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1 **Development and validation of an LC–MS/MS method for the**
2 **simultaneous determination of Bisphenol A and its**
3 **chlorinated derivatives in adipose tissue**

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29

30

31

32 **Abstract**

33 Bisphenol A (BPA) and its chlorinated derivatives (Clx-BPA) are environmental pollutants
34 exhibiting endocrine-disrupting (ED) properties suspected to be involved in the pathogenesis
35 of hormone-dependent cancers, such as breast and prostate cancers. Due to their lipophilic
36 properties, they may accumulate in adipose tissue which could therefore be a suitable matrix
37 to assess long-term exposure to these compounds and relationships with the tumorigenesis of
38 these cancers.

39 An LC-MS/MS assay for the determination of BPA and Clx-BPA in adipose tissue samples was
40 developed and fully validated according to current bioanalytical validation guidelines. Ionization
41 was achieved using an electrospray source operating in the negative mode and quantification
42 of target analytes was obtained in the multiple reaction monitoring mode. Both standard and
43 quality control (QC) samples were prepared in blank adipose tissue samples. Linearity was
44 demonstrated over the ranges 0.125 to 8.000 and 0.0125 to 0.8000 ng/mL for BPA and Clx-
45 BPA, respectively. Accuracy and precision were demonstrated over the whole concentration
46 range: intra and inter-day bias values were in the 85 – 114% range and imprecision of the
47 method did not exceed 14%. Lower limits of quantification were validated using QCs at 0.1250
48 and 0.0125 ng/mL for BPA and Clx-BPA, respectively. Internal standard-corrected matrix
49 effects were comparable in breast and prostate adipose tissues, demonstrating that this
50 method could be used to reliably assay BPA and Clx-BPA in both tissues. The method was
51 sensitive enough to determine BPA and Clx-BPA in breast adipose tissue obtained from
52 women undergoing breast surgery, enabling identification of different patterns of exposure to
53 these ED chemicals.

54 The method enables the reliable quantification of BPA and Clx-BPA in adipose tissue and
55 could be used to assess long-term exposure to these compounds and potential associations
56 with hormone-dependent cancers.

57

58 **Keywords:**

59 Adipose tissue; Bisphenol A and its chlorinated derivatives; Endocrine disruptors; Breast ;

60 Prostate; Hormone-dependent cancer.

61

62 1. Introduction

63 Bisphenol A (BPA) is a well-known ubiquitous environmental pollutant exhibiting endocrine-
64 disrupting properties [1]. Chlorinated derivatives of bisphenol A (Clx-BPA) are formed by
65 chlorination of bisphenol A present in water during water-disinfection process. As a result Clx-
66 BPA have essentially been observed in various aqueous media such as tap water [2,3],
67 sewage sludge [4] and wastewater of paper recycling plant [5]. Clx-BPA have also been found
68 in human biological matrices: urine [6–8], placental tissue [9], breast milk [10] and colostrum
69 [11]. Clx-BPA exhibit endocrine-disrupting properties: *in vitro* they show higher (up to 100
70 times) estrogenic activities than BPA [12]. Riu et al. have demonstrated that Clx-BPA are
71 human PPAR γ ligands and that activation of PPAR γ depends on the degree of halogenation
72 [13]. In humans, Clx-BPA exposure has been associated with the occurrence of type 2 diabetes
73 [14] and obesity [15].

74 Due to direct activation of the estrogen receptor, BPA may have an impact on carcinogenesis
75 in hormone-dependent cancers such as breast [16] and prostate cancers [17]. This is also the
76 case for Clx-BPA [18]. The agonist effect of BPA and Clx-BPA on PPAR γ receptors may also
77 have an impact on tumorigenesis in hormone-dependent cancer [19,20] and they may exert
78 indirect tumorigenesis activity through obesity and inflammation [21,22].

79 Assessing human exposure to environmental pollutants is a major component of health risk
80 assessment [23]. For that purpose, several approaches have been proposed and
81 determination of exposure can be either indirect or direct. However, direct measurement of
82 environmental pollutants in human body fluids, commonly urine or blood, is now considered as
83 the method of reference. This is the so-called Human Biomonitoring (HBM) that reflects the
84 total body burden resulting from all routes of exposure [24].

85 As a means of relating BPA and Clx-BPA exposure to cancer pathogenesis, blood or urine
86 concentrations are probably poor biomarkers of exposure due to short biological half-life, at
87 least for BPA [25]. It has been demonstrated that persistent [26] as well as non-persistent

88 pollutants [27,28] may accumulate in adipose tissue due to their lipophilic properties. These
89 results suggest that concentration in adipose tissue may be a suitable biomarker in
90 assessment of long-term exposure [28].

91 Not many methods have been reported for the determination of BPA in adipose tissue. One is
92 based on gas chromatography-mass spectrometry (GC-MS) [29] while others are based on
93 LC-MS/MS [27,28,30]. To our knowledge, no LC-MS/MS method has been reported for the
94 simultaneous determination of BPA and Clx-BPA. Fernandez et al. have briefly reported on a
95 GC-MS method [31].

96 Therefore the objectives of this study were to develop and validate an LC-MS/MS assay for
97 simultaneous determination of BPA and Clx-BPA in adipose tissue and to apply the validated
98 method to a set of real samples in view of demonstrating its suitability and relevance.

99

100 **2. Materials and methods**

101 2.1. Chemicals and reagents

102 Bisphenol A (BPA) and its deuterated analogue (BPA-d₁₆) were obtained from Sigma-Aldrich®
103 (St Louis, USA). Chlorinated derivatives of BPA (Clx-BPA) and the deuterated analogue of
104 dichlorobisphenol A (2,2'-DCBPA-d₁₂) were purchased from @rtMolecule® (Poitiers, France).
105 Acetonitrile, methanol and water were acquired from Carlo Erba® (Val de Reuil, France). They
106 were of Ultra-High Performance Liquid Chromatography (UHPLC) grade and we demonstrated
107 that they were free of BPA and Clx-BPA. Materials in glass or polypropylene were used to
108 avoid any BPA contamination. MIX I (MgSO₄, NaCl, Na₂H citrate - 1.5 H₂O, Na₃ Citrate - 2
109 H₂O) and MIX VI (MgSO₄, Diamino PSA (Primary Secondary Amine), C₁₈ ec (Octadecyl
110 modified silica phase, endcapped)) were purchased from Machery-Nagel® (Hoërdt, France).

