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Assessment of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry based methods for investigating 206 dioxin-like micropollutants in animal-derived food matrices

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1 **Highlights**

2

3 • GC×GC-TOF/MS enabled 206 dioxin-related micropollutants in solvent to be
4 separated.

5 • A sample preparation protocol was set up for the analysis of complex food matrices.

6 • A powerful food spiking method was proposed.

7 • The LODs ranged between 50-227 pg/g.

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Abstract

This paper evaluates different multiresidue methods based on comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOF/MS) to analyze dioxin-related micropollutants in complex food matrices. In a first step, the column sets Rtx-PCB/BPX-50 and Rtx-Dioxin2/BPX-50 were compared in terms of peak shape (width and symmetry) and resolution for the separation of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) in solvent. A satisfactory separation of 206 dioxin-related micropollutants including the 17 toxic PCDD/Fs was achieved in 75min with the column set Rtx-dioxin2/BPX-50. In a second time, the GC×GC-TOF/MS method was spread to the analysis of dioxin-related micropollutants in complex food matrices. An extraction procedure including accelerated solvent extraction (ASE), centrifugal evaporation and gel permeation chromatography (GPC) was optimized. Starting with meat as a model matrix, a micropollutant spiking method was then set up by comparing seven methods in terms of recoveries and reproducibility. The method combining immersion of the meat in a large volume of solvent containing micropollutants followed by homogenization by blender induced recoveries in the acceptable range of 70–130% and satisfactory standard deviations ($\leq 10\%$) for most of the compounds studied. Limits of detection of the GC×GC-TOF/MS method ranged between 50 and 100 pg/g of spiked fresh meat for PCBs and between 65 and 227 pg/g for PCDD/Fs. Potential applications of this method are discussed.

Keywords

Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOF/MS); food spiking; multiresidue method; Polychlorinated biphenyls (PCBs); Polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs)

66 1. Introduction

67 The principal source of human exposure to persistent organic pollutants (POPs) is
68 food, and most particularly, the consumption of animal products [1,2]. With the safety of
69 health in mind, it is therefore important to accurately assess the risk the presence of these
70 substances in food has on human health. This risk is currently assessed by determining the
71 micropollutant content in raw food, yet knowing solely the content in the raw material is not
72 enough to assess the amount actually assimilated by consumers. In actual fact, the
73 transformations undergone by the food whether they be technological such as cooking [3,4] or
74 physiological such as digestion [5,6] should also be taken into consideration. To assess the
75 impact of these transformations on the contaminants contained in food, multiresidue methods
76 (MRM) are particularly valuable as they allow for simultaneous monitoring of a large number
77 of molecules in a single analysis. Of the toxic contaminants potentially present in food and
78 remaining a current concern [7], polychlorinated biphenyls (PCBs), polychlorinated dibenzo-
79 *p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are interesting candidates to
80 monitor as these families of molecules have a wide variety of congeners whose structure is
81 not affected by the different stages of the analytical protocol due to their thermoresistance.
82 PCBs, used on a massive scale as insulating materials in industry up until the 1980's and
83 PCDD/Fs, whose release is related to human and industrial activities and particularly
84 combustion phenomena, are also persistent in the environment [8,9]. In addition, these
85 molecules are carcinogenic, mutagenic and reprotoxic, mainly *via* their action on the aryl
86 hydrocarbon receptor (AhR) [10,11]. Thus the World Health Organization has defined toxic
87 equivalency factors (WHO-TEF) to assess the toxicity of dioxin-like PCBs and PCDD/Fs [9].
88 The European Regulations laying down methods of sampling and analysis for the EU official
89 control of levels of PCDD/Fs and PCBs in food and feed have been recently amended by EU
90 Regulations No 589/2014 and 709/2014.

91 To analyze PCBs and PCDD/Fs, several studies have shown the relevance of using
92 two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass
93 spectrometry (TOF-MS) [12,13]. This method is particularly useful on complex matrices
94 because the high resolution provided by GC×GC enables to reduce any overlap between
95 target analytes and matrix interferences, and to get lower LODs than with GC-TOF/MS
96 [14,15]. Additionally, even if sensitivity and linearity range of GC×GC-TOF/MS are lower
97 than that of GC-HRMS [13] which is used by reference laboratories, it could be a relevant
98 alternative to study the impact of technological or physiological transformation on the level of

99 contaminants in food. Although, to our knowledge, no study to date has explored the
100 simultaneous analysis of all the congener profiles of PCBs and PCDD/Fs, several authors
101 have worked on optimising the separation of 209 PCB congeners or 17 toxic dioxin congeners
102 in solvent. For the PCBs, 188, 192 and 196 of the 209 congeners were respectively able to be
103 separated with the Rtx-PCB/Rxi-17, HT8/BPX-50, and SPB-Octyl/SLB-IL59 columns sets
104 [16-18]. For the dioxins, the 17 toxic congeners were able to be separated by GC×GC-
105 TOF/MS coupled with an Rtx-Dioxin2/Rtx-PCB column set [19]. The Rtx-dioxin2 column
106 was also used coupled to a Rtx-500 column to analyze 17 toxic PCDD/F congeners and 4
107 dioxin-like PCB congeners [12]. In view of this research, the Rtx-PCB, HT8, SPB-Octyl and
108 Rtx-dioxin2 columns in first dimension seem to be the most promising to simultaneously
109 analyze the existing 209 PCB congeners and the 17 toxic dioxin congeners by GC×GC-
110 TOF/MS. For coupling with these different columns, the use of a BPX-50 type column in
111 second dimension appears to be the preferred and classic choice [17,20].

112 To be able to study the health risk associated to the presence of PCBs and PCDD/Fs in
113 food, the analytical methods assessed in pure solvent should then be validated on real and
114 complex matrices the contaminants of which should be extracted with good recovery rates
115 [21]. To do this, homogeneous and reproducible multicontaminated products are required
116 [22]. Several studies have explored spiking with contaminants in environmental matrices such
117 as soils [23], even if the spiking methods used were highly varied and the descriptions often
118 incomplete. The literature relating to spiking food matrices with contaminants is virtually nil.
119 Efficient methods for food spiking must therefore be found.

