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ION TRAP MS/MS *vs.* HRMS FOR THE ANALYSIS OF PCDDs/Fs AND DIOXIN-LIKE PCBs IN FOOD SAMPLES

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Introduction

Polychlorodibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) constitute a class of ubiquitous toxic environmental pollutants of great concern since they exhibit potential risks for human health. As a result of their physical and chemical properties, these compounds are extremely resistant to chemical and biological degradation and therefore they can bio-accumulate and bioconcentrate in the environmental and easily enter into the feed and food chain. In addition, PCDD/Fs and dl-PCBs are present in environmental and food samples at a very low concentration that makes necessary the development of powerful sensitive analytical methods for their determination. Normally, gas chromatography coupled with high-resolution mass spectrometry (GC-HRMS) is used for the determination of these compounds as described in US EPA Method 1613 and 1668 and the European Standard Method EN1948-1/2/3, but this technique is relatively expensive. Recently, the establishment of new maximum residue values for these compounds in food and feed samples [1] lead to an enhancement of the regular monitoring of dioxins and dl-PCBs. In this sense, the development of less expensive but reliable methods is required [2]. In the recent years, mass spectrometry based on ion-trap analysers (ITMS) have become an interesting alternative to high-resolution mass spectrometry (HRMS) for the analysis of these compounds in environmental and food samples [3,4].

The aim of this work is to demonstrate the suitability of the ion-trap tandem mass spectrometry for the analysis of PCDD/Fs and dl-PCBs in food samples. For this purpose, a comparative study using different food samples such as vegetable oil, clean fish extract, milk and fish oil, was performed on the framework of the European research project DIFFERENCE ("Dioxins in food and feed-reference methods and new certified reference materials"). The results and conclusion of the evaluation study is presented here.

Methods and Materials

Analytical method

All food samples (vegetable oil, clean fish extract, milk and fish oil) were supplied from RIVO (Netherlands Institute for Fisheries Research) in the framework of the European Project DIFFERENCE. The samples remained frozen until they were processed. Extraction procedures varied depending on the sample nature: (a) the lipid fraction contained in milk samples was recovered by liquid-liquid extraction using organic solvents (diethyl ether and petroleum ether);

(b) the two oil samples were directly dissolved in n-hexane. Then, the samples were spiked with known amounts of a $^{13}C_{12}$-PCDDs/PCDFs and dioxin-like $^{13}C_{12}$-PCBs mixture. Then, organic matrix was removed by a sulphuric acid treatment. Finally, the extracts were rotary concentrated and filtrated prior to 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, respectively, were obtained and subsequently analysed

GC-ITMS/MS instrumentation

All analysis of PCDD/Fs and dioxin-like PCBs 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. Ion-trap mass spectrometer operating conditions were: 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.

GC-HRMS instrumentation

Analysis 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 in EI+ (32 eV) mode and at a 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 was carried out using 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

GC-Ion trap MS/MS optimisation

For ITMS/MS analysis, the precursor ions of native and $^{13}C_{12}$-labelled PCDD/Fs and dioxin-like PCBs were selected in different acquisition segments for each group of chlorination over the chromatographic run. The mass isolation window of the precursor ion was set to 1 m/z in order to obtain a high selectivity and a good sensitivity. Different CID parameters such as excitation time, excitation voltage and the stability *q* parameter were optimised. The optimum resonance excitation voltage for each homologue group were determined from 0.2 to 2.5 V in 0.1 V steps. In this conditions, CID excitation time and stability *q* were fixed to 15 ms and 0.45, respectively. The optimum excitation voltage for TeCDDs and PeCDDs was 1.3 V, for TeCDFs was 1.4 V, for

PeCDFs was 1.5 V, for HxCDDs and HxCDFs was 1.6, for HpCDDs, HpCDFs and OCDD was 1.7 V and for OCDF was 1.9V. In the case of dl-PCBs, the excitation voltage was fixed to 1.4 V for Tetra-, Penta- and Hexa-CBs, and 1.5 V for Hepta-CBs. The two product ions from each precursor ion corresponding to the loss of two chlorine atoms for dl-PCBs and COCl fragment for PCDD/Fs were selected for quantification and confirmation purposes. Quality parameters of the GC-ITMS/MS technique were determined. Repeatability and long-term precision was between 5% and 12%, with LODs between 0.6 and 0.9 pg g^{-1} of oil or fat.

Comparative study

After optimisation of the ion-trap MS/MS for each family of compounds, the food samples were analysed using both determination methods, GC-ITMS/MS and GC-HRMS. The results obtained with the two techniques are summarised in Table 1, where the mean of total WHO-TEQ values, upper and lowerbound, expressed as pg WHO-TEQ/g are given. As can be seen, good agreement between both methods was achieved with low relative standard deviations. In addition, the individual concentration obtained using both techniques for each toxic congener-specific also showed a good concordance. As an example, the results of PCDD/Fs and dl-PCBs in a fish oil sample is given in Figure 1. From this study, it can be concluded that ion-trap tandem mass spectrometry can be considered as an attractive alternative to HRMS at low cost for the determination of PCDD/Fs and dl-PCBs in food samples. Further studies are being performed in order to confirm the suitability of this MS/MS technique.

