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## Quantitative method for conjugated metabolites of bisphenol A and bisphenol S determination in food of animal origin by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry.

Yoann Deceuninck, Emmanuelle Bichon, T. Gény, Bruno Veyrand, Flore C. Grandin, Catherine Viguié, Philippe Marchand, B Le Bizec

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1 **Quantitative method for conjugated metabolites of bisphenol A**  
2 **and bisphenol S determination in food of animal origin by Ultra**  
3 **High Performance Liquid Chromatography – Tandem Mass**  
4 **Spectrometry**

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7 **Y. Deceuninck\*<sup>1</sup>, E. Bichon<sup>1</sup>, T. Gény<sup>1</sup>, B. Veyrand<sup>1</sup>, F. Grandin<sup>2</sup>, C. Viguié<sup>2</sup>, P.**  
8 **Marchand<sup>1</sup> and B. Le Bizec<sup>1</sup>**

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11 <sup>1</sup>LABERCA, Oniris, INRA, Université Bretagne-Loire, 44307, Nantes, France

12 <sup>2</sup>UMR 1331 TOXALIM INRA/ENVT/INP/UPS, 31076 Toulouse, France

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20  
21

22 \* Corresponding author

23 Yoann DECEUNINCK, ONIRIS, École nationale vétérinaire, agroalimentaire et de  
24 l'alimentation Nantes-Atlantique, Laboratoire d'Etude des Résidus et Contaminants dans les  
25 Aliments (LABERCA), Atlanpole - La Chantrerie, CS50707, Nantes, F-44307, France, tel :  
26 +33 2 40 68 78 80, fax : +33 2 40 68 78 78, email : [laberca@oniris-nantes.fr](mailto:laberca@oniris-nantes.fr).

27

28 ABSTRACT

29 With the objectives of both generating bisphenols (BPs) conjugates occurrence data in food  
30 from animal origin but also investigating the origin of associated contamination, the present  
31 study deals with the development of an efficient analytical method aiming at monitoring both  
32 BPA and BPS conjugated metabolites in food from animal origin. The objective of such  
33 monitoring is to determine the origin of BPs contamination (FCM or animal contamination).  
34 The targeted compounds were BPA-monoglucuronide (BPA-1G), BPA-diglucuronide (BPA-  
35 2G), BPA-monosulfate (BPA-1S), BPA-disulfate (BPA-2S) and BPS-monoglucuronide  
36 (BPS-1G). The developed standard operating procedure includes a preliminary solid-liquid  
37 extraction step followed by two successive solid phase extraction (SPE) stages, using  
38 successively a non-polar phase and a strong cation exchange polymer. Quantification was  
39 achieved according to both the isotopic dilution and surrogated quantification methods, using  
40  $^{13}\text{C}$ -BPA-1G and BPA- $\text{d}_6$ -1S as internal standards. Linearity was validated ( $R^2 > 0.99$ ) for each  
41 molecule within the concentration range  $[0 - 10] \mu\text{g kg}^{-1}$ . Detection limits ranged from  $0.02$   
42  $\mu\text{g kg}^{-1}$  (BPA-1G in muscle, BPA-1S and BPA-2G in liver) to  $0.50 \mu\text{g kg}^{-1}$  (BPA-2S in  
43 muscle). The strategy was then proven on liver samples collected from pregnant ewes  
44 subcutaneously exposed to BPA during 105 days, at  $50 \mu\text{g kg}^{-1}$  per day. BPA-1G, BPA-2G  
45 and BPA-1S were detected and quantified at a concentration of  $3.81 \mu\text{g kg}^{-1}$ ,  $0.80 \mu\text{g kg}^{-1}$  and  
46  $0.09 \mu\text{g kg}^{-1}$ , respectively. The analytical method was finally implemented on fifty unpacked  
47 food samples from animal origin in which significant free BPA concentrations were  
48 previously measured. Since no metabolites of BPA could be measured ( $< \text{LOD}$ ), it means that  
49 such free BPA present in the samples originates from direct contact of the food item with a  
50 material containing BPA.

51 *KEYWORDS*

52 *Bisphenol A, Bisphenol S, conjugated metabolites, foodstuffs, UHPLC-MS/MS*

54 Bisphenol A (BPA) is a high production volume industrial chemical used in the production of  
55 polycarbonate and epoxy resins which have a large panel of applications, such as plastic food  
56 containers and epoxy food-can coatings. Its migration from packaging to food is well  
57 documented [1-5]. Currently authorized as food contact material within the European Union,  
58 the European Food Safety Authority (EFSA) has recently set a regulatory specific migration  
59 limit at 50  $\mu\text{g kg}^{-1}$  [6] and many countries, in the world, have adopted restrictions regarding  
60 the use of BPA in baby products or in food contact materials, in particular in the case of infant  
61 feeding bottles [7].

62 Estrogenic properties of BPA have been highlighted in both animals and humans, together  
63 with a range of adverse effects on brain, behavior, mammary glands, metabolism, obesity and  
64 reproduction [8-15]. Therefore, potential alternatives or BPA substitutes have been  
65 considered; among them, bisphenol F (BPF) and mostly, bisphenol S (BPS) are commonly  
66 chosen as monomer in the production of epoxy resins used as food contact material, while  
67 adverse effects of both BPS and BPF have been reported in several articles [16-21]. In  
68 parallel, biomonitoring data, dealing with the total (enzymatic hydrolysis) bisphenol  
69 analogues determination have been recently published [22-25], showing that BPA and BPS  
70 are the predominant bisphenol chemicals in biological samples.

71 Metabolism and kinetics of BPA in humans have been exhaustively studied by Dekant *et al.*  
72 and Völkel *et al.* who identified BPA monoglucuronide (BPA-1G) and BPA monosulfate  
73 (BPA-1S) as predominant BPA conjugated metabolites in urine [26-28]. They also described  
74 both rapid biotransformation and excretion of BPA. Similar metabolism mechanisms have  
75 been described leading for BPS and BPF to the following conjugated metabolites: BPF-1G,  
76 BPF-1S, BPS-1G and BPS-1S [29-31].

77 Several approaches have been reported for the determination of BPA-1G, BPA-2G [32],  
78 BPA-1S [32, 33] and BPA-2S [34], BPS-1G [35] in biological matrices, such as in plasma,  
79 serum and/or urine. Sample preparation procedures are usually based on solid phase  
80 extraction (SPE) such as solid-phase mixed-mode reverse-phase/anion-exchange extraction  
81 [32, 36], a combination of two successive SPE cartridges such as an amino-propyl silica-  
82 based cartridge ( $\text{NH}_2$ ) and a reverse-phase  $\text{C}_{18}$  [34], a polymeric reversed-phase sorbent with  
83 hydrophilic-lipophilic balance properties (HLB) [33], on-line SPE [35]. Finally, LC-MS/MS  
84 is commonly used as detection technique using the negative electrospray ionization [28, 32,

85 34-36] and the Selected Reaction Monitoring (SRM) mode. All reported analytical methods  
86 enable the quantification of BPA-1G which is identified as the main conjugated metabolites of  
87 BPA. Its quantification is generally performed according to the isotopic dilution method using  
88 to the labeled standard <sup>13</sup>C-BPA-1G while other targeted metabolites quantification is carried  
89 out using <sup>13</sup>C-BPA-1G, BPS-1G-d<sub>8</sub> and/or BPA-d<sub>6</sub>-1S as surrogated internal standards.  
90 Therefore, direct determination of conjugated metabolites of BPA in biological matrices, such  
91 as blood, serum or urine has been investigated in the framework of human exposure studies  
92 [32-34, 36-39]. Nevertheless, no analytical method focused simultaneously on the four  
93 conjugated metabolites of BPA, no more than conjugated metabolites of BPS. Additionally,  
94 these studies deal with biological matrices, and most of the reported method are implemented  
95 for urine samples.

96 Regarding foodstuff investigations, numerous data of BPA concentrations have been reported  
97 for several years, among them, results obtained in the framework of the second French total  
98 diet study (TDS2) dealing with a large scale of food items[40-42]. In this study, significant  
99 BPA concentration levels have been reported in non-canned foodstuffs, especially in food of  
100 animal origin. Therefore, the question of the origin of BPA contamination was raised: Is the  
101 contamination *ante-* or *post-mortem*? The answer could be found if the presence of conjugated  
102 metabolites of BPA is confirmed. While, to our knowledge, no analytical strategy has been  
103 already published for the direct analysis of conjugated metabolites of BPs in food, an  
104 analytical method targeting direct determination of these compounds, namely, BPA-1G, BPA-  
105 2G, BPA-1S, BPA-2S and BPS-1G in a wide range of foodstuff items of animal origin, was  
106 undertaken in the present work. An adequate strategy was developed and optimized for the  
107 determination of the five targeted metabolites using UHPLC-MS/MS. The corresponding  
108 analytical performances were assessed and the method was implemented to food samples in  
109 order to determine the origin of BPA contamination.

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**2 Material and methods**

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*2.1 Materials, reagents and reference substances*

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Acetonitrile and water (HPLC gradient-grade quality) were purchased from Carlo Erba (Val de Reuil, France) and from Panreac (Castellar del Vallès, Spain), respectively. Cyclohexane, acetic acid, formic acid, methanol and both ammonium acetate and ammoniac were obtained

117 from VWR (Fontenay-sous-Bois, France). Both solid phase extraction columns, i.e.  
118 Chromabond HR-X and SAX Cuqax were purchased, respectively, from Macherey-Nagel  
119 (Hoerdts, France) and UCT (Bristol, United States).

120 Bisphenol S  $\beta$ -D-glucuronide (BPS-1G), Bisphenol A  $\beta$ -D-glucuronide (BPA-1G), Bisphenol  
121 A bis sulfate (BPA-2S), Bisphenol A bis  $\beta$ -D-glucuronide (BPA-2G), Bisphenol A mono  
122 sulfate (BPA-1S),  $^{13}\text{C}_{12}$ -Bisphenol A  $\beta$ -D-glucuronide ( $^{13}\text{C}$ -BPA-1G) and BPA- $\text{d}_6$  mono  
123 sulfate (BPA- $\text{d}_6$ -1G) were obtained from Toronto Research Chemicals *Inc.* (Toronto,  
124 Canada). The chemical structures, molecular formulae and mono isotopic masses of the  
125 targeted molecules are shown in **Table 1**.

126 Individual stock standard solutions were prepared at a concentration of  $100 \text{ ng } \mu\text{L}^{-1}$  by  
127 dissolving an appropriate amount of each substance in the recommended solvent, i.e.  
128 methanol for BPA-1G, BPA-2G,  $^{13}\text{C}$ -BPA-1G, BPA-1S and BPA- $\text{d}_6$ -1S, and water for BPA-  
129 2S. Working solutions were obtained by tenfold successive dilution in the appropriate solvent  
130 at concentrations ranging from  $10 \text{ ng } \mu\text{L}^{-1}$  to  $0.01 \text{ ng } \mu\text{L}^{-1}$ . All the standard solutions were  
131 stored at  $4^\circ\text{C}$ , in the dark.

