Determination of PCBs in human blood using solid-phase extraction with on-column lipid decomposition

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Introduction

Biomonitoring of polychlorinated biphenyls (PCBs) has been widely used to determine the human body burden of these abundant and toxic environmental pollutants (1-7). From the human tissues investigated, blood seems to be best suited compared to breastmilk and adipose tissue as it is readily available from all parts of the general population. However, the analysis of PCBs in blood is demanding due to the low PCB concentrations, limited sample amount and complex matrix. Previously applied methods have mostly been based on liquid-liquid extraction followed by fat separation or decomposition (2,3). In order to reduce solvent consumption and sample handling, we developed a method based on solid-phase extraction (SPE) of PCBs from whole blood and direct decomposition of lipids and other non-persistent components by concentrated sulphuric acid on the SPE column.

Materials and Methods

Blood samples

Venous blood was collected in either EDTA- or heparin-treated glass containers. Plasma was obtained by centrifugation of EDTA-treated blood. Serum was isolated by centrifugation of untreated blood samples 2 hrs after the collection.

Extraction and Analyses

EDTA-treated whole blood (5ml) was spiked with isooctane solutions of CB-29, CB-143, CB-155, CB-181 and CB-207 as internal standards (IS). Blood samples used for accuracy and recovery studies were further spiked with a mixture of 32 CBs at a level of 0.13 ng/ml. After dilution with a mixture of formic acid and isopropanol, samples were transferred to the SPE column (Isolute ENV+; International Sorbent Technology). Proteins and polar lipids were washed out with dilute isopropanol and methanol. The lipids were decomposed by applying concentrated sulphuric acid directly on the SPE column. After washing the SPE column with water, dilute sodium bicarbonate and dilute methanol, the CBs were eluted with dichloro-methane. The eluate was further purified by chromatography on two Pasteur pipettes in series

filled with acid silica and basic alumina. Finally, individual CB congeners were determined by capillary gas chromatography with electron capture detection (GC-ECD).

The same procedure was applied to plasma and serum samples, however, the following modifications were necessary for heparin-treated blood: After addition of all reagents, the blood samples were fortified with a small amount of sodium dodecylsulphate (SDS) and centrifuged at 7000 rpm. Further, a washing step using dilute potassium hydroxide was used after the application of the blood sample on the SPE column.

Results and Discussion

The method is applicable to both plasma, serum and whole blood (see chromatograms in Fig. 1). It turned out that formic acid in isopropanol was best suited to released CBs from lipoproteins and that the transfer of the CBs from the whole blood matrix to the SPE material was enhanced by addition of a small amounts of isooctane. Application of sulphuric acid directly onto the SPE column resulted in an efficient decomposition of the lipids enabling direct GC analysis of the dichloromethane eluate (Fig.1a). However, additional sample clean-up using two Pasteur pipettes in series, resulted in a somewhat lower background, which facilitated peak identification of minor congeners.

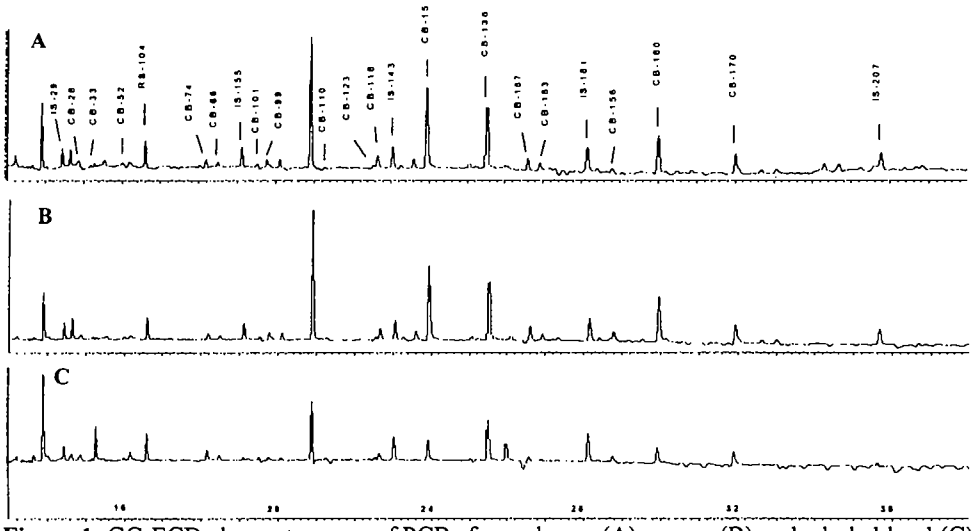

Figure 1. GC-ECD chromatograms of PCBs from plasma (A), serum (B) and whole blood (C).

Using heparin as anticoagulant for whole blood samples resulted in heparin-lipoprotein precipitates which clogged the SPE columns. Centrifugation at 7000 rpm was necessary to obtain clear solutions which passed through the columns. In some cases, small amounts of precipitates could be washed from SPE columns with diluted KOH without any effect on CB recoveries. Extraction of CBs from heparin-treated blood was found to be supported by addition of SDS.

