ELSEVIER

Journal of Chromatography B, 734 (1999) 219–227

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of polychlorinated biphenyls in human blood by solid-phase extraction including on-column lipid decomposition

Karel Janák[a,*], Espen Jensen[b], Georg Becher[b,c]

[a]Department of Environmental Medicine, National Institute of Public Health, P.O. Box 4404 Torshov, N-0403 Oslo, Norway
[b]Department of Chemistry, University of Oslo, Oslo, Norway
[c]Norwegian Institute for Water Research, P.O. Box 173 Kjelsås, N-0411 Oslo, Norway

Received 8 March 1999; received in revised form 16 July 1999; accepted 22 July 1999

Abstract

A method for the isolation of polychlorinated biphenyls (PCBs) from human blood using solid-phase extraction (SPE) has been developed. The procedure incorporates decomposition of lipids by concentrated sulphuric acid directly on the SPE column. Conditions for transferring PCBs onto the SPE column and washing the decomposed blood components from the SPE column were optimised. After clean-up the extracts were analysed using gas chromatography with electron capture detection. An average recovery of PCBs from spiked blood samples was about 78±8% and an average precision was about 109±7%. Quantitation has been done using four internal standards and calibration curves based on five concentration levels. Low procedural blanks made it possible to determine PCBs in blood quantitatively at a level down to 2–10 pg g^{-1}. The integrated method for blood is fast, less laborious than methods using liquid–liquid extraction and has a low consumption of organic solvents. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Polychlorinated biphenyls

1. Introduction

The environmental impact of polychlorinated biphenyls (PCBs) has been known for many years. Though PCBs have been banned in many countries they still present a worldwide pollution problem because of their persistence in the environment. They are lipophilic and bioaccumulate in the food chain. Humans are exposed to PCBs mainly through their consumption of fatty foods [1]. Biomonitoring of PCBs has been widely used to determine the human body burden of these pollutants [1,2]. In this respect, blood represents a readily available and renewable human tissue.

Several publications describing congener specific analysis of PCBs in human blood samples have appeared. Standard methods generally comprise the release of the pollutants from lipoproteins, the extraction of pollutants together with lipids from the organic matrix, a clean-up step and gas chromatography (GC) with electron-capture detection (ECD) or mass spectrometry (MS).

Formic acid [3,4], methanol [5–7], formic acid with 2-propanol [8] or ethanol with saturated aqueous ammonium sulphate [9–11] have been found most useful for protein precipitation. For the extraction step, repeated liquid–liquid extraction (LLE)

*Corresponding author.

0378-4347/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(99)00348-5

applying hexane [9–13], or hexane in mixture with other solvents (diethyl ether [3,5–7,14], methyl-*tert*.-butyl ether [15], dichloromethane (DCM) [16]) is most commonly used. Recently, the accelerated solvent extraction has been introduced resulting in faster and more efficient extraction [17]. Good extraction efficiency was reported with supercritical fluid extraction [18] and solid-phase extraction (SPE). Several SPE materials have been used; e.g., C_{18}-bonded-silica cartridges [19,20], diatomaceous earth (Hydromatrix) with sodium chloride [2], Lipidex 5000 gel [8], Empore extraction C_{18}-silica disks [21], and even restricted-access reversed-phase high-performance liquid chromatography (HPLC) columns [22] for sorption of PCBs from blood. These advanced modes of extraction use less solvents, are faster, and sometimes more selective than LLE. Most of these extraction methods aim at a complete extraction of the lipids in order to determine the lipid content gravimetrically. However, the lipid content of blood is rather low. A minimum volume of blood of about 50 ml has been recommended for gravimetric lipid determination to achieve sufficient accuracy [23]. Furthermore, different extraction methods may result in different efficiencies of extraction of different lipid classes [3,13,24]. Thus, it seems better to either express the concentration of the pollutants on wet mass basis or to use a summation procedure based on the analytically determined concentrations of the individual lipid classes. Anyhow, the lipids must be separated from the sample prior to GC separation. This may be done either before extraction by saponification [14], or, which is more frequent, after the extraction. In the latter case decomposition with concentrated sulphuric acid [2,3,7,10,11,16,21,25] or by adsorption chromatography using aluminium oxide [4,8], silica gel [8,12], or Florisil [5,23] or by gel-permeation chromatography [25] has to be applied.

These procedures involve several time consuming operations with a high risk for contamination of the sample. Thus, there is a need for more rapid, cost effective, integrated methods. In this paper, a method for determination of PCBs in blood using SPE is described. SPE columns packed with hyper-cross-linked styrene–divinylbenzene copolymer were selected with respect to their sorption properties (non-polar interactions, high capacity), flow properties (high flow-rates even at low back pressures), high purity and mechanical and chemical stability. An integrated procedure including decomposition of lipids and other non-persistent components by concentrated sulphuric acid directly on the SPE column was employed. The aim of the study was to create a simple, efficient, fast and reliable method for the determination of low levels of PCBs in blood.