## 132 *2.2 Foodstuffs of animal origin*

133 Food items used for development, method performance evaluation and generation of  
134 preliminary data regarding conjugated metabolites of BPA and BPS detection in food, were  
135 collected in the framework of the second French Total Diet Study dealing with the  
136 investigation of BPA level in food items from animal origin [40, 41, 43]. Therefore, a wide  
137 range of matrices were included in the scope of the developed method, including various fish  
138 products, such as pollock, cod, salmon or hake, delicatessen meats, such as terrine, merguez  
139 sausage, raw/smoked or Parma ham, bovine/porcine/ovine meat, liver, muscle, turkey breast,  
140 roast pork and chicken. Samples were prepared according the procedure described by Sirot et  
141 *al.* [44] and were ground for homogenization using a food mixer.

## 142 *2.3 Incurred liver sample*

143 Liver samples were originating from a previous experiment looking at the effect of long term  
144 bisphenol A gestational exposure on the thyroid function in the pregnant ewe [45]. Animal  
145 procedures were carried out under agreement 311155515 for animal experimentation from the

146 French Ministry of Agriculture and validated by the local ethical committee for animal  
147 experimentation (project number: 2016 0323 10542323).

148 Briefly, pregnant ewes (n=5) were exposed to BPA, administered subcutaneously, at a dose of  
149  $50 \mu\text{g kg}^{-1}$  per day during 105 days. Plasma concentrations of BPA, BPAG and BPAS were  
150 assayed using a previously developed different method [37]. Results are reported in the study  
151 referenced above. Liver samples from the same area were collected in average 24h after the  
152 last BPA administration et were immediately snap frozen in liquid nitrogen. Samples were  
153 stored at  $-80^{\circ}\text{C}$  until assay in the current study to confirm the identity of bisphenol  
154 metabolites.

#### 155 *2.4 Standard Operating Procedure*

156 The analytical strategy was developed for the determination of BPA-1G, BPA-2G, BPA-1S,  
157 BPA-2S and BPS-1G in a wide range of food items from animal origin (**Figure 1**). The  
158 equivalent of 5 g of sample homogenized after a milling step was accurately weighted in  
159 polypropylene tube of 50 mL. Then, 25  $\mu\text{L}$  of internal standards ( $^{13}\text{C}$ -BPA-1G and BPA- $d_6$ -  
160 1S,  $1 \text{ ng } \mu\text{L}^{-1}$ ) were directly added to the samples, mixed and left in contact with the matrix at  
161 least 2 h. Afterwards, 20 mL water/acetonitrile (50:50, v/v) were added to the fresh sample  
162 before shaking during 1 min with the vortex. The extraction mixture was kept around 12 h at  
163 room temperature ( $20^{\circ}\text{C}$ ) before centrifugation at 4500 rpm during 20 min. The supernatant  
164 was collected into two tubes and a second extraction was directly performed on the matrix  
165 without any contact duration using 10 mL of the same mixture of water /acetonitrile (50:50,  
166 v/v). The supernatants were combined and acetonitrile was evaporated under a gentle stream  
167 of nitrogen at  $50^{\circ}\text{C}$ . For clean-up purposes, two successive solid phase extractions (SPE) were  
168 carried out. The first SPE was performed using a polystyrene-divinylbenzene polymer with a  
169 moderate/high specific surface ( $>1000\text{m}^2 \text{ g}^{-1}$ ). The SPE cartridge was conditioned  
170 successively with 6 mL methanol and 6 mL water. After loading the extracted sample (around  
171 10 mL after evaporation), the stationary phase was successively washed with 5 mL water and  
172 8 mL cyclohexane. Then, a strong vacuum was applied and a first elution containing sulfate  
173 compounds was carried out using 4 mL acetonitrile. Afterwards, the stationary phase was  
174 washed another time with 10 mL acetonitrile and glucuronide molecules were eluted with 20  
175 mL methanol. Both organic eluted fractions were evaporated to dryness under a gentle stream  
176 of nitrogen, at  $50^{\circ}\text{C}$  and were reconstituted in 5 mL water. These aqueous phases  
177 corresponding to glucuronide and sulfate fractions, were separately loaded onto a second

178 quaternary ammonium SPE SAX cartridge previously activated with 6 mL methanol and 6  
179 mL water. After washing with 5 mL water and 10 mL methanol, the glucuronide fraction (G)  
180 was eluted using 10 mL methanol with 2% formic acid (v/v). An additional washing step was  
181 carried out using 10 mL methanol and sulfate fraction (S) was finally eluted using 10 mL  
182 methanol with 15% of ammonia 32% (v/v). The two collected fractions (G and S) were  
183 evaporated to dryness under a nitrogen stream and reconstituted each in 100  $\mu$ L of  
184 water/acetonitrile (90:10, v/v) for injection. For one sample, solvent consumption was  
185 estimated to: 31 mL water, 29 mL acetonitrile, 72 mL methanol, 8 mL cyclohexane, 0.2 mL  
186 formic acid and 1.5 mL ammonia. In the experimental conditions implemented in our  
187 laboratory, an operator was able to analyse from one to twenty-four samples within two  
188 working days.

### 189 *2.5 UHPLC-MS/MS measurement*

190 Liquid chromatography separation was achieved on an Acquity Ultra Performance Liquid  
191 Chromatography (UPLC) System (Waters, Milford, MA, USA) equipped with a Thermo  
192 Hypersil Gold column (100 $\times$ 2.1 mm, 1.9 $\mu$ m) (Thermo Fisher Scientific, San José, CA, USA)  
193 maintained at 40°C. Mobile phases consisted in 0.1% formic acid in water (MP A) and 0.1%  
194 formic acid in acetonitrile (MP B) for glucuronide fraction. For sulfate fraction analysis,  
195 mobile phases were 20 mM ammonium acetate in water (MP A') and 20 mM ammonium  
196 acetate in acetonitrile (MP B'). The flow rate was set at 0.4 mL min<sup>-1</sup> for both analyses. The  
197 starting gradient was set at 90% of mobile phase A or A', for 1 min before performing a  
198 gradual increase of the organic phase (MP B or MP B') gradient to reach 20% at 3 min, 30%  
199 at 6.5 min and 100% at 7.5 min. Then the gradient was restored to its initial conditions within  
200 0.5 min and remained as such for 2 min.

201 Detection was carried out using a XEVO TQ-S instrument (Waters, Milford, MA, USA)  
202 operating in the negative Electrospray Ionisation mode (ESI-). Capillary voltage was set at 2  
203 kV, source temperature at 150°C, desolvation temperature at 500°C, desolvation gas (N<sub>2</sub>)  
204 at 600 L h<sup>-1</sup> and collision gas flow at 0.15 mL min<sup>-1</sup>. The acquisition was performed using the  
205 Selected Reaction Monitoring (SRM). Diagnostic SRM transitions were first generated using  
206 waters Intellistart<sup>TM</sup> software and all the parameters were optimized individually for each  
207 diagnostic signal, as indicated in **Table 2**. Data acquisition and data processing were  
208 performed using MassLynx, version 4.1 software.



210 BPA-1G and BPA-1S quantification was achieved according to isotopic dilution method  
211 using  $^{13}\text{C}$ -BPA-1G and BPA- $\text{d}_6$ -1S, respectively (25 ng directly added to each homogenized  
212 sample). Area signals of BPA-1G (403>113),  $^{13}\text{C}$ -BPA-1G (415>113), BPA-1S (307>227)  
213 and BPA- $\text{d}_6$ -1S (313>233) were determined and both ratio, i.e. BPA-1G area/  $^{13}\text{C}$ -BPA-1G  
214 area and BPA-1S area/ BPA- $\text{d}_6$ -1S area were calculated. For the other targeted molecules, i.e.  
215 BPA-2G, BPA-2S and BPS-1G, quantification was achieved using  $^{13}\text{C}$ -BPA-1G and BPA- $\text{d}_6$ -  
216 1S as surrogated internal standards. Therefore, area signals of BPA-2G (579>403) and BPS-  
217 1G (425>249) were determined and the corresponding ratio BPA-2G area/  $^{13}\text{C}$ -BPA-1G area  
218 and BPS-1G area/  $^{13}\text{C}$ -BPA-1G area were calculated. Finally, BPA-2S area signal (445>307)  
219 was measured and the ratio BPA-2S area/ BPA- $\text{d}_6$ -1S area was calculated. In parallel, a  
220 calibration curve was performed in the range [LOQ-10  $\mu\text{g kg}^{-1}$ ] and the equation of linear  
221 regression was determined for each molecule of interest. The concentrations of metabolite  
222 compounds were determined regarding the calculated quantity of the molecule and its  
223 corresponding test sample weight.

225 Detection and quantification limits, specificity, linearity and efficiency of the extraction step  
226 were assessed before method implementation and food items subsequent characterisation.  
227 Limits of detection (LOD) and quantification (LOQ) were systematically determined in each  
228 analysed sample for BPA-1G and BPA-1S, on the basis of the observed signals of  $^{13}\text{C}$ -BPA-  
229 1G and BPA- $\text{d}_6$ -1S, from which the concentrations levels leading to an observed  $S/N$  ratio of  
230  $S/N=3$  (LOD), on the first SRM transition and  $S/N=3$  (LOQ), on the second transition. This  
231 strategy is defined in the JRC guidance document [46], as “the concentration of an analyte in  
232 the extract of a sample which produces an instrumental response at two different ions to be  
233 monitored with a  $S/N$  ratio of 3:1 for the less intensive raw data signal”.

234 For the other targeted molecules, i.e. BPA-2G, BPA-2S and BPS-1G, their corresponding  
235 LODs and LOQs were calculated on the basis of three different categories of fortified  
236 samples, in the range [0-0.5  $\mu\text{g kg}^{-1}$ ]. Therefore, three different LODs and LOQs were  
237 assessed for these three compounds of interest corresponding to the three investigated  
238 matrices, i.e. bovine liver, Pollock sample and porcine muscle. These three selected matrices  
239 were considered to be representative of all the samples of interest. LODs and LOQs were then

240 defined as the lowest concentration point of the calibration curve that gives an acceptable and  
241 consistent signal for the principal transition and the second one, respectively.  
242 Specificity was assessed by checking the absence of interfering compounds responding to the  
243 diagnostic signals of the targeted molecules, in the range of their expected retention times.  
244 This parameter was also evaluated on the basis of the results obtained for the three  
245 representative matrices used in the framework of the validation process. Linearity was first  
246 evaluated for external calibration curves using standard solutions at six increasing  
247 concentration levels (namely 0, 0.5, 2.5, 5, 10 and 50 ng of the different analytes on-column).  
248 Secondly, the linearity for extracted calibration curves (liver, fish and meat) was evaluated  
249 within the 0-10  $\mu\text{g kg}^{-1}$  concentration range. The quality of the linear regressions was assessed  
250 through their related coefficient of determination ( $R^2$ ).  
251 Finally, liquid/solid extraction and analytical strategy efficiency were checked using an ovine  
252 liver sample originating from an animal experiment.

