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Trace analysis of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and WHO polychlorinated biphenyls in food using comprehensive two-dimensional gas chromatography with electron-capture detection

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Abstract

Trace analysis of 2,3,7,8-polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and the 12 WHO-PCBs (four non-*ortho* and eight mono-*ortho* congeners that have been assigned toxic equivalence factors, TEFs, by the World Health Organisation) was conducted by comprehensive two-dimensional gas chromatography with a micro electron-capture detector (GC × GC-μECD). Four food matrices (fish oil from herring, spiked cows' milk, vegetable oil and an eel extract) were analysed by two GC × GC laboratories, and four GC–HRMS laboratories generated reference values. The two GC × GC laboratories used different column combinations for separating the target analytes. For the first dimension, non-polar DB-XLB and VF-1 columns were used, and for the second dimension, an LC-50 liquid crystalline column with unique selectivity for planar compounds. The congener-specific and total toxic equivalence (TEQ) data obtained using DB-XLB × LC-50 were in good agreement with results obtained by the GC–HRMS laboratories. The WHO-PCB data obtained with the VF-1 × LC-50 combination was also good, but the PCDD/F concentrations were sometimes overestimated due to matrix interferences. GC × GC-μECD using DB-XLB × LC-50 seems to fulfil the European Community requirements of a screening method for PCDD/F and WHO-PCB TEQ in food.

Keywords: Comprehensive two-dimensional gas chromatography; GC×GC; μECD; PCB; PCDD/F; TEF; TEQ; Quantification; Food; Dioxins; GC–HRMS

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) constitute three classes of structurally related chlorinated aromatic hydrocarbons. Due to their hydrophobic character and resistance to metabolic degradation these substances have been found in a wide range of biological samples, where they exist as complex congener mixtures. Seven 2,3,7,8-substituted PCDDs and 10 PCDFs are generally considered the most persistent and toxic PCDD/F congeners, since they have toxic properties similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is the most toxic congener of this group of compounds [1]. Their toxic properties include carcinogenicity, immunotoxicity and the induction of diverse adverse effects in reproductive, developmental and endocrine systems. To facilitate evaluation of the risks they pose, the concept of toxic equivalence factors (TEFs) has been developed. TEFs can be used to establish the total toxic equivalence (TEQ) of PCDD/Fs (from here on also referred to as dioxins) mixtures present in various matrices such as animal tissues, soil, sediments and water. A basic assumption underlying the TEF concept is that the contributions to the total toxicity made by all congeners are additive. All compounds included in the TEF scheme should fulfil the following criteria: they must have a structural relationship to dioxins, bind to the Ah-receptor and induce Ah-receptor-mediated biochemical and toxic responses [2]. Furthermore,

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the compounds must be persistent and accumulate in the food chain. Four non-*ortho* and eight mono-*ortho* substituted polychlorinated biphenyls (the 'WHO-PCBs': CBs 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189) also show dioxin-like effects and have been assigned TEF values by the World Health Organisation (WHO) [2,3]. The TEFs of most WHO-PCBs are much lower than those of the most toxic dioxins. However, since they are generally present at much higher concentrations, they can contribute significantly to the total TEQ.

The determination of dioxins and WHO-PCBs in food is complicated by the fact that they are usually present in pg/g concentrations, while the matrices involved are complex. Therefore, both highly selective and sensitive analytical techniques are required. Due to the large difference in concentrations between the groups, the planar compounds are often separated from less planar compounds. The concentrations of the most toxic dioxins and non-*ortho* CBs are often three orders of magnitude lower than those of the most abundant CBs, 138, 153 and 180 [4].

The analysis of dioxins and WHO-PCBs is currently expensive. This is partly due to the time-consuming pre-treatment and clean-up steps required, which are commonly based on more than one chromatographic separation, and partly because it involves the use of gas chromatography–high-resolution mass spectrometry (GC–HRMS). The notion that GC–HRMS is needed for accurate and congener-specific determination of dioxins and dioxin-like CB analysis is well established. However, although the sensitivity and selectivity offered by GC–HRMS is unrivalled, throughput is low and cost is high.

The European Community (EC) has recently set maximum residue limits (MRLs) for dioxins in various foods and feedstuffs. In order to monitor dioxin levels on a routine basis, a higher-frequency method of analysis is needed. Consequently, there is a clear need for methods that can generate results comparable to GC–HRMS, but at a lower cost [5].

GC × GC is a powerful separation technique that was invented in the late 1980s and has been further developed in the last decade [6,7]. Much higher peak capacities can be obtained than in conventional GC, because each successive small fraction eluting from the conventional-size first-dimension column is subjected, in real time, to a second, orthogonal separation, on a relatively short (ca. 1 m) second-dimension column with different separation characteristics. The key element of a GC × GC system is the modulator, which accumulates and focuses fractions eluting from the first column and re-injects them into the second column. Developments in recent years have resulted in numerous modulators based on thermal heating, cryogenic cooling or valve switching [8–14]. As a result of the focusing effect achieved from the modulation, the signal-to-noise ratios (S/N) increase and limits of detection (LODs) decrease compared to one-dimensional GC (normally, S/N increases 5–10-fold). Despite this S/N enhancement only the micro electron-capture detector (μECD) provides enough sensitivity for trace-level organochlorine pollutant analysis. However, although GC × GC-μECD is suitable for the analysis of the target compounds, its application to WHO-PCBs and dioxins has mainly focused on the separation of standards or technical mixtures or, in some cases, the identification of CBs present in a few biological samples [14–19].

As regards modulation, a recent study showed that, from a chromatographic point of view, all cryo-modulators are suitable for dioxin analysis [13]. As for the selection of first- and second-dimension columns, a liquid crystalline column (LC-50) column is to be preferred as the second-dimension column, because of its unique selectivity for planar compounds resulting in the excellent separation of the target analytes from matrix constituents. When combined with a highly efficient non-polar (DB-XLB) column, complete separation of the 29 target compounds is achieved [19]. The step presented in this study, is the validation of the procedure through quantitative analysis of the 17 2,3,7,8-substituted PCDD/Fs and the 12 WHO-PCBs in food samples (fish oil from herring, spiked cows' milk, vegetable oil and an eel extract) using GC × GC-μECD and GC–HRMS. Preliminary results of this study were previously presented in brief by Danielsson et al. [20].

