High throughput GC-HRMS acquisition methods for the analysis of PCDD/Fs and PCBs in biological matrices

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) have been recognized as highly toxic and ubiquitous environmental contaminants. They have structure-related toxicity at low exposures and hence sample preparation and instrumental quantification requires determination of individual congeners at very low levels (ppb and ppt). For this reason sample preparation involves sophisticated and delicate multistage steps, which can also require several days. In the last few years a lot of effort has been expended in the development of high throughput methods to increase lab productivity and food safety, in the context of enhanced capacity to cope with potential dioxin crises [1]. In this paper, two Gas Chromatography coupled with High Resolution Mass Spectrometry (GC-HRMS) alternative acquisition methods have been developed for confirmatory analysis of Dioxins and PCBs in biological samples (food, feed and serum). The first method proposed uses a classical GC method and Dual Data Acquisition, a new system developed by Thermo Fisher Scientific, (Bremen, Germany). The second method is based on Fast GC using smaller and narrower chromatographic columns. Both methods increase lab productivity while maintaining similar performance of our ISO17025 validated method for the confirmatory analysis of Dioxins and PCBs in biological matrices. When coupling one of these acquisition methods with high throughput sample preparation already available in our lab [1], the quantitative analysis of one sample can be completed within 4 hours.

Materials and methods

Sample preparation in our laboratory provides two fractions per sample, one containing regulated PCDDs, PCDFs and coplanar-PCBs (co-PCBs, #77, 81, 126, 169), here referred to as "Dioxin fraction", and the other one containing mono-*ortho* (MO-, #105, 114, 118, 123, 156, 157, 167, 189) and indicator (I-, #28, 52, 101, 138, 153, 180) PCBs, here referred to as "PCB fraction". Analytical methods were developed accordingly. Nonane puriss analytical-reagent grade standard for GC, purchased from Fluka (Steinheim, Germany) was the injection solvent. A six point calibration curve for PCDD/Fs and co-PCBs, ranging from 0.05 to 10 pg/µL for Tetra- and Penta-congeners, was prepared from the following standard solutions: native PCDD/Fs and native co-PCBs standards were respectively the NK-ST-B4 and the BP-CP81 solutions, both purchased from Wellington (Wellington Laboratories, Guelph, Canada); ^{13}C-labelled internal standard (ISTD) for PCDD/Fs and co-PCBs was the EDF-4144 solution and ^{13}C-labelled recovery standard (RS) was the EDF-4145, both obtained from CIL (Cambridge Isotope Laboratories, Tewksbury, Massachusetts, United States). The EDF-4144 standard was also diluted and used as spiking solution for the quantification of native congeners with Isotope Dilution (ID) mass spectrometry; while the EDF-4145 was diluted and used to assess recoveries. An eight point calibration curve for MO- and I-PCBs, ranging from 0.4 to 140 pg/µL, was prepared using the following standards: EC-4987 (native MO-PCBs), EC-5179 (native I-PCBs), EC-4058 (^{13}C-labelled ISTDs for I-PCBs) and EC-1414 (^{13}C-labelled PCB 80 RS) were

