo Alto, CA, USA) was used in the negative electrospray ionization mode. Mass spectra were acquired in SIM or MRM mode. In the latter case, fragmentor voltage and collision energy were optimized for each compound and two diagnostic signals were monitored for each target PFC (Table 1). Whatever the acquisition mode, the common optimized source parameters were as follows: 3 kV for capillary voltage, 10 L/min for desolvation gas flow rate, 45 psi for nebulisation gas pressure, and respectively 250°C and 300°C for source and desolvation gas temperatures.

High resolution MS and MS/MS on LTQ-Orbitrap

An LTQ-Orbitrap^{TM} Discovery (Thermo Fisher Scientific, Bremen, Germany) system was used in the negative electrospray ionization mode. Full scan or product ion scan experiments were performed at a 30,000 resolution (FWHM) in the range *m/z* [200-900]. General source parameters were optimized for 5 model compounds (2 carboxylic acids, 2 sulfonic acids and 1 fluorotelomer acid). The drying gas (N_2) temperature was set to 280°C. The sheath and auxiliary gas flow values were respectively optimized at 40 and 10 (arbitrary units). The electrospray voltage was 4 kV. Capillary and tube lens voltages were respectively set to -14 V and -85 V. Quantitative analysis was performed using extracted mass chromatograms recorded in the full scan mode, applying the mass-to charge values given in table 1.

High resolution MS and MS/MS on Q-TOF

A Q-TOF (Agilent 6530, Palo Alto, CA, USA) instrument was used in the negative electrospray ionization mode. The source parameters used were the same as those optimised on the previous triple quadrupole, the source configuration being the same on both instruments. Product ion scan experiments were performed in *m/z* range from 50 to 1100 at a resolution of 8,000 (FWHM). Quantitative analysis was performed using extracted mass chromatograms recorded in product ion mode, applying the mass-to-charge values given in Table 1.

Limit of detection and linearity

The estimated instrumental limits of detection, based on the typical 3:1 signal-to-noise ratio principle, as well as a linear dynamic range from 0.1 to 1000 pg injected were assessed on standard solutions on the three instruments. All these assays were carried out in triplicates for each tested concentration level.

RESULTS AND DISCUSSION

Mass spectrometric behavior of PFCs in MS mode

Seven target compounds belonging to different PFC sub-families were investigated (Figure 1). The pseudo-molecular ions $[M-H]^-$ were observed as the main generated ionic species on the 3 different MS systems tested. On the whole, the full scan experiments confirmed the high physico-chemical stability of these compounds, with a poor in-source fragmentation phenomenon for the tested fragmentor values, ranging from 20 up to 160 V. Mass spectra were acquired for PFOS, PFOA, PFOSi, PFDPA, FOUEA, FOEA, and PFOSA. For PFOS, PFOSi, PFDPA and PFOSA, the spectra revealed that $[M-H]^-$ was the main ion produced. For PFOA, the in-source fragmentation appeared slightly higher with $[M-H]^-$ and $[M-CO_2-H]^-$ ions formed. Finally, for FOUEA, $[M-CO_2-HF-H]^-$ ion was observed and, for FOEA, other minor ionic species were formed. The chemical formulae corresponding to these different ions are reported in table 2. As a conclusion, the observed high stability of PFCs (that will be further confirmed in MS/MS too, see below) was a first indication that it would probably be difficult to achieve a good signal specificity in low resolution MS.

Mass spectrometric behavior of PFCs in MS/MS mode

The MS/MS fragmentation pathways observed for the previous 7 model PFCs belonging to different sub-families are presented in Figure 2 and Table 2. For PFOS (Figure 2a), a loss of the hydrophilic sulfonate group was observed, leading to $[SO_3]^-$ (*m/z* 80) and/or $[FSO_3]^-$ (*m/z* 99) ions. However, these fragment ions remained of poor intensity and limited specificity. For PFOA (Figure 2b), the observed fragmentation appeared slightly more effective, with the loss of CO_2 and subsequent fragmentation on the alkyl chain leading to $[CF_3-(CF_2)_n]$ ions with n equal to 2, 3 or 5. In particular, the comparison of the PFOA and PFOS MS^2 mass spectra confirmed the higher stability of the latter, even at a higher collision energy (15 *versus* 40 eV), which could be explained by the high electronegativity of the sulfonate group. PFOSi (Figure 2c) seemed to be less stable than sulfonate, with a highest fragmentation observed for lower collision energy as compared to PFOS. Regarding FOEA and FOUEA (Figures 2d and 2e), a significant difference of behavior was observed between saturated and unsaturated forms, which can be attributed to the stabilisation of the FOUEA structure due to its double bond. The PFDPA (Figure 2f) showed the highest stability, with only one observed fragment ion corresponding to the loss of the hydrophilic group $[PO_3]^-$. Finally, PFOSA (Figure 2g) also presented a main loss of the hydrophilic group $[SO_2N]$, as well as some additional fragment ions following the fragmentation of its alkyl chain. To conclude, the LC-MS/MS detection strategy on low resolution triple quadrupole instruments can be based on a limited number of diagnostic signals combined with weak specificity. Although LC-MS/MS provides good quantification performances and high efficiency for multiresidue analyses, this strategy may then encounter some limitations in terms of trace analysis in complex biological matrices. Furthermore, in some cases the low resolution LC-MS/MS approach appeared not fully compliant with strict regulatory purposes due to insufficient identification criteria, since a “pseudo” MRM transition ($[M-H]^- > [M-H]^-$) has to be used as diagnostic signal. For instance, for phosphonic acid family, which are the most stable class of PFCs, the use of pseudo-MRM transition is required since only one transition is available. Moreover also for other class of compounds (sulfonic acid), the aim of reaching the highest sensitivity leads to the choice of the pseudo-MRM transition instead of $[M-H]^- > [FSO_3]^-$.