2. Experimental

2.1. Samples

Three samples of human blood were studied: two were obtained from volunteers at the National Institute of Public Health. Blood was drawn directly into EDTA-treated vacutainer tubes (Becton Dickinson, Meylan, France). The third sample was obtained from the Blood Center (City Hospital Ullevål, Oslo, Norway). Samples were stored at −20°C until analysis.

2.2. Solvents, reagents and glassware

All solvents were pesticide grade from Labscan (Dublin, Ireland) and used as supplied. Water was purified using Elga, Option 4 Water Purifier device (Elga, Bucks, UK). Formic acid, sulphuric acid and sodium carbonate, were of analytical-reagent grade quality (Merck, Darmstadt, Germany). Basic aluminium oxide 60, silica gel 60 and sodium sulphate, all chromatography grade (Merck) were activated by overnight heating at 190°C, 130°C and 600°C, respectively, and stored in a desiccator.

All glassware, except volumetric glassware, was washed in 2.5% RSB 25 foaming alealine cleaner (Chemical Products, Brussels, Belgium), rinsed with distilled water and then heated at 450°C for 4 h.

2.3. Standards

The method was evaluated for PCB congeners which are commonly present in human blood in detectable amounts. Standards of PCBs were obtained in solution (99% purity) from Restek (Sulzbach, Germany). Analytes: IUPAC Nos. 18, 28, 33, 47, 52, 66, 74, 81, 99, 101, 105, 110, 114, 118, 122,

123, 128, 138, 141, 153, 156, 157, 167, 170, 180, 183, 187, 189, 194, 206, 209; internal and reference standards (I.S.s): IUPAC Nos. 29, 143, 155, 181 and 207; recovery standard (RS) IUPAC No. 104. All standard solutions were prepared in 2,2,4-trimethylpentane.

2.4. Analytical procedure

2.4.1. Spiking

Spiked samples were prepared by fortifying blood samples (5 g) with 100 μl of PCB standard solution (31 congeners) at a concentration of 3.27–32.7 ng g^{-1} and with 30 μl of the solution containing internal standards at a concentration of 21.875 ng g^{-1}. The same amount of internal standard solution was added to non-spiked samples together with 100 μl of 2,2,4-trimethylpentane and samples were kept overnight at 4°C before the analysis.

2.4.2. Extraction

A flow scheme of the whole method including blood precipitation, SPE, lipid decomposition, elution of PCBs and sample clean-up before the GC analysis is shown in Fig. 1. A 10-ml volume of formic acid–2-propanol (4:1, v/v) was added to the blood sample (5 g). The sample was sonicated in an ultrasonic bath (Transsonic 460, Elma, Singen, Germany) for 5 min. After about 30 min, 10 ml of water–2-propanol (17:3, v/v) was added and the sample sonicated again for 5 min. After 30 min, the mixture was gradually transferred to a washed and preconditioned (1 ml methanol, 8 ml DCM, 5 ml methanol, 3 ml water) SPE column Isolute ENV+ (International Sorbent Technology, Hengoed, UK). The SPE column consisted of 200 mg hyper-cross-linked styrene–divinylbenzene copolymer (average particle diameter 91.9 μm, specific surface area 1000 m^2 g^{-1}) filled in the bottom of a column reservoir (6 ml). The SPE column was mounted in a 12-port Visiprep solid-phase extraction Vacuum Manifold (Supelco, Bellefonte, PA, USA). The maximum flow-rate of sample through the SPE column was 1 ml min^{-1}. The SPE column was washed with 15 ml of water–2-propanol (19:1, v/v) and with 10 ml of water–methanol (9:1, v/v) at a flow-rate up to 10 ml min^{-1}. Then it was run dry for about 20 s to expel most of the non-adsorbed water from the column and

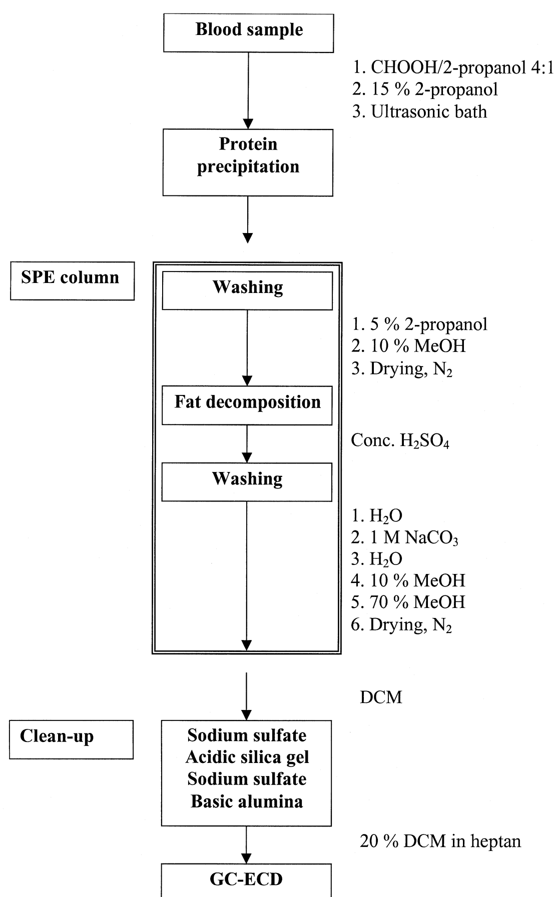

Fig. 1. Schematic outline of the method.