2. Materials and methods

2.1. Chemicals

Two sets of standard solutions were used, one for method development and one for quantification. The former set of solutions consisted of one solution with all 209 CBs (a mixture of C-CS-01 through C-CS-09 from Accustandards, New Haven, CT, USA) and one containing the 2,3,7,8-substituted dioxins (EPA-1613CSS; Wellington Laboratories, Guelph, ONT, Canada) and CBs 77, 126 and 169 (Cambridge Isotope Laboratories, Andover, MA, USA) which was utilised as a retention time reference standard. For quantification, the same solution as described above was used for dioxins and non-*ortho* CBs, but for the mono-*ortho* CBs, a solution containing the 12 WHO-PCBs was used (EC-4935; Cambridge Isotope Laboratories). An internal standard (IS) solution, containing CB 79, 1,2,3,4,6,7,9-HpCDD, 1,2,3,4-TCDD (each at 5 pg/μL) and CB 159 (at 100 pg/μL) and a syringe spike solution, containing 1,2,3,4-tetrachloronaphthalene (TCN; at 16 pg/μL) and octachloronaphthalene (OCN; at 8 pg/μL) were also used. All standards were made up in toluene.

The solvents used in sample preparation were of high purity: analytical grade diethyl ether from Merck KgaA (Darmstadt, Germany), spectrographic grade ethanol 99.5% from Kemetyl (Haninge, Sweden) and glass-distilled acetone, dichloromethane, *n*-hexane and toluene from Burdick & Jackson (Muskegon, MI, USA). Silica (Kieselgel 60, 0.063–0.200 mm) was acquired from Merck, and Celite® 545 from Fluka (Buchs, Switzerland).

2.2. Samples

Four sample types were analysed, fish oil from herring, spiked cows' milk, vegetable oil and an eel extract. The herring was caught in May 2000, west of the Shetland Islands. The milk and vegetable oil were purchased from a supermarket in The Netherlands and were fortified with the 17 2,3,7,8-substituted PCDD/Fs using a standard solution containing all congeners (Wellington). The non-*ortho* CBs were added from standard solutions of the individual congeners and the mono-*ortho* CBs were spiked using a mixture from The Netherlands Institute of Food Safety (RIKILT, Wageningen, The Netherlands). The following individual congeners were added to the milk to better represent the congener profile normally found in milk: 2,3,4,7,8-PeCDF, OCDF, 1,2,3,4,6,7,8-HpCDD and OCDD. The milk was fortified with a total TEQ of about 10 pg TEQ/g lipid. The vegetable oil was spiked in a similar way to obtain a congener profile resembling the profile found in herring oil. For this purpose, additional amounts of 2,3,4,7,8-PeCDF, 1,2,3,4,6,7,8-HpCDF, OCDF, 1,2,3,4,6,7,8-HpCDD and OCDD were added. The target level was around 3 pg WHO-PCB-TEQ and 3 pg PCDD/F-TEQ per gram of oil. The eel extract was obtained by extracting a composite sample derived from eels from various freshwater locations in The Netherlands. After extraction, 5 g aliquots of fat were cleaned over a silica column impregnated with sulphuric acid. The solvent was evaporated and the residue re-dissolved in *n*-heptane. Each laboratory received 5 ml of eel extract, equivalent to 4 g of fat. All samples were prepared by RIVO and were distributed to the six participating laboratories (denoted laboratories A, B, C, D, E and F) as coded samples. Thus, the contaminant levels were unknown to the analysts.

2.3. Sample preparation

Each laboratory used its own extraction, clean-up and instrumental analysis methods with the exception of laboratory F that obtained aliquots of the final extracts of laboratory E. All four laboratories (A through D) that generated GC–HRMS reference data used isotope-labelled ISs and isotope dilution quantification. These methods will not be discussed further as they have all been thoroughly validated through participation in laboratory intercalibrations. However, the sample preparation method of laboratory E will be described in some detail as it was combined with GC × GC-μECD (in laboratories E and F).

Roughly the same extraction and sample pre-treatment was used for GC × GC-μECD as for conventional GC–HRMS. However, other ISs had to be added, as the μECD does not differentiate between ^{13}C-labelled and unlabelled congeners. All samples were fortified with 40 μL of IS before extraction (milk) or clean-up (oils and eel extracts). Milk aliquots ($n = 6$) of about 150 ml each were mixed with 50 ml of sodium oxalate-saturated ethanol and then liquid-liquid extracted three times with 225 ml diethyl ether/*n*-hexane (7/10) [21]. Ethanol (99.5%; 50 ml) was added and the solvents were removed by rotary evaporation at reduced pressure and a temperature of 30 °C. If an aqueous residue was observed towards the end of the evaporation, more ethanol was added and the evaporation was repeated. The fat content was determined gravimetrically. For the fish oil ($n = 6$) and the vegetable oil ($n = 1$) analyses, portions of about 3 g oil were used. The milk fat and the oils were dissolved in *n*-hexane and transferred to a multilayer silica column (Ø 35 mm) containing (from the bottom): glass wool, 6 g KOH-silica, 3 g silica, 17 g 40% H_2SO_4 on silica (w/w), 7 g 20% H_2SO_4 on silica (w/w), 3 g silica and 7 g Na_2SO_4. Prior to use, the silica columns were washed with *n*-hexane (2 ml × 100 ml). The samples were eluted with 200 ml *n*-hexane, and then the volumes were reduced to approx. 1 ml by rotary evaporation. In the next step, an activated carbon column was used to fractionate the target compounds according to planarity. From this point onwards, the eel extracts ($n = 2$) were also included. The method used was a modified version of the EPA method 1613, Revision B [22]. Activated AX-21 carbon (Anderson Development, MI, USA) was mixed with Celite in the proportions 7.9/92.1. The mixture was cleaned by Soxhlet extraction in toluene for 12 h and dried at 130 °C overnight. The carbon/Celite mixture (0.5 g) was packed in the centre of a glass pipette (10 ml, cut at both ends) with glass wool on either side. Before use, the column was washed with 4 ml dichloromethane (DCM)/methanol/toluene 15/4/1 (v/v/v), 1 ml DCM and 5 ml *n*-hexane. The extracts were transferred to the column with 3 × 1 ml *n*-hexane and eluted with 30 ml *n*-hexane followed by 40 ml *n*-hexane/DCM, 1/1 (v/v) and then 40 ml toluene. Before the elution of fraction 3, the column was turned upside down. This elution scheme resulted in three fractions. Most of the di- through tetra-*ortho* CBs were recovered in fraction 1, the mono-*ortho* CBs in fraction 2, and the non-*ortho* CBs and dioxins in fraction 3. After reducing the solvent volume to approx. 1 ml, the samples were transferred to washed, miniature, multilayer silica columns (Ø 5 mm) containing KOH-silica, silica, 40% H_2SO_4-silica and Na_2SO_4, and then eluted with 8 ml *n*-hexane. Prior to injection, 40 μL of the solution containing TCN and OCN were added to serve as both syringe spike and retention reference standards. Finally, the samples were evaporated to ca. 30 μL under a gentle stream of nitrogen.

