EXTENSION OF A MULTICOMPONENT METHOD TO INCLUDE THE DETERMINATION OF OH-PCBs AND OH-PBDEs IN BIOLOGICAL MATRICES

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Introduction

Halogenated organic contaminants (HOCs) such as the polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), are persistent lipophilic substances which can accumulate in biota. Many of the HOCs are shown to possess toxicological effects in varying degrees. PCBs can cause both immunosuppressive, neurotoxic and endocrine disrupting effects, as well as impairment of reproduction[1]. PBDEs are shown to be able to cause both neurotoxic and immunosuppressive effects, and to have endocrine disrupting properties[1].

It has been shown that species in varying degrees can metabolise these anthropogenic compounds, e.g. by insertion of hydroxyl (OH) or methyl sulfonyl ($MeSO_2$) groups[2]. Recently, focus has been directed towards the compounds which are directly responsible for the various toxicological effects observed. In many cases some metabolites of the anthropogenic compounds possess higher toxicological potencies than their parent compound. Thus, in the risk assessment of the various compounds it is of most importance to gain knowledge also of possible metabolism and metabolites.

The diversity of HOCs used in the industry, and the recent interest in levels of metabolites makes it desirable to have multicomponent methods for the analysis of various biological matrices. The Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science has a well established multimethod for the determination of PCBs, chlordanes, toxaphenes, DDT and other organochlorine pesticides, PBDEs and hexabromocyclododecane (HBCD)[3]. The aim of the present study was to extend the multicomponent method and include determination of OH-PCBs and OH-PBDEs[4]. The developed method was validated and found applicable for all compound classes, but to limit this presentation only the validation results for the OH-PCBs and the OH-PBDE are presented.

Materials and Methods

The multicomponent analytical method is based on the method originally described by Brevik[5] and later modified by Bernhoft & Skaare[6].

The following OH-PCB congeners were purchased from Wellington Laboratories Inc. (Ontario, Canada): 2,3,3',4',5-pentachloro-4-biphenylol (4-OH-CB107), 2,2',3',4,4',5-hexachloro-3-biphenylol (3'-OH-CB138), 2,2',3,4',5,5'-hexachloro-4-biphenylol (4-OH-CB146), 2,2',3,4',5,5',6-heptachloro-4-biphenylol (4-OH-CB187) and 2',3,3',4',5,5'-hexachloro-4-[$^{13}C_{12}$]biphenylol (4'-OH-[$^{13}C_{12}$]CB159). 2',3,3',4',5'-pentachloro-4-biphenylol (4'-OH-CB106) and 2',3,3',4',5,5'-hexachloro-4-biphenylol (4'-OH-CB159) were purchased from Larodan Fine Chemicals AB (Malmö, Sweden). 6-hydroxy-2,2',4,4'-tetraBDE (6-OH-BDE47) was purchased from Cambridge Isotope Laboratories (Massachusetts, USA). From the solutions of the pure compounds mixtures with the different compound classes were prepared in isooctane.

Blood and plasma samples from sheep were provided by the Laboratory Animal Unit at NVH, and liver from cattle and chickens were left-overs from the National Residue Monitoring Plan. These were used as blank matrices in the validation experiments. All the glassware used in the analyses were washed with cyclohexane:acetone (1:1) prior to use. For all evaporation purposes nitrogen gas (99.6%) was used. A summary of the sample preparation procedure is shown in the flow chart in Figure 1.