then washed with 4 ml of concentrated sulphuric acid at a flow-rate of 0.2 ml min^{-1}. Subsequently, it was washed with water (10 ml), 1 *M* Na_2CO_3 (3 ml), water (10 ml), and 10 ml of water–methanol (9:1, v/v) at a flow-rate of up to 10 ml min^{-1}. Finally, the sorbent bed was run dry and ca. 0.2 ml of water–methanol (3:7, v/v) was added (a few drops to make drying easier). Then the sorbent was dried with suction under a nitrogen atmosphere for about 15 min. PCBs were eluted with 5 ml of DCM at a flow-rate of 0.5 ml min^{-1}.

2.4.3. Final clean-up and preparation for GC analysis

The solvent was evaporated to approximately 10 μl and then diluted with 100 μl of *n*-heptane. The solution was transferred to a Pasteur pipette filled

successively from the tip with a small plug of glass wool, and dry packed with aluminium oxide (height: 30 mm), anhydrous sodium sulphate (5 mm), silica gel impregnated with 60% (w/w) sulphuric acid (10 mm) and anhydrous sodium sulphate (5 mm). Before loading of the sample, the column was washed with 10 ml of *n*-heptane. After the application of the sample, the column was washed again with 3 ml of *n*-heptane. PCBs were eluted using 3 ml of heptane–DCM (4:1, v/v). The sample was concentrated and transferred to a microvial. After addition of the recovery standard, CB-104, the sample was subjected to GC–ECD analysis.

2.4.4. Gas chromatography

A Perkin-Elmer Auto System gas chromatograph equipped with an electron-capture detector and operated by Turbochrom 4, PE Nelson system (Perkin-Elmer, Beaconsfield, UK) was used. The splitless injection inlet of the GC instrument was fitted with a deactivated retention gap, 1.0 m×0.53 mm I.D. (Hewlett-Packard, Avondale, PA, USA), coupled to a CP-Sil 8 CB fused-silica capillary column (50 m×0.25 mm I.D., 0.25 μm film, Chrompack, Middelburg, The Netherlands). Hydrogen was used as carrier gas at a linear velocity of 41 cm s^{-1} at 90°C and argon–methane (5%) as make-up gas at flow-rate of 50 ml min^{-1}. The injector temperature was set to 250°C and the detector temperature to 350°C. A 1-μl volume of sample was injected in splitless mode (1 min) at a initial column temperature 90°C (1 min). The column temperature was raised at a rate of 10°C min^{-1} to 200°C, then at 2°C min^{-1} to 260°C and further at 10°C min^{-1} to 290°C which was held for 5 min.

2.5. Assessment of the analytical quality

Recoveries of CB-congeners were investigated in blood samples fortified with a standard solution at three different concentration levels; 0.065, 0.131 and 0.654 ng of each congener per g blood, respectively.

All analyses were performed in triplicate. Peak identification was based on the retention of the congener relative to one of the five internal standards. Calibration curves based on peak heights were created using standard solution containing 31 PCB congeners at five concentration levels and based on four internal standards. The detection limits were 0.5–3 pg g^{-1} blood and quantitation limits were set to concentration levels about three-times higher.

Reagent blanks comprising the whole procedure with blood samples substituted by a corresponding amount of water were analysed in parallel with six blood samples. Each step of the analytical procedure was carefully checked for possible contaminants. Nevertheless, sometimes the gas chromatogram of the blank showed responses at retention times of CB-congeners. In these cases the blank response was subtracted from the analyte response in the sample.

3. Results and discussion

This paper considers the determination of PCBs in whole blood samples. Previously, we have briefly reported [26] on the application of the method to plasma and serum. It is generally believed, that the concentration of persistent organochlorine pollutants in plasma and serum, when related to the extractable lipid content, is similar to that in whole blood and representative of the concentration in adipose tissue [27]. However, there is evidence, that the distribution of CB congeners [23] and of chlorinated dibenzodioxins and dibenzofurans [28] in serum, plasma and whole blood, respectively, is not the same even when expressed on the lipid mass basis. Further, the amount and type of lipids extracted are strongly dependent on the composition of the solvents used [24]. Body burden is reflected by the PCB levels in the whole blood. Therefore, the development of the procedure was done with samples of whole blood.

