# Determination of PCBs in biological samples using SFE in combination with GC-MS. Results of method validation studies.

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## Abstract
An SFE - GC/MS method has been developed and validated for the determination of polychlorinated biphenyls (PCBs) in biological samples. The application of this method showed no significant differences with those obtained with a previously applied method using NP-LC in combination with GC/ECD. In addition, results appeared to be fairly well comparable with assigned values established in two interlaboratory comparison studies.

## 1 Introduction

The environmental and biological impact of polychlorinated biphenyls (PCBs) is already known for many years. Many countries restricted or even ceased the production and use of formulations containing PCBs in the 1970s. Despite of this, PCBs are still a matter of concern because of their persistence in the environment, the food chain and human tissues. PCBs are therefore still incorporated in various monitoring programmes. Standard methods for the analysis of foodstuffs and human tissues generally involve extraction of lipids, various clean-up steps to remove the bulk of the lipids and other co-extractants, and final analysis with gas chromatography with either ECD or MS. In the last few years, the possibilities for selective extractions with supercritical fluid extraction (SFE) have been investigated. SFE conditions selected may not be too extreme to prevent co-extraction of lipids and some publications describe high mixing ratios for fat retainers from 1:6 to 1:20 [1,2]. So, only relatively small amounts of fat can be handled, resulting in increased limits of determination.
In this paper, we report on the progress achieved in the validation of an SFE-GC/MS method for the determination of environmentally and toxicologically relevant PCBs in fatty samples [4]. The performance of this method has been compared with a previously applied method based on normal phase liquid chromatography (NP-LC) in combination with GC/ECD [3]. In addition, results will be shown from participation in two recent interlaboratory comparison studies.

## 2 Materials and methods

*Supercritical fluid extraction*
Supercritical fluid extractions were performed on a Hewlett Packard SFE 7680T (Wilmington, DE, USA) instrument using $CO_2$ (Hoek Loos 5.3, Amsterdam, The Netherlands) as supercritical fluid. Samples were mixed in a weight ratio of at least 1:3 with silica/$AgNO_3$ 10% or alumina basic, depending on the type of fat. SFE parameters were optimized giving the following final SFE conditions: extractions were performed using a pressure of 134 bar at 50°C resulting in a

supercritical density of 0.65 g/ml with a flow of 4 ml/min, using 10 min static and a variable dynamic extraction time depending on the amount of fat and the selected PCBs (i.e. 60 min dynamic extraction time for 1 g of fat for PCB 180). All analytes were trapped on a solid phase of Florisil (Merck, Darmstadt, Germany). The nozzle and trap temperature were 45°C and 20°C, respectively. Analytes were recovered by elution with 1.5 to 1.8 ml of hexane. Samples were concentrated or directly injected on GC-ECD [4] or GC-HRMS.

*Analysis*

GC/MS analyses of PCBs were carried out on a VG Autospec GC/MS instrument (VG Analytical, Manchester, UK) equipped with a CTC A200S autosampler and a VG OPUS data system using a 50 m x 0.25 mm I.D. CP-Sil 8 CB-MS fused silica capillary column with a film thickness of 0.25 µm (Chrompack, Middelburg, NL). Ionization of the sample was performed under electron impact (EI) conditions using 31 eV electrons with a filament emission current of 0.25 mA. The source temperature was 250°C. The resolving power of the analyzer was set at 5000:1. The components were detected by monitoring of the two most abundant ions of the chlorine isotope cluster of the molecular ions of native and $^{13}C_{12}$-labeled PCBs as well as the internal sensitivity standards. The sampling time and delay time were 50 and 10 ms, respectively, for each selected ion. Samples were injected in the splitless mode (1.0-2.5 µl) at an injector temperature of 275°C. Columns were directly connected to the source of the mass spectrometer. The GC/MS interface temperature was set at 290°C. Helium was used as the carrier gas with a mean linear velocity through the column of 29 cm/s at a column temperature of 200°C. Identification and quantification was performed using $^{13}C_{12}$-labeled surrogate standards that were added prior to the SFE-extraction of the sample. The method showed limits of determination as low as 0.05 to 0.1 ng/g of fat.

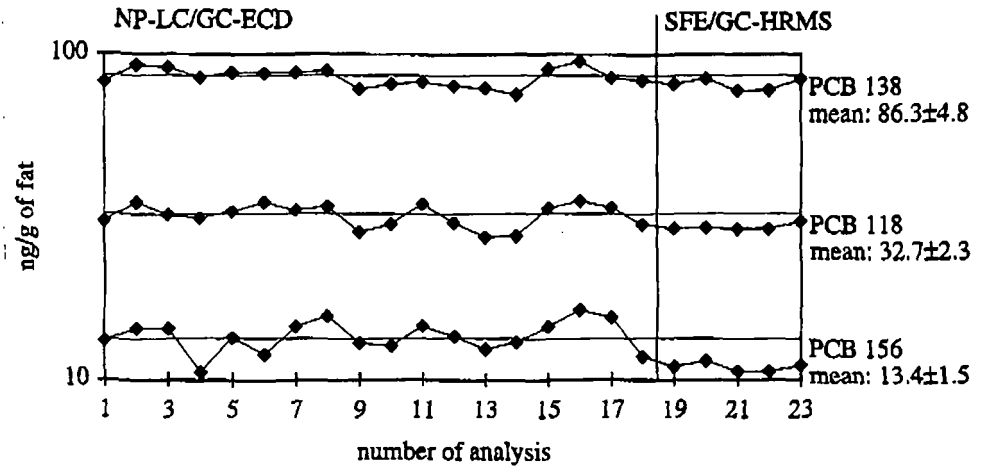


**Figure 1**

Results of PCBs in quality control samples of human milk, analysed with NP-LC and GC-ECD (sample nos. 1-18), and with SFE and GC-MS (sample nos. 19-23).

