MULTICOMPONENT ANALYSIS OF ORGANOCHLORINE AND ORGANOBROMINE CONTAMINANTS IN HUMAN MILK, BLOOD PLASMA, LIVER AND ADIPOSE TISSUE

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Introduction

The development of industry and extended use of synthetic products cause emissions of new organohalogen pollutants into the environment. If these substances are lipophilic they may accumulate in animals and humans. The toxicity of such compounds and their metabolites often is not well known and may depend on interactions between several compounds. With the increasing number of pollutants there is a need for multicomponent methods for simultaneous analysis of a range of pollutants in the same sample. Such analysis will give possibility to record temporal trends of different compounds and to find relations in occurrence and sources.
The method based on extraction with Lipidex 5000 has previously been used for analysis of a range of persistent organohalogen compounds: polychlorinated biphenyls (PCBs), polychlorinated naphtalens (PCNs), hexachlorobenzene (HCB), 1,1-dichloro-2,2-bis (4-chlorophenyl) ethene (*p,p'*-DDE), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (*p,p'*-DDT), methylsulphonyl metabolites of chlorinated biphenyls and *p,p'*-DDE ($MeSO_2$-CBs and $MeSO_2$-DDE), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)[1-3]. The aim of the present study was to extend the multicomponent method and include analysis of polybrominated diphenyl ethers (PBDEs) and hydroxylated polychlorinated biphenyls (OH-PCBs) in blood.

Materials and Methods

Samples

Blood plasma was bought at the Karolinska Hospital Blood Donor Center, Stockholm.

Standards

The standards of PBDEs and OH-PCBs were from Larodan Fine Chemicals AB (Sweden) or were kind gifts from Prof. Åke Bergman, Wallenberg Laboratory, Stockholm University, Sweden.

Instruments

The mass spectrometer used was a VG 70-250 instrument equipped with Hewlett-Packard gas chromatograph HP 5890A. A fused silica, methyl 5% phenyl silicone, capillary column (25 m x 0.32 mm, 0.25 μm film thickness (Quadrex, New Haven, CT, USA)) was used for gas chromatographic separations.
The oven temperature for analysis of PBDEs was:
190°C for 0.1 min, programmed to 230°C at 5°C/min, hold for 0.2 min, programmed to 235°C at 1°C/min, hold for 0.2 min, programmed to 270°C at 3.5°C/min, and hold for 8 min.
The oven temperature for analysis of OH-PCBs was:
180°C for 0.1 min, programmed to 235°C at 4°C/min, hold for 0.2 min, programmed to 273°C at 3.5 °C/min, hold for 8 min.

Electron ionization was performed in an "EI-only" ion source at the electron energy of 31 eV and the trap current of 500 μA. The source temperature was 260°C. The acceleration voltage was 6 kV and the resolution at m/z 293 was 5000-6000. The MS was operated in a selected ion monitoring mode.

Analytical procedure

Analysis of PCBs, PCNs, PCDD/F, HCB, *p,p'*-DDE, *p,p'*-DDT, PBDEs and methylsulphonyl metabolites of PCBs and DDE in different tissues have been previously reported[1-5]. Recently the method has also been used for analysis of OH-PCBs human liver and adipose tissue[6]. In the present study we modified the method for analysis of PBDEs and OH-PCBs in blood plasma. The scheme of the analytical procedure is shown in Figure 1.

The blood plasma sample (10 ml) was weight into the 100 ml flask with PTFE-lined screw cap and internal standards (100 μl of $^{13}C_{12}$-3,3',4,4'-BDE 0.5 pg/μl and 100 μl of 4-OH-CB162 0.5 pg/μl) were added. A blank sample (10 ml Milli Q water) was analyzed in parallel. Formic acid (10 ml) was mixed with the sample and left for 15 min. 2-Propanol (4 ml), water (4 ml) and Lipidex 5000 (3 g) were added and the mixture was shaken at 35°C for 3 hours in a water bath. After the extraction the gel was transferred to a glass column (2 cm ID), the solvent was drained and the gel eluted with 30% and 50% methanol in water (10 ml each). Organohalogen compounds and part of lipids were eluted from the Lipidex gel with acetonitrile (50 ml) and the rest of lipids with a mixture of methanol/hexane/chloroform (1:1:1, 20 ml). The dry weight of the two last fractions was used for lipid content determination. PBDEs and OH-PCBs were separated by chromatography on an aluminium oxide column. PBDEs were eluted in the first fraction with hexane (20 ml). Further purification was made on silica gel and Bio-Beads S-X3[5]. OH-PCBs were eluted from aluminium oxide column after the elution of $MeSO_2$-CBs and $MeSO_2$-DDE (dichloromethane/hexane 1:1, 20 ml) with acidified methanol (0.1 ml conc. H_2SO_4 in 100 ml methanol, 10 ml) followed by methanol (40 ml). This fraction was concentrated under reduced pressure to about 200 μl, 100 μl hexane and 1 ml diazomethane solution were added and the mixture was kept over night. After the derivatization, the solvent was evaporated with the gentle flow of nitrogen. The residue was dissolved in hexane (3 ml), injection standard (100 μl of decachlorobiphenyl 1.1 pg/ μl) was added and the solution was treated with sulfuric acid (3 ml).