2.4. Instrumental analysis

Six replicate analyses of the spiked milk and the fish oil were performed. Two samples of each type were analysed on three different days to generate information on within-lab repeatability and reproducibility. The vegetable oil that was supposed to contain approx. 3 pg PCDD/F TEQ/g and 3 pg CB TEQ/g was analysed once as a quality control solution. The eel extract was analysed in duplicate in order to assess the analytical performance, excluding the contribution from the extraction, and to examine possible matrix effects during detection. Laboratories A, B, C, E and F analysed all four

sample types, while laboratory D only analysed the fish and vegetable oil.

2.5. GC × GC systems

The GC × GC systems were built from HP6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatographs equipped with a longitudinally modulating cryogenic system (LMCS) (lab E) or a loop-type carbon dioxide jet modulator (KT2002 CO_2 system; Zoex, Lincoln, NE, USA) (lab F), a split/splitless injector and two detectors, a μECD and an FID. For both systems the flow of cryogen was regulated using a needle valve. The LMCS was adjusted to maintain a trapping temperature of ca. 130 °C below the oven temperature and the KT2002 cold-jet temperature was adjusted to 0–10 °C, at an oven temperature of 90 °C. The KT2002 hot-jet temperature and time were 400 °C and 200 ms, respectively. Helium was used as carrier gas at, in most cases, a constant flow of 1.0 ml/min. Lab F, however, used a programmed flow – 1.3 ml/min (21 min), at 0.4 ml/min to 1.6 ml/min (5 min), then at 0.4 ml/min to 1.3 ml/min – for non-*ortho* CB and dioxin analyses. The injection volume and temperature were set at 2 μl and 280 °C, respectively, and all injections were carried out in the splitless mode with the split valve opened 2 min after injection. The μECD was operated at 300 °C with a nitrogen make-up gas flow of 150 ml/min (lab F; 100 ml/min) and a data acquisition rate of 50 Hz. In lab E the modulation period (P_M) was 5 s during method development runs and 6 s during sample runs. Lab F used a modulation period of 8 s for all analyses.

Laboratory E used a 30 m × 0.25 mm × 0.25 μm DB-XLB (proprietary) fused-silica column from J&W Scientific (Folsom, CA, USA) in the first dimension and a 0.15 mm narrow-bore LC-50 (50% liquid crystalline-methylpolysiloxane) from J&K Environmental (Milton, ONT, Canada) with a film thickness of 0.1 μm in the second dimension. A 1.4 m section of LC-50 was used to separate the 209 CBs and for the analysis of mono-*ortho* CBs. For the analysis of dioxins and non-*ortho* CBs, the length of the LC-50 was reduced to 0.9 m because of the strong retention of the dioxins on this column. Because of the high bleed of the LC-50 and the thick walls of the DB-XLB column, a thin-wall 0.15 m × 0.1 mm × 0.1 μm 007-1 (dimethyl polysiloxane) from Quadrex (Woodbridge, CT, USA) was installed as a modulation capillary (between the first- and second-dimension columns). All connections were made using press-fits that were glued using polyimide resin to ensure a durable seal. Both columns were placed in the same oven. For the CB method development the oven temperature programme was: 80 °C (2 min), at 20°/min to 160 °C, at 2 °C/min to 220 °C, then at 3 °C/min to 240 °C and finally at 30°/min to 270 °C, which is the upper temperature limit of the LC-50 column. For the mono-*ortho* CB sample runs, the oven temperature programme was: 80 °C (2 min), at 10°/min to 180 °C, at 1.5 °C/min to 240 °C (2 min), then at 30°/min to 270 °C. In all dioxin and non-*ortho* CB runs, the temperature programme was: 80 °C (2 min), at 30°/min to 210 °C, at 1 °C/min to 230 °C, 0.5 °C/min to 242 °C, and at 40°/min to 270 °C (16 min).

Laboratory F used a 30 m × 0.25 mm × 0.25 μm VF-1 ms (100% methylpolysiloxane) fused-silica column from Varian (Middelburg, The Netherlands) as the first-dimension column and a 0.9 m × 0.18 mm × 0.15 μm LC-50 as the second-dimension column. The columns were coupled via a 1.5 m × 0.1 mm i.d. uncoated fused-silica deactivated column (BGB Analytik, Aldiswil, Switzerland), which served as the modulator loop. Mini press-fits (Techrom, Purmerend, The Netherlands) were used for the connections. The temperature programme for analyses of mono-*ortho* CBs was 80 °C (2 min), at 10°/min to 180 °C, at 1.5°/min to 240 °C (2 min), and at 15°/min to 270 °C (40 min) and for analyses of non-*ortho* CBs and dioxins, 90 °C (2 min), at 30°/min to 210 °C, at 1°/min to 230 °C, at 0.5°/min to 236 °C, and at 40°/min to 270 °C (16 min).

2.6. GC × GC quantification

HP Chemstation software (Agilent Technologies) was used to control the GC instruments and to acquire data. The raw HP Chemstation files were exported as csv-files, which were converted to ASCII text matrix files using software developed by Marriott and Kinghorn (RMIT University, Melbourne, Australia). These files were read into Transform v. 3.3 (Research Systems, Boulder, CO, USA) and presented as contour plots. Both GC × GC laboratories identified peaks by overlaying contour plots of standards and samples. Quantification was performed using the IS technique and raw peak areas from HP Chemstation. The areas of the modulated peaks were integrated and added manually. The following ISs were used: 1,2,3,4-TCDD for tetra- and penta-CDD/Fs and non-*ortho* CBs; 1,2,3,4,6,7,9-HpCDD for hexa- through octa-CDD/Fs; and CB 159 for mono-*ortho*-CBs. CB 81 was not present in the quantification solution and had to be quantified using the relative response factor of CB 77.

