AN IMPROVED CLEAN-UP STRATEGY FOR SIMULTANEOUS ANALYSIS OF PCDDs, PCDFs AND PCBs IN BIOLOGICAL SAMPLES.

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Introduction

Polyhalogenated aromatic compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are ubiquitous environmental polluants. Owing to their structural similarity with dioxins, non and mono-ortho PCBs present the same mechanism of toxicity than 2,3,7,8-TCDD and have been attributed a Toxic Equivalent Factor (1). The importance of non-ortho PCBs contribution in the I-TEQ evaluation have already been demonstrated (2). Nevertheless neither non-ortho nor mono-ortho PCBs has been part to most monitoring programmes. For instance in Belgium, following the so called "dioxin crisis", the government imposed a norm of 5 pg TEQ/g fat for 17 dioxins and furans (2,3,7,8-substitued congeners) and 200 ng/g fat for some PCBs , only the 7 marker PCBs (Aroclor 1260). Currently, these analyses are performed using two separate protocols having their own sample preparation steps and analytical tools.

The aim of this study was to demonstrate the feasibility of the 17 dioxins, 12 non and mono-ortho PCBs and the 7 marker PCBs isolation using the Power-Prep System™, an automated purification system for which efficiency in dioxins analysis has already been demonstrated last years (3,4,5), from the same extract in a single step of clean-up. The analysis is carried out separately using high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC/HRMS) for dioxins and gas chromatography coupled to low resolution (ion trap) tandem in time mass spectrometry (GC/MS/MS) for PCBs.

Methods and Materials

All methods and materials are described in detail elswhere (6). Nevertheless here is a summary.

Reference method for PCBs. Accelerated solvent extraction (ASE) were performed on an ASE™ 200 extractor (Dionex ,Sunnyvale, CA, USA) using hexane as solvent. Extracted fat is melted and is directly laid down on the top of a multilayer column (acidified silicagel, aluminium oxide and sodium sulphate) and is eluted by hexane. The final extract is evaporated with nonane as keeper.

Multi-analytes method. Accelerated solvent extraction (ASE) were performed on an ASE™ 200 extractor (Dionex ,Sunnyvale, CA, USA) using hexane as solvent. Extracted fat were then processed through gel permeation chromatography to allow the lipids reduction, evaporated in a Turbovap™ II Concentration Workstation (Zymark Corporation, Hopkinton, MA, USA), diluted in hexane and purified using the Power-Prep System™ (Fluid Management Systems, Waltham, MA, USA). The description of this system has previously been made (3,4,5). Briefly, this is an automated clean-up system which uses disposable silica, alumina and carbon columns following in order to separate analytes of interest from matrix interferences following reported results (7). The configuration of the system allows the operator to collect different fractions at different stage of

the purification. The collected fractions can therefore be concentrated and analysed by mass spectrometry.

PCDD/Fs and coplanar PCBs analysis were performed by GC-HRMS using a MAT95XL high-resolution mass spectrometer (Finnigan, Bremen, Germany) and a Hewlett-Packard (Palo Alto, CA, USA) 6890 Series gas chromatograph.
Other PCBs analysis were carried out with a Saturn 2000 GC/MS/MS coupled with a Star 3400CX gas chromatograph and a 8200CX autosampler (Varian, Walmut Creek, USA).

Results and Discussion

The classical sequence of clean-up events constituting the program for a run on the automated system is depending of the solvent type, the flow rate and the utilisation or by-pass of the different columns. The idea in this study was to maintain the same program as used for dioxins isolation and sequentially collect the eluting fractions to determine the fractionation pattern for the 7 marker and the 8 mono-ortho PCBs.

