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Hanane Kadar, Bruno Veyrand, Jean-Philippe Antignac, Sophie Durand,
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Comparative study of low versus high resolution liquid chromatography - mass spectrometric strategies for measuring perfluorinated contaminants in fish

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Abstract:	<p>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 (perfluoroalkylsulfonate, 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 multiresidues 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</p>

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.

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3 1 **Comparative study of low versus high resolution liquid chromatography -**
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5 2 **mass spectrometric strategies for measuring perfluorinated contaminants in**
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7 3 **fish**
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13 6 Hanane KADAR^{1,3}, Bruno VEYRAND^{1,3*}, Jean-Philippe ANTIGNAC^{1,2}, Sophie DURAND^{1,3},
14 7 Fabrice MONTEAU^{1,3}, Bruno LE BIZEC^{1,3}.
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18 9 ¹ONIRIS, USC 2013 LABERCA, Atlanpole - La Chantrerie, BP 50707, Nantes, F-44307
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20 10 ² INRA, Nantes, F-44307
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22 11 ³ Université Nantes Angers Le Mans
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29 15 Abbreviations: Multiple Reaction Monitoring (MRM), Quadrupole Time-of-flight (Q-TOF),
30 16 High Resolution Mass spectrometry (HRMS), Low Resolution Mass Spectrometry (LRMS)
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20 * Corresponding Author:

21 LABERCA, ONIRIS, Site de la Chantrerie

22 Route de Gachet, BP 50707, 44307-NANTES Cedex 3, FRANCE

23 Tel: 00 33 2 40 68 78 80

24 Fax: 00 33 2 40 68 78 78

25 E-Mail: laberca@oniris-nantes.fr
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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 (perfluoroalkylsulfonate, 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 were 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.

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

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63 INTRODUCTION

64 Perfluorinated compounds (PFCs) are synthetic chemical substances produced and used for their
65 hydrophobic and lipophobic properties, as anti-sticking material or surfactant related products
66 (Kissa, 2001). PFCs are used in many applications, including oil- and water-repellent coatings for
67 carpets, textiles, leather, paper, cardboard and food packing materials, electronic and
68 photographic devices, and surfactants in some cleaning agents, cosmetics and fire-fighting foams.
69 They are also used as an essential processing aid in the manufacture of some fluoropolymers such
70 as polytetrafluoroethylene (PTFE) and to a lesser extent as antistatic additives in industrial
71 applications and in the electronics industry (Hansen et al. 2002). Consequently, consumers from
72 industrialised countries are daily in contact with these chemicals, through a high number of
73 manufactured products. Furthermore, as many other chemicals of anthropogenic origin, PFCs
74 may be released into the environment at each step of their life cycle and found in various food
75 chain components. Food, especially through particular vectors of chemical exposure such as fish,
76 represents a main source of consumers' exposure to PFCs (Fromme et al. 2007; Simcik and
77 Dorweiler 2005; Taniyasu et al. 2003; Giesy and Kannan 2001; Rylander et al. 2009; Hölzer et
78 al. 2009).

79
80 Perfluorinated compounds include a large group of chemicals which are characterized by a fully
81 fluorinated hydrophobic linear or branched carbon chain attached to various hydrophilic moieties.
82 This chemical structure explains their physico-chemical characteristics such as chemical and
83 thermal stability, low surface free energy and surface active properties. The C–F bond is
84 particularly strong, and is resistant to various degradation modes, including reaction with acids
85 and bases, oxidation, and reduction. While some PFCs undergo chemical transformations, these
86 reactions occur mainly on the hydrophilic group of the molecule rather than on the perfluorinated
87 alkyl chains moiety (3M 1999). The most commonly studied PFC substances are
88 perfluoroalkylsulfonates and carboxylates. Among these, perfluorooctane sulfonate (PFOS) and
89 perfluorooctanoic acid (PFOA) are two main representative of the PFCs family (Figure 1).
90 PFOS is classified as a persistent, bioaccumulative and toxic chemical substance, thus fulfilling
91 the criteria for being considered as a persistent organic pollutant (POP) under the Stockholm
92 Convention (Wang et al. 2009). Moreover, recommendation 2010/161/EU was adopted to
93 encourage member states to develop methods for the monitoring of perfluorinated compounds in

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2
3 94 food. The toxicity of PFOS and PFOA has been studied mainly in rodents. On the whole,
4
5 95 hepatotoxicity, developmental toxicity, immunotoxicity, hormonal and neuroendocrine effects, as
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7 96 well as carcinogenic potency are the observed effects of main concern (Fei et al. 2009, Olsen et
8
9 97 al. 2009, Wolf et al. 2007; Austin et al. 2003). More recently, some *in vitro* genotoxicity studies
10
11 98 indicated that PFOS and/or PFOA may induce oxidative stress, apoptosis or increase the potential
12
13 99 genotoxicity of other chemicals in a multi-exposure context (Liu et al. 2007a, 2007b, Yao and
14
15 100 Zong 2005, Jernbro et al. 2007). Moreover, PFOS and PFOA have been suspected to have an
16
17 101 effect on human reproduction and development during pregnancy (Trudel et al. 2008, Fromme et
18
19 102 al. 2009). Recent studies on fluorotelomer acids – another class of fluorinated compounds – have
20
21 103 revealed that their toxicity was higher than that of carboxylic acids on crustacean (Philipps et al.
22
23 104 2007).

