DETERMINATION OF POLYCHLORINATED BIPHENYLS AND P',P-DDE IN HUMAN BLOOD PLASMA

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Introduction

Human exposure to halogenated persistent pollutants can either be estimated by assessment following occupational, geographical, dietary and other known factors related to the exposure, or the exposure may be determined by analytical chemical determinations of actual levels in human tissues. It is important to have robust, low resource and high performance analytical methods in screening, and other high sample volume applications. Various methods have been employed for extraction of polyhalogenated compounds in blood. The extractions used have been e.g. liquid-liquid extraction[1, 2], solid phase extraction[3, 4] and supercritical fluid extraction[5]. We have developed a method for human samples, e.g. blood, serum and plasma, based on a water adsorbing hydromatrix column. The method is validated here.

Materials and Methods

A validation of an analytical method for chlorinated POPs in blood plasma, based on hydromatrix extraction, multicolumn clean-up and GC-MS determination, was performed. A similar extraction method, applied for dioxins, has been described earlier[6].

Samples

Plasma stabilised with CPD (citrate solution) was provided by the Blood Central at Örebro University Hospital, Sweden. Samples were stored at -20 ºC until analysis. The plasma was divided into 10 g portions. Three fortified (spiked) samples at three different levels were used for the evaluations as shown in Table 1. Three non-spiked plasma samples were analysed to determine the levels of the analytes. Before usage, the samples were incubated over night at room temperature. Laboratory blanks (water) were extracted and analysed in the same way as the plasma.

Clean-up

In short, the plasma samples were applied to hydromatrix (Chem-Elut) columns and eluted with a mixture of isopropanol and hexane. Excess water was removed with sodium sulphate and the lipid weight was determined gravimetrically. Lipids were removed with multilayer silica columns. Solvents were from Labscan, Sweden (Enviroscan grade).

Analysis

Analyses were performed on a HP 6890 gas chromatograph equipped with a HP 7683 autoinjector. Injector temperature was held at 250 ºC and 2 ml sample was injected in splitless mode. A HP-5MS 5 % phenyl methyl siloxane capillary column was used (30 m x 0.25 mm ID, 0.25 mm film thickness). Helium was used as carrier gas with a constant flow of 1.1 ml/min. The temperature program 180 ºC (2 min), 15 ºC/min to 205 ºC (0 min), 4 ºC/min to 285 ºC (6 min) was employed in all analyses. Detection

Table 1. Fortification levels for the plasma samples in ng/g (lipid weight).

	Level 1	Level 2	Level 3
PCB #28	0.96	96	192
PCB #52	0.96	96	192
PCB #101	0.96	96	192
PCB #153	0.96	96	192
PCB #138	0.96	96	192
PCB #180	0.96	96	192
p,p'-DDE	0.96	96	192

was made with a HP 5973 low resolution-mass spectrometer in EI (electron impact) mode with the molecular ions detected in single ion monitoring (SIM) analyses.

Results and Discussion

All analytes were found in non-spiked plasma at levels exceeding LOD except for PCB #52. Table 2 lists concentrations and limits of determination for non-spiked plasma samples. The levels are similar to current reports of PCB levels in unexposed human blood[7, 8]. Blank levels were all below 6 % of native sample concentrations.

Limit of determination was defined as a signal to noise ratio of 3. The first unspiked replicate was used to determine LODs (Table 2) for the analytes. All LODs were below 1.0 ng/g (l.w.) except for p,p'-DDE.

Table 2. Concentrations and limits of determination in unspiked plasma expressed in ng/g (l.w).

	Replicate 1	Replicate 2	Replicate 3	Mean conc.	SD	LOD
PCB #28	1.0	1.2	0.8	1.0	0.2	0.4
PCB #52	<0.9	<0.9	<0.9	-	-	0.9
PCB #101	0.9	0.9	0.6	0.8	0.1	0.2
PCB #153	102	95	111	103	7.8	0.5
PCB #138	61	56	69	62	6.6	0.5
PCB #180	209	193	214	206	11	0.6
p,p'-DDE	87	84	95	89	5.7	2.8

Linearity was estimated by plotting the detected concentration as a function of spiked concentration (Figure 1). The linearities in the interval between 0.96 and 192 ng/g (l.w.) were satisfying with correlation values (R^2) of 0.983 (DDE) or higher.