3.1. Comments on the procedure

The optimisation of the whole procedure resulted in isolation and concentration of persistent, non-polar, low-molecular-mass compounds from blood. Using GC–ECD for separation of isolated pollutants, rather simple, easily quantifiable chromatograms with mostly fully separated peaks were obtained, as demonstrated in Fig. 2. Our attention was focused on the following steps: release of pollutants from lipoproteins by protein precipitation, transfer of analytes from the coagulated blood onto the sorbent

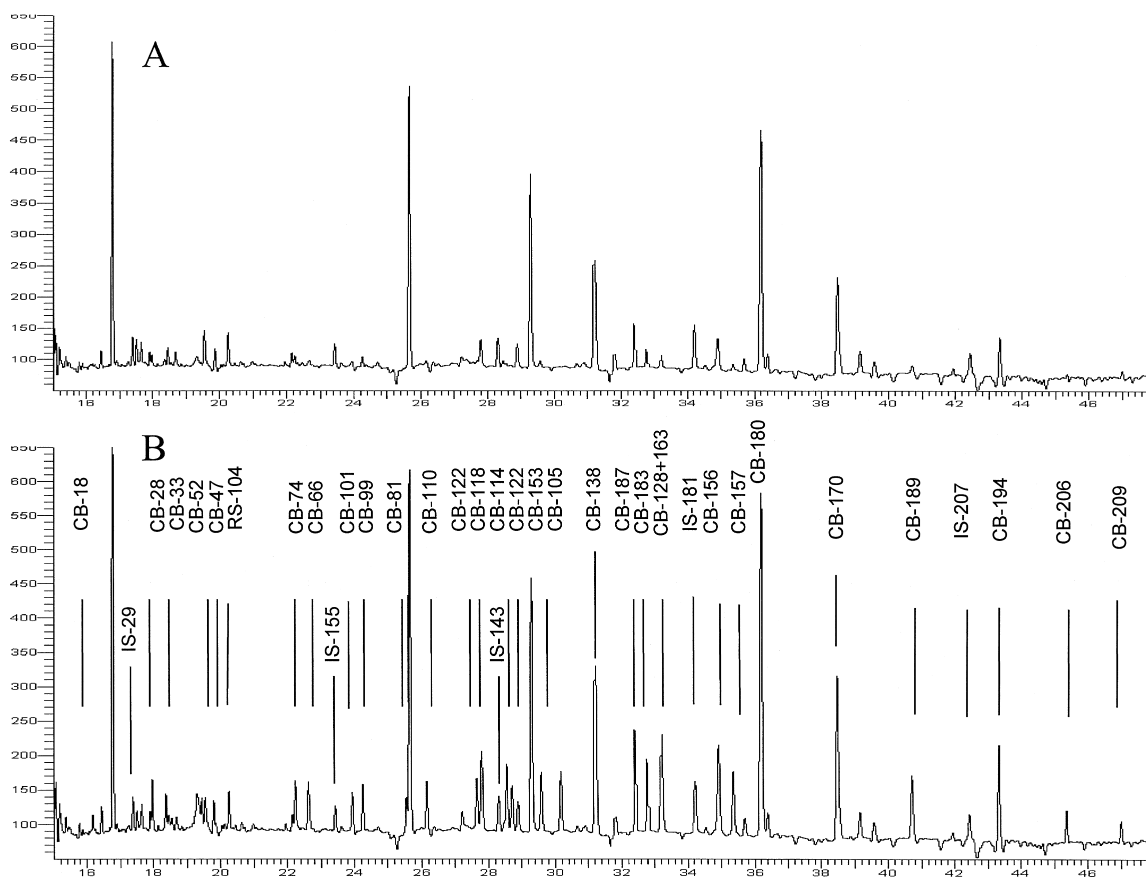

Fig. 2. Gas chromatographic separation of PCBs (GC–ECD) in whole blood. (A) Non-spiked sample, (B) sample spiked at a level of 0.131 ng g^{-1} per congener.

and decomposition and washing of the endogenous components from the sorbent.

3.1.1. Sample preparation prior to SPE

It has been reported [29], that mixtures of an acid and an organic solvent like acetonitrile, methanol or 2-propanol are very efficient for denaturation of blood proteins. Methanol and 2-propanol were selected on the basis of the preliminary sorption tests. The application of methanol–formic acid for blood denaturation resulted in difficulties with blood throughput. Blood proteins precipitated in coarse particles that gradually clogged the column. No such effect was observed with plasma [26]. Using 2-propanol instead of methanol resulted in fine precipitates running smoothly through the SPE column. A dilution of the sample by water–2-propanol after protein precipitation and ultrasonication of the mixture was found to be important to maintain a low flow resistance of the SPE column during sample loading. 2-Propanol (12% in the sample) was also found to be superior to methanol (14% in the sample) when testing the sorption efficiency from salt water solutions spiked with PCBs (1.09 ng of each CB congener). While the average recoveries were about 59±8% when using methanol, they raised to 86±10% with 2-propanol. Thus, it was important to optimise the solvent composition with respect to sorption of PCBs.

As briefly reported elsewhere [26], addition of a small amount of 2,2,4-trimethylpentane improved the recoveries of internal standards from blood samples. This might be due to an improved transfer of solutes from the precipitated blood to the sorbent or due to

partitioning of solutes into a layer of 2,2,4-trimethylpentane sorbed on the sorbent surface.

