DEVELOPMENT OF A GC×GC-TOF/MS BASED METHOD TO STUDY THE FATE OF 206 DIOXIN-LIKE MICROPOLLUTANTS IN MEAT DURING COOKING

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Abstract – The aim of the present paper was to develop a multiresidue method based on GC×GC-TOF/MS in order to investigate changes induced by cooking in the composition of a complex food matrix spiked with 226 dioxin-related micropollutants. In a first step, a GC×GC-TOF/MS method was developed to achieve a satisfactory separation of the 209 PCBs and the 17 toxic PCDD/Fs in hexane. The best GC×GC-TOF/MS conditions determined according to peak shape (width and symmetry) and resolution enabled to separate 206 dioxin-related micropollutants. Starting with meat as a model matrix, the second step enabled to set up procedures for both micropollutant spiking and sample preparation. The later included accelerated solvent extraction (ASE), centrifugal evaporation and gel permeation chromatography (GPC). Recoveries in the acceptable range of 70−130% and satisfactory standard deviations (≤10%) were obtained 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. Finally, the multiresidue method was implemented to assess the modulating influence of cooking on meat content on the 206 dioxin-related micropollutants.

Key Words – Food spiking, Multiresidue method, Polychlorinated biphenyls (PCBs), Polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs).

I. INTRODUCTION

Food-producing animals can be exposed to various dioxin-related compounds like polychlorobiphenyls (PCBs), polychlorodibenzo-p-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs). Due to their lipophilic nature, these micropollutants are rapidly transferred from the environment to animal edible tissues where they are bioaccumulated, thus representing a public health risk. Only a fraction of these micropollutants is bioaccessible to the consumer due to technological processes applied to the food matrix before ingestion. Therefore, worldwide food safety agencies encourage residue chemists to investigate their fate during processes like cooking in order to upgrade their risk assessment procedures. To assess the impact of these transformations on the contaminants potentially 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. For example, to analyze simultaneously the existing 209 PCBs and the 17 toxic 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) [1-4]. In view of these researches, the Rtx-PCB and Rtx-Dioxin2 gas chromatography columns in first dimension seem to be the most promising to simultaneously analyze PCBs and PCDD/Fs by GC×GC-TOF/MS [2,3]. 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. This strategy required homogeneous and reproducible multicontaminated products, and efficient methods for food spiking must therefore be found.

In order to ultimately improve the assessment of the risk related to the occurrence of chemical contaminants in food, this study is aimed at proposing an analysis method to monitor PCBs and PCDD/Fs in meat. A first study was designed to assess the relevance of GC×GC-TOF/MS to monitor these compounds in solvent by comparing different column combinations on the basis of the peak shape and the peak resolution. When the analytical method in pure solvent is setup, it was then proven in complex matrices taking meat as a model. To do this, an extraction, concentration and

clean-up method was proposed. Different spiking scenarios were then compared based on the standard deviations and recovery rates obtained for the different contaminants studied. The performances of the analytical method were then assessed in terms of linearity (R^2) and sensitivity (LOD) on a spiked matrix. Finally, the impact of cooking on the level of PCBs and PCDD/Fs in meat was studied.

II. MATERIALS AND METHODS

Sample spiking. Ground beef samples (15% fat) were purchased from a French supplier. Seven methods for ground beef spiking were compared with a PCB and PCDD/F concentration of 2 ng/g of fresh meat. These methods combined micropollutant addition to 120 g of ground meat and matrix homogenization. Two approaches for micropollutant addition were tested: (i) addition of a micro-volume (1mL) of dichloromethane containing micropollutants to ground beef, (ii) immersion of ground beef in a large volume of dichloromethane (20mL) containing micropollutants followed by evaporation under hood. 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.

Extraction, clean-up and concentration. All samples were extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA, USA). Hexane was used as extraction solvent at a temperature of 100°C and pressure of 1500 psi. The extract was evaporated (Rocket, GenevacLtd.) 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.) then 100µL of hexane were added prior to analysis.