24
25 106 Numerous PFCs have been detected in nearly all environmental media and biota, reflecting the
26
27 107 widespread global pollution in all parts of the ecosystem (Pistocchi et al. 2009). Two recent
28
29 108 surveys of PFCs in food samples, carried out in the UK and Sweden (EFSA 2008), provided
30
31 109 some European country-related data. According to the EFSA (European Food Safety Agency)
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33 110 expert panel, “*Data on PFAS in food from monitoring activities in the EU countries are on the*
34
35 111 *whole insufficient and the contamination of most foodstuffs cannot be characterised at present*”
36
37 112 (EFSA 2008). However, fish and fisheries products have been identified as particularly
38
39 113 contaminated products. Several studies report PFCs’ presence in fish at concentrations varying
40
41 114 from some ppb to hundreds of ppb (Taniyasu et al. 2005, Furdui et al. 2007, Nania et al. 2009). In
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43 115 this matrix, PFOS concentrations are almost invariably higher than PFOA concentrations and the
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45 116 PFOS concentrations in fish liver are consistently higher than those in fillet. PFOS has been
46
47 117 shown to bioaccumulate in fish, with an estimated time clearance of 50% in fish around 100 days
48
49 118 (EFSA 2008). Thus, fish seems to be an important source of human exposure to PFOS, as
50
51 119 confirmed by EFSA in 2008: “*Based on the limited information available, fish and fishery*
52
53 120 *products seem to be one important source of human exposure to PFOS and PFOA*”. A specific
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55 121 population characterised by high fish consumption such as the Inuit community was also
56
57 122 confirmed as particularly exposed to PFOS (Dallaire et al. 2009).
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2
3 124 The main measurement technique for perfluorinated compounds remains liquid chromatography
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5 125 coupled to tandem mass spectrometry on triple quadrupole instruments after negative
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7 126 electrospray ionization. However, the need for an unambiguous quantification of an extended
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9 127 range of monitored compounds justified the evaluation of alternative approaches, especially those
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11 128 based on high resolution instruments. Indeed, Guo et al. 2008 reported the benefits of using other
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13 129 instruments such as a Quadrupole –time of flight (Q-TOF). Besides, Llorca et al. 2009 suggested
14
15 130 a method based on an hybrid mass analyzer QqLIT (Quadrupole-Linear Ion trap) system to
16
17 131 achieve unequivocal identification and quantification of PFCs compounds. So far, no comparison
18
19 132 of the performances for different systems has yet been performed. For that purpose, the present
20
21 133 study has compared three different LC-MS related instruments and various signal acquisition
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23 134 modes, from low resolution full scan and selected ion monitoring acquisition on triple quadrupole
24
25 135 (QqQ) to high resolution full scan or product ion scan mode on an orbital trap (LTQ-Orbitrap) or
26
27 136 a quadrupole-time-of-flight (Q-TOF). After having characterized the global spectrometric
28
29 137 behavior of 7 PFCs in MS and MS/MS modes, the different tested technologies were discussed in
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31 138 terms of performances (specificity, sensitivity, dynamic range, linearity...), advantages and
32
33 139 limitations, and possible drawbacks in the scope of a multi-residue monitoring.

140

141 **Materials and methods**

142

143 **Reagents and Chemicals**

144 All the following reference substances were purchased from BCP Instruments (Wellington
145 Laboratories, Guelph, Ontario, Canada): PFOA (perfluoro-n-octanoic acid) ; PFDA (perfluoro-n-
146 decanoic acid); PFOS (potassium perfluorooctanesulfonate) ; FOEA (2-perfluorooctylethanoic
147 acid) ; FOUEA (2H-perfluoro-2-decenoic acid); PFOSi (sodium perfluoro-1-octanesulfinate
148 acid); PFDPA (perfluorodecylphosphonic Acid) ; FOSA (perfluoro-1-octanesulfonamide) and
149 mass labeled standards PFOA $^{13}\text{C}_4$ (perfluoro-n-[1,2,3,4- $^{13}\text{C}_4$]octanoic acid); PFOS $^{13}\text{C}_4$ (sodium
150 perfluoro-1-[1,2,3,4- $^{13}\text{C}_4$]octanesulfonate); FOUEA $^{13}\text{C}_2$ 2H-perfluoro-[1,2- $^{13}\text{C}_4$]-2-decenoic
151 acid; PFOSi $^{13}\text{C}_4$ sodium perfluoro-1-[1,2,3,4- $^{13}\text{C}_4$]-octanesulfinate; FOSA $^{13}\text{C}_8$ perfluoro-1-
152 [$^{13}\text{C}_8$]octanesulfonamide. Taurochenodeoxycholic acid, tauroursodeoxycholic acid,
153 taurodeoxycholic acid, taurohyodeoxycholate acids were purchased from Sigma-Aldrich (Saint-
154 Quentin Fallavier, France). Methanol (picograde quality) was provided by UGC Promochem

1
2
3 155 (Wesel, Germany). Ammonium acetate and glacial acetic acid were from Merck (Darmstadt,
4
5 156 Germany). Deionised water (<18 MΩ.cm) was obtained from nanopure system (Barnstead,
6
7 157 Germany). Supelclean envicarb® was acquired from Supelco (Sigma-Aldrich, Saint-Quentin
8
9 158 Fallavier, France).

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11 12 160 **Sampling and sample preparation**

13
14 161 As a prerequisite, all materials used for sampling and sample preparation were washed with
15
16 162 acetone in order to avoid cross-contamination between samples. Fish samples were freeze-dried
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18 163 to obtain a minimum of 2 g for each sample, further ground and homogenized. Then, 1 g of dried
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20 164 sample was transferred in a 50 mL polypropylene tube, in which 2 ng of each internal standard
21
22 165 were added before vortexing. A liquid solid extraction (LSE) using 15 mL of MeOH was
23
24 166 performed. The sample was then mechanically agitated for 15 minutes. After centrifugation,
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26 167 3 mL of supernatant were purified using dispersive solid phase extraction with Envicarb
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28 168 stationary phase, according to a method described by Powley et al. (2005). An adaptation of this
29
30 169 procedure included the addition of 200 µL of glacial acetic acid to 150 mg of the graphitized
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32 170 carbon stationary phase (Envicarb®). Finally, 2 mL of the resulting extracts were evaporated to
33
34 171 dryness under nitrogen, and reconstituted in 200 µL MeOH/H₂O 0.02M ammonium acetate,
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36 172 (50/50, v/v). A final centrifugation step was performed to discard most of the lipid fraction
37
38 173 present in fish, and the supernatant was transferred into a polypropylene vial vessel.

37 174

38 39 175 **HPLC separation**

40 176 An identical liquid chromatographic system was used for the three different tested MS systems,
41
42 177 based on a 1200 series binary pump from Agilent (Palo Alto, CA, USA). The separation was
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44 178 performed on a Gemini C₁₈ reverse phase column (3 µm, 50 x 2.0 mm) fitted with a guard
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46 179 column (3 µm, 10 x 2.0 mm) (Phenomenex, Torrance CA, USA). The mobile phase consisted of
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48 180 MeOH (Solvent A) and ammonium acetate 20 mM (Solvent B). The elution gradient started with
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50 181 30% A for 2 min, followed by a 7 min linear gradient to 100%, then 5 min hold at 100%, and
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52 182 returned back to 30% in 3 min. The column, kept at 40°C was equilibrated during 3 min prior to
53
54 183 the next injection. Injection volume was set at 20 µL for both LTQ-Orbitrap and Q-TOF systems
55
56 184 and 50 µL on the triple quadrupole system.

56 185

186 **Low resolution MS and MS/MS on triple quadrupole**

187 A triple quadrupole instrument (Agilent 6410, Palo Alto, CA, USA) was used in the negative
188 electrospray ionization mode. Mass spectra were acquired in SIM or MRM mode. In the latter
189 case, fragmentor voltage and collision energy were optimized for each compound and two
190 diagnostic signals were monitored for each target PFC (Table 1). Whatever the acquisition mode,
191 the common optimized source parameters were as follows: 3 kV for capillary voltage, 10 L/min
192 for desolvation gas flow rate, 45 psi for nebulisation gas pressure, and respectively 250°C and
193 300°C for source and desolvation gas temperatures.