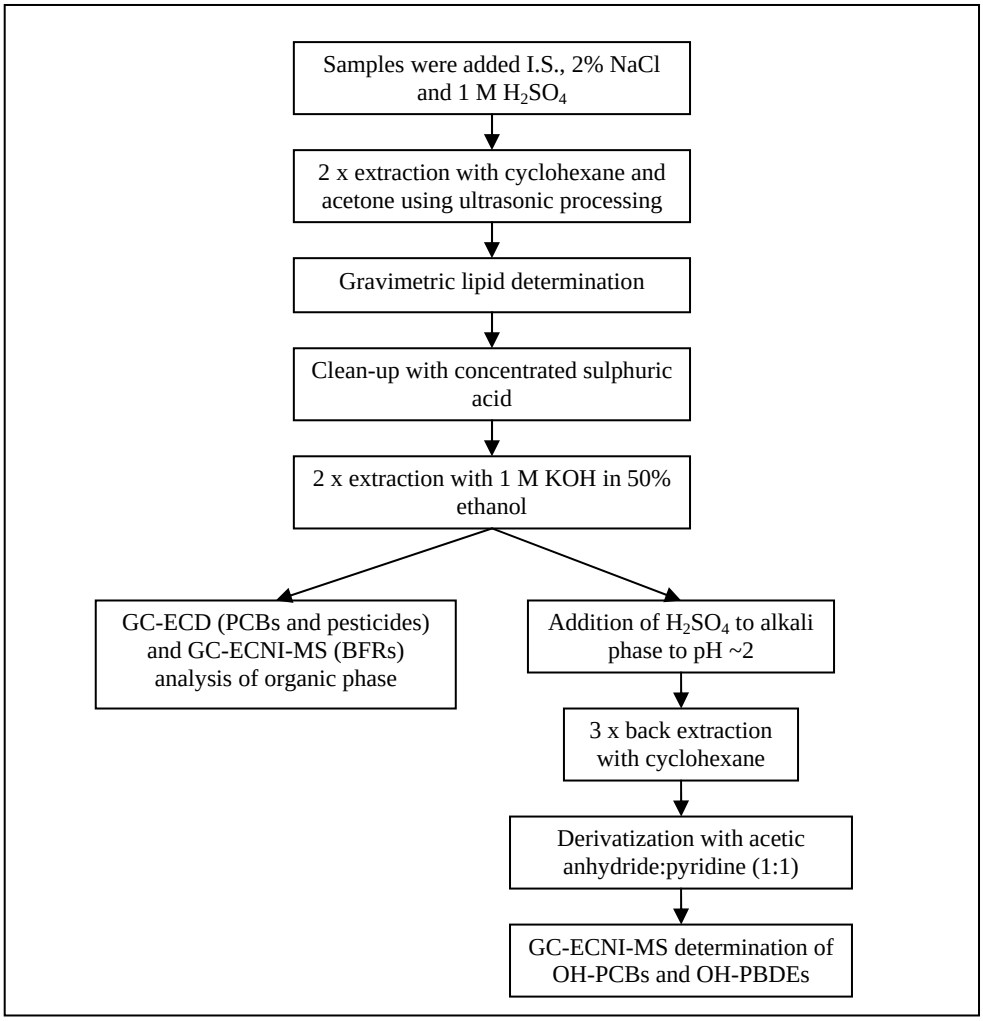

Figure 1*: Flow chart of the multicomponent method for analysis of blood and liver.*

2-5 g blood/plasma or 1-7 g liver were weighed in 80 ml centrifuge tubes, and added the following internal standards: 4'-OH-[$^{13}C_{12}$]CB159, PCB-29, -112 and -207 and BDE-77, -119 and -181. Before extraction the samples were added 4 ml 2% NaCl and 10 ml 1 M H_2SO_4. All samples were then extracted twice with cyclohexane and acetone using an ultrasonic processor. The liver samples were in addition homogenised for ~15s with a Ultra Turrax homogeniser (IKA Labortechnik Staufen, Germany) after addition of the solvents for the first extraction. Lipid contents were determined gravimetrically.

The lipid-extracts were cleaned up with 4 or 6 ml concentrated H_2SO_4 (depending on expected lipid content). The organic supernatants were transferred to 10 ml glass tubes, before extraction with 2 x 5 ml 1 M KOH in 50% ethanol. The organic phases from this extraction were analysed by GC-ECD (PCBs and pesticides) and GC-ECNI-MS (brominated flame retardants). The alkaline phases were acidified with concentrated H_2SO_4 to pH between 1 and 2, and re-extracted with 3 x 5 ml cyclohexane. These organic phases, containing the OH-metabolites, were then evaporated to ~1 ml, before derivatization with acetic anhydride:pyridine (1:1). The samples were subsequently analysed by the GC-ECNI-MS system described below.

An Agilent 6890 Series GC system with Agilent autosampler, Agilent 7683 Series split/splitless injector and Agilent 5673 quadropole mass spectrometer was used. The injector temperature was 210ºC and samples of 2 µl were injected in pulsed splitless mode with a pulse pressure of 28 psi for 1 min. The purge flow was 50 ml/min and the purge time was 1 min. The capillary column was a J&W Scientific DB-5 MS from Agilent Technologies, USA, with dimensions 0.25 mm i.d., 60 m length and 0.25 µm film thickness. The carrier gas used was helium (99.999%) and the reagent gas was methane (99.995%). The separation was performed at a constant flow of 1.5 ml/min. The temperature program was as follows; 90ºC for 2 min, then raised by 10ºC/min to 220ºC (held for 5 min), 5ºC/min to 250ºC (held for 1 min) and 20ºC/min to 300 (held for 1 min). The mass spectrometer was operated in the electron capture negative ionization mode. The analytes were monitored at two ions each, and the temperatures in the ion source and the quadropole were 150ºC and 106ºC, respectively. MSD Chemstation Version D.01.00 (Agilent) was used both for instrument control and data analysis.

