RAPID ANALYSIS OF PLANAR PCBS USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

Peter Haglund[1], Mikael Harju[1], Ruby Ong[2] and Philip Marriott[2]

[1]Environmental Chemistry, Umeå University, SE-90187 Umeå, Sweden. [2]Applied Chemistry, Royal Melbourne Institute of Technology, Melbourne 3001, Australia

Introduction

The toxicity of polychlorinated biphenyls (PCBs) differs dramatically depending on the substitution pattern, especially the number of *ortho*-chlorine substituents. PCBs with many *ortho*-chlorines possess phenobarbital type (PB) induction, whilst PCBs without *ortho*-chlorines (non-*ortho* PCBs) possess methylcholanthrene type induction and "dioxin-like" effects. Mono-*ortho* PCBs induces both systems, but to a lesser extend[1].

The shape of the PCB molecules also depends on the substitution in *ortho*-position. The non-*ortho* substituted PCBs does more easily adopt a planar configuration than the other congeners, as reflected by the rotational energy barriers that decrease in the order non-*ortho* < mono-*ortho* < di-*ortho* << tri-*ortho* < tetra *ortho*.

In the present study we have adopted comprehensive two-dimensional gas chromatography (GC×GC) in the quest for a simple and cost-effective analytical procedure for non- and mono-*ortho* PCBs. The GC×GC system was a longitudinally modulated cryogenic system (LMCS). A smectic liquid-crystal stationary column was selected as the first column since liquid-crystal phases exhibit a particular strong retention of planar compounds, or rather, discrimination of non-planar compounds[2]. The other column was a non-polar column. In this way, the non-*ortho* PCBs elute very fast from the second (non-polar) column and sensitivity is maximised. If ultimate sensitivity is required the whole peak volume of each PCB may be sampled using the LCMS trap and presented to the second column as a single package.

Methods and Materials

The GC was an Agilent HP6890 system retrofitted with an Everest LMCS system (Chromatography Concepts, Doncaster, Australia) and equipped with a flame ionisation detector (FID). A 10m × 0.18mm, 0.1μm film, liquid crystalline column (LC-50; J&K Environ. Ltd, Canada) was used as 1st column, followed by a 0.25m × 0.1mm, 0.1μm film, non-polar 2nd column (BPX-5; SGE international, Australia). Hydrogen was used as the carrier gas at a constant flow of 1.0 ml/min. The CO_2 flow into the LCMS cryo-trap was adjusted using a needle valve to a temperature ≈100°C below the oven temperature. One microliter aliquots of toluene solutions of the PCBs were splitless injected using an autosampler, and the oven temperature was ramped as follows: 90°C for 1 minutes, raise at 30°C/min to 170°C, then raise at 18°C/min to 275°C. The modulation period was 2s. The FID was operated either at 5 or 100Hz depending on whether the GC or GC×GC mode was utilised. The Agilent ChemStation software was used for system control and data acquisition. The resulting data was exported as a comma separated value file, converted to a text matrix format using the Comprehensive GC File Converter 2.0 software of Chromatography Concepts, and imported to Transform 3.3 (Fortner Research, USA) for data visualisation.

Results and Discussion

The PCBs elute rapidly from the liquid crystal column that was used as 1st dimension column, i.e. at relatively low temperatures. To avoid excessive retention in the second column, the length of it was reduced as much as practically possible. Even after reducing the effective length of the 2nd column to 0.25m the PCBs were strongly retained. However, using a fast temperature ramp it was possible to obtain appropriate second column retention times.

According to our previous retention behaviour studies of the non-*ortho* PCBs should be most strongly retained by the LC-50 column[2], followed by the mono-*ortho* PCBs, the di-ortho PCBs, and the multi-*ortho* PCBs. As a consequence, these congeners are expected to elute at the highest temperature within each homologue group and, thus, to have the shortest 2nd dimension retention times. This was confirmed by analysing a technical PCB (Clophen A50). The non-*ortho* PCBs 77 (3,3',4,4'-tetrachlorobiphenyl), 126 (3,3',4,4',5-pentachlorobiphenyl) and 169 (3,3',4,4',5,5'-hexachlorobiphenyl) (**boldfaced**) are all found in the lower right hand corner of the GC×GC contour plot, c.f. **Figure 1**. Close to these the mono-ortho PCBs 105 (2,3,3',4,4'-pentachlorobiphenyl), 118 (2,3',4,4',5- pentachlorobiphenyl), and 156 (2,3,3',4,4',5-hexachlorobiphenyl) (***boldfaced/italic***) can be seen. Furthermore, no peaks can be seen at the coordinates of PCBs 77, 126, and 169 in the contour plot of unspiked Clophen A50 (data not shown).