120 In order to ultimately improve the assessment of the risk related to chemical
121 contaminants of food, this study is aimed at proposing an analysis method to monitor toxic
122 dioxin-like PCB and PCDD/F congeners in complex food matrices such as meat products with
123 a high water, protein and fat content. A first study will be conducted to assess the relevance of
124 GC×GC-TOF/MS to simultaneously monitor the PCBs and PCDD/Fs. In order to obtain the
125 best possible chromatographic separation of these contaminants, the performance of different
126 column combinations will initially be compared in pure solvent on the most toxic compounds
127 [24] and most frequently found in meat [25] based on peak shape [14] and peak resolution
128 [18]. When the pure solvent analytical method is set up, it will then be proven in complex
129 matrices taking high fat content ground meat as a model. To do this an extraction,
130 concentration and clean-up method will be proposed working on a matrix destructured by
131 freeze-drying. Different spiking scenarios will then be compared to a structured matrix based
132 on the standard deviations and recovery rates obtained on the different contaminants studied.

133 The performance of the analytical method will then be assessed in terms of linearity (R^2) and
134 sensitivity (LOD) on a spiked matrix.

135

136 **2. Material and Methods**

137 **2.1 Chemicals and standards**

138 Hexane, dichloromethane and toluene were organic trace analysis grade solvents (Sigma-
139 Aldrich, Saint-Quentin Fallavier, France). Activated aluminum oxide (acidic, Brockmann I)
140 and diatomaceous earth used for the preparation of ASE cells were obtained from Sigma-
141 Aldrich. Micropollutant reference standards were obtained from AccuStandard Europe
142 (Niederbipp, Switzerland) and LGC Standards (Molsheim, France) for the 209 PCBs and the
143 17 PCDD/Fs, respectively. The 209 PCBs were divided into nine stock solutions concentrated
144 to 10 ng/ μ L. For the PCDD/Fs, the concentration of the stock solution was different according
145 to the congeners: tetra's 2.5 ng/ μ l, penta's, hexa's and hepta's 6.25 ng/ μ l and octa's 12.5
146 ng/ μ l. Internal standards were used for the accurate quantification of target compounds: 3'-F-
147 PCB-28 (Chiron, Trondheim, Norway), 3'-F-PCB-81 (Chiron), 13 C-labelled PCB-111
148 (Wellington laboratories, Guelph, ON, Canada) and 13 C-labelled PCB-194 (Wellington).

149

150 **2.2 Sample spiking**

151 Ground beef samples (15% fat) were purchased from a French supplier. Matrix blanks
152 consisting in spiking meat with pure dichloromethane without micropollutant were made on
153 five different unspiked meat samples to determine the level of PCBs or PCDD/Fs in fresh
154 meat. Seven methods for ground beef spiking were compared with a concentration of 2 ng/g
155 of fresh meat chosen to give concentration in ready-to-run samples within the range of
156 linearity of GC \times GC-TOF/MS for these compounds. These methods combined micropollutant
157 addition to 120g of ground meat and matrix homogenization. Two approaches for
158 micropollutant addition were tested: (i) addition of a micro-volume of dichloromethane (1mL)
159 containing micropollutants to ground beef, (ii) immersion of ground beef in a large volume of
160 dichloromethane (20mL) containing micropollutants followed by evaporation under hood.
161 Dichloromethane (DCM) was used as solvent in the spiking method because of the high
162 solubility of PCBs and PCDD/Fs in DCM and because of the high volatility of this solvent.
163 After micropollutant addition, four homogenization methods were tested: 2 min with a stand

164 mixer, 2 min with a blender, 2 min with an Ultra-turrax and 3 min with a liquid nitrogen
165 grinder.

166

167 **2.3 Extraction, clean-up and concentration**

168 Extraction and clean-up were performed according to Saito *et al.* with slight modifications
169 [26]. Briefly, all samples were extracted by Accelerated Solvent Extraction (ASE) using a
170 Dionex ASE 350 extractor (Sunnyvale, CA, USA). 34 mL stainless-steel extraction cells were
171 filled from bottom to top with 15 g of acidic alumina and 5 g of ground beef dispersed with
172 diatomaceous earth according to Bjorklund *et al.* [27]. Paper filters were placed at the bottom
173 of the cell and between the layers. ASE extraction included heating (5 min), static time (5
174 min) and purging (90 sec) with two extractions per sample. Hexane was used as extraction
175 solvent at a temperature of 100°C and pressure of 1500 psi. After filtration through a 0.2 µm
176 filter (Phenomenex, Torrance, CA), the extract (approximately 40 mL) was evaporated
177 (Rocket, Genevac Ltd.) using toluene as keeper in order to minimize loss of analytes during
178 the evaporation step, then 4 mL of dichloromethane were added. Gel Permeation
179 Chromatography (GPC) (Gilson, Middleton, WI, USA) purification was carried out on a S-X3
180 Bio-Beads column using dichloromethane as eluting solvent at a flow rate of 5 mL/min. The
181 fraction obtained was evaporated to dryness (Rocket, Genevac Ltd.) and redissolved in 100
182 µL of hexane prior to analysis. All samples were spiked with internal standards at 100 ng/mL
183 at different steps (ASE, evaporation and GC×GC analysis).

