Biomonitoring of 21 endocrine disrupting chemicals in human hair samples using ultra-high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT Rapid industrial growth has increased human exposure to a large variety of chemicals with adverse health effects. These industrial chemicals are usually present in the environment, foods, beverages, clothes and personal care products. Among these compounds, endocrine disrupting chemicals have raised concern over the last years. In the present work, the determination of 21 EDCs in human hair samples is proposed. An analytical method based on the digestion of the samples with a mixture of acetic acid/methanol (20:80, v/v) followed by a solid-liquid microextraction and analysis by ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) was developed and validated. The most influential parameters affecting the extraction method were optimized. The method was validated using matrix-matched calibration and recovery assays. Limits of detection ranged from 0.2 to 4 ng g^{-1}, limits of quantification from 0.5 to 12 ng g^{-1}, and inter- and intra-day variability was under 15% in all cases. Recovery rates for spiked samples ranged from 92.1 to 113.8%. The method was applied for the determination of the selected compounds in human hair. Samples were collected weekly from six randomly selected volunteers (three men and three women) over a three-month period. All the analyzed samples tested positive for at least one of the analyzed compounds.

KEYWORDS: Endocrine disrupting chemicals; Biomonitoring; Human hair analysis; UHPLC–MS/MS

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1. Introduction

Humans are constantly exposed to new chemicals with adverse health effects. These potentially hazardous compounds, are ubiquitous in the environment, foods, beverages, and personal care products. Among these emerging contaminants, endocrine disrupting chemicals (EDCs) have raised concern over the last years. EDCs are synthetic and natural substances that can interfere with the hormonal systems in wildlife and humans. The biological effects of EDCs are related to their ability to mimic/antagonize endogenous hormones, or alter the synthesis and metabolism of endogenous hormones and receptors (Sonneschein and Soto, 1998). Bisphenol A (BPA) and its chlorinated derivatives, parabens (PBs), benzophenone-UV filters (BPs) and perfluoroalkyl compounds (PFCs) are included in this group of compounds (Fei et al., 2009; Kavlock et al., 2013; Liao et al., 2009; Liu et al., 2007; Paris et al., 2002; Rivas et al., 1997; U.S. Environmental Protection Agency, 2003).

BPA is mainly used for the manufacturing of epoxy resin and polycarbonate plastics, with an annual production of over 2-3 million tons. BPA has attracted considerable attention of governments and the scientific and medical community (Bisphenol A website). The European Food Safety Authority (EFSA) and the US-Environmental Protection Agency (US-EPA) have established the maximum acceptable level of exposure to BPA of 50 $\mu g\ kg^{-1}$ body weight/day (Nicolucci et al., 2013). There are many sources of human exposure to BPA, with diet considered the main source (Calafat et al., 2008). Interestingly, literature has reported the formation of chlorinated derivatives of BPA during the chlorination process of drinking water before consumption (Yamamoto and Yasuhara, 2002). PBs are the alkyl esters of *p*-hydroxybenzoic acid. They are used as antimicrobial preservatives, mainly against mold and yeast. PBs are used as additives in the manufacture of cosmetic products, pharmaceuticals, food and beverages. In the last years, several studies have demonstrated the ability of PBs to disrupt physiological functions

57 in both in vitro (van Meeuwen et al., 2008), and in vivo models (Boberg et al., 2010; Soni et al., 58 2005). Inhalation, dermal contact and ingestion are the main sources of exposure to PBs (El 59 Houssein et al., 2010). The existing European Union (EU) limit for total PB concentration in 60 cosmetics is 0.8 % (w/w) and 0.4 % (w/w) as acid for mixtures or individually, respectively 61 (European Union Regulation No. 1223/2009). BPs are one of the most common UV filters used in 62 sunscreens because they absorb UVA (320 to 400 nm) and UVB (290 to 320 nm) radiation. BPs 63 have been reported to enter the human body through the food chain (Cuderman and Heath, 2007) 64 and skin absorption (Jiang et al., 1999). Finally, PFCs are commonly used in the manufacture of 65 finished products such as paints, adhesives, waxes, polishes, electronics, fire-fighting foams and 66 caulks, as well as grease-proof coatings for food packaging (Corsini et al., 2014; Giesy and 67 Kannan, 2001; Henkster et al., 2003; Rivière et al., 2014). Although food is the major exposure 68 route for PFCs, drinking water, indoor air and house dust can also be potential sources of PFC 69 exposure (Domingo, 2011).

70 Hair analysis has been traditionally used for drug testing, especially in cases of drug abuse 71 (Olivezira et al., 2007). In addition, hair has been used as a bio-indicator of exposure to pesticides 72 in children and exposure to organochlorine pollutants in adults. Although human milk, placental 73 tissue, serum, blood or urine are conventionally used to determine exposure to EDCs, hair analysis 74 could be used as a complementary tool, especially in cases of chronic exposure or when traditional 75 matrices are not available. The main advantage of hair analysis is its large window of detection, 76 which allows us to establish a chronological profile of exposure based on hair length (Cooper et 77 al., 2012). Additional advantages of hair analysis include non-invasive collection and easy 78 monitoring, difficult tampering with samples, storage and transportation at room temperature, and 79 most contaminants are chemically stable (Caplan and Golberger, 2001). Therefore, the analysis 80 of hair samples can might be of great interest for the assessment of exposure to different EDCs 81 (Król et al., 2013), but not many analytical methods have been validated for the determination of

82 the EDCs mentioned in hair samples. Moreover, the methods described in the literature focused
83 on the individual determination of BPA (Tzatzarakis et al., 2015), PBs (Sakol et al., 2015), or both
84 chemicals (Martín et al., 2015).
85 The aim of the present work was to develop a multi-class method for the determination of 21
86 EDCs in human hair samples. A sensitive multi-residue method based on the digestion of hair
87 samples followed by a solid-liquid microextraction prior to the UHPLC–MS/MS analysis was
88 developed. BPA and its chlorinated derivatives (mono- bi- tri- and tetrachorobisphenol A), four
89 PBs (methyl-, ethyl-, n-propyl- and butylparaben), six BP-UV filters (benzophenone-1,
90 benzophenone-2, benzophenone-3, benzophenone-6, benzophenone-8 and 4-
91 hidroxybenzophenone) and six PFCs (perfluorobutanoic to perfluorooctanoic acid and
92 perfluorooctane sulfonate) were selected as target analytes. After validation, the method was
93 applied to determine the free pollutant content in samples from six randomly selected volunteers
94 (three men and three women) from Granada, Spain.
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96
97 ## 2. Experimental
98
99 *2.1. Chemicals and reagents*
100
101 The reagents used for the experiments were of high analytical grade and purity. A Milli-Q
102 system from Millipore (Bedford, MA, USA) was used for water purification (18.2 MΩ cm).
103 Perfluorobutanoic acid (PFBuA) (98%), perfluoropentanoic acid (PFPeA) (97%),
104 perfluorohexanoic acid (PFHxA) (≥97%), perfluroroheptanoic acid (PFHpA) (99%),
105 perflurorooctanoic acid (PFOA) (96%) and perfluorooctanesulfonic acid (PFOS) (≥98%),
106 perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid (MPFOA), methylparaben (MPB), ethylparaben (EPB), n-

