Preliminary data on the optimisation of the clean-up procedures of acetylated and methylated hydroxy-PCBs with solid-phase extraction

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ABSTRACT

*Clean-up of acetylated and methylated hydroxy-polychlorinated biphenyls (PCB-AcO, PCB-MeO) was studied according to several methods: alumina column, acidic-basic columns, silica and florisil columns.
*The florisil column appeared to be the best of the methods tested.

INTRODUCTION

For the clean-up of hydroxy-metabolites of halogenated benzenes, polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs) and -furans (PCDFs) generated in a microsomal assay (*in vitro*), various methods have been applied. In one case, the extracts of microsomal incubations received no clean-up[1]. Several authors used thin layer chromatography (TLC) usually followed by scintillation counting of radioactive compounds or high performance liquid chromatography (HPLC)[2-5]. The latter two used GC-MS and GC-ECD as detection methods. Gel permeation chromatography (GPC) was used to clean up faeces and to separate the parent compound and sulphur containing metabolites from the hydroxy-PCDF (PCDF-OH) and PCDD-OH[6,7]. From both fractions, only the former fraction received additional clean-up with an alumina column. The PCDF-OH/PCDD-OH fraction was trimethyl-silylated and received no additional clean-up prior to GC-MS analysis. Bile extracts were made suitable for GC-MS analysis with a C_{18}-column followed by a silica cartridge[8].

Next to these specially developed methods, some clean-up procedures for the parent compounds exist as well. These could also be well applicable. The PCBs are separated from fat by a simple alumina column[9] whereas the PCDD-PCDF extracts are treated with an acidic-basic column and a subsequent alumina column[10].

So several methods are available. From these methods, the alumina column, Acidic-Basic column and silica column were choosen for initial optimisation of the clean up. All these were choosen because they seem the most convenient. The florisil cartridge was also included since similar results as with the silica were expected.

MATERIAL AND METHODS

Chemicals

Silica and florisil cartridges were Sep-Pak PLUS cartridges, obtained from Waters The 3-chloro-4-hydroxy-biphenyl (MCB-OH) was obtained from Fluka AG, 2,2',6,6'-tetrachlorol-4,4'-dihydroxy-biphenyl (TCB-OH) was obtained from Aldrich. The acetylated compounds were made as follows: the PCB-OHs, dissolved in ethanol, were added to 10 ml of water. Subsequently, 1 ml of 1 N NaOH and 1 ml of acetic acid anhydrid were added. This was incubated for 1 hour. After addition of hexachlorobenzene (HCB) in trimethylpentane as a recovery standard, the acetylated compounds and the HCB were extracted 3 times with 4 ml hexane. All 3 aliquots were combined. From this solution, samples were taken for clean up by column chromatography.

The methylated compounds were made as follows: The PCB-OHs, dissolved in ethanol, were added to 10 ml of water. After addition of HCB, the compounds were extracted 3 times with 4 ml hexane. The solvent was evaporated under N_2 until 100 μl. Next, 50 ml aceton, 2.6 g K_2CO_3 and 2 ml CH_3I were added. The mixture was refluxed under stirring for at least 6 hs. Next, the mixture was filtered, evaporated and dissolved in hexane. This solution was filtered once again. From this solution, samples were taken for clean up by column chromatography.

Columns and cartridges

The alumina columns and acidic-basic columns were prepared as described before[9,10]. The acidic-basic columns were tested combined as well as separately. Samples of 1 ml of a mixture of the PCB-MeO or PCB-AcO and HCB were applied to the column and subsequently eluted with different volumes of hexane and/or dichloromethane.

The florisil and silica cartridges were attached to a 5 ml syringe and rinsed 3 times with 5 ml hexane. After each volume, ± 2 ml of air (as an air lock) was passed through (rate ± 10 ml/min). The sample (1 ml) was loaded and passed through with an air lock. Next, 3 ml hexane was passed through with an air lock. Both fractions were combined. Next, several fractions of either hexane, dichloromethane or methanol were passed through and collected. Between the fractions, an air lock was passed through.

To test the cartridges in an actual situation, 4 microsomal suspensions (eiderducklings, 50 mg microsomal protein each) were extracted (4 times with 4 ml hexane): two after an acetylation procedure (3 hs instead of 1 h) and two without pretreatment. Before the extraction, a solution of TCB-MeO was added. After evaporation until 1 ml, the fractions were passed through either a florisil or a silica cartridge. All samples were analysed with either GC-ECD, GC-FID or GC-MSD.

RESULTS AND DISCUSSION

Alumina column

To elute all PCB-MeO and PCB-AcO, at least 29 ml hexane was required. This is 16 ml more than the volume required to elute PCBs and chlorinated pesticides[9]. With dichloromethane as eluent, 15 ml was enough to obtain 100% recovery. According to Th. Hillebrand (pers. comm.), the risk for contamination with lipids or fatty acids is still present in this case.

Acidic-basic column

The acetylated compounds showed low recoveries from acidic, basic and combined colums (0-2%). The methylated compounds showed high recoveries from the basic column (100%) whereas the acidic and combined columns showed recoveries of 0-3%. This clean-up procedure does not seem suitable for these types of derivatised compounds.

Table 1: Recovery of TCB-MeO after separation from a microsomal suspension extraction by florisil or silica cartridges. Type of procedure: Acetylation means after acetylating the microsomal suspension and adding standard solution to the extract, untreated means just plain extraction and adding of standard solution before treating.

Column	Type of procedure	Recovery
Florisil	Acetylation	89%
	Untreated	99%
Silica	Acetylation	95%
	untreated	84%

Sep-Pak cartridges

After the initial passing of the sample (1 ml) and the first 3 ml hexane, both silica and florisil columns could be eluted with 4 ml dichloromethane. This yielded high recoveries. As an additional check, an extra 4 ml methanol was passed through as well. Methanol completely destroys the retention and separating characteristics of the columns so any retained TCB-AcO should appear in this fraction. In case of the silica column, some TCB-AcO could be detected in this last fraction, whereas the florisil column had retained nothing.

In the actual situation (extraction of microsomal suspensions), both types of cartridges displayed a satisfactory performance (table 1). Both cartridges showed high recoveries, comparable to results obtained with PCDF-MeO[8]. The differences in recovery between the two types of cartridges were not obvious, though the performance of florisil cartridges tends to be better. An additional advantage is that in case of the acetylation, florisil does retain some of the disturbing ECD-detectable compounds whereas silica lets them pass through. For the methylation, a similar phenomanon was observed. GC-FID showed no signs of remaining lipids or fatty acids for both cartridges.

CONCLUSIONS

*Alumina columns seem less suitable due to the high volume of eluent required.
*Acidic-basic columns are not suitable for the clean-up of hydroxy-metabolites.
*Florisil cartridges, eluted with dichloromethane, offer the best solution for clean-up of PCB-MeO and PCB-AcO, of the methods tested.

LITERATURE

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