ANALYSIS OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN FISH PRODUCTS AND BY-PRODUCTS BY GAS CHROMATOGRAPHY-ION TRAP TANDEM MASS SPECTROMETRY

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Introduction

GC-HRMS is currently considered the reference technique for the analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs)[1]. Nevertheless, HRMS has a high operating cost and requires personnel with a great technical skill. The major challenge for the analysis of these compounds is to reduce costs per analysis by improving the speed of extraction and clean-up and by introducing less expensive alternatives to GC-HRMS, while maintaining the same level of performance. GC-MS-MS using ion-trap mass analyzers is one of the most promising technique[2]. The lack of selectivity of the ion-trap due to the unit mass resolution is compensated by operating the instrument in the tandem mode. In addition, low enough limits of detection can be achieved because of the significant increase of the signal-to-noise ratio provided by this operating mode. This technique has been applied to the analysis of PCDD/Fs and dl-PCBs in environmental and food samples. However, in spite of the legislative measures adopted in food and feed, the number of papers demonstrating the real applicability of this technique to the analysis of these compounds, especially for PCDD/Fs, in food and feed is still limited[3,4]. The aim of present paper was the evaluation of the performance of a GC-MS system equipped with an ion-trap as mass analyzer working in tandem mode, in external ionization configuration, in fish and aquaculture products and by-products, in order to obtain an available low-cost methodology to accomplish European Regulations requirements.

Materials and methods

Three matrix samples were used: fish, fish meal and/or feed, and fish oil. A number of 5 samples from each group were analyzed in duplicate. Fish products were *Pangasius* fillets, smoked cod liver canned in oil, mackerel canned in oil and squid rings; fish oils were naturally contaminated or not contaminated; one sample of fish meal and one sample of feed were spiked at different levels (1.25 and 2.25 pg/g, respectively) with a sample of fish oil containing PCDD/Fs naturally present. Each sample was analyzed both by GC-HRMS and GC-MS-MS. Two extraction methods were applied: extraction on a chromatographic open column and accelerated solvent extraction (ASE). Purification steps were performed by automated procedures using the Power Prep system;extracts were cleaned up in disposable multilayer silica cartridges (CLDS-ABNSTD), alumina cartridges (CLDA-BAS-011) and carbon AX-21/celite (CLDC-CCE-034) from Fluid Management Systems, Inc.
Gas chromatograph Varian CP-3800coupled to an ion trap tandem mass spectrometer 4000 GC/MS from Varian, in external ionization configuration, was used. VF-5ms narrowbore column (Factor Four, 60m×0.25mm ID, 0.25 μm Film, Varian), was used. Helium Alphagaz He-2 (purity≥99.9999%) purchased from Air Liquide (Spain) was used as carrier gas. Standard solution from Wellington Laboratories Inc. EPA-1613LCS, EPA-1613ISS and EPA-1613CSS were used as surrogate, internal and cleanup standard. Dichloromethane, n-hexane, toluene, acetone, ethyl acetate of residue analysis grade were purchased from Merck. Nonane of high purity for GC was supplied from Fluka (Sigma-Aldrich). Sulphuric acid 95-97% was obtained from Merck.
Detection was performed based on the pattern of fragmentation of the congeners by MS/MS. Quantification was based on the isotope dilution method. Calibration was performed using native and labelled PCDD/Fs and dl-PCBs solutions from Wellington Laboratories (EPA-1613 CVS). Relative response factors (RRFs) of the native congeners to the corresponding $^{13}C_{12}$-labelled internal standards were also determined. RRFs were used to quantify the PCDD/F levels in the samples. The results were expressed as pg WHO TEQ g^{-1}, and were calculated for the non-detectable compounds using the limit of detection (upper bound values)[1].

Results and discussion:

Parameters affecting gas chromatography and isolation and fragmentation of precursor ions in the ion trap, such as excitation voltage and storage level, were optimized in order to achieve the highest level of robustness and sensitivity for all PCDD/Fs congeners. The optimized values applied for each compound are listed in Table 1.

