

## Validation of GC×GC-ECD for the Determination of Dioxins and Dioxin-like PCBs in Food and Feed

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### Introduction

Analysis of PCDD/Fs and WHO-PCBs in food and feed is challenging as the pollutants are present at ultra-trace levels together with much higher levels of other anthropogenic pollutants. This calls for use of highly selective and sensitive analytical methods. Traditionally these include extraction with mixtures of polar and apolar solvents followed by a multi-stage clean-up and fractionation and analysis with gas chromatography – high-resolution mass spectrometry (GC-HRMS). However, these methods are costly as the sample preparation is time consuming and the GC-HRMS instruments are expensive and require skilled operators. Thus, there is room for cost-efficient alternatives. This has been identified by the European Commission who funded two projects “Dioxin analysis by using comprehensive gas chromatography” (DIAC) and “Dioxins in food and feed – reference methods and new certified reference materials” (DIFFERENCE) in which alternative screening methods were developed and validated. In this paper, we present the validation of one of these; comprehensive two-dimensional gas chromatography (GC×GC) with electron capture detection.

### Materials and Methods

All samples were column extracted using acetone: *n*-hexane (2.5:1) followed by *n*-hexane: diethyl ether (9:1). The lipid weights were determined gravimetrically and the residues were taken up in *n*-hexane. Lipids and other polar or easily degraded compounds were removed using multi-layer silica columns packed with KOH-silica, H<sub>2</sub>SO<sub>4</sub>-silica, and silica; which were eluted with *n*-hexane. The halogenated aromatic compounds were then fractionated using activated charcoal into three fractions including 1) the bulk of PCBs, 2) primarily mono-*ortho* PCBs, and 3) non-*ortho* PCBs and PCDD/Fs [the dioxin fraction]. However, a few samples were fractionated using porous graphitized carbon (PGC) and 2-(1-pyrenyl) ethyldimethylsilylated (PYE) silica. In those cases, all WHO-PCBs were eluted in the second fraction. Finally, the PCDD/F and WHO-PCB containing fractions were analysed by GC×GC.

Two types of cryogenic modulators were used; laboratory A used a single-jet loop modulator from Zoex Corp. (Lincoln, NE, USA) and laboratory B used a longitudinally modulated cryogenic system (LMCS) from Chromatography Concepts (Doncaster, Australia). The GC×GC modulators were retrofitted on Agilent 6890 gas chromatographs, which were equipped with micro electron capture detectors ( $\mu$ ECDs). Both laboratories used 30-m non-polar first dimension columns with an ID of 0.25mm and a 0.25 $\mu$ m film thickness. Laboratory A used a VF-1 ms column from Varian and Laboratory B a DB-XLB column from J&W Scientific. As second dimension column a 0.9-m, or a 1.4-m, J & K Scientific LC-50 (Milton, Ontario, CA) liquid crystal column (0.15mm ID; 0.10 $\mu$ m film) was used. The longer secondary column was used for the dioxin fractions, the shorter for mono-*ortho* PCB containing fractions. Helium was used as carrier gas at a constant flow of 1.0 ml/min.