GC×GC-TOF/MS analysis. Samples were analyzed 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). Two 1D columns were tested in the study: Rtx-PCB (60 m × 0.18 mm × 0.18 µm) (Restek, Bellefonte, PA, USA) and Rtx-Dioxin2 (60 m × 0.25 mm × 0.25 µm) (Restek). The 1D column was connected to a BPX-50 (2 m × 0.1 mm × 0.1 µm) (SGE, Austin, TX, USA) 2D column. The primary oven temperature was initially set at 90°C for 1 min, then increased to 200°C at 20°C min^{-1}, then to 308°C at 2°C min^{-1} and at 5°C min^{-1} to 330°C for 10 min. The secondary oven temperature was set at 5°C higher than the primary oven temperature. The modulation period was 5 s. The run time for each sample was 75 min.

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 (T) defined by the formula $T = w_{0.05}/2f$, 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. Resolution factor (Rs) was calculated according to Zapadlo *et al*. [4]. Briefly, $Rs= \Delta t_R/w_b$, 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. 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.

Cooking tests. To study the fate of micropollutants during cooking, meat samples were cooked in a pan at 200°C at the bottom of the pan for 14 min according to WHO recommended cooking temperatures for meat.

III. RESULTS AND DISCUSSION

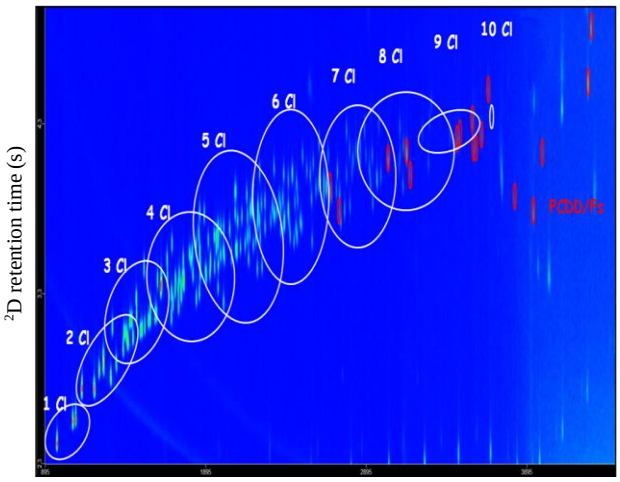
In order to compare the two column sets selected for this study, Rtx-Dioxin2/BPX-50 and Rtx-PCB/BPX-50, the width at half height and the tailing factor of the peaks obtained from analysis

of the 209 PCBs and 17 toxic PCDD/Fs in pure solvent were determined. Mean peak width at half height obtained for the 209 PCBs is 0.119 ± 0.021 s for the column set Rtx-PCB/BPX-50 and 0.087 ± 0.014 s for the Rtx-Dioxin2/BPX-50 column set. Peak width at half height for the 17 PCDD/Fs is greater than that obtained for the PCBs as it is 0.126 ± 0.005 and 0.099 ± 0.008 s for the column sets Rtx-PCB/BPX-50 and Rtx-Dioxin2/BPX-50, respectively. The results show that the width at half height of the peaks is lower for the Rtx-Dioxin2/BPX-50 column set than for the Rtx-PCB/BPX-50 column set, both for the 209 PCBs and the 17 PCDD/Fs.

The mean tailing factor of the peaks of PCBs is 1.08 ± 0.18 for the Rtx-PCB/BPX-50 column set and 1.21 ± 0.23 for the Rtx-Dioxin2/BPX-50 column set. Concerning the PCDD/Fs, the mean tailing factor obtained is 1.11 ± 0.17 and 1.32 ± 0.15 for the column sets Rtx-PCB/BPX-50 and Rtx-Dioxin2/BPX-50, respectively. The tailing factor obtained is therefore higher with the column set Rtx-Dioxin2/BPX-50 for the two families of micropollutants. The Rtx-Dioxin2/BPX-50 column set allows for finer peaks to be obtained but with a greater tailing factor than for the Rtx-PCB/BPX-50 set. The choice of the column set is therefore difficult from this criterion.

