SUITABILITY OF AN ION TRAP GC/MS/MS METHOD FOR ROUTINE ANALYSIS OF PCDD/Fs IN FISHERY PRODUCTS AND BY-PRODUCTS

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Introduction

Dioxins and furans are organochloride compounds which are considerated POPs (Persistent Organic Pollutants). Due to its persistence in environment, human population is exposed to these contaminants, mainly through the diet [1].
Since a considerable percentage of european citizens are exposed to higher than the tolerable weekly intake of 14 pg/kg (on a WHO-TEQ/body weight basis), the European Commission set legislation based on "maximum limits" and "action limits" of PCDDs/PCDFs in food and feedingstuffs[2-4]. But routine monitoring of dioxin levels in foods and feedinstuffs in the european market faces to the obvious lack of laboratories capable of providing an analytical service cheap and fast enough to the food and feedingstuff economic operators, due to the extremely high investment and the skills required to use the standard analytical instrumentation (HRGC-HRMS).

Fishery products and by-products constitute an important vector of dioxins towards humans due to bioacumulation and biomagnification, so fish processing industry is highly interested in the development of analytical procedures cheaper and faster than the the standard HRGC-HRMS.

The aim of this study was to demonstrate the suitability of a method of determination of the 17 congeners of dioxins and furans in fishery products and by-products, using a high resolution gas chromatography connected to an ion trap tandem mass spectrometer (HRGC-MS/MS) [5]based on the patern of fragmentation of the congeners by MS/MS and quantification by isotope dilution.

Methods and Materials

Reagents:
Hexane, Acetone, Toluene (Suprasolv grade, Merck); Sodium Sulphate (Suprasolv grade, Merck); Silica gel 60 (0.063-0.200 mm, Merck); Potasium Hidroxide (for analysis, Merck); Sulphuric Acid (for analysis, Merck); Carbon Cartridge (Supelclean-Envicarb, 3 mL, Supelco); Nonane (Purism Standard for GC, Fluka), CRM Carp-2 NRC (Canada).

Standard:
Native and labelled dioxin and furan standard were provided by Wellington Laboratories. Five calibration standard solutions (CS1-1613, CS2-1613, CS3-1613, CS4-1613, CS5-1613); labelled compound standard (LCS-1613) and internal standard solution (ISS-1613).

Apparatus:
Solvent evaporation was carried out in a Laborota 4000 connected to a Büchi V-503 vacuum pump. For solid phase clean-up cartridges, a Manifold J.T. Baker, spe 12-G, was used. Solvent evaporation was done with nitrogen in a Reacti-vap evaporator (18780, Pierce). Detection and quantification was carried out on a High Performance Gas Chromatography (Varian 3800) equipped with an Autosampler (Varian 8200), a CP-Sil 8 CB column (Varian, 50 m x 0.25 mm ID) and an Ion Trap Mass Spectrometer (Varian Saturn 2000). For adquisition and processing spectral information a Varian Star Saturn Work Station was used. To carry out the dialysis process a low density polyethylene bags is used.

Extraction and Purification

Depending on the fat content of the sample, two different procedures were used

- Low fat samples

Fat is extracted from 20 g of sample with addition of 10 µL of 15 labelled isomers solution using adsorption chromatography eluted with a 100 mL of a hexane: acetone solution (1:1) mixture. Samples with a high water content were previously dried with sodium sulphate during 24 h. Solvent was concentrated to 5 mL. Then fat is destroyed in chromatographic columns packed with silica and acid silica (modified with sulphuric acid) and eluted with 100 mL of hexane. After solvent evaporation to 5 mL, the sample is cleaned-up in a multi-layer column (packed with silica, acid silica modified with sulphuric acid and basic silica modified with potassium hydroxide) using 100 mL of hexane. Solvent mixture is evaporated to 1 mL. A solid phase extraction is carried out in the Carbon Cartridge. Dioxin and furans are separated eluting interferences in direct flow with hexane: toluene in different proportions (hexane: toluene 99:1, and hexane : toluene 75:25) and then dioxins and furans are eluted in inverse flow with toluene. Sample is evaporated to dryness and is redissolved in 5 µL of nonane and 5 µL of labelled internal standard solution.

