



**HAL**  
open science

# 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

Christelle Planche, Jérémy Ratel, Frédéric Mercier, Patrick Blinet, Laurent Debrauwer, Erwan Engel

## ► To cite this version:

Christelle Planche, Jérémy Ratel, Frédéric Mercier, Patrick Blinet, Laurent Debrauwer, et al.. 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. *Journal of Chromatography A*, 2015, 1392, pp.74-81. 10.1016/j.chroma.2015.02.054 . hal-03696520

**HAL Id: hal-03696520**

**<https://hal.science/hal-03696520v1>**

Submitted on 9 May 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

Title: 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

Author: Christelle Planche Jérémy Ratel Frédéric Mercier  
Patrick Blinet Laurent Debrauwer Erwan Engel



PII: S0021-9673(15)00313-1  
DOI: <http://dx.doi.org/doi:10.1016/j.chroma.2015.02.054>  
Reference: CHROMA 356312

To appear in: *Journal of Chromatography A*

Received date: 4-11-2014  
Revised date: 17-2-2015  
Accepted date: 17-2-2015

Please cite this article as: C. Planche, J. Ratel, F. Mercier, P. Blinet, L. Debrauwer, E. Engel, 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, *Journal of Chromatography A* (2015), <http://dx.doi.org/10.1016/j.chroma.2015.02.054>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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.

8

Accepted Manuscript



33  
34  
35  
36  
37

### 38 **Abstract**

39  
40 This paper evaluates different multiresidue methods based on comprehensive two-  
41 dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-  
42 TOF/MS) to analyze dioxin-related micropollutants in complex food matrices. In a first step,  
43 the column sets Rtx-PCB/BPX-50 and Rtx-Dioxin2/BPX-50 were compared in terms of peak  
44 shape (width and symmetry) and resolution for the separation of polychlorinated biphenyls  
45 (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) in solvent. A  
46 satisfactory separation of 206 dioxin-related micropollutants including the 17 toxic PCDD/Fs  
47 was achieved in 75min with the column set Rtx-dioxin2/BPX-50. In a second time, the  
48 GC×GC-TOF/MS method was spread to the analysis of dioxin-related micropollutants in  
49 complex food matrices. An extraction procedure including accelerated solvent extraction  
50 (ASE), centrifugal evaporation and gel permeation chromatography (GPC) was optimized.  
51 Starting with meat as a model matrix, a micropollutant spiking method was then set up by  
52 comparing seven methods in terms of recoveries and reproducibility. The method combining  
53 immersion of the meat in a large volume of solvent containing micropollutants followed by  
54 homogenization by blender induced recoveries in the acceptable range of 70–130% and  
55 satisfactory standard deviations ( $\leq 10\%$ ) for most of the compounds studied. Limits of  
56 detection of the GC×GC-TOF/MS method ranged between 50 and 100 pg/g of spiked fresh  
57 meat for PCBs and between 65 and 227 pg/g for PCDD/Fs. Potential applications of this  
58 method are discussed.

59

### 60 **Keywords**

61  
62 Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry  
63 (GC×GC-TOF/MS); food spiking; multiresidue method; Polychlorinated biphenyls (PCBs);  
64 Polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs)

65

## 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/ $\mu$ L. For the PCDD/Fs, the concentration of the stock solution was different according  
145 to the congeners: tetra's 2.5 ng/ $\mu$ l, penta's, hexa's and hepta's 6.25 ng/ $\mu$ l and octa's 12.5  
146 ng/ $\mu$ 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<sup>-1</sup>. Purge time was set to 60s with a  
197 flow rate of 50mL min<sup>-1</sup>. The primary oven temperature was initially set at 90°C for 1min,  
198 then increased to 200°C at 20°C min<sup>-1</sup>, then to 308°C at 2°C min<sup>-1</sup> and at 5°C min<sup>-1</sup> 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<sup>-1</sup>. 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<sup>-1</sup> 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  $0.119 \pm 0.021$   
245 second for the column set Rtx-PCB/BPX-50 and  $0.087 \pm 0.014$  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  $0.126 \pm 0.005$  second and  $0.099 \pm 0.008$  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  $1.08 \pm 0.18$  for the column set Rtx-PCB/BPX-50  
258 and  $1.21 \pm 0.23$  with the column set Rtx-dioxin2/BPX-50. Concerning the 17 PCDD/Fs, the  
259 mean tailing factor obtained is  $1.11 \pm 0.17$  and  $1.32 \pm 0.15$  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

## 429 **Acknowledgements**

430 This study was supported by the French National Research Agency, project SOMEAT,  
431 Contract No. ANR-12-ALID-0004. Safety of Organic Meat. Available at <http://www.someat.fr>  
432