Limits of LC-MS/MS analysis of PFCs in fish samples

The low resolution MS/MS approach in MRM mode was confirmed to be suitable for the quantitative determination of PFCs in biological samples. However, as previously mentioned, the lack of specificity of the main diagnostic signal in MRM mode, the poor sensitivity of potential additional signals when they exist, sound as potential limitations of this strategy. Therefore, HRMS was envisaged as a potentially valuable alternative. Indeed, the mass defect typically associated to these compounds due to the presence of fluorinated atoms was expected to provide a way to enhance the signal specificity in high resolution.

Comparison of low *versus* high resolution MS and MS/MS measurement in fish

High resolution MS on an orbital trap system was expected to combine a good signal specificity due to the mass defect typically observed for these halogenated substances and a good sensitivity in full scan mode. Indeed, the exact mass of fluorine atom (m=18.9984 uma) is leading to a slight mass defect compared to the expected nominal mass (m=19 uma), whereas the exact mass of hydrogen atom (m=1.00794 uma) is leading to a slight mass excess. Finally, this mass defect induced for the target PFCs permits to discriminate them from isobaric matrix components characterised by a CxHyOz formula using HRMS and accurate mass analysis. Figure 3 presents the typical extracted ion chromatograms obtained for PFDA (a perfluoroalkylcarboxylic acid) with a mass accuracy of 0.500 Da in LRMS and 0.003 Da in HRMS. These results indicated that most interfering signal contributions can be discarded when the mass accuracy was reduced to 0.003 Da, confirming the valuable interest of high resolution MS for monitoring these fluorinated compounds. However, this approach is still facing some difficulties to fulfil strict regulatory criteria in terms of unambiguous identification of the target analytes, as only one ion being available with sufficient intensity to be used as diagnostic signal.

Comparison of product ion scans on QTOF and LTQ-Orbitrap in a scope of a multiresidue analysis

In a perspective of a multi-residue analysis based on high resolution MS/MS, the product ion scan mode was tested on the two mass filters (QTOF and LTQ-Orbitrap). This acquisition mode was expected to obtain two diagnostic signals for each target compound in high resolution. As shown in figure 4, the obtained chromatograms presented a sufficient number of points per peaks for QTOF whereas it lacked the apex of the peaks for the chromatograms acquired with the LTQ-Orbitrap. Results indicated that due to a faster scan rate (0.05s/scan vs 0.5 s/scan), Q-TOF appeared more efficient in this mode compared to the tested first generation of LTQ-Orbitrap for a comparable resolution. This observation was also expected considering the respective characteristics of beam- *versus* trap-based instruments. In conclusion, LC-HRMS^2 on Q-TOF could appear as a good alternative to LC-LRMS^2 on triple quadrupole in terms of sensitivity / specificity balance and for regulatory purpose.

Distinction between PFOS and a bile acid interfering compound in fish: the added value of high resolution

A particular signal specificity issue was identified and encountered during the analysis of several fish samples. Basically, the two diagnostic signals monitored for PFOS in LC-LRMS^2 on triple quadrupole in MRM mode correspond to $[M-H]^- > [M-H]^-$ and $[M-H]^- > [SO_3]^-$, i.e. 499>499 and

499>80, respectively. The 499>499 has been preferred to the 499>99 transition since it provided better sensitivity with a signal to noise ratio fifteen times higher for the fish sample presented in Figure 6. Thus, an interfering compound further identified as a bile acid was found to (1) coelute with PFOS in the used LC separation conditions and (2) share the same diagnostic signals. This interference was first described by Benskin et al. (2007) and identified as one of the 4 cholic acids isomers presented in Figure 5. We identified taurochenodeoxycholic acid as the isomer disturbing the PFOS signal. As a consequence, the fish sample preparation procedure was particularly pointed out as the probable origin of this compound in the analyzed samples, the gall-bladder containing all these bile acids being possibly mixed to some extent with fish muscle. As shown in Figure 6a, the presence of this interfering compound could lead to a significant overestimation of the determined PFOS concentration level when LC-LRMS^2 is used as the measurement technique. One way to circumvent this overestimation is first to include in the acquisition method an additional diagnostic signal specific to taurochenodeoxycholic acid (e.g. 499>124 corresponding to the loss of the taurine group) in order to reveal its presence in the considered sample (Figure 6c). Then, if this presence is confirmed, unbiased quantification of PFOS can be performed on the basis of the 499>99 diagnostic signal which remains specific to this compound and unaffected by the interference (Figure 6b). However, this strategy is clearly penalizing performances in terms of sensitivity due to the poor intensity of the 499>99 diagnostic signal (around 15 fold lower compared to the pseudo-MRM transition). Finally, this particular real case illustrated a limit of the LC-LRMS^2 approach for perfluorinated compounds analysis. Although high resolution MS appears to be suitable to prevent quantification error coming from unexpected matrix interference, others possible ways to cope with this issue does exist. For instance, the improvement of the sample preparation procedure could be one way to get rid of interferences. However the associated effort, time and cost will have to be renewed for each particular matrix. Another level of action is to modify the chromatographic conditions (elution gradient and/or stationary phase). Unfortunately,. the separation of co-eluting compounds always remains challenging, and even if a given particular situation could be solved by this way it will not ensure the absence of troubles with other sample or matrix.

Conversely, the LC-HRMS approach offered a valuable and more sustainable alternative way to avoid such quantification error, since the target signal of interest can be distinguished from the signal of the interference on the basis of the accurate mass measurement. As illustrated in Figure 7, no significant quantitative difference is observed between LRMS (0.500 Da mass accuracy) and HRMS (0.001 Da mass accuracy) measurement for a fish sample without the interference (Figures 7c and d). Whereas in presence of taurochenodeoxycholate, an overestimation of the PFOS signal may occurs with LRMS, which is not observed with HRMS (Figures 7a and b), and that could reach twice the amount of PFOS contained in the fish.. Therefore, high resolution MS was chosen as a method of choice for measuring PFCs with high specificity, this statement being found valid either for the LTQ-Orbitrap (30,000 resolution) or Q-TOF (8,000 resolution) instruments.

