GCxGC-IDTOFMS for the measurement of dioxins and PCBs in foodstuffs: a comparison with GC-IDHRMS, GC-IDQISTMS/MS, and DR-CALUX.

Jef Focant[1], Gauthier Eppe[1], Marie-Louise Scippo[2], Anne-Cécile Massart[1], Catherine Pirard[1], Guy Maghuin-Rogister[2], Edwin De Pauw[1]

[1]CART Mass Spectrometry Laboratory, Chemistry Department, University of Liège
[2]CART Laboratory of Analysis of Foodstuffs of Animal Origin, University of Liège

Introduction

The measurement of PCDD/Fs and PCBs at the ultra-trace level is challenging. The reasons are the low levels to measure (ppb to ppq) and the large number of compounds to consider (>35 out of a total of >400). GC coupled to ^{13}C-labelled isotope dilution (ID) sector selected ion monitoring (SIM) HRMS is the reference method used for measurement of PCDD/Fs and PCBs. Today, alternatives do exist.

In the MS area, GC coupled to low resolution quadrupole ion storage mass spectrometer (QISTMS) operated in multiple reaction monitoring (MRM) tandem-in-time mode (MS/MS) has been extensively studied. Both sector SIM-HRMS and MRM-QISTMS however suffers from scan rate limitations and are not compatible with the measurement of a large number of compounds characterized by different masses inside a same acquisition window. Time-of-flight mass spectrometry (TOFMS) offers the advantage of a comprehensive mass analysis in a broad dynamic range. GC-TOFMS limited sensitivity can be improved by operating in fast GC mode (peak are compressed and give higher signals) but the already limited chromatographic resolution is further reduced and undesirable co-elution problems arise. Comprehensive two-dimensional gas chromatography (GCxGC) also offers zone compression but without scarifying chromatographic resolution[1]. GCxGC-IDTOFMS has recently been successfully used to analyze soil and ashes for PCDD/Fs[2], as well as large sets of analytes (PCBs, OCPs, and PBDEs) in human body fluids[3]. All analytes were separated chromatographically or by mean of mass spectral deconvolution. Biological assays like the dioxin-responsive chemical-activated luciferase gene expression (DR-CALUX) assay have also been largely used for dioxin testing and for global toxicity measurement[4].

The study focused on the development and comparison of a GCxGC-IDTOFMS method with other alternatives for the measurement of 7 PCDDs, 10 PCDFs, 4 non-*ortho*-PCBs, 8 mono-*ortho*-PCBs, and 6 indicator PCBs (Aroclor 1260) in foodstuffs for which regulatory levels have been set by the European Union[5].

Materials and Methods

Fish, pork, and cow's milk samples issued from the DIFFERENCE European project. QC samples and method BCs were regularly run for QA/QC purposes. For the DR-CALUX, matrix-specific reference samples were analyzed by both GC-IDHRMS and DR-CALUX. For MS analysis of PCDD/Fs and dioxin-like PCBs, samples were PLE extracted (Dionex ASE 200) and cleaned-up using the automated Power-Prep™ system (Fluid Management Systems Inc.)[6]. For indicator PCBs, samples were LLE extracted and cleaned-up according to the Beltest I014 method. For DR-CALUX analysis, samples were LLE extracted and cleaned-up according to the method proposed by BioDetection System (BDS)[7]. **GC-IDHRMS.** PCDD/Fs and non-*ortho*-PCBs were measured on an Autospec Ultima (Micromass) coupled to an Agilent 6890 Series GC. The column was a 50m VF-5MS (0.20 mm ID x 0.33 µm df) (Varian). Mono-*ortho*-PCBs and indicator PCBs were measured on an MAT95XL (ThermofinniganMAT) coupled to an Agilent 6890 Series GC. The column was a 25m HT-8 (0.22 mm ID x 0.25 µm df) (SGE)[6]. **GC-IDQISTMS/MS.** PCDD/Fs and non-*ortho*-PCBs were measured using a low resolution Finnigan PolarisQ ion trap mass spectrometer. The separation was performed on a 50m VF-5MS (0.20 mm ID x 0.33 µm df) (Varian). Mono-*ortho*-PCBs and indicator PCBs were measured on a low resolution GCQ (Finnigan). Separation was performed on a 25m HT-8 (0.22 mm ID x 0.25 µm df) (SGE) column[8]. **GCxGC-IDTOFMS.** All analytes were measured on a Pegasus 4D (Leco Corp). A press-tight Restek Uniliner was used in the SSL injector. The column set was a 40m RTX-500