195 **High resolution MS and MS/MS on LTQ-Orbitrap**

196 An LTQ-OrbitrapTM Discovery (Thermo Fisher Scientific, Bremen, Germany) system was used
197 in the negative electrospray ionization mode. Full scan or product ion scan experiments were
198 performed at a 30,000 resolution (FWHM) in the range m/z [200-900]. General source parameters
199 were optimized for 5 model compounds (2 carboxylic acids, 2 sulfonic acids and 1 fluorotelomer
200 acid). The drying gas (N₂) temperature was set to 280°C. The sheath and auxiliary gas flow
201 values were respectively optimized at 40 and 10 (arbitrary units). The electrospray voltage was 4
202 kV. Capillary and tube lens voltages were respectively set to -14 V and -85 V. Quantitative
203 analysis was performed using extracted mass chromatograms recorded in the full scan mode,
204 applying the mass-to-charge values given in table 1.

206 **High resolution MS and MS/MS on Q-TOF**

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

214 **Limit of detection and linearity**

215 The estimated instrumental limits of detection, based on the typical 3:1 signal-to-noise ratio
216 principle, as well as a linear dynamic range from 0.1 to 1000 pg injected were assessed on

217 standard solutions on the three instruments. All these assays were carried out in triplicates for
218 each tested concentration level.

219

220 RESULTS AND DISCUSSION

221

222 Mass spectrometric behavior of PFCs in MS mode

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

236

237 Mass spectrometric behavior of PFCs in MS/MS mode

238 The MS/MS fragmentation pathways observed for the previous 7 model PFCs belonging to
239 different sub-families are presented in Figure 2 and Table 2. For PFOS (Figure 2a), a loss of the
240 hydrophilic sulfonate group was observed, leading to $[SO_3]^-$ (m/z 80) and/or $[FSO_3]^-$ (m/z 99)
241 ions. However, these fragment ions remained of poor intensity and limited specificity. For PFOA
242 (Figure 2b), the observed fragmentation appeared slightly more effective, with the loss of CO_2
243 and subsequent fragmentation on the alkyl chain leading to $[CF_3-(CF_2)_n]^-$ ions with n equal to 2, 3
244 or 5. In particular, the comparison of the PFOA and PFOS MS^2 mass spectra confirmed the
245 higher stability of the latter, even at a higher collision energy (15 *versus* 40 eV), which could be
246 explained by the high electronegativity of the sulfonate group. PFOSi (Figure 2c) seemed to be
247 less stable than sulfonate, with a highest fragmentation observed for lower collision energy as

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2
3 248 compared to PFOS. Regarding FOEA and FOUEA (Figures 2d and 2e), a significant difference
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5 249 of behavior was observed between saturated and unsaturated forms, which can be attributed to the
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7 250 stabilisation of the FOUEA structure due to its double bond. The PFDPA (Figure 2f) showed the
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9 251 highest stability, with only one observed fragment ion corresponding to the loss of the
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11 252 hydrophilic group $[\text{PO}_3]^-$. Finally, PFOSA (Figure 2g) also presented a main loss of the
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13 253 hydrophilic group $[\text{SO}_2\text{N}]$, as well as some additional fragment ions following the fragmentation
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15 254 of its alkyl chain. To conclude, the LC-MS/MS detection strategy on low resolution triple
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17 255 quadrupole instruments can be based on a limited number of diagnostic signals combined with
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19 256 weak specificity. Although LC-MS/MS provides good quantification performances and high
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21 257 efficiency for multiresidue analyses, this strategy may then encounter some limitations in terms
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23 258 of trace analysis in complex biological matrices. Furthermore, in some cases the low resolution
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25 259 LC-MS/MS approach appeared not fully compliant with strict regulatory purposes due to
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27 260 insufficient identification criteria, since a “pseudo” MRM transition ($[\text{M}-\text{H}]^- > [\text{M}-\text{H}]^-$) has to be
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29 261 used as diagnostic signal. For instance, for phosphonic acid family, which are the most stable
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31 262 class of PFCs, the use of pseudo-MRM transition is required since only one transition is
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33 263 available. Moreover also for other class of compounds (sulfonic acid), the aim of reaching the
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35 264 highest sensitivity leads to the choice of the pseudo-MRM transition instead of $[\text{M}-\text{H}]^- > [\text{FSO}_3]^-$.

36 266 **Limits of LC-MS/MS analysis of PFCs in fish samples**

37 267 The low resolution MS/MS approach in MRM mode was confirmed to be suitable for the
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39 268 quantitative determination of PFCs in biological samples. However, as previously mentioned, the
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41 269 lack of specificity of the main diagnostic signal in MRM mode, the poor sensitivity of potential
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43 270 additional signals when they exist, sound as potential limitations of this strategy. Therefore,
44
45 271 HRMS was envisaged as a potentially valuable alternative. Indeed, the mass defect typically
46
47 272 associated to these compounds due to the presence of fluorinated atoms was expected to provide
48
49 273 a way to enhance the signal specificity in high resolution.

50 274 51 275 **Comparison of low versus high resolution MS and MS/MS measurement in fish**

52 276 High resolution MS on an orbital trap system was expected to combine a good signal specificity
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54 277 due to the mass defect typically observed for these halogenated substances and a good sensitivity
55
56 278 in full scan mode. Indeed, the exact mass of fluorine atom ($m=18.9984$ uma) is leading to a slight

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2
3 279 mass defect compared to the expected nominal mass ($m=19$ uma), whereas the exact mass of
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5 280 hydrogen atom ($m=1.00794$ uma) is leading to a slight mass excess. Finally, this mass defect
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7 281 induced for the target PFCs permits to discriminate them from isobaric matrix components
8
9 282 characterised by a $C_xH_yO_z$ formula using HRMS and accurate mass analysis. Figure 3 presents
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11 283 the typical extracted ion chromatograms obtained for PFDA (a perfluoroalkylcarboxylic acid)
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13 284 with a mass accuracy of 0.500 Da in LRMS and 0.003 Da in HRMS. These results indicated that
14
15 285 most interfering signal contributions can be discarded when the mass accuracy was reduced to
16
17 286 0.003 Da, confirming the valuable interest of high resolution MS for monitoring these fluorinated
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19 287 compounds. However, this approach is still facing some difficulties to fulfil strict regulatory
20
21 288 criteria in terms of unambiguous identification of the target analytes, as only one ion being
22
23 289 available with sufficient intensity to be used as diagnostic signal.