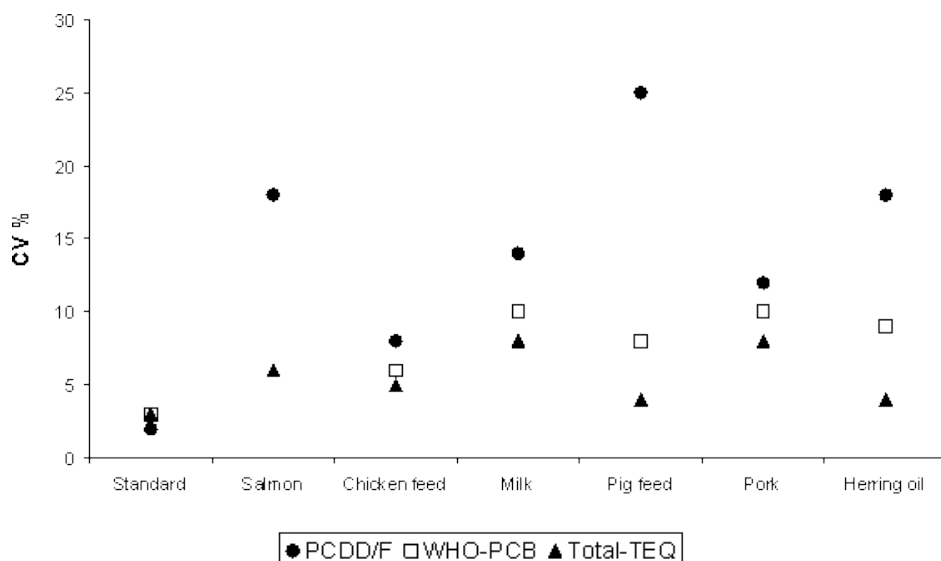
The modulation period and oven temperature program rates were optimized at each laboratory to obtain good separation between target analytes and between target analytes and matrix components. Generally, the modulation periods were between 5 and 8s. The  $\mu$ ECDs were operated at 50Hz and with the high make-up gas flow rates, 100 - 150 ml/min, to minimize detector band broadening.

### Results and Discussion

The sensitivity and linearity of the GC×GC-ECD system have been evaluated and the results are presented elsewhere<sup>1,2</sup>. It was shown that the limits of detection (LODs) for standard solutions of PCDD/Fs and WHO-PCBs are comparable to those of GC-HRMS; 40–150 fg and 30–60 fg, respectively. Good linearity ( $r^2 > 0.998$ ) and dynamic

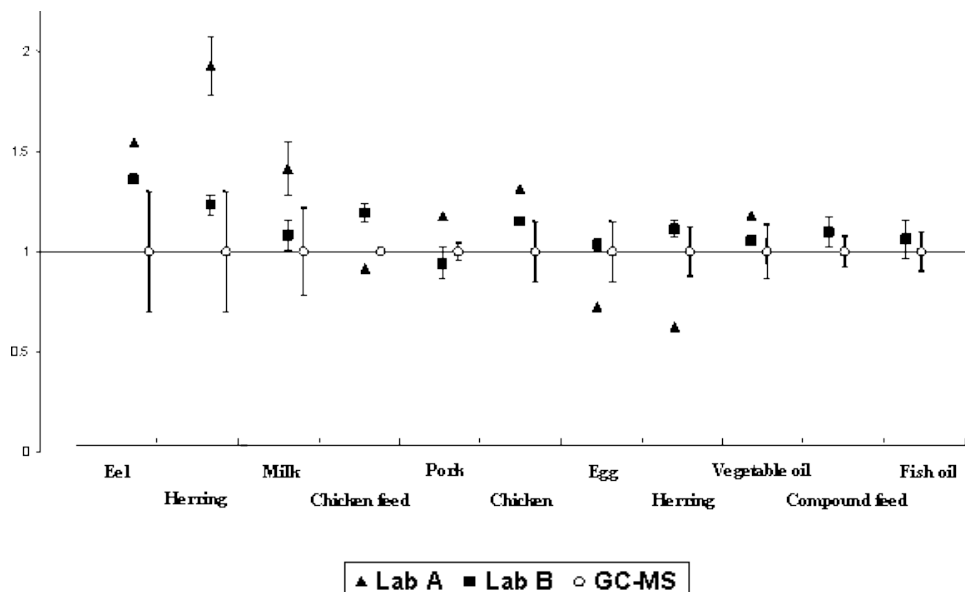
range (0.1–40 pg) was also reported. More important, however, is the LODs for low level samples, which have been assessed through analysis of vegetable oil, spiked with PCDD/Fs at levels close to the European Community (EC) maximum levels (0.75 pg WHO-TEQ/g oil). The LOD of the GC×GC-ECD method was estimated to 0.5 pg total PCDD/F-WHO-TEQ/g oil. Thus, with a reasonable sample intake (>5g) it would be possible to determine whether the levels of dioxins in a lot of vegetable oil are above or below the maximum level and, thus, determine if it is compliant or non-compliant with the current legislation.