107 propylparaben (PPB), and n-butylparaben (BPB) were supplied by Alfa Aesar (Massachusetts,
108 MA, USA). Bisphenol A (BPA), tetrachlorobisphenol A (Cl_4-BPA), deuterium labelled bisphenol
109 A (BPA-d_{16}), benzophenone-1 (BP-1), benzophenone-2 (BP-2), benzophenone-3 (BP-3),
110 benzophenone-6 (BP-6), benzophenone-8 (BP-8), 4-hydroxybenzophenone (4-OH-BP),
111 deuterium labelled benzophenone (BP-d_{10}), deuterium labelled ethylparaben (EPB-d_5), LC–MS
112 grade methanol, water and acetonitrile, acetic acid and ammonia (25%, w/v) were supplied by
113 Sigma-Aldrich (Madrid, Spain). Mono-, di- and trichlorobisphenol A (Cl-BPA, Cl_2-BPA, Cl_3-
114 BPA) were synthesized in our laboratory (purity > 99 %) by chlorination of BPA (Vílchez et al.,
115 2003). Stock solutions of 1000 mg L^{-1} of each compound, prepared in methanol, were stored in a
116 freezer at –20 ºC. Working solutions were prepared fresh weekly by diluting the stock standard
117 solutions in methanol or in the initial mobile phase. They were maintained at 4 ºC in the
118 refrigerator. All of the solutions were stored in the dark, in order to prevent photodegradation.
119
120 *2.2. Instrumentation and software*
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122 A chromatographic system Acquity UPLC™ H-Class (Waters, Manchester, UK), provided
123 with a binary solvent manager was used for chromatographic separation. Analyte detection was
124 carried out using a triple quadrupole mass spectrometer (Waters) Xevo TQS with an orthogonal
125 Z-spray™ electrospray ionization (ESI) source. The stationary phase was an Acquity UPLC® BEH
126 C_{18} column (50 mm × 2.1 mm i.d., 1.7 µm particle size). Other laboratory equipment such as a
127 vortex-mixer (IKA, Staufen, Germany), an ultrasound-HD bath (Selecta, Barcelona, Spain), a
128 Spectrafuge™ 24D centrifuge from Labnet International, Inc. (New Jersey, USA) and a sample
129 concentrator (Stuart, Staffordshire, UK) were also used. Statistical analysis of data was performed
130 with Statgraphics Plus version 5.1 (Statpoint Technologies Inc., Virginia, USA).
131

132 *2.3. Sample collection and storage*

133

134 Hair samples were collected from six healthy volunteers (three men and three women) aged
135 18 or older from the city of Granada, Spain. Hair samples were collected weekly for a three-month
136 period. All volunteers were informed about the scope and nature of the study. Hair specimens were
137 cut from the posterior vertex region of the head, as this region is associated with the smallest
138 variation in growth rate, and as close as possible to the scalp. Sample length was of roughly 3–5
139 cm. Samples were anonymized and stored in aluminum foil, at room (ambient) temperature until
140 further processing and analysis. One of the concerns in hair analysis is the need to differentiate
141 between external contamination (from air or dust) and internally incorporated chemicals. The ideal
142 decontamination procedure should remove external contamination (deposited on the hair cuticle)
143 without affecting internally incorporated compounds (present in the hair bulk).

144 To remove endogenous substances as well as adsorbed chemicals from the surface of the hair
145 (Schramm, 2008), samples were washed twice with MilliQ water (ultra-sonicated for 5 min),
146 washed with SDS (for 5 min), and finally rinsed with MilliQ water (for 5 min). In forensic
147 protocols, SDS has shown to be significantly less effective for removing external contamination
148 of hair with lipophilic substances compared with more aggressive substances such as MeOH or
149 DCM. For this work, however, water and SDS were used since, as reported by the scarce literature
150 available, the use of organic solvents could extract internally deposited contaminants (Kucharska
151 et al., 2015), especially polar organic pollutants like PBs or BPA. SDS and water are used by most
152 authors to determine other organic pollutants like polybrominated diphenyl ethers (PBDEs) (Tadeo
153 et al., 2009; Zheng et al., 2011; Krol et al., 2014), polychlorinated biphenyls (PCBs) (Zhao et al.,
154 2008; Wielgomas et al., 2012; Liang et al., 2014) or pesticides (Altshul et al., 2004; Cuong et al.,
155 2012) in hair samples. After washing, samples were cut in 2–3 mm pieces, dried at room

156 temperature and wrapped in aluminum foil until analysis. Thus, all pollutant concentrations
157 determined are related to the weight of the dry hair.
158
159 *2.4. Basic procedure*
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161 *2.4.1. Preparation of spiked samples*
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163 No certified reference materials are commercially available for the determination of these
164 compounds in hair. Therefore, spiked samples were prepared for optimization and validation of
165 the method. The samples were spiked at eight concentration levels (from 0.5 to 500.0 ng g^{-1}) for
166 calibration standards, validation and quality control, and at 100.0 ng g^{-1} for method optimization.
167 Aliquots of 0.05 g were weighted into 2 mL Eppendorf tubes and spiked with 100 μL of a methanol
168 solution (50.0 ng mL^{-1}) of the four surrogates: MPFOA, BPA-d_{16}, EPB-d_5 and BP-d_{10} (100.0 ng g^{-}
169 ^{1} final concentration in hair). After shaking for 1 min, the solvent was allowed to evaporate at room
170 temperature and the spiked samples were ready for the optimization and/or validation experiments.
171
172 *2.4.2. Sample treatment*
173
174 An aliquot of the hair sample (0.05 g) containing the surrogates was weighed in a 2 mL
175 Eppendorf tube, and incubated with 0.5 mL of a mixture acetic acid/methanol (20:80, v/v) at 38
176 ºC for 12 hours. After cooling to room temperature, 1 mL of acetonitrile was added for the
177 extraction of analytes. The mixture was shaken for 15 min and centrifuged for 5 min at 16,300 ×
178 *g*. The organic phase (acetonitrile) containing the analytes was separated into a glass vial and
179 evaporated to dryness under a nitrogen stream at room temperature. The residue was dissolved

180 with 250 µL of the initial mobile phase. After stirring for 30 s in a vortex and centrifugation for 5
181 min at 16,300 × *g*, the sample was injected into the UHPLC system.
182
183 *2.4.3. Ultra-high performance liquid chromatography–tandem mass spectrometry conditions*
184
185 Chromatographic separation of compounds was performed using an Acquity UPLC® BEH C_{18}
186 column (50 mm × 2.1 mm i.d., 1.7 µm particle size). A gradient mobile phase consisting of 0.1%
187 (v/v) ammonium in water (solvent A) and methanol (solvent B) was used. The optimized gradient
188 was: 0.0–2.0 min, 30% B; 2.0–5.0 min, 30-90% B; 5.0–5.1 min, 90–100% B; 5.1–7.0 min, 100%
189 B and back to 30% in 0.1 min (total run 10 min). Flow rate was 0.25 mL min^{-1}, injection volume
190 10 µL, and column temperature 40 ºC.
191 Selected reaction monitoring mode (SRM) was used for operation of the triple quadrupole
192 mass spectrometer. A unit mass resolution was set at Q1 and Q3 quadrupoles. ESI was performed
193 in negative ion mode. The ion source temperature was 150 ºC. Capillary voltage was 0.60 kV;
194 source temperature 150 ºC; desolvation temperature, 500 ºC; cone gas flow, 150 L h^{-1}; desolvation
195 gas flow, 500 L h^{-1}; collision gas flow, 0.15 mL min^{-1}; and nebulizer gas flow, 7.0 bars. Nitrogen
196 (99.995%) was used as cone and desolvation gas. The collision gas was argon (99.999%). Dwell
197 time was fixed in 25 ms. Table 1 shows the parameters and mass transitions for each compound.
198 **Table 1**
199
200 *2.4.4. Quality assurance and quality control*
201
202 To assure the validity of the results obtained, simple quality assurance and quality control
203 (QA/QC) measurements were carried out. First, to test for background contamination, procedural
204 blanks were injected. Blanks were processed in the same way as the samples and injected into the

UHPLC–MS/MS system. No quantifiable amounts of target compounds were detected. To evaluate potential contamination and variability in the chromatographic system, spiked samples at two concentration levels, 0 and 250 ng g^{-1}; and a standard in the initial mobile phase (100 ng mL^{-1}) were injected in triplicate every 20 samples.