3. Results and discussion

3.1. GC × GC method optimisation

Among the mono-*ortho* CBs there are two problematic congener pairs, CBs 118/123 and CBs 156/157, which require a slow temperature ramp for complete separation. A slow ramp is also beneficial for the separation of target compounds from matrix components. In the dioxin fraction, the most problematic congener pairs are: 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD, 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDF, 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDD, and OCDD and OCDF. With the relatively slow temperature ramp used in this study these pairs are almost baseline-separated except OCDD and OCDF on VF-1 × LC-50. A faster ramp would cause the Hx-CDD/F pairs to coelute. However, since they have the same TEF value and the analysis time can be reduced by about

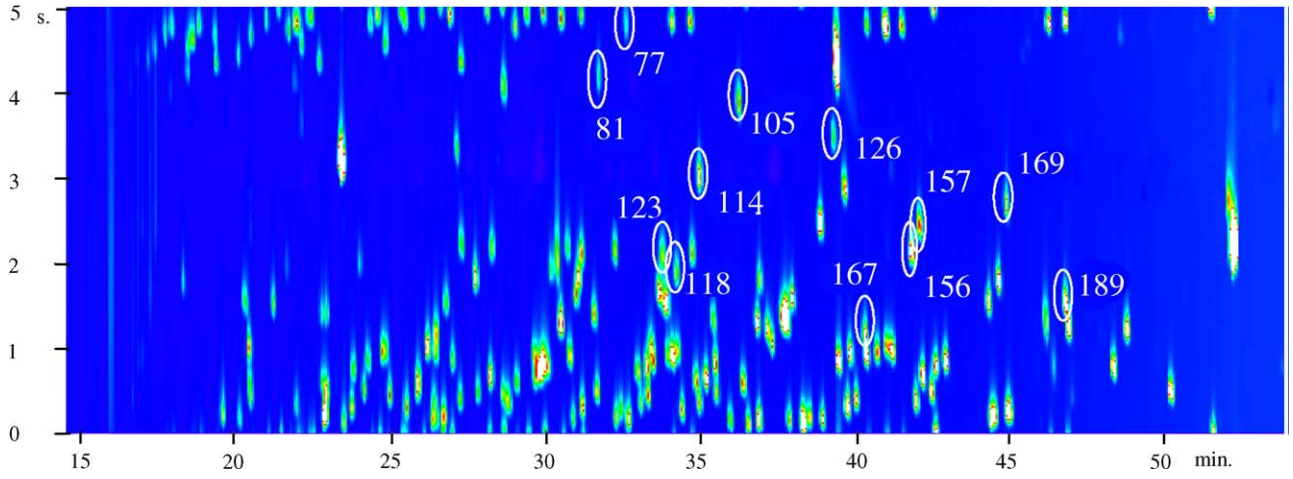

Fig. 1. GC × GC-μECD contour plot of the 209 CB congeners mixture with DB-XLB × LC-50 column combination.

10 min with a somewhat faster temperature ramp, an increase could be useful if a high sample throughput is needed. Nevertheless, for this study, a slow ramp was chosen, as the analysis-time still was acceptable and it was important to separate all analytes in order to obtain congener-specific data for the comparison of GC × GC-μECD and GC–HRMS. One important difference between DB-XLB and VF-1 as the first-dimension column is the ca. 20 °C lower elution temperature of VF-1. This means, that when VF-1 is used in the first dimension, the analytes have significantly higher retention in the second-dimension column. This allows the use of a 0.9 m long LC-50 column in the second dimension for analysis of the CB fraction to achieve the same separation as with a 1.4 m long column coupled to DB-XLB. However, for the analysis of dioxins on VF-1 × LC-50, despite raising the modulation period to 8 s, co-elutions due to wrap-around were observed. In order to avoid this, the second-dimension separation was accelerated by programming the flow of the carrier gas to higher values (1.3 ml/min (21 min), at 0.4 ml/min^2 to 1.6 ml/min (5 min), then at 0.4 ml/min^2 to 1.3 ml/min), since faster temperature programming and use of a second dimension oven was not an option due to the temperature limit of the LC-50 column.

3.2. Separation of WHO-PCBs

On VF-1 × LC-50 one WHO-PCB pair, CBs 118 and 123, coelutes with each other and with a number of other CBs. On DB-XLB × LC-50 all WHO-PCBs are resolved and nine out of the 12 WHO-PCBs were also completely separated from all other CBs in the 209-CB mixture (Fig. 1). The plot also clearly shows that the LC-50 column exhibits shape selectivity, as the non-*ortho* CBs are the most strongly retained congeners in the second dimension, followed by the mono-*ortho* CBs. On DB-XLB × LC-50, CBs 189 and 167 partially overlapped with CBs 195 and 181, respectively, and CB 123 coeluted with CBs 106 and 109. However, CBs 181 and 106 are absent and CBs 109 and 195 have a low abundance in technical PCB formulations (Aroclors) [23]. By using a 60 m × 0.18 mm × 0.18 μm DB-XLB column connected to a 2 m × 0.15 mm × 0.15 μm LC-50 column, and even slower oven temperature ramping, complete separation of CBs 189 and 167 from partially coeluting CBs is possible [16]. However, CB 123 was still only partially separated (second-dimension resolution, $^2R_s = 0.9$) from CBs 106/109. Interestingly, CBs 123 and 106/109 were almost baseline separated ($^2R_s = 1.4$) when an FID detector was used instead of the μECD (see Fig. 2). This is due to the relatively large post-column band broadening of the μECD. However, since a short analysis time is desired, the shorter columns with faster oven temperature ramps were selected for analysis, despite the consequent loss of resolution. The separation of mono-*ortho* CBs in a fish oil sample is shown in Fig. 3.

3.3. Separation of dioxins

All 17 2,3,7,8-substituted CDD/Fs and the non-*ortho* CBs 77, 126 and 169 were almost completely separated from each other on both column combinations (Fig. 4). On DB-XLB × LC-50, the two 1,2,3,4,7,8- and 1,2,3,6,7,8-substituted congener pairs (6F1/6F2 and 6D1/6D2 in Fig. 4) slightly overlapped in the first dimension, while 2,3,7,8-TCDD and CB 126 coeluted in the first dimension, but were separated in the second due to the difference in planarity.

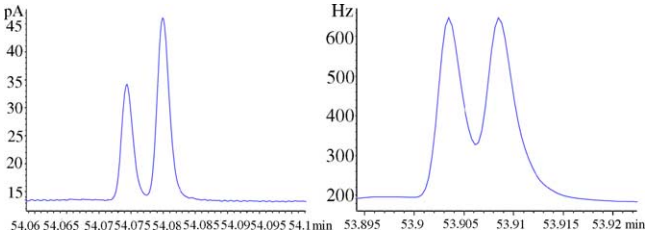

Fig. 2. Sections of the raw chromatograms from the analysis of the 209 CB congeners mixture, illustrating the separation in 2D of CBs 106/109 and 123, when using a FID (left) or a μECD (right).