PBDEs and methylated OH-PCBs were analyzed by GC/MS.

Results and Discussion

The method for multicomponent analysis gives possibility to analyze a broad spectrum of organohalogen compounds. The liquid-gel partitioning for extraction is a suitable method and has an advantage over liquid-liquid partitioning since emulsions are not formed and less amount of solvent is needed. By modification of the liquid-gel partitioning technique organohalogen compounds from different matrices can be transferred into the gel. Different adsorbents can be used for subsequent purifications.

The previously used method for analysis of organochlorinated compounds in human blood plasma[1] was modified for analysis of PBDEs and OH-PCBs. The sample size was reduced to 10 ml, which resulted in the use of smaller solvent amounts. Subsequent sample purifications using silica gel and Bio-Beads S-X3 were performed as described previously[5]. OH-PCBs were collected in the additional fraction from aluminium oxide column.

The recovery of internal standard, added to the blood plasma before extraction, was 60% and 75% for $^{13}C_{12}$-3,3',4,4'-BDE and 4-OH-CB162, respectively.

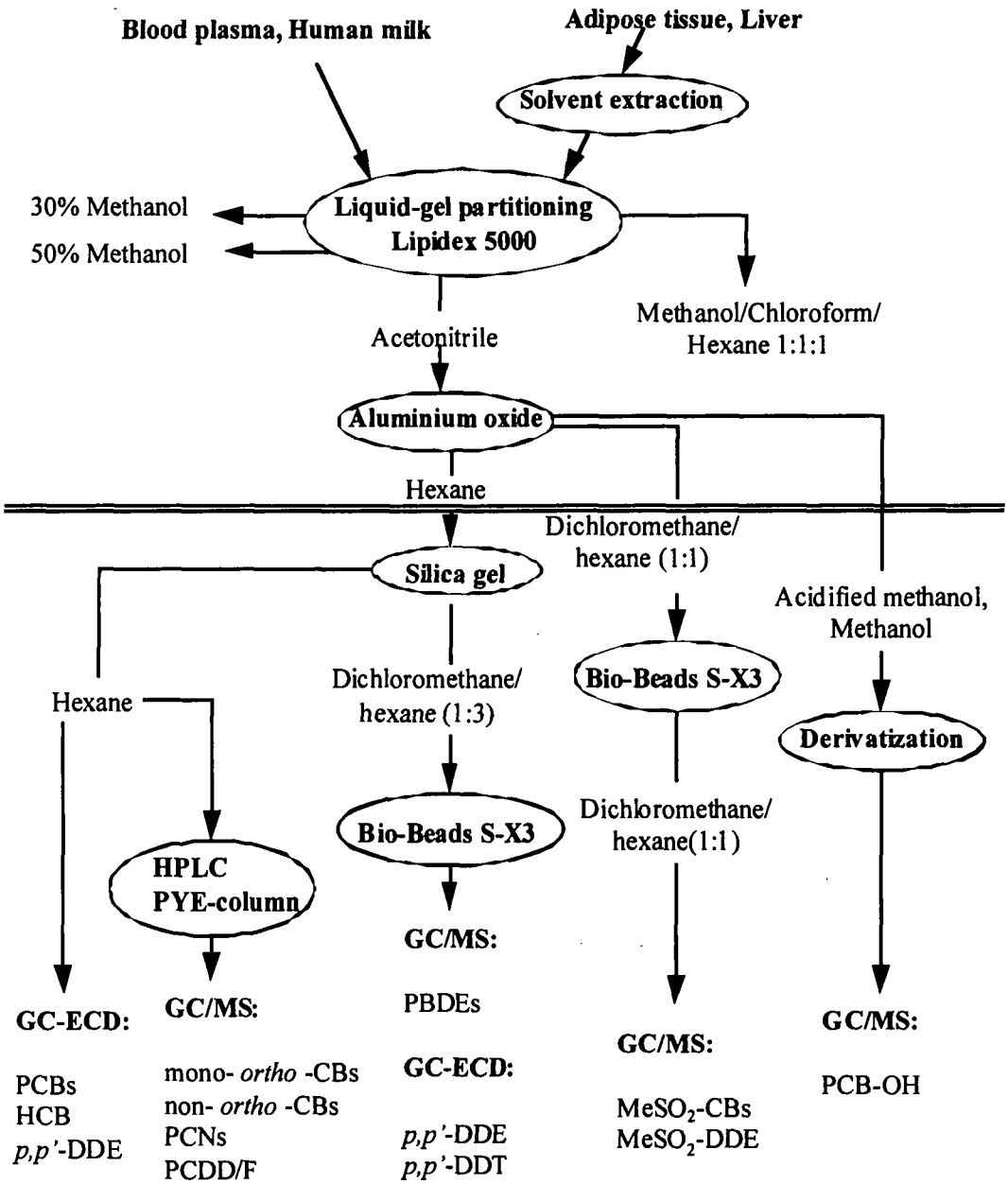

Figure 1. The flow scheme of the multicomponent analysis of organohalogen compounds in human tissues.

References

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