Synthesis of instrumental performances

A summary of the instrumental performances obtained on the different tested systems is reported in Table 3. On the whole, the highest sensitivity was obtained on the Orbitrap system, followed by Q-TOF and triple quadrupole systems. If we compare the results for PFOS, the limits of detection (LOD) are 0.5, 0.7 and 3.8 pg injected respectively for Orbitrap, Q-TOF and QqQ. If we compare with the results obtained by Berger et al. (2004), the LODs are 2, 10 and 25 pg injected respectively for TOF, QqQ and ion trap systems. We notice the same order in sensitivity for Q-TOF and QqQ, but with lower values for our study, which could be due to the novelty of the instruments used and the different construction of the ion source.

The dynamic linear ranges are shown in Table 3. The lower limit was usually the limit of quantification set to a signal-to-noise ratio of 10:1. Ranges were found higher for both triple quadrupole and Orbitrap systems compared to the Q-TOF. For instance, for PFOA, the linear range covers more than 3 orders of magnitudes for both MRM (QqQ) and full scan mode (Orbitrap), whereas it covers only 2 orders of magnitude for product ion mode on Q-TOF.

CONCLUSION

Low and high resolution MS or MS/MS measurement strategies on three different instruments (triple quadrupole, Q-TOF and LTQ-Orbitrap) were compared for the analysis of perfluorinated compounds in fish. Ions generated in the source were first characterized by a high stability which penalizes the performances of the LRMS^2 approach on triple quadrupole in MRM mode due to the very limited fragmentation. This approach was not fully satisfying with regard to the unambiguous identification criteria defined by the regulation, only one diagnostic ion being monitored with sufficient intensity for each target compound. Moreover, a risk of quantitative overestimation was observed with this approach for PFOS in some fish samples due to the presence of a co-eluting and isobaric bile acid interfering compound. Alternatively, HRMS on LTQ-Orbitrap system in full scan mode appeared as the most powerful approach in terms of sensitivity and specificity due to the typical mass defect characterizing these halogenated compounds. Its higher specificity also allowed circumventing the risk of overestimation. However, this system provided only one diagnostic ion and consequently may not fit with common regulatory requirements in terms of unambiguous identification (at least two diagnostic signal needed). Therefore, HRMS^2 on Q-TOF system in product ion scan mode offered a good compromise in terms of sensitivity / specificity balance, with two possible diagnostic ions monitored for each target analyte, medium sensitivity compared to that achieved on QqQ and Orbitrap devices, and sufficient resolution to circumvent the specific interference issue revealed for some fish samples. However, the linear range is more limited on QTOF compared to the other systems.

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Figure captions

Figure 1 : Chemical structure of 7 fluorinated compounds belonging to different sub-families

Figure 2: Typical LC-(ESI-)-MS^2 product ion scan mass spectra obtained on the QqQ instrument for a) PFOS (Ec=40eV) b) PFOA (Ec=10eV), c) PFOSi (Ec=15eV) and d) FOUEA (Ec=40eV), e) FOEA (Ec=15 eV), f) PFDPA (Ec=40 eV), g) PFOSA (Ec=35 eV).

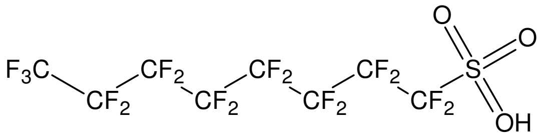
Figure 3 : LTQ-Orbitrap diagnotic chromatograms extracted from a full scan analysis with a mass accuracy of (up) 0.500 Da (LRMS) or (down) 0.003 Da (HRMS) for PerFluoro-n-decanoic Acid in fish sample

Figure 4 : Typical LC-(ESI-)-HRMS^2 diagnostic ion chromatograms of 7 perfluorinated compounds obtained for a standard solution (0.5 ng injected on-column) on the LTQ-Orbitrap (R = 7500), or Q-TOF (R= 8000) systems in daughter scan mode.

Figure 5: Chemical structures of taurochenodeoxycholic acid (TCDCA) (a), taurodeoxycholic acid (TDCA) (b), Tauroursodeoxycholic acid (TUDCA) (c) and taurohyodeoxycholic acid (THDCA) (d)

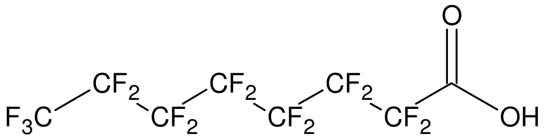
Figure 6: Examples of diagnostic chromatograms obtained for a fish sample contaminated with taurochenodeoxycholate which disturb PFOS signal leading to an overestimation of quantitation a) Common transition of PFOS and taurochenodeoxycholate b) PFOS specific transition c) bile acid specific transition.

Figure 7: LC-MS diagnostic chromatograms of PFOS obtained in a mackerel sample without (a & b) or with (c & d) bile acid interferent extracted with 0.500 Da (a & c) or 0.001 Da (b & d) mass accuracy, respectively.