184 **2.4 GC×GC-TOF/MS analysis**

185 Samples were analysed on a time-of-flight mass spectrometer (Pegasus 4D, Leco, St Joseph,
186 MI) coupled to a two-dimensional gas chromatograph (6890, Agilent Technologies) equipped
187 with a dual stage jet cryogenic modulator (licensed from Zoex). Four 1D columns were tested
188 in the study: SPB-Octyl (60 m x 0.25 mm x 0.25 µm) (Sigma-Aldrich), HT8 (50 m x 0.25 mm
189 x 0.25 µm) (SGE, Austin, TX, USA), Rtx-PCB (60 m x 0.18 mm x 0.18 µm) (Restek,
190 Bellefonte, PA, USA) and Rtx-Dioxin2 (60 m x 0.25 mm x 0.25 µm) (Restek). The 1D
191 column was connected by a deactivated ultimate union (Agilent Technologies, Santa Clara,
192 CA) to a BPX-50 (2 m x 0.1 mm x 0.1 µm) (SGE) 2D column. A splitless injection of 1 µL of
193 sample extract was performed through a CTC CombiPal autosampler (CTC Analytics,
194 Zwingen, Switzerland) with an inlet temperature set at 280°C. A split/splitless inert liner
195 (restek, sky® 4.0 mm ID liner) was used. Ultra-pure grade helium (purity 99.9995%) was

196 used as carrier gas with a constant flow rate of 1.5 mL min⁻¹. Purge time was set to 60s with a
197 flow rate of 50mL min⁻¹. The primary oven temperature was initially set at 90°C for 1min,
198 then increased to 200°C at 20°C min⁻¹, then to 308°C at 2°C min⁻¹ and at 5°C min⁻¹ to 330°C
199 for 10min. The secondary oven temperature was set at 5°C higher than the primary oven
200 temperature. The modulator temperature was set at 15°C higher than the primary oven
201 temperature and the modulation period was 5s with 1.20 and 1.30 s for the hot and cold
202 pulses, respectively. The transfer line temperature was set at 280°C. The mass spectrometer
203 was operated with an ionization energy of 70eV, a detector voltage of 1800V and a data
204 acquisition rate of 100 spectra s⁻¹. The run time for each sample was 75 min. Analytical blank
205 samples of pure solvent were run to check the absence of targeted analytes. GC×GC data were
206 processed using the LECO ChromaTOF software (version 4.50.8.0).

207 **2.5 Peak shape, resolution factor, and limit of detection**

208 The peak shape of targeted compounds was studied through the peak width at half height and
209 the tailing factor. The mass fragments used for identification and quantification of PCBs and
210 PCDD/Fs are given in supporting information (Table SI). Peak width at half height is defined
211 as the width of a peak (in time units) at half peak height. Tailing factor (T) provides a peak
212 symmetry metric defined by the formula $T = w_{0.05}/2 \times f$, where $w_{0.05}$ is the peak width defined
213 at 5% of peak height and f is the distance from the peak maximum to the leading edge of the
214 peak, the distance being measured at a point 5% of the peak height from the baseline [14].
215 Resolution factor (R_s) was calculated according to Zapadlo *et al.* [18]. Briefly, $R_s = \Delta t_R/w_b$
216 where t_R is the retention time and w_b is the mean peak width at the base. Neighboring peaks
217 were considered as resolved for a resolution factor $R_{s,1D} \geq 0.6$ in the 1D or $R_{s,2D} \geq 0.4$ in the
218 2D. Calibration curves were generated for 19 PCBs and 7 PCDD/Fs from seven concentration
219 levels: 10, 25, 50, 100, 250, 500 and 1000 ng mL⁻¹ prepared both in solvent and in matrix.
220 Each concentration level was injected in triplicate. Calibration curves were constructed by
221 plotting the analyte/internal standard peak area ratio against the concentration. The linearity of
222 the calibration curves was assessed for each compound by calculating the coefficients of
223 determination (R^2). The limit of detection (LOD), using the definition $3s/m$ (where s is the
224 standard deviation of the intercept, and m is the slope of the linear calibration curve), was
225 determined from the calibration curves for each individual compound studied [14]. The “lm”
226 (linear model) function of R (release 2.14.0) [28] was used on the calibration curve data for
227 the determination of R^2 as well as s and m requested for LOD calculation.

228

229 3. Results and Discussion

230 3.1 Comparison of column sets in pure solvent

231 Preliminary analyses with the SPB-Octyl and HT8 columns revealed for the first,
232 significant bleeding as well as a maximum operating temperature (280°C) limited for the
233 study of the PCDD/Fs and for the second, a low separation capacity (data not shown). Rtx-
234 PCB and Rtx-Dioxin2 were therefore used as a first dimension with a run time determined to
235 get the best separation of dioxin-like micropollutants without wrap-around.

236

237 3.1.1 Peak shape

238 In order to compare the two column sets selected for this study, Rtx-dioxin2/BPX-50
239 and Rtx-PCB/BPX-50, the width at half height and the tailing factor of the peaks obtained on
240 analysis of the 209 PCBs and 17 toxic PCDD/Fs in pure solution were determined [14].

241 Table 1 shows the widths at half height obtained for the 17 PCDD/Fs and 18 PCBs
242 considered as the most relevant due to the frequency of their presence in meat (12 dioxin-like
243 and 6 PCB congeners which represent up to 50% of the PCBs found in food matrices) [25].
244 Mean width at half height of the peaks obtained on analysis of the 209 PCBs is 0.119 ± 0.021
245 second for the column set Rtx-PCB/BPX-50 and 0.087 ± 0.014 second with the Rtx-
246 dioxin2/BPX-50 set. Width at half height of the peaks obtained for the 17 PCDD/Fs is greater
247 than that obtained for the PCBs as it is 0.126 ± 0.005 second and 0.099 ± 0.008 second
248 respectively for the column sets Rtx-PCB/BPX-50 and Rtx-dioxin2/BPX-50. The results
249 show that the width at half height of the peaks obtained with the column set Rtx-
250 dioxin2/BPX-50 is less than that obtained with the column set Rtx-PCB/BPX-50 both for the
251 209 PCBs and the 17 PCDD/Fs. Despite optimizations of hot/cold pulses, modulation period
252 and temperature offset (data not shown), peaks are wider than 0.06 s of peak width at half
253 height obtained for PCBs and PCDD/Fs by Focant et al. [13].