24 25 291 **Comparison of product ion scans on QTOF and LTQ-Orbitrap in a scope of a multiresidue** 26 27 292 **analysis**

28 293 In a perspective of a multi-residue analysis based on high resolution MS/MS, the product ion
29
30 294 scan mode was tested on the two mass filters (QTOF and LTQ-Orbitrap). This acquisition mode
31
32 295 was expected to obtain two diagnostic signals for each target compound in high resolution. As
33
34 296 shown in figure 4, the obtained chromatograms presented a sufficient number of points per peaks
35
36 297 for QTOF whereas it lacked the apex of the peaks for the chromatograms acquired with the LTQ-
37
38 298 Orbitrap. Results indicated that due to a faster scan rate (0.05s/scan vs 0.5 s/scan), Q-TOF
39
40 299 appeared more efficient in this mode compared to the tested first generation of LTQ-Orbitrap for
41
42 300 a comparable resolution. This observation was also expected considering the respective
43
44 301 characteristics of beam- *versus* trap-based instruments. In conclusion, LC-HRMS² on Q-TOF
45
46 302 could appear as a good alternative to LC-LRMS² on triple quadrupole in terms of sensitivity /
47
48 303 specificity balance and for regulatory purpose.

49 305 **Distinction between PFOS and a bile acid interfering compound in fish: the added value of** 50 51 306 **high resolution**

52
53 307 A particular signal specificity issue was identified and encountered during the analysis of several
54
55 308 fish samples. Basically, the two diagnostic signals monitored for PFOS in LC-LRMS² on triple
56
57 309 quadrupole in MRM mode correspond to $[M-H]^- > [M-H]^-$ and $[M-H]^- > [SO_3]^-$, i.e. 499>499 and

1
2
3 310 499>80, respectively. The 499>499 has been preferred to the 499>99 transition since it provided
4
5 311 better sensitivity with a signal to noise ratio fifteen times higher for the fish sample presented in
6
7 312 Figure 6. Thus, an interfering compound further identified as a bile acid was found to (1) coelute
8
9 313 with PFOS in the used LC separation conditions and (2) share the same diagnostic signals. This
10
11 314 interference was first described by Benskin et al. (2007) and identified as one of the 4 cholic
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13 315 acids isomers presented in Figure 5. We identified taurochenodeoxycholic acid as the isomer
14
15 316 disturbing the PFOS signal. As a consequence, the fish sample preparation procedure was
16
17 317 particularly pointed out as the probable origin of this compound in the analyzed samples, the gall-
18
19 318 bladder containing all these bile acids being possibly mixed to some extent with fish muscle. As
20
21 319 shown in Figure 6a, the presence of this interfering compound could lead to a significant
22
23 320 overestimation of the determined PFOS concentration level when LC-LRMS² is used as the
24
25 321 measurement technique. One way to circumvent this overestimation is first to include in the
26
27 322 acquisition method an additional diagnostic signal specific to taurochenodeoxycholic acid (e.g.
28
29 323 499>124 corresponding to the loss of the taurine group) in order to reveal its presence in the
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31 324 considered sample (Figure 6c). Then, if this presence is confirmed, unbiased quantification of
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33 325 PFOS can be performed on the basis of the 499>99 diagnostic signal which remains specific to
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35 326 this compound and unaffected by the interference (Figure 6b). However, this strategy is clearly
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37 327 penalizing performances in terms of sensitivity due to the poor intensity of the 499>99 diagnostic
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39 328 signal (around 15 fold lower compared to the pseudo-MRM transition). Finally, this particular
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41 329 real case illustrated a limit of the LC-LRMS² approach for perfluorinated compounds analysis.
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43 330 Although high resolution MS appears to be suitable to prevent quantification error coming from
44
45 331 unexpected matrix interference, others possible ways to cope with this issue does exist. For
46
47 332 instance, the improvement of the sample preparation procedure could be one way to get rid of
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49 333 interferences. However the associated effort, time and cost will have to be renewed for each
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51 334 particular matrix. Another level of action is to modify the chromatographic conditions (elution
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53 335 gradient and/or stationary phase). Unfortunately, the separation of co-eluting compounds always
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55 336 remains challenging, and even if a given particular situation could be solved by this way it will
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57 337 not ensure the absence of troubles with other sample or matrix.
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339 Conversely, the LC-HRMS approach offered a valuable and more sustainable alternative way to
340 avoid such quantification error, since the target signal of interest can be distinguished from the

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3 341 signal of the interference on the basis of the accurate mass measurement. As illustrated in Figure
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5 342 7, no significant quantitative difference is observed between LRMS (0.500 Da mass accuracy)
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7 343 and HRMS (0.001 Da mass accuracy) measurement for a fish sample without the interference
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9 344 (Figures 7c and d). Whereas in presence of taurochenodeoxycholate, an overestimation of the
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11 345 PFOS signal may occurs with LRMS, which is not observed with HRMS (Figures 7a and b), and
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13 346 that could reach twice the amount of PFOS contained in the fish.. Therefore, high resolution MS
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15 347 was chosen as a method of choice for measuring PFCs with high specificity, this statement being
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17 348 found valid either for the LTQ-Orbitrap (30,000 resolution) or Q-TOF (8,000 resolution)
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19 349 instruments.
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21 350
22 351 **Synthesis of instrumental performances**
23 352 A summary of the instrumental performances obtained on the different tested systems is reported
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25 353 in Table 3. On the whole, the highest sensitivity was obtained on the Orbitrap system, followed
26
27 354 by Q-TOF and triple quadrupole systems. If we compare the results for PFOS, the limits of
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29 355 detection (LOD) are 0.5, 0.7 and 3.8 pg injected respectively for Orbitrap, Q-TOF and QqQ. If
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31 356 we compare with the results obtained by Berger et al. (2004), the LODs are 2, 10 and 25 pg
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33 357 injected respectively for TOF, QqQ and ion trap systems. We notice the same order in sensitivity
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35 358 for Q-TOF and QqQ, but with lower values for our study, which could be due to the novelty of
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37 359 the instruments used and the different construction of the ion source.
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39 360
40 361 The dynamic linear ranges are shown in Table 3. The lower limit was usually the limit of
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42 362 quantification set to a signal-to-noise ratio of 10:1. Ranges were found higher for both triple
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44 363 quadrupole and Orbitrap systems compared to the Q-TOF. For instance, for PFOA, the linear
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46 364 range covers more than 3 orders of magnitudes for both MRM (QqQ) and full scan mode
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48 365 (Orbitrap), whereas it covers only 2 orders of magnitude for product ion mode on Q-TOF.
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50 366
51 367 **CONCLUSION**
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54 369 Low and high resolution MS or MS/MS measurement strategies on three different instruments
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56 370 (triple quadrupole, Q-TOF and LTQ-Orbitrap) were compared for the analysis of perfluorinated
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58 371 compounds in fish. Ions generated in the source were first characterized by a high stability which
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3 372 penalizes the performances of the LRMS² approach on triple quadrupole in MRM mode due to
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5 373 the very limited fragmentation. This approach was not fully satisfying with regard to the
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7 374 unambiguous identification criteria defined by the regulation, only one diagnostic ion being
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9 375 monitored with sufficient intensity for each target compound. Moreover, a risk of quantitative
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11 376 overestimation was observed with this approach for PFOS in some fish samples due to the
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13 377 presence of a co-eluting and isobaric bile acid interfering compound. Alternatively, HRMS on
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15 378 LTQ-Orbitrap system in full scan mode appeared as the most powerful approach in terms of
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17 379 sensitivity and specificity due to the typical mass defect characterizing these halogenated
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19 380 compounds. Its higher specificity also allowed circumventing the risk of overestimation.
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21 381 However, this system provided only one diagnostic ion and consequently may not fit with
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23 382 common regulatory requirements in terms of unambiguous identification (at least two diagnostic
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25 383 signal needed). Therefore, HRMS² on Q-TOF system in product ion scan mode offered a good
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27 384 compromise in terms of sensitivity / specificity balance, with two possible diagnostic ions
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29 385 monitored for each target analyte, medium sensitivity compared to that achieved on QqQ and
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31 386 Orbitrap devices, and sufficient resolution to circumvent the specific interference issue revealed
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33 387 for some fish samples. However, the linear range is more limited on QTOF compared to the other
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35 388 systems.
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3 480 **Figure captions**
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5 481
6
7 482 Figure 1 : Chemical structure of 7 fluorinated compounds belonging to different sub-families
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9 483
10 484 Figure 2: Typical LC-(ESI)-MS² product ion scan mass spectra obtained on the QqQ instrument
11 for a) PFOS (Ec=40eV) b) PFOA (Ec=10eV), c) PFOSi (Ec=15eV) and d) FOUEA (Ec=40eV),
12 485
13 486 e) FOEA (Ec=15 eV), f) PFDPA (Ec=40 eV), g) PFOSA (Ec=35 eV).
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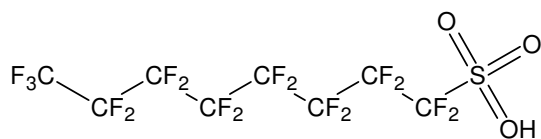
15 487
16 488 Figure 3 : LTQ-Orbitrap diagnostic chromatograms extracted from a full scan analysis with a mass
17 489 accuracy of (up) 0.500 Da (LRMS) or (down) 0.003 Da (HRMS) for PerFluoro-n-decanoic Acid
18
19 490 in fish sample
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21 491
22 492 Figure 4 : Typical LC-(ESI)-HRMS² diagnostic ion chromatograms of 7 perfluorinated compounds obtained for a
23 493 standard solution (0.5 ng injected on-column) on the LTQ-Orbitrap (R = 7500), or Q-TOF (R= 8000) systems in
24 494 daughter scan mode.
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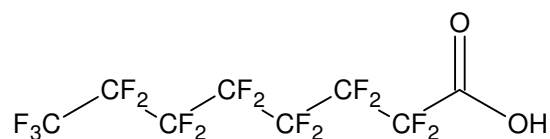
26 495
27 496 Figure 5: Chemical structures of taurochenodeoxycholic acid (TCDCA) (a), taurodeoxycholic
28 497 acid (TDCA) (b), Tauroursodeoxycholic acid (TUDCA) (c) and taurohyodeoxycholic acid
29 498 (THDCA) (d)
30