(0.18 mm ID x 0.10 µm df) (Restek Corp.) in the first dimension (^{1}D) and a 1.5m BPX-50 (0.10 mm ID x 0.10 µm df) (SGE) in the second dimension (^{2}D). The modulation period (P_M) was 4s. The hot pulse duration was 750ms. The modulator and secondary oven temperature offset were 40 °C and 20 °C. The collected mass range was 100–550amu. The scan rate was 60 scans per sec. **DR-CALUX.** It originated from BioDetection System (BDS, NL). Analyses were performed by exposing the cells (triplicates, 96-well plates) during 24h to sample extracts and to 2,3,7,8-TeCDD standard solutions in DMSO diluted in culture medium. See previous report for details[9].

Results and Discussion

GCxGC separation and calibration

GCxGC separation has been optimized to enable all analytes to be separated in a single injection (Figure 1). However, we decided to consider two separate injections because data handling and processing were major issues for the GCxGC-TOFMS instrument and also because the sample preparation we use produces two separate fractions (indicator PCBs + mono-*ortho*-PCBs and PCDD/Fs + non-*ortho*-PCBs). The high thermal stability of the carborane and 50% phenyl phases permitted temperatures as high as 370°C, ensuring the production of narrow ^{2}D peaks (w_b=150ms). All PCDD/Fs and PCBs were baseline-separated with the exception of 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD for which attention was needed for the assignment of potentially overlapping ^{2}D peaks. Due to mass spectral similarities, the two traces cannot be automatically deconvoluted. The chromatographic separation was still in agreement with the European Commission Directive 2002/69/EC[10]. PeCB-123 and PeCB-118, as well as HxCB-163 (not monitored here) and HxCB 138 (Aroclor 1260) were successfully separated. Two ions were summed for quantification of all compounds (details available upon request). For the low end of the calibration curve, only one ^{2}D peak was produced, due to the small amount of compound. Calibration correlation coefficients were greater than 0.999. For mono-*ortho* and indicator PCBs, lowest iLODs were 0.4 pg/µl (S/N > 5). For PCDD/Fs, lowest iLODs were 0.2 pg/µl (S/N > 2). Peaks were only assigned after isotope ratio verification (20% deviation range accepted). The dynamic working range spanned three orders of magnitude.

Comparison of methods on a congener basis

For non- and mono-*ortho*-PCBs, all methods performed similarly (Figure 2). Lower RSDs were reproducibly observed for the reference GC-IDHRMS method. For PCDD/Fs, levels were much lower than for PCBs and can be considered as the background levels currently encountered in the EU. For fish, both GCxGC-IDTOFMS and GC-IDQISTMS/MS compared well with GC-IDHRMS. However, although the RSDs for GC-IDHRMS were 7–14%, GCxGC-IDTOFMS and GC-IDQISTMS/MS RSDs ranged from 10 to 60% and from 5 to 30%, respectively. The calibration standard concentrations were selected to cover as much as possible of the working range but out-of-calibration situations can always arise, depending on the congener distribution in the sample. From this study, it appeared that GCxGC-IDTOFMS was more affected by this type of out-of-calibration situation. In the case of pork (30 g sample size) and milk (130 g sample size), which are characterized by low background levels, the RSDs were higher (up to 90%). Despite the limited precision, the congener distribution was still well defined for all matrices and can be used to describe specific matrix patterns for contamination source tracking or fingerprinting of sets of samples.

