Title: Tetrahydrofuran-water extraction, in-line clean-up and selective liquid chromatography/tandem mass spectrometry for the quantitation of perfluorinated compounds in food at the low picogram per gram level

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30 **Abstract**

31 A new solvent extraction system was developed for extraction of PFCs from food. The
32 extraction is carried out with 75:25 (v/v) tetrahydrofuran:water, a solvent mixture that
33 provides an appropriate balance of hydrogen bonding, dispersion and dipole–dipole
34 interactions to efficiently extract PFCs with chains containing 4–14 carbon atoms from
35 foods. This mixture provided recoveries above 85% from foods including vegetables,
36 fruits, fish, meat and bread; and above 75% from cheese. Clean-up with a weak anion-
37 exchange resin and Envi-carb SPE, which were coupled in line for simplicity, was
38 found to minimize matrix effects (viz. enhancement or suppression of electrospray
39 ionization). The target analytes (PFCs) were resolved on a perfluorooctyl phase column
40 that proved effective in separating mass interferences for perfluorooctane sulfonate
41 (PFOS) in fish and meat samples. The mass spectrometer was operated in the negative
42 electrospray ionization mode and used to record two transitions per analyte and one per
43 mass-labelled method internal standard. The target PFCs were quantified from solvent
44 based calibration curves. The limits of quantitation (LOQs) were as low as 1–5 pg
45 analyte g^{-1} food; by exception, those for C_4 and C_5 PFCs were somewhat higher (25–30
46 pg g^{-1}) owing to their less favourable mass response. To the best of our knowledge
47 these are the best LOQs for PFCs in foods reported to date. The analysis of a variety of
48 foods revealed contamination with PFCs at levels from 4.5 to 75 pg g^{-1} in 25% of
49 samples (fish and packaged spinach).C_{10}–C_{14} PFCs were found in fish, which testifies to
50 the need to control long chain PFCs in this type of food. The proposed method is a
51 useful tool for the development of a large-scale database for the presence of PFCs in
52 foods.

53

54

55 **1. Introduction**

56

57 Human exposure to perfluorinated compounds (PFCs) is currently receiving
58 considerable attention from scientists and policy makers owing to the ubiquity of these

59 substances in human blood and tissue samples worldwide, but particularly in
60 industrialized areas [1,2]. The most abundant PFC in human samples is perfluorooctane
61 sulfonate (PFOS), which was widely used; however, other perfluoroalkyl sulfonates
62 (PFASs) and carboxylic acids (PFACs) are also frequently detected [1-3]. PFCs are
63 toxic, highly persistent and bioaccumulative; this has led the European Union [4], North
64 America [5] and major manufacturers such as 3M [6] and DuPont [7] to impose
65 stringent restrictions on the production and use of compounds such as PFOS and
66 perfluorooctanoic acid (PFOA).

67 Although humans are exposed to PFCs from a number of sources, food (drinking
68 water included) could bethe dominant intake pathway. PFCs can contaminate food by
69 bioaccumulation of ,especially, longer chain members in fish and shellfish (a result of
70 oceans acting as contaminant sinks) [8] or contact with packaging material. Few
71 systematic investigations on PFC levels in food are conducted to date mostly in North
72 America and Western Europe [9-14].The European Food Safety Authority (EFSA) has
73 completed a risk assessment on PFOS and PFOA in the food chain and established a
74 tolerable daily intake (TDI) of 150 and 1500 ng kg^{-1} body weight day^{-1}, respectively
75 [15]. EFSA has noted an urgent need for data on PFC levels in various food items in
76 order to better understand contamination routes and monitor trends in exposure levels.

77 Analysing PFCs in such complex and variable matrices as foods is a rather
78 challenging task in many ways. The PFCs typically encountered in food include ionic,
79 water-soluble short chain and non-polar long-chain compounds (*viz.* C_4–C_{14} PFACs and
80 C_4–C_8 PFASs), the extraction efficiency of which is strongly dependent on solvent
81 polarity. The PFC concentrations measured so far suggest their presence at low levels
82 (pg g^{-1} to low ng g^{-1} range) in primary foods such as meat, milk, cereals, oil, fruits and
83 vegetables, but higher levels (ng g^{-1} to µg g^{-1}) for some specific compounds in fish and
84 offal foods [1]. Whereas the quantitation of PFCs in fish is generally straightforward
85 and has improved considerably in recent years [19-21], scaling down to the pg g^{-1} level
86 requires using highly efficient extraction methods in addition to extensive, complex
87 clean-up and time-consuming solvent reduction procedures.

88 Only a limited number of methods are currently available to investigate dietary
89 exposure to PFCs [10,11,17,18]. Food samples (1–10 g, wet weight) are usually serially
90 extracted with medium-polar solvents such as methanol or acetonitrile, whether directly
91 or following alkaline digestion. Commonly, a clean-up procedure involving successive
92 treatment with dispersive graphitized carbon (ENVI-carb) and/or filtration through a
93 weak anion-exchange (WAX) SPE material is needed. Liquid chromatography
94 (LC)/negative electrospray ionization (ESI)/triple quadrupole mass spectrometry
95 (MS/MS) has become the *de facto* standard for quantifying PFCs inasmuch as it
96 provides detection limits in the range 1–100 pg. Most LC separation procedures for this
97 purpose use standard C_{18} or C_8 phases; however, use of fluorinated stationary phases to
98 separate PFCs by fluorine content and conventional reversed phase mechanisms is being
99 fostered to prevent co-elution of known biological mass interferences with PFOS [22]
100 and PFHxS [23].

101 These methods, however, are still confronted with many problems. Thus, they
102 provide low recoveries which are strongly dependent on the chain length and polar
103 groups present in the particular PFC, as well as on the sample matrix components. For
104 example, the absolute recoveries from lamb liver provided by the most sensitive method
105 reported so far (detection limits 1–650 pg g^{-1}) [11] are in the range 83–72% for C_{4-8}
106 PFASs and 65–17% for C_{6-12} PFACs; these recoveries are matrix-dependent and differ
107 from those for other foods (*e.g.* 30–70% for $^{13}C_4$-PFOS and 60–133% for $^{13}C_4$-PFOA).
108 In addition, little information is available about the concentrations of the shorter- (C_{4-5})
109 and longer-chain (C_{13-14}) PFACs in foods owing to their poor extraction by medium-
110 polar solvents.

111 In this work, we developed a simple, fast, and efficient method for the
112 quantitative extraction of C_4–C_{14} PFACs and C_4–C_8 PFASs from a variety of
113 representative food items prior to their LC–ESI-MS/MS determination. Mixtures of
114 tetrahydrofuran (THF) and water were used for this purpose on the grounds of their
115 large differences in dielectric constant (ε) and Hildebrand solubility parameter (δ), and
116 hence of the ability to prepare mixed solvents spanning a wide range of dispersion,

117 dipole–dipole and hydrogen bonding forces [24] which were examined with a view to
118 facilitating solubilization of all PFCs. Sample clean-up was done by using an in-line
119 coupled anion exchange resin and graphitized carbon SPE; and LC was done on a
120 perfluorooctyl stationary phase to prevent matrix mass interferences for PFASs. The
121 results are discussed below.
122
123 **2. Material and methods**
124
125 *2.1. Chemicals*
126
127 All chemicals were analytical reagent-grade and used as supplied. Both target
128 and mass-labeled PFCs were supplied by Wellington Laboratories, in 50 µg mL^{-1}
129 solutions. The fourteen target PFCs studied were as follows: perfluorobutanoic acid
130 (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA),
131 perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA);
132 perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA),
133 perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA),
134 perfluorotetradecanoic acid (PFTeDA), potassium perfluoro-1-butanesulfonate (PFBS),
135 potassium perfluoro-1-hexanesulfonate (PFHxS) and PFOS. The stable isotope
136 analogues $^{13}C_4$PFBA, $^{13}C_2$PFHxA, $^{18}O_2$PFHxS, $^{13}C_4$PFOA, $^{13}C_5$PFNA, $^{13}C_2$PFDA,
137 $^{13}C_4$PFOS and $^{13}C_2$PFUdA were used as method standards (ISs) to control for potential
138 losses during extraction and clean-up and MS performance (incl. ion suppression and
139 enhancement). $^{13}C_8$PFOS, $^{13}C_8$PFOA and $^{13}C_7$ PFUdA were used as injection ISs and
140 added just prior to injection. The injection ISs were only used to *monitor* MS
141 performance and were not used for correction of the results. Sodium taurodeoxycholate
142 hydrate (TDCA) and ammonium formate were supplied by Sigma. Tetrahydrofuran
143 (THF) was obtained from Sigma-Aldrich (Steinheim, Germany) and methanol (MeOH)
144 and LC-grade water were supplied by JT Baker (Deventer, The Netherlands). Stock
145 standard solutions each containing a mixture of target PFCs, method ISs or injection