254 Two or three modulations per peak could be observed for the 17 PCDD/Fs and 18 PCBs
255 most relevant in meat showing no significant tailing in the first dimension. The tailing factors
256 obtained for these compounds on the second dimension are shown in Table 1. The mean
257 tailing factor of the peaks of the 209 PCBs is 1.08 ± 0.18 for the column set Rtx-PCB/BPX-50
258 and 1.21 ± 0.23 with the column set Rtx-dioxin2/BPX-50. Concerning the 17 PCDD/Fs, the
259 mean tailing factor obtained is 1.11 ± 0.17 and 1.32 ± 0.15 respectively for the column sets

260 Rtx-PCB/BPX-50 and Rtx-dioxin2/BPX-50. The tailing factor obtained is therefore higher
261 with the column set Rtx-dioxin2/BPX-50 regardless of the family of compounds.

262 The column set Rtx-dioxin2/BPX-50 allows for finer peaks to be obtained but with a
263 greater tailing factor than the Rtx-PCB/BPX-50 set. It is therefore difficult to choose the
264 column set on this criterion.

265

266 3.1.2 Peak resolution

267 In order to objectively compare the separation capacity of the two column sets studied,
268 resolution factors were calculated as per Zapadlo *et al.* [18]. Out of the existing 209 PCB
269 congeners, 189 were thus resolved with the column set Rtx-Dioxin2/BPX-50 compared to 194
270 with the column set Rtx-PCB/BPX-50. In both cases, PCBs 126 and 169 with the highest
271 WHO-TEFs are not coeluted. These results are similar to those obtained by Focant *et al.* [17]
272 and Megson *et al.* [16] who managed to respectively separate 192 PCBs with the column set
273 HT8/BPX-50 and 188 PCBs with the Rtx-PCB/Rxi-17 set. However, the analysis time was
274 146 and 153 minutes respectively in these two studies compared to only 75 minutes in our
275 study. Even if the proposed analytical method led to wider peaks than former studies, it
276 enables a separation comparable to literature data while reducing the analysis time
277 significantly. With regard to the 18 most relevant PCBs for our meat model matrix, the two
278 column sets have an equivalent separation capacity with 15 PCBs resolved out of the 18. Of
279 these 18 PCBs, the coelution between PCBs 101 and 90 found with the two column sets were
280 already reported by Megson *et al.*, who used a column set Rtx-PCB/Rxi-17 and by Zapadlo *et*
281 *al.* with a column set SPB-Octyl/SLB-IL59 [16,18]. In addition, the coelutions found between
282 PCBs 138 and 163 with the column set Rtx-dioxin2/BPX-50 and between PCBs 123 and 107
283 with the Rtx-PCB/BPX-50 set were already described in other studies [17].

284 Out of the 17 toxic PCDD/Fs, the column set Rtx-Dioxin2/BPX-50 allows for all the
285 compounds to be separated. In fact, although the calculation of the resolution factors shows
286 coelution between 2,3,4,7,8-PeCDF and PCB 203, these two compounds were considered as
287 resolved due to the presence of specific ions in their respective mass spectra allowing for
288 individual monitoring. These results are consistent with those obtained by Focant *et al.* [12]
289 and Hoh *et al.* [19] who succeeded in correctly separating the 17 toxic PCDD/F congeners *via*
290 GC×GC-MS with the column set Rtx-dioxin2 in the first dimension. With the column set Rtx-
291 PCB/BPX-50, 2 congeners out of the 17 PCDD/Fs are coeluted, 2,3,4,7,8-PeCDF and

292 1,2,3,7,8-PeCDD. With a TEF=1, 1,2,3,7,8-PeCDD is however very relevant to analyze as it
293 is part of the most toxic PCDD/Fs and the most monitored in food. Figure 1 shows the
294 coelution of this compound obtained with the Rtx-PCB/BPX-50 set compared to the Rtx-
295 Dioxin2/BPX-50 set allowing for it to be monitored. It is interesting to also point out that, out
296 of the toxic PCDD/Fs, TCDD which has already been found coeluting with PCB 126 [12], is
297 resolved here with the two column sets tested.

298 As the two column sets are the same to monitor the 18 most relevant PCBs in meat, the
299 column set Rtx-dioxin2/BPX-50 which allows for the 17 toxic PCDD/Fs to be separated was
300 therefore chosen for the rest of this study. Figure 2 shows the contour plot obtained with this
301 column set which therefore allows for 189 PCBs and 17 PCDD/Fs to be separated, i.e. 206
302 dioxin-like micropollutants (supporting information, Table SI). Further studies might enable
303 to compare BPX-50 separation capacity with other 2D columns complementary from the Rtx-
304 Dioxin2 in order to optimize the use of the 2D space for PCBs while maintaining the
305 separation obtained for dioxins.

306

307 **3.2 Assessment of an extraction, concentration and clean-up protocol**

308 In order to be able to use the GC×GC-TOF/MS method to monitor toxic PCBs and
309 PCDD/Fs in a high-fat complex matrix such as ground beef, a protocol of extraction of
310 contaminants by ASE, defatting and concentration of the extract obtained was proposed
311 according to Saito *et al.* [26] with slight modifications concerning the solvent used for
312 extraction, the type of in-cell fat retainer, the clean-up of the resulting extract and evaporation
313 time. Firstly, PCBs and PCDD/Fs were quantified in matrix blanks. Then, considering the
314 high fat matrix used in this study, different clean-up procedures (cold extraction with
315 acetonitrile, in-cell clean-up, GPC, ASE followed by GPC) were tested. Only the combination
316 of in-cell clean-up with GPC enabled to concentrate the final extract and thus to get
317 satisfactory reliability and sensitivity (data not shown). To assess the relevance of the protocol
318 in concrete terms, a matrix contaminated at a known concentration was required. In order to
319 initially overcome recovery and homogeneity problems posed by impregnation of the
320 structured meat matrix, the extraction/concentration/clean-up protocol was from a spiked
321 freeze-dried matrix according to Reid *et al.* [29]. The contaminant recovery rates after the
322 ASE, GPC and concentration steps were measured on 5 PCBs spread over the chromatogram
323 obtained after analysis of 5 extracts by GC×GC-TOF/MS. These recovery rates were within
324 the 70%-130% range generally considered as acceptable [21], with $92\pm 13\%$ for BZ-1,