Repeatabilities at the two highest concentration levels had relative standard deviations below 10% except for PCB #138 (14 %).

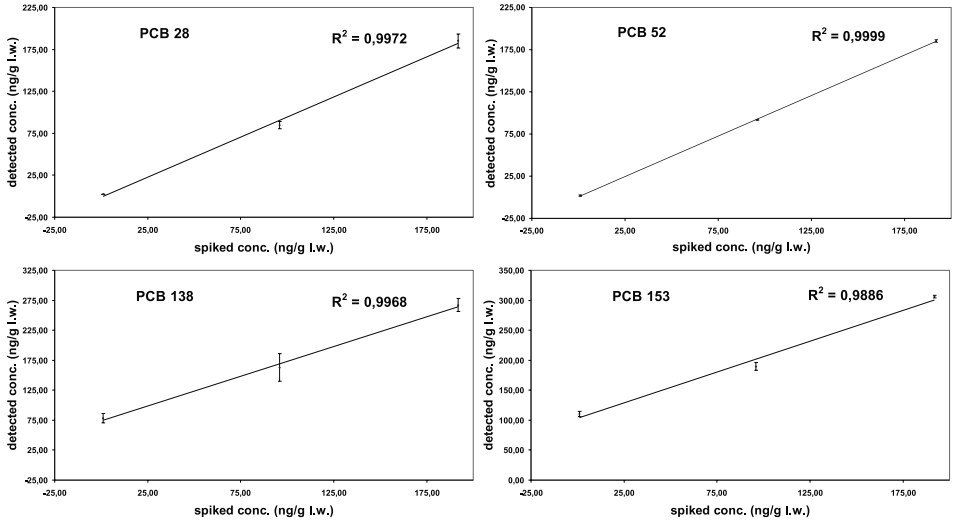

Figure 1. Linearity for PCB #28, #52, #138 and #153. Standard deviations (n=3) for each level are presented as error bars.

Recovery

The concentrations in the unspiked samples were subtracted from the spiked ones in the recovery calculations. Recoveries were not calculated for the first level, since this level was within the SD range for the native analytes. Recovery values were between 87 and 128 % (Table 3). Similar recoveries for PCBs in human blood have been obtained by Janak et.al (1999)[3] and Turrio-Baldassarri et.al. (1999)[5] when using solid phase extraction and supercritical fluid extraction, respectively.

Table 3. Recoveries at the two spiking levels. Relative standard deviation in parenthesis.

	96 ng/g l.w.		192 ng/g l.w.	
	n	Recovery (%)	n	Recovery (%)
PCB #28	3	87 (5.1)	3	96 (4.6)
PCB #52	3	95 (0.7)	3	96 (0.9)
PCB #101	3	100 (2.4)	3	98 (5.8)
PCB #153	3	91 (7.7)	3	106 (1.1)
PCB #138	3	105 (23)	3	107 (5.3)
PCB #180	3	97 (3.7)	3	92 (6.1)
p,p'-DDE	3	115 (21)	3	128 (8.2)

In conclusion, the results from the validation show that the method is well suited for extracting PCBs and DDE from blood plasma. High recoveries together with high repeatability and low LODs are obtained.

ANALYSIS I

Further validation of the method regarding PBDEs (polybrominated diphenyl ethers) will be performed. A pilot study with PBDEs has already been done with promising results.

Acknowledgements

The authors thank Dr Päpke from ERGO for fruitful discussions and suggestions and Brock Chittim, Wellington Standards, Guelph, Canada for making ^{13}C-labelled standards available. We would also like to thank the Knowledge Foundation for financial support.

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