3.1.2. Sorption on the SPE column

The flow of the liquid through the column during the sorption of the analytes did not seem to be critical as long as it was below 1 ml min^{-1}. Fortifying the reagent mixture used for protein denaturing with PCB standards, the sorption of PCBs on the SPE column was proven to be independent of the concentration in the range of 0.06 to 1.64 ng of each CB congener per ml of salt water (5 ml in total). Although the recoveries (87% in average) varied between individual congeners (10–15% SD), the differences in recoveries between the different levels of the same congener were much lower and no trend could be seen.

3.1.3. Lipid decomposition, washing and drying

The major procedural simplification consisted in the direct decomposition of the lipids sorbed onto the SPE column. Subsequently, the more polar (oxidised) decomposition products could be selectively washed out from the sorbent. Thus, the selection of a sorbent for SPE was limited to those materials which could withstand concentrated sulphuric acid. In this respect Isolute ENV+, a styrene–divinylbenzene copolymer, proved to work well. Treatment with concentrated sulphuric acid affected neither the consistency of the sorbent, nor the ability of the

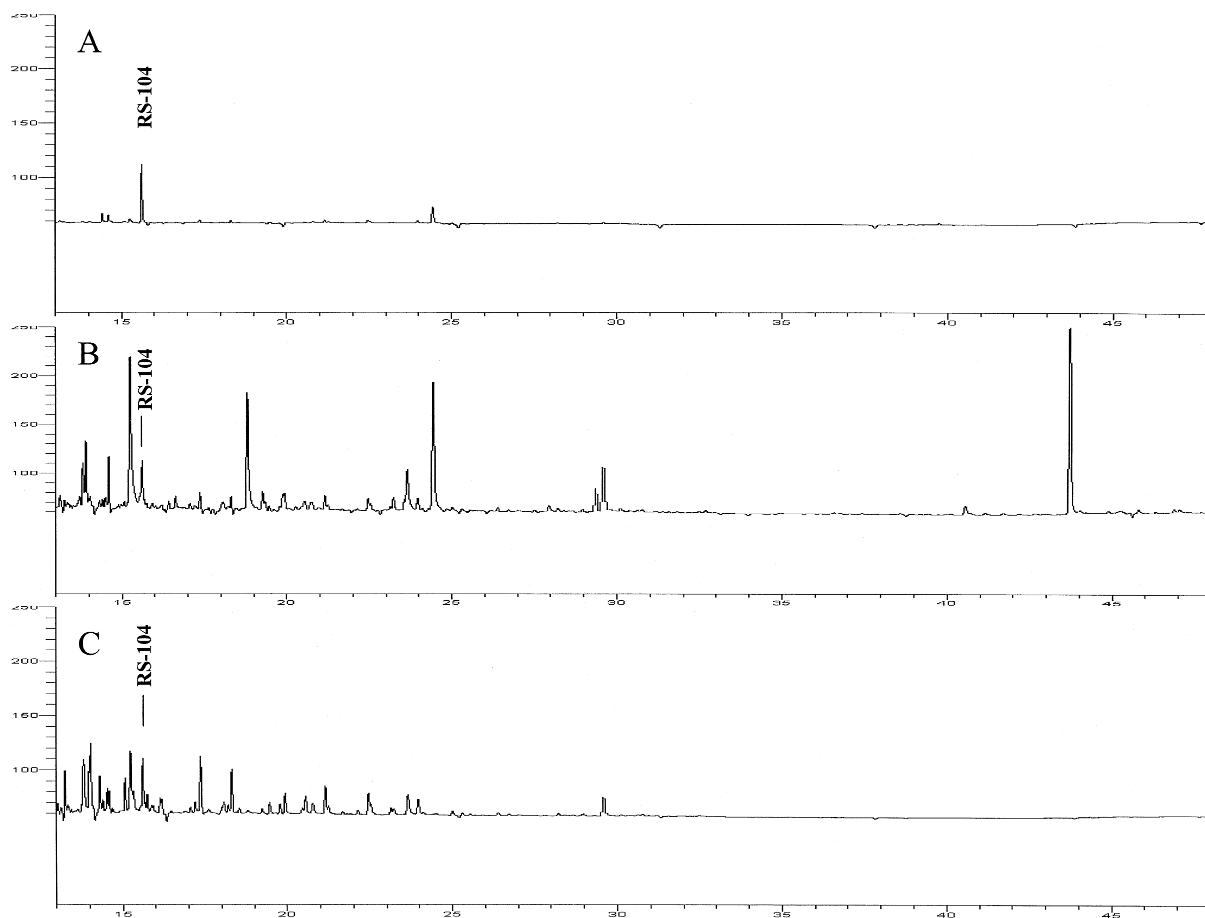

Fig. 3. Chromatograms of procedural blanks. (A) Optimised blank, (B) sulphuric acid treatment not included, (C) the same as (A), but laboratory air used for drying instead of nitrogen.

sorbent to retain adsorbed solutes. Further, it resulted in a more complete elution of the PCBs from the sorbent. 94% of the sum of PCBs were recovered from the SPE column with only 3 ml of DCM after concentrated sulphuric acid treatment compared to only ca. 65% recovery (using the same amount of solvent) if the sulphuric acid was not applied. Finally, it was found that the direct washing with concentrated sulphuric acid resulted in very clean blanks (see for comparison Fig. 3A and B).