111 2.2. Preparation of stock solutions, working solutions and spiking solutions

112 Analyte stock solutions were prepared in methanol at 10 µg/mL for BPA and 1 µg/mL for Clx-
113 BPA. Deuterated internal standard (IS) stock solutions were at 10 µg/mL for BPA-d₁₆ and 1
114 µg/mL for 2,2'-DCBPA-d₁₂. Stock solutions were stored at -20°C before use. Each day of
115 analysis, two working solutions were freshly prepared in methanol: one with analytes at 80
116 ng/mL and 8 ng/mL for BPA and Clx-BPA, respectively, and one at 40 ng/mL and 4 ng/mL for
117 BPA-d₁₆ and 2,2'-DCBPA-d₁₂, respectively. Working solutions were diluted with methanol to
118 obtain spiking solutions ranging from 1.25 ng/mL to 80.00 ng/mL and from 0.125 to 8.000
119 ng/mL ng/mL for BPA and Clx-BPA, respectively. These solutions were used to prepare
120 calibration standards in adipose tissue.

121 2.3. Sample collection

122 Samples of adipose tissue were obtained from patients during breast or prostate surgery at
123 the university hospital of Poitiers, France. Patients who had given their informed consent after
124 having been fully informed could be enrolled in this study. Samples were collected in glass
125 tubes to avoid contamination with BPA. All samples were stored at -20 °C before use.

126 2.4. Sample extraction

127 Each sample of adipose tissue (100 mg) was homogenized in a clean glass tube with 500 μ L
128 of water using an Ultra-Turrax® (IKA® T18 Basic), and spiked with 50 μ L of IS working
129 standard solution. Calibrant samples were prepared in adipose tissue by spiking tissue
130 homogenate with 50 μ L of spiking solutions to obtain final concentrations ranging from 8.000
131 ng/mL to 0.125ng/mL and 0.8000 to 0.0125 ng/mL, for BPA and Clx-BPA, respectively. For
132 blank and unknown samples, spiking solution was replaced by 50 μ L of pure methanol. For
133 double-blank samples, spiking and IS solutions were replaced by 100 μ L of pure methanol.
134 Then 500 μ L of acetonitrile were added to homogenates. Samples were vortex-mixed for one
135 minute and 100 mg of MIX I were added. pH was adjusted to 5.5 with NaOH 1M, vortex-mixed
136 again for one minute and centrifuged at 2330 \times g for five minutes. The upper organic layer was
137 transferred into a clean glass tube, 100 mg of MIX VI were added, vortexed again for 30
138 seconds and centrifuged at 2330 \times g for five minutes. The upper organic layer was transferred
139 into a clean glass-tube and evaporated to dryness at 37°C under a gentle nitrogen stream. Dry
140 residues were dissolved in 200 μ L of a water/methanol (70/30, v/v) solution, vortex-mixed and
141 transferred into glass vials for injection. Finally, 20 μ L of extract were injected into the LC–
142 MS/MS system.

143 2.5. Liquid chromatography

144 A UHPLC Shimadzu® Nexera X2 (Kyoto, Japan) pump was equipped with a Waters® Acquity
145 CSH C18 (3.5 μ m particle size, 2.1 \times 100 mm, Milford, USA) column heated at 40°C.
146 Chromatographic separation was achieved using a binary mobile phase (water (A)/methanol
147 (B)) delivered in the gradient mode at a flow rate of 350 μ L.min⁻¹. Gradient elution started at
148 30% of B (0-0.5 minute); then linearly increased to 90% of B (0.5-7 min); then to 99% of B (7-
149 7.5 min); maintained at 99% of B for 5 minutes (7.5-12.5 min); then decreased to 30% of B

150 (12.5-13 min) and finally maintained at 30% of B for 2.5 minutes (13-15.5 min) for re-
151 equilibration of the column.

152 2.6. Mass spectrometry

153 Analyte quantification was performed with an API6500+ mass spectrometer from Sciex®
154 (Concord, Canada) using an electrospray ionization (ESI) interface, operating in negative
155 ionization mode. Ion source and MS/MS conditions were optimized with infusion of a 1 µg/mL
156 solution of the individual analytes at 10 µL/min. For each compound multiple reaction
157 monitoring mode (MRM) with two specific daughter fragment transitions for one parent
158 precursor ion was used. The first was used for quantification while the second was used for
159 confirmation. Dwell and cycle times were optimized using the Scheduled MRM® algorithm.
160 Data were acquired with Analyst® software and quantification was obtained with MultiQuant®
161 software.

162 2.7. Validation

163 Bioanalytical method validation was performed according to EMA guidelines [32]. Selectivity,
164 calibration curve linearity, accuracy, precision, lower limits of the method and matrix effect
165 were determined.

166 To determine selectivity, for each analyte and deuterated standard we examined
167 chromatograms at their corresponding retention time in blank adipose tissue samples.
168 Acceptable selectivity was defined by the absence of any detectable peak.

169 Calibrant samples were prepared using a pool of adipose tissue obtained from donors.
170 Calibration curves, ranging from 0.125 to 8 (0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0) ng/mL, and
171 from 0.0125 to 0.8 (0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8) ng/mL for BPA and Clx-BPA,
172 respectively, were constructed with 1/x weighting. For each analyte five sets of calibration
173 curves were examined. R squared, slope and y-intercept were determined. Linearity was
174 demonstrated when correlation coefficient was over 0.99.