Comparison of methods on a TEQ basis

Samples were run on the DR-CALUX and data were compared to the GC-IDHRMS (WHO TEFs). All DR-CALUX tests met the European guidelines[10]. The quantification limits were 0.08, 0.02 and 0.03 pg DR-CALUX TEQ/g product for fish, pork and milk, respectively. Data were corrected using matrix-specific reference samples to ensure similar congener distributions in both the reference and the unknown samples and reduce the effect of the differences between TEFs and REPs. The ratio of the total TEQ (sum of PCDD/Fs and dioxin-like PCBs) concentration measured by GC-IDHRMS over the DR-CALUX response was used as a correction factor applied to the raw DR-CALUX data. For milk, the reference sample pattern was different from a classical background congener distribution and this influenced the raw data correction. The direct consequence was erroneous low recovery for the milk sample, inducing an over-estimation of the corrected CALUX data. The GCxGC-IDTOFMS and GC-IDQISTMS/MS responses for PCDD/Fs and PCBs compared favorably with GC-IDHRMS (Figure 3). The lower GCxGC-IDTOFMS value for pork was due to the lower reported concentration for PeCB-126, high contributor

(TEF=0.1) to the TEQ. The MS-based method TEQs and the DR-CALUX reference sample corrected TEQ compared well, although DR-CALUX RSDs were significantly higher (10–28%), which is still acceptable for a screening method[10].

Comparison of method costs

The cost estimate depends on several parameters, the comparison is based on similar operating conditions. Costs of alternative techniques are not much lower than for GC-IDHRMS (Table 1). The cost distribution is, however, different. For the three alternative methods, if the cost contribution related to the measurement itself is reduced, the cost for scientific employment is increased (data processing, reviewing, and reporting). For GCxGC-IDTOFMS, multi-group analyte measurement can be carried out in 45min (0.8 analyte per min) but additional efforts are needed to speed up data processing and reviewing. Because TOFMS does not operate in SIM mode, all masses included in the defined mass range are collected. This permits the screening of mass spectral data for other unknown compounds. The DR-CALUX approach does not offer congener-specific data and pattern description but permits a price cut of a factor of 2, making it the most economically efficient screening method.

Conclusions

The future integration of dioxin-like PCBs into the EU regulation will help the DR-CALUX assay and the related simple sample preparation scheme to become truly the screening method of choice for global toxicity evaluation at a moderate cost. Both of the MS-based alternative methods are capable of describing PCB and PCDD/F congener profiles with reasonable accuracy (trueness and precision) when source identification is required for contamination tracking. GCxGC-IDTOFMS and GC-IDQISTMS/MS should therefore be defined as complementary methods to GC-IDHRMS, rather than screening methods. The study showed that the particularly large ion volume in the TOFMS source makes it unlikely to be as influenced by sample extract quality as other classical small source types (HRMS and QISTMS), a significant advantage for routine use.

Acknowledgements

The authors acknowledge the University of Liège, the Walloon Region, and the FEDER and FSE funds for their financial support. Leco Corp. is acknowledged for technical support.

References

[1] Dimandja J.-M.D. (2004) *Anal. Chem.* 76: 167A.

[2] Focant J.-F., Reiner E.J., MacPherson K., Kolic T., Sjödin A., Patterson D.G.Jr., Reese S.L., Dorman F.L. and Cochran J.W. (2004) *Talanta* 63: 1231.

[3] Focant J.-F., Sjödin A., Turner W.E. and Patterson D.G.Jr. (2004) *Anal. Chem.* 76: 6313.

[4] Behnisch P., Hosoe K. and Sakai S.-I. (2001) *Environ. Int.* 27: 413.

[5] Council Regulation No 2375/2001 of 29 November 2001, setting maximum levels for certain contaminants in foodstuffs, Official Journal of the European Communities L32 (2001) 1.

[6] Focant J.-F., Eppe G., Pirard C. and De Pauw E. (2001) *J. Chromatogr. A* 925: 207.

[7] Training-manual BDS' DR-CALUX® bioassay, Version BDS-031/b/2002, BioDetection Systems, NL.

[8] Eppe G., Focant J.-F., Pirard C. and De Pauw E. (2004) *Talanta* 63: 1135.