325 110±7% for BZ-19, 118±10% for BZ-172, 130±10% for BZ-206 and 130±8% for BZ-209.
326 These results are of the same proportions as those obtained by Wang *et al.* who also explored
327 the recoveries measured after extraction by ASE then defatting by GPC on samples of soil
328 freeze-dried than spiked with PCBs [30]. The recoveries obtained therefore vary from 87 to
329 160% but only PCBs BZ-153 and 180, not quantified in our study, had very high recoveries,
330 outside the 70%-130% range. In our study, the standard deviations were also measured for the
331 same previously stated 5 PCBs. The values obtained are between 7.2% and 13.1%, only 2-
332 chlorobiphenyl has a standard deviation greater than 10%. This may be explained by its less
333 repeatable extraction due to being the most water soluble congener of the 209 PCBs [31].
334

335 **3.3 Comparison of different spiking methods and performance of GC×GC-** 336 **TOF/MS with the column set Rtx-Dioxin2/BPX-50**

337 In order to be able to monitor the future of PCBs and PCDD/Fs during the technological
338 and physiological processes the food matrices are subjected to, a homogeneous and
339 reproducible multicontaminated, fixed concentration meat matrix is required. Based on
340 protocols found in the literature for spiking contaminants in a real matrix *via* the addition of
341 an organic solvent [23,32], 7 different spiking methods were tested and compared. Each
342 method includes a step where the matrix is impregnated with a contaminant solution (injection
343 of a small volume or immersion) and a homogenizing step (ultra-turrax, blender or mixer).
344 Although the use of an organic solvent to spike can alter the properties of the matrix, it was
345 required due to the low water solubility of the POPs studied [33]. For the 7 different spiking
346 methods, solvent choice, volume of solvent used, evaporation time, homogenization method
347 and homogenization time were optimised in order to have the best recoveries and standard
348 deviations for targeted analytes. Table 2 shows the recovery rates and standard deviations of
349 the areas corrected for 5 PCBs spread over the GC×GC-TOF/MS chromatogram with the 7
350 spiking methods tested. Only the methods coupling the injection of a small volume of
351 contaminant solution diluted in a solvent and homogenizing by blender or mixer don't allow
352 for recoveries within the traditionally accepted limit of 70%-130% to be obtained [21].
353 Among the other methods, the one coupling the injection of a small volume of solvent and
354 mixing by ultra-turrax is the one inducing the least efficient homogenizing of the spike with
355 standard deviations between 57% and 60%. The methods coupling an immersion step with
356 homogenizing by blender or ultra-turrax are alternatively those allowing for the lowest
357 standard deviations to be obtained, therefore the best homogeneity of the spike, which is

358 essential for the statistical validity of future findings which could be conducted on this matrix
359 [22]. In order to further assess the reliability of these two spiking methods, their
360 reproducibility was then tested. Table 3 shows the standard deviations and recovery rates
361 which were then obtained after extraction of the contaminants and analysis *via* GC×GC-
362 TOF/MS for 20 different PCBs, among which the 15 most relevant PCBs in meat and not
363 coeluted with the column set chosen in this study. For the majority of the PCBs, the recovery
364 rates are greater when homogenizing with a blender is performed. Only PCB 28 has a
365 recovery rate outside the 70%-130% limits with this spiking method. Regarding the standard
366 deviations obtained, they remain lower than 10% for the majority of the PCBs quantified
367 when homogenizing with a blender is performed. Only PCBs 77 and 114 have greater
368 standard deviations with this spiking method. In addition to these two compounds, 7 other
369 PCBs and particularly the 4 PCBs quantified as the heaviest have standard deviations greater
370 than 10% with the method using the ultra-turrax. These results may be compared to those of
371 Suedel *et al.* who implemented, in samples of sediments, a protocol coupling the injection, in
372 1 g of matrix, of a few microlitres of fluoranthene dissolved in a very small volume of
373 acetone, an evaporation step of the solvent for two minutes then a manual mixing step for 60
374 seconds [34]. A standard deviation of 10.3% of the fluoranthene was then reported which
375 corresponds to the mean standard deviation obtained in our study with homogenizing by ultra-
376 turrax. Reid *et al.* compared eleven different spiking protocols to contaminate soil samples
377 with phenanthrene and benzo[a]pyrene [29]. In the eleven protocols, the standard deviations
378 recorded could go up to 43% which is much greater than those obtained in our study.
379 Alternatively, the best homogeneity (2.4% of standard deviation) was obtained on dehydrated
380 samples. Among the protocols tested on non-dehydrated samples as is the case in our study,
381 the lowest standard deviation (4.1%) was obtained using a small volume of ethanol (1µL for
382 1g of sample) to perform the spike solution before manual mixing coupled to mixing of the
383 spiked matrix with a blender. This standard deviation is less than the lowest mean standard
384 deviation obtained in our study on a structured matrix which was therefore 7.8% with
385 homogenizing by blender. It is, however, to be pointed out that these results were obtained
386 with polycyclic aromatic hydrocarbons which can explain for the differences found with our
387 results relating to the PCBs. For the rest of this study, the method coupling a step of
388 immersing the meat into a solvent solution containing the contaminants and a homogenizing
389 step with a blender is the one retained after the recoveries obtained with the PCBs were
390 validated on some dioxins (99.9±12% for 2,3,7,8-TeCDD, 84.1±9% for 2,3,7,8-TeCDF,
391 73.6±11% for 2,3,4,7,8-PeCDF, 88.8±12% for 1,2,3,7,8-PeCDD). In order to validate the

392 method for official control procedures, these recovery results will have to be confirmed at a
393 second spiking level.

