QUALITY ASSESMENT FOR THE ANALYSIS OF PCDDs/PCDFs IN INDIVIDUAL HUMAN SERUM SAMPLES

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Introduction

At present, it is highly recognized the existence of a wide number of anthropogenic processes responsible for the presence of toxic pollutants such as chlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/PCDFs) in the environment. Great efforts and investments were undertaken to know the behaviour of these xenobiotics and major consensus about the hypothesis that food chain is the most important route for human exposure was established [1, 2]. Furthermore, recent dioxin contamination episodes demonstrated the link between dioxin releases to the environment and their presence in humans. Reported data revealed detectable concentrations of PCDDs/PCDFs in general population. Commonly, dioxin body burden is evaluated by the analysis in whole blood, plasma or adipose tissues [3].

The aim of this work was to optimise a relevant methodology for the ultratrace analysis of PCDDs/PCDFs in individual human serum samples. In order to carry out the study, different strategies including the elaboration of quality control samples, parallel sample analysis, control blanks and a number of quality assurance measures were implemented as analytical current practices. Some of the main drawbacks in the analysis of PCDDs/PCDFs in these kind of samples come from two conflicting aspects: the small sample size and the low levels expected to be found. Taking this into account, an unavoidable compromise between the sample amount and the minimum analytical requirements, mainly the detection limit (LOD), is mandatory.

To reach this goal C_{18} solid phase extraction was used to remove the analytes from the matrix[4]. Clean up was perfomed by solid-liquid adsorption chromatography using a variety of adsorbents[5]. Instrumental analysis was achieved by high-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC/HRMS).

Finally, the optimised methodology was applied to evaluate the potential impact in general population living in the surroundings of an obsolete municipal waste incinerator plant (MWI). Thus, more than 400 individuals serum samples potencially exposed to the emission of the incinerator and people not exposed were considered in this study.

Materials and Methods

Sampling

Blood sampling campaigns and serum isolation were performed through the services of a specific hospital placed in the area. Detailed protocol was provided by the Laboratory of Dioxins based on reported literature[5].

Serum samples provided periodically by the *Hospital Doctor Josep Trueta* in Girona (Spain) were employed to prepare different pools used to optimise the methodology as well as a quality control samples.

Serum lipid determination

Serum lipid determinations were carried out through the Biochemistry Services of the *Hospital Clinic* in Barcelona (Spain). Total serum lipid value consisted on the sum of total colesterol total cholesterol, triglycerid, free cholesterol and phospholipids[5].

Sample preparation method

Teflon bottles containing approximately 30 g of serum samples were spiked with a known amount of labelled standards daily prepared in acetone7 (EPA-1613LCS, Wellington Lab., Canada) and shaked for half an hour to homogenize the standard solution with the serum. Then, 30 mL formic acid and 30 mL water were sequentally added and shaked again. Now, samples were ready for the extraction process.

Extraction

Extraction was based on solid phase extraction techniques (SPE) using 10 g of non-endcapped C_{18} cartridges (IST, Isolute SPE) in batches formed by real serum samples, quality control samples (QA/QC) and blanks. Prior to the extraction SPE cartridges were conditioned by adding methanol and water. Later on, samples were applied to the top of the C_{18} cartridges and less retained biogenic material passed through the cartridge to the waste basin.

Next, sorbent was dried first with methanol and finally with a nitrogen current. At this point, analytes were eluted with n-hexane into a conic bottom tube.

Clean up
Clean up was carried out using an automated system (PowerPrep™, Fluid Management System Inc., USA), which employs three different columns: multilayer silica, basic alumina and PX-21 carbon adsorbents. After purification, extracts were concentrated under a N_2 current in an automatic system (Turbo Vap, Zymark). Finally, samples were transferred to vials and the extracts were reconstituted with 5 µl of nonane and fortified with 5 µl of a diluted internal standard mixture formed by two labelled congeners (EPA-1613ISS, Wellington Lab.).

Instrumental analysis
Instrumental analysis was based on the use of HRGC-HRMS (Autospec Ultima NT, Waters, Manchester, UK) equipped with a CTC 200S autosampler. Fragmentograms were obtained in the selected ion-monitoring (SIM) mode, using an EI+ source and at 10000 resolving power. Chromatographic separation was achieved using a DB-5 ms GC capillary column (0.18 mm film thickness, 0.18 µm I.D., 40 m length). The temperature program was: 140 ºC (1min) to 200 ºC (1min) at 20 ºC/min, then at 5ºC/min to 310 ºC and held isothermally for 6 min at 310 ºC. Quantification was performed by the isotopic dilution method. Relative Response Factors (RRF) were achieved from the analysis of six standard calibration points prepared specially for this study considering the low espected levels.

Quality Control/Quality Assurance
Quality control measures included the analysis of quality control, blanks and spiked samples or the participation in intercalibration exercises as a current quality policy of the Laboratory of Dioxins. Other common practices included sensitivity of MS, chromatographic separation, power resolution or recovery rates of labelled standards.