3. Results and discussion

3.1. Extraction procedure optimization

An extraction with organic solvents was used for the isolation of the analytes from the previously digested hair samples. Key variables of the extraction step such as type of solvent and technique used were accurately optimized using aliquots of 0.1 g of sample spiked with the compounds at a concentration level of 100 ng g^{-1}. All experiments were carried out in triplicate.

3.1.1. Selection of the extraction solvent

Four extraction solvents (acetone, acetonitrile, ethyl acetate and methanol), which are widely used in the literature for the extraction of different families of EDCs from biological samples, were assayed (Rodríguez-Gómez et al., 2015). After incubation, spiked hair samples were mixed with 1 mL of each of the studied solvents. The highest extraction efficiencies (> 80% in all cases) were obtained using acetonitrile, followed by acetone, ethyl acetate and methanol. Therefore, acetonitrile was selected for optimization.

3.1.2. Selection of the extraction procedure

230

231 One of the main difficulties traditionally found in hair analysis is to distinguish between
232 external contaminants and chemicals incorporated from the human body. In order to overcome
233 this, hair samples were thoroughly washed to remove contaminants from the surface and biogenic
234 compounds, such as fatty acids, sphingolipids or steroids, which might affect analysis. Sample
235 washing protocol has been described in Section 2.3. After washing, the key variables of the
236 extraction procedure to be optimized are sample amount, solvent for extraction volume, and
237 incubation time. A 15-run Box–Behnken experimental design including three replicates at the
238 center point was used for fitting a second-order response surface. Three factors and three levels
239 for each one were selected: amount of sample (0.05, 0.125 and 0.2 g), acetonitrile volume (0.5, 1.0
240 and 1.5 mL) and incubation time (0.6 and 12 h). The data were analyzed using ANOVA, which
241 provided determination coefficients (R^2) > 0.90 in all cases. P-values for the *lack-of-fit* test were
242 >0.05, which makes the model satisfactory with a 95% confidence level. Pareto charts were also
243 obtained and statistically significant effects of the variables were screened using a Student's t-test.
244 Variables having a confidence > 95% were considered to have a significant effect on the extraction
245 efficiency. The desirability function was studied for the combination of the optimized
246 experimental values for each compound. First, the responses for each analyte in the Box–Behnken
247 design were normalized (0 to 1). The global desirability function is the geometric mean for each
248 response. Figure 1 shows the plot of this function when sample amount *vs.* solvent volume, with
249 fixed extraction time, is represented. The optimal values were 12 h for incubation time, 0.05 g for
250 sample amount and 1.0 mL for solvent volume.

251

252 **Figure 1**

253

254 *3.1.3. Optimization of the re-dissolution step*

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256 After isolation of analytes from digested samples, the extraction solvent is removed by
257 evaporation under a nitrogen stream, and the dry extract is then re-dissolved in the initial UHPLC
258 mobile phase for chromatographic analysis. The effect of the volume of reconstitution solvent is
259 an important aspect to take into account when mass spectrometry is used as the detection
260 technique. Selectivity aspects, including ion suppression, are critical in this case. Volumes of 50,
261 100, 250, 500 and 1000 µL were tested, and it was observed that the use of volumes > 250 µL
262 provided a cleaner extraction solution with less background, while with 50 and 100 µL the
263 extraction solution appeared cloudy and the noise was extremely high, making quantification
264 difficult. Since a constant response was observed for volumes > 250 µL in all cases, this was the
265 selected volume.

266

267 *3.2. Analytical performance*

268

269 A calibration curve at eight concentration levels was built for each compound.
270 Analyte/surrogate peak area ratio *versus* concentration of analyte was used for establishing the
271 calibration function. Surrogates (MPFOA, BPA-d_{16}, EPB-d_5 and BP-d_{10}) were added at a final
272 concentration of 100 ng g^{-1} of hair. The presence/absence of matrix effect was estimated using two
273 calibration curves for each EDC. The first curve was built in the initial mobile phase, and the
274 second in samples of natural dark hair free of analytes obtained from one of the volunteers. A
275 Student's *t*-test was used for comparison of the two calibration curves. Previously, a Snedecor's
276 *F*-test was applied to compare the estimated variances ($S^2_{y/x}$). The Student's *t*-test showed, in most
277 of the cases, statistically significant differences between the slopes of the two calibration curves,
278 which indicated significant matrix effects. Therefore, the validation and the application of the

279 method was carried out using matrix-matched calibration for all the studied analytes. The
280 analytical and statistical parameters are summarized in Table 2.
281
282 **Table 2**
283
284 *3.3. Validation of the method*
285
286 Linearity, sensitivity (limits of detection, LODs, and quantification, LOQs), accuracy (in
287 terms of trueness and precision) and selectivity were determined. The US Food and Drugs
288 Administration (FDA) guideline for Bioanalytical Method Validation was used.
289 *Linearity.* The concentration range selected was from the minimal quantified amount (LOQ)
290 to 125.0 ng g^{-1} for all compounds except for BPA, its chlorinated derivatives and PFBuA which
291 was 250.0 ng g^{-1} (see Table 2). Linearity of the calibration curves was assessed with the
292 determination coefficients (% R^2) and the P-values (% P_{lof}) of the *lack-of-fit* test (Analytical
293 Method Committee, 1994). The values obtained for R^2 ranged from 99.4% for PFOA to 99.9% for
294 Cl-BPA, Cl_2-BPA, Cl_4-BPA, MPB, EPB, BPB, BP-1, BP-2, BP-3, BP-6, BP-8, 4-OH-BP, PFBuA,
295 PFPeA, PFHpA and PFOS. P_{lof} values > 5% were found for all compounds. Therefore, a good
296 linearity within the stated ranges can be assured.
297 *Sensitivity.* LODs and LOQs were calculated in order to check the sensitivity of the analytical
298 method. These parameters were calculated from the calibration function, using the standard
299 deviation of residual, $S_{y/x}$, the slope of the curve, b, and an estimation of the standard deviation of
300 the blank (S_0), (Currie, 1999). The LOD is defined as $3 \cdot S_0$ and the LOQ as $10 \cdot S_0$. The values
301 obtained for the LODs ranged from 0.2 to 4 ng g^{-1}, and the LOQs from 0.5 to 12 ng g^{-1} for MPB
302 and PFBuA respectively. The results are summarized in Table 2.

303 *Accuracy (precision and trueness).* Since no certified materials are commercially available to
304 evaluate the precision and the trueness of the proposed method, a recovery assay with spiked hair
305 samples (free of the analytes), at three concentrations levels for each compound, was proposed.
306 For BPA and its chlorinated derivatives and PFBuA, 25.0, 125.0 and 250.0 ng g^{-1} were the
307 concentrations assayed; and 5.0, 25.0 and 125.0 ng g^{-1} for to the rest of the EDCs analyzed. The
308 analyses were carried out in triplicate over six days. The relative standard deviation (RSD) was
309 used for precision determination, and % recovery was applied for trueness evaluation. Precision
310 and trueness results are shown in Table 3. The recoveries obtained were close to 100% and RSDs
311 were < 15% for all compounds. The values obtained for both parameters are within the acceptable
312 limits defined by the Analytical Method Committee guidelines (≤15%, except for the LOQ which
313 should not exceed 20%) which indicate that the method is highly accurate.