### 253 **3 Results and discussion**

#### 254 *3.1 Analytical LC-MS/MS development*

255 Chromatographic conditions were investigated with the objective of providing simultaneous  
256 efficient separation between all the targeted molecules combined with a good resolution of the  
257 different peaks. Preliminary experiments were carried out using water + 0.1% formic acid and  
258 acetonitrile + 0.1% formic acid as mobile phases on a Hypersil Gold column. Solvent gradient  
259 was then optimized within a 10 min run time, including a stabilization duration of 3 min. The  
260 flow rate and the column temperature were set to 0.4  $\text{mL min}^{-1}$  and 40°C, respectively. While  
261 both efficient separation and resolution were obtained for the glucuronide compounds, these  
262 experimental chromatographic conditions were not adequate for the sulfate fraction. Peak  
263 tailing was indeed observed for compounds BPA-1S and BPA-d<sub>6</sub>-1S. Additionally, BPA-2S  
264 was not detected with these initial conditions. These first results are illustrated in **Figure 2** on  
265 a salmon extract fortified with 5 ng of BPA-1S, BPA-2S (equivalent to 1  $\mu\text{g kg}^{-1}$ ) and 10 ng  
266 of BPA-d<sub>6</sub>-1S (equivalent to 2  $\mu\text{g kg}^{-1}$ ). As the most acidic compound (BPA-2S) is not  
267 detected, its retention was probably very weak on reversed phase using acid modifier in the  
268 mobile phase. Furthermore, the mono-sulfated compounds would be stabilized under a neutral  
269 form to improve their peak shape. Therefore, the composition of mobile phases was modified

270 as follows: 0.1% of formic acid was substituted with 20 mM ammonium acetate for both  
271 mobile phases, i.e. water + 20 mM ammonium acetate (MP A') and acetonitrile + 20 mM  
272 ammonium acetate (MP B'), in accordance with our previous works [47]. Initial developed  
273 gradient was then implemented in order to compare both conditions. Peak shape and peak  
274 tailing were significantly improved for both BPA-1S and its corresponding internal standard  
275 using ammonium acetate as a modifier. Moreover, an efficient retention of BPA-2S was also  
276 observed with ammonium acetate modifier, allowing its analysis under these new  
277 chromatographic conditions.

278 The resulting chromatographic run was 10 min for each fraction of interest. Regarding  
279 glucuronide fraction, the first (more polar) compound (BPS-1G) eluted at 3.61 min in a first  
280 (glucuronide) fraction analysis and a last (less polar) one (BPA-1G) eluted at 6.77 min. on the  
281 same way, the first eluted compound for sulfate fraction was BPA-2S at 5.00 min and the  
282 second one (BPA-1S) at 7.36 min. These optimized chromatographic conditions were  
283 subsequently used in this study. In comparison with the previous published articles, the  
284 proposed chromatographic strategy allows the detection of a greater number of conjugated  
285 metabolites of both BPA and BPS, without any peak tailing or peak fronting observations.

286 Then, the mass spectrometric parameters were optimized in the Selected Reaction Monitoring  
287 mode (SRM) for reaching high confidence level in terms of unambiguous identification of the  
288 molecules of interest at low concentrations according to 2002/657/EC decision requirements  
289 [48] (identification points >4) . Therefore, three or four diagnostic SRM transitions per  
290 targeted compound were monitored and optimized using Water's Intellistart system and then  
291 checked manually. Additionally, two SRM transitions were also monitored for each internal  
292 standard, namely <sup>13</sup>C-BPA-1G and BPA-d<sub>6</sub>-1S. Final acquisition method was sequenced in  
293 two different time windows for glucuronide fraction (G-TW1 and G-TW2) and in a single  
294 time window for sulfate fraction (S-TW1) (**Table 2**). Thanks to this analytical approach,  
295 selectivity, chromatographic efficiency and resolution were concluded compatible with  
296 expectations for food from animal origin characterization. While two diagnostic transitions  
297 were generally monitored in most of the published articles, the monitoring acquisition method  
298 was developed in order to strengthen the identification of the targeted molecules.

### 299 3.2 Standard Operating Procedure

300 The standard operating procedure (**Figure 1**) was developed and optimized for the direct  
301 determination of conjugated metabolites of bisphenol A and bisphenol S, in a wide range of  
302 food items from animal origin. Particular attention was given to the first extraction step  
303 efficiency. Therefore, different solid-liquid extractions have been evaluated on different  
304 foodstuffs. Water, water/acetonitrile (50:50, v/v), Folch [49] or Bligh and Dyer [50]  
305 procedures were compared on the basis on results obtained with liver, fish and muscle  
306 samples fortified with 5 ng of each compound of interest. Solid-liquid extraction carried out  
307 using water/acetonitrile mixture was preferred because acetonitrile allows efficient protein  
308 precipitation, enhancing thus the sample treatment facility. Indeed, no clogging was observed  
309 with subsequent SPE when using this strategy whereas a solid-liquid extraction driven using  
310 only water resulted in problematic SPEs. Additionally, no significant differences were  
311 observed between water/acetonitrile, Folch or Bligh and Dyer procedures, neither during  
312 sample treatment nor on the recovery yields. Therefore, two successive extractions using  
313 water/acetonitrile (50:50, v/v) were finally chosen for enhanced recovery  
314 yields/performances.

315 Afterwards, two consecutive orthogonal SPE purification stages were envisaged, *i.e.* a non-  
316 polar phase combined to a strong anion exchange sorbent. Regarding the non-polar SPE  
317 phase, several trials have been performed to optimize the washing step. Initially, various  
318 mixtures of water/methanol (from 100% water to 0%) were tested on reference standard  
319 solutions. A washing step using 5 mL water/methanol (90:10, v/v) was considered as suitable  
320 conditions. These experimental conditions were then implemented for the analysis of different  
321 matrices, such as liver, muscle and fish. For each tested matrix, both washing and elution  
322 fractions were collected and analyzed. No repeatable results were obtained insofar as variable  
323 recoveries of all targeted compound were observed in the washing fraction depending on the  
324 analyzed matrix. Actually, the eluting strength necessary for the elution of both glucuronide  
325 and sulfate compounds was observed as matrix-dependent. Therefore, cyclohexane was  
326 successfully tested in order to remove non polar interferences and then elution step was  
327 optimized as follows: sulfate compounds were eluted using acetonitrile while glucuronide  
328 molecules were secondly eluted using methanol. Because of lipid residual occasionally  
329 observed for fatty samples, a second SPE step using a strong anion exchange (SAX) phase  
330 was also included in the analytical workflow in order to improve the purification efficiency.  
331 Initially two different stationary phases have been investigated: the first one was a  
332 copolymeric strong anions exchanger bonded on a HR-X phase (HR-XA, Macherey Nagel)

333 and the second one was a strong anions exchanger on a functionalized silica (SAX, UCT).  
334 Regarding HR-XA cartridge, satisfactory recoveries were obtained for glucuronide  
335 compounds using an elution mixture of methanol + 2% formic acid (>90%). Nevertheless,  
336 sulfate compounds were not eluted in the corresponding fraction using methanol + ammonia,  
337 even with 15% ammonia, as described by Anizan et al. [47]. This kind of stationary phase  
338 presents too strong ionic affinities with BPA-1S, BPA-1S-d<sub>6</sub> and BPA-2S to allow their  
339 elution regarding the determination of conjugated metabolites of steroids in urine. Moreover,  
340 recovery yields obtained using SAX cartridge were excellent (>95%) for both glucuronide  
341 molecules eluted with methanol / formic acid (98:2, v/v) and sulfate compounds eluted with  
342 methanol / ammonium hydroxide 32% (85:15, v/v). Therefore, the additional step based on a  
343 SAX cartridge was chosen to achieve the purification before analysis. **Figure 3** illustrates an  
344 example of SRM chromatograms obtained for a salmon analysis, with the following analytical  
345 procedures: Solid-liquid extraction + filtration (A), Solid-liquid extraction + non polar SPE  
346 (B) and solid-liquid extraction + non polar SPE + SAX SPE (C). SRM chromatograms were  
347 slightly better using HR-X SPE in comparison to the single solid-liquid extraction: both  
348 intensities and S/N ratios were improved, especially regarding BPA-1S detection (307>227).  
349 Finally, the addition of SAX SPE purification improved significantly all results, especially in  
350 terms of S/N ratios. SRM chromatograms and its corresponding S/N calculation were  
351 investigated for the main diagnostic transition of BPA-1S (307>227) regarding the three  
352 different analytical strategies. S/N=8 was obtained for a single solid-liquid extraction, S/N=12  
353 for solid-liquid extraction combined with non-polar SPE, and S/N=104 for the developed and  
354 optimized analytical method. This standard operating procedure is quite innovative in  
355 comparison with the analytical methods already published which focus on biological matrices,  
356 such as blood or urine. Indeed, the determination of conjugated metabolites of BPA and BPS  
357 in foodstuffs, at very low levels (from 0.02 to 0.50 µg/kg), require an efficient sample  
358 preparation which is fulfilled with the combination of both consecutive orthogonal SPE steps.

### 359 3.3 Validation results

#### 360 3.3.1 Background contamination

361 The control of in-laboratory environmental contamination with BPA was considered as a  
362 major potential critical issue, as already mentioned in several articles [42, 51-55]. Despite  
363 their non-ubiquitous status, the potential background contamination of conjugated metabolites

364 of bisphenol A and bisphenol S was still necessary to be assessed. All materials and analytical  
365 measurement devices were tested. Moreover, one procedural blank sample was systematically  
366 included in each series of samples in the framework of the experimental plan implementation.  
367 The results demonstrated neither background contamination nor traces in more than thirty  
368 blank analyzed samples (<LOD) for the five metabolites of interest, allowing to conclude that  
369 no particular attention had to be paid to the background contamination for the determination  
370 of conjugated metabolites of both BPA and BPS, in the experimental conditions implemented  
371 in this analytical development.

### 372 3.3.2 Linearity – calibration curve

373 The linearity of the developed analytical method was determined on calibration curves made  
374 of standard solutions and extracted spiked foodstuffs (liver, muscle and fish). Eight  
375 concentration levels were used in the range of [0 – 10]  $\mu\text{g kg}^{-1}$ , with a majority of calibration  
376 points (n=6) included in the range 0 – 1  $\mu\text{g kg}^{-1}$ . The intercept was not forced through the  
377 origin due to the possible presence of the targeted compounds in the non-fortified sample.  
378 Excellent linearity values were obtained for all the compounds of interest, as all resulting  
379 coefficients of determination ( $R^2$ ) were found higher than 0.99 with residuals below 20% on  
380 the relative response factors (RRF). For each quantified analyte, both calibration curves (i.e.,  
381 external and internal calibration) were found mimetic according to the Student statistical test,  
382 with equivalent slope. Therefore, sample quantification was carried out using the standard  
383 calibration curve.