146 ISs, at a 100 ng mL^{-1} concentration each were prepared separately in methanol and
147 stored in closed polypropylene bottles at room temperature.
148
149 *2.2. Determination of PFCs in foods*
150
151 *2.2.1. Sample preparation and preservation*
152
153 Muscle fillets of raw fish (herring, pangasius, salmon and flounder) and meat
154 (pork and chicken), whole-grain bread, vegetables (spinach and carrot), fruits (orange
155 and apple), cheese (*Gouda*) and sunflower oil samples were bought at local
156 supermarkets in Amsterdam (The Netherlands) in August–September 2009. An amount
157 of 50 g of fish, meat or fruit was homogenized in an Ultra-Turrax homogenizer T25
158 equipped with S25N-8g and S25N-25g dispersing elements (Ika Werke, Germany); on
159 the other hand, cheese and vegetables were homogenized in a crushing machine. About
160 10 g of each homogenized sample (2.5 g for cheese) was weighed in a 50 mL
161 polypropylene (PP) tube, freeze-dried for 24 h (freeze-drier Lyph lock 1 L, Labconco,
162 Kansas City, MO, USA) and stored frozen at –20 ºC until analysis. Sunflower oil
163 samples (5 g) were used untreated.
164
165 *2.2.2. Tetrahydrofuran–water extraction*
166
167 Freeze-dried vegetable, fruit, meat and fish samples were fortified at a 125 pg g^{-1}
168 wet weight (w.w.) concentration level with method ISs and extracted with 20 mL of
169 75:25 (v/v) THF:water by shaking in 50 mL PP tubes with an orbital shaker (SM 30,
170 Edmund Buhler Gmbh, Hechingen, Germany) at 500 rpm for 10 min. Cheese samples
171 were spiked with 500 pg g^{-1} concentrations of the method ISs. The volume of
172 THF:water mixture used to extract bread was 30 mL. Sunflower oil samples (5 g fresh
173 weight, 125 pg g^{-1} IS) were cleaned up directly. After extraction, samples were
174 centrifuged (centrifuge SW 12, Firlabo, Meyzieu, France) at 3500 rpm for 10 min and

175 10 mL of their clear supernatant (15 mL for bread) was transferred to 15 mL PP tubes
176 and allowed to evaporate down to 6 mL at 50 ºC under a nitrogen stream; because only
177 THF evaporated, the concentrated solution contained 42% water. The samples were
178 then diluted to 15 mL with distilled water and centrifuged again at 3500 rpm for 5 min
179 to facilitate precipitation of solids and phase separation of lipids.
180
181 *2.2.3. Clean-up by anion exchange and in-line coupled graphitized carbon SPE*
182
183 Oasis WAX (6cc, 150 mg) and Supelclean ENVI-carb (6cc, 250 mg) cartridges
184 supplied by Waters and Supelco (Zwijndrecht, The Netherlands), respectively, were
185 used for sample clean-up. Diluted extracts (15 mL, solid material and lipids discarded)
186 or sunflower oil (5 g) were transferred onto preconditioned [25] weak anion exchange
187 (WAX) SPE cartridges at a rate of 1 drop/s. After loading, the cartridges were washed
188 with 4 mL of 25 mM acetate buffer at pH 4 and 8 mL of a 50:50 (v/v) THF:acetonitrile
189 mixture at a rate of 2 drop/s. Further cleaning was achieved by using larger volumes of
190 THF:acetonitrile mixture (12 mL for vegetables and fruits, and 16 mL for sunflower
191 oil). Then, the SPE WAX cartridge was coupled to the ENVI-carb cartridge via a
192 suitable polyethylene (PE) adaptor cap and a volume of 6 mL of methanol containing
193 0.1% NH_4OH passed through both SPE materials. Cartridges were dried under vacuum
194 to ensure maximal recovery of the eluates, which were evaporated to dryness (50 ºC,
195 N_2) and reconstituted with 250 µL of a mixture of 1:1 methanol and aqueous
196 ammonium formate (6.3 mM, pH 4) containing a 5 ng mL^{-1} concentration of injection
197 ISs. Finally, the extracts were transferred to 1.5 mL PP Eppendorf tubes and centrifuged
198 (ultracentrifuge Biofuge 28RS, Heraeus Sepatech, Lelystad, Netherlands) at 13.000 rpm
199 for 10 min, after which a 200 µL aliquot of supernatant was transferred to a PP LC vial.
200
201 *2.2.4. Quantitation of PFCs by LC-ESI-MS/MS*
202

203 The target PFCs were separated and quantified by using Agilent 1200 Series LC
204 system (Palo Alto, CA, USA) coupled with an Agilent 6410 electrospray interface (ESI)
205 operating in the negative ion mode prior to triple-quadrupole mass spectrometric
206 detection. A Fluorosep RP Octyl column (particle size 5 µm, i.d. 2.1 mm, length 15 cm)
207 supplied by ES Industries (West Berlin, NJ, USA) was used as stationary phase. A
208 Waters Symmetry C_{18} guard column (particle size 5 µm, i.d. 3.9 mm, length 20 mm)
209 obtained from Waters (Milford, Massachusetts, USA) was inserted before the LC
210 column. A Water Symmetry C_{18} column (particle size 5 µm, i.d. 2.1 mm, length 50 mm)
211 also supplied by Waters was used to assess the advantages of the fluorinated column in
212 terms of selectivity. The injection volume used was 20 µL. The mobile phase consisted
213 of 6.3 mM aqueous ammonium formate at pH 4 and methanol, and was passed at a flow
214 rate of 0.3 mL min^{-1}. The column temperature was set at 25 ºC. The gradient elution
215 program was 65% water during the first 2 min, a linear gradient from 65% to 5% water
216 over the next 53 min and 100% methanol for another 10 min. Reconditioning the
217 column took about 10 min. As recommended by the supplier, the Fluorosep column was
218 cleaned after each batch of runs. For this purpose, the column was flushed with water
219 for 15 min to remove the buffer and then with a 30:70 THF:ACN (v/v) mixture for 2 h
220 to remove highly hydrophobic compounds. The operating conditions for the ESI source
221 were as follows: capillary voltage 1000 V, source temperature 325 ºC, gas flow rate 6 L
222 min^{-1} and nebulizer gas pressure 25 psi. Table 1 of supplementary data shows the
223 quantifier and qualifier ions coming from two selected transitions used for each target
224 PFC, the internal standards together with their corresponding quantifier ions, and the
225 associated values for the fragmentor voltage and collision energy. The quantifier and
226 qualifier ions for TDCA (a common interference for PFOS) are also given. The selected
227 reaction monitoring transitions for each analyte and internal standard were acquired by
228 using a dwell time of 0.02 s for each. Solvent based calibration curves were constructed
229 from standard solutions containing the target PFCs at concentrations over the ranges
230 stated in Table 1 and 5 ng mL^{-1} concentrations of method and injection ISs and were
231 prepared by appropriate dilution of methanolic stock solutions with 1:1

232 methanol/aqueous ammonium formate (6.3 mM, pH 4). Instrument control, file
233 acquisition and peak integration were done with the software Mass Hunter (Agilent).
234 PFC concentrations in sample extracts (containing the method IS at 5 ng mL^{-1} that is
235 added before extraction) were calculated from the calibration curve obtained by plotting
236 the ratio of analyte peak area to method IS peak area against the analyte concentration.
237

238 **3. Results and discussion**

239

240 *3.1. Control of background contamination*

241

242 One typical problem encountered in determining PFCs is background
243 contamination arising from the presence of a variety of fluoropolymer materials in the
244 components of LC equipment or labware [21,26]. Inlet solvent Teflon tubes were
245 identified as the main source of contamination with PFCs (at the low ng mL^{-1} level) in
246 our LC system. Its effect, however, was effectively suppressed by replacing the Teflon
247 tubes with PEEK tubes. As a precautionary measure, an additional column (Water
248 Symmetry 5 μm, 2.1 mm × 50 mm) was inserted between the pump and injector in
249 order to trap PFCs (mainly PFOA) released from the instrument. Contamination arising
250 from labware was prevented by using disposable PP tubes, vials and pipettes. Eluates
251 were evaporated with 99.999% pure nitrogen. No contaminating PFC was detected
252 above its detection limit. In any case, appropriate blanks were routinely injected into the
253 instrument during sample processing sequences in order to check for potential
254 procedural or instrumental contamination.

255

256 *3.2. Solvent extraction method*

257

258 Efficient extraction of amphiphilic molecules requires the use of solvents
259 capable of establishing properly balanced polar and non-polar interactions. The
260 hydrocarbon chains of PFCs investigated here contain 4–14 carbon atoms and are

261 highly hydrophobic owing to the presence of fluorine. The polar groups in PFACs
262 include hydrogen donors and acceptors, whereas those in PFASs include anions and
263 hydrogen acceptors. It is difficult to obtain strong enough polar (hydrogen bonding,
264 dipole–dipole) and non-polar (dispersive) interactions with all PFCs to ensure efficient
265 extraction from food by using an individual solvent. A solvent mixture must thus be
266 used instead.