Except for low chlorinated PCBs (tri-CBs, tetra-CBs), the recoveries were not dependent on the methanol content of the water–methanol mixture used for washing in the range between 10 to 70% (v/v) methanol. 10% methanol in water was selected because of the higher recoveries of the lower chlorinated congeners as the chromatographic purity of the eluates was similar despite of the methanol concentration.

No PCBs were detected in the eluates from the washing steps, neither before nor after the treatment with concentrated sulphuric acid.

3.1.4. Elution and clean-up

Efficient elution of solutes from the Isolute ENV+ column in a small volume of an appropriate solvent requires that the sorbent is totally dry before applying the eluent. Cyclohexane, DCM, toluene and their mixtures were tested as eluents. DCM appeared to be the best choice. More then 90% of the solutes were eluted with only 3 ml of DCM, and the solvent itself contributed very little to the blank and was easily evaporated.

As already shown [26], it was possible with this procedure to analyse samples eluted from SPE column directly on GC–ECD. Nevertheless, a simple clean-up, of the sample by adsorption chromatography, as described above, resulted in lower background and in chromatograms more suitable for data processing.

3.1.5. Procedural blanks

The whole procedure was optimised in order to get as clean blank chromatograms as possible. The sorbent was very stable and did not give rise to peaks in the blank; all possible impurities were washed out before the sorption of the samples. Little contribution to the blank was found from solvents and the reagents applied on the SPE column. However, laboratory air sucked through the SPE column during the drying period contributed rather much to an elevated background level, see Fig. 3. Large improvements in minimising the blank response were achieved when the vacuum manifold was shielded with nitrogen (99.996% purity) during the drying period, see Fig. 3A.

Table 1
Mean recoveries related to addition of CB-104 as a recovery standard and relative standard deviations (%) of PCBs spiked into whole blood samples

Congener	Mean recovery (RSD)[a]		
	Spike (ng CB per g blood)		
	0.654[b]	0.131[b]	0.065[c]
CB-018	82 (8)	59 (3)	72 (2)
CB-028	98 (9)	75 (10)	85 (6)
CB-033	101 (9)	71 (8)	87 (5)
CB-052	97 (8)	84 (4)	66 (13)
CB-047	83 (11)	66 (16)	62 (12)
CB-074	119 (8)	95 (10)	92 (6)
CB-066	103 (8)	87 (11)	81 (2)
CB-101	100 (8)	97 (11)	80 (6)
CB-099	97 (8)	83 (8)	77 (3)
CB-081	103 (12)	91 (11)	47 (1)
CB-110	87 (7)	95 (8)	64 (5)
CB-123	96 (7)	90 (9)	80 (10)
CB-118	101 (8)	87 (11)	68 (6)
CB-114	95 (8)	89 (2)	70 (3)
CB-122	99 (7)	88 (10)	79 (4)
CB-153	91 (8)	95 (14)	73 (6)
CB-105	103 (9)	93 (11)	83 (3)
CB-141	89 (7)	84 (17)	68 (5)
CB-138	95 (7)	98 (5)	75 (2)
CB-187	83 (8)	80 (21)	66 (5)
CB-183	81 (7)	84 (19)	58 (7)
CB-128	87 (6)	87 (2)	78 (3)
CB-156	74 (8)	86 (9)	45 (5)
CB-157	65 (7)	79 (12)	61 (3)
CB-180	73 (7)	108 (1)	54 (11)
CB-170	80 (7)	83 (8)	56 (3)
CB-189	72 (7)	76 (15)	58 (4)
CB-194	51 (6)	60 (14)	41 (3)
CB-206	45 (18)	44 (31)	31 (3)
CB-209	46 (20)	24 (25)	35 (15)

[a] Each blood sample was spiked with each of the 29 congeners at each level.
[b] Average of five samples.
[c] Average of three samples.

3.2. Method evaluation

The method was evaluated by spiking blood samples at three different levels in the concentration range commonly found in human blood [23]. In Table 1, recoveries of individual PCBs are given relative to CB-104. Generally, good recoveries were obtained at all spiking levels, however, some differences between the congeners were observed. The highest chlorinated (octa-, nona- and deca-) congeners were recovered at much lower degree than the other congeners. Regarding quantification, this might be compensated by the selection of a suitable internal standard for the particular PCB. A typical pattern of recoveries of the internal standards used is shown in Fig. 4. The internal standard CB-29 reflected well the recovery of CB-18 congener. Most of the other PCBs could be quantitated using either CB-143 or CB-181 as internal standard depending on the closeness of the retention. The reason for the low recovery of CB-155 is not known. It was therefore used only as a reference compound and not as an internal standard. The observed lower recoveries of the highly chlorinated biphenyls were compensated by the similar recovery of the internal standard CB-207.