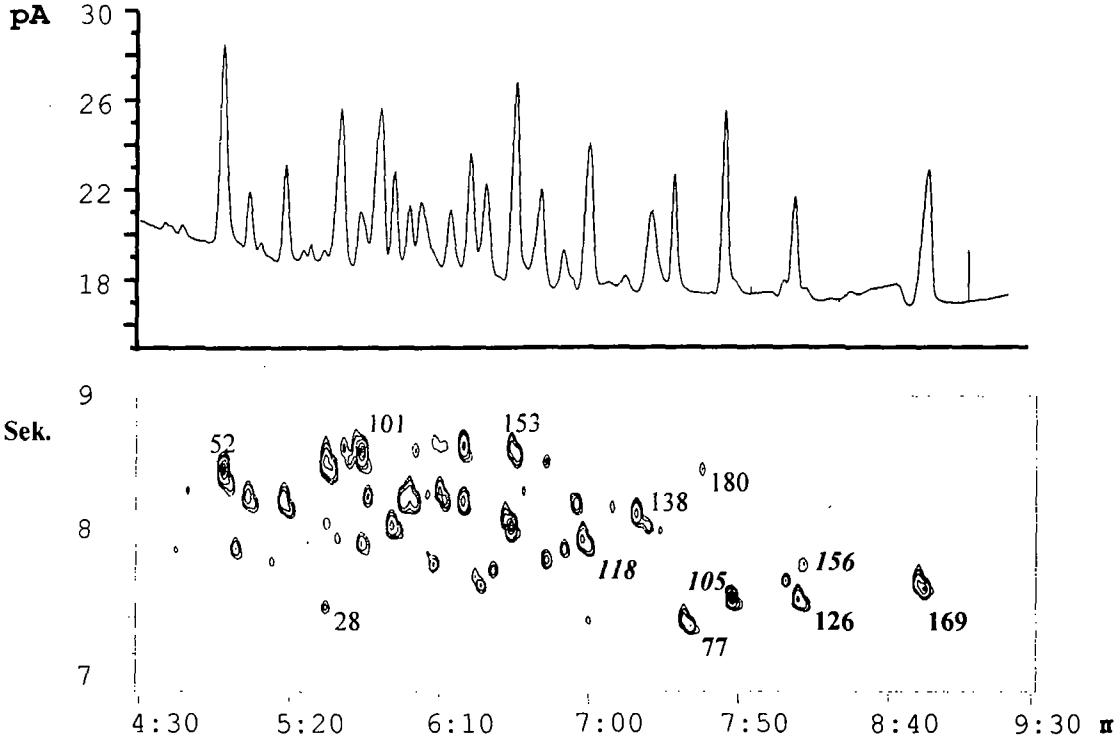

Figure 1: Clophen A50 analysed using normal GC (top panel) and GC×GC (lower panel). The contour levels of the GC×GC plot were 23, 30, 40, 80, 120, 160, and 200 pA (baseplane at 16 pA).