[9] Scippo M.-L., Eppe G., De Pauw E. and G. Maghuin-Rogister (2004) *Talanta* 63: 1193.

[10] Commission Directive 2002/69/EC of 26 July 2002, Official Journal of the European Communities, (6.8.2002) L209/5.

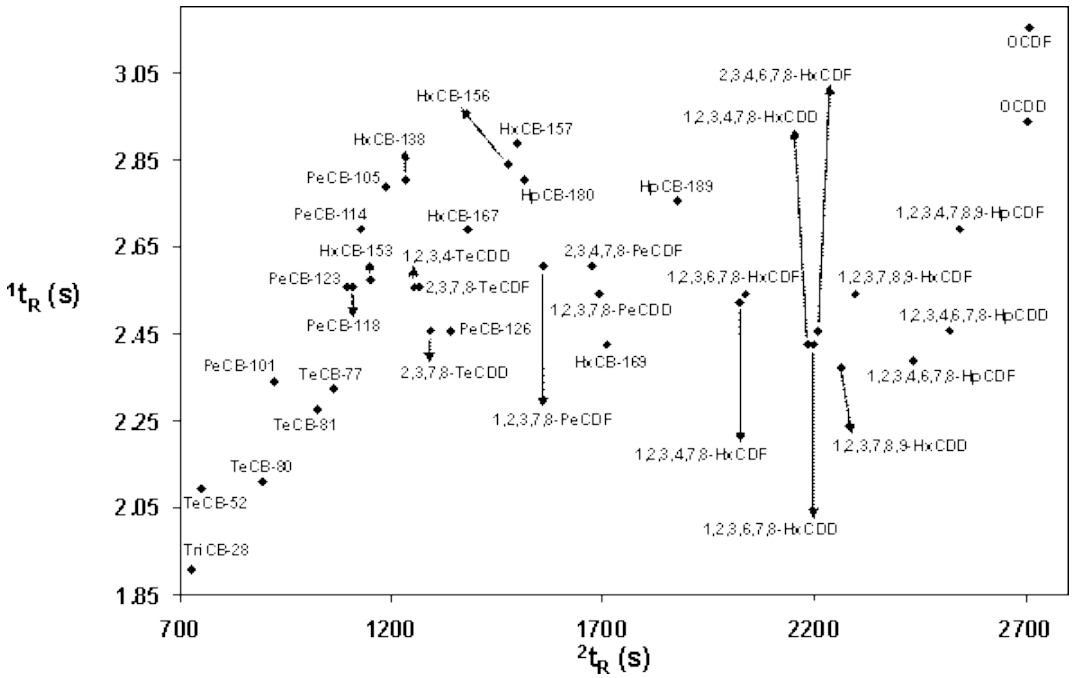

Figure 1: GCxGC-IDTOFMS apex plot based on the retention data of the 37 compounds.