### 384 3.3.3 Detection limits

385 The limit of detection (LOD) was estimated as the concentration from which a significant  
386 signal to noise ratio ( $S/N=3$ ) was obtained. LODs were initially determined in three food  
387 matrices, namely liver, muscle and fish for the five targeted molecules. Regarding BPA-G and  
388 BPA-S, considered as the principal conjugated metabolites of BPA, LODs ranged from 0.02  
389 to 0.10  $\mu\text{g kg}^{-1}$  for the three investigated matrices. LODs obtained for BPA-2G and BPS-1G  
390 ranged from 0.02  $\mu\text{g kg}^{-1}$  for BPA-2G in liver to 0.18  $\mu\text{g kg}^{-1}$  for BPS-1G in fish sample.  
391 Detailed LOD results obtained for the five compounds of interest and the three investigated  
392 matrices are reported in **Table 3**. Additionally, LODs were systematically calculated for each  
393 analyzed sample for both BPA-1G and BPA-1S on the basis of the major transition of the  
394 corresponding internal standard, i.e.: 415>113 for  $^{13}\text{C}$ -BPA-1G and 313>233 for BPA-d<sub>6</sub>-1S.

395 Regarding, BPA-2S, observed LODs were significantly higher than the other targeted  
396 compounds, ranging from 0.10  $\mu\text{g kg}^{-1}$  in liver sample to 0.50  $\mu\text{g kg}^{-1}$  in bovine muscle. To  
397 our knowledge, no earlier studies have reported LODs of conjugated metabolites of both BPA  
398 and BPS, in foodstuffs. Nevertheless, the obtained LODs are consistent with BPA  
399 contamination levels reported in the framework of the second French total diet study [41].

#### 400 3.4 Proof of the concept

401 The developed and validated analytical method was applied to various foodstuffs of animal  
402 origin.

403 Firstly, an incurred liver sample originating from an animal exposed to BPA on the basis of  
404 50  $\mu\text{g kg}^{-1}$  per day during 105 days was analyzed. The results show that BPA-1G, BPA-1S  
405 and BPA-2G were detected ( $>\text{LOD}$ ) in this sample, unambiguously identified and quantified  
406 at a concentration of 3.81  $\mu\text{g kg}^{-1}$ , 0.80  $\mu\text{g kg}^{-1}$  and 0.09  $\mu\text{g kg}^{-1}$ , respectively, as illustrated in  
407 **Figure 4**. Therefore, BPA-1G appears as the main conjugated metabolite of BPA in ovine  
408 liver representing 81% of the conjugated forms. BPA-1S was detected with a proportion of  
409 17% and BPA-2G represented 2% of the conjugated forms. BPA-2S was not detected,  
410 ( $<\text{LOD}$ ). This animal experiment results demonstrates that (i) BPA is extensively metabolized  
411 in the ovine liver, and, (ii) the efficiency of both extraction and purification efficiencies.  
412 Moreover, to the best of our knowledge it is the first time that BPA-2G has been identified in  
413 a liver sample originating from an animal exposed to BPA.

414 Secondly, fifty different foodstuffs of animal origin were selected as previously characterized  
415 for their contamination with free BPA [56]. The main objective was to investigate possible /  
416 potential presence of conjugated metabolites of BPA. As none of the investigated molecules  
417 (BPA-1G, BPA-2G, BPA-1S and BPA-2S) could be detected ( $<\text{LOD}$ ) in any of the fifty  
418 selected foodstuffs samples, it means that BPA contamination was the result of direct contact  
419 with material containing BPA, and was not the result of living animal exposure to BPA which  
420 would have led to *in-vivo* metabolism and the subsequent presence of metabolites.

421 Therefore, the proof of the strategy efficiency was demonstrated insofar as the developed  
422 analytical method was successfully applied to a large variety of foodstuffs of animal origin  
423 and the observed results allows (i) to characterize BPA metabolism in ovine liver and (ii)  
424 determine the origin of BPA contamination.

425

#### 4 Conclusion

426 Analytical method based on a first efficient solid/liquid extraction followed by the  
427 combination of two successive solid phase extraction (SPE) proved to be a suitable strategy  
428 for the direct detection and quantification of BPA-1G, BPA-2G, BPA-1S, BPA-2S and BPS-  
429 1G in a large scale of foodstuffs of animal origin. Conjugated bisphenol concentrations are  
430 usually deduced from “total” and “free” bisphenol concentrations with the major critical issue  
431 consideration of ubiquitous molecules. To the best of our knowledge, and according to the  
432 previous standard operating procedures already published, it is the first analytical  
433 methodology allowing the simultaneous determination of the four conjugated metabolites of  
434 BPA and mono glucuronide BPS. Targeted compounds were easily separated onto the  
435 UHPLC system and the Selected Reaction Monitoring mode of the triple quadrupole mass  
436 analyzer allowed the unambiguous detection and quantification of the five targeted molecules.  
437 Detection limits ranged from  $0.02 \mu\text{g kg}^{-1}$  for BPA-1G in muscle or both BPA-1S and BPA-  
438 2G in liver, to  $0.50 \mu\text{g kg}^{-1}$  for BPA-2S in muscle. Quantification was performed using both  
439  $^{13}\text{C}$ -BPA-1G and BPA- $\text{d}_6$ -1S as internal standards; corresponding linearity was quite  
440 satisfying with all coefficients of determination above 0.99. While no targeted compound was  
441 detected in any of the analyzed samples (<LODs), The developed analytical method was  
442 successfully implemented to the determination of conjugated metabolites of BPA and BPS in  
443 a first heterogeneous set of 50 foodstuffs of animal origin, previously investigated regarding  
444 their BPA levels. The comparison between both results, i.e. free BPA concentrations and  
445 conjugated forms validated the *post mortem* origin of BPA in these samples. Additionally, an  
446 incurred liver sample originating from pregnant ewes administered subcutaneously with BPA  
447 ( $50 \mu\text{g kg}^{-1}$  per day) during 105 days, was analyzed using the developed analytical method.  
448 BPA-1G, BPA-1S and BPA-2G were detected (>LOD), unambiguously identified and  
449 quantified at a concentration of  $3.81 \mu\text{g kg}^{-1}$ ,  $0.80 \mu\text{g kg}^{-1}$  and  $0.09 \mu\text{g kg}^{-1}$ , respectively.  
450 These results confirm that BPA-1G is the main conjugated metabolite of BPA, since BPA-1S  
451 and BPA-2G were also observed.  
452 Therefore, this analytical method could be used in the framework of investigations regarding  
453 both BPA and BPS conjugated metabolites, in food items but also in biological matrices.

454



- 456 [1] S. Errico, M. Bianco, L. Mita, M. Migliaccio, S. Rossi, C. Nicolucci, C. Menale, M. Portaccio, P.  
457 Gallo, D.G. Mita, N. Diano, Migration of bisphenol A into canned tomatoes produced in Italy:  
458 dependence on temperature and storage conditions, *Food chemistry*, 160 (2014) 157-164.
- 459 [2] A. Goodson, H. Robin, W. Summerfield, I. Cooper, Migration of bisphenol A from can coatings--  
460 effects of damage, storage conditions and heating, *Food additives and contaminants*, 21 (2004) 1015-  
461 1026.
- 462 [3] E.M. Munguia-Lopez, S. Gerardo-Lugo, E. Peralta, S. Bolumen, H. Soto-Valdez, Migration of  
463 bisphenol A (BPA) from can coatings into a fatty-food simulant and tuna fish, *Food additives and*  
464 *contaminants*, 22 (2005) 892-898.
- 465 [4] M.I. Santillana, E. Ruiz, M.T. Nieto, J. Bustos, J. Maia, R. Sendon, J.J. Sanchez, Migration of  
466 bisphenol A from polycarbonate baby bottles purchased in the Spanish market by liquid  
467 chromatography and fluorescence detection, *Food additives & contaminants. Part A, Chemistry,*  
468 *analysis, control, exposure & risk assessment*, 28 (2011) 1610-1618.
- 469 [5] M.L. Spagnuolo, F. Marini, L.A. Sarabia, M.C. Ortiz, Migration test of Bisphenol A from  
470 polycarbonate cups using excitation-emission fluorescence data with parallel factor analysis, *Talanta*,  
471 167 (2017) 367-378.
- 472 [6] Commission Regulation (EU) 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes  
473 and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as  
474 regards the use of that substance in plastic food contact materials, *Off. J. Eur. L41/6* (2018).
- 475 [7] S.A. Baluka, W.K. Rumbelha, Bisphenol A and food safety: Lessons from developed to developing  
476 countries, *Food and Chemical Toxicology*, 92 (2016) 58-63.
- 477 [8] S. Eladak, D. Moison, M.J. Guerquin, G. Matilionyte, K. Kilcoyne, T. N'Tumba-Byn, S. Messiaen, Y.  
478 Deceuninck, S. Pozzi-Gaudin, A. Benachi, G. Livera, J.P. Antignac, R. Mitchell, V. Rouiller-Fabre, R.  
479 Habert, Effects of environmental Bisphenol A exposures on germ cell development and Leydig cell  
480 function in the human fetal testis, *PLoS one*, 13 (2018) e0191934.
- 481 [9] C. Desdoits-Lethimonier, L. Lesne, P. Gaudriault, D. Zalko, J.P. Antignac, Y. Deceuninck, C. Platel,  
482 N. Dejucq-Rainsford, S. Mazaud-Guittot, B. Jegou, Parallel assessment of the effects of bisphenol A  
483 and several of its analogs on the adult human testis, *Hum Reprod*, 32 (2017) 1465-1473.
- 484 [10] X.H. Xu, J. Zhang, Y.M. Wang, Y.P. Ye, Q.Q. Luo, Perinatal exposure to bisphenol-A impairs  
485 learning-memory by concomitant down-regulation of N-methyl-D-aspartate receptors of hippocampus  
486 in male offspring mice, *Hormones and behavior*, 58 (2010) 326-333.
- 487 [11] X. Xu, Y. Ye, T. Li, L. Chen, D. Tian, Q. Luo, M. Lu, Bisphenol-A rapidly promotes dynamic  
488 changes in hippocampal dendritic morphology through estrogen receptor-mediated pathway by  
489 concomitant phosphorylation of NMDA receptor subunit NR2B, *Toxicology and applied pharmacology*,  
490 249 (2010) 188-196.
- 491 [12] R. Moral, R. Wang, I.H. Russo, C.A. Lamartiniere, J. Pereira, J. Russo, Effect of prenatal  
492 exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression  
493 signature, *The Journal of endocrinology*, 196 (2008) 101-112.
- 494 [13] M. Ben Maamar, L. Lesne, C. Desdoits-Lethimonier, I. Coiffec, J. Lasserguere, V. Lavoue, Y.  
495 Deceuninck, J.P. Antignac, B. Le Bizec, E. Perdu, D. Zalko, C. Pineau, C. Chevrier, N. Dejucq-  
496 Rainsford, S. Mazaud-Guittot, B. Jegou, An investigation of the endocrine-disruptive effects of  
497 bisphenol a in human and rat fetal testes, *PLoS one*, 10 (2015) e0117226.
- 498 [14] E.F. Efsa Panel on Food Contact Materials, A. Processing, Scientific Opinion on the risks to public  
499 health related to the presence of bisphenol A (BPA) in foodstuffs, *EFSA Journal*, 13 (2015) 3978-n/a.
- 500 [15] M.T. Do, V.C. Chang, M.A. Mendez, M. de Groh, Urinary bisphenol A and obesity in adults:  
501 results from the Canadian Health Measures Survey, *Health Promot Chronic Dis Prev Can*, 37 (2017)  
502 403-412.
- 503 [16] D.G. Skledar, J. Schmidt, A. Fic, I. Klopčič, J. Trontelj, M.S. Dolenc, M. Finel, L.P. Masic,  
504 Influence of metabolism on endocrine activities of bisphenol S, *Chemosphere*, 157 (2016) 152-159.
- 505 [17] V. Le Fol, S. Ait-Aissa, M. Sonavane, J.M. Porcher, P. Balaguer, J.P. Cravedi, D. Zalko, F. Brion,  
506 In vitro and in vivo estrogenic activity of BPA, BPF and BPS in zebrafish-specific assays,  
507 *Ecotoxicology and environmental safety*, 142 (2017) 150-156.
- 508 [18] L. Ivry Del Moral, L. Le Corre, H. Poirier, I. Niot, T. Truntzer, J.F. Merlin, P. Rouimi, P. Besnard, R.  
509 Rahmani, M.C. Chagnon, Obesogen effects after perinatal exposure of 4,4'-sulfonyldiphenol  
510 (Bisphenol S) in C57BL/6 mice, *Toxicology*, 357-358 (2016) 11-20.