314

315 **Table 3**

316

317 *Selectivity.* The chromatograms of the procedure blank and the corresponding blank of sample
318 were analyzed. Taking into account the retention time of all analytes, no interferences from
319 endogenous substances were detected. The spectrometric conditions used ensured high selectivity
320 of the UHPLC-MS/MS method. Figure 2A shows the SRM chromatograms obtained from a blank
321 hair sample spiked with the analytes (75.0 ng g^{-1}).

322 **Figure 2**

323

324 *3.4. Biomonitoring of EDCs in human hair. Method application*

325

326 After validation, the method was used for the determination of the selected EDCs in hair
327 samples from six volunteers. In order to study the persistence of compounds over a long period of

328 time, a hair sample from each volunteer was collected weekly over a three-month period (12
329 samples per volunteer). Three replicates were analyzed for each day. The results obtained,
330 expressed as means of concentrations for each volunteer, are summarized in Table 4. In all the
331 analyzed samples at least one of the compounds studied was found. Figure 2B shows the
332 chromatograms of one of the samples (M04).

333

334 **Table 4**

335

336 As shown in Table 4, 13 of the 21 analyzed compounds were detected in almost one of the
337 samples but were not quantified in all of them. BPA was detected in only three of the samples.
338 Chlorinated derivatives of BPA were not detected in any of the analyzed samples. For PBs, at least
339 one of the compounds was detected and quantified in five of the samples. Regarding BPs, only
340 four of the six compounds were detected and/or quantified in at least one sample. BP-1 and BP-3
341 showed the highest concentration values. Finally, among the six PFCs analyzed, PFOA was the
342 most commonly found compound, followed by PFOS. Both compounds were quantified in 83%
343 of the samples. PFPHxA and PFPHpA were also detected in five of the samples but quantified in
344 one, with concentrations close to the LOQs for both compounds.

345 Because of the limited information available on the selected pollutants in human hair, the
346 comparison of the results previously published in hair and other biological matrices is of great
347 interest. Perez et al. (2012) studied the presence of PFCs in human hair and urine and found a
348 similar pattern of PFCs to ours, but they found much higher concentration in urine than in hair
349 samples . PFOS and PFOA were the compounds more frequently quantified. PFOA was present
350 in the 0.1–6 ng g^{-1} range among the positive samples, whereas PFOS was between 3.7 and 7.0 ng
351 g^{-1}, while concentrations up to 49 ng L^{-1} were reported in urine samples. In a large study by
352 Vassiliadou et al. (2010), concentrations ranging from 7.5 to 15 ng mL^{-1} for PFOS and from 2 to

4 ng mL^{-1} for PFOA were found in blood samples from three different groups of adults living in Greece. Some of the selected analytes have also been previously analyzed by the authors of the present work in human milk (Rodríguez-Gómez et al., 2014). BPA, PBs and BPs were detected and quantified in almost one of the analyzed samples. However, none of the chlorinated derivatives of BPA were detected. BPA was quantified at concentrations ranging from 3.2 to 10.8 ng mL^{-1}. Among the four PBs analyzed, EPB and PPB were the most abundant quantified in most milk samples, with concentrations ranging from 0.8 to 43.5 ng mL^{-1} for PPB and from 0.8 to 15.0 ng mL^{-1} for EPB. Martín et al. (2015) reported concentration levels of up to 1980 ng g^{-1} for EPB and PPB in human hair from German individuals. Hines et al. (2015) reported concentrations in multiple matrices (milk, urine and serum) of PBs and BPA. These data suggest that urine is the best matrix for measurements, with concentration levels similar to those found in this study, 993 and 279 ng mL^{-1} for MPB and PPB respectively. In a recent study to estimate the burden of BPA in urban and rural populations, Tzatzarakis et al. (2015) reported concentration levels in hair between 17.7–192.8 ng g^{-1} in urban population and between 13.1–72.8 ng g^{-1} in rural population.

4. Conclusions

A new analytical method for the biomonitoring of 21 EDCs (BPA and its chlorinated derivatives, 4 PBs, 6 BP-UV filters and 6 PFCs) in human hair samples has been successfully validated with good analytical features using a simple extraction procedure with acetonitrile after incubation of samples, and UHPLC-MS/MS analysis. The method was successfully validated, obtaining low LODs (between 0.2 ng g^{-1} for MPB; and 4 ng g^{-1} for PFBuA), high recoveries (>90% and <115%) and precision (RSD <15%). The proposed procedure of sample preparation was considered optimal because of its high extraction yield and easy operation.

The method has been applied to hair samples obtained from six volunteers over a three-month period. To our knowledge, this is the first validated method that allows the biomonitoring of such a large number of EDCs. At least one of the compounds analyzed was found in 100% of the samples, although quantification was not always possible.

The main advantage of the present study is the possibility of performing a multiresidue and multiclass analytical determination in only two steps while providing the sensitivity and selectivity necessary for the detection of compounds in human hair samples. Since hair is a representative sample for providing evidences of EDC exposure, the sampling protocol is crucial. Hair should be cut from the posterior vertex region of the head, as close as possible to the scalp (root) to minimize external exposure, with a length of roughly 3–5 cm. The main limitation of hair analysis is distinguishing between internal and external exposure. For this reason, hair samples were carefully washed. After washing, samples were cut in 2-3 mm pieces. Hair natural pigmentation and hair treatments are also possible limitations of hair analysis.

Finally, one of the limitations of the present work is the small amount of samples, and a larger cohort is required for the determination of global exposure to the target analytes. Nonetheless, this method is a powerful analytical tool that can be used in further determinations of human exposure to EDCs.

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404 # References

405

406 Altshul, L., Covaci, A., Hauser, R., 2004. The relationship between levels of PCBs and
407 pesticides in human hair and blood: preliminary results. Environ. Health Perspect. 112,
408 1193–1199.

409

410 Analytical Methods Committee, 1994. Is my calibration linear? Analyst 119 2363–2366.

411

412 Bisphenol A Website. Document available on line at: http://www.bisphenol-a.org
413 Visited: 13/06/2016.

414

415 Boberg, J., Taxvig, C., Christiansen, S., Hass, U., 2010. Possible endocrine disrupting effects of
416 parabens and their metabolites. Reprod. Toxicol. 30, 301–312.

417

418 Calafat, A.M., Ye, X.Y., Wong, L.Y., Reidy, J.A., Needham, L.L., 2008. Exposure of the U.S.
419 population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environ. Health Perspect.
420 116, 39–44.

421

422 Caplan, Y.H., Goldberger, B.A., 2001. Alternative specimens for workplace drug testing. J. Anal.
423 Toxicol. 25, 396–399.

424

425 Cooper, G.A.A., Kronstrand, R., Kintz, P., 2012. Society of Hair Testing guidelines for drug
426 testing in hair. Forensic Sci. Int. 218, 20–24.

427

428 Corsini, E., Luebke, R.W., Germolec, D.R., DeWitt, J.C., 2014. Perfluorinated compounds: emerging POPs with potential immunotoxicity. Toxicol. Lett. 230, 263–270.