267 In this work, we used mixtures of THF and water on the grounds of their
268 substantial difference in solvation capability this being given by their different
269 Hildebrand solubility parameter values (δ_{water} = 23.3 $cal^{1/2}$ $cm^{-3/2}$, δ_{THF} = 9.5 $cal^{1/2}$ $cm^{-3/2}$
270). The Hildebrand parameter (δ_T) provides a measure of the overall intermolecular
271 forces resulting from the additive effect of dispersion (δ_d), dipole–dipole (δ_p) and
272 hydrogen bonding (δ_h) forces. Individual values for these forces (Hansen parameters,
273 $cal^{1/2}$ $cm^{-3/2}$) are easily available from the literature [24]; based on their values for water
274 (δ_d = 7.6, δ_p = 7.8, δ_h = 20.6) and THF (δ_d = 8.2, δ_p = 2.8, δ_h = 3.9), hydrogen bonding
275 and dispersion forces are the major components in water and THF, respectively. Hansen
276 parameters can be used to calculate fractional Teas parameters in order to construct a
277 Teas diagram, i.e. a triangular plot that graphically represents the solubility of a solvent
278 in terms of these three forces [24]. Solvents spanning a wide range of solvation can be
279 obtained simply by changing the composition of the THF:water mixture as shown in
280 Figure 2B.

281

282 The ability of THF:water mixtures in v/v ratios from 100:0 to 0:100 to extract
283 C_4–C_{14} PFACs and C_4–C_8 PFASs from food was assessed by using freeze-dried
284 pangasius fillet samples (10 g, wet weight, blank material) fortified at a 10 ng g^{-1} w.w.
285 concentration of target PFCs. After extraction, the target compounds were directly
286 measured in the untreated solvent extract. Before development of the method was
287 completed, matrix-matched calibration was used in all tests to ensure accurate
288 quantitation. Figure 1A shows the PFC recoveries obtained with the different
289 THF:water mixtures studied (solvent volume = 40 mL).

No extraction of the most polar (< C_6) and non-polar (>C_{10}) PFCs was achieved with water; also, the recoveries obtained with pure THF never exceeded 40% (Figs 1A,a,h). The balance between polar (dipole–dipole and hydrogen bonding) and non-polar (dispersion) forces for both solvents (Fig. 1B,a,h) resulted in inadequate solubilization of PFCs. The recoveries obtained with most of the solvent mixtures were strongly dependent on the length of the hydrocarbon chain of the PFCs (Fig. 1A,b,e,f,g), the lowest values invariably being those for the most hydrophobic compounds. Using a 75:25 (v/v) THF:water mixture (Fig. 1A,c) suppressed the dependence of recoveries on the PFC structure and raised them above 94%. Similar results were obtained with THF:water mixture compositions around the previous value (e.g. Fig. 1A,d).

Based on these results, the ideal solvent for extracting C_4–C_{14} PFCs is one with a Hildebrand parameter value around 12–14 (Fig. 1B); and Teas parameters with (f_h + f_p)/non-polar (f_d) force ratios of about 1.2. For example, a 25:75 mixture of water and THF has $f_h = 34$, $f_p = 20$ and $f_d = 46$ (Fig. 1B,c). A solvent mixture consisting of 75:25 (v/v) THF:water was finally chosen as optimal for extraction.

Because water content varies among food types (*e.g.* ~70–75% for fish and meat, but ~90–95% for fruits and vegetables), obtaining quantitative recoveries and reproducible results entails freeze-drying food samples for analysis. The effectiveness of this procedure was assessed by freeze-drying 10 g (wet weight) of pangasius fillets fortified at a 10 ng g^{-1} w.w. concentration of target C_4–C_{14} PFACs and C_4–C_8 PFASs for 24 h, and extracting the freeze-dried samples with 40 mL of 75:25 (v/v) THF:water. These tests were conducted in triplicate. The recoveries obtained from the freeze-dried samples exceeded 95% for all PFCs, and standard deviations were 2–5%. Therefore, freeze-drying the samples caused no significant PFC losses.

Since water is a major component of food and can be expected to influence the solvation behaviour of common solvents for PFC extraction (methanol, acetonitrile), it is advisable to estimate its influence via a Teas diagram; irrespective of the particular solvent used for extraction, freeze-dried samples can be expected to give more reproducible, less matrix-dependent results in PFC quantitation.

319 The optimal ratio of solvent volume to sample amount was determined by
320 extracting a variety of foods containing variable proportions of proteins, carbohydrates
321 and lipids (*e.g.* herring and flounder fillets, cheese, pork, green pepper and bread).
322 Freeze-dried samples (10 g, wet weight, fortified with at 10 ng g^{-1} w.w. concentration
323 of method ISs) were extracted with 10, 20, 30 and 40 mL of 75:25 (v/v) THF:water in
324 50 mL PP tubes with shaking at 500 rpm for 10 min and subsequent centrifugation at
325 3500 rpm for 10 min. An aliquot of each extract was used for analysis. The recoveries
326 thus obtained were independent of the solvent volume within the studied range. A 2:1
327 ratio of solvent (mL) to sample (g) was selected as optimal for most samples in order to
328 be able to analyse an aliquot of the extract representing half the solvent volume used for
329 extraction. Higher solvent (mL)/sample (g) ratios were selected for cheese and bread
330 (4:1 and 3:1) owing to the high fat content of cheese (~27%) —which resulted in phase
331 separation and precluded the use of a homogeneous solution at a 2:1 ratio— and the
332 high porosity of bread, which hindered sample dispersion and solvent recovery after
333 extraction.

334 The influence of matrix components on PFC recoveries was investigated by
335 extracting representative food samples fortified at a 10 ng g^{-1} w.w. concentration of
336 method ISs. Figure 2 shows the results, as well as the extraction conditions. Matrix-
337 matched calibration was used to correct recoveries for potential suppression or
338 enhancement of IS ionization. As can be seen from Fig. 2, recoveries were matrix-
339 independent and exceeded 85% in all samples except cheese, where they were around
340 75% for some ISs (*e.g.* $^{13}C_4$PFOA, $^{13}C_2$PFDA and $^{13}C_2$PFUdA). The high extraction
341 efficiency of this method for PFCs spanning a wide polarity range in any type of food is
342 one clear advantage over existing methods [11,17].

343 The extraction method was validated by analysing flounder fillets previously
344 used in the Second International Interlaboratory Study on PFCs [20] and containing the
345 following concentrations of the target compounds, expressed as mean values and their
346 standard deviations: 18.0 ± 4.1, 17.5 ± 4.6, 21.1 ± 4.6, 15.9 ± 4.1, 17.3 ± 5.2 and 150 ±
347 44 ng g^{-1} for PFOA, PFNA, PFDA, PFUdA, PFDoA and PFOS, respectively. Sample

348 aliquots (10 g, wet weight) were freeze-dried and spiked with the corresponding mass-
349 labeled PFCs (see Table 1 of supplementary data). The mean values and standard
350 deviations thus obtained with the proposed method were 18.5 ± 0.8, 19.2 ± 0.3, 21.7 ±
351 0.3, 18.0 ± 0.9, 21.9 ± 0.8 and 166 ± 7 ng g^{-1} for PFOA, PFNA, PFDA, PFUdA,
352 PFDoA and PFOS, respectively. These results are all within the range of reported mean
353 ± *s* values for the reference material.
354

355 *3.3. Sample clean-up and concentration*
356

357 Individual or serial weak anion exchange (WAX) SPE and dispersive
358 graphitized carbon are currently the most widely used clean-up choices for quantifying
359 PFCs in food [11,17,18]. Because the amount of sample used for analysis was 10 g, the
360 use of both SPE materials was highly advisable in order to have clean enough sample
361 extracts —and avoid MS signal suppression or ionization caused by matrix interferences
362 and sensitivities at the pg g^{-1} level as a result. In this work, two serial clean-up steps
363 involving WAX and graphitized carbon materials were applied to the THF:water
364 extracts; investigations were intended for getting quantitative recovery of the target
365 PFCs and simplify the overall procedure.
366 Similarly to the methanol extracts, dilution with water was required to obtain
367 good recoveries [11,23]; in fact, direct filtration of 20 mL of the 75:25 (v/v) THF:water
368 extracts through WAX SPE resulted in very low retention of PFCs (Table 2). Retention
369 greatly improved by effect of partially or completely evaporating the THF in the extract
370 with nitrogen at 50 ºC and adding water to obtain a final THF:water volume of 20 mL
371 —at this point, centrifugation was needed to remove matrix components which had thus
372 been rendered insoluble. Table 2 shows the most salient results. Quantitative retention
373 of PFCs on WAX was only achieved with THF proportions below 25%, which was thus
374 chosen as optimal —evaporation of the required volume of THF took only around 5–10
375 min by virtue of the high volatility of the solvent. Retention of PFCs was also favored
376 by filtering lower sample volumes [*e.g.* 10 mL of sample extract in 75:25 (v/v)

377 THF:water], but was still inadequate for most PFCAs (Table 2). Samples of sunflower
378 oil (5 g, fresh weight) were directly filtered through WAX SPE cartridges.