511 [19] N. Ahsan, H. Ullah, W. Ullah, S. Jahan, Comparative effects of Bisphenol S and Bisphenol A on  
512 the development of female reproductive system in rats; a neonatal exposure study, *Chemosphere*, 197  
513 (2018) 336-343.

514 [20] S. Kolla, M. Morcos, B. Martin, L.N. Vandenberg, Low dose bisphenol S or ethinyl estradiol  
515 exposures during the perinatal period alter female mouse mammary gland development, *Reprod*  
516 *Toxicol*, 78 (2018) 50-59.

517 [21] J.C. Park, M.C. Lee, D.S. Yoon, J. Han, M. Kim, U.K. Hwang, J.H. Jung, J.S. Lee, Effects of  
518 bisphenol A and its analogs bisphenol F and S on life parameters, antioxidant system, and response  
519 of defensome in the marine rotifer *Brachionus koreanus*, *Aquatic toxicology*, 199 (2018) 21-29.

520 [22] Y. Chen, J. Fang, L. Ren, R. Fan, J. Zhang, G. Liu, L. Zhou, D. Chen, Y. Yu, S. Lu, Urinary  
521 bisphenol analogues and triclosan in children from south China and implications for human exposure,  
522 *Environmental pollution*, 238 (2018) 299-305.

523 [23] G. Russo, F. Barbato, E. Cardone, M. Fattore, S. Albrizio, L. Grumetto, Bisphenol A and  
524 Bisphenol S release in milk under household conditions from baby bottles marketed in Italy, *J Environ*  
525 *Sci Health B*, 53 (2018) 116-120.

526 [24] L.H. Wu, X.M. Zhang, F. Wang, C.J. Gao, D. Chen, J.R. Palumbo, Y. Guo, E.Y. Zeng, Occurrence  
527 of bisphenol S in the environment and implications for human exposure: A short review, *The Science*  
528 *of the total environment*, 615 (2018) 87-98.

529 [25] D. Chen, K. Kannan, H. Tan, Z. Zheng, Y.L. Feng, Y. Wu, M. Widelka, Bisphenol Analogues Other  
530 Than BPA: Environmental Occurrence, Human Exposure, and Toxicity-A Review, *Environmental*  
531 *science & technology*, 50 (2016) 5438-5453.

532 [26] W. Volkel, T. Colnot, G.A. Csanady, J.G. Filser, W. Dekant, Metabolism and kinetics of bisphenol  
533 a in humans at low doses following oral administration, *Chemical research in toxicology*, 15 (2002)  
534 1281-1287.

535 [27] W. Dekant, W. Volkel, Human exposure to bisphenol A by biomonitoring: methods, results and  
536 assessment of environmental exposures, *Toxicology and applied pharmacology*, 228 (2008) 114-134.

537 [28] W. Volkel, N. Bittner, W. Dekant, Quantitation of bisphenol A and bisphenol A glucuronide in  
538 biological samples by high performance liquid chromatography-tandem mass spectrometry, *Drug*  
539 *metabolism and disposition: the biological fate of chemicals*, 33 (2005) 1748-1757.

540 [29] V. Le Fol, S. Ait-Aissa, N. Cabaton, L. Dolo, M. Grimaldi, P. Balaguer, E. Perdu, L. Debrauwer, F.  
541 Brion, D. Zalko, Cell-specific biotransformation of benzophenone-2 and bisphenol-s in zebrafish and  
542 human in vitro models used for toxicity and estrogenicity screening, *Environmental science &*  
543 *technology*, 49 (2015) 3860-3868.

544 [30] N. Cabaton, D. Zalko, E. Rathahao, C. Canlet, G. Delous, M.C. Chagnon, J.P. Cravedi, E. Perdu,  
545 Biotransformation of bisphenol F by human and rat liver subcellular fractions, *Toxicology in vitro : an*  
546 *international journal published in association with BIBRA*, 22 (2008) 1697-1704.

547 [31] N. Cabaton, M.C. Chagnon, J.C. Lhuguenot, J.P. Cravedi, D. Zalko, Disposition and metabolic  
548 profiling of bisphenol F in pregnant and nonpregnant rats, *Journal of agricultural and food chemistry*,  
549 54 (2006) 10307-10314.

550 [32] G. Provencher, R. Berube, P. Dumas, J.F. Bienvenu, E. Gaudreau, P. Belanger, P. Ayotte,  
551 Determination of bisphenol A, triclosan and their metabolites in human urine using isotope-dilution  
552 liquid chromatography-tandem mass spectrometry, *Journal of chromatography. A*, 1348 (2014) 97-  
553 104.

554 [33] R.R. Gerona, T.J. Woodruff, C.A. Dickenson, J. Pan, J.M. Schwartz, S. Sen, M.W. Friesen, V.Y.  
555 Fujimoto, P.A. Hunt, Bisphenol-A (BPA), BPA glucuronide, and BPA sulfate in midgestation umbilical  
556 cord serum in a northern and central California population, *Environmental science & technology*, 47  
557 (2013) 12477-12485.

558 [34] C. Liao, K. Kannan, Determination of free and conjugated forms of bisphenol A in human urine  
559 and serum by liquid chromatography-tandem mass spectrometry, *Environmental science &*  
560 *technology*, 46 (2012) 5003-5009.

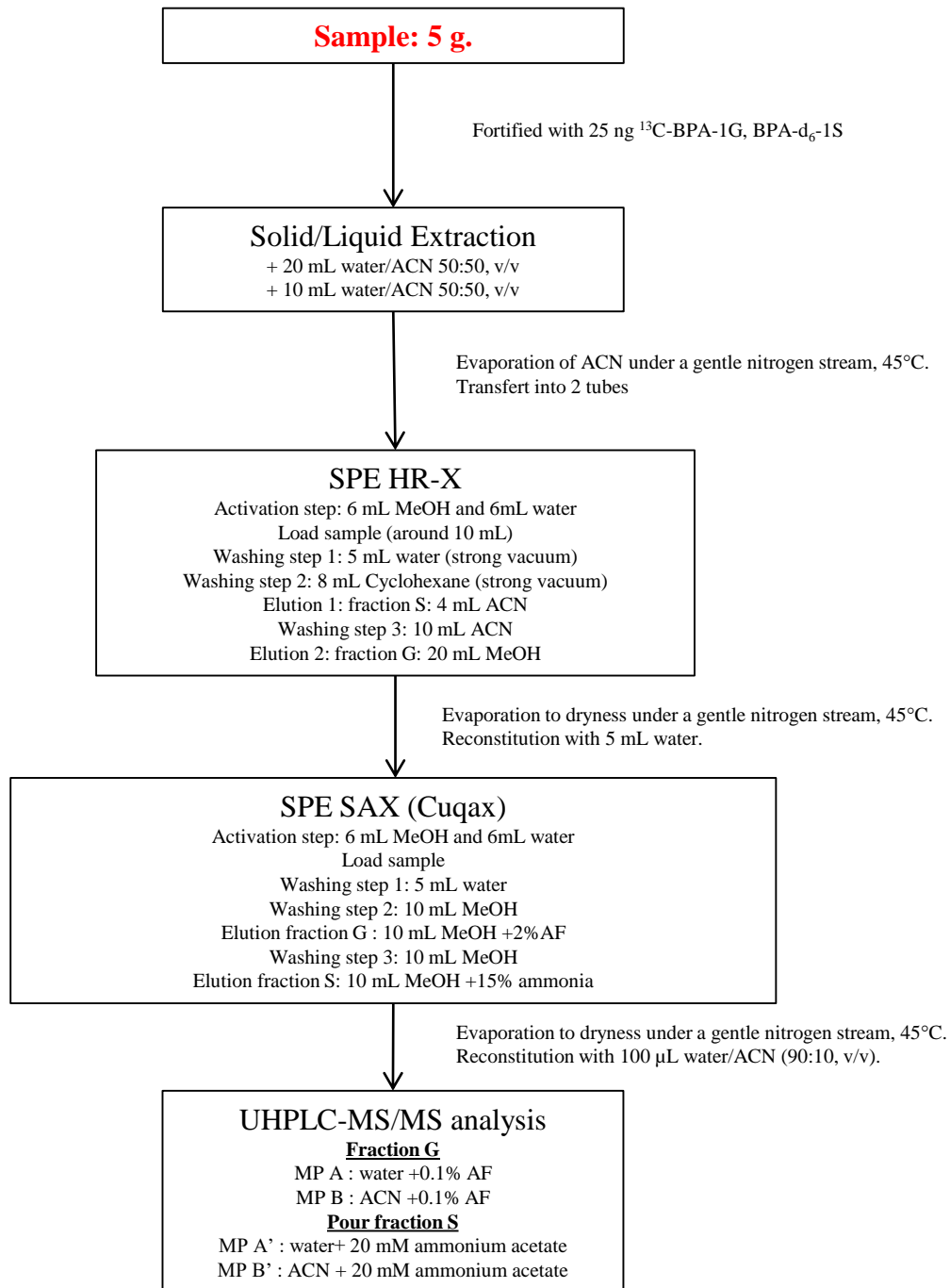
561 [35] F. Grandin, N. Picard-Hagen, V. Gayraud, S. Puel, C. Viguie, P.L. Toutain, L. Debrauwer, M.Z.  
562 Lacroix, Development of an on-line solid phase extraction ultra-high-performance liquid  
563 chromatography technique coupled to tandem mass spectrometry for quantification of bisphenol S and  
564 bisphenol S glucuronide: Applicability to toxicokinetic investigations, *Journal of chromatography. A*,  
565 1526 (2017) 39-46.

566 [36] Z.Z. Hauck, K. Huang, G. Li, R.B. van Breemen, Determination of bisphenol A-glucuronide in  
567 human urine using ultrahigh-pressure liquid chromatography/tandem mass spectrometry, *Rapid*  
568 *communications in mass spectrometry : RCM*, 30 (2016) 400-406.