430

431 Cuderman, P., Heath, E., 2007. Determination of UV filters and antimicrobial agents in environmental water samples. Anal. Bioanal. Chem. 387, 1343–1350.

433

434 Cuong, L.P., Evgen'ev, M.I., Gumerov, F.M., 2012. Biomonitoring of organic and inorganic chemicals in the hair of Vietnamese people via spectral and chromatographic analysis. J. Supercritical Fluids 61, 86–91.

437

438 Currie, L.A., 1999. Detection and quantification limits: origins and historical overview. Anal. Chim. Acta 391, 127–134.

440

441 Domingo, J.L., 2011. Health risks of dietary exposure to perfluorinated compounds. Environ. Int. 40, 187–195.

443

444 El Hussein, S., Muret, P., Berard, M., Makki, S., Humbert, P., 2007. Assessment of principal parabens used in cosmetics after their passage through human epidermis–dermis layers (ex-vivo study). Exp. Dermatol. 16, 830–836.

447

448 European Parliament and of the Council of 30 November 2009 on cosmetic products, European Union Regulation No. 1223/2009 (2009) 59–209.

450

451 Fei, C., McLaughlin, J.K., Lipworth, L., Olsen, J., 2009. Maternal levels of perfluorinated chemicals and subfecundity. Human. Reprod. 24, 1200–1205.

453

454 Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife.
455 Environ. Sci. Technol. 35, 1339–1342.

456

457 Guidance for Industry, Bioanalytical Method Validation, U.S. Department of Health and Human
458 Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER),
459 Center for Veterinary Medicine (CVM), 2001.

460

461 Hekster, F.M., Laane, R.W.P.M., de Voogt, P., 2003. Environmental and toxicity effects of
462 perfluoroalkylated substances. Rev. Environ. Contam. Toxicol. 179, 99–121.

463

464 Hines, E.P., Mendola, P., von Ehrenstein, O.S., Ye, X., Calafat, A.M., Fenton, S.E., 2015.
465 Concentrations of environmental phenols and parabens in milk, urine and serum of lactating
466 North Carolina women. Reprod. Toxicol. 54, 120–128.

467

468 Jiang, R., Roberts, M.S., Collins, D.M., Benson, H.A.E., 1999. Absorption of sunscreens across
469 human skin: an evaluation of commercial products for children and adults. Br. J. Clin.
470 Pharmacol. 48, 635–637.

471

472 Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., Lucier, G.,
473 Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan,
474 D.M., Sinks, T., Tilson, H.A., 1996. Research needs for the risk assessment of health and
475 environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored
476 workshop. Environ. Health Perspect. 104, 715–740.

477

478 Król, S., Namieśnik, J., Zabiegała, B., 2014. Occurrence and levels of polybrominated diphenyl
479 ethers (PBDEs) in house dust and hair samples from Northern Poland; an assessment of
480 human exposure. Chemosphere. 110, 91–96
481
482 Król, S., Zabiegała, B., Namiesnik, J., 2013. Human hair as a biomarker of human exposure to
483 persistent organic pollutants (POPs). Trends Anal. Chem. 47, 84−98.
484
485 Liang, B., Liu, X., Hou, J., Liang, G., Gong, W., Xu, D., Zhang, L., 2014. PCBs levels and
486 indicator congeners in children's and adolescent's hair. Environ. Pollut. 185, 10–15.
487
488 Liao, C., Wang, T., Cui, L., Zhou, Q., Duan, S., Jiang, G., 2009. Changes in synaptic transmission,
489 calcium current, and neurite growth by perfluorinated compounds are dependent on the chain
490 length and functional group. Environ. Sci. Technol. 43, 2099–2104.
491
492 Liu, C., Du, Y., Zhou, B., 2007. Evaluation of estrogenic activities and mechanism of action of
493 perfluorinated chemicals determined by vitellogenin induction in primary cultured tilapia
494 hepatocytes. Aquat. Toxicol. 85, 267–277.
495
496 Martín, J., Möder, M., Gaudl, A., Alonso, E., Reemtsma, T., 2015. Multi-class method for
497 biomonitoring of hair samples using gas chromatography-mass spectrometry. Anal. Bioanal.
498 Chem. 407, 8725–8734.
499
500 Multi-Year Plan (FY2000-2012) for Endocrine Disruptor, Office of Research and Development.
501 U.S. Environmental Protection Agency. 2003.
502 Available at: http://www.epa.gov/osp/myp/edc.pdf (Visited: 11/04/2016).

503

504 Nicolucci, C., Rossi, S., Menale, C., Miraglia del Giudice, E., Perrone, L., Gallo, P., Mita, D.G.,
505 Diano, N., 2013. A high selective and sensitive liquid chromatography–tandem mass
506 spectrometry method for quantization of BPA urinary levels in children. Anal. Bioanal.
507 Chem. 405, 9139–9148.

508

509 Oliveira, C.D.R., Roehsig, M., Almeida, R.M., Rocha, W.L., Yonamine, M., 2007. Recent
510 advances in chromatographic methods to detect drugs of abuse in alternative biological
511 matrices. Curr. Pharm. Anal. 3, 95–109.

512

513 Paris, F., Balaguer, P., Térouanne, B., Servant, N., Lacoste, C., Cravedi, J.P., Nicolas, J.C., Sultan,
514 C., 2002. Phenylphenols, biphenols, bisphenol-A and 4-tert-octylphenol exhibit α and β
515 estrogen activities and antiandrogen activity in reporter cell lines. Mol. Cell. Endocrinol.
516 193, 43–49.

517

518 Perez, F., Llorca, M., Farre, M., Barcelo, D., 2012. Automated analysis of perfluorinated
519 compounds in human hair and urine samples by turbulent flow chromatography coupled to
520 tandem mass spectrometry. Anal. Bioanal. Chem. 402, 2369–2378.

521

522 Rivas, A., Olea, N., Olea-Serrano, F., 1997. Human exposure to endocrine-disrupting chemicals:
523 assessing the total estrogenic xenobiotic burden. Trends Anal. Chem. 16 (1997) 613–619.

524

525 Rivière, G., Sirot, V., Tard, A., Jean, J., Marchand, P., Veyrand, B., Le Bizec, B., Leblanc, J.C.,
526 2014. Food risk assessment for perfluoroalkyl acids and brominated flame retardants in the

527 French population: results from the second French total diet study. Sci. Total Environ. 491–
528 492, 176–183.
529
530 Rodríguez-Gómez, R., Dorival-García, N., Zafra-Gómez, A., Camino-Sánchez, F.J., Ballesteros,
531 O., Navalón, A., 2015. New method for the determination of parabens and bisphenol A in
532 human milk samples using ultrasound-assisted extraction and clean-up with dispersive
533 sorbents prior to UHPLC–MS/MS analysis. J. Chromatogr. B 992, 47–55.
534
535 Rodríguez-Gómez, R., Zafra-Gómez, A., Camino-Sánchez, F.J., Ballesteros, O., Navalón, A.,
536 2014. Gas chromatography and ultra high performance liquid chromatography tandem mass
537 spectrometry methods for the determination of selected endocrine disrupting chemicals in
538 human breast milk after stir-bar sorptive extraction. J. Chromatogr. A. 1349, 69–79.
539
540 Sako1, A.V.F., Dolzan1, M.D., Micke, G.A., 2015. Fast and sensitive method to determine
541 parabens by capillary electrophoresis using automatic reverse electrode polarity stacking
542 mode: application to hair samples. Anal. Bioanal. Chem. 407, 7333–7339.
543
544 Schramm, K.W., 2008. Hair biomonitoring of organic pollutants. Chemosphere 72, 1103 –1111.
545
546 Soni, M.G., Carabin, I.G., Burdock, G.A., 2005. Safety assessment of esters of p-hydroxybenzoic
547 acid (parabens). Food Chem. Toxicol. 43, 985–1015.
548
549 Sonnenschein, C., Soto, A.M., 1998. An updated review of environmental estrogen and androgen
550 mimics and antagonists. J. Steroid. Biochem. Mol. Biol. 65, 143–150.
551