379 The cartridges were initially eluted with 4 mL of acetate buffer (25mM, pH 4),
380 which is allegedly required to remove interfering biomolecules (lipids, proteins) and
381 improve adsorption of the target anions [25], and then with 8 mL of a 50:50 (v/v)
382 mixture of THF and acetonitrile. This hydrophobic solvent mixture (ε_{THF} = 7.5, ε_{ACN} =
383 36) proved more efficient than another commonly used solvent —methanol [11,25]— in
384 removing highly hydrophobic compounds such as dyes or lipids, and rendered the PFC
385 extracts less coloured. The low solubility of PFCs in pure THF and ACN —neither is a
386 hydrogen donor, so neither can solubilize the typical anionic polar groups of PFCs—
387 ensured the absence of PFC losses during the washing step. In order to obtain cleaner
388 extracts, a volume of THF:ACN mixture of 12 mL was used with vegetables and fruits;
389 as a result, the internal standard recoveries for $^{13}C_8$PFOA,$^{13}C_7$PFUdA and $^{13}C_8$PFOS
390 from final orange sample extracts rose from 67%, 64% and 80% to 110%, 94% and
391 105%, respectively, as the amount of washing solvent was increased from 8 to 12 mL.
392 A volume of 16 mL of solvent mixture was used for sunflower oil samples, which were
393 subjected to no pretreatment.

394 Elution of PFCs from the WAX material with 4 mL of methanol containing
395 0.1% NH_4OH was quantitative and independent of the particular food type; however,
396 the most hydrophobic PFCs were strongly adsorbed to the insoluble matrix components
397 produced during evaporation to dryness of the eluates and could not be completely
398 recovered by reconstitution with 250 µL of a 50:50 (v/v) mixture of 6.3 mM ammonium
399 formate and methanol; for example, salmon extracts exhibited losses of 50–60% for C_{13}
400 and C_{14} acids. This led us to subject the extracts to additional clean-up with ENVI-carb
401 as proposed by other authors elsewhere [11,12].

402 In order to simplify the clean-up procedure with ENVI-carb, which is usually
403 applied in a dispersive format [11,12,18] —and thus requires transfer and centrifugation
404 of the extracts 2–4 times and causes the loss of some solvent as a result—, we chose to
405 conduct this step with Supelclean ENVI-carb cartridges (6cc, 250 mg). The cartridges

406 were previously conditioned with 4 mL of methanol and connected at the bottom with
407 Oasis WAX cartridges through a suitable PE adaptor cap at the time of elution of PFCs
408 —using 6 mL of methanol containing 0.1% NH_4OH is recommended to completely
409 elute PFCs from both types of cartridges. The addition of acetic acid when using ENVI-
410 carb [27] is recommended to avoid potential losses of anionic compounds by effect of
411 the slightly basic nature of graphitized carbon. However, we checked that no losses of
412 PFCs occurred in methanolic solutions containing 0.1% NH_4OH (fortified with 5 ng
413 mL^{-1} concentrations of the PFCs) on passage thorough cartridges containing 250 mg of
414 ENVI-carb. After elution, cartridges were vacuum-dried in order to maximize recovery
415 of the eluate, which was evaporated to dryness (50 ºC, N_2) and reconstituted with 250
416 μL of a mixture of aqueous 50:50 (v/v) ammonium formate (6.3 mM, pH4):methanol
417 containing a 5 ng mL^{-1} concentration of injection internal standards (Table 1 of
418 supplementary data). Ultracentrifugation of this extract is recommended because,
419 although suitable for standard solutions, filtration causes losses of the most hydrophobic
420 PFCs in some foods (*e.g.* around 20% for $^{13}C_2$PFUdA in pork sample extracts passed
421 through Waters GHP syringe filters, 0.2 μm, 13 mm).
422
423 *3.4. Total method recoveries*
424
425 The lack of mass-labeled homologs for some PFCs at the time of this study
426 (namely for PFPeA, PFHpA, PFTrDA, PFTeA and PFBS) led us to examine the
427 suitability of the proposed sample treatment for efficiently recovering the target PFCs
428 throughout the procedure (extraction, WAX/ENVI-carb SPE, evaporation,
429 reconstitution). Pangasius fillets samples —which were previously found to be free of
430 detectable levels of PFCs—; were fortified with target C_4-C_{14} PFACs and C_4, C_6 and C_8
431 PFASs at two different concentration levels (100 and 2000 pg g^{-1}) prior to freeze-
432 drying. The recoveries ranged from 88 to 110%, and their relative standard deviations
433 from 5 to 10% (n = 3). No significant differences in recovery between PFCs were

434 found. This justifies using the recommended method ISs for PFCs having no
435 commercially available mass-labeled homologs (Table 1 of supplementary data).

436 The ability of the proposed sample treatment to effectively extract PFCs from a
437 variety of foods was assessed by spiking representative samples of each of the major
438 food groups (*viz.* fruit and vegetables, meat, fish, cereals and fat-rich foods) following
439 freeze-drying with a 125 pg g^{-1} concentration of method ISs, and calculating the total
440 method recoveries. Table 3 shows the results. Recoveries exceeded 80% for fruits and
441 vegetables —spinach excepted—, and also for meat, fish and cereals. Therefore, the
442 proposed method is the first reported method capable of quantitatively recovering PFCs
443 at the low picogram-per-gram level from a variety of foods. Recoveries were lower for
444 PFACs in fat-rich foods, but were still above 60% in all instances. Losses of PFACs
445 from these matrices occurred mainly in their extraction with the THF:water mixture
446 (*e.g.* in the longest chain PFACs from cheese, Fig. 2) or during treatment with
447 WAX/ENVI-carb SPE for oil directly subjected to SPE. The decreased recoveries for
448 spinach were ascribed to matrix effects on the signals for the injection ISs. An increase
449 in the volume of solvent used for the washing SPE WAX step (maybe up to 16 mL as
450 used for sunflower oil) could help to reduce matrix effects in this more complex matrix.

451

452 *3.5. Analytical performance*

453

454 *3.5.1. Sensitivity*

455

456 Table 1 lists the analytical figures of merit of the proposed method. The
457 instrumental limits of quantitation (LOQs) and detection (LODs) were calculated from
458 50:50 (v/v) MeOH:aqueous ammonium formate (6.3 mM, pH 4) blanks containing a 5
459 ng mL^{-1} concentration of method and injection ISs at a signal-to-noise ratio of 10 and 3,
460 respectively. The LOQs and LODs were 0.7 and 0.5 ng mL^{-1} for short-chain PFCs
461 (PFBA, PFPeA and PFBS); 0.025 and 0.01 ng mL^{-1} for long-chain acids (PFDoA,
462 PFTrA and PFTeA); and 0.05 and 0.02 ng mL^{-1} for all other compounds except PFOA

463 and PFUdA (LOQ = 0.2 ng mL^{-1}, LOD = 0.12 ng mL^{-1}) and PFHxS (LOQ = 0.5 ng
464 mL^{-1}, LOD = 0.3 ng mL^{-1}). The increased LODs and LOQs for the latter compounds
465 were a result of their presence as impurities in proportions below 1% in the mass-
466 labeled reagents used as internal standards and could be improved by decreasing the
467 amount of ISs added to samples.

468 Method LOQs and LODs were calculated on the basis of sample blanks taking
469 into account a signal-to-noise ratio of 3 and 10, respectively, and the concentration
470 factor obtained with the method. Carrot, orange, pork, bread and sunflower oil sample
471 extracts were used for this purpose. No significant differences in background noise
472 between matrices were observed. This led us to adopt general LOQs and LODs for all
473 types of foods based on worst-case calculations assuming 70% total average recovery
474 for each PFC for the concentration factor since total method recoveries ranged from
475 about 60 to 120% in all samples. Table 1 shows the estimated LOQs and LODs, which
476 are the lowest reported so far to the best of our knowledge for this type of determination
477 [11,12,17]. Values of LOQs and LODs for cheese are four times higher due to the use of
478 a smaller sample size (2.5 g). A smaller volume of solvent at reconstitution step could
479 be used to compensate this loss of sensitivity.

480

481 *3.5.2. Selectivity*

482

483 PFC quantitation is reportedly subject to substantial matrix effects even after
484 thorough SPE clean-up [11,12,17]. Such effects, which result from co-eluting matrix
485 components, are compound- and food type-dependent, and may cause signal
486 enhancement or suppression, or even PFAS peak misidentification by effect of mass
487 interferences in samples of animal origin. The effect of these interferences on the
488 quantitation of PFCs with the proposed method were investigated in depth by using both
489 the proposed column (Fluorosep RP Octyl), which provides selective retention, and the
490 most widely used stationary phase (C_{18}).

