



# 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-03696520>**

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.

**Highlights**

- GC×GC-TOF/MS enabled 206 dioxin-related micropollutants in solvent to be separated.
- A sample preparation protocol was set up for the analysis of complex food matrices.
- A powerful food spiking method was proposed.
- The LODs ranged between 50-227 pg/g.

**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<sup>1,2</sup>, Jérémy Ratel<sup>1</sup>, Frédéric Mercier<sup>1</sup>, Patrick Blinet<sup>1</sup>, Laurent Debrauwer<sup>2</sup>,  
Erwan Engel<sup>1\*</sup>

<sup>1</sup> INRA, UR370 QuaPA, MASS group, 63122 Saint-Genès-Champanelle, France

<sup>2</sup> INRA, UMR 1331 TOXALIM, AXIOM platform, 31027 Toulouse, France

Submitted to

Journal of Chromatography A

\* Author for Correspondence

Erwan Engel: Email : [erwan.engel@clermont.inra.fr](mailto:erwan.engel@clermont.inra.fr) Tel: +33 4 73624589

**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)

## 1. Introduction

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

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

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

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

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

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

## 2. Material and Methods

### 2.1 Chemicals and standards

Hexane, dichloromethane and toluene were organic trace analysis grade solvents (Sigma-Aldrich, Saint-Quentin Fallavier, France). Activated aluminum oxide (acidic, Brockmann I) and diatomaceous earth used for the preparation of ASE cells were obtained from Sigma-Aldrich. Micropollutant reference standards were obtained from AccuStandard Europe (Niederbipp, Switzerland) and LGC Standards (Molsheim, France) for the 209 PCBs and the 17 PCDD/Fs, respectively. The 209 PCBs were divided into nine stock solutions concentrated to 10 ng/ $\mu$ L. For the PCDD/Fs, the concentration of the stock solution was different according 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 ng/ $\mu$ L. Internal standards were used for the accurate quantification of target compounds: 3'-F-PCB-28 (Chiron, Trondheim, Norway), 3'-F-PCB-81 (Chiron),  $^{13}$ C-labelled PCB-111 (Wellington laboratories, Guelph, ON, Canada) and  $^{13}$ C-labelled PCB-194 (Wellington).

### 2.2 Sample spiking

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



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

### 2.3 Extraction, clean-up and concentration

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

### 2.4 GC×GC-TOF/MS analysis

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

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 flow rate of 50mL min<sup>-1</sup>. The primary oven temperature was initially set at 90°C for 1min, 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 for 10min. The secondary oven temperature was set at 5°C higher than the primary oven temperature. The modulator temperature was set at 15°C higher than the primary oven temperature and the modulation period was 5s with 1.20 and 1.30 s for the hot and cold pulses, respectively. The transfer line temperature was set at 280°C. The mass spectrometer was operated with an ionization energy of 70eV, a detector voltage of 1800V and a data acquisition rate of 100 spectra s<sup>-1</sup>. The run time for each sample was 75 min. Analytical blank samples of pure solvent were run to check the absence of targeted analytes. GC×GC data were processed using the LECO ChromaTOF software (version 4.50.8.0).