433

## 434 **References**

- 435 [1] H.W. Vallack, D.J. Bakker, I. Brandt, E. Brostrom-Lunden, A. Brouwer, K.R. Bull, C. Gough, R.  
436 Guardans, I. Holoubek, B. Jansson, R. Koch, J. Kuylenstierna, A. Lecloux, D. Mackay, P.  
437 McCutcheon, P. Mocarelli, R.D.F. Taalman, Controlling persistent organic pollutants—what next?,  
438 *Environ. Toxicol. Pharmacol.* 6 (1998) 143-175.
- 439 [2] B.E. Fisher, Most unwanted, *Environ. Health Perspect.* 107 (1999) A18-23.
- 440 [3] J.L. Domingo, Influence of cooking processes on the concentrations of toxic metals and various  
441 organic environmental pollutants in food: a review of the published literature, *Crit. Rev. Food*  
442 *Sci. Nutr.* 51 (2011) 29-37.
- 443 [4] G. Perelló, R. Martí-Cid, V. Castell, J.M. Llobet, J.L. Domingo, Influence of various cooking  
444 processes on the concentrations of PCDD/PCDFs, PCBs and PCDEs in foods, *Food Control* 21  
445 (2010) 178-185.
- 446 [5] G.H. Xing, Y. Yang, J.K.Y. Chan, S. Tao, M.H. Wong, Bioaccessibility of polychlorinated biphenyls  
447 in different foods using an in vitro digestion method, *Environ. Pollut.* 156 (2008) 1218-1226.
- 448 [6] A. Marques, H.M. Lourenco, M.L. Nunes, C. Roseiro, C. Santos, A. Barranco, S. Rainieri, T.  
449 Langerholc, A. Cencic, Principles and applications of comprehensive two-dimensional gas  
450 chromatography, *Food Res. Int.* 44 (2011) 510-522.
- 451 [7] R. Malisch, A. Kotz, Dioxins and PCBs in feed and food—Review from European perspective, *Sci.*  
452 *Total Environ.* 491-492 (2014) 2-10.
- 453 [8] R. Weber, C. Gaus, M. Tysklind, P. Johnston, M. Forter, H. Hollert, E. Heinisch, I. Holoubek, M.  
454 Lloyd-Smith, S. Masunaga, P. Moccarelli, D. Santillo, N. Seike, R. Symons, J. P. Machado Torres,  
455 M. Verta, G. Varbelow, J. Vijgen, A. Watson, P. Costner, J. Woelz, P. Wycisk, M. Zennegg, Dioxin-

- 456 and POP-contaminated sites—contemporary and future relevance and challenges, *Environ. Sci.*  
457 *Pollut. Res.* 15 (2008) 363-393.
- 458 [9] K. Srogi, Levels and congener distributions of PCDDs, PCDFs and dioxin-like PCBs in  
459 environmental and human samples: a review, *Environ. Chem. Lett.* 6 (2008) 1-28.
- 460 [10] T.M. Crisp, E.D. Clegg, R.L. Cooper, W.P. Wood, D.G. Anderson, K.P. Baetcke, J.L. Hoffmann, M.S.  
461 Morrow, D.J. Rodier, J.E. Schaeffer, L.W. Touart, M.G. Zeeman, Y.M. Patel, Environmental  
462 endocrine disruption: an effects assessment and analysis, *Environ. Health Perspect.* 106 (1998)  
463 11-56.
- 464 [11] J.P. Whitlock, Induction of cytochrome P4501A1, *Annu. Rev. Pharmacol. Toxicol.* 39 (1999) 103-  
465 125.
- 466 [12] J.F. Focant, E.J. Reiner, K. MacPherson, T. Kolic, A. Sjodin, D.G. Patterson, S.L. Reese, F.L.  
467 Dorman, J. Cochran, Measurement of PCDDs, PCDFs, and non-ortho-PCBs by comprehensive  
468 two-dimensional gas chromatography-isotope dilution time-of-flight mass spectrometry (GC×  
469 GC-IDTOFMS), *Talanta* 63 (2004) 1231-1240.
- 470 [13] J.F. Focant, G. Eppe, M.L. Scippo, A.C. Massart, C. Pirard, G. Maghuin-Rogister, E. De Pauw,  
471 Comprehensive two-dimensional gas chromatography with isotope dilution time-of-flight mass  
472 spectrometry for the measurement of dioxins and polychlorinated biphenyls in foodstuffs:  
473 Comparison with other methods, *J. Chromatogr. A* 1086 (2005) 45-60.
- 474 [14] E. Engel, J. Ratel, P. Blinet, S.T. Chin, G. Rose, P.J. Marriott, Benchmarking of candidate detectors  
475 for multiresidue analysis of pesticides by comprehensive two-dimensional gas chromatography,  
476 *J. Chromatogr. A* 1311 (2013) 140-148.
- 477 [15] P.Q. Tranchida, F.A. Franchina, P. Dugo, L. Mondello, Comprehensive two-dimensional gas  
478 chromatography-mass spectrometry: Recent evolution and current trends, *Mass Spectrom. Rev.*  
479 XX (2014) 1-11.
- 480 [16] D. Megson, R. Kalin, P.J. Worsfold, C. Gauchotte-Lindsay, D.G. Patterson, M.C. Lohan, S. Comber,  
481 T.A. Brown, G. O'Sullivan, Fingerprinting polychlorinated biphenyls in environmental samples  
482 using comprehensive two-dimensional gas chromatography with time-of-flight mass  
483 spectrometry, *J. Chromatogr. A* 1318 (2013) 276-283.
- 484 [17] J.F. Focant, A. Sjodin, D.G. Patterson, Improved separation of the 209 polychlorinated biphenyl  
485 congeners using comprehensive two-dimensional gas chromatography–time-of-flight mass  
486 spectrometry, *J. Chromatogr. A* 1040 (2004) 227-238.
- 487 [18] M. Zapadlo, J. Krupcik, T. Kovalczuk, P. Majek, I. Spanik, D.W. Armstrong, P. Sandra, Enhanced  
488 comprehensive two-dimensional gas chromatographic resolution of polychlorinated biphenyls  
489 on a non-polar polysiloxane and an ionic liquid column series, *J. Chromatogr. A* 1218 (2011) 746-  
490 751.