The reproducibility was excellent for both standards and samples. The coefficients of variation (CV) for individual PCDD/Fs and WHO-PCBs, based on medians, and for total-TEQ are shown in **Figure 1**. The CVs for WHO-PCBs and total-TEQ were generally below 10%, while somewhat higher CVs were observed for the individual PCDD/Fs. This is most likely due to the lower levels of PCDD/Fs as compared to WHO-PCBs. Overall, the results are satisfactory as the CVs for total-TEQs are significantly lower than the 30% and 15% that is stipulated by the EC for food and feed screening and confirmatory methods, respectively.



**Figure 1.** The graph shows coefficients of variation (CV) for GC×GC-ECD analysis of PCDD/Fs, WHO-PCBs and total-TEQ in various food and feed analysed at laboratory B.

The accuracy was assessed by parallel analysis of a wide variety of food and feed items by GC×GC-ECD and GC-HRMS. The samples generally contained PCDD/F and WHO-PCB at realistic levels, i.e. close to the EC maximum levels. The feed samples had the following total TEQs (pg/g product): 1.2 for compound feed, 1.6 for chicken feed, 9.9 for herring oil and 11 for unspecified fish oil. The food samples had the following total TEQs (pg/g lipids): 1.4, 1.9, 4.4 and 21 for pork, herring, chicken and eel tissue, respectively; 5.6 for vegetable oil; 6.4 for egg and 14 for spiked milk. The GC×GC and GC-HRMS results were generally in good agreement (**Figure 2**). The results of Laboratory A showed some variability, and were sometimes higher than GC-HRMS, sometimes lower, while the results from Laboratory B were close to the GC-HRMS data. Generally, the mean levels of the GC×GC-ECD analyses by Laboratory B were within the spread of the GC-HRMS results. Overall, there seems to be a slight overestimation in the total-TEQ values, as compared to GC-HRMS. However, an overestimation is much better than an underestimation for a screening method, as false negative results are much worse than false positive results.



**Figure 2.** Total-TEQ concentration ratios; GCxGC-ECD (laboratory A and B) vs GC-MS, from the determination of PCDD/Fs and WHO-PCBs in various food and feed items. The error bars correspond to one standard deviation.

It is, however, conceivable that other classes of halogenated organic compounds may interfere in the determination of PCDD/Fs and WHO-PCBs. This was investigated within the DIFFERENCE project by analysis of spiked portions of a vegetable oil. All oils were spiked with a realistic mixture of PCDD/Fs at 3 pg WHO-TEQ/g oil, and most oils were also fortified with an excess of potentially interfering compounds at a total concentration 200 ng/g. The interferences included PCBs, polychlorinated naphthalenes (PCNs), and mono- through decachlorodiphenyl ethers (PCDEs). In the GCxGC-ECD analyses, no significant bias was detected.

In our opinion, GCxGC-ECD is a realistic alternative to GC-HRMS for dioxin and dioxin-like PCB analyses of food and feed. It appears to be robust, offers sufficient sensitivity and selectivity, and produce both accurate and precise results. It does fulfil the European Community requirements for dioxin and dioxin-like PCB analyses<sup>3</sup>. In contrast to other common screening methods, such as bioassays, GCxGC-ECD produce full congener profiles. The congener pattern of GCxGC-ECD has been shown to faithfully reproduce those obtained by GC-HRMS. Almost without exception the confidence limits of the two techniques overlap. Thus, GCxGC-ECD may be used as a routine method for the congener-specific analysis of 2,3,7,8-PCDD/Fs and WHO-PCBs in food and feed. Unfortunately, the current EC legislation requires HRGC-HRMS as confirmatory method for official control of such samples. This might, however, change in the near future as not only GCxGC-ECD, but also several other realistic alternatives have emerged in recent years, e.g. GC low-resolution (LR) MS-MS<sup>4</sup> and GCxGC-LRMS<sup>5,6</sup>.

### Acknowledgements

The DIAC and DIFFERENCE projects were financially supported by the European Community (contracts G6RD-CT-2001-00623 and G6RD-CT-2001-00572). The analytical contribution of the participating GC-HRMS laboratories is gratefully acknowledged.

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