394 In order to determine method performance, the limits of detection (LOD) and
395 quantification (LOQ) were measured in spiked meat for 7 PCDD/F congeners and 19 PCB
396 congeners spread over the whole chromatogram (Table 4). The linearity of the calibration
397 lines allowing for these LODs and LOQs to be estimated was assessed by calculating
398 coefficients of determination. All these values are greater than 0.980 except for one of the
399 dioxin congeners (0.974). As shown in Table 4, the range of linearity spans from 50 to 5 000
400 pg/g for the majority of the constituents. The LODs obtained vary from 50 to 100 pg/g for the
401 PCBs and 65 to 227 pg/g for the PCDD/Fs. Excluding sample preparation that includes a
402 concentration by a factor of about 100, our results may be compared to those obtained by Van
403 der Lee *et al.* for several PCBs contained in different food matrices and analysed on a
404 GC×GC-TOF/MS with a column set RTX-CL pesticides/BPX-50 [35]. Of the seven PCBs
405 considered, 5 therefore had LODs less than those obtained in our study (from 1.25 to 2.5 ng/g)
406 whereas two PCBs had LODs comparable to our results (10 ng/g). The results obtained for the
407 PCBs are therefore consistent with those obtained by Van der Lee *et al.* which enables our
408 method to be validated. With LOQs at pg level [36], TOF/MS has a lower sensitivity for PCB
409 and PCDD/F analysis [13] than HRMS or MS/MS whose LOQs range between 30 and 80 fg
410 for tetra- to hexa-chlorinated compounds and between 80 and 320 fg for hepta- to octa-
411 chlorinated compounds [37]. However, GC×GC and adapted sample preparation procedures
412 may help to partially compensate the lower sensitivity of TOF/MS detection in case of trace
413 contaminants in complex matrices [14,15].

414

415 **4. Conclusion**

416 The GC×GC-TOF/MS method assessed in this study allows for simultaneous analysis
417 of 206 dioxin-like micropollutants thanks to the use of a column set Rtx-Dioxin2/BPX-50. To
418 be able to study these compounds present in trace form in a complex and high fat matrix such
419 as ground beef, this method was coupled to accelerated solvent extraction, GPC clean-up and
420 concentration protocols allowing for good recovery rates to be obtained. This work has also
421 allowed for a sample preparation optimization to obtain a matrix of ground beef
422 homogeneously spiked with these contaminants. Before reference laboratories use it, more
423 extensive investigations will be necessary to validate reproducibility and robustness of the
424 spiking method proposed by the present study. The aim is now to use these methodological

425 breakthroughs to monitor these contaminants during technological or physiological
426 transformations which the food may be subjected to. These data could be incorporated into the
427 analyses of risks relating to the presence of these contaminants in food.

428

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432

433

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- 543

543

544 **Figure captions**

545

546 **Fig.1** Separation of 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF by GC×GC-TOF/MS with Rtx-
547 PCB/BPX-50 (A) and Rtx- Dioxin2/BPX-50 (B).

548 **Fig.2** GC×GC-TOF/MS contour plot of the 209 PCBs and 17 PCDD/Fs with the Rtx-
549 Dioxin2/BP-X50 column set.

550

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550 **Table 1.** Comparison of the peak shape obtained by GC×GC-TOF/MS for 18 most relevant
 551 PCBs and 17 PCDD/Fs with Rtx-dioxin2/BPX-50 and Rtx-PCB/BPX-50, respectively.

Compound	Congener	Rtx-dioxin2/BPX50		Rtx-PCB/BPX50	
		W_h^1	T^2	W_h	T
2,4,4'-TCB	BZ-28	0.077	1.214	0.100	1.000
2,2',5,5'-TCB	BZ-52	0.125	0.947	0.121	1.100
2,2',4,5,5'-PCB	BZ-101	0.085	1.127	0.116	1.031
3,4,4',5-TCB	BZ-81	0.092	1.250	0.120	0.833
3,3',4,4'-TCB	BZ-77	0.088	1.417	0.124	0.752
2',3,4,4',5-PeCB	BZ-123	0.097	1.143	0.145	0.562
2,3',4,4',5-PeCB	BZ-118	0.089	1.106	0.116	1.027
2,3,4,4',5-PeCB	BZ-114	0.117	1.261	0.128	0.875
2,2',4,4',5,5'-HxCB	BZ-153	0.088	1.563	0.128	0.875
2,3,3',4,4'-PeCB	BZ-105	0.090	1.308	0.128	0.794
2,2',3,4,4',5'-HxCB	BZ-138	0.148	0.994	0.130	1.416
3,3',4,4',5-PeCB	BZ-126	0.083	1.278	0.059	0.969
2,3',4,4',5,5'-HxCB	BZ-167	0.040	1.071	0.107	1.071
2,3,3',4,4',5-HxCB	BZ-156	0.085	1.042	0.110	0.978
2,3,3',4,4',5'-HxCB	BZ-157	0.092	1.091	0.110	1.161
2,2',3,4,4',5,5'-HpCB	BZ-180	0.091	1.500	0.135	1.079
3,3',4,4',5,5'-HxCB	BZ-169	0.082	0.891	0.109	0.762
2,3,3',4,4',5,5'-HpCB	BZ-189	0.092	0.979	0.122	1.133
2,3,7,8-TCDF		0.092	1.409	0.118	0.974
2,3,7,8-TCDD		0.081	1.318	0.119	1.067
1,2,3,7,8-PeCDF		0.095	1.219	0.125	1.068
2,3,4,7,8-PeCDF		0.100	1.577	0.124	1.068
1,2,3,7,8-PeCDD		0.094	1.269	0.127	0.972
1,2,3,4,7,8-HxCDF		0.100	1.367	0.127	0.947
1,2,3,6,7,8-HxCDF		0.096	1.417	0.131	0.865
2,3,4,6,7,8-HxCDF		0.102	1.567	0.121	1.079
1,2,3,4,7,8-HxCDD		0.106	1.375	0.129	1.263
1,2,3,6,7,8-HxCDD		0.098	1.115	0.131	1.000
1,2,3,7,8,9-HxCDD		0.100	1.071	0.125	1.357
1,2,3,7,8,9-HxCDF		0.107	1.500	0.134	1.294
1,2,3,4,6,7,8-HpCDF		0.091	1.292	0.128	1.025
1,2,3,4,6,7,8-HpCDD		0.091	1.286	0.129	1.361
1,2,3,4,7,8,9-HpCDF		0.097	1.333	0.121	1.071
OCDD		0.109	1.200	0.121	1.452
OCDF		0.118	1.139	0.126	1.056

552

553 ¹ Peak width at half height in 2D seconds, determined from the main peak modulation

554 ² Tailing factor defined by the formula $T = w_{0.05}/2 \times f$, where $w_{0.05}$ is the peak width defined at 5% of peak
 555 height and f is the distance from the peak maximum to the leading edge of the peak, the distance being measured
 556 at a point 5% of the peak height from the baseline [14].