Recoveries of CBs from spiked plasma samples are shown in Fig. 2. Similar results were obtained for serum and whole blood samples as well. Recoveries are good except for the highest chlorinated CBs, which are usually not present in human blood.

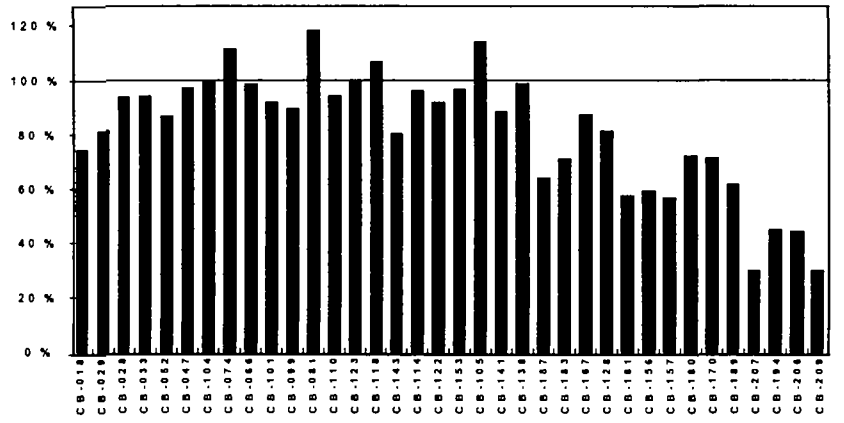

Figure 2. Recoveries of CBs from plasma spiked with 0.13 ng/ml per CB (average of 3 samples).

High accuracy was obtained for the determination of all congeners in spiked plasma (Fig. 3) and serum and somewhat lower for whole blood (average 84%).

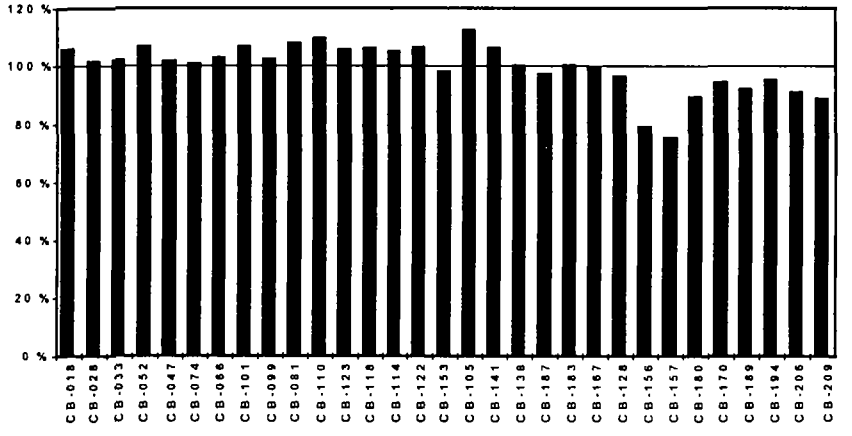

Figure 3. Realtive accuracy for the determination of CBs in plasma spiked with 0.13 ng/ml for each CB (average of 3 samples).

The recoveries of IS from non-spiked plasma and serum samples were similar to those from spiked samples. In contrast, much lower recoveries of IS were found for non-spiked whole blood samples as compared to spiked samples (Fig. 4). However, addition of 100 µl of iso-

octane to the whole blood mixture resulted in recoveries similar to those found for serum and plasma.

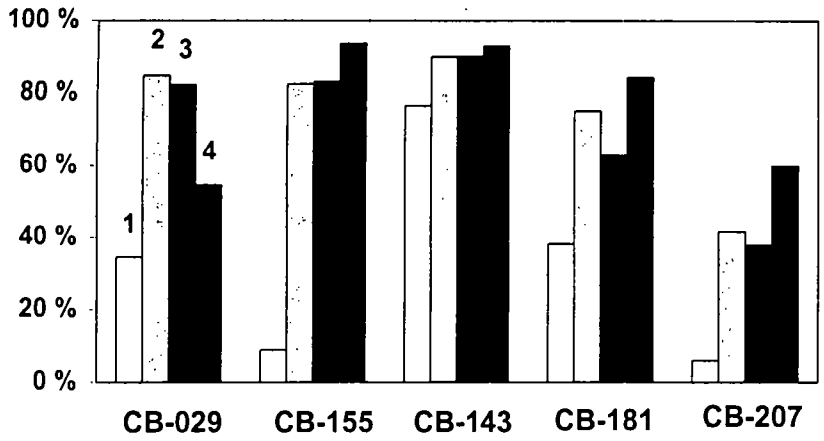

Figure 4. Recoveries of IS from (1) non-spiked blood, (2) spiked blood, (3) non-spiked serum, and (4) non-spiked blood with addition of isooctane (average of 3 samples).

In conclusion, the presented method is well reproducible, fast, easy to perform, necessitates much lower amount of organic solvents and presents a lower risk of contamination than traditional extraction methods.

References

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