- High fat samples

In liquid and semiliquid samples with a high fat content a dialysis process is carried out in 10 g of sample previous addition of 10 µL of 15 labelled isomers solution, in 300 mL of hexane for 60 hours [6,7]. Then solven was concentrated to 5 mL and cleaned up in a multi-layer column as described above.

1. GC-MS/MS method

1 µL of sample is injected in HRGC/MS/MS. Sample introduction was achieved by splitless injection at 300º C using He as carrier gas. A capillary column was temperature programmed as follows: 90º C initial hold for 2 minutes, increase at a rate of 20º C min^{-1} to 200º C and hold for 1.3 minutes followed by an increase of 1º C min^{-1} to 230º C and hold 7 minutes. Finally, increase at a rate of 10º C min^{-1} to 300º C and hold 20 minutes.

The GC-MS transferline was kept at a temperature of 280º C, manifold temperature at 80º C and ion trap at 230º C. The ionisation is carry out by automatic electronic ionisation. Selected conditions in the ion trap for MS/MS are:

Compound	m/z Molecular Ion	m/z Daughter Ions	Excitation Storage Level	Excitation Amplitude
TCDD	321,9	257+259	145,0	2,60
^{13}C-TCDD	333,9	268+270	145,0	2,60
PeCDD	355,9	291+293	155,0	2,50
^{13}C-PCDD	367,9	302+304	155,0	2,50
HxCDD	390,8	327+329	170,0	1,90
^{13}C-HxCDD	402,8	338+340	170,0	1,90
HpCDD	424,8	361+363	185,0	2,00
^{13}C-HpCDD	436,8	372+374	185,0	2,00
OCDD	458,7	395+397	195,0	2,29
^{13}C-OCDD	470,7	406+408	195,0	2,20
TCDF	305,9	241+243	135,0	2,70
^{13}C-TCDF	317,9	252+254	135,0	2,70
PCDF	338,8	275+277	165,0	2,20
^{13}C-PCDF	350,8	286+288	165,0	2,20
HxCDF	374,8	311+313	165,0	2,20
^{13}C-HxCDF	386,8	322+324	165,0	2,10
HpCDF	408,8	345+347	180,0	2,40
^{13}C-HpCDF	420,8	356+358	180,0	2,40
OCDF	442,7	379+381	195,0	2,40