557

557 **Table 2.** Comparison of 7 spiking methods on the basis of the recovery rates and the standard
 558 deviations of the extraction of 5 PCBs in meat. These methods differed in their ways to add
 559 the micropollutants into the meat matrix and to homogenize the spiked matrix after
 560 micropollutant addition.

561

PCB	Immersion								Micro-volume addition					
	Blender		LN ₂ grinder		Ultra-turrax		None		Blender		Stand mixer		Ultra-turrax	
	RR ¹	SD ²	RR	SD	RR	SD	RR	SD	RR	SD	RR	SD	RR	SD
BZ-1	83.6	8.5	87.9	11.7	93.6	7.1	97.0	21.2	37.7	51.0	21.3	12.5	93.1	59.3
BZ-19	90.0	7.3	93.2	10.2	91.9	4.1	104.4	18.1	35.2	48.4	19.5	10.7	94.1	58.8
BZ-172	117.2	7.6	119.1	12.3	109.3	7.3	121.5	9.1	41.4	49.9	26.4	19.5	95.4	59.4
BZ-206	110.2	6.8	107.4	8.2	98.4	5.8	112.7	9.8	39.7	44.5	28.3	15.1	91.0	59.2
BZ-209	101	3.6	104.0	8.8	96.6	5.1	104.3	9.3	39.0	48.3	24.0	12.2	88.3	57.0

562

563 ¹ Recovery rates (%)

564 ² Standard deviations (%)

565

566

567

567 **Table 3.** Comparison of the recovery rates and reproducibility for the two best spiking
 568 methods selected for 20 PCBs (n= 5 replicates).

	Immersion + blender		Immersion +569 ultra-turrax	
	RR ¹	SD ²	RR	SD
BZ-1	87.0	4.7	81.2	9.7
BZ-19	92.0	8.6	82.3	8.7
BZ-28*	133.4	6.1	120.0	7.6
BZ-52*	112.2	9.4	101.9	8.2
BZ-77*	116.2	20.8	124.6	20.5
BZ-81*	102.5	8.0	92.0	10.7
BZ-105*	114.6	3.1	106.4	3.2
BZ-114*	110.7	10.1	111.8	10.8
BZ-118*	112.3	8.5	101.3	13.0
BZ-123*	105.1	5.8	100.4	7.8
BZ-126*	92.4	7.1	82.6	4.6
BZ-153*	123.4	9.1	109.7	21.6
BZ-156*	107.9	5.0	112.6	10.3
BZ-157*	91.3	8.6	91.4	5.5
BZ-167*	106.5	3.8	103.3	8.5
BZ-169*	96.1	6.1	87.2	6.1
BZ-172	119.2	7.8	116.1	11.1
BZ-189*	94.3	6.1	93.0	15.7
BZ-206	120.7	9.7	116.3	10.4
BZ-209	111.4	8.3	104.9	12.0

570

571 ¹ Recovery rates (%)

572 ² Standard deviation (%)

573 * Non coeluted PCBs forming part of the 18 most relevant in meat

574

575

576

576 **Table 4.** Performance of GC×GC-TOF/MS used with a Rtx-Dioxin2/BPX50 column set for
 577 quantification of 19 PCBs and 7 PCDD/Fs.

578

Compound	¹ t _R ^a (s)	² t _R ^b (s)	Linearity (pg g ⁻¹)	Correlation coefficient R ²	LOD ^c (pg g ⁻¹)	LOQ ^d (pg g ⁻¹)
BZ-1	970	2.42	50-5 000	0.995	66	218
BZ-3	1085	2.57	50-5 000	0.991	98	327
BZ-8	1260	2.89	50-5 000	0.992	62	205
BZ-19	1315	3.10	50-5 000	0.991	66	220
BZ-18	1385	3.08	50-5 000	0.995	64	214
BZ-15	1440	3.04	50-5 000	0.988	77	256
BZ-54	1510	3.45	50-5 000	0.986	83	277
BZ-28	1580	3.17	50-5 000	0.991	86	288
BZ-33	1615	3.34	50-5 000	0.994	55	182
BZ-22	1655	3.39	50-5 000	0.996	50	155
BZ-52	1705	3.35	50-5 000	0.980	100	333
BZ-49	1725	3.34	50-5 000	0.990	70	234
BZ-74	1970	3.61	50-5 000	0.986	85	283
BZ-87	2220	3.79	50-5 000	0.993	60	201
BZ-151	2300	3.73	50-5 000	0.990	70	232
BZ-77	2320	3.76	50-5 000	0.990	71	238
BZ-149	2350	3.85	50-5 000	0.981	99	329
BZ-123	2390	3.72	50-5 000	0.996	50	142
BZ-118	2415	3.70	50-5 000	0.993	58	194
2,3,7,8-TCDF	2670	3.90	50-2 500	0.989	75	251
2,3,7,8-TCDD	2725	3.77	50-2 500	0.977	118	393
1,2,3,7,8-PeCDF	3030	4.08	50-5 000	0.985	120	399
2,3,4,7,8-PeCDF	3145	4.11	50-5 000	0.997	65	217
1,2,3,4,7,8-HxCDF	3460	4.20	50-2 500	0.992	65	218
1,2,3,6,7,8-HxCDF	3475	4.21	50-2 500	0.986	84	279
1,2,3,6,7,8-HxCDD	3575	4.16	50-5 000	0.974	227	757