552 Tadeo, J.L., Sánchez-Brunete, C., Miguel, E., 2009. Determination of polybrominated diphenyl
553 ethers in human hair by gas chromatography–mass spectrometry. Talanta. 78, 138–143.
554
555 Tzatzarakis, M.N., Vakonaki, E., Kavvalakis, M.P., Barmpas, M., Kokkinakis, E.N., Xenos, K.,
556 Tsatsakis, A.M., 2015. Biomonitoring of bisphenol A in hair of Greek population.
557 Chemosphere 118, 336–341.
558
559 van Meeuwen, J.A., van Son, O., Piersma, A.H., de Jong, P.C., van den Berg, M., 2008. Aromatase
560 inhibiting and combined estrogenic effects of parabens and estrogenic effects of other
561 additives in cosmetics. Toxicol. Appl. Pharmacol. 230, 372–382.
562
563 Vassiliadou, I., Costopoulou, D., Ferderigou, A., Leondiadis, L., 2010. Levels of
564 perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in blood samples from
565 different groups of adults living in Greece. Chemosphere. 80, 1199–1206.
566
567 Vílchez, J.L., del Olmo, M., González-Casado, A., Navalón, A., 2003. Spanish Patent ES 2 190
568 852 B1 (2005). Patent application number ES 20 190 852.
569 Available at: http://digibug.ugr.es/handle/10481/1114 (ES 2190852 B1).
570 Visited: 11/06/2016.
571
572 Wielgomas, B., Czarnowski, W., Jansen, E.H.J.M., 2012. PCBs levels and indicator congeners in
573 children's and adolescents' hair. Chemosphere. 89, 975–981.
574

575 Yamamoto, T., Yasuhara, A., 2002. Chlorination of bisphenol A in aqueous media: formation of
576 chlorinated bisphenol A congeners and degradation to chlorinated phenolic compounds.
577 Chemosphere 46, 1215–1223.
578
579 Zhao, G., Wang, Z., Dong, M.H., Rao, K., Luo, J., Wang, D.I., Zha, J., Huang, S., Xu, Y., Ma, M.,
580 2008. PBBs, PBDEs, and PCBs levels in hair of residents around e-waste disassembly sites
581 in Zhejiang Province, China, and their potential sources. Sci. Total Environ. 397, 46–57.
582
583 Zheng, J., Luo, X.J., Yuan, J-G., Wang, J., Wang, Y.T., Chen, S.J., Mai, B.X., Yang, Z.Y., 2011.
584 Levels and sources of brominated flame retardants in human hair from urban, e-waste, and
585 rural areas in South China. Environ. Pollut. 159, 3706–3713.

586 ## Figure captions

587

588 **Fig. 1.** Optimization of the sample amount, solvent volume and incubation time. Response
589 surfaces obtained for Box–Behnken design: (A) BPA and chlorinated derivatives; (B)
590 PBs; (C) BPs and (D) PFCs. Time of digestion was fixed in 12 hours.

591

592 **Fig. 2.** SMR chromatograms of: (A) a spiked sample standard mixture (75.0 ng g^{-1} of each
593 analyte), (B) a natural hair sample (Sample M04).