- 491 [19] E. Hoh, K. Mastovska, S.J. Lehotay, Optimization of separation and detection conditions for  
492 comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry analysis  
493 of polychlorinated dibenzo-p-dioxins and dibenzofurans, *J. Chromatogr. A* 1145 (2007) 210-221.
- 494 [20] M. Herrero, E. Ibanez, A. Cifuentes, J. Bernal, Multidimensional chromatography in food analysis,  
495 *J. Chromatogr. A* 1216 (2009) 7110-7129.
- 496 [21] EPA Method 8000C, Determinative Chromatographic Separations, March 2003.
- 497 [22] G.L. Northcott, K.C. Jones, Spiking hydrophobic organic compounds into soil and sediment: a  
498 review and critique of adopted procedures, *Environ. Toxicol. Chem.* 19 (2000) 2418-2430.
- 499 [23] G.L. Northcott, K.C. Jones, Developing a standard spiking procedure for the introduction of  
500 hydrophobic organic compounds into field-wet soil, *Environ. Toxicol. Chem.* 19 (2000) 2409-  
501 2417.
- 502 [24] M. Van den Berg, L. Birnbaum, A.T.C. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J.P. Giesy, A.  
503 Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X.R. van Leeuwen, A.K.D. Liem, C.  
504 Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern,  
505 T. Zacharewski, Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife,  
506 *Environ. Health Perspect.* 106 (1998) 775-792.
- 507 [25] V. Sirot, A. Tard, A. Venisseau, A. Brosseau, P. Marchand, B. Le Bizec, J.C. Leblanc, Dietary  
508 exposure to polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and  
509 polychlorinated biphenyls of the French population: Results of the second French Total Diet  
510 Study, *Chemosphere* 88 (2012) 492-500.
- 511 [26] K. Saito, A. Sjodin, C.D. Sandau, M.D. Davis, H. Nakazawa, Y. Matsuki, D.G. Patterson,  
512 Development of a accelerated solvent extraction and gel permeation chromatography analytical  
513 method for measuring persistent organohalogen compounds in adipose and organ tissue  
514 analysis, *Chemosphere* 57 (2004) 373-381.
- 515 [27] E. Bjorklund, T. Nilsson, S. Bowadt, Pressurised liquid extraction of persistent organic pollutants  
516 in environmental analysis, *Trac-Trends Anal. Chem.* 19 (2000) 434-445.
- 517 [28] R.C. Team, (2012 <http://www.R-project.org>).
- 518 [29] B.J. Reid, G.L. Northcott, K.C. Jones, K.T. Semple, Evaluation of spiking procedures for the  
519 introduction of poorly water soluble contaminants into soil, *Environ. Sci. Technol.* 32 (1998)  
520 3224-3227.
- 521 [30] P. Wang, Q. Zhang, Y. Wang, T. Wang, X. Li, L. Ding, G. Jiang, Evaluation of Soxhlet extraction,  
522 accelerated solvent extraction and microwave-assisted extraction for the determination of  
523 polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples, *Anal.*  
524 *Chim. Acta* 663 (2010) 43-48.