175 Inter- and intra-day accuracy and precision were determined using QCs prepared with blank
176 breast adipose tissue samples. QC concentrations were 0.125, 1.0, 4.0 ng/mL and 0.0125,
177 0.10, 0.40 ng/mL for BPA and Clx-BPA, respectively. Trueness values within the 85-115%
178 range were accepted for validation of accuracy; a maximal 80-120% range was accepted for
179 low QC. Precision was evaluated and expressed as coefficient of variations (CV%). Values
180 had to be lower or equal than 20% for low QC and 15% for medium and high QCs.

181 In each batch of analysis (for method validation and routine analysis), five blank samples were
182 analyzed. The corresponding mean and standard deviation of the peak area were calculated
183 for each target analyte. The limit of detection (LOD) was set to three times the standard
184 deviation for each target analyte [8].

185 The limit of quantification (LOQ), defined as the lowest level of the calibration standards (0.125
186 ng/mL for BPA and 0.0125 ng/mL for Clx-BPA) was fully validated with low-level QCs prepared
187 with breast adipose tissue spiked at this concentration [32].

188 Matrix effect (ME) and recovery (RE) were also determined over the whole concentration range
189 according to the method described by Matuszewski et al. [33]. Briefly, the signals from the
190 analysis of three sets of samples were compared: (A) extracted QCs prepared in breast
191 adipose tissue, (B) blank breast adipose tissue samples spiked at the same concentrations
192 after extraction and (C) corresponding neat standards prepared in mobile phase, all at three
193 levels of concentration (low, medium and high). IS corrected matrix effect was also calculated
194 [33].

195 A complementary set of experiments was conducted in order to demonstrate that this method
196 could be applied to other types of adipose tissues for example prostate adipose tissue.
197 Calibrant and QC samples were prepared using blank prostate adipose tissue. Matrix effect
198 and recovery were assessed and compared to results obtained with breast adipose tissue.
199 Linearity and accuracy were also assessed in prostate adipose tissue samples.

200

201

202 **3. Results**

203 3.1. LC-MS/MS conditions

204 Optimal conditions for the electrospray ionization source operating in the negative mode were
205 determined as follows: ion spray voltage at -4500V, source temperature at 650°C, curtain gas
206 pressure at 30.0 psi, collision gas set as high, ion source gas 1 pressure at 40.0 psi and ion
207 source gas pressure 2 at 60.0 psi. Specific mass spectrometer parameters (declustering
208 potential, entrance potential, collision energy and collision cell exit potential) for each
209 quantification and ion transition pairs are reported in Table 1.

210 Chromatographic conditions allowed peak separation for each target analyte (Figure 1) and
211 corresponding retention times are reported in Table 1.

212 3.2. Method validation

213 No significant chromatographic peaks were observed at the retention times of Clx-BPA in blank
214 breast adipose tissue samples (Figure 2), thereby demonstrating the specificity of the method.
215 A peak of BPA was present due to baseline contamination of human matrices with this
216 ubiquitous pollutant. Linearity of the method was demonstrated over the whole concentration
217 range with correlation coefficients >0.99 (Table 2). The high value of y-intercept found for
218 bisphenol A was due to basal occurrence of this compound in adipose tissue (Figure 2). The
219 accuracy and precision of the method were demonstrated over the whole range of
220 concentration. Intra and inter-day bias values were in the range 85 – 114% in accordance with
221 bioanalytical validation guidelines. Imprecision of the method (both intra- and inter-day) did not
222 exceed 14%. The limits of quantification, corresponding to the lowest standards of the
223 calibration curves, were validated at 0.1250 and 0.0125 ng/mL for BPA and Clx-BPA,
224 respectively. At the LOQ, good accuracies and precisions were demonstrated (Table 3) and
225 high signal-to-noise peaks were obtained (Figure 2). Matrix effects in breast tissue were low
226 to moderate since ion suppression and enhancement did not exceed 31 and 2%, respectively

227 (Table 4). They were satisfactorily corrected by internal standards (Table 4). Recoveries were
228 moderate but, importantly, were consistent over the whole range of concentration (Table 4).
229 Slightly higher absolute matrix effects were measured in prostate adipose tissue but they were
230 also satisfactorily corrected by IS (Table 5). Recoveries of target analytes from prostate
231 adipose tissues were in the same range as those from breast adipose tissues (Table 5).
232 Linearity was also demonstrated using this matrix (Table 6).

233 3.3. Application of the method

234 This method enables the determination of BPA and Clx-BPA in adipose tissue samples from
235 women receiving breast surgery for breast lesions. BPA was systematically quantified in these
236 samples with concentrations ranging from 0.238 to 1.745 ng/mL (1.19 - 8.73 ng/g) (Table 7).
237 Exposure to Clx-BPA was variable among participants: overall detection frequency was 45%.
238 Table 7 shows that some patients are exposed to several of these compounds (patient BE)
239 whereas others are not (patient RS).

240

241 4. Discussion

242 For the first time, an LC-MS/MS method enabling the simultaneous determination of BPA and
243 Clx-BPA in adipose tissue has been developed and validated according to well-established
244 bioanalytical validation guidelines. Methods for assaying Bisphenol A in adipose tissue had
245 previously been developed using LC-MS/MS [27,28,30] or GC-MS [29]. A GC-MS method for
246 analysis of BPA and Clx-BPA initially developed for water and plasma samples has been
247 succinctly described as a means of analysis of adipose tissue [31]

248 Several attempts were made to optimize extraction procedure. We started with a method
249 similar to the one used for the analysis of Clx-BPA in milk samples [10] using either methanol
250 or acetonitrile as a precipitation reagent. This method enabled to obtain high recovery rates of
251 target analytes but also led to high matrix effects with adipose tissue. One key step to reduce
252 matrix effect was removal of lipid content from adipose tissue samples. The method proposed
253 by Cariou et al. [34] was not chosen due to a labor-intensive protocol. We also avoided the
254 use of freezing for lipid removal, with freezing time ranging from 15 min to overnight [28,30],
255 to shorten experimentation time. Our final method involves a very simple sample preparation
256 step with QuEChERS reagents. While QuEChERS-based extraction has frequently been used
257 in food analysis [35,36], to our knowledge this is the first time that it is considered as a means
258 of extracting environmental pollutants from human adipose tissue. Our extraction method
259 involves two simple and rapid steps: the first is a salting-out extraction step with acetonitrile
260 and the second is a clean-up step using dispersive solid-phase extraction with a C18 sorbent
261 to remove lipids. Using our method, recoveries were moderate but did not impact the overall
262 performances. Indeed, aside from its simplicity and low cost, our method proves to be effective.