The clean-up scheme on the Power-Prep System™ consists in a succession of 3 different types of columns: multilayer silica, basic alumina and PX-21 carbon. After conditioning steps using classical solvents, the extract is diluted in hexane and loaded on the silica column for fat removal and then eluted to the alumina column with hexane (F1). Once on the alumina column, the sample is eluted with a mixture of hexane/dichloromethane 98:2 to a collection tube (F2) and with hexane/dichloromethane 50:50 through the carbon column (F3). Once the carbon column has been loaded, an ethyl acetate/benzene 1:1 mixture is applied in the forward direction (F4) Afterwards, toluene is applied in the back flush direction on carbon column and collected as F5, called the "dioxin fraction". The summarised fractionation scheme is depicted in Fig.1

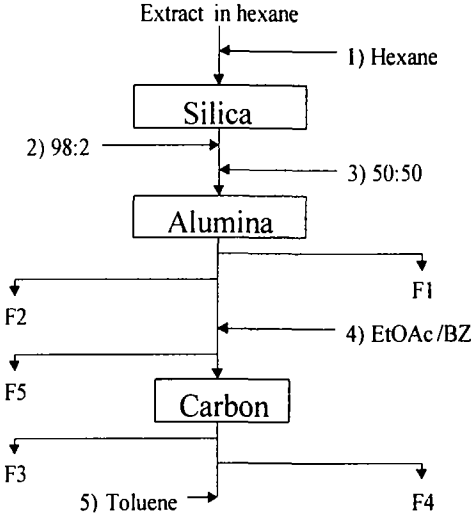


Fig. 1 Scheme for fraction collection

Although non-ortho PCBs (cPCBs) having a planar geometry are collected with good recoveries (Fig.2) in the PCDD/Fs fraction from the toluene back-flush on the carbon column (F5), other PCBs are dispersed in the different fractions (Fig.3) following the degree of substitution and the position of the chlorine atoms. Both figures show that all the desired congeners are quantitatively recovered in collected fractions. Recoveries are between 70 and 110%, which can be considered as effective.

Structural and electronical correlations : Carbon which is actually known to fractionate organic molecules according to their structural properties allows the elution of coplanar species retained between its planar layers by displacement using structurally related solvents like toluene (8). Other congeners with chlorine atoms in ortho position can't take this planar geometry and pass through

the carbon. Their separation is performed upstream, on the basic alumina column, where they are selectively desorbed in function of their polarity and the one of the selected solvent.

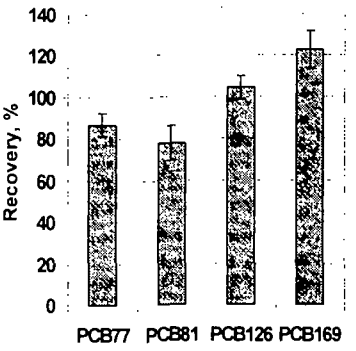


Fig. 2 Recovery of coplanar PCB congeners in the «dioxin fraction» (F5)

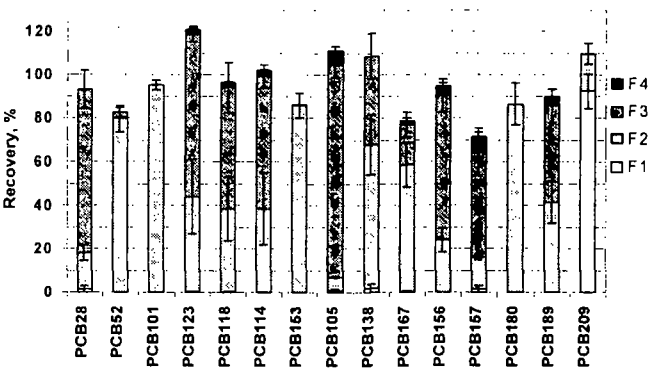
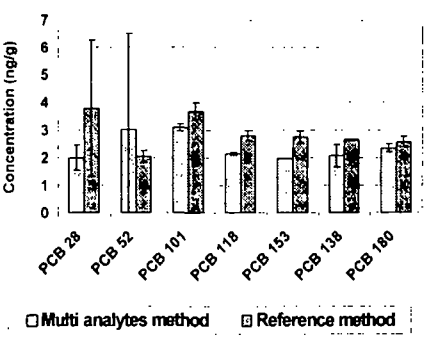