Results and Discussion

In this work, an improved analytical methodology for the analysis of PCDDs/PCDFs in individual human serum samples was exhaustively assessed against current quality control and assurance procedures with the aim to participate in a comprehensive study covering more than 400 human serum samples.

To this end, different quality control approaches were attended. Firstly, a quality control sample was prepared mixing individual human serum subsamples to obtain a pool. Thus, more than 80 quality control samples allowed us to evaluate the overall goodness of the method. Moreover, different PCDDs/PCDFs concentrations were also considered by the analysis of spiked quality control samples. Criteria for spiking consisted on the addition of a dilution mixture of 17 toxic congeners (EPA1613 PAR solution) increasing the at levels 2 and 3 times with respect the levels of 2,3,7,8-TCDD found in the non-spiked quality control sample. Table 1 summarises the results of the spiked and non-spiked quality control sample analysis.

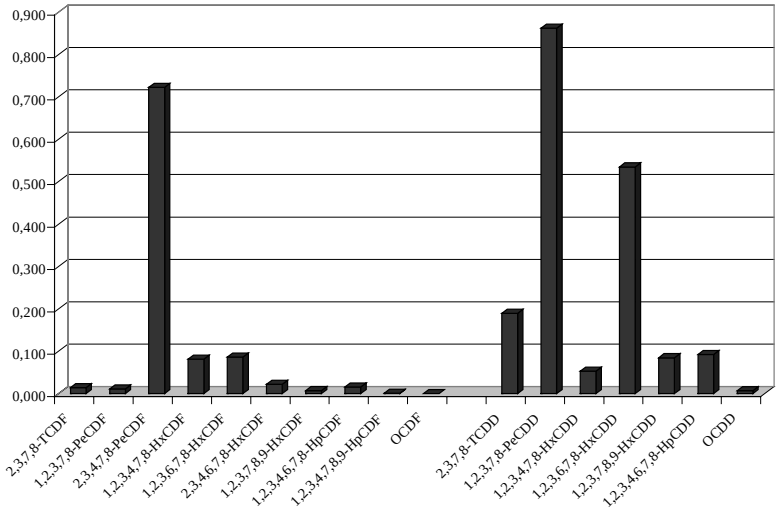
An important tool to assess the table comes from the characterization of the congener distribution of the toxic PCDDs/PCDFs in real serum samples. Fingerprint analysis showed that, in general, OCDD presented the highest concentration followed by HpCDD and 1,2,3,6,7,8-HxCDD; the rest of congeners remained in a minor proportion, close the detection limit (Figure 1). Major contribution to TEQ comes from 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, and 1,2,3,6,7,8-HxCDD with a 30%, 25% and 20% respectively. Therefore, quality parameters of the analysis were as follows: smallest RSD% between 6 and 10% were obtained for the most concentrated congeners, meanwhile the congeners with concentration close to the LOD presented RSD% up to 49%. Nevertheless, better RSD values were obtained in spiked samples ranging from 3 to 12%. This could be explained because the concentrations of PCDDs/PCDFs in spiked samples were far from the detection limit.

Regarding the study, more than 550 samples were performed following the described procedure including quality control samples, blank samples and real samples. The pattern found in real samples did not vary from those obtained in quality control pools. As an example, in Figure 2 a chromatogram of 1,2,3,6,7,8-HxCDD (m/z=389.5157) is given. Overall results found in the study showed no significant differences on the population despite of the potencial exposure to the PCDDs/ PCDFs released from the incinerator plant.

Acknowledgements
The authors would like to acknowledge to Prof. Olaf Päpke from Ergo and to Dioxin Laboratory from Liege specially to Jean-François Focant and the for the help given. Special thanks for their unselfish collaboration to Doctor J.M. Mauri from Hospital Doctor Josep Trueta (Girona) and Doctor Elena Casals from The Hospital Clinic (Barcelona). And finally to the laboratory members who work hardly on this study.

Figure 1.

a) Fingerprint for TEQ values (total pg in 30g of serum)



b) Figerprint for concentration values (total pg in 30g of serum)