Compound		Precursor ions (m/z)	CID rf (m/z)	CID voltage (V)	Product ions (m/z)
TCDF	^{12}C	306.0	135	6.40	241+243
	^{13}C	318.0	140	6.40	252+254
TCDD	^{12}C	322.0	142	5.40	257+259
	^{13}C	334.0	147	5.50	268+270
PeCDFs	^{12}C	340.0	150	6.60	275+277
	^{13}C	352.0	155	6.60	286+288
PeCDD	^{12}C	355.9	157	5.80	291+293
	^{13}C	367.9	162	5.85	302+304
HxCDFs	^{12}C	373.9	165	6.40	309+311
	^{13}C	385.9	170	6.40	320+322
HxCDDs	^{12}C	389.9	172	4.30	325+327
	^{13}C	401.9	177	4.50	336+338
HpCDFs	^{12}C	407.9	180	6.40	345+347
	^{13}C	419.9	185	6.50	356+358
HpCDDs	^{12}C	423.9	187	4.20	361+363
	^{13}C	435.8	192	4.20	372+374
OCDD	^{12}C	459.7	203	4.00	395+397
	^{13}C	471.8	208	4.00	406+408
OCDF	^{12}C	443.7	196	5.40	379+381

Table 1.- Optimized MS-MS parameters for the analysis of PCDD/Fs and dl-PCBs by the ion-trap mass analyzer GC/MS 4000 in external ionization configuration.

Performance of the whole analytical method was evaluated. Limits of detection were defined as the concentration of the analyte that produces a peak with a signal-to-noise ratio of 3, and were determined using samples and standards of low concentrations, establishing the relationship between concentration values and standard deviations obtained. Values of 0.10-0.15 pg in tetra- and penta-chlorinated congeners, 0.20-0.50 pg in hexa- and hepta-chlorinated congeners and 1.20-1.50 in octa-chlorinated congeners were obtained.
Linearity was tested during the period of the study (several months) evaluating results from 7 calibration data sets. Relative response factors (RRFs) standard deviation was lower than 15% in most congeners, and lower than 25% in all of them, except in OCDD, showing a higher value (48%). No false negatives were obtained during the study, coextracted interferences caused some problems in the most complex matrices, but the clean-up process combined with modifications in MS detector parameters, such as high mass ejection voltage, allowed the suitability of the whole preparation and determination method.

Values obtained from GC-MS-MS method (Table 2) are higher than values obtained from HRMS (when calculated based on the upper bound values) whenever the individual PCDD/F congeners are lower than the method detection limits. Nevertheless, the results obtained are in good agreement with the reference values when PCDD/Fs levels are nearby the regulated maximum limits, showing that this GC-MS-MS method is a useful

method to discriminate among samples that do not accomplish present regulations and samples that do, and at the same time, reaching most of the criteria required to confirmation methods .

WHO-dioxin-TEQ				
Sample	EU Regulation[5,6]		MS-MS Method	HRMS or Ref. Value
	Food	Feeding stuffs	Mean ± SD	
	pg TEQ PCDD/F g-1			
Pangasius (fillet)	4.0	1.25	0.45 ± 0.14	0.01
Cod (liver)	4.0	1.25	14.21 ± 0.17	12.91
Scomber	4.0	1.25	0.39 ± 0.00	0.03
Squid (ring)	4.0	1.25	0.45 ± 0.00	0.05
Carp (CARP-2)*	4.0	1.25	14.28 ± 0.88	15.68
Feed 1	-	2.25	0.51 ± 0.17	0.16
Feed 2	-	2.25	2.67 ± 0.11	2.25
Feed 3	-	2.25	0.87 ± 0.06	0.36
Fish meal 1	-	1.25	0.50 ± 0.06	0.07
Fish meal 2	-	1.25	1.94 ± 0.05	1.53
Fish oil 1	2.0	6.0	8.51 ± 0.27	9.09
Fish oil 2	2.0	6.0	2.52 ± 0.09	2.99
Fish oil 3	2.0	6.0	1.54 ± 0.10	1.60
Fish oil 4	2.0	6.0	2.63 ± 0.33	3.05
Cod liver oil FAPAS 2009**	2.0	6.0	1.63	1.75
Cod liver oil FAPAS 2010**	2.0	6.0	2.95	2.70

* TEQ calculated from congeners with a reference value as stated in reference material CARP-2.
** TEQ lower bound value.

Table 2.- WHO-dioxin-TEQ values obtained after replicate analysis by ASE extraction (except fish oils), Power Prep purification and GC-MS-MS determination, and reference values obtained from HRMS, or assigned values (FAPAS, reference material CARP-2).

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References:

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