## 2.5 Peak shape, resolution factor, and limit of detection

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

### 3. Results and Discussion

#### 3.1 Comparison of column sets in pure solvent

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

##### 3.1.1 Peak shape

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

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

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

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

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

### 3.1.2 Peak resolution

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

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

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

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

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

In order to be able to use the GC×GC-TOF/MS method to monitor toxic PCBs and PCDD/Fs in a high-fat complex matrix such as ground beef, a protocol of extraction of contaminants by ASE, defatting and concentration of the extract obtained was proposed according to Saito *et al.* [26] with slight modifications concerning the solvent used for extraction, the type of in-cell fat retainer, the clean-up of the resulting extract and evaporation time. Firstly, PCBs and PCDD/Fs were quantified in matrix blanks. Then, considering the high fat matrix used in this study, different clean-up procedures (cold extraction with acetonitrile, in-cell clean-up, GPC, ASE followed by GPC) were tested. Only the combination of in-cell clean-up with GPC enabled to concentrate the final extract and thus to get satisfactory reliability and sensitivity (data not shown). To assess the relevance of the protocol in concrete terms, a matrix contaminated at a known concentration was required. In order to initially overcome recovery and homogeneity problems posed by impregnation of the structured meat matrix, the extraction/concentration/clean-up protocol was from a spiked freeze-dried matrix according to Reid *et al.* [29]. The contaminant recovery rates after the ASE, GPC and concentration steps were measured on 5 PCBs spread over the chromatogram obtained after analysis of 5 extracts by GC×GC-TOF/MS. These recovery rates were within the 70%-130% range generally considered as acceptable [21], with  $92\pm13\%$  for BZ-1,

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

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

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

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

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

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

#### 4. Conclusion

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



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

## Acknowledgements

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

## References

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

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

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

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

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

**Table 1.** Comparison of the peak shape obtained by GC×GC-TOF/MS for 18 most relevant 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

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

<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 height and  $f$  is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline [14].

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

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

<sup>1</sup> Recovery rates (%)

<sup>2</sup> Standard deviations (%)

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

	Immersion + blender		Immersion + 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

<sup>1</sup> Recovery rates (%)

<sup>2</sup> Standard deviation (%)

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



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

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

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

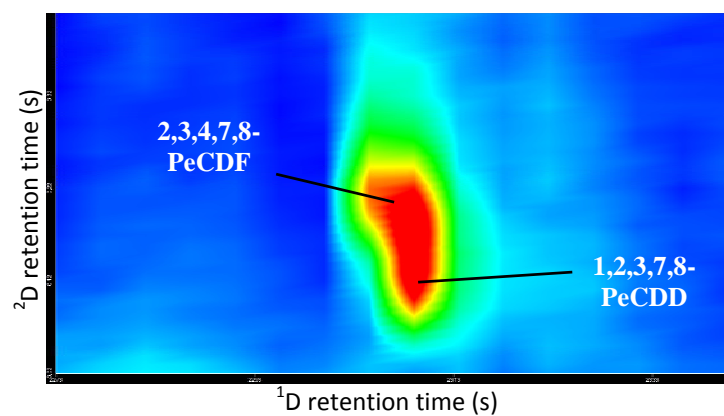
<sup>b</sup> Retention time in second dimension (s)

<sup>c</sup> Limit of detection. LOD is defined by the formula  $3s/m$  ( $s$  is the standard deviation of the intercept, and  $m$  is the slope of the linear calibration curve)

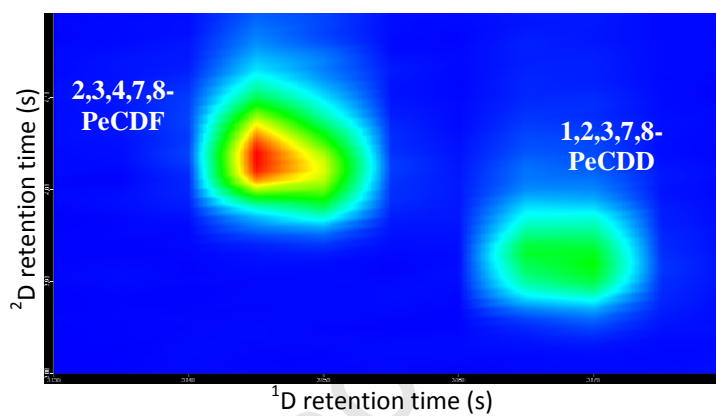
<sup>d</sup> Limit of quantification

586 Fig.1

587 A) Rtx-PCB/BPX-50



B) Rtx-Dioxin2/BPX-50



595

596

597

598

599

600

Fig.2