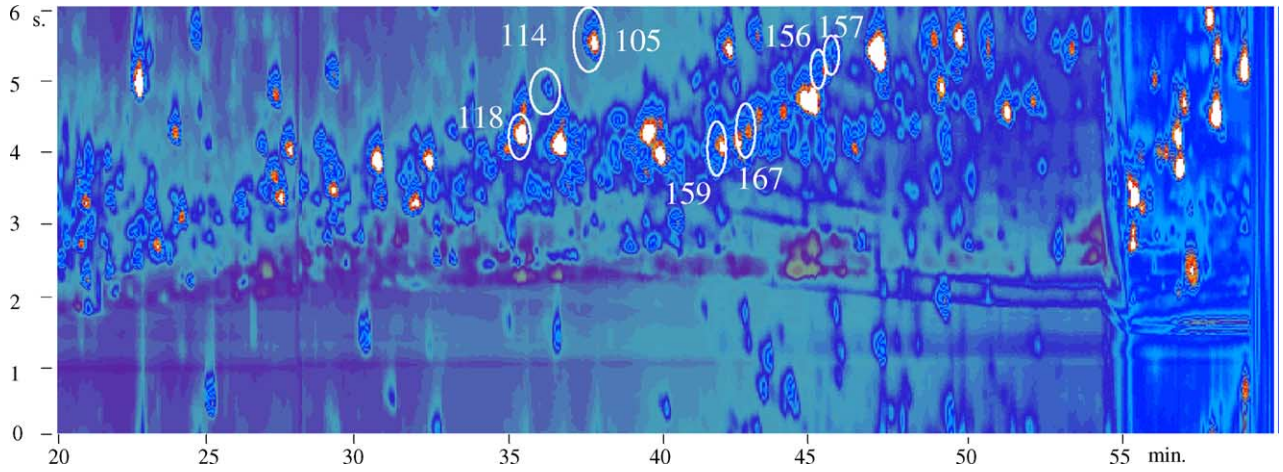

Fig. 3. GC × GC-μECD contour plot of a fish oil analysis (mono-*ortho* CB fraction) with DB-XLB × LC-50 column combination.

2,3,7,8-TCDD is more planar than CB 126 and is therefore retained more strongly on the LC-50 column. There is almost no separation at all of the dioxins in the second dimension, indicating (not surprisingly) that they have similar planarity. The shape selectivity is however very efficient in discriminating against matrix interferences and column bleed. The interferences are generally much less strongly retained in the second dimension than the analytes (Fig. 5). On VF-1 × LC-50 the separation of the dioxins was similar except that OCDD could not be separated from OCDF.

3.4. Evaluation of internal standards

1,2,3,4-TCDD and 1,2,3,4,6,7,9-HpCDD proved to be suitable ISs for the planar fraction, as they were resolved from the target analytes and matrix components in all sample types studied. CB 79 could not be resolved from interfering compounds and therefore 1,2,3,4-TCDD were used for the non-*ortho* CBs. CB 159 was chosen as IS for the mono-*ortho* CBs, since it is not present in technical CB mixtures or, as far as we know, in biological samples. It is therefore suitable as IS as long as a full separation from matrix compounds is achieved. The recoveries of the ISs were in the range of 74–130% for all four sample types.

3.5. Congener-specific results

The average concentration of each congener, for each sample type and participating laboratory, is shown in Table 1, along with coefficients of variation (CV) for one GC × GC-μECD and one GC–HRMS laboratory. Table 1 shows that the WHO-PCB data produced by both GC × GC-μECD systems (laboratories E and F) are similar and agree very well with the GC–HRMS data. Dioxin data produced by laboratory E (using DB-XLB × LC-50 column combination) shows also remarkably good agreement, but data of labo-

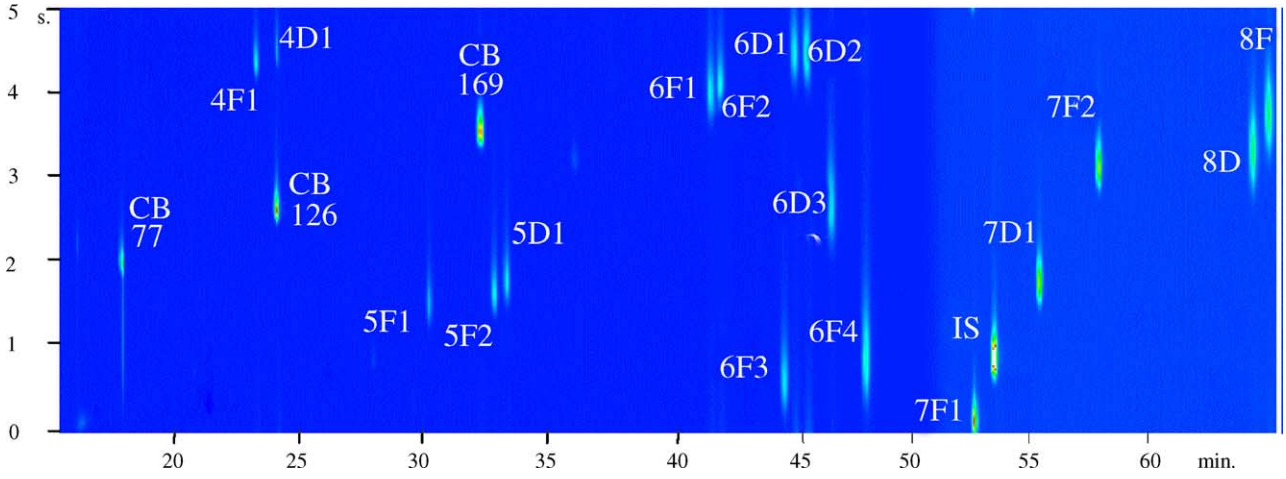

Fig. 4. GC × GC-μECD contour plot of a mixture containing all 2,3,7,8-substituted PCDD/Fs with DB-XLB × LC-50 column combination. Assignment: 4F1, 2,3,7,8-TCDF; 4D1, 2,3,7,8-TCDD; 5F1, 1,2,3,7,8-PeCDF; 5F2, 2,3,4,7,8-PeCDF; 5D1, 1,2,3,7,8-PeCDD; 6F1, 1,2,3,4,7,8-HxCDF; 6F2, 1,2,3,6,7,8-HxCDF; 6F3, 2,3,4,6,7,8-HxCDF; 6D1, 1,2,3,4,7,8-HxCDD; 6D2, 1,2,3,6,7,8-HxCDD; 6D3, 1,2,3,7,8,9-HxCDD; 6F4, 1,2,3,7,8,9-HxCDF; 7F1, 1,2,3,4,6,7,8-HpCDF; IS, 1,2,3,4,6,7,9-HpCDD; 7D1, 1,2,3,4,6,7,8-HpCDD; 7F2, 1,2,3,4,7,8,9-HpCDF; 8D, OCDD; 8F, OCDF.