491 Signal enhancement or suppression was estimated by comparing the response for
492 injection ISs (5 ng mL^{-1}) in the final reconstituted extracts with the average response of
493 these standards in the calibration solutions on the assumption that signal changes due to
494 instrument fluctuations would be negligible. Table 2 of supplementary data shows the
495 results, expressed as recoveries for the three injection ISs and two stationary phases
496 used. Most of the samples analysed with the Fluorosep column (~85%) exhibited little
497 signal suppression or enhancement (≤ 10%); the greatest effects in this respect were
498 those on $^{13}C_8$ PFOS in spinach (25% suppression) and $^{13}C_7$ PFUdA in bread (15%
499 enhancement). Matrix effects were generally more marked with the C_{18} column; thus,
500 signal suppression was 36% and 57% for $^{13}C_8$PFOA in spinach and herring,
501 respectively, and 37% for $^{13}C_8$ PFOS in spinach, whereas signal enhancement amounted
502 to 40% for $^{13}C_8$ PFOS in apple and cheese. Therefore, the combination of the proposed
503 sample treatment and the selective chromatographic retention mechanism for PFCs is
504 effective towards preventing signal enhancement or suppression by co-eluting matrix
505 components.

506 Overestimating the proportion of PFOS by misreporting co-eluting cholic acids
507 is a major problem here and continues to detract from accuracy in the determination of
508 PFCs in samples of animal origin. For example, TDCA bile salts, which contain a
509 sulfonate group, have been found to co-elute with PFOS on C_{18} columns [22,28,29].
510 The mass difference of these compounds (498.2968) is not large enough from that of
511 PFOS (498.9297) and compromise accuracy in their QQQ tandem mass spectrometry
512 determination; therefore, co-elution, especially in the presence of high levels of the
513 interferents, can lead to overestimation at the 499–80 transition. This problem has been
514 addressed in various ways including the use of other column types providing more
515 selective retention mechanisms (*e.g.* perfluorooctyl [17] or a ion-exchange phase
516 column [29]), the use of a more selective —but also less sensitive— transition (the 499–
517 99 transition, mainly [11]) or that of time-of-flight (TOF) mass spectrometry, which
518 affords accurate mass discrimination between PFOS and interferents [17].

519 PFOS peak misidentification was investigated by analysing a number of samples
520 (n = 10) of animal origin including muscle meat from pork and chicken, and fish fillets
521 from herring, salmon and pangasius, all of which were fortified at a 125 pg g^{-1} w.w.
522 concentration of method ISs. To this end, the chromatographic separation of PFOS and
523 TDCA on the Fluorosep column was optimized, the retention times being 43.8 and 38.0
524 min, respectively. Although the C_{18} column also provided well-resolved peaks for the
525 TDCA reagent (37 min) and PFOS (36 min), TDCA isomers are commonly present in
526 these samples, and co-elute with PFOS. Characteristic transitions for TDCA (498.3–80,
527 498.3–107 and 498.3–124) were also optimized, the fragmentor voltage and collision
528 energy values being quite different from those used for PFOS (see Table 1 of
529 supplementary data); this rendered TDCA undetected at the 499–80 transition used to
530 quantify PFOS at concentrations below ~100 ng mL^{-1} (equivalent to 5 ng g^{-1} in the
531 samples at an arbitrary extraction efficiency of 100% for TDCA). This had no effect on
532 the Fluorosep column, but increased the tolerance of TCDA by the C_{18} columns.
533 TCDA was present in all meat (chicken and pork) and fish samples (herring,
534 salmon and pangasius), its estimated concentrations in them ranging from 2.5 to 350 ng
535 g^{-1}. Quantifying PFOS with the Fluorosep column (499–80 transition) provided
536 concentrations of 57 ± 5 and 16 ± 1 pg g^{-1} in herring and salmon, respectively, but
537 undetectable levels in the other samples (including chicken). These values are consistent
538 with those obtained by using the C_{18} column with the 499–99 selective transition,
539 namely: *viz.* 61 ± 6 and 14 ± 1 pg g^{-1} for herring and salmon, respectively, and
540 undetectable levels for the other samples. However, using the more sensitive 499–80
541 transition provided PFOS concentrations of 251 ± 50, 17 ± 1 and 33 ± 2 pg g^{-1} in
542 herring, salmon and chicken, respectively, thus revealing that TCDA concentrations
543 above 100 ng mL^{-1} in the extracts led to PFOS overestimation (~440% in herring) and
544 misidentification (*e.g.* in chicken) with the C_{18} column. Although both the Fluorosep
545 column and the C_{18} column can be used to quantify PFOS with accuracy, use of the
546 former is recommended because it affords measurement at the 499–80 transition, which
547 is more sensitive than the 499–99 transition. By way of example, Fig. 3 shows the

548 chromatographic peaks for TDCA, PFOS and the method IS $^{13}C_4$ PFOS in the herring
549 sample as obtained with the two columns. As can be seen, the cholic acid signal at the
550 transition of interest for PFOS (499–80) was rather low and the measurement of PFOS
551 with the C_{18} column was interfered with by this transition.
552
553 *3.6. Analysis of food samples*
554
555 The proposed method was used to analyse a variety of food samples (n = 12)
556 including fish (herring, pangasius and salmon) and meat (pork and chicken) muscle
557 fillets, whole-grain bread, vegetables (spinach and carrot), fruits (orange and apple),
558 cheese (*Gouda*) and sunflower oil, all in duplicate.
559 Figure 3 shows the selected ion chromatograms for PFCs extracted from (A) a
560 standard solution containing a 1 ng mL^{-1} concentration and (B) a salmon muscle
561 sample. Only three samples (25% of the foods studied) were found to be contaminated
562 with PFCs (salmon, herring and spinach). Among PFASs, only PFOS was present in
563 fish (at 57 ± 5 pg g^{-1} in herring and 16 ± 1 pg g^{-1} in salmon); also, PFHxS was detected
564 in spinach, albeit at levels below its LOQ. As noted earlier, PFOS was clearly
565 distinguished from the bile salts present in fish. Long-chain PFACs were present in fish,
566 which confirms that bioaccumulation of these compounds involves mainly those with
567 the longest carbon chains [30] and the need for simple methods to quantify C_{12}–C_{14}
568 PFACs. The concentrations found were 27 ± 2, 7.7 ± 0.5, 31 ± 2, 11.3 ± 0.5 and 27 ± 6
569 pg g^{-1} for PFOA, PFNA, PFUdA, PFDoA and PFTrDA, respectively, in herring; and
570 5.6 ± 0.6, 10.3 ± 0.7, 75 ± 4, 16 ± 1, 31.3 ± 2 and 4.5 ± 0.6 pg g^{-1} for PFNA, PFDA,
571 PFUdA, PFDoA, PFTrDA and PFTeDA, respectively, in salmon. Spinach samples,
572 which were bought packaged, were also contaminated with PFACs: PFHpA at 8.8 ± 0.4
573 pg g^{-1} and PFOA at 31 ± 2 pg g^{-1}. PFC contamination in packaged lettuce was
574 previously reported by other authors [12]. Transfer from soil to crops [31] is a possible
575 source of contamination with PFCs in these foods. In addition, contamination may also
576 originate from processing the foods (e.g. washing) and packaging. The generally low

577 levels of PFCs found in this study are consistent with previous results of other authors
578 [9,11,12].
579

580 **Conclusions**

581

582 The following method is proposed for PFCs in food: freeze drying of the
583 samples; extraction using THF-water 75:25%; SPE enrichment and clean-up using
584 combined Oasis WAX and graphitised carbon followed by LC-ESI-MS/MS using a
585 fluorinated stationary phase column. The main assets of the proposed method for
586 determining PFCs in a variety of dietary foods include (*a*) very low detection limits (1–
587 5 pg g^{-1} except for C_4 and C_5 PFCs), which should enable accurate estimation of current
588 food contamination levels; (*b*) the ability to quantitatively extract PFCs spanning a
589 wider polarity range (C_4–C_{14}) relative to most existing methods (C_6–C_{12}); (*c*) the
590 matrix-independence of recoveries for a variety of samples (lipid-, protein- and
591 carbohydrate-rich food) by effect of the high extraction efficiency and minimal matrix
592 effects —a result of a smart combination of efficient extraction and clean-up with the
593 use of a highly selective perfluorooctyl phase column for PFC separation—; and (*d*) a
594 high accuracy and precision.
595