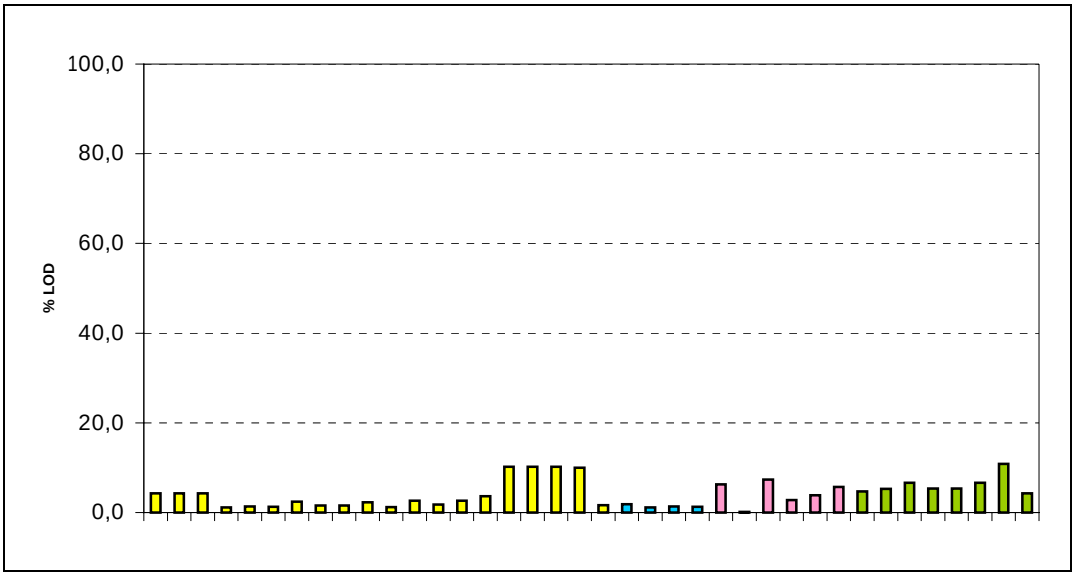
2. Confirmation of results

Analite identity is confirmed by comparing retention time between native and labelled analog isomers and the ion ratio is fulfilled between M and M+2 considering an interval ± 25 % of the theoretical ion ratio. Besides, the match of mass spectra similarity between the sample peak and standard peak should be at least 700 per 1000.

Results and discussion

In order to demonstrate the effectiveness of dioxins and furans analysis in fishery products and by-products it is necessary to carry out the validation of the method.

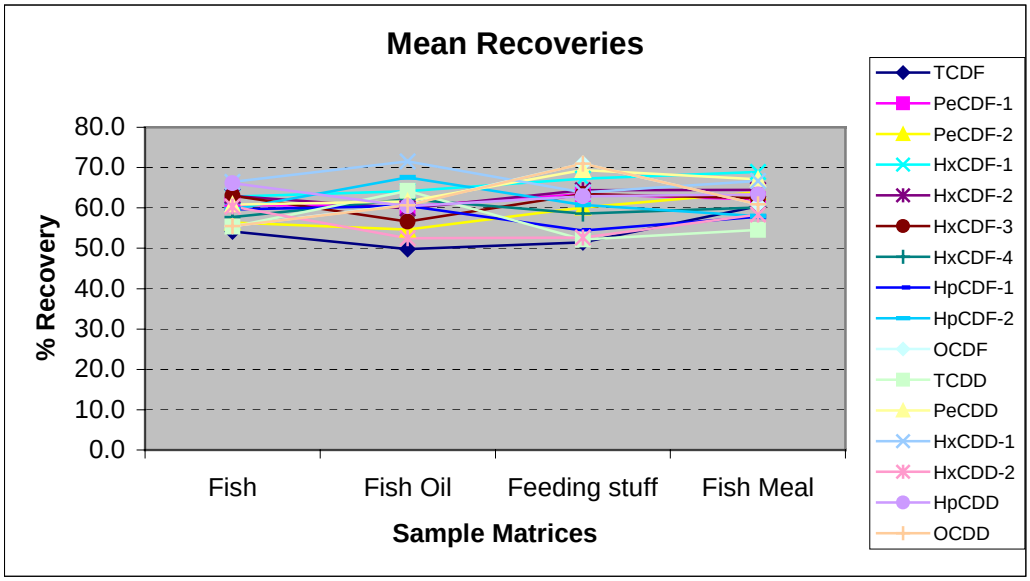
- *Limit of detection* The instrumental limit of detection established for each compound is estimated as the concentration providing a signal-to-noise ratio equal to 3. The limit of detection on the sample also depends on the sample matrix. Diverse products have been analysed which are classified in four groups: fish, aquaculture feeding stuffs, fish meal and fish oil. Results demonstrate that the contribution of limit of detection percentage respect to the maximum legal limit is minor than 10 %.



- *Recoveries*

Recoveries for each labelled congener have been studied in diverse matrix samples. It is observed that recovery average values are around 60 %. Besides, it is possible to emphasize that values between different matrices are practically constant. Also is important to

considerate that fish, fish meal and feeding stuffs are analysed like low fat samples, however fish oil is a high fat sample.



Statistical studies of recovery values for each congener in diverse samples. Results show that recovery is quite constant among congeners and also among the sample matrices tested in this study, according to the observed recovery of labelled congeners.