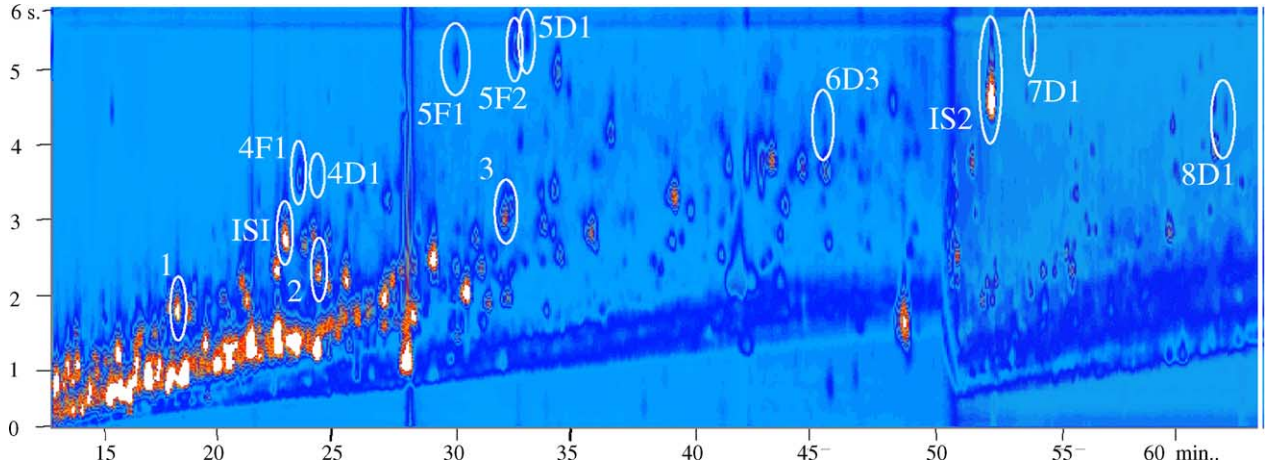

Fig. 5. GC × GC-μECD contour plot of a fish oil analysis (PCDD/F and non-*ortho* CB fraction) with DB-XLB × LC-50 column combination. Assignment: 1, CB 77; 2, CB 126; 3, CB 169; IS1, 1,2,3,4-TCDD; 4F1, 2,3,7,8-TCDF; 4D1, 2,3,7,8-TCDD; 5F1, 1,2,3,7,8-PeCDF; 5F2, 2,3,4,7,8-PeCDF; 5D1, 1,2,3,7,8-PeCDD; 6D3, 1,2,3,7,8,9-HxCDD; IS2, 1,2,3,4,6,7,9-HpCDD; 7D1, 1,2,3,4,6,7,8-HpCDD; 8D1, OCDD.

ratory F (obtained on VF-1 × LC-50 column combination) are for some congeners significantly higher. There are several explanations for the unsatisfactory performance of the VF-1 × LC-50 combination. First, chromatography on this combination was performed under flow programming conditions that resulted in retention-time instability (different retention-time shifts for IS and analyte peaks) and possible peak misidentification. Second, higher flows adversely affected the separation power of the first-dimension column (operated in non-optimum region of *H–u* curve). Third, the 0.18 mm i.d. LC-50 column (coupled to VF-1) provided lower resolution than the 0.15 mm i.d. column (coupled to DB-XLB). Fourth, the modulation period of 8 s allowed three modulations per first-dimension peak, which is not enough to satisfactorily plot the first-dimension peak shape. Finally, the low μECD make-up gas flow of 100 ml/min caused additional peak broadening, resulting in a degradation of the second-dimension separation. Thus, it can be concluded that proper tuning of the GC × GC-μECD system is absolutely necessary to obtain good results. Once properly tuned (as in the case of laboratory E), GC × GC-μECD is a highly suitable method for assessing congener distributions, which is not possible with screening methods such as bioassays.

Although the properly tuned GC × GC-μECD method gives comparable average concentrations to HRMS, the CVs are larger. As expected, the GC × GC-μECD variation seems to be concentration-dependent, i.e. CVs were high mainly when analytes were at concentrations close to or equal to their limits of detection (LODs), such as: 2,3,7,8-TCDD (40%), 1,2,3,7,8,9-HxCDD (37%), 1,2,3,4,6,7,8-HpCDD (30%) and PCB 167 (36%) in the fish oil, and HxCDD/Fs in the milk (18–42%). For the other dioxins, comparable CVs were obtained with the two techniques. The CVs of the CBs were generally lower for GC–HRMS than for GC × GC-μECD, although they were acceptable for both techniques (generally <20%).

As described above, six replicates were analysed as three pairs to obtain estimates of within-laboratory repeatability and reproducibility. Better repeatability (data not presented) than reproducibility was obtained for most of the congeners, especially for those close to their LODs One source of variation for compounds present in concentrations close to their LODs is the relative 'position' of the modulation over the respective peaks, referred to as the phase of modulation by Ong and Marriott [24]. If one modulation occurs just before and one just after the peak apex, and with the same difference in time to the apex, a symmetrically modulated peak distribution is obtained (so-called "in-phase" modulation), which results in an intense, centrally modulated peak. On the other hand, if one modulation occurs precisely at the peak apex, another type of symmetric peak distribution is obtained, with two equally large central peaks ("180°-out-of-phase" modulation). In-phase and 180°-out-of-phase modulation will result in the highest and lowest possible peak intensities, respectively. Every modulation phase in-between these cases will give non-symmetric profiles, in which the amplitude of the highest peak will be somewhere between the two extremes. In any analysis, many analytes may be present at low concentration, so it is not possible to control the modulation of all peaks, i.e. to adjust the phase of modulation for each solute in order to optimise sensitivity. Furthermore, matrix effects may cause shifts in the 1D-retention times, and thus in the modulation phase. The difference between the areas of the highest corresponding in-phase peak and the highest 180°-out-of-phase peak was some 40% in the quoted study [24]. Small variations in column head pressure and phase ageing will also affect the modulation phase. These considerations may partly explain why the repeatability was found to be significantly better than the reproducibility.