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580 ^a Retention time in first dimension (s)

581 ^b Retention time in second dimension (s)

582 ^c Limit of detection. LOD is defined by the formula 3s/m (s is the standard deviation of the
 583 intercept, and m is the slope of the linear calibration curve)

584 ^d Limit of quantification

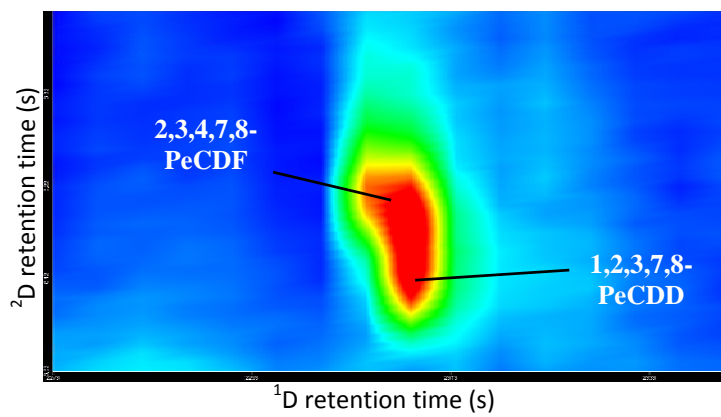
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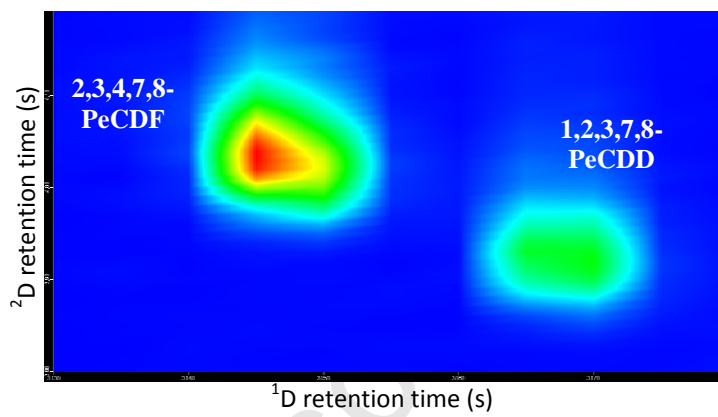
586 Fig.1

587 A) Rtx-PCB/BPX-50

B) Rtx-Dioxin2/BPX-50



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600 Fig.2

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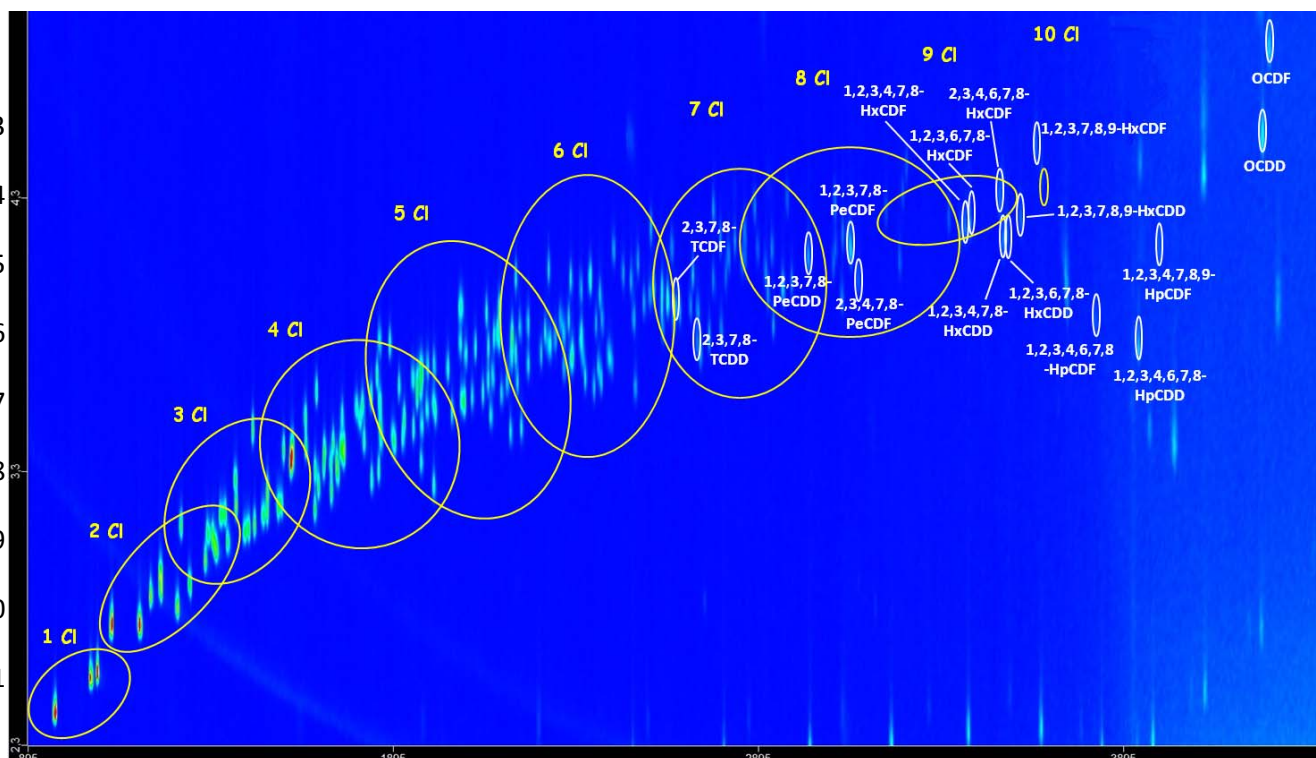
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 ^1D retention time (s)

Accepted