As a result, satisfactory accuracy for the determination of PCBs from the spiked blood samples was obtained at all spiking levels (Table 2). The spiked PCBs were recovered at a somewhat higher amount then the actual spiking level. This might be a result of generally lower recoveries of the PCB congeners used as internal standards. Though this phenomenon was found insignificant for most of the analysed PCB congeners, it has been subjected for further studies.

4. Conclusion

Using the developed method based on SPE with on-column lipid decomposition, 31 PCB congeners were detected and quantified in spiked human blood. Hitherto, we have utilised this procedure for analyses of over 100 whole blood samples, as well as 27 plasma and 18 serum samples. The simple operational procedure and the capacity for running simultaneously up to 12 samples on SPE columns using the Visiprep manifold increased the sample throughput significantly compared to procedures based on LLE. The method decreased the risk for sample contamination and gave low sample background. It is both user and environment friendly as it reduces the use of organic solvents.

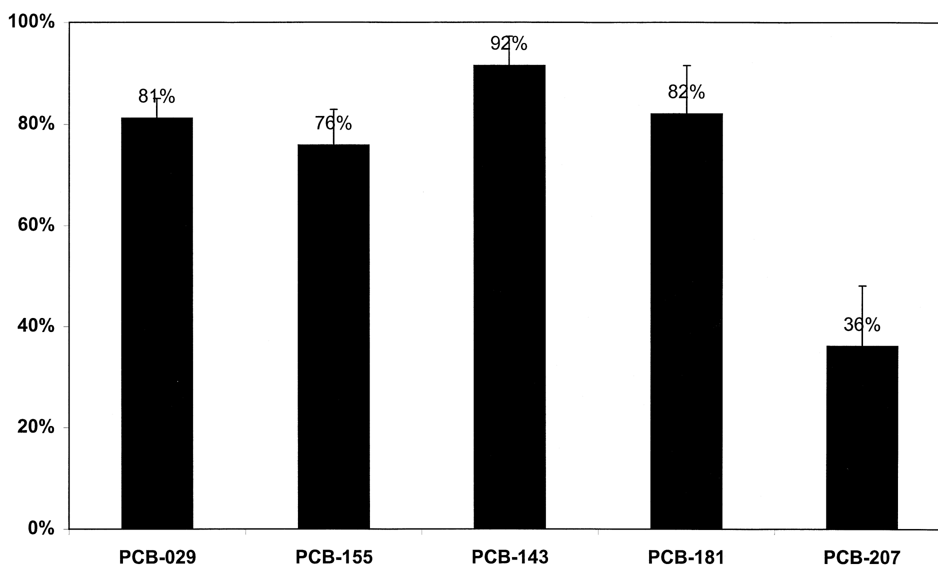

Fig. 4. Average recoveries of internal standards from three non-spiked blood samples.

Table 2
Accuracy and relative standard deviation (%) for the determination of PCBs spiked into whole blood samples

Congener	Mean accuracy (RSD)[a]		
	Spike (ng CB per g blood)		
	0.654[b]	0.131[c]	0.065[b]
CB-018	91 (15)	90 (3)	114 (14)
CB-028	103 (8)	107 (10)	118 (10)
CB-033	113 (5)	107 (8)	125 (16)
CB-052	110 (5)	103 (4)	109 (10)
CB-047	105 (7)	115 (16)	96 (5)
CB-074	115 (4)	108 (10)	120 (19)
CB-066	123 (3)	116 (11)	127 (3)
CB-101	123 (4)	121 (11)	111 (3)
CB-099	115 (3)	118 (8)	125 (2)
CB-081	100 (16)	100 (11)	65 (10)
CB-110	114 (4)	126 (8)	78 (11)
CB-123	109 (4)	112 (9)	120 (4)
CB-118	113 (4)	115 (11)	104 (7)
CB-114	106 (4)	119 (2)	105 (2)
CB-122	116 (2)	119 (10)	122 (8)
CB-153	108 (1)	130 (14)	131 (10)
CB-105	116 (6)	126 (11)	118 (2)
CB-141	103 (2)	100 (17)	102 (2)
CB-138	113 (1)	100 (5)	121 (3)
CB-187	98 (4)	99 (21)	104 (3)
CB-183	108 (5)	114 (19)	106 (3)
CB-128	114 (8)	135 (2)	135 (5)
CB-156	84 (21)	120 (9)	73 (3)
CB-157	87 (22)	134 (12)	105 (11)
CB-180	91 (10)	109 (1)	91 (5)
CB-170	101 (10)	90 (8)	97 (3)
CB-189	89 (12)	123 (15)	100 (3)
CB-194	72 (11)	98 (14)	162 (8)
CB-206	63 (14)	114 (31)	123 (13)
CB-209	68 (16)	98 (25)	106 (16)

[a] Each blood sample was spiked with each of the 29 congeners at each level.
[b] Average of five samples.
[c] Average of three samples.