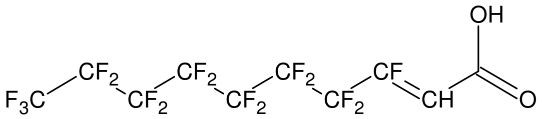
PFOS

$C_8H_1F_{17}SO_3$ – mi = 499.9380 uma



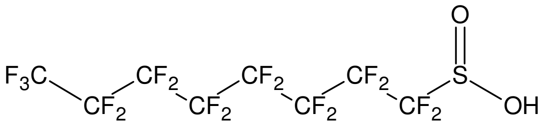
PFOA

$C_8H_1F_{15}O_2$ – mi = 413.9742 uma



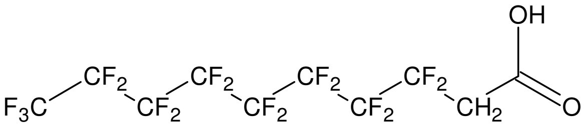
FOUEA

$C_{10}H_2F_{16}O_2$ – mi = 457.9805 uma



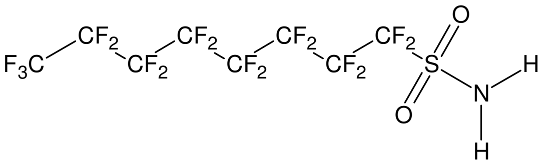
PFOSi

$C_8H_1F_{17}SO_2$ – mi = 483.9431 uma



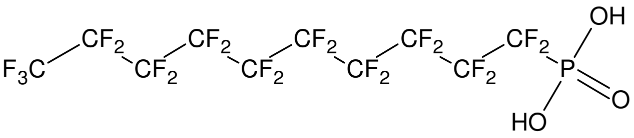
FOEA

$C_{10}H_3F_{17}O_2$ – mi = 477.9867 uma



PFOSA

$C_8H_2F_{17}N_1O_2S$ – mi = 498.9540 uma



PFDPA

$C_{10}H_2F_{21}PO_3$ – mi = 599.9412 uma

Figure 1

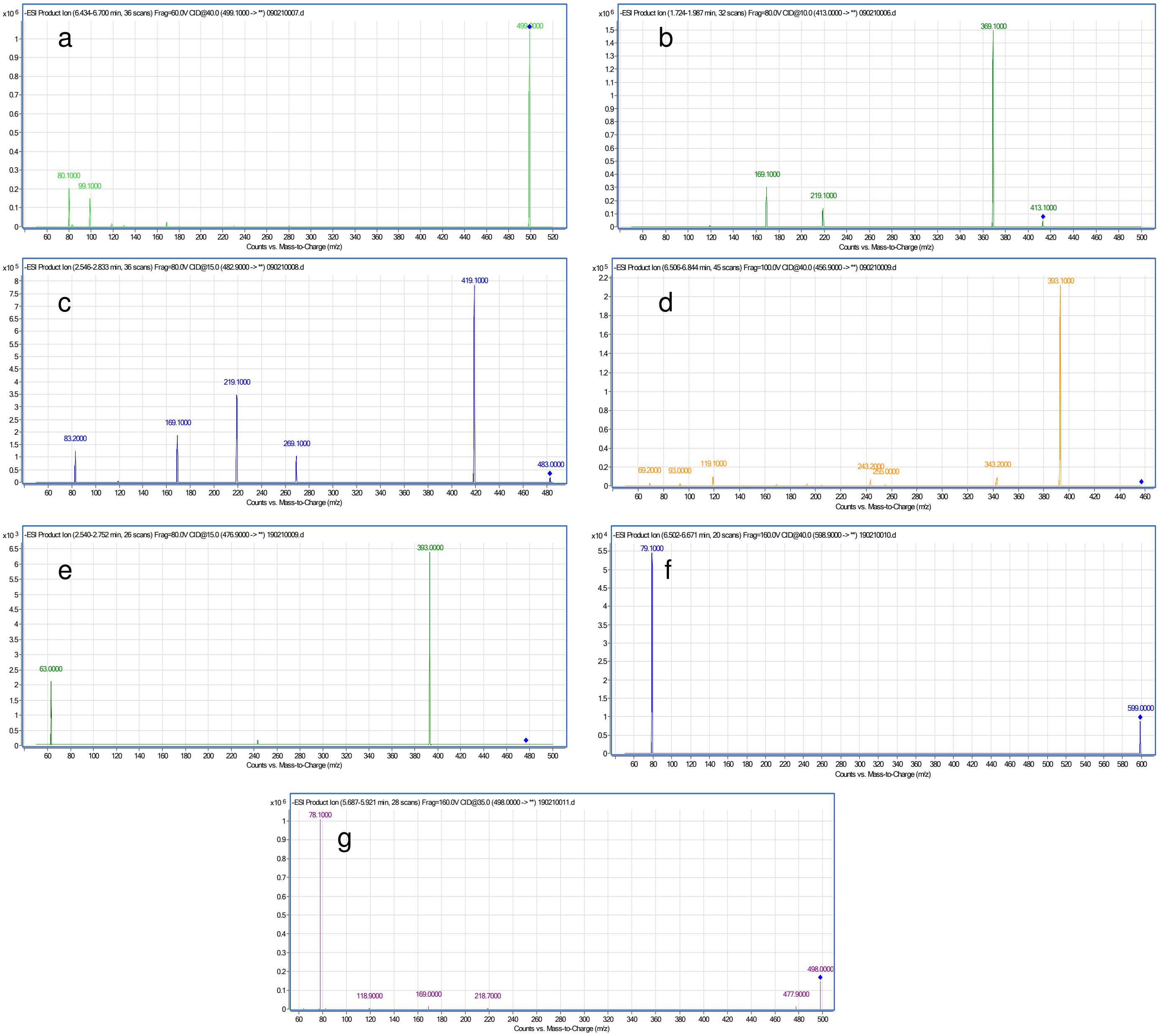

a
x10 6
-ESI Product Ion (6.434-6.700 min, 36 scans) Frag=60.0V CID@40.0 (499.1000 -> **) 090210007.d
499.1000
80.1000
99.1000
Counts vs. Mass-to-Charge (m/z)
b
x10 6
-ESI Product Ion (1.724-1.987 min, 32 scans) Frag=80.0V CID@10.0 (413.0000 -> **) 090210006.d
369.1000
169.1000
219.1000
413.1000
Counts vs. Mass-to-Charge (m/z)
c
x10 5
-ESI Product Ion (2.546-2.833 min, 36 scans) Frag=80.0V CID@15.0 (482.9000 -> **) 090210008.d
419.1000
219.1000
169.1000
83.2000
269.1000
483.0000
Counts vs. Mass-to-Charge (m/z)
d
x10 5
-ESI Product Ion (6.506-6.844 min, 45 scans) Frag=100.0V CID@40.0 (456.9000 -> **) 090210009.d
393.1000
69.2000
93.0000
119.1000
243.2000
255.0000
343.2000
Counts vs. Mass-to-Charge (m/z)
e
x10 3
-ESI Product Ion (2.540-2.752 min, 26 scans) Frag=80.0V CID@15.0 (476.9000 -> **) 190210009.d
393.0000
63.0000
Counts vs. Mass-to-Charge (m/z)
f
x10 4
-ESI Product Ion (6.502-6.671 min, 20 scans) Frag=160.0V CID@40.0 (598.9000 -> **) 190210010.d
79.1000
599.0000
Counts vs. Mass-to-Charge (m/z)
g
x10 6
-ESI Product Ion (5.687-5.921 min, 28 scans) Frag=160.0V CID@35.0 (498.0000 -> **) 190210011.d
78.1000
118.9000
169.0000
218.7000
477.9000
498.0000
Counts vs. Mass-to-Charge (m/z)