569 [37] M.Z. Lacroix, S. Puel, S.H. Collet, T. Corbel, N. Picard-Hagen, P.L. Toutain, C. Viguie, V.  
570 Gayraud, Simultaneous quantification of bisphenol A and its glucuronide metabolite (BPA-G) in plasma  
571 and urine: applicability to toxicokinetic investigations, *Talanta*, 85 (2011) 2053-2059.  
572 [38] C. Kubwabo, I. Kosarac, K. Lalonde, W.G. Foster, Quantitative determination of free and total  
573 bisphenol A in human urine using labeled BPA glucuronide and isotope dilution mass spectrometry,  
574 *Analytical and bioanalytical chemistry*, 406 (2014) 4381-4392.  
575 [39] I. Kosarac, C. Kubwabo, K. Lalonde, W. Foster, A novel method for the quantitative determination  
576 of free and conjugated bisphenol A in human maternal and umbilical cord blood serum using a two-  
577 step solid phase extraction and gas chromatography/tandem mass spectrometry, *Journal of*  
578 *chromatography. B, Analytical technologies in the biomedical and life sciences*, 898 (2012) 90-94.  
579 [40] ANSES, Opinion and expert appraisal report of the French Agency for Food, Environmental and  
580 Occupational Health & Safety on the assessment of the risks of bisphenol A (BPA) for human health.  
581 *Maisons Alfort, Volume 1* (2013).  
582 [41] N. Bemrah, J. Jean, G. Riviere, M. Sanaa, S. Leconte, M. Bachelot, Y. Deceuninck, B.L. Bizec, X.  
583 Dauchy, A.C. Roudot, V. Camel, K. Grob, C. Feidt, N. Picard-Hagen, P.M. Badot, F. Fources, J.C.  
584 Leblanc, Assessment of dietary exposure to bisphenol A in the French population with a special focus  
585 on risk characterisation for pregnant French women, *Food and chemical toxicology : an international*  
586 *journal published for the British Industrial Biological Research Association*, 72 (2014) 90-97.  
587 [42] Y. Deceuninck, E. Bichon, S. Durand, N. Bemrah, Z. Zendong, M.L. Morvan, P. Marchand, G.  
588 Dervilly-Pinel, J.P. Antignac, J.C. Leblanc, B. Le Bizec, Development and validation of a specific and  
589 sensitive gas chromatography tandem mass spectrometry method for the determination of bisphenol A  
590 residues in a large set of food items, *Journal of chromatography. A*, 1362 (2014) 241-249.  
591 [43] ANSES, Second French Total Diet Study (TDS2) – Volume 1: Inorganic contaminants, minerals,  
592 persistent organic pollutants, mycotoxins and phyto-oestrogens. Expert appraisal report. Scientific  
593 Editions. Maisons-Alfort, ANSES: 305., (2011).

594 [44] V. Sirot, J.L. Volatier, G. Calamassi-Tran, C. Dubuisson, C. Menard, A. Dufour, J.C. Leblanc,  
595 Core food of the French food supply: second Total Diet Study, *Food additives & contaminants. Part A,*  
596 *Chemistry, analysis, control, exposure & risk assessment*, 26 (2009) 623-639.  
597 [45] D. Guignard, V. Gayraud, M.Z. Lacroix, S. Puel, N. Picard-Hagen, C. Viguie, Evidence for  
598 bisphenol A-induced disruption of maternal thyroid homeostasis in the pregnant ewe at low level  
599 representative of human exposure, *Chemosphere*, 182 (2017) 458-467.  
600 [46] E.C. Joint Research Centre, Guidance Document on the Estimation of LOD and LOQ for  
601 Measurements in the Field of Contaminants in Feed and Food, Publications Office of the European  
602 Union, (2016).  
603 [47] S. Anizan, D. Di Nardo, E. Bichon, F. Monteau, N. Cesbron, J.P. Antignac, B. Le Bizec, Targeted  
604 phase II metabolites profiling as new screening strategy to investigate natural steroid abuse in animal  
605 breeding, *Analytica chimica acta*, 700 (2011) 105-113.  
606 [48] Commission Decision of 12 August 2002 (2002/657/EC) implementing Council Directive 96/23/EC  
607 concerning the performance of analytical methods and the interpretation of results.  
608 [49] F.N. Lebaron, J. Folch, The effect of pH and salt concentration on aqueous extraction of brain  
609 proteins and lipoproteins, *Journal of neurochemistry*, 4 (1959) 1-8.  
610 [50] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, *Can J Biochem*  
611 *Physiol*, 37 (1959) 911-917.  
612 [51] R. Carabias-Martinez, E. Rodriguez-Gonzalo, P. Revilla-Ruiz, J. Hernandez-Mendez, Pressurized  
613 liquid extraction in the analysis of food and biological samples, *Journal of chromatography. A*, 1089  
614 (2005) 1-17.  
615 [52] B. Shao, H. Han, X. Tu, L. Huang, Analysis of alkylphenol and bisphenol A in eggs and milk by  
616 matrix solid phase dispersion extraction and liquid chromatography with tandem mass spectrometry,  
617 *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 850 (2007)  
618 412-416.  
619 [53] Y. Watabe, T. Kondo, M. Morita, N. Tanaka, J. Haginaka, K. Hosoya, Determination of bisphenol  
620 A in environmental water at ultra-low level by high-performance liquid chromatography with an  
621 effective on-line pretreatment device, *Journal of chromatography. A*, 1032 (2004) 45-49.  
622 [54] K. Inoue, K. Kato, Y. Yoshimura, T. Makino, H. Nakazawa, Determination of bisphenol A in human  
623 serum by high-performance liquid chromatography with multi-electrode electrochemical detection,  
624 *Journal of chromatography. B, Biomedical sciences and applications*, 749 (2000) 17-23.  
625 [55] W. Volkel, M. Kiranoglu, H. Fromme, Determination of free and total bisphenol A in human urine  
626 to assess daily uptake as a basis for a valid risk assessment, *Toxicology letters*, 179 (2008) 155-162.

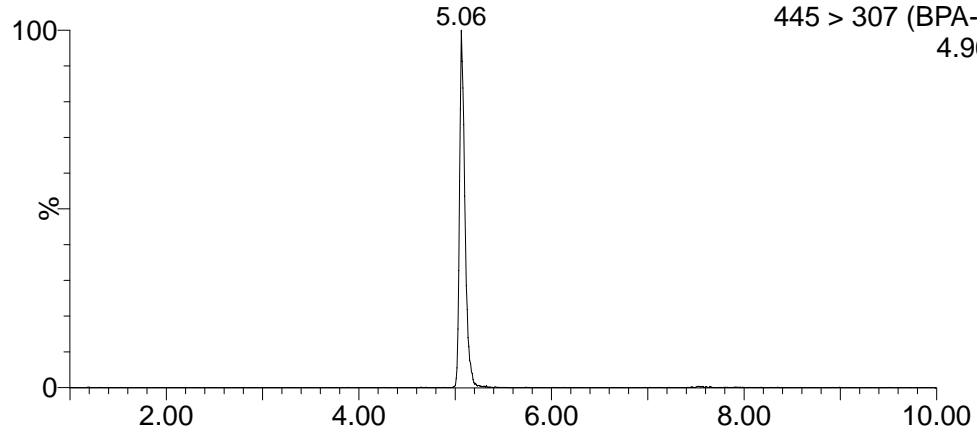
627 [56] S. Gorecki, N. Bemrah, A.C. Roudot, E. Marchioni, B. Le Bizec, F. Faivre, M. Kadawathagedara,  
628 J. Botton, G. Riviere, E.m.-c.c.s. group, Human health risks related to the consumption of foodstuffs of  
629 animal origin contaminated by bisphenol A, Food and chemical toxicology : an international journal  
630 published for the British Industrial Biological Research Association, 110 (2017) 333-339.



15.403.1\_ELS+HRX+SAX

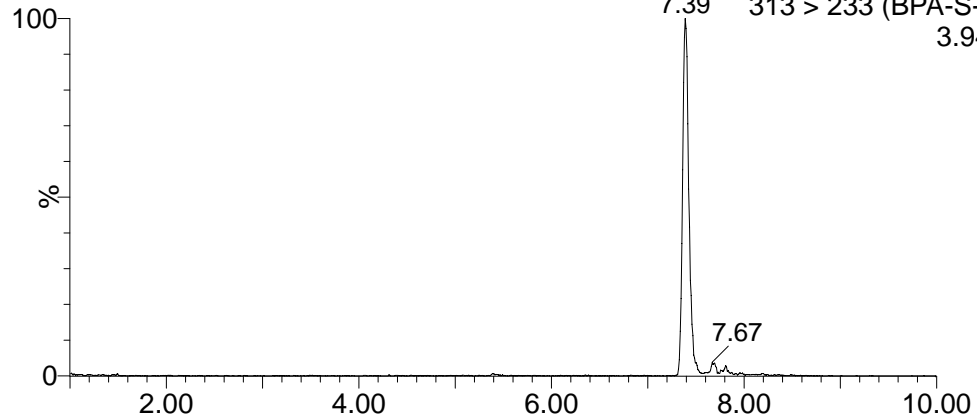
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MRM of 8 Channels ES-  
445 > 307 (BPA-2S)  
4.96e5



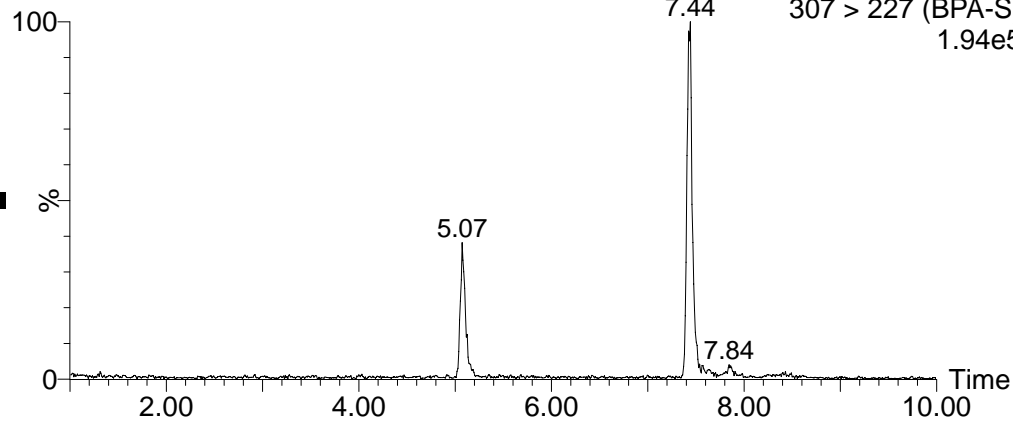
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MRM of 8 Channels ES-  
313 > 233 (BPA-S-d6)  
3.94e5