purchased from CIL, and MBP-MXK ^{13}C-labelled ISTDs for MO-PCBs was obtained from Wellington. ^{13}C-labelled ISTD solutions and ^{13}C-labelled PCB 80 RS solution were diluted and used to quantify the native compounds and assess recoveries for this fraction. GC-HRMS was used for the identification and quantification of all the congeners. For all the experiments in this study, a Double Focusing Sector (DFS) mass spectrometer from Thermo was connected by two heated transfer lines (270°C) to two GCs (Trace 1310 Series), each one provided with Split/Splitless (SSL) injector and connected to a TriPlus RSH autosampler. The HRMS instrument was operated in selected ion monitoring (SIM) mode with mass resolution at least 10,000 at a 10 % valley, using Perfluorotributylamine (PFTBA or FC-43) as the reference compound. The ion source was maintained at 260°C. Each chromatographic peak was defined by the acquisition of a minimum of 10 mass spectra. For part of this study, Dual Data Acquisition modules were installed on each GC. A DB-5ms 60 m × 0.25 mm × 0.25 µm column from Agilent (Agilent Technologies, Santa Clara, California United States) was used to separate PCDD/Fs and co-PCBs. Injection volume was 1 µL and the SSL injector was operated in splitless mode at 290 °C, with split flow 70 mL/min for 2 minutes and purge flow 5 mL/min. Helium carrier gas was maintained at constant flow rate of 1 mL/min. The oven temperature was maintained at 120 °C for 5 min, ramped at 25 °C/min to 250 °C and held for 5 minutes, then ramped at 2.5 °C/min to 285 °C for 16 minutes, and eventually at 10°C/min to 300°C for 5 minutes. The total temperature program took 51.7 min. An HT-8 25 m × 0.22 mm × 0.25 µm column from SGE (by Trajan Scientific and Medical, Ringwood, Victoria, Australia) was deployed for the separation of MO- and I-PCBs. A volume of 1 µL was injected in splitless mode at 290 °C, with split flow 70 mL/min for 2 minutes and purge flow 5 mL/min. Helium was used as the carrier gas at constant flow rate of 0.8 mL/min. The oven temperature was maintained at 140 °C for 2 min, ramped at 15.0 °C/min to 220 °C held for 7.5 min, ramped at 6.0 °C/min to 250 °C, ramped at 2.0 °C/min to 265 °C, and finally ramped at 28 °C/min to 320 °C, for a total separation time of 30 min. In this instance, mass spectrometric traces of two ions, for each native, ^{13}C-labelled ISTD and RS compounds were recorded in SIM. For the Fast GC experiments, both Dual Data modules were disconnected from the GCs. An Rtx-5 20 m × 0.18 mm × 0.20 µm column from Restek (Bellefonte, Pennsylvania, United States) was used for PCDD/Fs and co-PCBs. A volume of 0.7 µL was injected on the SSL injector in splitless mode at 290 °C, with split flow 70 mL/min for 2 minutes and purge flow 5 mL/min. Helium carrier gas was maintained at constant flow rate of 1 mL/min during all the temperature program reported in Table 1 (left side), for a total run time of 17 minutes. For the PCB fraction, an HT-8 10 m × 0.10 mm × 0.10 µm column from SGE was deployed. Injection volume for this narrow bore column was 0.3 µL. SSL injector was heated at 290 °C and working in splitless mode at 70 mL/min for 2 minutes and purge flow of 5 mL/min. Helium carries gas flow was 0.45 mL/min during the temperature program reported in Table 1 (right side), for a total run time of 11.5 minutes.

Table 1: Temperature programs for the analysis of Dioxins (left) and PCBs (right) in Fast GC

Dioxin fraction	Rate (°C/min)	Temp (°C)	Hold time (min)
Initial		120	1.3
1	60	225	2.8
2	20	232	1.6
3	30	245	0.8
4	2.5	253	0
5	20	283	2.5
6	80	310	0.5

PCB fraction	Rate (°C/min)	Temp (°C)	Hold time (min)
Initial		60	0.35
1	45	200	0
2	5	215	0
3	7	235	0
4	20	255	0
5	40	300	0

Results and discussion

***Dual Data Acquisition*:** The Dual Data module consists of a switching valve where two columns are connected: the chromatographic column and a restriction, where only Helium as the carrier gas is flowing. The valve is able to divert the flow entering the ion source: during method waiting time (from the injection to the elution of the first analyte) pure Helium enters the ion source and column flow is directed to the purge. As soon as the first eluting compound approaches, the Dual Data valve switches and diverts column flow into the ion source for MS acquisition (measuring time), and Helium from the restriction to the purge. DFS equipped with two GCs, both with Dual Data modules, allows optimization of the acquisition rate, as the waiting time of one method overlaps with the measuring time of the other method, and *vice versa*. As described in the Materials and Methods section, in this case, the DB-5 60 m column and HT-8 25 m column were used for the analysis of Dioxins and PCBs respectively. The two chromatographic methods used in these experiments were part of an already validated procedure for the analysis of Dioxins and PCBs in biological matrices in our laboratory with proved chromatographic performances for biological matrices [2]. In particular, our Reference chromatographic method for the Dioxin fraction was divided into ~15 minutes waiting time and 37 minutes acquisition time; while the method for PCBs consisted of 7.5 minutes waiting time and 17.5 minutes acquisition time. Thus the total run time required per sample (two fractions) was around 76.7 minutes and 18 samples could be run per day. The introduction of the Dual Data Module allowed the measurement of one sample in 54.5 minutes and 26 samples per day, with almost 45 % productivity increase (Table 2). The Dual Data modules did not exert a discernible effect on peak shape and calibration curves, while procedural blanks and Quality Control samples (QCs, prepared in house) injected when modules were installed gave comparable results to our classical acquisition method. Full validation of the method involving Dual Data modules was not carried out, but our results showed that this new technology is valuable tool for faster quantification of Dioxins and PCBs in biological matrices.