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603

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672
673

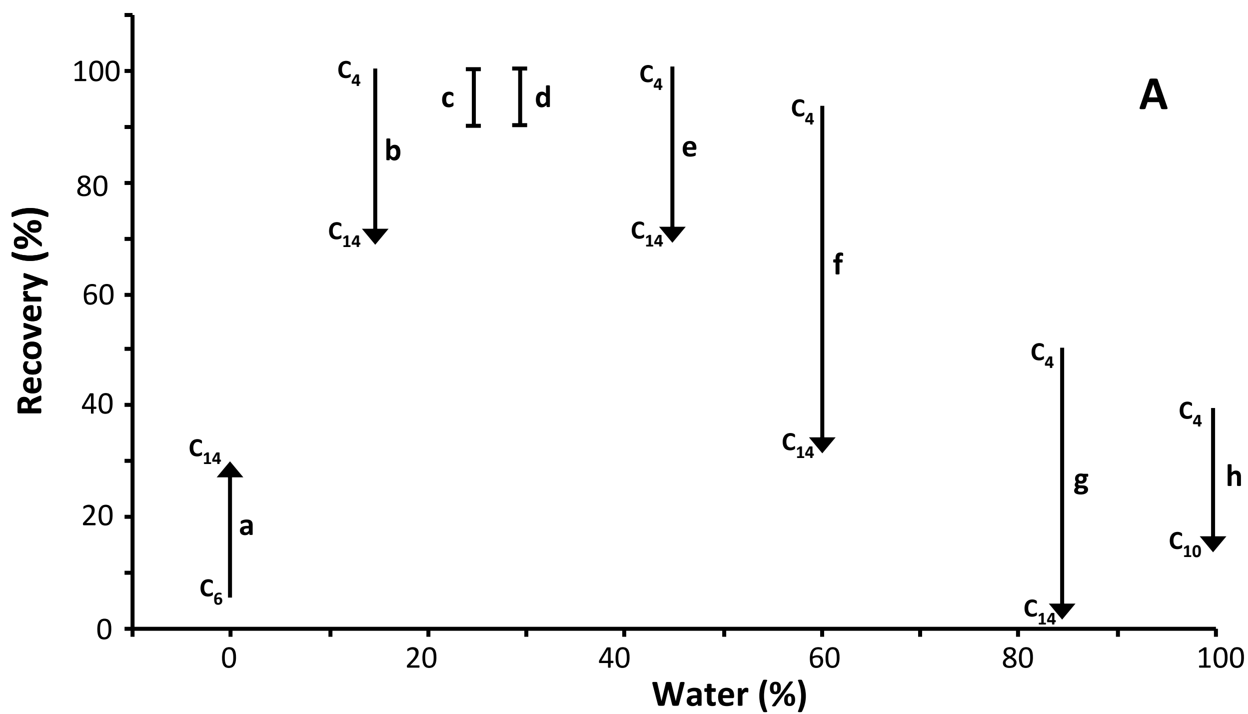
FIGURE CAPTIONS

Figure 1. (A) Recovery ranges obtained by extracting of frozen-dried pangasius fillet samples (10 g, wet weight, blank material) fortified with C_4–C_{14} PFCs (10 ng g^{-1} w.w.), using water:THF mixtures in variable (v/v) ratios: (*a*) 0:100, (*b*) 15:85, (*c*) 25:75, (*d*) 30:70, (*e*) 45:55, (*f*) 60:40, (*g*) 85:15 and (*h*) 0:100. (B) Teas graph showing the Hildebrand solubility (δ) and Hansen (f_d, f_p and f_h) parameters for each THF:water mixture.

Figure 2. Recoveries and standard deviations ($n = 2$) obtained by extraction with 20 mL of 75:25 (v/v) THF:water of a variety of food samples (10 g, wet weight, and 2.5 g for Gouda cheese) fortified with method internal standards at a 10 ng g^{-1} concentration. Matrix-matched calibration was used to calculate recoveries.

Figure 3. LC–ESI–MS/MS selected ion chromatograms for PFCs extracted from (A) a standard solution containing a 1 ng mL^{-1} concentration and (B) a salmon muscle sample.

Supplementary data Figure 1. LC–ESI–MS/MS selected ion chromatograms for PFOS, TDCA, PFOS and the surrogate IS $^{13}C_4$ PFOS (125 pg g^{-1}) used in the analysis of herring fillet samples with (A) a Fluorosep RP Octyl column and (B) a C_{18} column.