20161212010

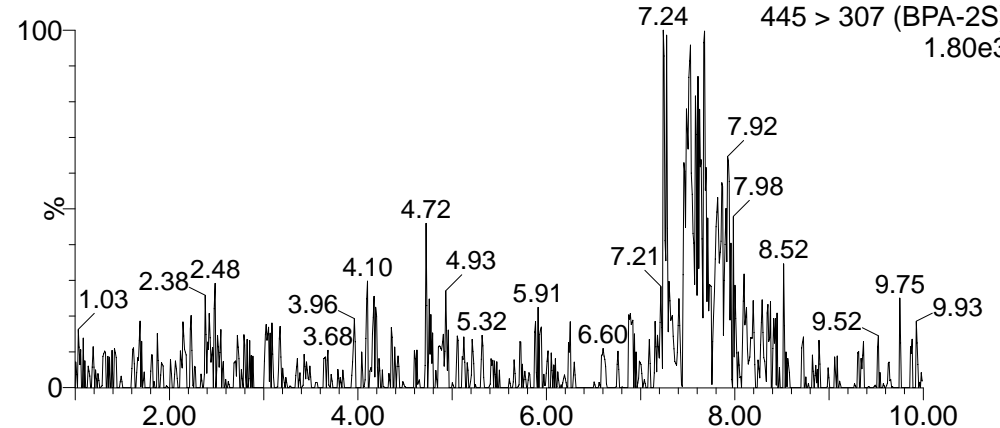
MRM of 8 Channels ES-  
307 > 227 (BPA-S)  
1.94e5



15.403.1\_ELS+HRX+SAX

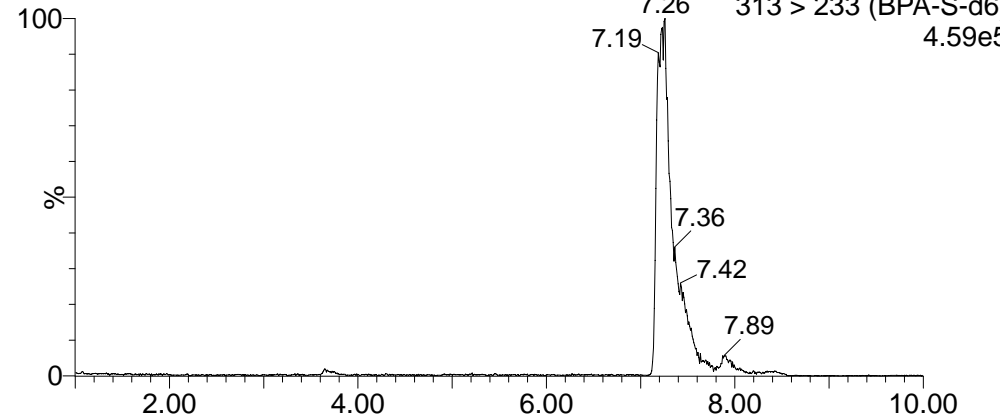
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MRM of 8 Channels ES-  
445 > 307 (BPA-2S)  
1.80e3



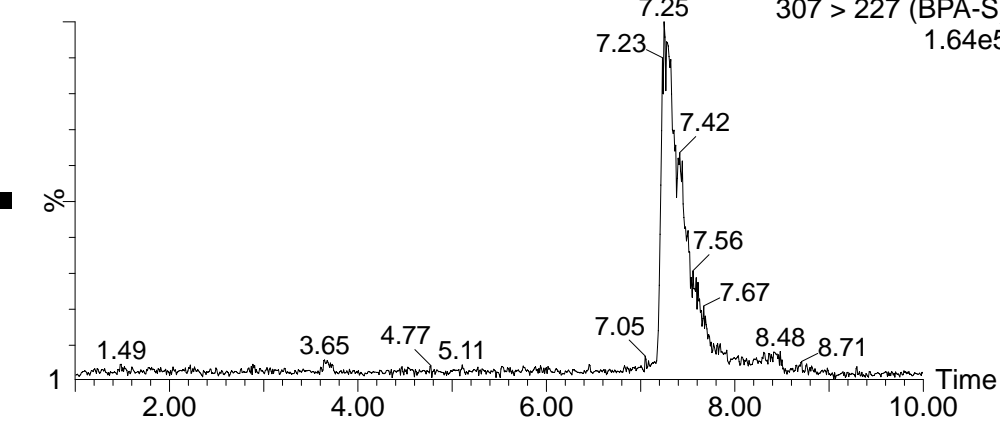
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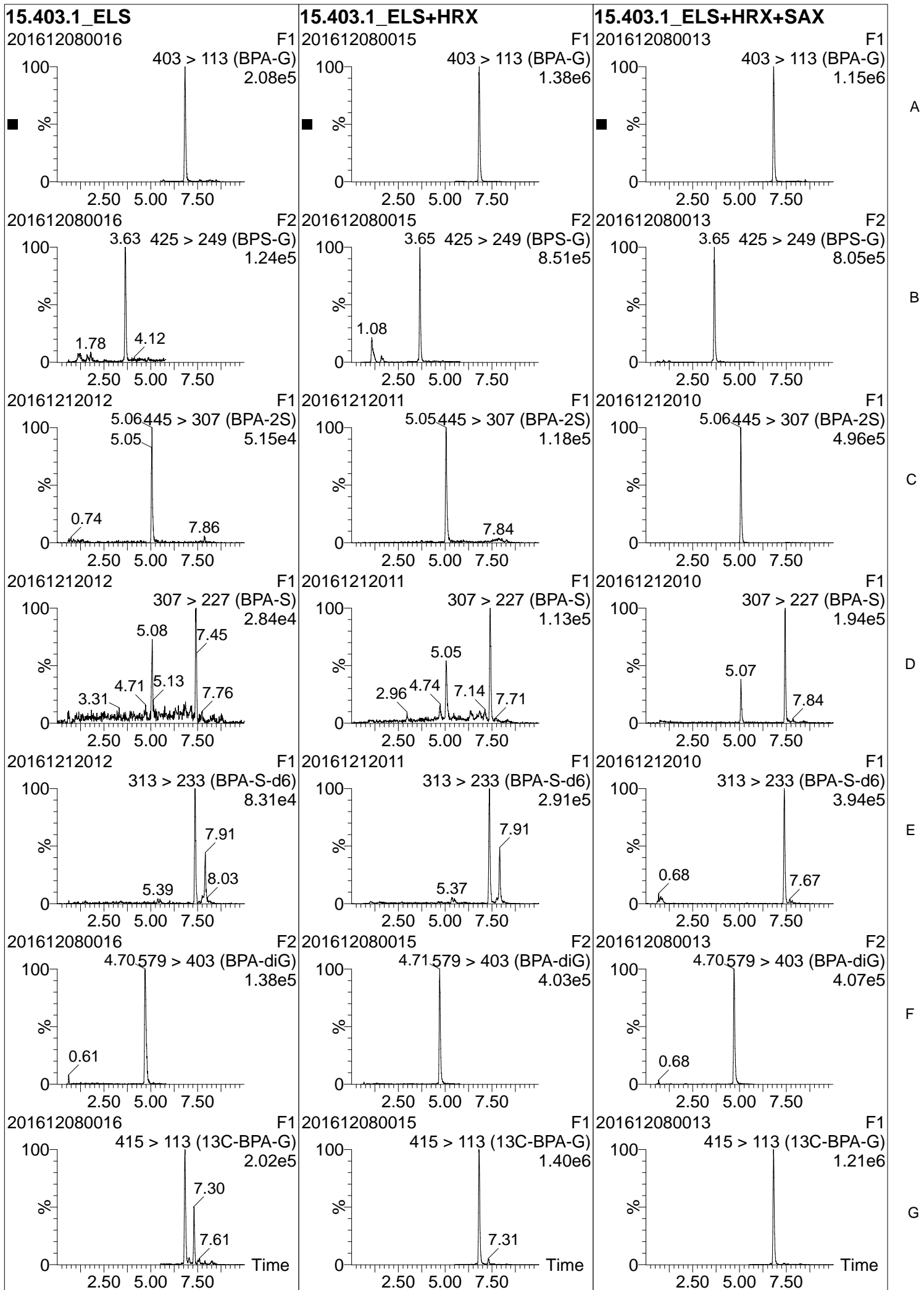
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4.59e5