Figure 2

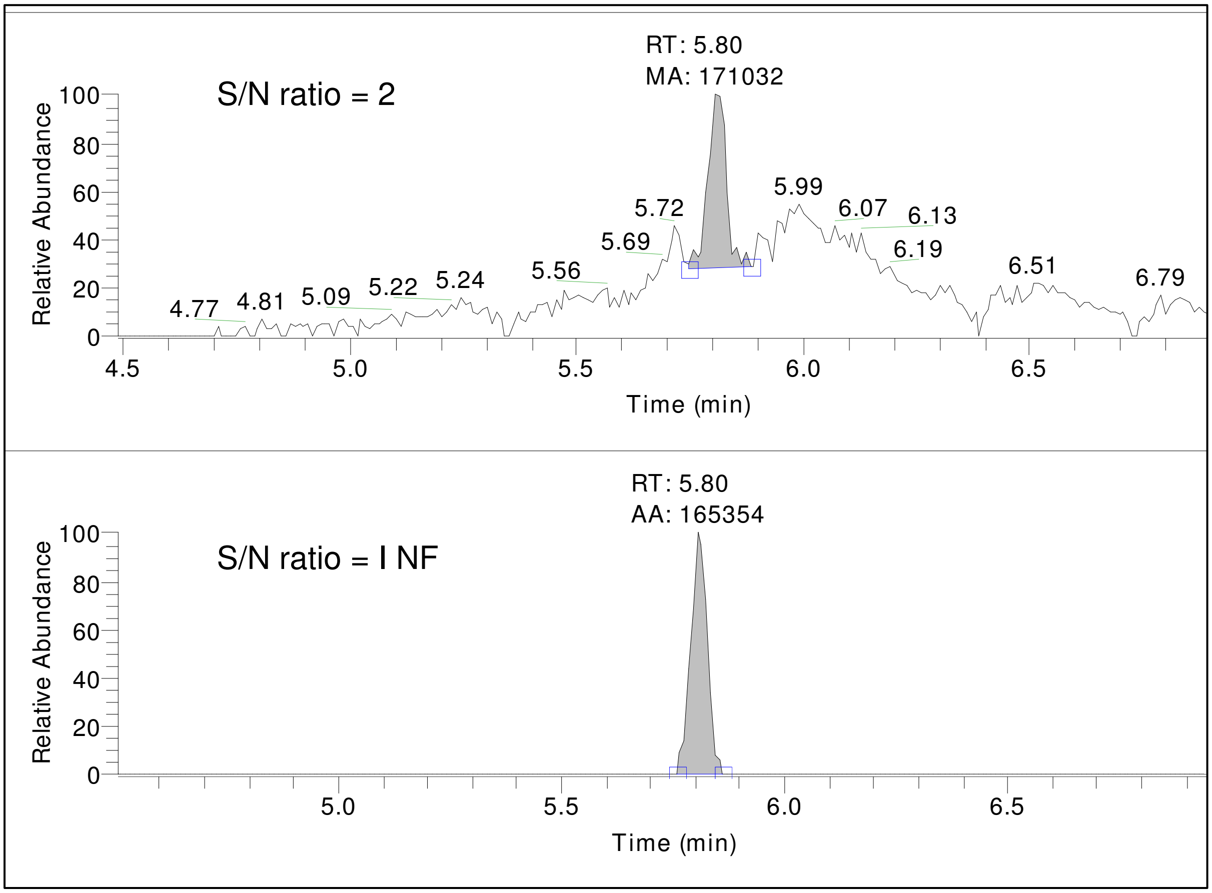