31 499
32 500 Figure 6: Examples of diagnostic chromatograms obtained for a fish sample contaminated with
33 501 taurochenodeoxycholate which disturb PFOS signal leading to an overestimation of quantitation
34 502 a) Common transition of PFOS and taurochenodeoxycholate b) PFOS specific transition c) bile
35 503 acid specific transition.
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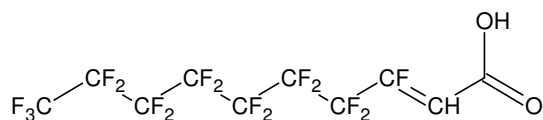
37 504
38 505 Figure 7: LC-MS diagnostic chromatograms of PFOS obtained in a mackerel sample without (a
39 506 & b) or with (c & d) bile acid interferent extracted with 0.500 Da (a & c) or 0.001 Da (b & d)
40 507 mass accuracy, respectively.
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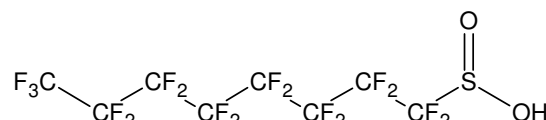
PFOS

 $\text{C}_8\text{H}_1\text{F}_{17}\text{SO}_3 - \text{mi} = 499.9380 \text{ uma}$


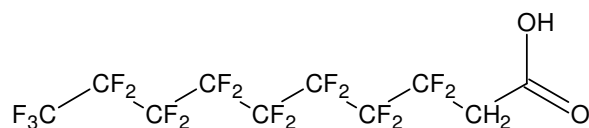
PFOA

 $\text{C}_8\text{H}_1\text{F}_{15}\text{O}_2 - \text{mi} = 413.9742 \text{ uma}$


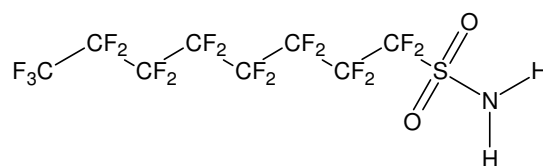
FOUEA

 $\text{C}_{10}\text{H}_2\text{F}_{16}\text{O}_2 - \text{mi} = 457.9805 \text{ uma}$


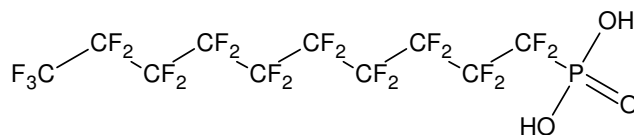
PFOSi

 $\text{C}_8\text{H}_1\text{F}_{17}\text{SO}_2 - \text{mi} = 483.9431 \text{ uma}$


FOEA

 $\text{C}_{10}\text{H}_3\text{F}_{17}\text{O}_2 - \text{mi} = 477.9867 \text{ uma}$


PFOSA

 $\text{C}_8\text{H}_2\text{F}_{17}\text{N}_1\text{O}_2\text{S} - \text{mi} = 498.9540 \text{ uma}$


PFDPA

 $\text{C}_{10}\text{H}_2\text{F}_{21}\text{PO}_3 - \text{mi} = 599.9412 \text{ uma}$