263 Separation of target analytes was achieved using a 3.5 μm particle size column. Although the
264 HPLC system was suitable for UHPLC, this particle size was chosen for better column life time.

265 Our assay has been fully validated according to well-established bioanalytical validation
266 guidelines: linearity, repeatability and reproducibility have been demonstrated using calibration

267 standards and QCs prepared in adipose tissue, which is recommended by guidelines but not
268 always performed. Special emphasis was put on the study of matrix effect and overall matrix
269 effects were low for all target analytes in both tested matrices. In an ideal situation each target
270 analyte would have its own corresponding stable isotope labelled internal standard (SIL-IS) to
271 correct matrix effect. However, to our knowledge only 2,2'-dichlorobisphenol A is commercially
272 available and other deuterated compounds should be custom synthesized which represents a
273 significant cost. Moreover, our data demonstrates that deuterated DCBPA enables to correct
274 matrix effect for other chlorine derivatives. Furthermore, IS-corrected matrix effects were
275 comparable in breast and prostate adipose tissues, demonstrating that this method could
276 reliably assay BPA and Clx-BPA in both tissues.

277 BPA is ubiquitous in the environment, therefore specific precautions were implemented to
278 avoid contamination with target analytes that may cause misleading results: tissue samples
279 were collected and stored in glass vials, ultrapure solvents were used throughout the study
280 and all reagents and material were tested for the absence of target analytes

281 Calibration standards and QCs were prepared using a pool of blank matrix as recommended
282 by bioanalytical validation guidelines. However, target analytes may be present in blank
283 matrices due to basal contamination of individuals since these compounds may be ubiquitously
284 found in the environment and consequently in an individual's organisms. This was particularly
285 the case for BPA. As a result, the mean signal corresponding to 5 blank samples determined
286 for each series was systematically subtracted from signal of standard and QC samples to
287 improve the accuracy of BPA in the low range.

288 Our LOQ for BPA was validated using QCs and was in the same range as the one obtained
289 by Reeves et al. [30] (0.38 ng/g). No LOQ was defined in the paper by Artacho-Cordon et al.
290 but the lowest QC tested for BPA was 2 ng/g [28]. No LOQ was validated for Clx-BPA in the
291 paper by Fernandez et al. [31] but values defined as LOD were high (0.5 to 3.0 ng/mL) in
292 comparison to our LOQ. The sensitivity of our assay was adequate to detect and quantify BPA
293 and Clx-BPA in real samples. Bisphenol A was systematically quantified with BPA

294 concentration in the 0.238-1.745 ng/mL range (1.19 - 8.73 ng/g). These concentrations were
295 of the same order of magnitude as those measured in adipose tissue originating in various
296 sources [27,29,31] but higher than those found by Reeves et al. in breast adipose tissue [30].
297 Our method enables to identify different patterns of exposure to Clx-BPA, with some patients
298 being exposed whereas others are not. Concentrations of Clx-BPA in our samples were lower
299 than those found by Fernandez et al. [31] who measured median concentrations equal to 3.14,
300 7.77 and 0.74 ng/g for CBPA, DCBPA and TCBPA , respectively.

301 As suggested by others [28], adipose tissue may be a suitable biological matrix for assessment
302 of mid to long-term exposure to environmental pollutants, especially non-persistent
303 compounds presenting short half-lives. Collection of urine is undoubtedly more convenient and
304 non-invasive but it may not reflect exposure over a prolonged period when half-life of target
305 compound is short [37]. This is the case for bisphenol A of which the half-life is about 5 hours
306 whereas half-lives for Clx-BPA remain unknown. BPA and Clx-BPA are lipophilic compounds
307 with logP values ranging from 3.8 to 6.4 for BPA and TCBPA, respectively [2], thereby
308 suggesting that they may accumulate in adipose tissue.

309 This method could also be used to assess the relationships between exposure to BPA and
310 Clx-BPA and local tumorigenesis effects of these endocrine-disrupting compounds. Indeed,
311 adipose tissue is important in tumor microenvironments and is now recognized as a major
312 factor in the development, growth and progression of cancer [38]. Adipose tissue inflammation
313 may be a key process in promotion of cancer [39] and it has been shown in *ex vivo* experiments
314 that low doses of BPA may promote secretion of inflammatory adipokines such as interleukin-
315 6 and tumor necrosis factor α [40]. Obesity is also considered as a risk factor for breast adipose
316 tissue inflammation suggesting an indirect effect of obesogenic pollutants [15,41]. Moreover,
317 it has been shown *in vitro* that BPA and TCBPA presented an affinity to the estrogen receptor
318 and expressed a proliferative potential in the human breast cancer cell line MCF-7 [18]. Finally,
319 as a biomarker of long-term exposure, this method could be applied in epidemiological studies.

320

321 **5. Conclusion**

322 In conclusion, this method enables the reliable determination of BPA and Clx-BPA in adipose
323 tissue and could help to assess long-term exposure to these compounds as well as their
324 possible associations with hormone-dependent cancers. For instance, this method will be
325 applied to a study aiming to compare BPA and Clx-BPA exposure in women undergoing breast
326 surgery for different breast lesion classes (benign, epithelial atypia and confirmed breast
327 cancer).

328

329

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332 **Contributors:**

333 Conception or design of the work: NV, SR, CN, SR, VM, AD

334 Data collection: NV, GC, JR, CN, TC

335 Data analysis and interpretation: NV, GC, JR, AD

336 Drafting the article: NV, GC

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1 Figure captions:

2 Figure 1: Chromatogram of a blank adipose tissue sample. BPA: bisphenol A, MCBPA: monochlorobisphenol A; DCBPA: dichlorobisphenol A;
3 TCBPA: trichlorobisphenol A; TTCBPA: tetrachlorobisphenol A.