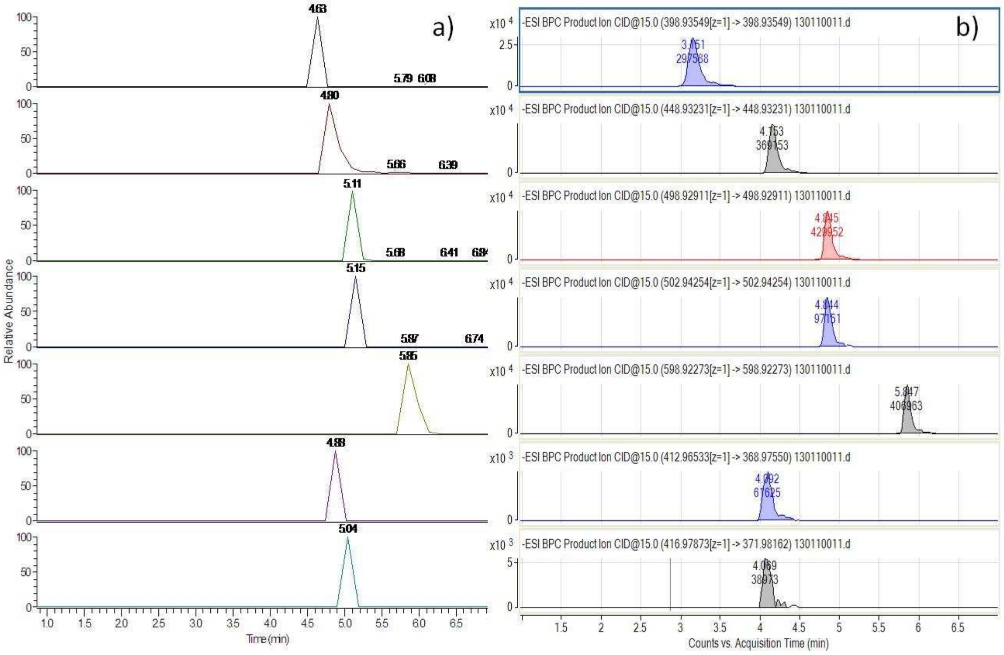
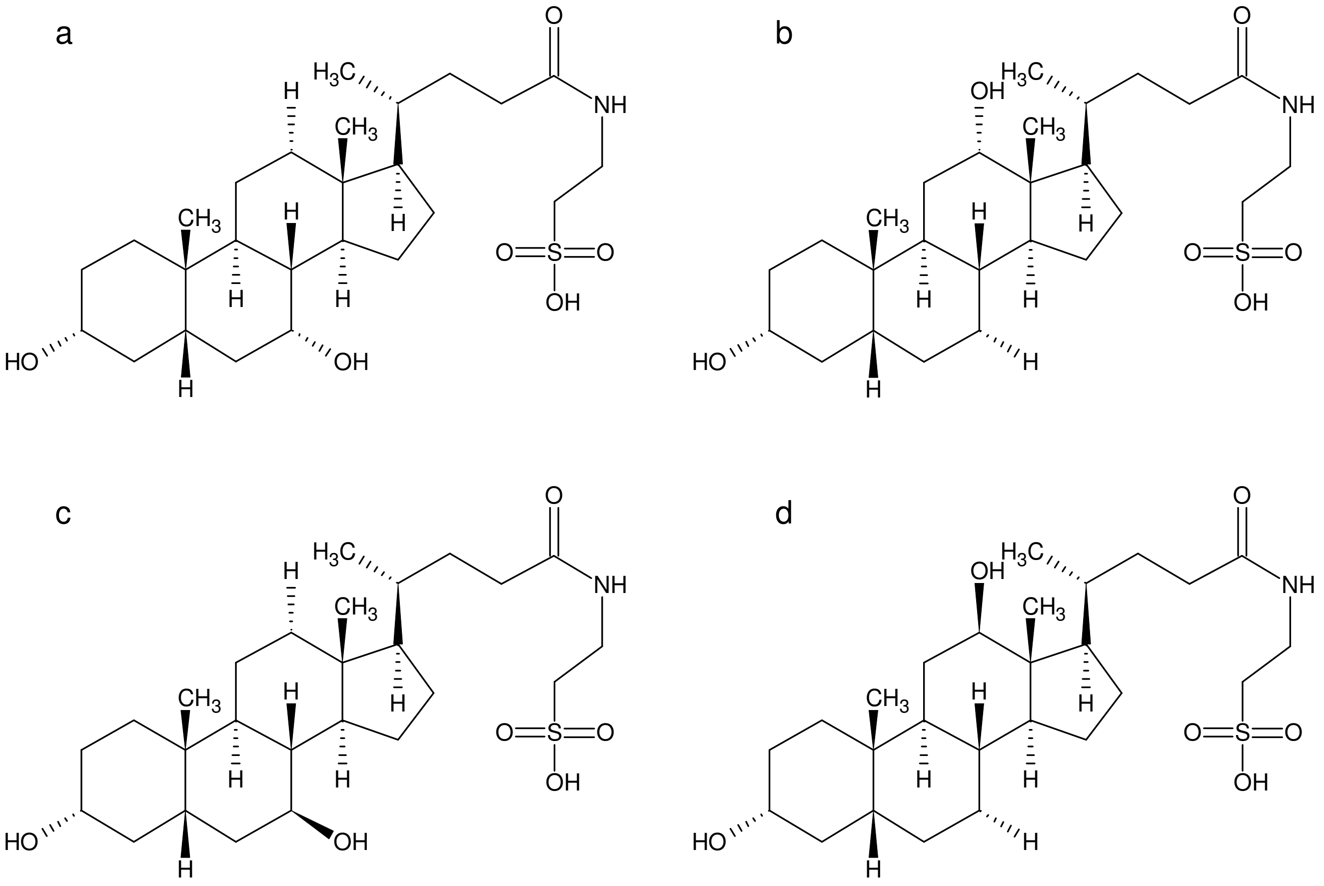