- 525 [31] Y.B. Wang, C.S. Hong, Effect of hydrogen peroxide, periodate and persulfate on photocatalysis of  
526 2-chlorobiphenyl in aqueous TiO<sub>2</sub> suspensions, *Water Res.* 33 (1999) 2031-2036.
- 527 [32] U.C. Brinch, F. Ekelund, C.S. Jacobsen, Method for spiking soil samples with organic compounds,  
528 *Appl. Environ. Microb.* 68 (2002) 1808-1816.
- 529 [33] M. Howsam, R.A. Verweij, N.M. Van Straalen, A solvent-free method for spiking terrestrial algae  
530 (*Desmococcus* spp.) with pyrene for use in bioassays, *Chemosphere* 53 (2003) 253-262.
- 531 [34] B.C. Suedel, J.H. Rodgers, P.A. Clifford, Bioavailability of fluoranthene in freshwater sediment  
532 toxicity tests, *Environ. Toxicol. Chem.* 12 (1993) 155-165.
- 533 [35] M.K. Van der Lee, G. Van der Weg, W.A. Traag, H.G. Mol, Qualitative screening and quantitative  
534 determination of pesticides and contaminants in animal feed using comprehensive two-  
535 dimensional gas chromatography with time-of-flight mass spectrometry, *J. Chromatogr. A* 1186  
536 (2008) 325-339.
- 537 [36] J.-F. Focant, C. Pirard, G. Eppe, E. De Pauw, Recent advances in mass spectrometric  
538 measurement of dioxins, *J. Chromatogr. A* 1067 (2005) 265–275.
- 539 [37] A.M. Ingelido, G. Brambilla, A. Abballe, A. Di Domenico, A.R. Fulgenzi, N. Iacovella, A.L. Iamiceli, S.  
540 Valentini, E. De Felip, PCDD, PCDF, AND DL-PCB analysis in food: performance evaluation of the  
541 high-resolution gas chromatography/low-resolution tandem mass spectrometry technique using  
542 consensus-based samples, *Rapid Commun. Mass Spectrom.* 26 (2012) 236–242.
- 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

Accepted Manuscript

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 <sup>1</sup> Peak width at half height in 2D seconds, determined from the main peak modulation

554 <sup>2</sup> 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 <sub>2</sub> grinder		Ultra-turrax		None		Blender		Stand mixer		Ultra-turrax	
	RR <sup>1</sup>	SD <sup>2</sup>	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 <sup>1</sup> Recovery rates (%)

564 <sup>2</sup> 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 <sup>1</sup>	SD <sup>2</sup>	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 <sup>1</sup> Recovery rates (%)

572 <sup>2</sup> 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	<sup>1</sup> t <sub>R</sub> <sup>a</sup> (s)	<sup>2</sup> t <sub>R</sub> <sup>b</sup> (s)	Linearity (pg g <sup>-1</sup> )	Correlation coefficient R <sup>2</sup>	LOD <sup>c</sup> (pg g <sup>-1</sup> )	LOQ <sup>d</sup> (pg g <sup>-1</sup> )
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

579

580 <sup>a</sup> Retention time in first dimension (s)

581 <sup>b</sup> Retention time in second dimension (s)

582 <sup>c</sup> 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 <sup>d</sup> Limit of quantification