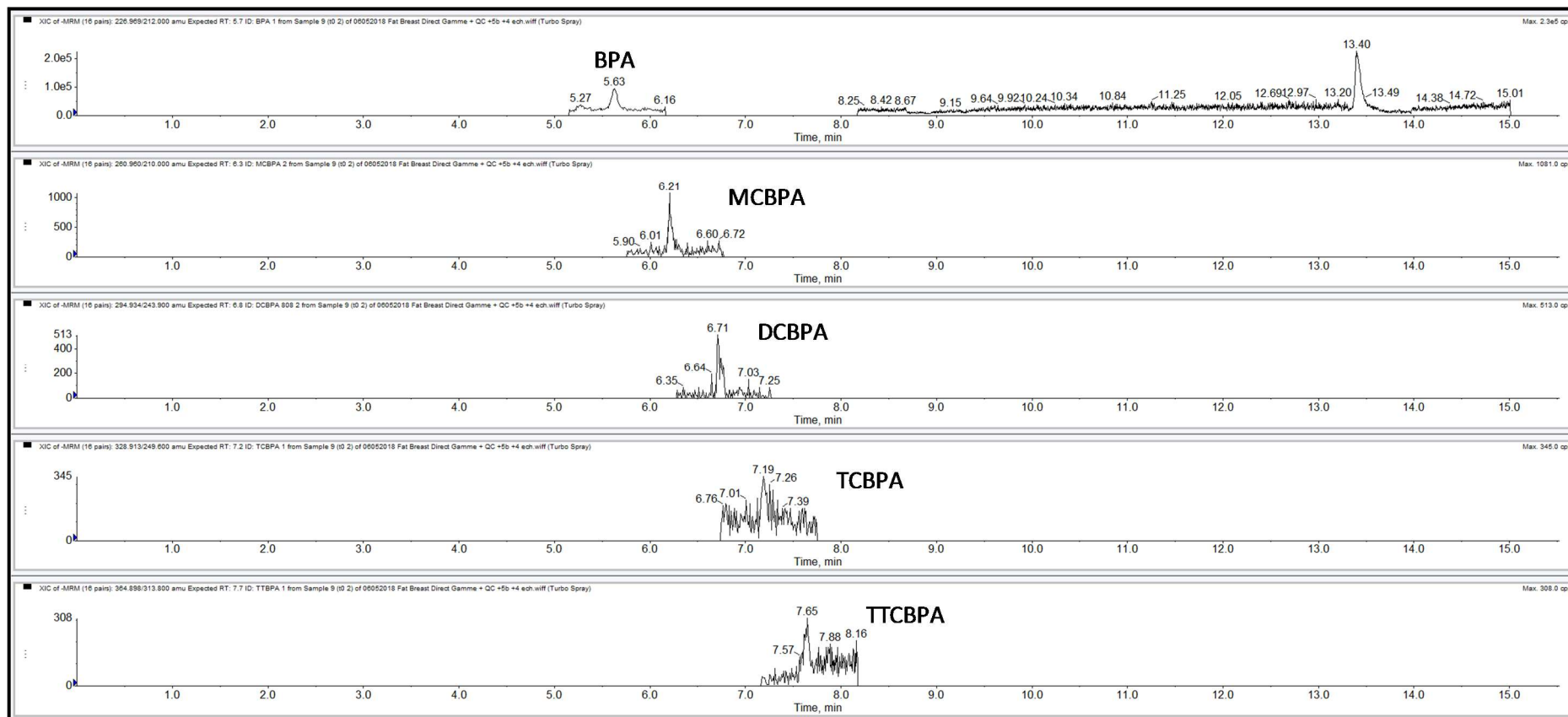
4 Figure 2: Chromatogram corresponding to the lower limit of quantification (LOQ): lowest standard sample prepared in adipose tissue sample
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7 Figure 3: Chromatogram of the adipose tissue sample from individual PN. BPA and TTCBPA were quantified while MCBPA, DCBPA and
8 TCBPA were not detected (<LOD) (also see Table 5). BPA: bisphenol A, MCBPA: monochlorobisphenol A; DCBPA: dichlorobisphenol A;