## 3 Results

*Internal validation*

The comparability of the developed method with the conventional extraction method, consisting of a clean-up of the raw fat extract by fractionation on normal phase HPLC with column switching followed by GC/ECD analysis [3], has been tested using a quality control sample of human milk (23 samples over a 2 years period). In both methods, a liquid-liquid extraction of the milk is used as a first step. Figure 1 shows the results for three representative PCBs. The repeatability of the SFE - GC/MS method was 2 - 10% for most PCBs. The SFE results showed no significant deviations from those obtained with the NP-LC method.

*IUPAC intercalibration exercise*

In the Second Round of an IUPAC intercalibration exercise on PCBs [5], participants were asked to analyse two fish oils , each in five-fold. In our lab, two sets of samples were analysed, one set with SFE in combination with ECD and another set also with SFE, but followed by GC/HRMS. For both sets, the reported data of our lab were within $2S_R$ of the assigned values for most congeners (Figure 2). The within-lab repeatability with GC/HRMS was better than with GC/ECD which is probably a result of the higher selectivity and sensitivity of the MS. The ECD responses of the less chlorinated PCBs in particular appeared to be disturbed by matrix (fat) interferences. No such interefences could be observed in GC/MS.

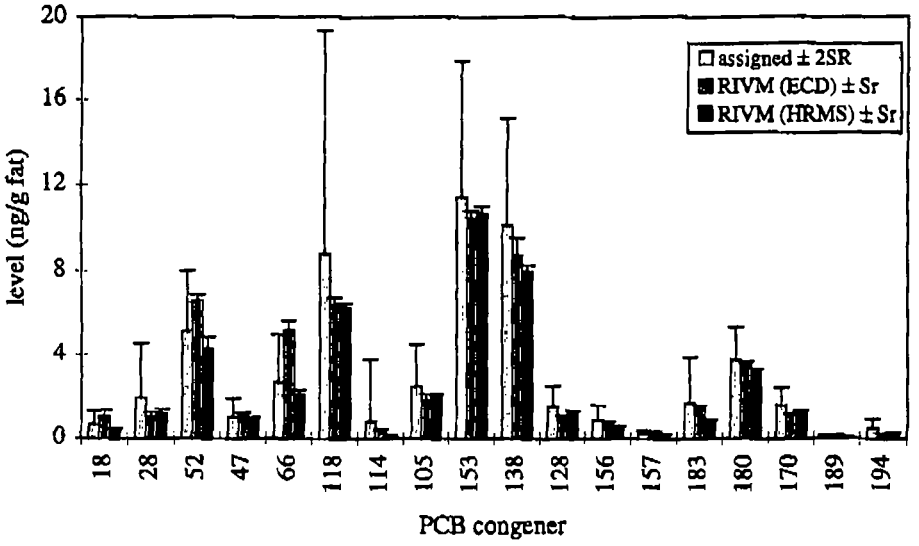


**Figure 2**

Results of our participation in an IUPAC Intercalibration Exercise [5]: comparison of assigned values (SR denotes standard deviation of reproducibility) and the mean values of our lab (Sr denotes standard deviation of repeatability) for both GC/ECD and GC/HRMS (further details, see text). PCB levels expressed in ng/g of oil).

*WHO's Interlaboratory Quality Assessment Study on Human Milk and Blood*
In the fourth round of the WHO coordinated interlaboratory studies, the levels of PCDDs, PCDFs and PCBs had to be determined in 8 samples of human milk and 8 samples of blood plasma [6]. In Figure 3, our results are compared with the consensus values for the eight samples of blood plasma. For both human milk (not shown) and blood plasma, our results appeared to be well comparable with the consensus values. The largest deviations were found for PCB congeners detected at lower levels.

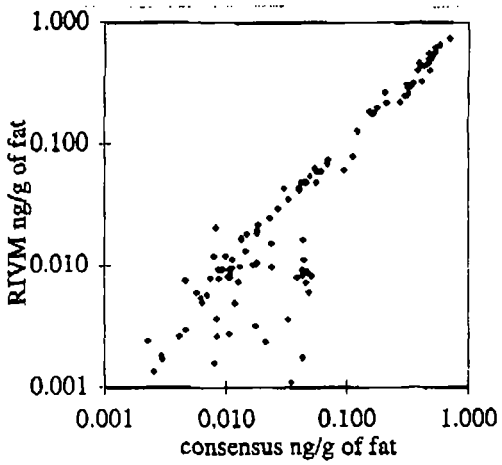


**Figure 3**
Results WHO Intercalibration Study: reported versus consensus values for PCBs in 8 plasma samples.

## References


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