Table 1. Results of the comparative study on PCDD/Fs and dl-PCBs analysis in food samples

	Upperbound		Lowerbound	
	GC-ITMS/MS	GC-HRMS	GC-ITMS/MS	GC-HRMS
Vegetable oil				
pg PCDD/Fs WHO-TEQ/g	2.94	2.54	2.70	2.53
pg dl-PCBs WHO-TEQ/g	2.88	2.97	2.88	2.97
Total pg WHO-TEQ/g	5.82	5.50	5.58	5.50
Clean-fish extract (n=2)				
pg PCDD/Fs WHO-TEQ/g	7.76 ± 0.15	7.11 ± 0.38	7.44 ± 0.18	7.04 ± 0.33
pg dl-PCBs WHO-TEQ/g	20.69 ± 0.06	21.83 ± 0.05	20.69 ± 0.06	21.83 ± 0.05
Total pg WHO-TEQ/g	28.44 ± 0.08	28.94 ± 0.33	28.13 ± 0.11	28.87 ± 0.28
Milk (n=6)				
pg PCDD/Fs WHO-TEQ/g fat	3.92 ± 0.24	3.43 ± 0.37	3.67 ± 0.24	3.43 ± 0.37
pg dl-PCBs WHO-TEQ/g fat	6.91 ± 0.45	6.19 ± 0.94	6.91 ± 0.45	6.19 ± 0.94
Total pg WHO-TEQ/g fat	10.83 ± 0.46	9.62 ± 0.96	10.58 ± 0.46	9.61 ± 0.96
Fish oil (n=6)				
pg PCDD/Fs WHO-TEQ/g	4.27 ± 0.27	4.73 ± 0.49	3.95 ± 0.33	4.66 ± 0.49
pg dl-PCBs WHO-TEQ/g	4.81 ± 0.40	4.17 ± 0.48	4.81 ± 0.40	4.17 ± 0.48
Total pg WHO-TEQ/g	9.08 ± 0.57	8.90 ± 0.74	8.76 ± 0.64	8.83 ± 0.74

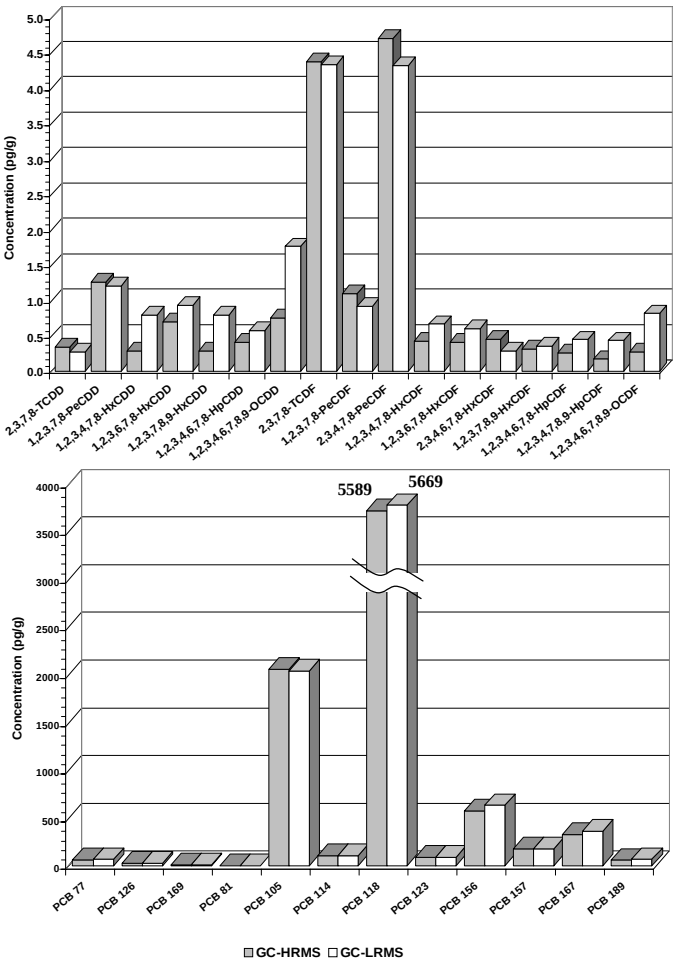

Figure 1. Concentration of PCDD/Fs and dioxin-like PCBs obtained with GC-ITMS/MS and GC-HRMS in a fish oil sample (mean values of six replicates)

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