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10 Figure 4: Chromatogram of the adipose tissue sample from individual LC. BPA and TTCBPA were quantified while MCBPA, DCBPA and
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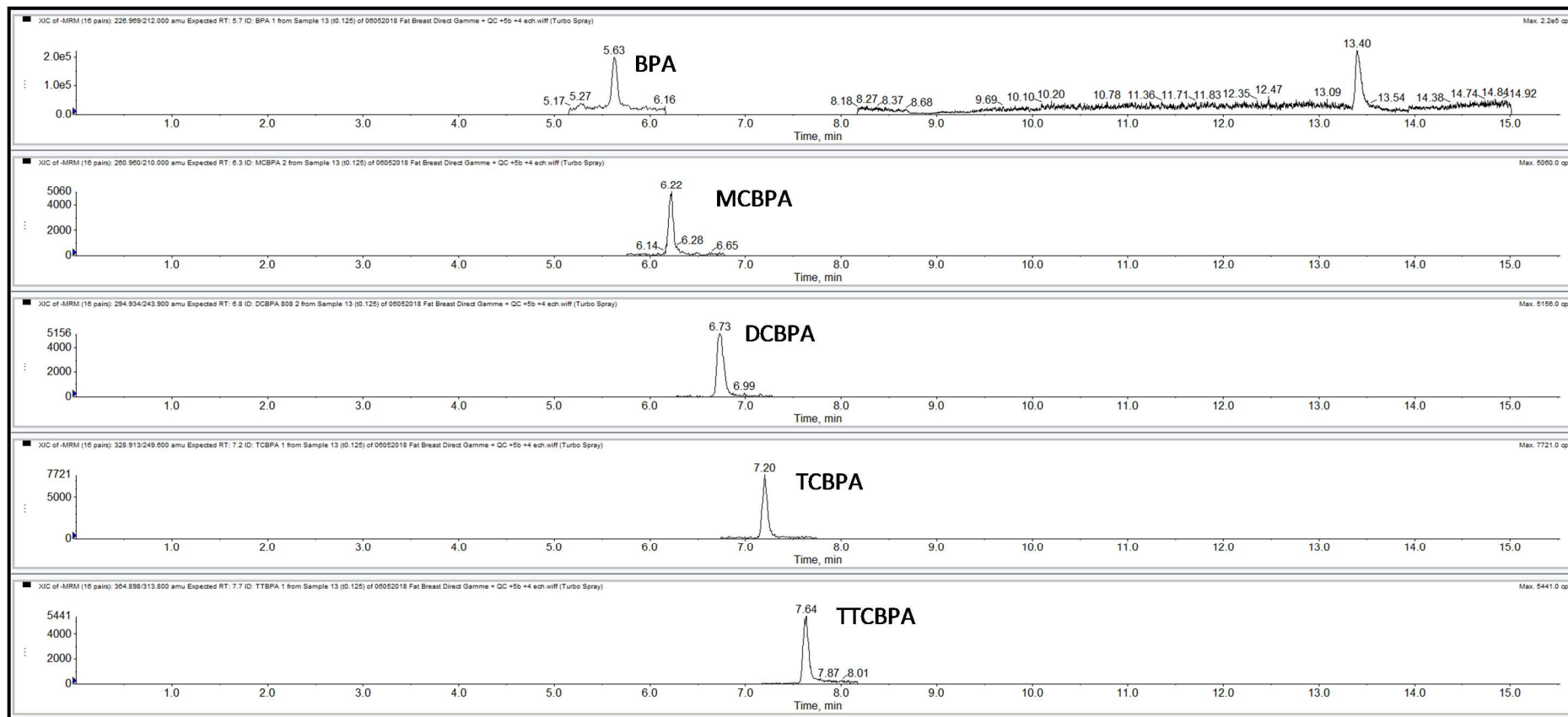
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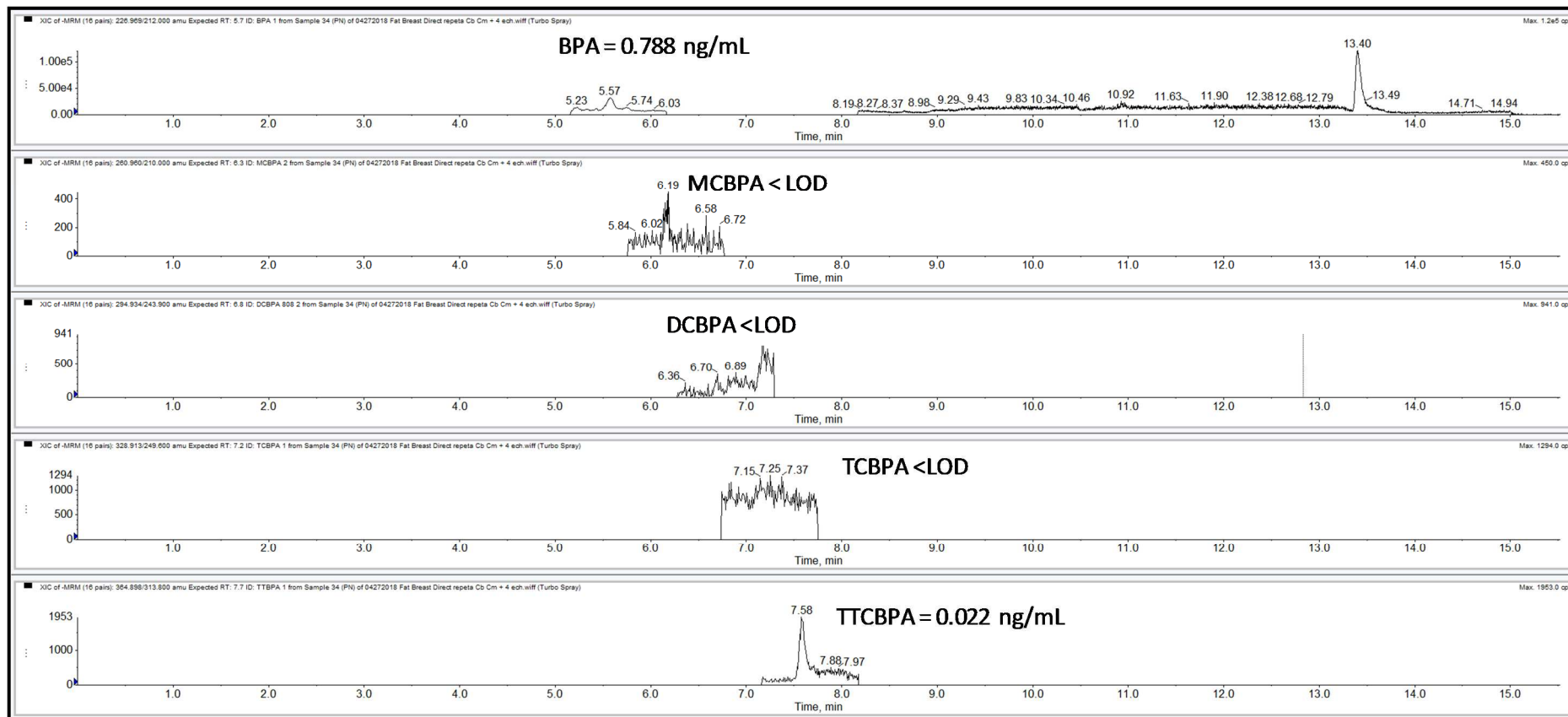
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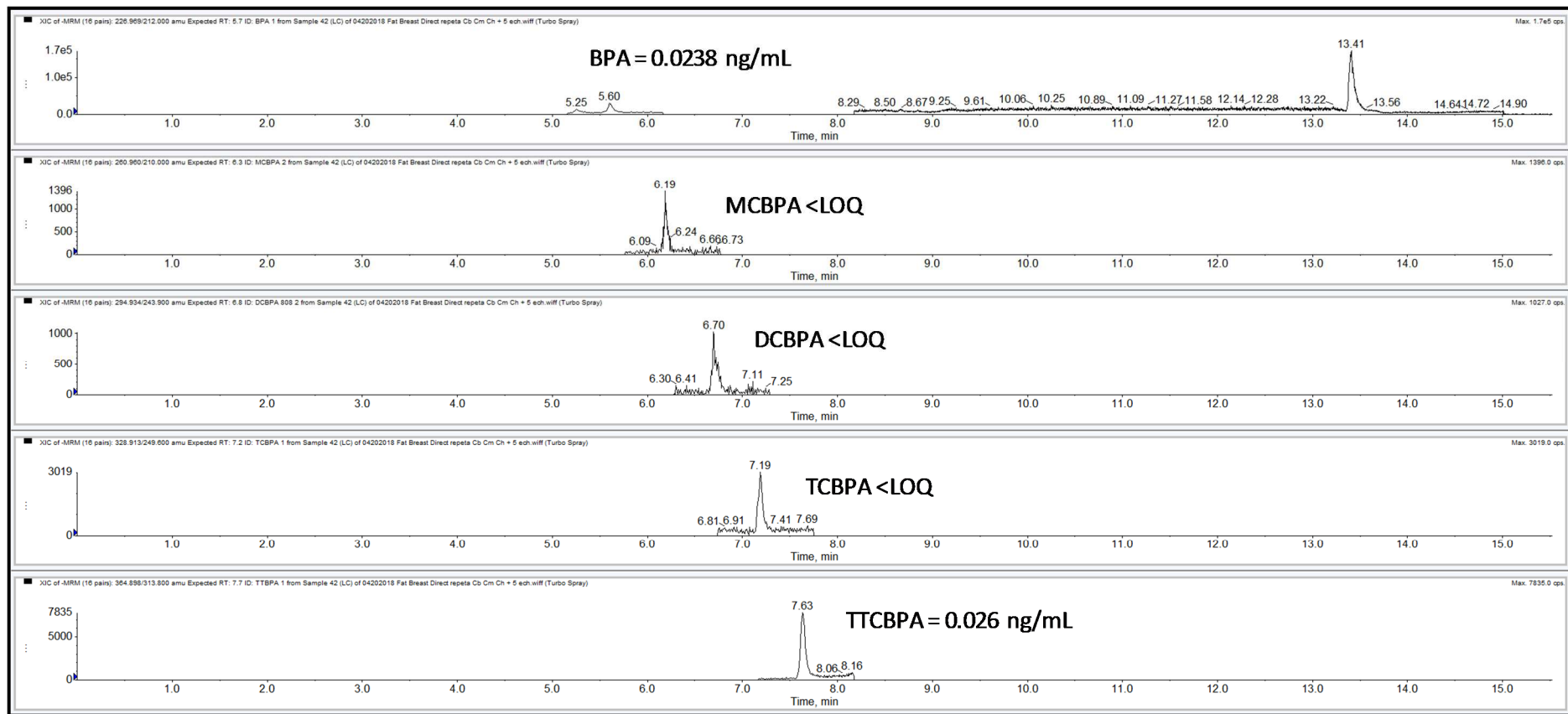
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38 dichlorobisphenol A; TCBPA: trichlorobisphenol A; TTCBPA: tetrachlorobisphenol A.