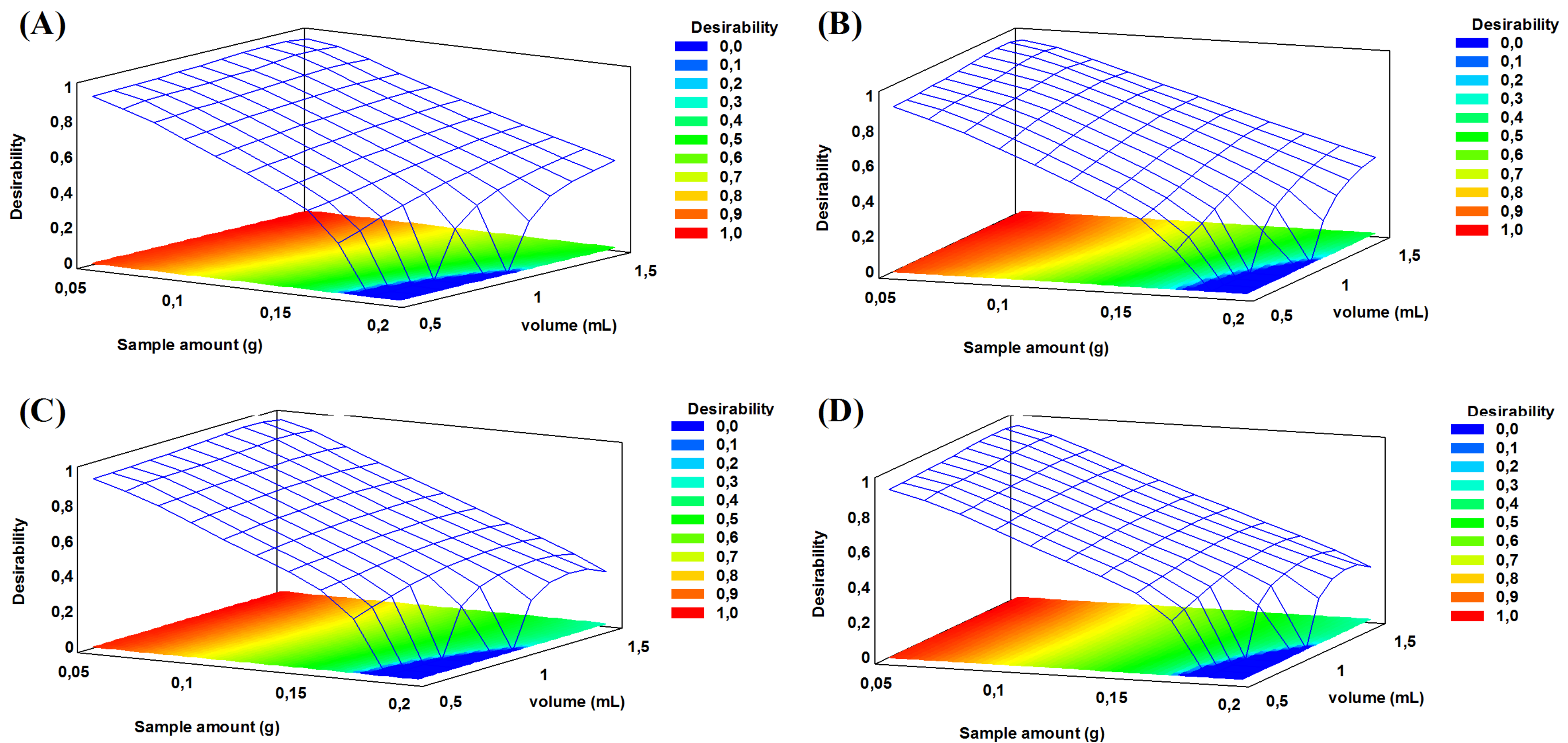

Figure 1

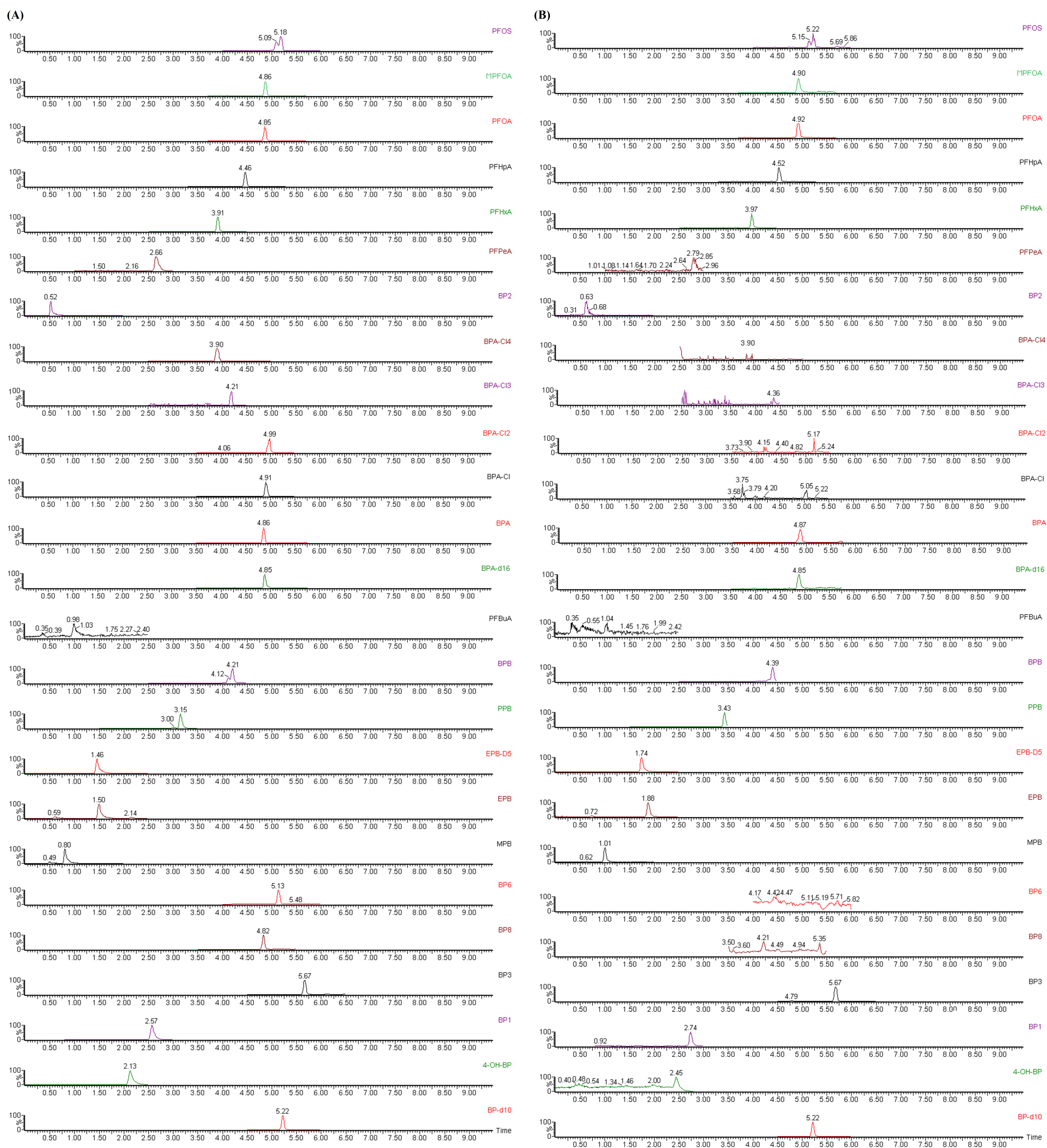

Figure 2

Table 1

Transitions and optimized potentials for UHPLC–MS/MS analysis

	Transitions	CV	CE		Transitions	CV	CE
BPA	227.2 → 211.9[a]	-50	-22	BP-8	245.0 → 120.9[a]	14	20
	227.2 → 132.9[b]	-50	-26		245.0 → 150.9[b]	14	20
Cl-BPA	261.1 → 182.0[a]	-56	-30	4-OH-BP	199.0 → 120.8[a]	36	20
	261.1 → 210.0[b]	-56	-22		199.0 → 104.8[b]	36	18
Cl_2-BPA	295.0 → 215.9[a]	-74	-30	PFBuA	213.0 → 169.0[a]	36	20
	295.0 → 243.9[b]	-74	-24		213.0 → 5.6[b]	36	20
Cl_3-BPA	329.0 → 249.8[a]	-52	-32	PFPeA	263.0 → 219.0[a]	36	20
	329.0 → 277.9[b]	-52	-24		263.0 → 89.7[b]	36	20
Cl_4-BPA	365.0 → 313.9[a]	-50	-28	PFHxA	313.0 → 269.0[a]	36	20
	365.0 → 285.9[b]	-50	-32		313.0 → 119.0[b]	36	20
MPB	151.1 → 91.8[a]	-38	-22	PFHpA	363.0 → 319.0[a]	36	20
	151.1 → 135.8[b]	-38	-14		363.0 → 333.0[b]	36	20
EPB	165.1 → 91.9[a]	-38	-24	PFOA	413.0 → 369.0[a]	36	20
	165.1 → 136.6[b]	-38	-16		413.0 → 194.0[b]	36	20
PPB	179.1 → 91.8[a]	-42	-24	PFOS	499.0 → 80.0[a]	36	20
	179.1 → 136.1[b]	-42	-16		499.0 → 52.0[b]	36	20
BPB	193.1 → 91.4[a]	-42	-24	BPA-d_{16}	241.2 → 223.0[a]	-46	-22
	193.1 → 136.1[b]	-42	-16		241.2 → 141.9[b]	-46	-32
BP-1	214.9 → 136.8[a]	2	18	EPB-d_5	170.1 → 92.1[a]	-38	-24
	214.9 → 105.1[b]	2	32		170.1 → 136.0[b]	-38	-16
BP-2	245.1 → 134.8[a]	-40	-16	BP-d_{10}	193.1 → 109.8[a]	18	16
	245.1 → 108.9[b]	-40	-22		193.1 → 81.8[b]	18	30
BP-3	229.0 → 150.8[a]	4	20	MPFOA	417.0 → 371.0[a]	36	20
	229.0 → 104.9[b]	4	18		417.0 → 168.0[b]	36	20
BP-6	275.0 → 150.9[a]	14	18				
	275.0 → 94.9[b]	14	34				

[a] SRM transition used for quantification; [b] SRM transition used for confirmation

CV: Cone voltage (V); CE: Collision energy (eV)