Validation was performed by spiking twenty blood samples with the OH-PCBs and the OH-PBDE at five different levels ranging from 0.025 to 0.5 ng/g blood. The levels were 0.025 (n = 6), 0.1 (n = 1), 0.25 (n = 6), 0.4 (n = 1) and 0.5 (n = 6) ng/g blood. Two replicates of non-spiked samples (blank matrix) were also analysed, for investigation of analytes initially present. In addition to these blood samples eight liver samples from cattle were spiked at two different levels. The low level (n = 4) constituted 0.063 ng/g liver of the OH-PCBs/OH-PBDE, while the high level (n = 4) constituted 0.625 ng/g liver of the OH-PCBs/OH-PBDE. Between assay repeatability was tested by analysing a laboratory reference material consisting of blood from one environmentally exposed hooded seal (*Cystophora cristata*) on different days throughout the time course of the study[4].

Calibration solutions (without matrix) were used to create calibration curves (12 levels ranging from 0.1-100 ng/ml), and the quantifications were done using peak area relative to the internal standard. Identification of the analytes were done by comparing the retention times with the calibration solutions, and confirmed by controlling the ion abundancy ratio. All peaks were manually integrated, since the software often drew poor baselines. The validation results were based on one injection per sample.

Results and Discussion

The Laboratory of Environmental Toxicology is accredited according to the requirements of NS-EN ISO/IEC 17025 (TEST 051). A full validation of the analytical method was performed to ensure that it was suitable for its intended use. Accuracy was determined by measuring recovery relative to internal standard in samples added a known amount of all the analytes. The amounts of analytes initially present in the blood and liver were measured and subtracted before the accuracies were calculated. Table 1 shows the mean recoveries of the OH-PCBs and the OH-PBDE in the blood and liver, respectively.

Table 1: *Accuracy measured as mean recovery relative to the internal standard of the OH-PCBs and the OH-PBDE from the spiked blood samples and liver samples, respectively. Repeatability is given as standard deviations and variation coefficients (C_v%, relative standard deviation in %).*

	BLOOD			LIVER		
Analytes	**Mean recovery %**	**Standard deviation**	**C_v%**	**Mean recovery %**	**Standard deviation**	**C_v%**
4-OH-CB107	106	4.8	5.1	120	3.9	3.4
4-OH-CB146	91	4.3	5.5	107	2.3	2.1
4'-OH-CB106	114	3.3	2.7	110	2.6	2.4
3'-OH-CB138	93	5.9	6.7	99	2.1	2.2
4-OH-CB187	46	6.3	20.4	50	0.9	1.7
6-OH-BDE47	106	11.7	12.1	52	3.4	6.5
4'-OH-CB159	99	1.6	1.7	96	1.6	1.6

Mean recoveries were in the range 46-114% for blood (n=20) and 50-120% for liver (n=8). The variation coefficients were within the requirements of the laboratorys quality system. The reason for the low recoveries and the somewhat high variation coefficient of 4-OH-CB187 is still unknown, but the low recovery of 6-OH-BDE47 was most likely due to chromatographic degradation. Unfortunately, an internal standard that would be more suitable for the brominated compound was not obtained at the time of the analyses. The between assay repeatability (n=15) expressed as variation coefficients was in the range 12-53%, where the high value was assigned to 3'-OH-CB138 which was on the detection limit of the method. The methods detection limits ranged from 0.001 ng/g for 4'-OH-CB159 to 0.036 ng/g for 6-OH-BDE47. The method linearity was in the range 0.996-0.999 for all the analytes[4].

The analytical method developed for the trace determination of OH-PCBs, OH-PBDEs and neutral HOCs in blood and liver, has been shown to be reliable, robust and convenient to apply. The method is suited for a wide range of analytes, which is a huge advantage when several classes of compounds are to be determined. In addition the method will most likely also be applicable for the analysis of other biological matrices (e.g. muscle tissue, egg and milk). A few issues are left to be resolved to obtain accurate results for all the analytes, especially the OH-PCB 4-OH-CB187, which only gave ~50 % recovery relative to the internal standard in the validation experiments. In addition, the OH-PBDE 6-OH-BDE47 gave low recovery values in the validation experiment with liver, although not in the validation experiment with blood. This could be an indication of the need for a more suitable internal standard for the OH-PBDE.

It is assumed that an increase in number of the hydroxylated analytes will be easy to incorporate in the method. Because the congener patterns can vary considerably among species, it is worthwhile to expand the analyte selection. This will be pursued in future studies.

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References

1. AMAP 2004. *AMAP assessment 2002: Persistent pollutants in the arctic*. Arctic Monitoring and Assessment Programme, Oslo, Norway.
2. Letcher RJ, Klasson-Wehler E, Bergman Å. *The Handbook of Environmental Chemistry* 2000; Vol. 3 Part K Chapter 11.
3. Murvoll KM, Skaare JU, Anderssen E, Jenssen BM. *Environmental Toxicology and Chemistry* 2006; 25:190.
4. Løken K. Thesis for the Master's degree in chemistry, Department of chemistry, University of Oslo, Oslo, Norway, 2006.
5. Brevik EM. *Bulletin of Environmental Contamination & Toxicology*, 1978;19:281.
6. Bernhoft A, Skaare JU. *Environmental Pollutants*, 1994;86:99.