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1 Tables

2 Table 1: Descriptive table of retention times, precursor ions, fragment ions and MS/MS
 3 optimization conditions (declustering potential, DP; entrance potential, EP; collision energy,
 4 CE; collision cell exit potential, CXP) for all analytes. For CE and CXP, first and second
 5 values are for quantification and confirmation ions, respectively

Analyte	Retention time (min)	Precursor ion (m/z)	Quantification ion (m/z)	Confirmation ion (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
BPA	5.62	227.0	211.8	132.8	-65	-10	-24 /-34	-19 /-11
BPA-d16	5.55	241.1	222.9	141.9	-90	-10	-28 /-34	-25 /-15
MCBPA	6.20	260.9	181.7	209.7	-120	-10	-38 /-30	-19 /-21
DCBPA	6.72	294.9	215.8	279.8	-75	-10	-40 /-30	-24 /-13
TCBPA	7.20	329.0	249.7	277.7	-85	-10	-42 /-34	-27 /-31
TTCBPA	7.64	364.8	313.7	285.6	-110	-10	-36 /-44	-33 /-29
DCBPA-d12	6.65	307.0	224.9	252.8	-120	-10	-42 /-34	-23 /-25

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8 Table 2: Mean (+/- SD) slope, correlation coefficient (r^2) and y-intercept of calibration curves
 9 prepared with breast adipose tissue (n=5).

Compound	BPA	MCBPA	DCBPA	TCBPA	TTCBPA
Slope (mean \pm SD)	1.082 \pm 0.148	1.514 \pm 0.496	3.574 \pm 1.148	3.321 \pm 0.900	3.011 \pm 1.203
Correlation coefficient r^2 (mean \pm SD)	0.999 \pm 0.000	0.997 \pm 0.003	0.997 \pm 0.003	0.997 \pm 0.003	0.997 \pm 0.002
Y-intercept (mean \pm SD)	0.487 \pm 0.058	0.007 \pm 0.004	0.003 \pm 0.005	0.009 \pm 0.011	0.023 \pm 0.025

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11

12 Table 3: Intra and inter-day accuracy and precision for each analyte in breast adipose tissue
 13 at each level of QC (n=5)

Compound	Concentration of QC (ng/mL)	Accuracy (%)		Precision (%)	
		Intra-day	Inter-day	Intra-day	Inter-day
BPA	0.125 (LOQ)	110	95	8	3
	1.0	106	101	6	4
	4.0	109	104	4	6
MCBPA	0.0125 (LOQ)	114	112	3	14
	0.10	97	102	10	8
	0.40	96	104	2	5
DCBPA	0.0125 (LOQ)	99	104	4	13
	0.10	96	102	5	6
	0.40	102	104	3	5
TCBPA	0.0125 (LOQ)	99	101	4	7
	0.10	100	102	8	4
	0.40	101	102	3	5
TTCBPA	0.0125 (LOQ)	85	109	8	7
	0.10	101	103	8	7
	0.40	106	108	6	13