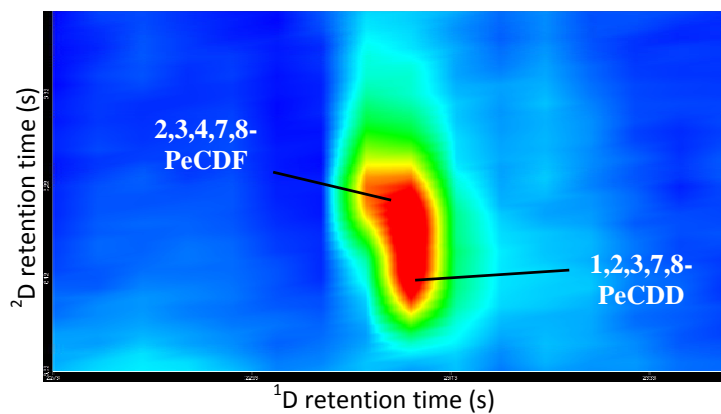
585

586

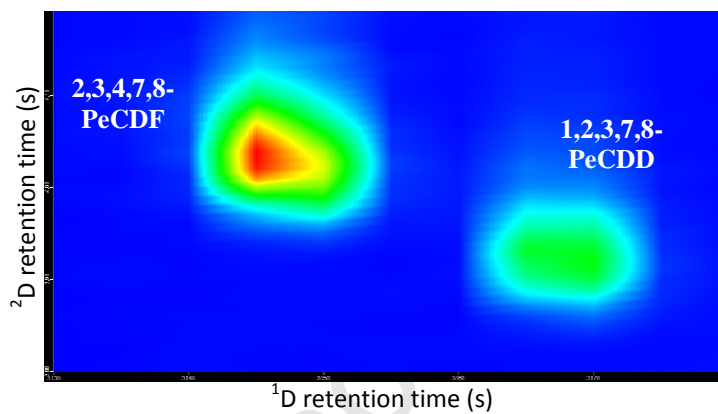
586 Fig.1

587 A) Rtx-PCB/BPX-50

B) Rtx-Dioxin2/BPX-50



594



595

596

597

598

599

600

Accepted Manuscript

600 Fig.2

601

602

603

604

605

606

607

608

609

610

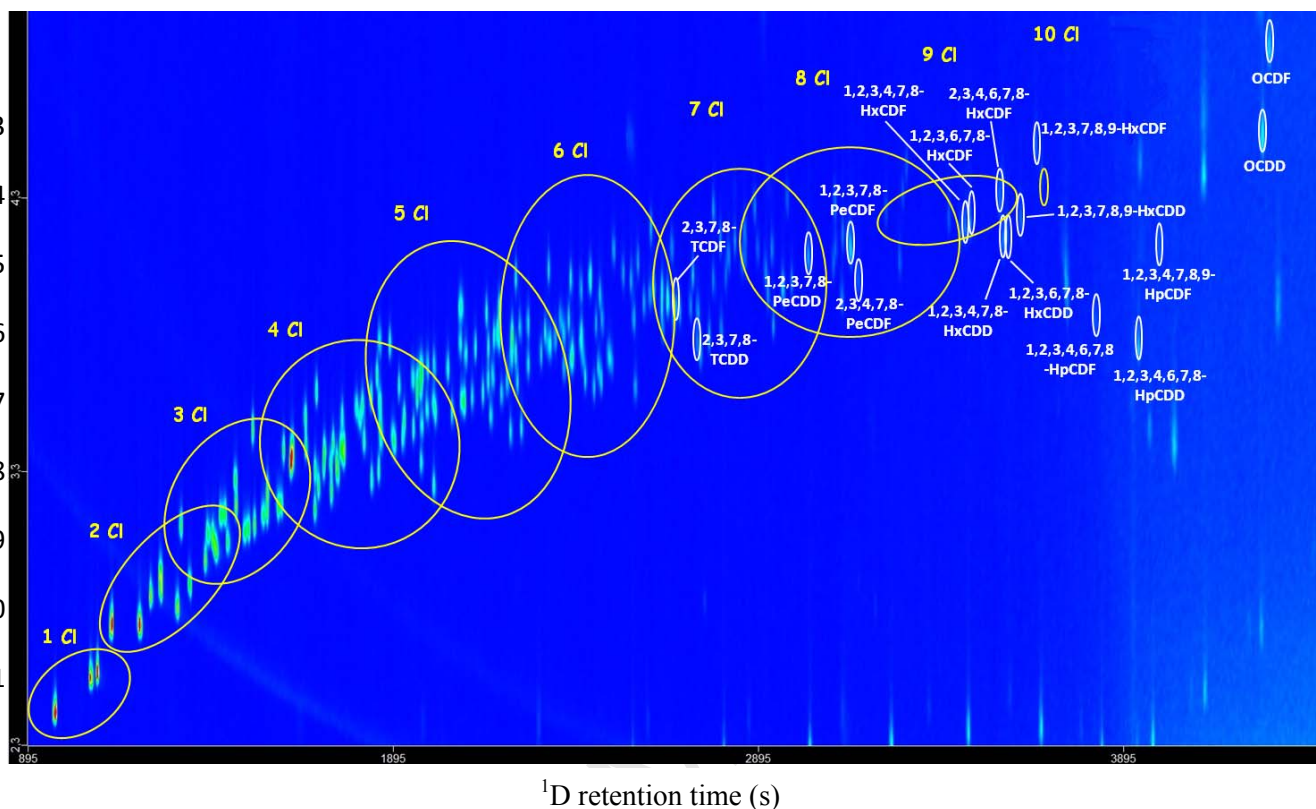
611

612

613

614

615



ACCEPTED