Another possible source of variation for compounds that are close to their LODs is baseline instability, as observed in the mono-*ortho* CB and HxCDD/F regions of the GC × GC chromatograms, probably due to interfering sample con-

Table 1
Average concentrations (pg/g) of PCDD/Fs and WHO-PCBs in four sample types determined using GC×GC-μECD and GC–HRMS, and CV (%) for laboratories D and E for fish oil and milk

Matrix	Fish oil ($n=6$)						Eel ($n=2$)					Vegetable oil ($n=1$)						Milk ($n=6$)					Fish oil		Milk
Technique	HRMS				GC × GC		HRMS			GC × GC		HRMS				GC × GC		HRMS			GC × GC		CV	CV	CV
Laboratory	A	B	C	D	E	F	A	B	C	E	F	A	B	C	D	E	F	A	B	C	E	F	D	E	E
2,3,7,8-TCDD	0.32	0.34	0.4	0.29	0.29	2.2	0.91	0.91	1.1	0.97[a]	1.0	0.33	0.33	0.37	0.25	<0.33	0.32[a]	0.59	0.53	0.77	0.87	0.85	13	40	14
1,2,3,7,8-PeCDD	1.3	1.3	1.7	1.5	1.2	1.5[a]	1.8	1.8	1.9	1.7	1.8[a]	0.58	0.66	0.66	0.57	<0.30	0.62[a]	1.2	1.0	1.6	1.1	1.3[a]	6.9	16	18
1,2,3,4,7,8-HxCDD	0.24	0.29	0.26	<1.0	<0.24	0.33	0.25	0.34	0.32	<0.40	1.1	0.72	0.72	0.82	1.6	<0.47	5.5	1.2	0.9	1.4	0.65	0.48	NA	NA	37
1,2,3,6,7,8-HxCDD	0.75	0.7	1.1	<0.81	<0.25	5.4	1.4	1.3	1.7	0.76	4.8	0.68	0.64	0.86	1.3	<0.49	2.6	1.4	1.2	1.7	0.85	4.1	NA	NA	33
1,2,3,7,8,9-HxCDD	0.16	0.29	I	<0.74	0.3	0.35	0.27	0.34	0.31	<0.41	0.3	0.64	0.65	0.81	1.1	0.67	1.8	1.0	0.84	1.4	1.3	0.37	NA	37	26
1,2,3,4,6,7,8-HpCDD	0.33	0.41	0.72	<1.3	0.58	4.3	0.69	0.64	0.85	1.1	3.0	3.8	2.9	4.5	1.4	3.9	9.1	6.5	3.7	6.6	5.7	7.6	NA	30	6
OCDD	0.62	0.76	3.7	<1.8	1.3	1.6	2.3	2.2	3.8	2.3	1.9	92	72	120	110	87	520	41	30	56	49	54	NA	19	8
2,3,7,8-TCDF	6.3	4.4	6.3	5.9	8.7	5.6	1.7	1.2	1.6	1.6	0.96	0.33	0.31	0.38	0.47	0.9	0.73	1.2	0.46	0.74	1.2	1.4	7	7	9
1,2,3,7,8-PeCDF	2.1	1.1	1.5	1.6	1.8	1.2	2.6	0.53	0.5	4.8	0.4	0.73	0.61	0.7	0.59	0.67	0.65	1.1	0.87	1.2	1.6	1.4	13	8	8
2,3,4,7,8-PeCDF	5.7	4.7	5.8	5.8	6.9	7.4	8.3	7.7	8.5	8.8	8.3	2.2	2	2	1.9	1.4	4.8	2.3	2	2.5	2.8	4.5	7	11	10
1,2,3,4,7,8-HxCDF	0.38	0.42	0.58	0.45	<0.30	0.59	0.54	0.71	0.83	<0.49	<0.38	0.68	0.68	0.74	0.53	<0.56	3.2	1.3	1.1	1.5	<0.47	0.49	14	NA	NA
1,2,3,6,7,8-HxCDF	0.65	0.41	0.53	0.45	<0.29	<0.22	0.78	0.72	0.94	<0.47	2.7	0.66	0.62	0.73	0.54	<0.54	0.54	1.2	1.0	1.3	<0.47	0.76	18	NA	NA
2,3,4,6,7,8-HxCDF	0.59	0.45	0.69	0.55	<0.37	0.78	0.8	0.66	0.94	<0.56	1.1	0.68	0.61	0.73	0.97	<0.69	7.9	1.1	0.91	1.4	1.3	1.8	19	NA	42
1,2,3,7,8,9-HxCDF	0.03	0.31	< 0.1	0.41	<0.31	0.33	<0.03	0.35	<0.10	1.6	2.0	<0.03	0.51	0.73	0.94	<0.55	I	0.93	0.77	1.1	1.6	1.2	27	NA	18
1,2,3,4,6,7,8-HpCDF	0.17	0.26	0.33	<0.81	<0.42	0.61	0.28	0.44	0.37	0.3	0.58	1.1	1.1	1.2	0.87	3.4	5.7	1.7	1.4	1.6	1.7	2.1	NA	NA	19
1,2,3,4,7,8,9-HpCDF	0.04	0.17	< 0.25	<0.74	<0.40	5.1	0.06	0.25	<0.25	<0.36	2.6	0.71	0.58	0.72	0.74	<0.89	<1.0	1.0	0.85	1.2	1.4	1.9	NA	NA	5
OCDF	<0.07	0.28	0.61	<1.7	<0.54	NA	0.22	0.45	<0.50	<0.40	NA	9.2	2	8.6	2.8	8.9	NA	7.1	3.3	7.3	8.9	NA	NA	NA	7
CB 77	80	63	88	73	100	80	91	89	84	100	70	1000	1100	1000	990	1000	1000	92	50	66	78	57	4	5	10
CB 126	39	28	37	38	48	33	180	160	170	180	150	22	20	21	21	27	23	36	25	39	58	36	5	6	9
CB 169	13	10	13	14	18	16	30	28	31	30	25	5.8	5.7	6.1	6.6	7.2	6.3	5.1	4.1	5.6	8.3	10	4	7	9
CB 81	2.5	2.2	3.6	3.2	I	I	6.3	5.9	6.1	7	I	3.1	2.9	2.5	2.6	0.56	I	10	7.3	11	21	I	14	NA	6
CB 105	2100	2100	2500	2100	1900	1800	8700	9300	9700	5300	5700	1200	1300	1400	1200	1300	1300	930	800	1100	850	980	2	10	12
CB 114	<100	110	100	88	75	63	390	580	500	350	410	<100	32	27	23	46	30	<100	86	100	96	110	3	21	18
CB 118	7400	5600	7800	6100	4500	4200	28000	27000	30000	15000	16000	5100	4800	5200	4100	4000	4600	57000	31000	66000	44000	42000	2	16	11
CB 123	<100	94	I	I	I	51	320	480	I	320	320	<100	32	32	I	38	34	<100	75	100	81	93	NA	NA	5
CB 156	730	580	710	700	540	530	3700	2700	3500	2000	2500	370	270	330	310	380	330	860	630	870	710	810	2	9	10
CB 157	250	180	220	210	220	190	740	630	810	430	500	<100	29	38	30	43	35	<100	78	120	94	110	4	18	12
CB 167	440	330	I	1800	620	290	2000	1500	1900	1000	1100	370	260	340	210	290	290	740	550	940	660	640	2	36	10
CB 189	<200	61	81	98	I	40	370	61	91	60	100	<200	27	34	51	44	39	<200	61	91	60	70	10	NA	10