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16 Table 4: Determination of matrix effect, IS-corrected matrix effect and recovery in breast
 17 adipose tissue at each level of QC (n = 3)

Compound	Concentration of QC (ng/mL)	Matrix effect (%)	IS-corrected matrix effect (%)	Recovery (%)
BPA	0.125	98	111	50
	1.0	77	83	22
	4.0	81	98	20
MCBPA	0.0125	80	100	19
	0.10	77	85	14
	0.40	69	83	15
DCBPA	0.0125	80	99	18
	0.10	94	98	15
	0.40	81	98	21
TCBPA	0.0125	92	114	17
	0.10	100	104	16
	0.40	97	116	20
TTCBPA	0.0125	100	126	17
	0.10	102	106	15
	0.40	94	112	17

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20 Table 5: Determination of matrix effect, IS-corrected matrix effect and recovery in prostate
 21 adipose tissue at each level of QC (n = 3)

Compound	Concentration of QC (ng/mL)	Matrix effect (%)	IS-corrected matrix effect (%)	Recovery (%)
BPA	0.125	61	79	40
	1.0	66	82	18
	4.0	72	86	18
MCBPA	0.0125	67	87	15
	0.10	62	85	14
	0.40	67	90	16
DCBPA	0.0125	74	95	14
	0.10	67	92	15
	0.40	72	97	16
TCBPA	0.0125	68	88	19
	0.10	91	123	10
	0.40	66	87	15
TTCBPA	0.0125	94	121	14
	0.10	93	113	9
	0.40	63	87	15

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24 Table 6: Slope, correlation coefficient (r^2) and y-intercept of calibration curves prepared with
25 prostate adipose tissue.

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Compound	BPA	MCBPA	DCBPA	TCBPA	TTCBPA
Slope	1.41	2.22	4.77	6.38	3.15
Correlation coefficient r^2	0.994	0.992	0.996	0.990	0.992
Y-intercept	0.480	0.002	0.004	-0.057	-0.023

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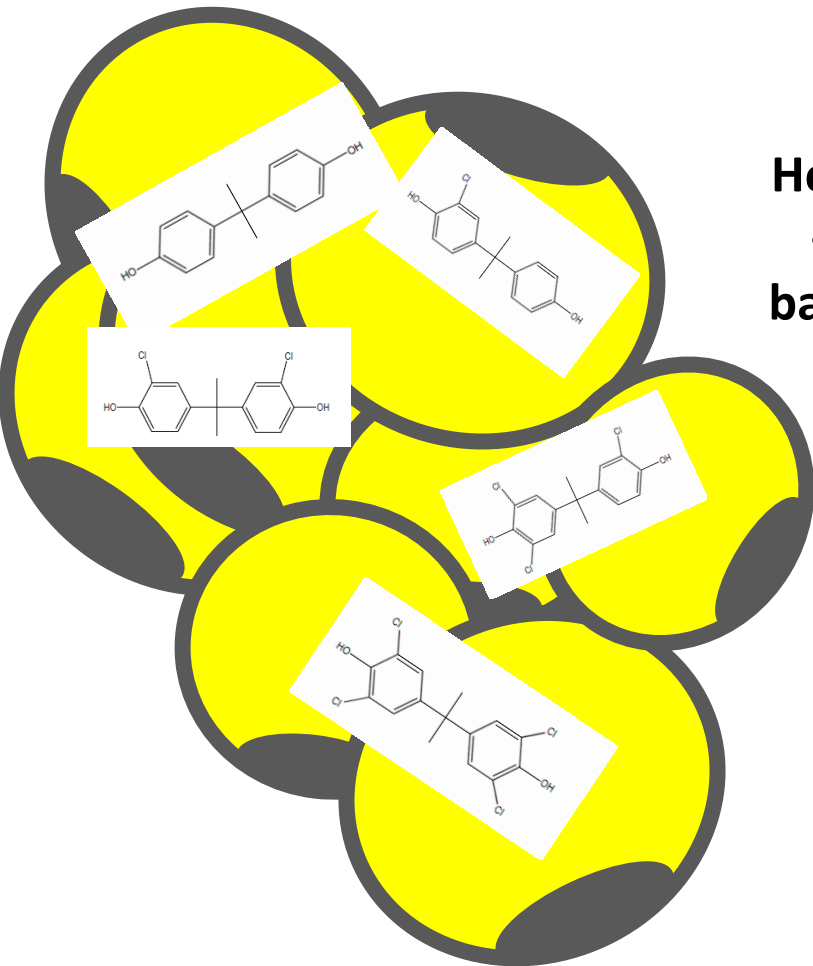
29 Table 7: BPA and Clx-BPA concentrations measured in 5 breast adipose tissue samples
30 obtained from women undergoing breast surgery. <LOQ: compound detected but not
31 quantified; <LOD: compound not detected.

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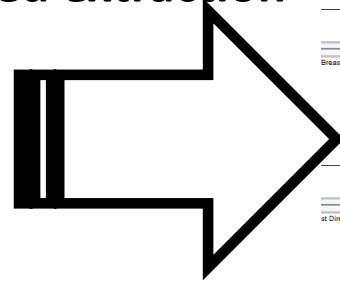
Endocrine disruptor concentrations (ng/mL)					
Patient ID	BPA	MCBPA	DCBPA	TCBPA	TTCBPA
RS	1.433	<LOD	<LOD	<LOD	<LOD
PN	0.788	<LOD	<LOD	<LOD	0,022
PC	1.510	0.046	<LOD	<LOD	<LOD
LC	0.238	<LOQ	<LOQ	<LOQ	0.026
BE	1.745	0.013	<LOD	0.013	0.014

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BPA and Clx-BPA in adipose tissue



Homogenization
+ QuEChERS-
based extraction



+ LC-MS/MS