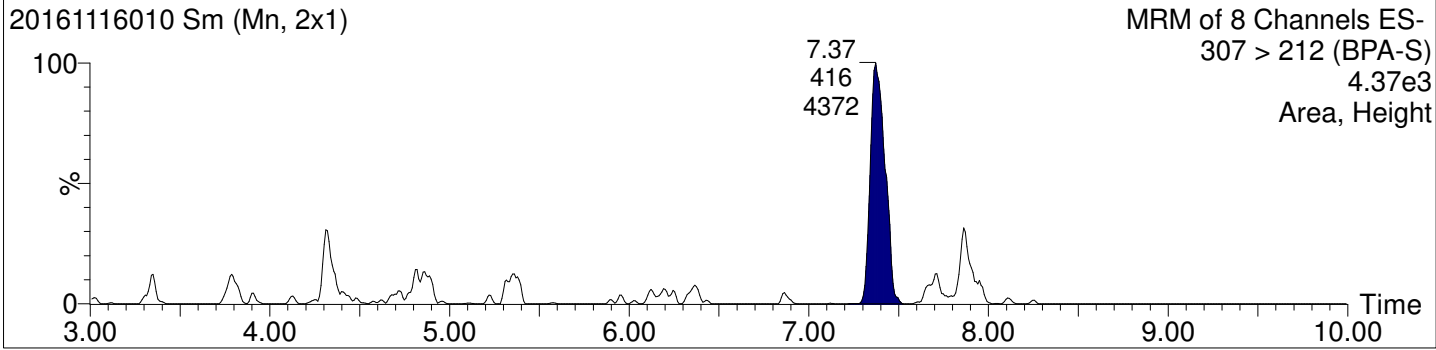
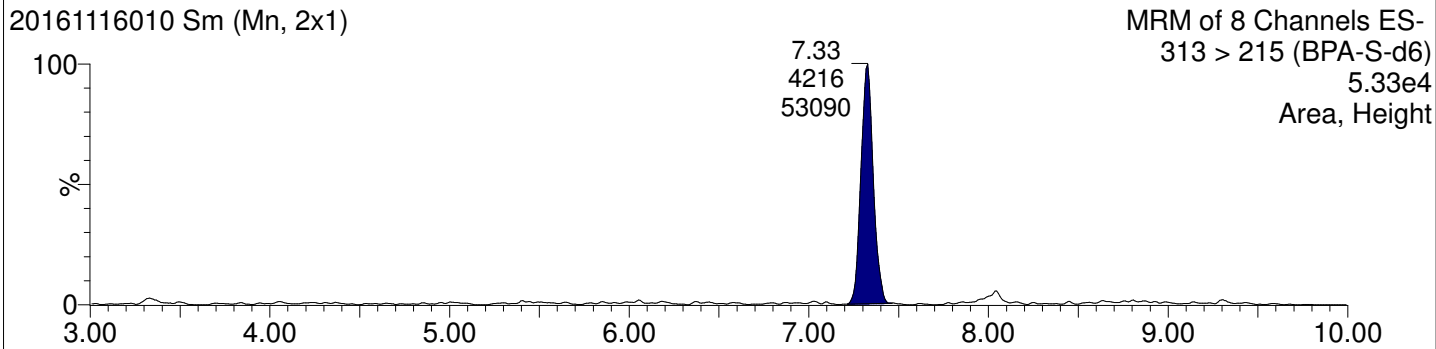
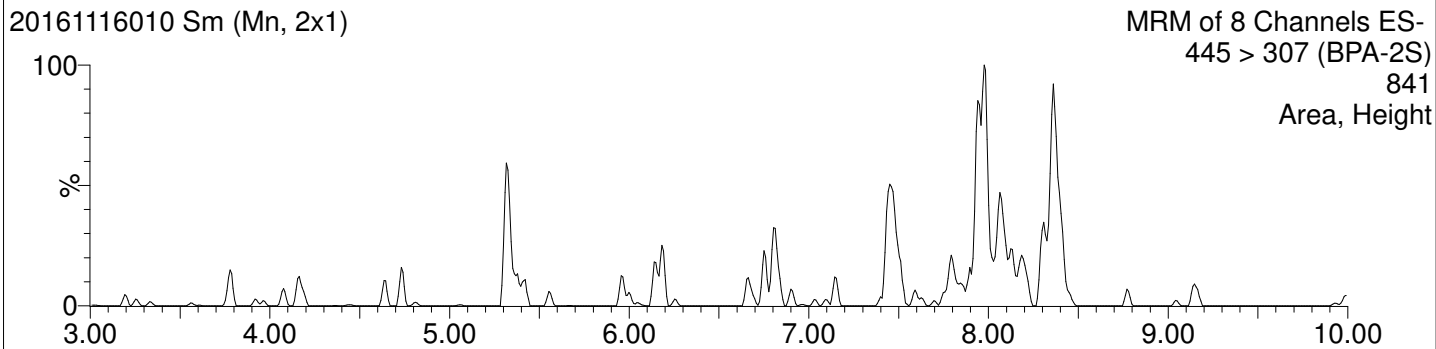
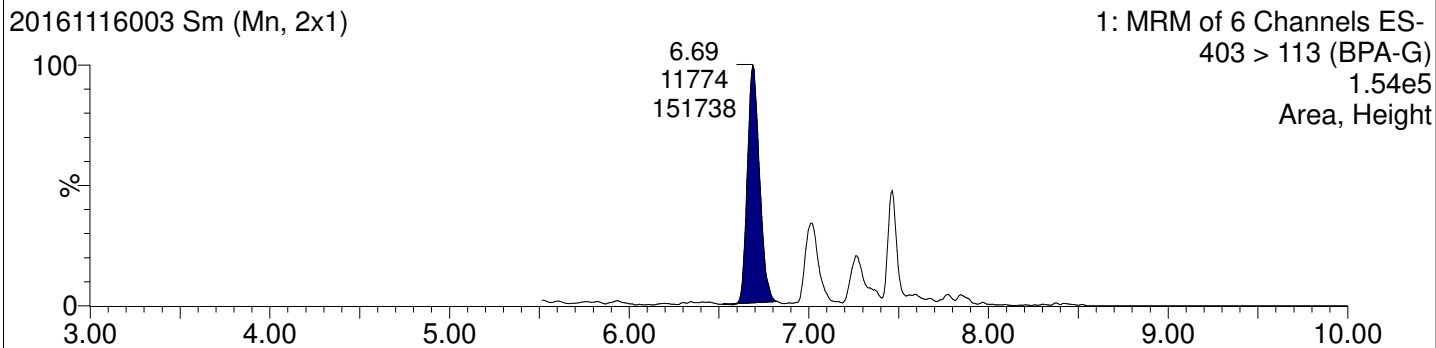
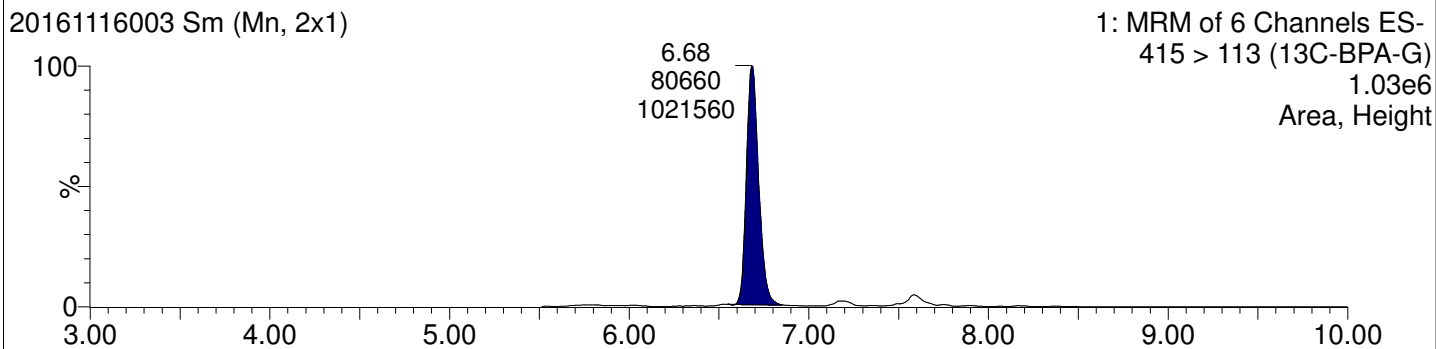
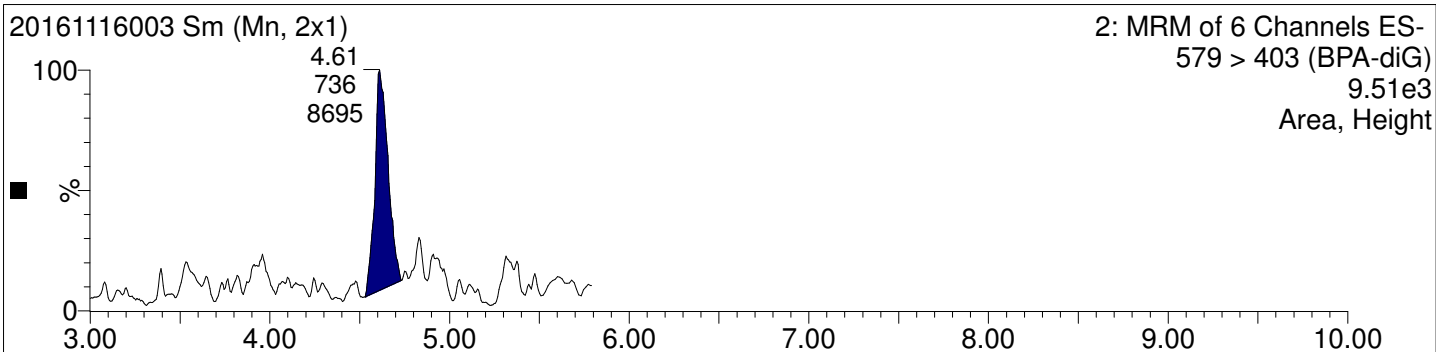


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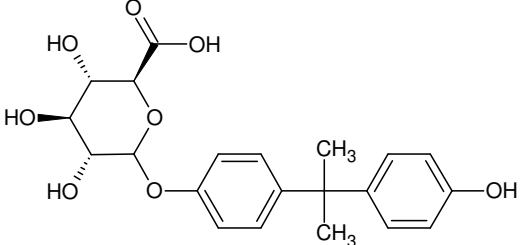
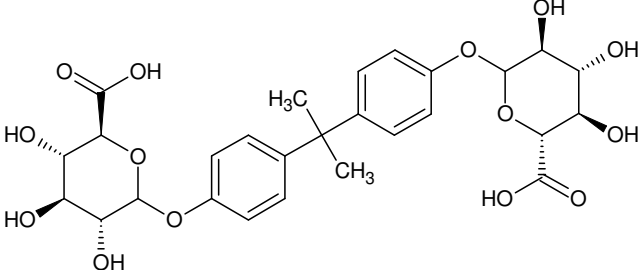
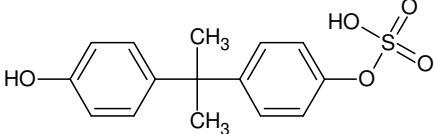
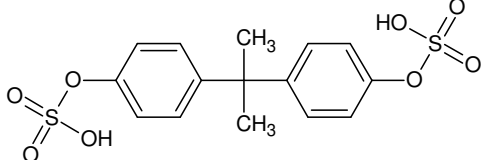
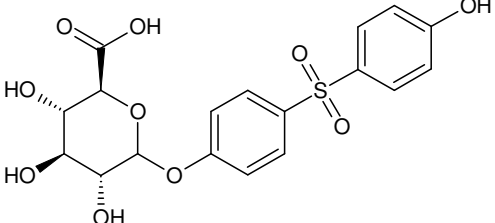
MRM of 8 Channels ES-  
307 > 227 (BPA-S)  
1.64e5









Molecule	Structure	Molecular formula	Molecular Weight (amu)
Bisphenol A $\beta$ -D-Glucuronide		$C_{21}H_{24}O_8$	404.41
Bisphenol A bis- $\beta$ -D-Glucuronide		$C_{27}H_{32}O_{14}$	580.53
Bisphenol A monosulfate		$C_{15}H_{16}O_5S$	308.35
Bisphenol A disulfate		$C_{15}H_{16}O_8S_2$	388.41
Bisphenol S $\beta$ -D-Glucuronide		$C_{18}H_{18}O_{10}S$	426.39

<b>Molecules</b>	<b>abbreviation</b>	<b>SRM transition s (m/z)</b>	<b>Cone voltage (V)</b>	<b>Collision energy (eV)</b>	<b>Retention time (min)</b>	<b>Fraction and time window (TW)</b>
BPA monoglucuronide	BPA-1G	403>227	52	20	6.77	G- TW2
		403>175	52	15		
		403>113	52	15		
		403>85	52	18		
<sup>13</sup> C-BPA monoglucuronide	<sup>13</sup> C-BPA-1G	415>175	52	20	6.77	G- TW2
		415>113	52	15		
BPA diglucuronide	BPA-2G	579>403	40	20	4.68	G- TW1
		579>175	40	20		
		579>113	40	20		
BPS monoglucuronide	BPS-1G	425>249	40	25	3.61	G- TW1
		425>175	40	15		
		425>113	40	15		
BPA monosulfate	BPA-1S	307>227	40	25	7.36	S- TW1
		307>212	40	30		
		307>133	40	40		
BPA monosulfate-d <sub>6</sub>	BPA-d <sub>6</sub> -1S	313>233	40	25	7.32	S- TW1
		313>138	40	40		
		313>215	40	30		
BPA disulfate	BPA-2S	445>307	40	20	5.00	S- TW1
		387>307	40	20		
		307>227	40	25		

	<b>Limits of detection - LOD (<math>\mu\text{g kg}^{-1}</math>)</b>				
	<b>BPA-1G</b>	<b>BPA-1S</b>	<b>BPS-1G</b>	<b>BPA-2G</b>	<b>BPA-2S</b>
<b>Liver</b>	0.04	0.02	0.10	0.02	0.10
<b>Muscle</b>	0.02	0.09	0.04	0.12	0.50
<b>Fish</b>	0.10	0.10	0.18	0.05	0.25