Table 2
Analytical and statistical parameters

	b ($g\ ng^{-1}$)	S_b ($g\ ng^{-1}$)	% R^2	LOD ($ng\ g^{-1}$)	LOQ ($ng\ g^{-1}$)	LDR ($ng\ g^{-1}$)
BPA	$8.40 \cdot 10^{-4}$	$2.72 \cdot 10^{-6}$	99.8	2	7	7 - 250
Cl-BPA	$1.00 \cdot 10^{-3}$	$8.72 \cdot 10^{-5}$	99.9	1	4	5 - 250
Cl_2-BPA	$3.30 \cdot 10^{-4}$	$7.09 \cdot 10^{-6}$	99.9	3	9	9 - 250
Cl_3-BPA	$6.47 \cdot 10^{-4}$	$3.43 \cdot 10^{-6}$	99.8	2	7	7 - 250
Cl_4-BPA	$9.50 \cdot 10^{-4}$	$4.02 \cdot 10^{-6}$	99.9	3	9	9 - 250
MPB	$5.24 \cdot 10^{-3}$	$2.09 \cdot 10^{-5}$	99.9	0.2	0.5	0.5 - 125
EPB	$3.50 \cdot 10^{-3}$	$1.70 \cdot 10^{-5}$	99.9	0.6	2	2 - 125
PPB	$7.20 \cdot 10^{-3}$	$1.80 \cdot 10^{-4}$	99.8	0.3	1	1 - 125
BPB	$6.00 \cdot 10^{-3}$	$8.92 \cdot 10^{-5}$	99.9	0.4	1	1 - 125
BP-1	$1.40 \cdot 10^{-1}$	$3.45 \cdot 10^{-3}$	99.9	0.5	2	2 - 125
BP-2	$8.00 \cdot 10^{-3}$	$3.48 \cdot 10^{-5}$	99.9	0.3	1	1 - 125
BP-3	$8.60 \cdot 10^{-2}$	$3.50 \cdot 10^{-4}$	99.9	0.5	2	2 - 125
BP-6	$3.96 \cdot 10^{-2}$	$7.40 \cdot 10^{-4}$	99.9	0.7	2	2 - 125
BP-8	$7.40 \cdot 10^{-2}$	$1.00 \cdot 10^{-3}$	99.9	0.9	3	3 - 125
4-OH-BP	$5.30 \cdot 10^{-1}$	$1.45 \cdot 10^{-3}$	99.9	0.8	3	3 - 125
PFBuA	$1.90 \cdot 10^{-3}$	$2.57 \cdot 10^{-5}$	99.9	4	12	12 - 250
PFPeA	$5.73 \cdot 10^{-3}$	$3.45 \cdot 10^{-5}$	99.9	1	4	4 - 125
PFHxA	$1.75 \cdot 10^{-2}$	$1.20 \cdot 10^{-3}$	99.5	2	5	5 - 125
PFHpA	$1.75 \cdot 10^{-2}$	$2.80 \cdot 10^{-4}$	99.9	2	5	5 - 125
PFOA	$3.00 \cdot 10^{-2}$	$2.30 \cdot 10^{-3}$	99.4	1	4	4 - 125
PFOS	$3.64 \cdot 10^{-2}$	$5.00 \cdot 10^{-4}$	99.9	0.6	2	2 - 125

b, slope; S_b, slope standard deviation; R^2, determination coefficient; LOD, limit of detection; LOQ, limit of quantification; LDR, linear dynamic range

Table 3
Recovery assay, precision and trueness of the method

	Spiked (ng mL^{-1})	Found[a] (ng mL^{-1})	RSD (%)	Recovery (%)
BPA	25.0	26.5	10.1	105.9
	125.0	128.8	7.4	103.0
	250.0	247.9	3.6	99.2
Cl-BPA	25.0	24.1	8.7	96.2
	125.0	130.9	13.4	104.7
	250.0	240.0	9.4	96.6
Cl_2-BPA	25.0	24.3	8.7	97.2
	125.0	121.3	4.3	97.1
	250.0	241.2	2.6	96.4
Cl_3-BPA	25.0	27.0	8.9	108.0
	125.0	135.9	5.6	107.1
	250.0	258.0	4.3	103.2
Cl_4-BPA	25.0	26.5	5.0	106.0
	125.0	124.9	8.3	99.9
	250.0	250.0	10.8	100.0
MPB	5.0	5.4	8.8	107.2
	25.0	25.0	10.8	100.1
	125.0	128.5	3.4	102.8
EPB	5.0	5.2	6.3	103.5
	25.0	23.8	7.0	95.3
	125.0	125.8	4.3	100.6
PPB	5.0	5.5	12.5	109.7
	25.0	26.0	6.5	104.1
	125.0	123.0	5.6	98.4
BPB	5.0	4.9	10.9	97.8
	25.0	24.7	7.8	98.7
	125.0	125.0	9.1	100.0
BP-1	5.0	4.9	14.5	98.5
	25.0	24.5	10.9	98.0
	125.0	127.4	11.9	101.9
BP-2	5.0	5.6	13.9	108.9
	25.0	27.0	12.7	107.8
	125.0	127.0	3.7	101.5
BP-3	5.0	4.6	9.3	92.1
	25.0	24.9	3.4	99.5
	125.0	126.3	3.8	101.0
BP-6	5.0	5.4	14.2	107.4
	25.0	26.8	2.5	107.2
	125.0	127.7	4.1	102.2
BP-8	5.0	5.7	5.3	113.8
	25.0	24.8	0.8	99.1
	125.0	119.7	12.2	95.8
4-OH-BP	5.0	5.0	5.3	99.0
	25.0	24.7	6.1	98.9
	125.0	128.0	9.7	102.0
PFBuA	25	24.6	1.1	98.4
	125	126.5	2.9	101.2
	250	249.5	10.0	99.8
PFPeA	5.0	4.9	4.9	98.5
	25.0	25.0	7.4	100.0
	125.0	129.5	5.1	103.6
PFHxA	5.0	4.4	7.8	88.0
	25.0	24.0	8.9	96.1
	125.0	126.7	3.3	101.3
PFHpA	5.0	5.5	10.3	110.1
	25.0	23.8	5.7	95.1
	125.0	125.2	5.3	100.1
PFOA	5.0	4.9	9.1	99.1
	25.0	26.7	5.1	106.9
	125.0	141.0	5.4	112.8
PFOS	5.0	5.5	2.0	109.4
	25.0	25.8	9.6	103.2
	125.0	128.6	2.5	102.9

[a] Mean of 18 determinations; RSD: relative standard deviation

Table 4
Application to hair samples

	*Mean amount for each volunteer (SD) ng g^{-1}						
	BPA	MPB	EPB	PPB	BPB	BP-1	BP-2
M01	ND	21 (1)	9.0 (0.3)	69 (4)	5.8 (0.3)	ND	2.8 (8.1)
M02	ND	ND	ND	ND	3.5 (0.3)	4.3 (0.2)	D
M03	45 (3)	33 (2)	ND	107 (4)	4.4 (0.3)	116 (4)	1.5 (5.0)
M04	12.3 (0.6)	18.7 (0.6)	D	80 (6)	9.4 (0.5)	29 (1)	5.8 (4.1)
M05	ND	ND	ND	ND	ND	ND	ND
M06	9.2 (0.6)	10.2 (0.6)	9.0 (0.6)	11.6 (0.6)	5.7 (0.3)	12.5 (0.6)	D
	BP-3	4-OH-BP	PFPHxA	PFPHpA	PFOA	PFOS	
M01	D	ND	D	D	10.6 (0.5)	4.9 (0.4)	
M02	7.0 (0.3)	ND	D	D	14.8 (0.6)	5.4 (0.2)	
M03	8.5 (0.5)	D	D	D	23.9 (0.7)	9.9 (0.5)	
M04	5.2 (0.2)	D	5.5 (0.4)	7.1 (0.4)	20.0 (0.9)	6.8 (0.3)	
M05	D	ND	ND	ND	D	D	
M06	10.5 (0.7)	ND	D	D	18.0 (0.7)	6.8 (0.4)	

* Mean of 36 analysis (12 samples per volunteer / 3 replicates a day); ND, not detected (< LOD); D: detected (>LOD and <LOQ); SD, standard deviation