References

[1] U.G. Ahlborg, A. Hanberg, K. Kenne, Risk Assessment of Polychlorinated Biphenyls (PCBs), Report NORD 1992:26, Nordic Council of Ministers, Copenhagen.

[2] H.R. Johansen, O.J. Rossland, S. Planting, M. Løvik, P.I. Gaarder, W. Gdynia, K.S. Bjerve, G. Becher, Environ. Health Perspect. 104 (1996) 756.

[3] L. Asplund, B.G. Svensson, A. Nilsson, U. Eriksson, B. Jansson, S. Jensen, U. Wideqvist, S. Skerfving, Arch. Environ. Health 49 (1994) 477.

[4] K. Norén, J. Sjövall, J. Chromatogr. 642 (1993) 243.

[5] A. Kocan, J. Petrík, B. Drobná, J. Chovancová, Chemosphere 29 (1994) 2315.

[6] C. Koopman-Esseboom, M. Huisman, N. Weisglas-Kuperus, C.G.V.d. Paauw, L.G.M.Th. Tuinstra, E.R. Boersma, P.J.J. Sauer, Chemosphere 28 (1994) 1721.

[7] M. Luotamo, J. Järvisalo, A. Aitio, Environ. Health Perspect. 60 (1985) 327.

[8] C. Weistrand, E. Jakobsson, K. Norén, J. Chromatogr. B 669 (1995) 207.

[9] K. Boggess, J. Paper, J. Wagner, R.E. Smith, Organohalogen Compounds 35 (1998) 89.

[10] E. Dewailly, J.J. Ryan, C. Laliberté, S. Bruneau, J.-P. Weber, S. Gingras, G. Carrier, Environ. Health Perspect. Suppl. 102 (1994) 205.

[11] D.G. Patterson Jr., C.R. Lapeza Jr., E.R. Barnhart, D.F. Groce, V.W. Burse, Chemosphere 19 (1989) 127.

[12] V.W. Burse, D.F. Groce, S.P. Caudill, P.M. Korver, D.L. Phillips, P.C. McClure, C.R. Lapeza Jr., S.L. Head, T.D. Miller, J.D. Buckley, J. Nassif, R.J. Timperi, P.M. George, Sci. Total Environ. 144 (1994) 153.

[13] R. Kallenborn, I.C. Burkow, E. Steene, T. Sandanger, M. Lund, H. Foshaug, E. Jensen, Organohalogen Compounds 35 (1998) 5.

[14] J.J. Ryan, E. Dewailly, Organohalogen Compounds 9 (1992) 207.

[15] E. Grimvall, L. Rylander, P. Nilsson-Ehle, U. Nilsson, U. Strömberg, L. Hagmar, C. Östman, Arch. Environ. Contam. Toxicol. 32 (1997) 329.

[16] U.S. Gill, H.M. Schwartz, B. Wheatley, Chemosphere 32 (1996) 1055.

[17] M. Davis, J. Paper, H. Shafiei, J. Stanley, Organohalogen Compounds 35 (1998) 105.

[18] E.G. van der Velde, S.H.M.A. Linders, W.C. Hijman, J.A. Marsman, R.S. den Hartog, A.K. Djien Liem, Organohalogen Compounds 35 (1998) 1.

[19] K. Abraham, U. Steuerwald, O. Päpke, M. Ball, A. Lis, P. Weihe, H. Helge, Organohalogen Compounds 26 (1995) 213.

[20] W.E. Turner, E.S. DiPietro, T.P. Cash, P.C. McClure, D.G. Patterson Jr., H. Shirkhan, Organohalogen Compounds 19 (1994) 31.

[21] A. Pauwels, D.A. Wells, A. Covaci, P.J.C. Schepens, Organohalogen Compounds 35 (1998) 167.

[22] N. Tanaka, K. Kimata, K. Hosoya, T. Araki, E.R. Barnhart, L.R. Alexander, P.C. McClure, C. Lapeza, D.G. Patterson Jr., Anal. Methods Instrument. 2 (1995) 41.

[23] J. Mes, L. Marchand, D. Turton, P.Y. Lau, Int. J. Environ. Anal. Chem. 48 (1992) 175.

[24] J.J. Ryan, P. Mills, Chemosphere 34 (1997) 999.

[25] L. Hovander, M. Athanasiadou, L. Asplund, E. Klasson-Wehler, Organohalogen Compounds 35 (1998) 115.

[26] K. Janák, E. Jensen, J.E. Aaseng, G. Becher, Organohalogen Compounds 35 (1998) 9.

[27] A. Schecter, O. Päpke, M. Ball, J.J. Ryan, Chemosphere 23 (1991) 1913–1919.

[28] E. Loshkina, Z. Amirova, R. Chalilov, R. Shukova, Organohalogen Compounds 35 (1998) 25.

[29] R.D. McDowall, J. Chromatogr. 492 (1989) 3.