RT: 5.80
MA: 171032
S/N ratio = 2
Relative Abundance
100
80
60
40
20
0
4.77
4.81
5.09
5.22
5.24
5.56
5.69
5.72
5.99
6.07
6.13
6.19
6.51
6.79
4.5
5.0
5.5
6.0
6.5
Time (min)
RT: 5.80
AA: 165354
S/N ratio = I NF
Relative Abundance
100
80
60
40
20
0
5.0
5.5
6.0
6.5
Time (min)

Figure 3



218x143mm (150 x 150 DPI)



mi= 498.2889

Figure 5

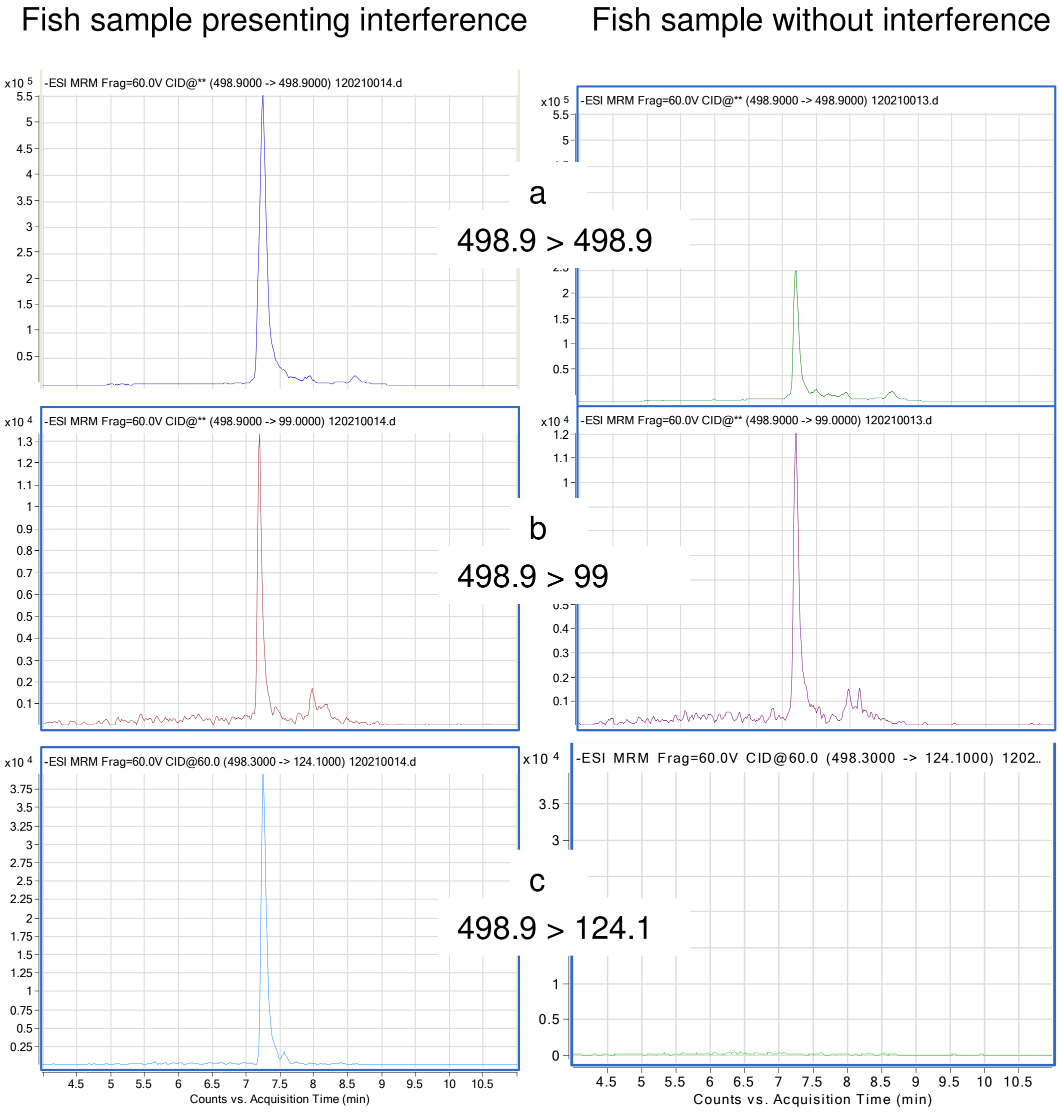

Fish sample presenting interference
Fish sample without interference
a
498.9 > 498.9
b
498.9 > 99
c
498.9 > 124.1
-ESI MRM Frag=60.0V CID@** (498.9000 -> 498.9000) 120210014.d
-ESI MRM Frag=60.0V CID@** (498.9000 -> 498.9000) 120210013.d
-ESI MRM Frag=60.0V CID@** (498.9000 -> 99.0000) 120210014.d
-ESI MRM Frag=60.0V CID@** (498.9000 -> 99.0000) 120210013.d
-ESI MRM Frag=60.0V CID@60.0 (498.3000 -> 124.1000) 120210014.d
-ESI MRM Frag=60.0V CID@60.0 (498.3000 -> 124.1000) 1202..
x10 5
x10 4
Counts vs. Acquisition Time (min)
4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5