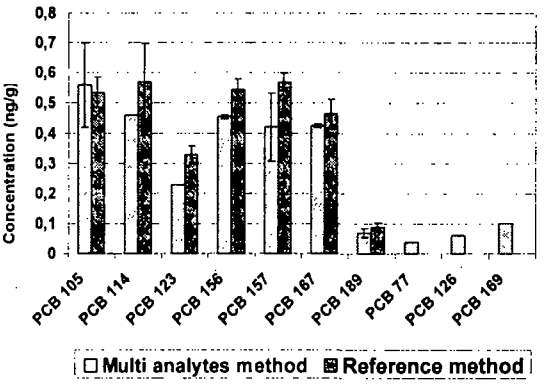
Fig. 3 Distribution of 8 mono-ortho and 7 marker PCBs

In order to correlate the fractionation pattern with polarities of analytes, dipolar moment have been calculated using molecular modelling program, SYBYL^{TM} 6.2 Molecular Modeling Software (Tripos, St. Louis, MO, USA). The geometry of each molecule has been optimised in order to reach the minimum potential energy. Dipolar moments were obtained by the Gasteiger-Hückel method and are expressed in Debby (D). Table 1 shows the correlation between the geometry and the dipolar moment (μ) of each congener with the fractionation distribution.

It clearly appears that for very low values of dipolar moment (between 0 and 0.03 D), elution of these congeners shift from F1 to F2 and, for higher values (below 2.34 D), shift to F3. This trend being less obvious for intermediate values of dipolar moment. Since congeners with same value of dipolar moment don't show the same distribution in the two fraction F2 and F3, other parameters have to be taken into account to explain the elution order of these PCBs. Assuming that part of PCBs interactions with alumina is due to hydrogen bonds, less chlorinated congeners should have more possibilities of making hydrogen bonds than more chlorinated ones. They should therefore interact more strongly with basic alumina and should be eluted later than high chlorinated congeners. Knowing that PCBs 180, 189, 167, 114,118 and 28 have the same value of dipolar moment of 1.01 D and considering PCB 180 and 189, which are the most chlorinated of them with seven substituting chlorine atoms, one can observe that they are the most abundant in the fraction F2 (80 % and 60 % respectively). The hexachlorinated CB 167 is equally distributed between F2 than F3, whereas the two pentachlorinated CBs 114 and 118 are mainly present in F3 (60 % and 70% respectively). In the case of the less chlorinated PCB 28, the effect of the poor availability of chlorine atoms for hydrogen bonding is even stronger and it end up almost only (80 %) in the fraction F3. The combined effect of dipolar moment and degree of chlorination can account for the very weak retaining of PCB 209 on basic alumina compared to PCB 101 which has nearly the same dipolar moment and ends up only in fraction F2.

Validation : To validate this cleanup, we applied this purification method to samples and compared results with an accredited method.
Analysis were carried out on 'home-made' Quality Control (QC) which were fortified beef fat commercially available. On one hand, two QC were analysed by the accredited method and on the other hand, two QC were purified by Gel Permeation Chromatography then by Power Prep where all fractions were pooled.
Fig 4 and 5 show results obtained in the two cases.





Results obtained with purification on Power Prep are slightly underestimated towards those obtained with classical method. Except for PCBs 28 and 52 standard deviations, both method are under 10% proving that both are reproducible.
Troubles were encountered for determination of PCBs 28, 52, 114 and 105. These congeners are very volatile and use of Turbovap as evaporating step could induce cross contamination.
Moreover, solvent used show high level of less chlorinated PCBs, especially tri-, tetra- and penta-CBs. That can explain their high standard deviation and the difference with expected values.
Knowing that large quantities of solvent are used (cyclohexane/ethylacetate for GPC; hexane, hexane/dichloromethane for Power Prep), high levels of PCBs 28, 52, 101, 118 and 138 are introduced with solvent during cleanup. Therefore to avoid an overestimation due to the large use of solvent, QC were every time followed by a "solvent blank" and the PCBs level of each congener was subtracted. In spite of that, errors can occur.

Conclusions

This new clean-up strategy allow to isolate in one sample prep, 34 compounds, that is 7 Polychlorinated Dibenzo-*p*-dioxins (PCDDs), 10 Polychlorinated Dibenzofurans (PCDFs),3 Coplanar Polychlorinated Biphenyls (cPCBs), 7 mono-ortho PCBs and 7 marker PCBs. This list can be extended to pesticides but optimisation is still needed because destruction occur in silica column. This kind of experiment is in progress in Control Disease Centre (Atlanta, USA).