I = interference; NA = not analysed.

[a] Average concentration of laboratories A, B and C.

Table 2
Upperbound TEQs (pg TEQ/g fat) in fish oil, spiked milk, vegetable oil and eel extract

	GC–HRMS				GC × GC-μECD	
	A	B	C	D	E	F
Fish oil						
CBs	5.5	4.2	5.4	5.8	6.0	4.4
PCDD/Fs	5.5	4.7	6.0	5.3	6.1	8.5
Total (CV %)	11.0 (3)	8.9 (10)	11.4 (7)	11.1 (6)	12.1 (4)	12.9 (22)
Spiked milk						
CBs	10.1	6.2	11.2	NA	10.9	8.6
PCDD/Fs	4.0	3.4	4.8	NA	4.3	5.6
Total (CV %)	14.1 (2)	9.6 (10)	16.0 (7)	NA	15.2 (7)	14.2 (9)
Vegetable oil						
CBs	3.3	3.0	3.1	2.9	3.6	3.6
PCDD/Fs	2.5	2.5	2.6	2.6	2.0	5.8
Total	5.8	5.5	5.7	5.5	5.6	9.4
Eel						
CBs	24.1	21.8	23.9	NA	21.5	18.9
PCDD/Fs	7.6	7.1	7.9	NA	7.9	8.4
Total	31.7	28.9	31.8	NA	29.4	27.3

NA = not analysed.

stituents. Indeed, the interferences were sometimes so severe that quantification was impossible (see Table 1). Better clean-up is one promising route. One way to improve the clean-up would be to use two planarity separation steps, e.g. activated carbon or PCG [25] for pre-separation and liquid chromatography (LC) on a pyrenyl-silica column for final fractionation. Admittedly, that would make clean-up more complicated and expensive. Partial automation is indicated to solve that problem. Various approaches to automation can be found in the literature; for example super-critical fluid extraction has been combined with carbon-trap fractionation [26] and pyrenyl fractionation has been combined on-line with GC determination (LC-GC) [27].

3.6. TEQ data

The GC × GC total TEQ results obtained by both laboratories compare well with those obtained by GC–HRMS, as is shown in Table 2. For the fortified milk, fish and vegetable oil, the total TEQ values calculated by laboratory E are in the range of results obtained by the four GC–HRMS laboratories. For the fish oil, the results were slightly higher than those obtained by the GC–HRMS laboratories, but within the 95% confidence interval for the average value of these four laboratories. In the GC × GC analysis of the eel extract, a matrix component interfered with the determination of 2,3,7,8-TCDD. As the other GC × GC data compared well with the GC–HRMS data, the average concentration of the GC–HRMS laboratories was used to calculate the 2,3,7,8-TCDD contribution to the total TEQ for GC × GC. The same was done for laboratory F for 2,3,7,8-TCDD in vegetable oil and for 1,2,3,7,8-PeCDD in all samples.

The CVs for laboratory E for the total TEQ of the six replicates of milk and fish oil were 7 and 4%, respectively, which are acceptable results and are comparable to the CVs reported by the GC–HRMS laboratories. The vegetable oil that was used as a quality control gave satisfactory values for all participating laboratories except laboratory F, with a total TEQ between 5.5 and 5.8 pg/g. The major contributing components in the samples analysed were CB 126, 2,3,4,7,8-PeCDF, and 1,2,3,7,8-PeCDD. These three congeners were responsible for 55–84% of the total TEQ.

4. Conclusions

For a WHO-PCDD/Fs-and-CBs screening method to meet the EC requirements, the false negative rate has to be less than 1% and the CV for TEQ less than 30%. On this basis, our results show that GC × GC-μECD has a high potential as a screening method for the determination of TEQs in food and feed. However, improved software will undoubtedly be needed to make quantification less time-consuming, while maintaining or enhancing reliability and allowing reasonable sample throughput. Problems associated with peak area integration might be particularly difficult to solve, since the baseline generated in GC × GC-μECD analyses of these complex matrices is rather irregular.

For a confirmatory method the EC sets even higher demands. The trueness (accuracy) of the measurement must be within 20% of the true value and the CV for TEQs should be less than 15% [5]. Although GC × GC-μECD appears to deliver data of such quality, more validation is needed. It is also desirable to improve the LODs to allow the sample input to be reduced. At present, the most sensitive GC–HRMS instruments are almost 10 times more sensitive than GC × GC-μECD. However, it should be noted that before GC × GC-μECD can be considered as a confirmatory method the EC

directives would have to be revised: currently mass spectrometry is the only mode of detection allowed for this purpose. It is worth noting that GC × GC-μECD may still be a feasible alternative to existing congener-specific GC–HRMS routine methods in other areas, for example, in environmental monitoring programmes.

Finally, to make GC × GC-μECD cost-effective, the sample preparation procedure will have to be improved (as discussed above). One attractive idea would be to combine and automate extraction and pre-separation, and LC separation and GC × GC-μECD. Such automation would not only reduce the amount of manual labour required and, thus, the cost of the analysis, but would also probably improve the quality of the data. Other areas where improvements can be effected are the GC column and the detector. Development of a μECD causing less band broadening and with increased sensitivity, and selective polar columns with higher temperature-stability, would help make GC × GC-μECD a big step forward.

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