Figure 6

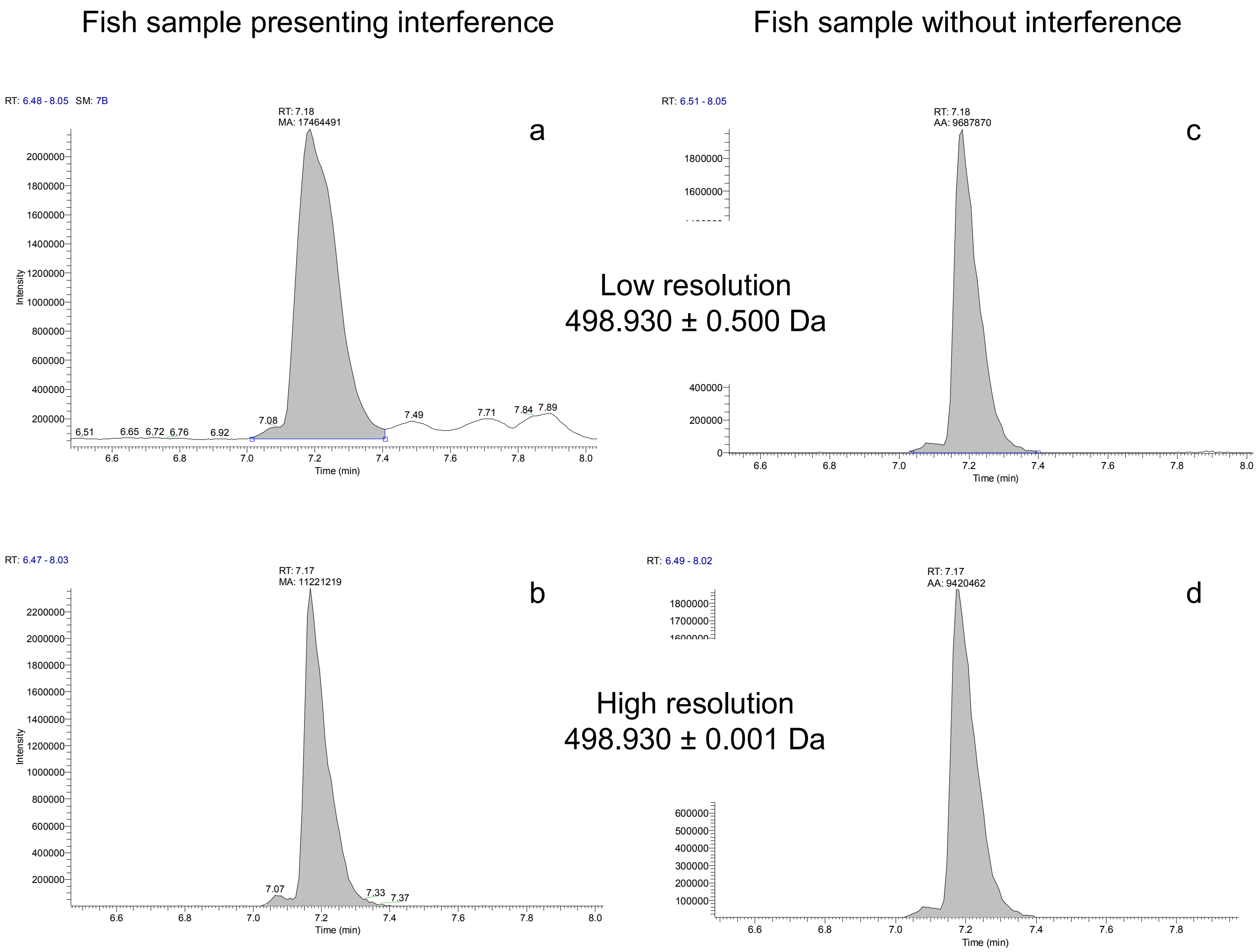

Fish sample presenting interference
Fish sample without interference
RT: 6.48 - 8.05 SM: 7B
RT: 7.18
MA: 17464491
a
RT: 6.51 - 8.05
RT: 7.18
AA: 9687870
c
Low resolution
498.930 ± 0.500 Da
RT: 6.47 - 8.03
RT: 7.17
MA: 11221219
b
RT: 6.49 - 8.02
RT: 7.17
AA: 9420462
d
High resolution
498.930 ± 0.001 Da
Intensity
Time (min)
6.51
6.65
6.72
6.76
6.92
7.08
7.49
7.71
7.84
7.89
7.07
7.33
7.37

Figure 7

Table1: Optimised parameters for the three instuments and for one analyte per family of perfluorinated compound.

Triple quadrupole MS				Orbitrap	QTOF	Orbitrap and QTOF	Orbitrap		QTOF		Orbitrap and QTOF
	SIM	MRM		Full scan							
fragmentor (V)	m/z	m/z > m/z	Collision energy (eV)	capillary voltage (V)	fragmentor (V)	m/z (± 10 ppm)	capillary voltage (V)	Collision energy (eV)	fragmentor (V)	Collision energy (eV)	m/z > m/z (± 10 ppm)
80	413	413>368,9	5	-14	80	412.966	-14	10	80	10	413,0>368.977
	369	413>169,1	15								413,0>168.989
60	499	499>499	15	-14	60	498.930	-14	20	60	20	498,9>498.930
		499>80	45					60		60	498,9>79.957
100	483	483>419	15	-14	100	482.935	-14	15	100	15	483,0>82.961
		483>219	5								483,0>218.986
100	457	457>393	15	-14	100	456.973	-14	15	100	15	456,9>456.973
	393	393>343	35								457>392.977
80	477	476,9>392,9	15	-14	80	476.979	-14	15	80	15	476,9>476.979
	393	476,9>63	30								476,9>392.977
160	599	599>599	40	-14	160	598.933	-14	15	160	15	598,9>598.933
	79	599>79	5								598,9>78.959
150	498	498>219	35	-14	150	497.946	-14	15	150	15	498,0>497.946
	219	498>78	25								498,0>218.986

Table 2: Structure of ions obtained after fragmentation obtained in MS and MS/MS mode

	m/z ratio	Ion structure elucidation
PFOA	885	$[2M+CH_3COO-H]^-$
	413	$[M-H]^-$
	369	$[M-CO_2-H]^-$
	219	$[M-CO_2-(CF_2)_3-H]^-$
	169	$[M-CO_2-(CF_2)_4-H]^-$
PFOS	499	$[M-H]^-$
	99	$[FSO_3]^-$
	80	$[M-C_8F_{16}]^-$
PFOSi	483	$[M-H]^-$
	419	$[M-SO_2-H]^-$
	269	$[M-SO_2-(CF_2)_3-H]^-$
	219	$[M-SO_2-(CF_2)_4-H]^-$
	169	$[M-SO_2-(CF_2)_5-H]^-$
	83	$[FSO_2]^-$
FOUEA	915	$[2M-H]^-$
	457	$[M-H]^-$
	393	$[M-CO_2-HF-H]^-$
	343	$[M-CO_2-CF_2-HF-H]^-$
PFDPA	599	$[M-H]^-$
	79	$[PO_3]^-$
FOEA	477	$[M-H]^-$
	413	$[M-CF_2=CH_2-H]^-$
	393	$[M-CF_2=CH_2-HF-H]^-$
	63	$[CF_2=CH]^-$
PFOSA	498	$[M-H]^-$
	478	$[M-HF-H]^-$
	219	$[M-SO_2-(CF_2)_4-H]^-$
	169	$[M-SO_2-(CF_2)_5-H]^-$
	119	$[M-SO_2-(CF_2)_6-H]^-$
	78	$[SO_2N]^-$

Table 3 : Summary of the main instrumental performances obtained on the three tested systems for six compounds

Compound	QQQ		ORBITRAP		Q-TOF	
	Calc.LOD [pg inj]	Linearity range [pg inj]	Calc.LOD [pg inj]	Linearity range [pg inj]	Calc.LOD [pg inj]	Linearity range [pg inj]
PFOA	0,1	0.3-1000	0,5	1.65-1000	0,1	0.330-500
PFOS	3,8	12.4-1000	0,5	1.65-1000	0,7	1.650-500
PFOSi	1,0	3.3-1000	0,5	1.65-1000	3,0	19.800-500
FOUEA	0,8	2.5-1000	2,0	6.60-500	3,0	9.900-500
FDEA	50,0	165.0-500	50,0	165-500	30,0	99-500
PFOSA	1,5	5.0-500	0,5	1.65-1000	1,0	3.300-500