Figure 1

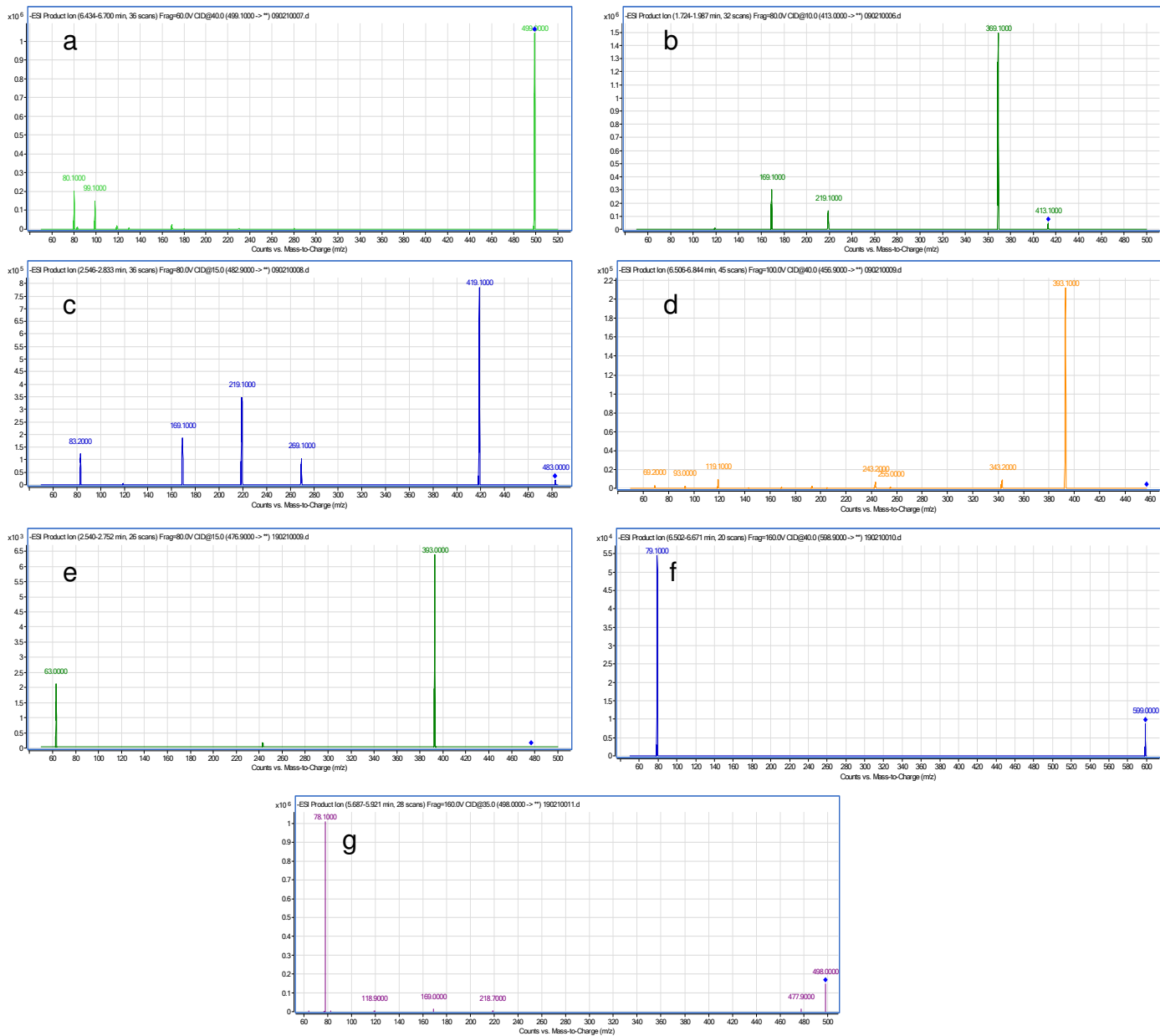


Figure 2

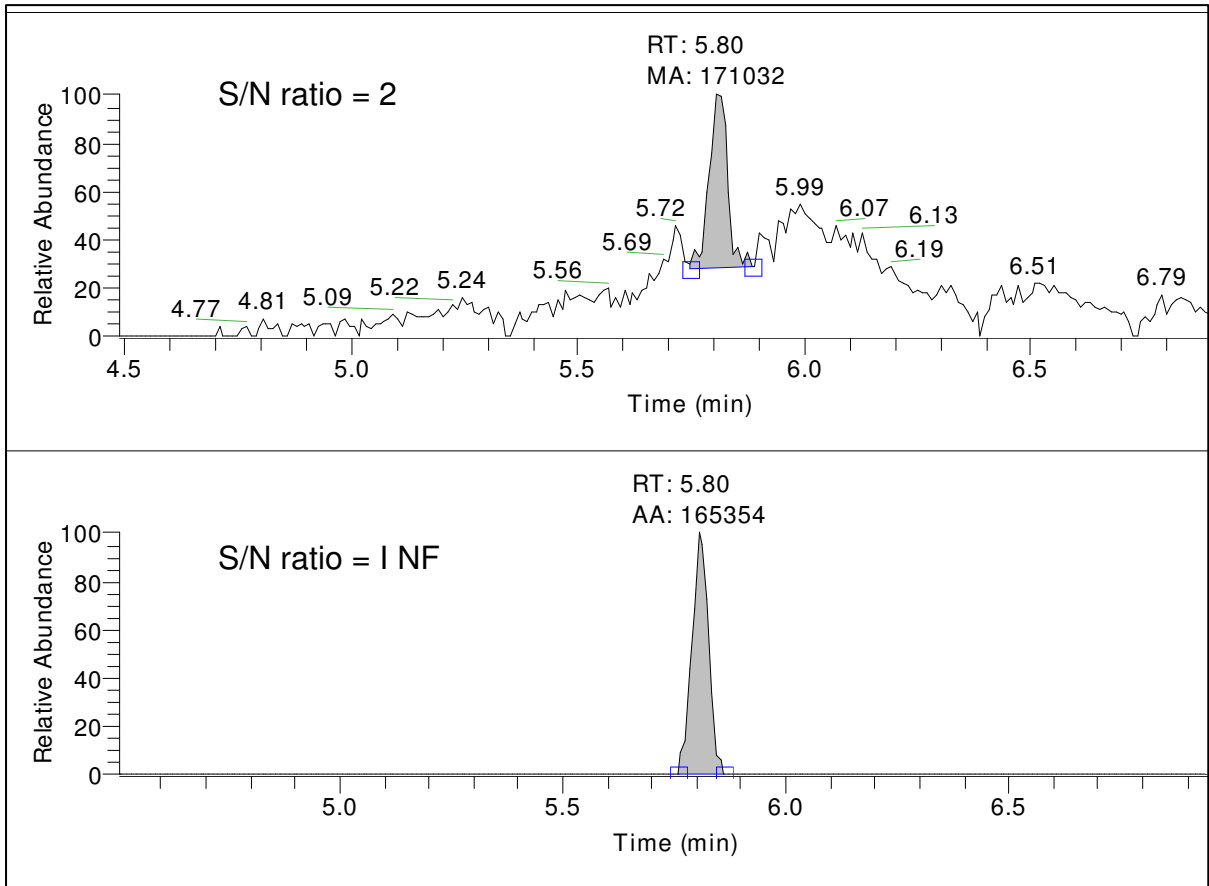