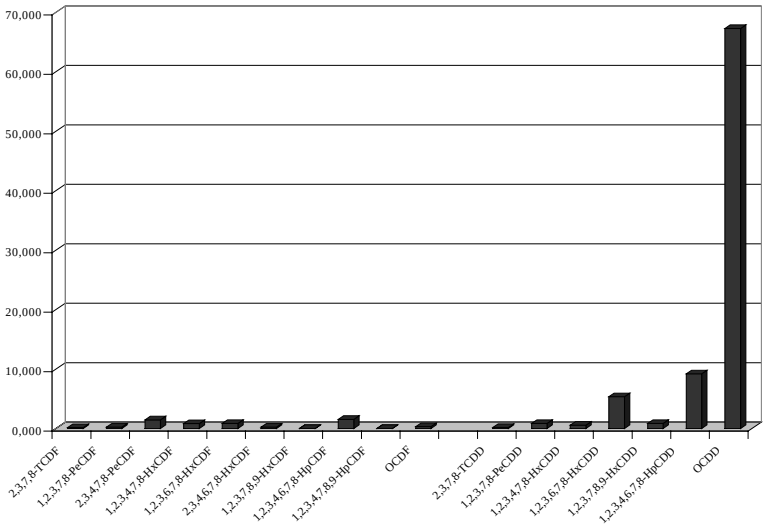
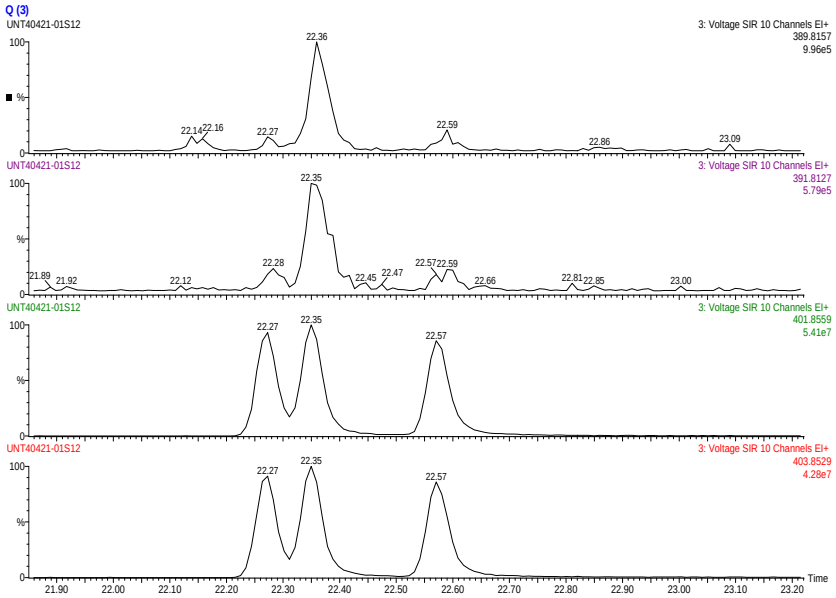


Table 1: Average, RSD, 95 percentil and minimum for quality control samples non spiked and spiked in two different level

	Non spiked sample (n=14)				1st Level of spiked sample (n=8)				2nd Level of spiked sample (n=10)			
	Average	*RSD%*	*95%*	*Min.*	*Average*	*RSD%*	*95%*	*Min.*	*Average*	*RSD%*	*95%*	*Min.*
2,3,7,8-TCDF	0,66	49,85	1,28	0,36	1,81	10,27	2,01	1,98	5,01	5,84	4,08	4,56
1,2,3,7,8-PeCDF	1,08	84,33	2,79	0,39	7,00	12,12	7,93	7,37	19,78	7,45	16,53	17,66
2,3,4,7,8-PeCDF	7,23	9,50	8,78	6,70	14,72	7,55	16,16	17,09	29,76	6,21	24,65	27,97
1,2,3,4,7,8-HxCDF	4,05	11,77	5,08	3,79	9,91	10,15	11,19	11,89	24,08	5,59	19,60	22,03
1,2,3,6,7,8-HxCDF	4,32	9,09	5,28	4,10	10,44	2,99	10,85	13,20	24,59	6,51	19,88	21,73
2,3,4,6,7,8-HxCDF	1,09	20,78	1,46	0,84	7,41	10,06	8,56	8,68	20,43	9,49	17,16	17,55
1,2,3,7,8,9-HxCDF	0,14	85,58	0,56	0,29	6,30	7,00	6,95	7,60	19,86	7,86	16,73	18,03
1,2,3,4,6,7,8-HpCDF	7,66	17,30	10,60	6,57	13,12	6,78	14,39	16,12	28,59	8,71	24,73	26,53
1,2,3,4,7,8,9-HpCDF	0,07	240,52	0,65	0,42	6,28	9,16	7,00	7,38	19,21	9,31	16,03	15,77
OCDF	1,42	26,86	2,04	0,80	13,08	10,47	14,64	14,85	37,49	11,04	31,81	30,50
2,3,7,8-TCDD	0,95	17,52	1,27	0,79	2,35	10,44	2,63	2,50	5,42	8,89	4,47	4,67
1,2,3,7,8-PeCDD	4,31	17,96	5,86	3,23	10,98	11,53	12,40	12,25	24,84	11,30	21,68	20,06
1,2,3,4,7,8-HxCDD	2,63	12,29	3,27	2,54	9,53	10,78	10,83	11,07	23,18	9,70	19,63	18,70
1,2,3,6,7,8-HxCDD	26,74	5,66	30,90	26,11	33,73	6,56	37,07	41,86	57,02	7,29	47,21	50,70
1,2,3,7,8,9-HxCDD	4,21	19,77	5,88	3,51	10,57	10,77	11,79	11,21	23,35	11,19	19,79	18,47
1,2,3,4,6,7,8-HpCDD	46,02	7,19	53,68	43,66	53,57	4,66	56,41	67,17	83,40	10,23	71,89	73,30
OCDD	336,30	7,55	395,25	317,08	361,33	4,76	386,80	452,00	511,66	5,72	416,30	471,45

Units in pg over 0.20 g fat

Figuere 2: Chromatograms of 1,2,3,6,7,8-HxCDD isomer for a serum sample



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