FRACTIONATION OF PCDD/F AND PCB IN SPE CARBON TUBES. COMPARISON TO OTHER FRACTIONATION METHODS IN HUMAN PLASMA ANALYSIS

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Introduction

Polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are three related families of toxic organochlorinated pollutants that are often found together in environmental and biological samples. However, these compounds cannot be analyzed in one single purified extract because PCB interfere in the analysis of PCDD/F, even though the analysis is performed by high resolution gas chromatography-high resolution mass spectrometry. Moreover, the concentration level of PCB is usually much higher (up to 10,000 fold) than that of PCDD/F, making necessary the separation of both types of compounds. In addition, some specific congeners of PCB, those with lack of chlorine in the *ortho* positions, elicit toxic responses similar to PCDD/F due to their planarity. As PCDD/F, these congeners, namely coplanar PCB, are also present in samples at very low concentrations. Furthermore, the separation from the bulk of PCB is also required to obtain correct quantifications.

Several methods have been described in literature to achieve this separation: open-chromatographic columns filled with activated carbon (Wako, AX-21, etc.), Carbopack C/Celite, Florisil, alumina, etc., and porous graphited carbon or pyrenyl-silica column, both used in preparative HPLC[1-4]. Each of them has advantages but also disadvantages. Methods using HPLC columns show a good separation and their automation is easy but they are very sensitive to the presence of lipids. On the other hand, open chromatographic columns filled with activated carbon need larger amounts of solvent and obtaining good procedure blanks can be hard work.

The use of solid phase extraction (SPE) tubes or disks to extract samples or purify extracts has increased in the last years, especially for pesticide analysis. Using this technique is simple, it needs relative small amounts of solvent and it can be easily automated. However, its use for PCB and PCDD/F analysis is not wide-spread. In one previous work[5], we developed a procedure to separate non-*ortho* and the bulk of PCB in SPE tubes pre-packed with Carbopack B. Now, we have improved the method to achieve the separation of PCDD/F, non-*ortho* PCB and the bulk of PCB in three fractions. In addition, this method has been compared to two others (open-chromatographic column filled with Florisil and HPLC equipped with a pyrenyl-silica column) to fractionate purified extracts of human plasma.

Methods and Materials

Fractionation methods

For fractionation in SPE carbon tubes, Supelclean ENVI-Carb SPE tubes (3 ml, 0.25 g of Carbopack B), supplied by Supelco (Bellefonte, PA, USA) were used. After washing and conditioning the carbon with 20 ml of toluene and 20 ml of hexane, the standard (when the method was developed) or the sample (when the three fractionation methods were compared) was loaded. The separation was performed under vacuum with the following eluents:

- Fraction C1+C2: Mono- to tetra-*ortho* PCB were eluted with 15 ml of hexane and 20 ml of hexane/toluene 99/1.
- Fraction C3: Non-*ortho* PCB were eluted with hexane/toluene 75/25.
- Fraction C4: PCDD/F elution was tested with several solvents (see "Results and discussion" below).

Fractionations in HPLC PYE column and in open-chromatographic columns filled with Florisil have already been described in literature[4,6].

Sample analysis

Six 100-ml aliquots of a pooled sample prepared from human plasma obtained from volunteers were analyzed. Prior to extraction, samples were spiked with a mixture of $^{13}C_{12}$ labeled 2,3,7,8-PCDD/F and $^{13}C_{12}$ labeled non-*ortho* PCB. Since it is known that concentrations of PCDD/F are low in plasma samples, they were also spiked with a mixture of native 2,3,7,8-PCDD/F. After spiking, samples were liquid-liquid extracted according to a modification of Patterson's method[7]. The extract was dried in a sodium sulfate column and concentrated under reduced pressure. After removing completely the solvent (60°C in oven, overnight), lipids were determined gravimetrically. The extract clean-up was performed with 2 ml of concentrated sulfuric acid and with a multilayer silica column. Two purified extracts were fractionated in HPLC PYE column, two in Florisil column and two in SPE carbon tubes. PCDD/F and non-*ortho* PCB fractions were concentrated up to 15 µL under nitrogen flow and the syringe standards ($^{13}C_{12}$-1234-TCDD and $^{13}C_{12}$-123789-HxCDD, for PCDD/F, and $^{13}C_{12}$-PCB 123 for PCB) were added. Di-*ortho* and higher PCB fractions were concentrated and 1234-TCN was added as syringe standard. All PCDD/F and non-*ortho* PCB were analyzed by HRGC-HRMS while di-*ortho* and higher PCB were analyzed in a HRGC-ECD system.

Results and Discussion

Development of carbon tubes method

The elution of PCB in this kind of SPE tubes were well-established in previous works of our group[5]. Therefore, this paper has focused on the elution of PCDD/F. Two main goals should be achieved: the complete elution of all PCDD/F congeners and their separation from non-*ortho* PCB.

