ION-TRAP TANDEM MASS SPECTROMETRY: A RELIABLE TECHNIQUE FOR THE ANALYSIS OF PCDD/Fs AND DIOXIN-LIKE PCBs IN FOOD AND FEED SAMPLES

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Introduction

The recent establishment of maximum residue limits for polychlorodibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in food [1] and feed [2] samples by the European Community and the future inclusion of dioxin-like polychlorinated biphenyls (dl-PCBs) in these values at the end of 2006, has led to an important increase on the routine analysis of these compounds. Therefore, there is a clear need to have powerful sensitive and selective methods for the analysis of these compounds at low concentration levels. Actually, gas chromatography coupled with high resolution mass spectrometry (GC-HRMS) is the technique of reference for the determination of these analytes in environmental and food samples due to its high sensitivity and selectivity. Nevertheless, this technique is relatively expensive and requires qualifier personnel. Therefore, the development of more economical but reliable methods that can deliver results comparable to GC-HRMS is required [3]. During the last years, gas chromatography coupled with ion trap mass spectrometry (GC-ITMS) working in MS/MS mode has become an interesting alternative technique to GC-HRMS for the analysis of PCDD/Fs and dl-PCBs [4,5].

The aim of the present work is to demonstrate the ability of the gas chromatography coupled with ion-trap tandem mass spectrometry (GC-ITMS/MS) for the analysis of PCDD/Fs and dl-PCBs in food and feed samples. This work was performed on the framework of the European research project called DIFFERENCE (Dioxins in Food and Feed - Reference methods and New Certified Reference Materials) with the objective to validate the GC-ITMS/MS method as alternative to HRMS in order to reduce the cost of dioxin analysis. The results and conclusions of the evaluation study are presented here.

Methods and Materials

Analytical method: A set of food and feed samples such as vegetable oil, chicken compound feed, pork tissue, chicken tissue, herring tissue and egg yolk and white were supplied by the Netherlands Institute for Fisheries Research (RIVO). Egg yolk and white and the chicken compound feed samples remained frozen until analysis. The other samples were stored at 4ºC or in a dry and dark place before use. Pork tissue, chicken tissue, herring tissue and egg yolk and white samples were lyophilised prior to the analysis. Extraction procedures applied to the matrices varied depending on the sample nature: (a) chicken compound feed and the lyophilised samples were spiked with known amounts of a $^{13}C_{12}$-PCDDs/PCDFs and dioxin-like $^{13}C_{12}$-PCBs mixture, and then were Soxhlet extracted for 24 h with toluene: cyclohexane (1:1) (b) the vegetable oil sample was directly dissolved in n-hexane and, then, spiked with the $^{13}C_{12}$-PCDDs/PCDFs and dioxin-like $^{13}C_{12}$-PCBs. The fat and organic matter were removed from the extracts using a sulphuric acid treatment. Finally, the extracts were rotary concentrated and filtrated before the clean-up process. Purification was accomplished by automated clean-up (Power-Prep/SPE system-FMS, Waltham, MA, USA) based on the use of multilayer silica, basic alumina and PX-21 carbon adsorbents. Two main fractions containing: (i) PCDD/Fs and non-ortho PCBs, and (ii) mono-ortho PCBs were obtained. After addition of the corresponding $^{13}C_{12}$-isotopically labelled congeners as syringe standard the extracts were analysed by GC-ITMS/MS and GC-HRMS.

GC-ITMS/MS instrumentation: Analysis of the target compounds were carried out on a TRACE GC 2000 Series gas chromatograph coupled with a GCQ/Polaris ion-trap mass spectrometer (ThermoFinnigan, Austin, TX, USA) equipped with an AS2000 autosampler. The chromatographic separation was performed using a DB-5MS (J&W Scientific, Folsom, USA) (5% phenyl, 95% methylpolysiloxane) fused-silica capillary column (60 m x 0.25 mm I.D., 0.25 μm film thickness). Oven temperature program was for PCDDs/Fs: 140ºC (held for 1 min) to 200ºC at 20ºC/min (held for 1 min) and to 300ºC at 3ºC/min (held for 20 min) and for dioxin-like PCBs: 140ºC (held for 2 min) to 180ºC at 20ºC/min (held for 1 min) and to 300ºC at 2.5ºC/min (held for 5 min). Helium was used as a carrier gas at a flow rate of 33 cm/s at 90ºC. Injector temperature was kept at 280ºC and splitless injection mode (1 min) was used. The operating conditions for the ion-trap mass spectrometer working at MS/MS mode were the following: positive electron ionisation (EI+) mode at an ionisation energy of 70 eV, ion source temperature