Fast GC: Fast GC methods, using a shorter and narrower column, were developed for high throughput analysis of PCDD/Fs and PCBs. No Dual Data module was installed in this set of experiments, because preliminary results showed poorer peak shape quality for these sharper peaks. Chromatographic resolution with such columns was lower in comparison with our classical acquisition method, but it was still fit-for-purpose. Possible MS interferences were resolved chromatographically, making sure that all ^{13}C-labelled PCDFs were separated from all native Dioxins, as well as co-PCBs (#126 from Tetra compounds and #169 from Pentas). GC separation of 1,2,3,4,7,8- HexaCDF and 1,2,3,6,7,8-HexaCDF isomers was better than 25 % peak to peak, as required by the EU Regulation for food and feed matrices [3] (Figure 1). The method developed for PCB fraction assured the separation of tri-chlorinated CBs, like #31 and 28, as well as of Penta-chlorinated congeners such as #163 and 138, and 123 and 118. Also the hexa-chlorinated CBs #156 and 157 were completely resolved. The DFS analyzer was required to work with high acquisition frequency because of peak squeezing. To assure the acquisition of at least 10 points for each chromatographic peak, two ions were recorded for each native compound, but only one for the ^{13}C-labelled ISTD and RS, as their concentration was between 5 to 50 times higher than native compounds in the calibration range. Dwell times for each congener were optimized to achieve the highest sensitivity while retaining good peak shape. Peak squeezing also improved the Signal-to-Noise ratio (S/N), allowing the detection of 19 fg TCDD on column, with S/N 202 (Figure 2). LOD and LOQ calculation is still ongoing, but they are expected to be lower than for our reference method. A six point calibration curve was injected for both fractions, and Relative Response Factor (RRF) relative standard deviation (RSD) was lower than 15 % for all the congeners, as required by EU Regulation. Fast GC methods allowed the analysis of a single sample, both fractions, in 28.5 min, and hence 50 samples per day. Productivity increase using Fast GC could be up to 177 % (Table 2).

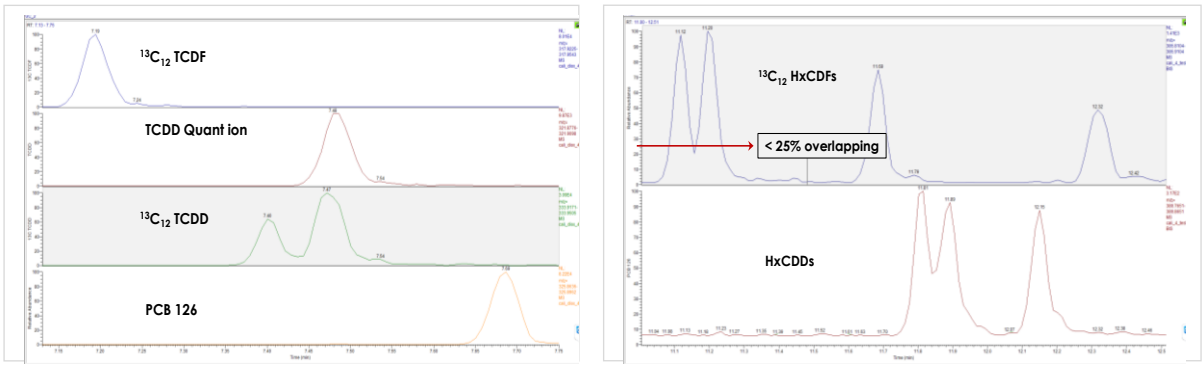

Figure 1: Chromatographic separation of Tetra- and Hexa-CDD/Fs using Fast GC

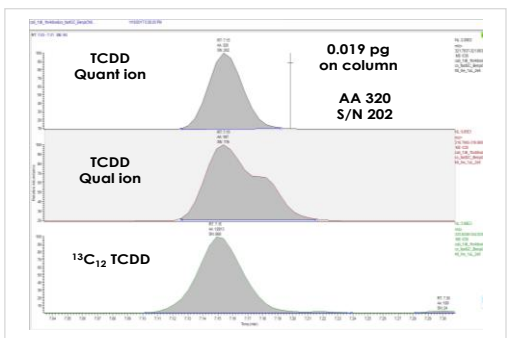

Figure 2: Signal of 19 fg of TCDD on Rtx-5 column analysed in Fast GC

Table 2: Comparison between productivity using our Reference method, Dual Data method, and Fast GC method

	Time/single sample(min)	Number of samples/day	Productivity increase
Reference method	76.7	18	
Dual Data method	54.5*	26	~45 %
Fast GC method	28.5	50	~177 %
* First sample of the series 62 min (waiting time of the first injection, 7.4 min, cannot be saved)			

Full validation of the Fast GC method is still ongoing, but these results show this technique could be used for the confirmatory analysis of Dioxins and PCBs in biological matrices.

References

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2. Focant J-F, Eppe G, Pirard C, De Pauw E (2001), *Journal of Chromatography A*, **925** 207-221
3. Commission Regulation (EU) No 589/2014 (2014) and Commission Regulation (EU) No 709/2014 (2014)