Firstly, several solvents and mixtures of them (toluene, toluene/acetone 95/5 and toluene/trifluoroacetic acid 99/1), described in literature for elution of PCDD/F from different kind of carbons, were tested. As it is shown in **figure 1a**, 100 ml of toluene in direct flow only eluted

TCDD and PeCDD/F, while the elution of HxCDD/F and HpCDD/F was only partial. The results obtained with toluene/acetone and toluene/trifluoroacetic acid were quite similar. Only when reverse-flow toluene (**figure 1b**) was used, the elution of all PCDD/F congeners, even OCDD/F, was achieved.

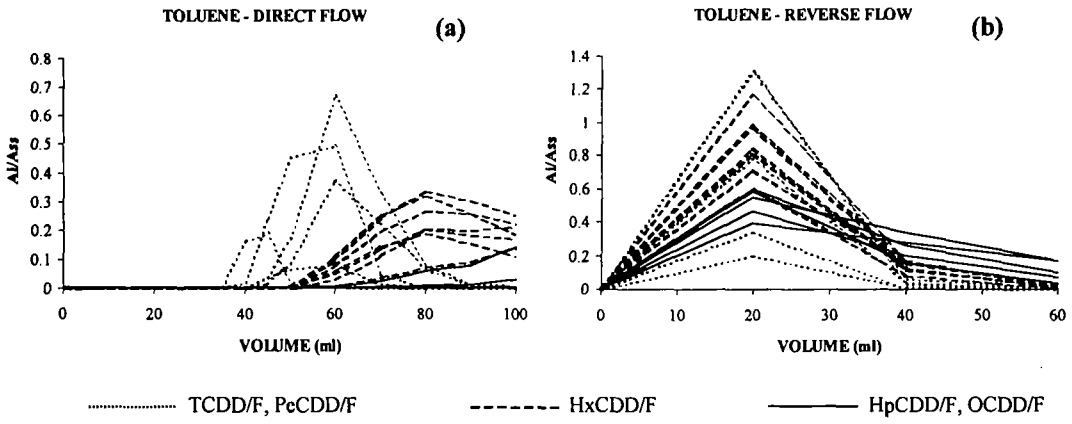

Figure 1. *Profile of PCDD/F eluted in SPE carbon tubes with (a) toluene in direct flow and (b) toluene in reverse flow (both are reconstructed from HRGC/HRMS analysis of the collected fractions: Ai/Ass is the ratio between the area of a single congener and the area of the syringe standard obtained in HRGC-HRMS).*

Secondly, in order to avoid the coelution of PCDD/F and non-*ortho* PCB, the fraction of these PCB was eluted with a mixture of hexane/toluene 75/25 instead of toluene (as it had been previously performed).

The results obtained in studying the accuracy and repeatability of the method showed that it was suitable for PCDD/F and PCB analysis in real-world samples.

Comparison to other fractionation methods

The results obtained in the fractionation of purified human plasma extracts with SPE carbon tubes, PYE column and open-chromatographic columns filled with Florisil are shown in **Table 1**. Not only methodological parameters, like number of fractions obtained or PCDD/F recoveries, are compared, but also other parameters are, like time, instrumentation and solvents required.

The best resolution is achieved with PYE column, which allows the separation of 4 fractions. However, PCDD/F and non-*ortho* PCB recoveries obtained in this procedure are too low. Florisil and ENVI-Carb tubes methods show similar PCDD/F recoveries but the first one does not allow the separation of non-*ortho* PCB from the bulk of PCB.

Time required to analyze a six-samples series is lower for Florisil method than for ENVI-Carb and much lower than for HPLC method because only one sample can be analyzed at the same time on PYE column. On the other hand, fractionation in columns filled with Florisil needs higher volumes of solvents (one of them chlorinated) than the other methods and it cannot be automated.

Table 1. Results of fractionation methods comparison.

Parameters	Florisil	ENVI-Carb	PYE
Number of fractions	2	3	4
Recoveries PCDD/F	50%-80%	50%-80%	20%-40%
Recoveries non-ortho PCB	----	51%-94%	10%-84%
Variability PCDD/F	<12% (<20%)	<7%	<10% (<16%)
Variability non-ortho PCB	----	<2%	<8%
Compounds detected in procedure blanks	OCDD	----	TCDF 83 & HxCDF 118
Solvents required	Hx 150 mL; DCM 100 mL	Hx 70 mL; Tol 85 mL	Hx 25 mL; Tol 35 mL
Samples at the same time	6-12	6	1
Time per six-sample series	2 h	3 h	8 h
Instrumentation required	oven	vacuum station & pump	HPLC chromatograph
Automation	No	Yes	Yes

In conclusion, we recommend the use of SPE pre-packed carbon tubes for the fractionation of PCB and PCDD/F due to the advantages that this novel method presents compared to others as Florisil or HPLC/PYE analysis.

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