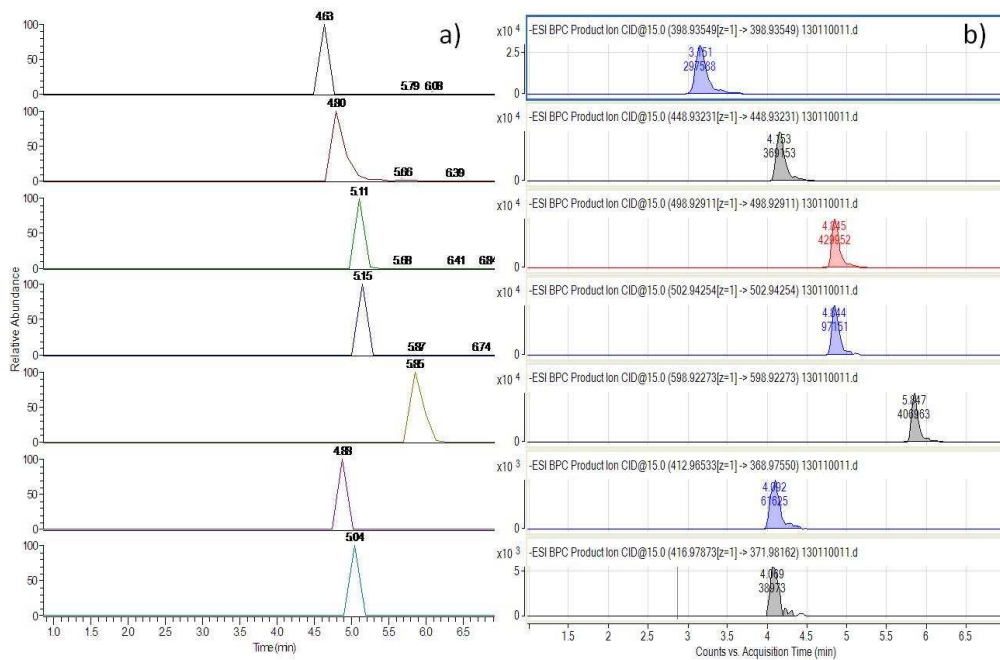
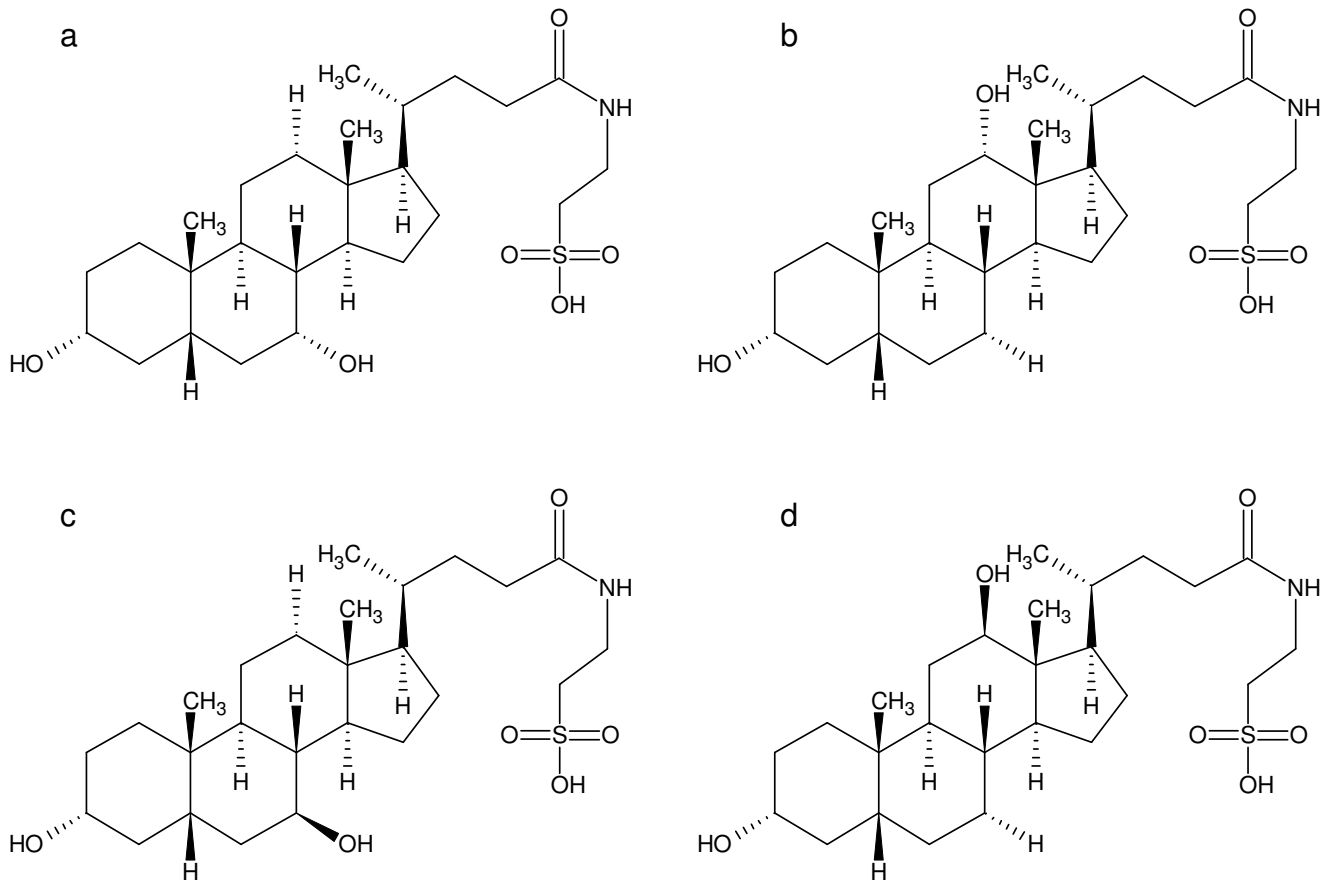


Figure 3



218x143mm (150 x 150 DPI)