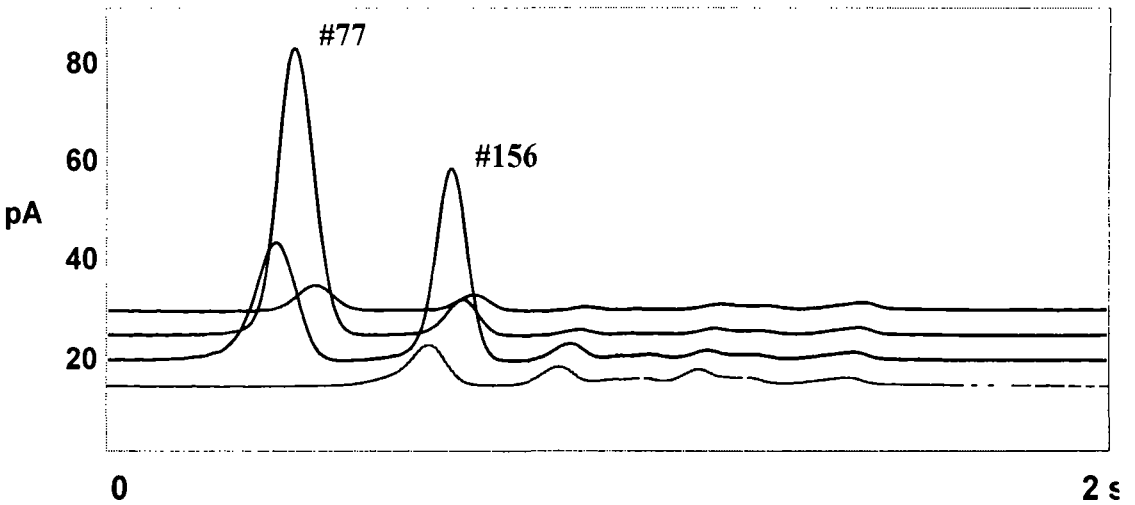

Figure 2: Individual 2nd dimension chromatograms from GC×GC analysis of Clophen A50spiked with PCBs 77 and 156. An offset of 5pA, between chromatogram, was used to enhance presentation.

Thus, no major PCB congeners elute at these retention times. The analyses did however pick up signals at the coordinates of the mono-*ortho* PCBs 105, 118, and 156. The relative intensities of these do however correlate with their proportions in the technical PCB formulations[3] and it was therefore concluded that these signals stem from PCBs 105, 118, and 156 and not from interfering PCBs.

Furthermore, PCBs 156 and 77 elute close to each other. To verify that they are resolved, a separate analysis was performed of a Clophen A50 standard spiked with two PCBs, c.f. **Figure 2**. The compounds are well resolved although a slight peak broadening (fronting) can be seen, indicating mass overload, which is often encountered using these narrow-bore thin film columns. It is therefore likely that PCB77 could be analysed without interferences of PCB157 even if the latter normally is present in much larger quantities. To overcome the overloading problem all samples should, ideally, be diluted and analysed by the much more sensitive micro-electron capture detector (μECD).

However, a considerable sensitivity increase was gained just by using the GC×GC mode of operation. The peak compression (in the LMCS trap), and short second column retention times of the mono- and non-*ortho* PCBs, resulted in a significant signal enhancement, e.g. the intensity of the PCB 105 signal increased from about 8 pA to > 160 pA. At the same time the noise level decreased. Consequently, a more than 20 fold increase in sensitivity was obtained.

We also run a mixture containing 7 major PCB congeners (PCBs 28, 52, 101, 118, 138, 153, and 180)[4] that are frequently analysed since they are both used for regulatory purposes and as indicator compounds. These all appears to be eluting as single components from the 2nd dimension column although all of them coelute with one or more congener from the 1st dimension column (**Figure 1**).

In single column GC analyses such coelutions of indicator PCBs and other PCB congeners is a large problem even if long highly efficient columns are used. The complete separation and unequivocal quantification of these congeners is a tedious task that requires analyses using multiple columns or at least a pair of columns and mass spectrometric (MS) detection.
Thus, it seems like this column set might be used not only to analyse non- and mono-*ortho* PCBs but also the 7 indicator PCBs. Eventually the large differences in concentrations between non- and mono-*ortho* PCBs and indicator PCBs in some environmental samples might pose a problem. To allow the detection of trace levels of non-*ortho* PCBs large sample portions have to be injected, which might lead to overloading of the 2nd dimension column with indicator PCBs. The use of a μECD instead of an FID would obviously improve the situation, but in some cases the linear range of this detector would be insufficient. Under such circumstances it would still be possible to perform the analysis by splitting the column flow after the LCMS trap. Two capillaries of same phase ratio but different internal diameter, connected to the split in one end and to an ECD detector (one per column) in the other, could then be used to analyse the two classes of PCBs. The narrow capillary would be used for indicator PCBs and the wide for planar PCBs.
A maximum throughput of 5 samples per hour could be obtained using the present column set and modulator design. This is about 10 times more than a standard PCB- mass spectrometry (MS) analysis, which requires a separate injection for each group of compounds.
Finally, although this system seems very promising for planar PCB as well as indicator PCB analyses more work is needed before it could be applied on a broad basis. Most important would be to carefully check for potential coelutions. And, before it could be used for the analysis of low level environmental samples the FID detector, used in this study, should be substituted by a more sensitive device such as the μECD or a time-of-flight MS. GC×GC-μECD is capable to detect sub-pg quantities of halogenated organic pollutants in complex environmental matrices[4].

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