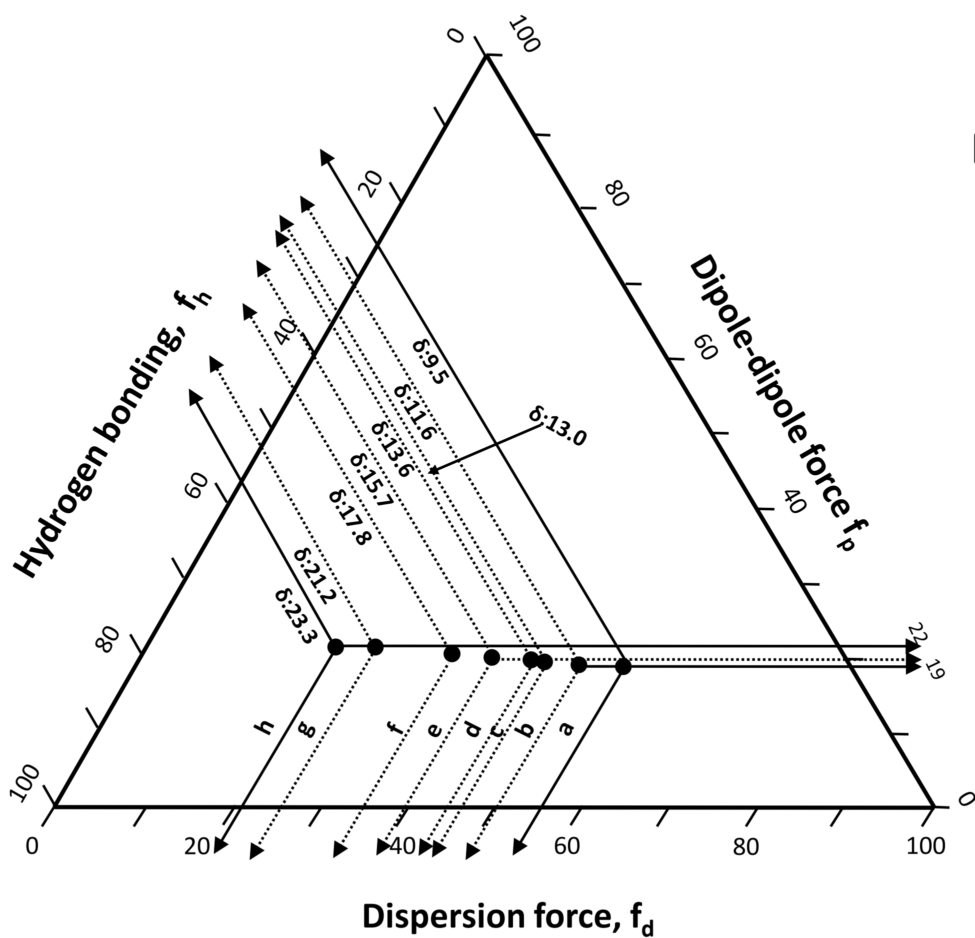


Figure 1

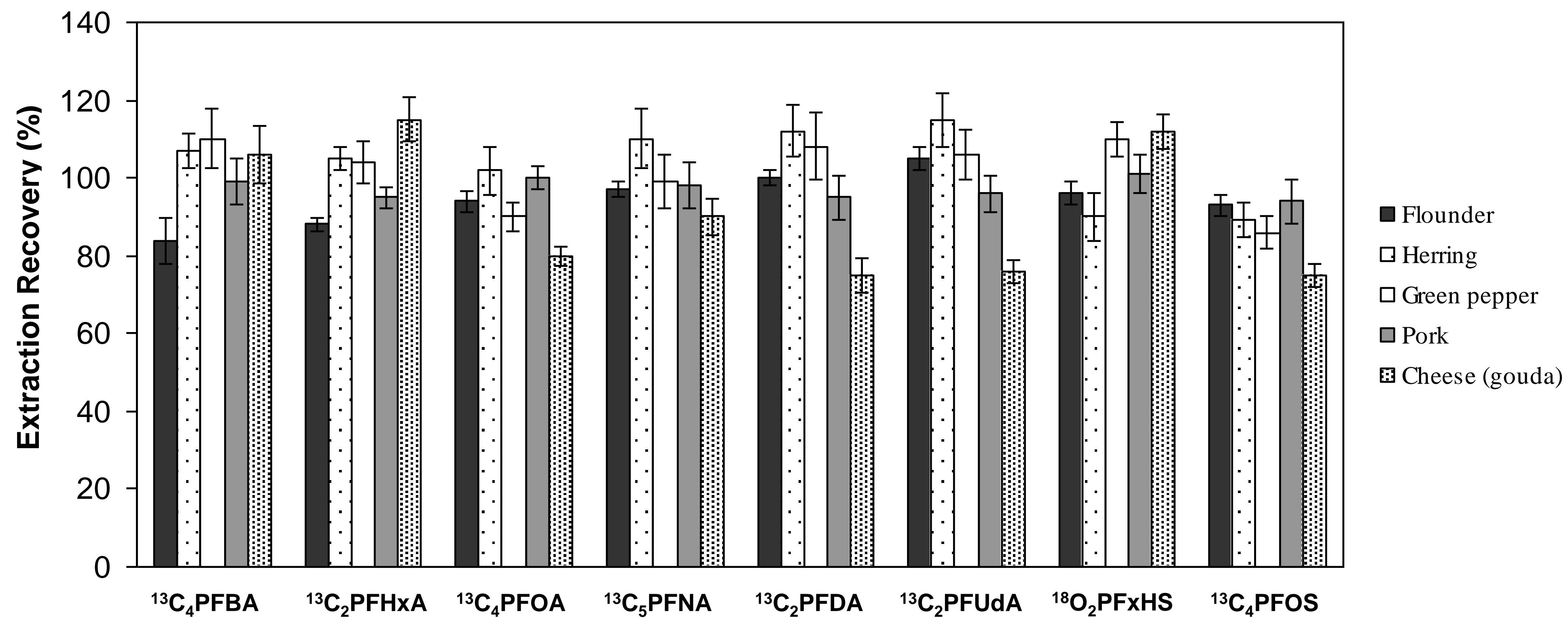
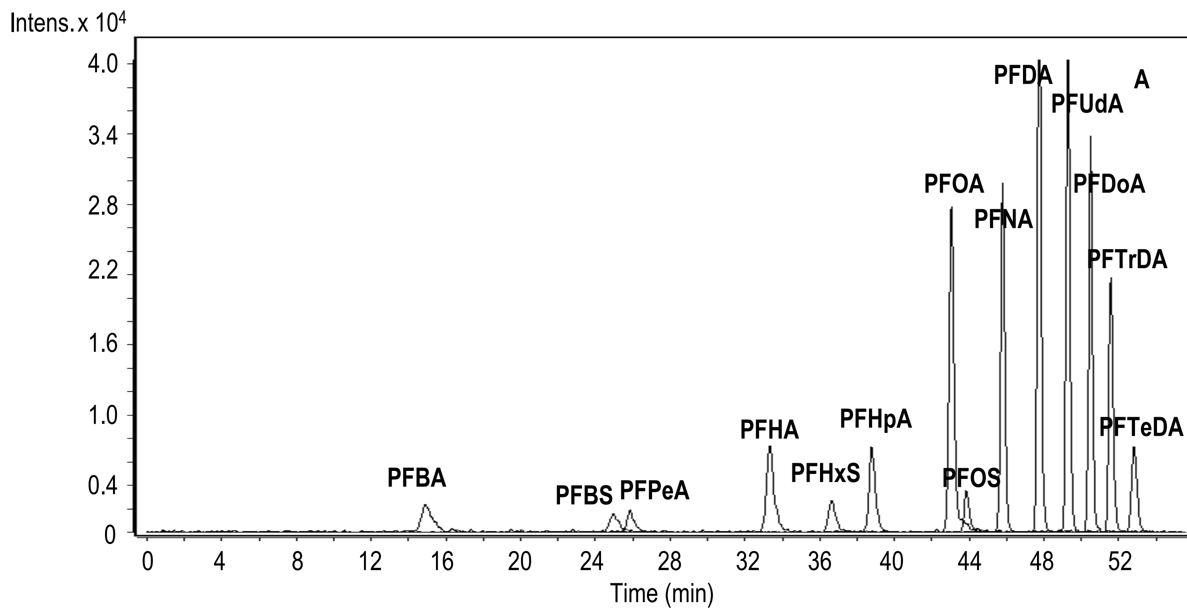


Figure 2



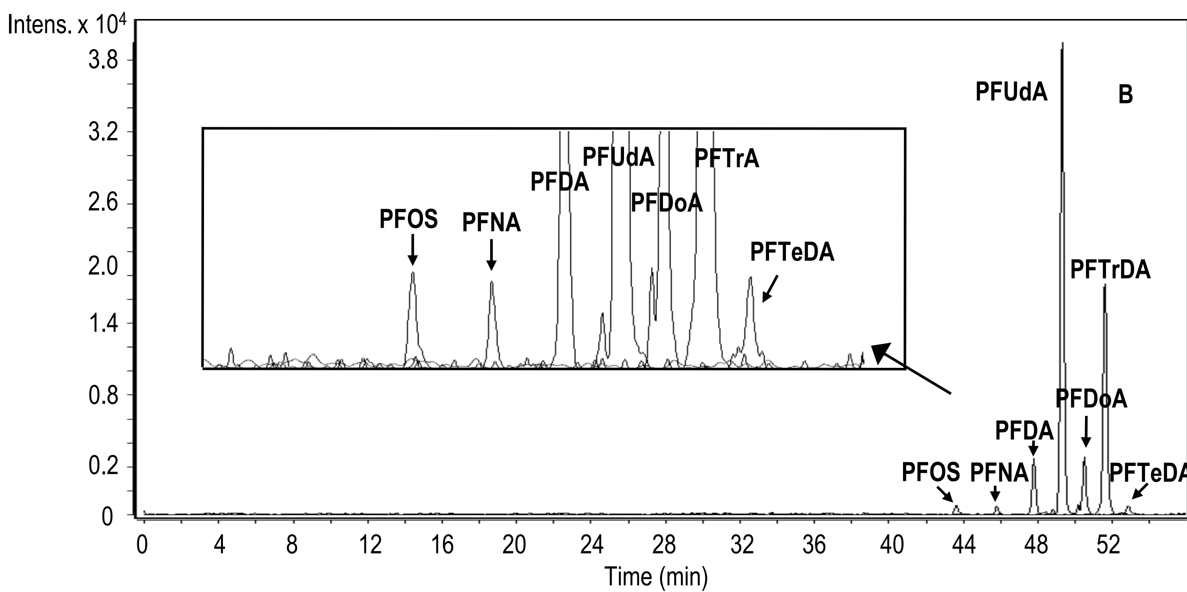
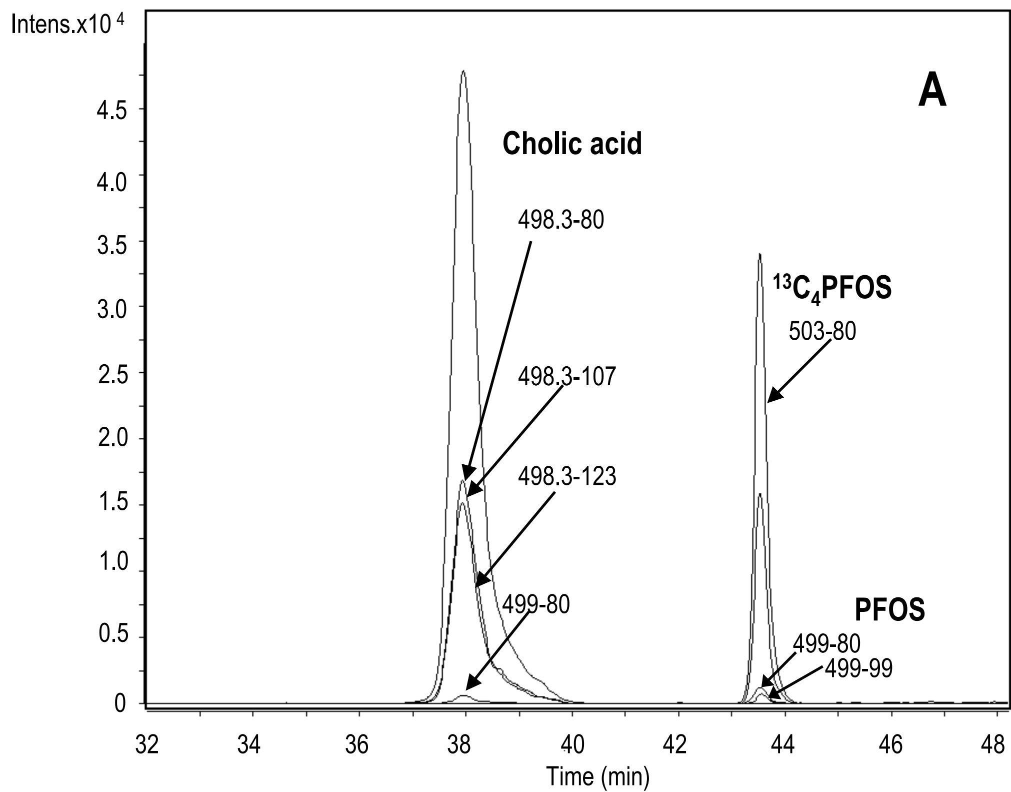