Review Only



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mi= 498.2889

Figure 5

Fish sample presenting interference Fish sample without interference

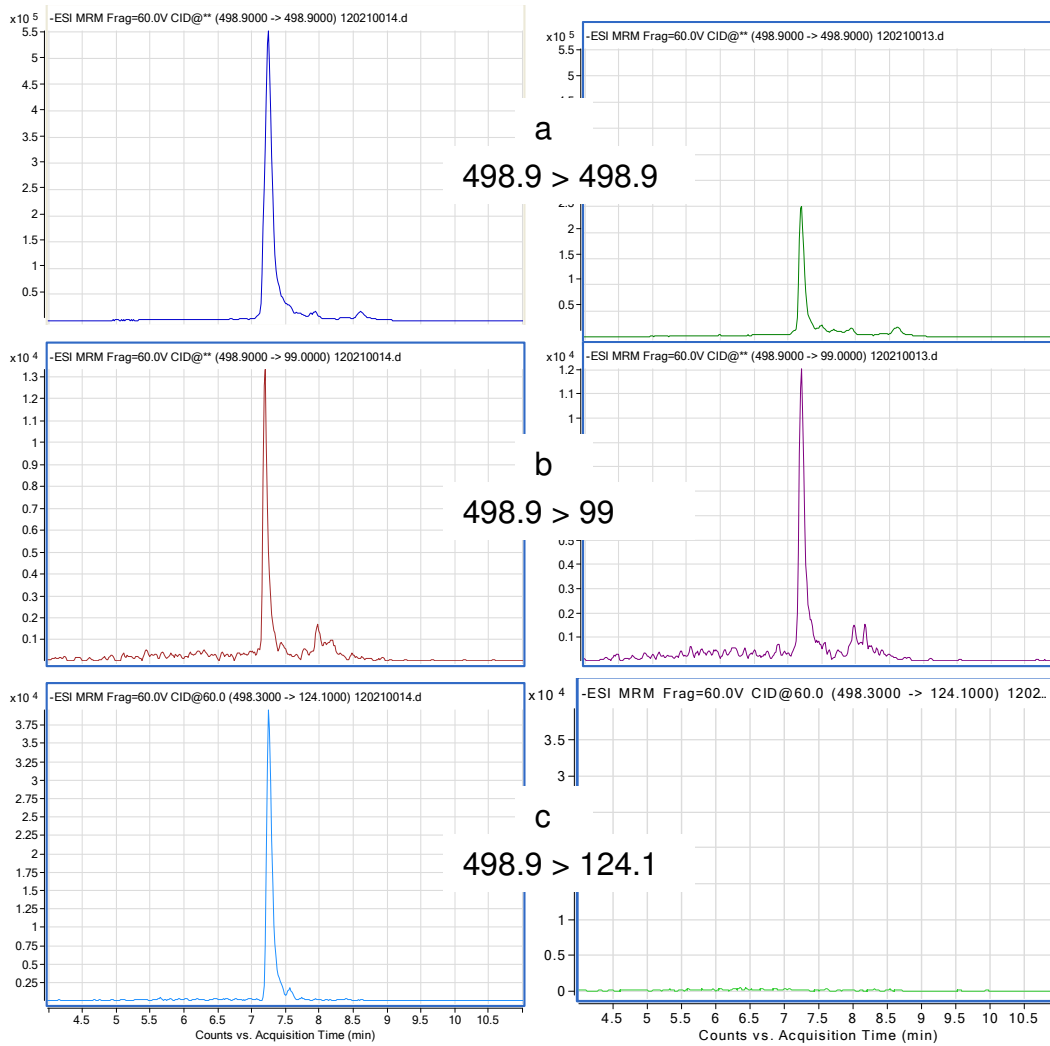
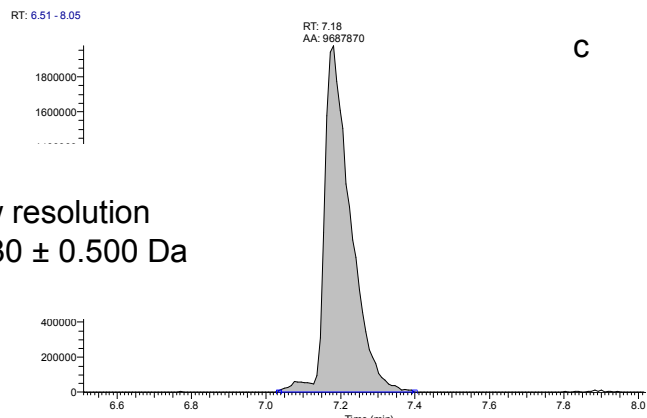
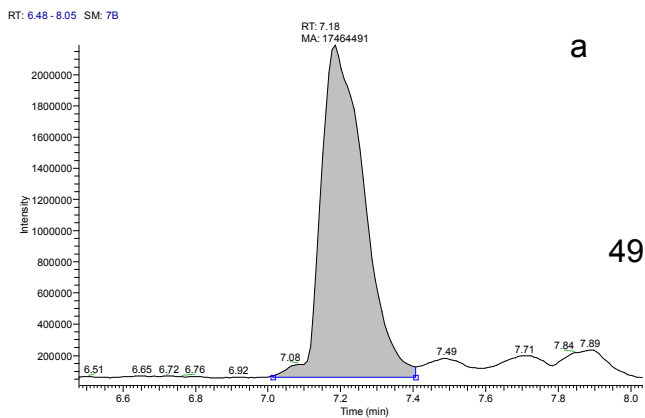


Figure 6

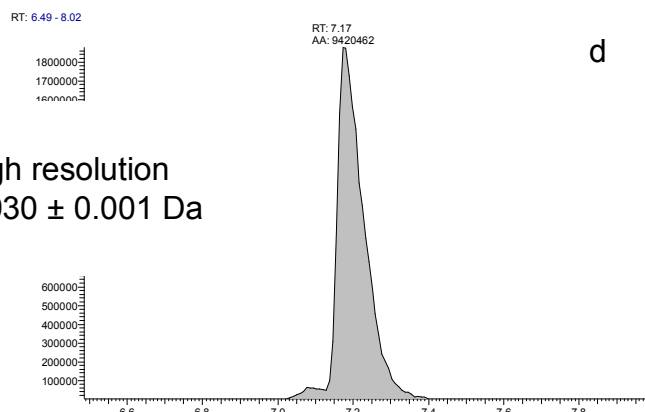
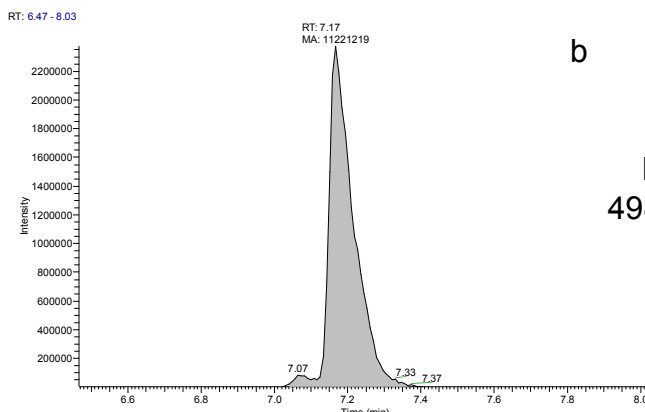
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Fish sample presenting interference

Fish sample without interference



Low resolution
 498.930 ± 0.500 Da



High resolution
 498.930 ± 0.001 Da

Figure 7

Table1: Optimised parameters for the three instruments 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 (\pm 10 ppm)	capillary voltage (V)	Collision energy (eV)	fragmentor (V)	Collision energy (eV)	m/z > m/z (\pm 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

	<i>m/z</i> 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	Linearity	Calc.LOD	Linearity	Calc.LOD	Linearity
	[pg inj]	range [pg inj]	[pg inj]	range [pg inj]	[pg inj]	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