Acknowledgements

The authors thank University of Liege and "Region Wallonne" for financial and technical support.

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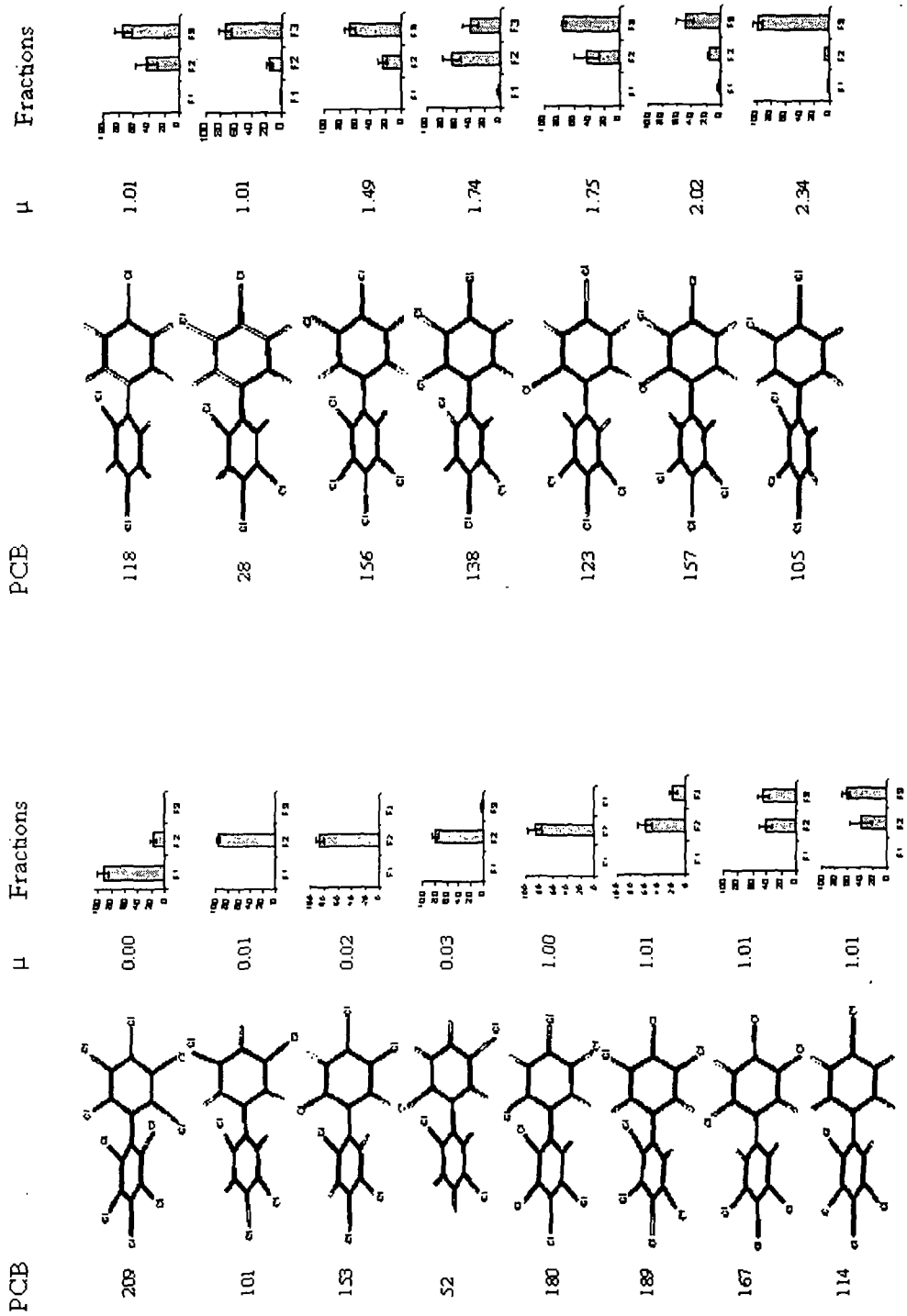

Table 1.Chemical structure, dipolar moment (μ) and distribution through the collected fractions of mono-ortho and marker PCBs.