Figure 3



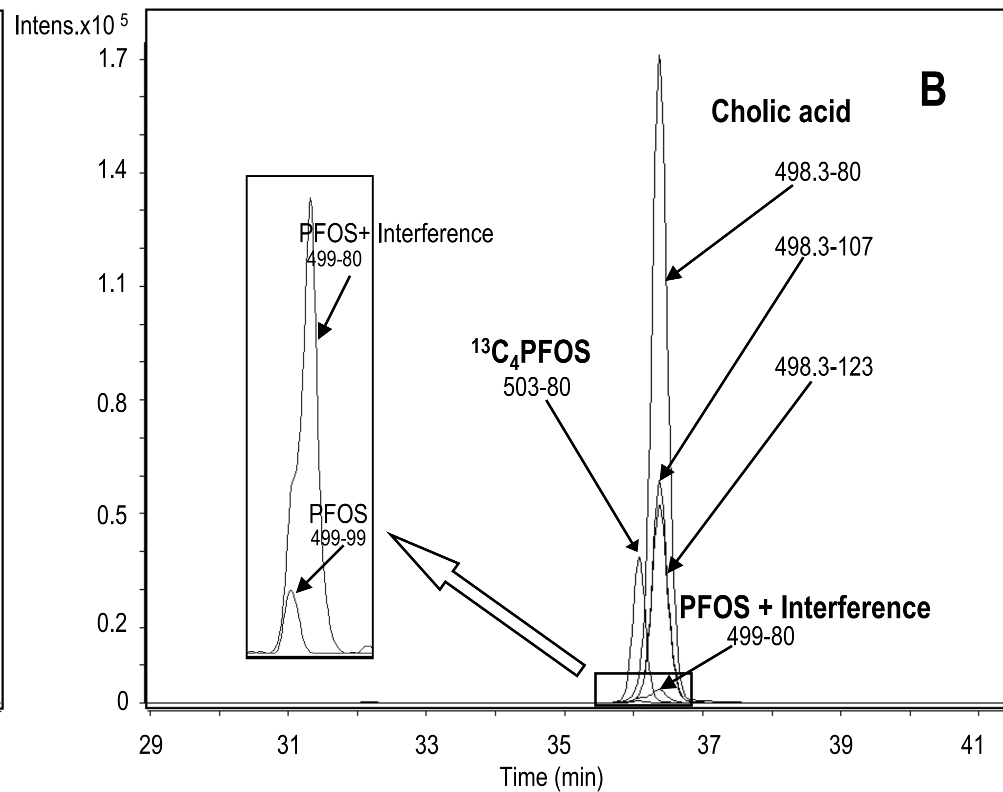


Figure 1

Table 1. Figures of merit of the quantitation of PFCs with the proposed method

PFCs	Calibration range (ng mL^{-1})	[a]Coefficient of determination (r^2)	Retention time (min)	[b]Method LOQ (pg g^{-1})	[b]Method LOD (pg g^{-1})
PFBA	0.7–20	0.995	14.4	60	30
PFBS	0.7–20	0.994	25.0	50	25
PFPeA	0.7–20	0.994	25.7	60	30
PFHxA	0.05–30	0.995	33.1	15	5
PFHxS	0.5–20	0.996	36.6	25	10
PFHpA	0.05–20	0.993	38.7	15	5
PFOA	0.15–20	0.994	42.8	10	5
PFOS	0.05–20	0.997	43.8	3.5	2
PFNA	0.05–20	0.996	45.8	5	3
PFDA	0.05–20	0.997	47.8	5	3
PFUdA	0.15–20	0.998	49.2	10	5
PFDoA	0.025–20	0.998	50.4	2.5	1
PFTrA	0.025–20	0.997	51.6	2.5	1
PFTeA	0.025–20	0.993	52.8	2.5	1

[a] $n = 8$; [b] Calculated on the basis of 70% recovery for each PFCs (a "worst-case scenario")

Table 2. Performance of the WAX SPE clean-up step as a function of the THF content of the sample extracts (expressed as recoveries of method ISs).

THF (%)	$^{13}C_4$ PFBA	$^{13}C_2$ PFHxA	$^{13}C_4$ PFOA	$^{13}C_5$ PFNA	$^{13}C_2$ PFDA	$^{13}C_2$ PFUdA	$^{13}C_4$ PFHxS	$^{13}C_4$ PFOS
75	6.4±0.2	4.8±0.6	2.3±0.2	1.9±0.1	1.51±0.04	1.3±0.1	5.9±0.4	1.5±0.4
35	90±4	97±4	85±14	87±18	79±25	75±31	96±4	94±2
25	103±6	99±3	95±5	102±5	99±3	102±4	97±4	98±2
15	100±4	99±1	105±3	105±6	104±5	102±3	95±4	105±3
7.5	107±7	100±3	101±4	105±1	103±1	102±2	105±1	105±5
0	108±6	98±3	95±2	98±4	90±5	89±5	100±3	91±4
[a]75	91±5	50±7	44±7	36±6	33±6	28±7	76±20	73±17

Blank herring sample extract (20 mL, except [a]10 mL) fortified with a 20 ng g^{-1} concentration of method IS PFCs before SPE; $n = 3$

Table 3. Method recoveries of the whole sample treatment (expressed as recovery for the method ISs)

	Fruits and vegetables				*Meat*		*Fish*			*Cereal*	*Fat-rich*	
	Carrot	**Spinach**	**Apple**	**Orange**	**Chicken**	**Pork**	**Salmon**	**Panga**	**Herring**	**Bread**	**Cheese**	**Oil**
	Recovery ± S[a] (%)											
$^{13}C_4$ PFBA	111±5	104 ±8	111 ±13	82±9	102 ±7	90 ±8	104 ±3	96±7	100 ±4	91 ±10	79±4	60 ±2
$^{13}C_2$ PFHxA	116 ±2	61 ±3	103 ±7	93 ±9	105 ±5	94 ±7	105 ±3	92±5	86 ±4	110 ±12	69±3	63 ±4
$^{13}C_4$ PFOA	82 ±6	90 ±6	98 ±7	82 ±4	97 ±5	105 ±4	102 ±3	84±4	78± 1	108 ±9	75± 4	70 ±5
$^{13}C_2$ PFNA	103± 6	78± 3	103 ±5	82 ±6	91 ±6	93 ±4	75 ±3	90 ±1	73± 6	100 ±4	76 ±7	73± 5
$^{13}C_2$ PFDA	87 ±6	70 ±5	96 ±4	84 ±6	89 ±5	103 ±7	70±5	92 ±1	77 ±3	114 ±7	70 ±7	75 ±4
$^{13}C_2$ PFUdA	90 ±7	72 ±6	106 ±8	82 ±5	82± 4	97 ±9	83 ±3	89 ±3	74 ±3	108 ±4	67 ±4	74 ±4
$^{13}C_4$ PFHxS	110 ±13	65 ±5	120 ±13	81 ±4	115 ±5	104 ±7	105± 6	104 ±1	106± 12	104± 6	96 ±6	98 ±7
$^{13}C_4$ PFOS	97 ±1	70 ±5	120 ±12	81 ±4	104 ±6	111 ±5	107 ±1	111 ±4	105± 12	100 ±2	87 ±5	89 ±7

[a] Standard deviation (n = 2)

Table SD1. Quantifier and qualifier ion transitions, and MS parameters, used to determine PFCs in foods

Target compound			Internal standards (ISs)				Fragmentor voltage (V)	Collision energy (V)
	Quantifier transition	*Qualifier transition*	*Method ISs*		*Injection ISs*			
PFBA	213–169	–	$^{13}C_4$ PFBA	217–172			60	3
PFBS	299–80	299–99	$^{18}O_2$ PFHxS	403–84			150	35
PFPeA	263–219	–	$^{13}C_4$ PFBA	217–172			60	3
PFHxA	313–269	313–119	$^{13}C_2$ PFHxA	315–270			80	4
PFHxS	399–80	399–99	$^{18}O_2$ PFHxS	403–84			150	45
PFHpA	363–319	363–169	$^{13}C_2$ PFHxA	315–270			80	4
PFOA	413–369	413–169	$^{13}C_4$ PFOA	417–372	$^{13}C_8$ PFOA	421–376	80	4
PFOS	499–80	499–99	$^{13}C_4$ PFOS	503–80	$^{13}C_8$ PFOS	507–80	200	48
PFNA	463–419	463–219	$^{13}C_5$ PFNA	468–423			100	5
PFDA	513–469	513–219	$^{13}C_2$ PFDA	515–470			100	5
PFUdA	563–519	563–269	$^{13}C_2$ PFUdA	565–520	$^{13}C_7$ PFUdA	570–525	100	6
PFDoA	613–569	613–319	$^{13}C_2$ PFUdA	565–520			100	7
PFTrA	663–619	–	$^{13}C_2$ PFUdA	565–520			100	7
PFTeA	713–669	713–369	$^{13}C_2$ PFUdA	565–520			100	4
TDC (PFOS interference)	498.3–107	498.3–80 498.3–124	–			–	290	65

PFCAs precursor ion $[M–H]^{–}$, quantifier product ion $[M–COOH]^{–}$
PFSAs precursor ion $[M–K]^{–}$, quantifier product ion $[SO_3]^{–}$

Table SD2. Recovery[a] of injection internal standards in different foods using the C_{18} and Fluorosep columns

	Fruits and vegetables				*Meat*		*Fish*			*Cereal*	*Lipid rich*	
	Carrot	**Spinach**	**Apple**	**Orange**	**Chicken**	**Pork**	**Salmon**	**Pangasius**	**Herring**	**Bread**	**Cheese**	**Oil**
	Injection internal standard recovery on C_{18} column (%) /recovery on Fluorosep column (%)											
$^{13}C_8$ PFOA	76/ 99	64/93	98/100	110/110	114/110	104/106	106/106	105/103	43/102	112/109	88/92	105/110
$^{13}C_8$ PFOS	117/95	63/75	140/108	94/109	115/113	92/106	112/100	113/110	100/110	92/95	140/110	101/100
$^{13}C_7$ PFUdA	82/84	90/85	109/102	105/93	117/109	114/97	92/92	112/114	101/101	115/115	82/89	103/105

[a] Average of two replicates