Thus, resolution factors were calculated. Out of the existing 209 PCB congeners, 189 were resolved with the Rtx-Dioxin2/BPX-50 column set compared to 194 with the Rtx-PCB/BPX-50 column set. 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. Out of the 17 toxic PCDD/Fs, the Rtx-Dioxin2/BPX-50 column set allows for all the congeners to be separated. These results are consistent with those obtained by Hoh *et al.* [3] who succeeded in correctly separating the 17 toxic PCDD/F congeners with the Rtx-Dioxin2/BPX-50 column set in the first dimension. With the Rtx-PCB/BPX-50 column set, 2 PCDD/F congeners, including 1,2,3,7,8-PeCDD, are coeluted. 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. As the two column sets are the same to monitor the 18 most relevant PCBs in meat, the Rtx-Dioxin2/BPX-50 column set which allows for the 17 toxic PCDD/Fs to be separated was therefore chosen for the rest of the study. Fig. 1 shows the contour plot obtained with this column set which allows for 189 PCBs and 17 PCDD/Fs to be separated, i.e. 206 dioxin-like micropollutants.

Fig. 1. GC×GC-TOF/MS contour plot of the 209 PCBs and 17 PCDD/Fs with the Rtx-Dioxin2/BPX-50 column set.



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 these contaminants by ASE, defatting and concentration of the extract obtained was proposed. To assess the relevance of this protocol, a matrix contaminated at a known concentration was required. In a first step, the extraction/concentration/clean-up protocol was set up from a spiked freeze-dried matrix to overcome recovery and homogeneity potential problems. The contaminant recovery rates were measured on 5 PCBs spread over the chromatograms obtained after the analysis of 5 extracts by GC×GC-TOF/MS. These recovery rates were within the 70–130% range generally considered as acceptable, with 92 ± 13% for BZ-1, 110 ± 7% for BZ-19, 118 ± 10% for BZ-172, 130 ± 10% for BZ-206 and 130 ± 8% for BZ-209. The standard deviations measured for the same 5 targeted PCBs were between 7.2% and 13.1%.

Secondly, the spiking protocol was applied to fresh ground meat. Seven 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). The two methods coupling an immersion step with a homogenization by blender or Ultra-turrax allowed for the lowest standard deviations to be obtained, and therefore the best homogeneity of the spike. In order to further assess the reliability of these two spiking methods, their reproducibility was then tested. For the majority of the PCBs, the recovery rates are greater when homogenization with a blender is performed. Regarding the standard deviations, the values remain lower than 10% for the majority of the PCBs quantified. More PCBs have standard deviations greater than 10% with the method using the homogenization by Ultra-turrax. The method coupling the immersion of the meat into a contaminant solution and the homogenization with a blender was thus selected for the rest of the study.

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. The linearity of the calibration lines allowing for LODs and LOQs to be estimated was assessed by calculating the coefficients of determination. All the values are greater than 0.980 except for one of the dioxin congeners (0.974). The range of linearity spans from 50 to 5000 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.

Losses of PCBs and PCDD/Fs during cooking were finally estimated. For medium meat, losses vary from 0.9% to 21.7% for PCBs and from 4.5% to 6.8% for PCDD/Fs. These results will be validated on rare and well done meat.

IV. CONCLUSION

The GC×GC-TOF/MS method assessed in this study allows 206 dioxin-like micropolluants to be simultaneously analyzed, thanks to the use of a Rtx-Dioxin2/BPX-50 column set. To be able to study these compounds at trace level 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 enabled also to obtain a matrix of ground beef homogeneously spiked with the dioxin-like micropolluants. Finally, these methodological breakthroughs were used to study the fate of PCBs and PCDD/Fs during cooking. These data may contribute to the improvement of risk assessment methodologies of chemicals in food.

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