- Linearity

Dioxins and furans are quantified by an internal standard method denominated isotopic dilution. The principle of the method is based on the linearity of the detector signal of each isomer, and the lack of variability of the relative response factor which relate the signal of each native isomer and the signal of the labelled analog. The stability of the response of the analytical instrument throughout the time is studied from the relative response factor of nine successive calibrations during a year have been made. It has been carried out a statistical study which shows that the relative standard deviation of the average relative response factors is in the most of cases minor than 15 %. Therefore we could conclude that the response of the detector is constant through the time, suporting the stability and the robustness of the method.

Compound	*Mean*	*SD*	*%RSD*	*Compound*	*Mean*	*SD*	*%RSD*
^{13}C-TCDF	1,565	0,119	7,60	TCDF	0,943	0,057	6,00
1,2,3,4-^{13}C-TCDD	1,000	0,000	0,00	TCDD	1,267	0,141	11,09
2,3,7,8-^{13}C-TCDD	1,207	0,080	6,61				
^{13}C-PCDF-1	0,975	0,140	14,34	PCDF-1	0,864	0,154	17,84
^{13}C-PCDF-2	0,882	0,129	14,67	PCDF-2	0,967	0,072	7,48
^{13}C-PCDD	0,993	0,161	16,24	PCDD	1,120	0,168	14,99
^{13}C-HxCDF-1	1,057	0,148	13,96	HxCDF-1	1,078	0,116	10,74
^{13}C-HxCDF-2	1,290	0,204	15,80	HxCDF-2	1,073	0,101	9,41
^{13}C-HxCDF-3	1,069	0,166	15,56	HxCDF-3	1,134	0,170	14,98
^{13}C-HxCDF-4	1,000	0,135	13,46	HxCDF-4	1,218	0,187	15,32
^{13}C-HxCDD-1	0,835	0,070	8,37	HxCDD-1	1,060	0,096	9,06
^{13}C-HxCDD-2	1,139	0,111	9,76	HxCDD-2	0,972	0,107	10,95
^{13}C-HxCDD-3	1,000	0,000	0,00	HxCDD-3	0,923	0,234	25,39
^{13}C-HpCDF-1	1,072	0,068	6,39	HpCDF-1	1,163	0,122	10,47
^{13}C-HpCDF-2	0,902	0,109	12,12	HpCDF-2	1,061	0,099	9,33
^{13}C-HpCDD	0,775	0,050	6,46	HpCDD	1,099	0,073	6,65
^{13}C-OCDD	0,598	0,081	13,62	OCDF	1,037	0,188	18,11
				OCDD	1,019	0,107	10,54

Table 1: Mean, standard deviation and relative standard deviation of nine average relative response factor from the calibrations.

- Accuracy

A certified reference material (carp muscle) is analised by low fat sample method. The most results are in the uncertainty interval of the material, but it is observed a lost of analites in the most chloride isomers.

	Certified material results	*Obtained results*	
Compound	**Concentration**	**Concentration**	**TEQ**
TCDF	18,2±1,6	19,72	1,972
PeCDF-1	5,6±0,3	5,42	0,221
PeCDF-2		14,51	7,253
HxCDF-1		2,88	0,288
HxCDF-2		1,58	0,158
HxCDF-3		0,00	0,000
HxCDF-4		0,00	0,000
HpCDF-1		2,12	0,021

HpCDF-2		0,00	0,000
OCDF		0,00	0,000
TCDD	7,4±0,7	7,74	7,745
PeCDD	5,3±1,3	5,05	5,052
HxCDD-1	1,6±0,3	2,11	0,211
HxCDD-2	5,8±0,8	5,31	0,531
HxCDD-3	0,78±0,12	1,38	0,138
HpCDD	8,4±0,9	4,82	0,048
OCDD	9,4±1,7	4,85	0,000

Table 2: Reference material results in a carp muscle

These results demonstrate the usefulness of the GC-MS/MS technique in routine tasks of dioxin monitoring for the fish industry, as a cheaper and faster alternatative to HRGC-HRMS, yielding detailed information on congener concentration through isotope dilution quantification and confirmation by MS/MS procedures.

Acknowledgments

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References

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