210ºC, transfer-line temperature 290ºC, trap-offset 10V. Xcalibur version 2.0 software was used for acquisition and treatment of the results. The most intense ion of the molecular cluster of each homologue group was selected as precursor ion, and the loss of COCl for PCDD/Fs and 2Cl for dl-PCBs were chosen as characteristic transition for MS/MS measurements. The MS/MS operating conditions were: isolation time and excitation time, 10 ms and 15 ms, respectively; stability parameter q_z was fixed to 0.45; the optimum excitation voltage for TeCDDs and PeCDDs was 1.3 V, for TCDFs was 1.4 V, for PeCDFs was 1.5 V, for HxCDDs and HxCDFs was 1.6 V, for HpCDDs, HpCDF and OCDD was 1.7 V and for OCDF was 1.9 V. In the case of dl-PCBs the excitation voltage was fixed to 1.4 V for all congeners excepting for hepta-PCBs with a value of 1.5 V.

GC-HRMS instrumentation: **The analyses of PCDD/Fs and dl-PCBs were performed on a GC 8000 Series gas chromatograph (Carlo Erba Instruments, Milan, Italy) coupled to an Autospec Ultima mass spectrometer (Micromass, Manchester, UK). Operating conditions were: EI+ (32 eV) mode, resolving power of 10,000. Source and transfer line temperatures were set at 275ºC and 290ºC, respectively. The chromatographic conditions were the same as for GC-ITMS/MS, working in selected ion monitoring mode.**

Quantification of PCDD/Fs and dioxin-like PCBs using both MS systems was carried out by mass isotopic dilution method. Relative response factors (RRF) were obtained for each individual 2,3,7,8-chlorosubstituted PCDDs/PCDFs and dioxin-like PCBs congeners by the analysis of different mixtures of labelled and unlabelled standards. WHO-TEQs values were calculated using the limit of detection (LOD) value for non-detectable compounds or values below to the LOD (upperbound).

Results and discussion

The developed GC-ITMS/MS method and the reference GC-HRMS method were applied to the analysis of PCDD/Fs and dl-PCBs in the above mentioned food and feed samples. As an example, in Fig.1 the GC-ITMS/MS chromatograms of Tetra-CDD/Fs and their $^{13}C_{12}$-isotopically labelled compounds are given. The results obtained with the two techniques are summarised in Table 1, where the mean and the standard deviation of the WHO-TEQ values (upperbound) for PCDD/Fs and dl-PCBs are given. As can

be seen, the results obtained with both methods are in agreement and show low standard deviations. In addition, the concentrations found in the samples for individual congeners using both techniques showed a good concordance. As an example, the concentrations of the individual toxic PCDD/F congeners obtained in the chicken compound feed using the proposed and the reference methods are compared in Fig.2. Similar concentration profiles were obtained using both techniques demonstrating the ability of the ion-trap mass spectrometry to provide good and reliable results. In summary, GC-ITMS/MS can be considered as a realistic alternative method to HRMS at relative low cost for the determination of PCDD/Fs and dl-PCBs in food and feed samples. Additional studies are being performed in order to demonstrate the applicability of this technique for the certification of food and feed reference materials.

Table 1. **WHO-TEQ values (upperboud) of PCDD/Fs and dl-PCBs obtained by GC-ITMS/MS and GC-HRMS in the food and feed samples analysed.**

	WHO-TEQ (Upperbound)			
	GC-ITMS/MS		GC-HRMS	
	Mean	S.D.	Mean	S.D.
Vegetable oil (n=4)				
pg PCDD/Fs /g oil	2.93	0.08	2.95	0.30
pg dl-PCBs WHO-TEQ /g oil	2.74	0.12	2.90	0.20
Total pg WHO-TEQ/g oil	5.67	0.08	5.85	0.35
Chicken compound feed (n=6)				
pg PCDD/Fs /g	0.90	0.04	0.84	0.02
pg dl-PCBs WHO-TEQ /g	0.85	0.03	0.87	0.05
Total pg WHO-TEQ/g	1.74	0.04	1.72	0.06
Pork tissue (n=6)				
pg PCDD/Fs /g fat	0.86	0.05	0.86	0.04
pg dl-PCBs WHO-TEQ /g fat	0.43	0.01	0.45	0.01
Total pg WHO-TEQ/g fat	1.29	0.06	1.31	0.05
Chicken tissue (n=2)				
pg PCDD/Fs /g fat	1.15	0.08	1.59	0.69
pg dl-PCBs WHO-TEQ /g fat	2.48	0.11	2.62	0.44
Total pg WHO-TEQ/g fat	3.63	0.03	4.20	1.13
Egg yolk and white n=2)				
pg PCDD/Fs /g fat	3.44	0.20	3.41	0.11
pg dl-PCBs WHO-TEQ /g fat	3.85	0.16	3.58	0.04
Total pg WHO-TEQ/g fat	7.28	0.04	6.98	0.07
Herring tissue (n=2)				
pg PCDD/Fs /g	0.99	0.01	0.88	0.01
pg dl-PCBs WHO-TEQ /g	1.12	0.04	1.16	0.02
Total pg WHO-TEQ/g	2.11	0.03	2.05	0.03