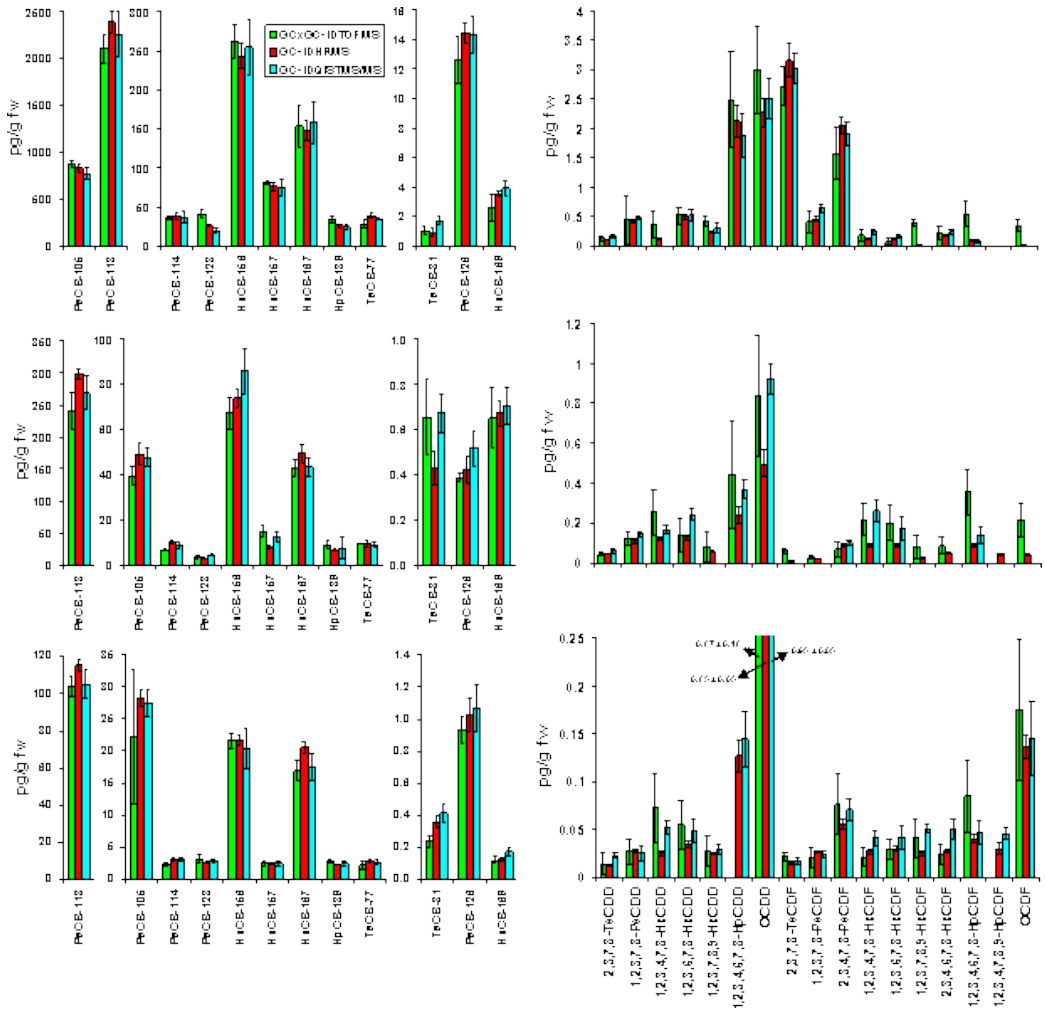

Figure 2: Comparison of GC-IDHRMS with GCxGC-IDTOFMS and GC-IDQISTMS/MS for the measurement of non-*ortho*, mono-*ortho*-PCBs, and PCDD/Fs in fish, in pork, and in milk (from top to bottom) samples (n=6).

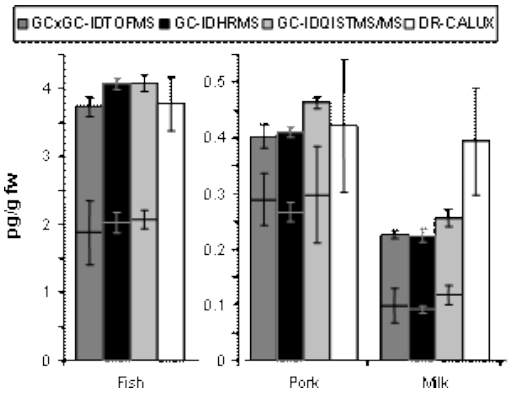

Figure 3: TEQ comparison of GC-IDHRMS with GCxGC-IDTOFMS, GC-IDQISTMS/MS, and DR-CALUX (reference sample corrected). Top: dioxin-like PCB. Bottom: PCDD/Fs.

Table 1 Estimated percent distribution of the cost of the measurement methods (feed samples).

	GC-IDHRMS	GCxGC-IDTOFMS	GC-IDQISTMS/MS	DR-CALUX[a]
Scientist employment	23	35	35	36
Extraction	11	8	11	7
Clean-up	28	27	33	29
Measurement	38	30	21	8
Licensing and royalties	-	-	-	20
Cost per sample (Relative)	+++++	+++++	++++	+++

[a]Cost based on duplicate measurements.