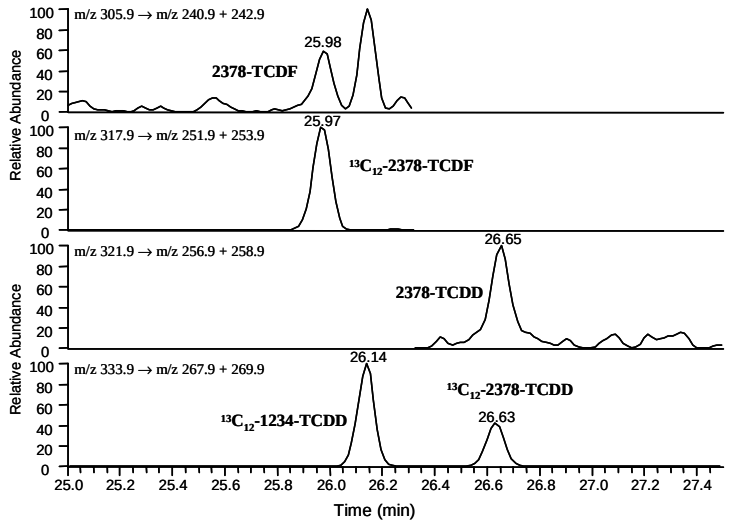

Figure 1. **GC-ITMS/MS chromatograms for the tetra-CDD/Fs and $^{13}C_{12}$-isotopically labelled compounds in a chicken compound feed sample.**

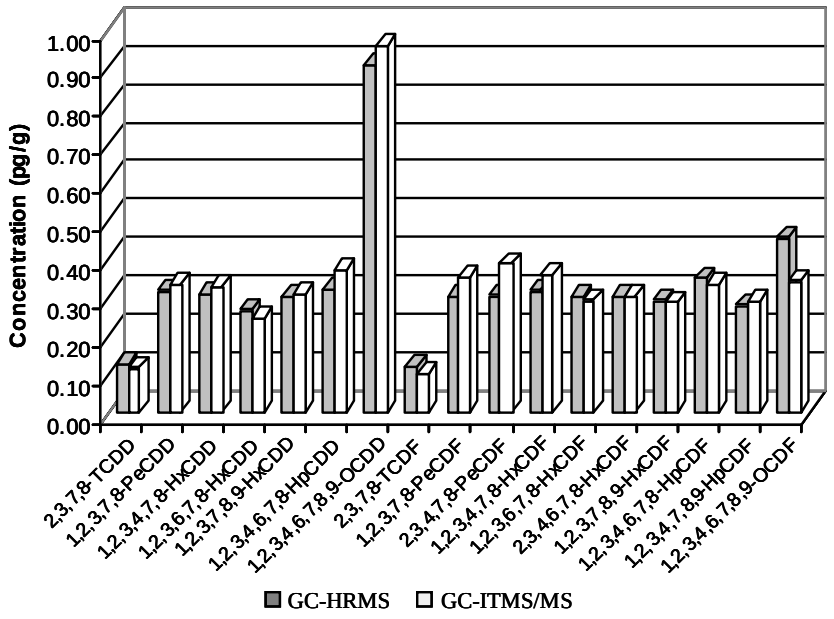

Figure 2. **Concentration of PCDD/Fs and dioxin-like PCBs obtained with GC-ITMS/MS and GC-HRMS in a chicken compound feed sample (mean values of six replicates).**

Acknowledgments

J. Malavia thanks to Ministerio de Ciencia y Tecnología for a FPI grant. The authors gratefully acknowledge the financial support by EU research project DIFFERENCE (G6RD-CT-2001-00623) and the *Ministerio de Ciencia y Tecnología* under the project no. REN2003-02692/ MAR..

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