ANALYSIS OF POLYCHLORINATED BIPHENYLS BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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Introduction

PCB analyses have been mainly focused on the determination of marker congeners (28, 52, 101, 118, 138, 153 and 180), which are the predominant PCB congeners found in humans and food stuffs of animal origin. In recent years, attention has been focused on the non- and mono-*ortho* substituted PCB congeners, because these CBs show the same type of toxicity as polychlorinated dibenzo-*p*-dioxins and dibenzofurans[1]. The separation of these congeners from the bulk of PCBs is complicated by their relatively low concentrations compared to the bulk of PCBs and by the high number of possible co-elutants[2]. As the WHO has recommended an acceptable daily intake for dioxins, furans and PCBs which include non-*ortho* (77, 81, 126, 169) and mono-*ortho* (105, 114, 118, 123, 156, 157, 167 and 189) substituted CBs, and this recommendation has been adopted by the European Scientific Committee for Food, it is likely that these non- and mono-*ortho* CBs will be included in food monitoring programs. Thus, there is a pronounced need for unambiguous measurements of these PCBs.

In recent years, comprehensive two-dimensional chromatography (GC×GC) has been shown to be very useful for the separation various complex samples[3-5]. In GC×GC two independent GC separations are applied to the sample. The sample is first separated on a high-resolution capillary GC column under programmed-temperature conditions. The effluent of this column then enters a thermal modulator, which traps each subsequent small portion of eluate, focuses these portions and introduces them into a second column for further separations. The second separation is made to be fast enough (e.g. 5-10s) to permit the continual introduction of small fractions from the first column without mutual interference. The most obvious advantage of GC×GC is the large peak capacity. Because retentions in the two dimensions are almost independent, the peak capacity that can be achieved is close to the product of the peak capacities of the two individual columns[3]. Another advantage of GC×GC system is the increase of the signal amplitude by a focusing effect of the thermal modulation, which leads to increase of S/N ratios and thus improvement of detection limit[6]. Finally, all peaks in the chromatogram are described by two time co-ordinates which makes the identification more reliable.

The above information indicates that the GC×GC should be a suitable separation technique for the analysis of PCBs. The goal of this paper is to examine the potential of GC×GC for the qualitative analysis and characterization of PCBs, with emphasis on the non- and mono-*ortho* PCB congeners.

Methods and Materials

The GC×GC system used for the experiments was a HP 6890 (Hewlett Packard, Wilmington, DE, USA) equipped with a thermal modulator assembly (Zoex Corp., Lincoln, NE, USA). Principles and working characteristics of the thermal modulator are extensively described elsewhere[7]. Helium

gas (Hoek Loos, Schiedam, The Netherlands) with a purity of 99.999% was used as carrier gas through the GC×GC system at the inlet pressure 50 psi. A micro electron capture detector (μ-ECD) was operated at 300°C, with 99.999% pure nitrogen (Hoek Loos, Schiedam, The Netherlands) as make-up gas at a flow rate of 60 ml/min. Samples were injected manually (1μl) into a split/splitless inlet port operated at 300°C in splitless mode. The purge time was set at 1 min. The first column was a 30m × 0.25mm i.d. × 0.25μm HP-1 (100%-dimethylpolysiloxane) fused silica column (Hewlett-Packard, Wilmington, DE, USA). Two different columns were used as a second dimension column: 1m × 0.1mm × 0.1μm HT-8 (8%-phenyl-polycarborane-siloxane) purchased from SGE International (Ringwood, Australia) and 1m × 0.1mm × 0.1μm SupelcoWax-10 (polyethylene glycol) purchased from Supelco, Bellefonte, PA, USA. A 7cm × 0.1mm × 3.5μm 007-1 (100%-methylpolysiloxane) capillary column (Quadrex Corp., Deerfield, IL, USA) was used as a modulator tube. The head of the first column was connected directly to the injector and the end to the modulator tube via a short piece of pre-column. The head of the second column was connected via a short piece of pre-column to the modulator tube and the end to the detector also via a pre-column. This installation enabled to place the whole second dimension column into the separate oven. The optimised temperature program of the 1st and the 2nd oven for HP-1 – HT-8 column combination was 90°C (2min), 5°C/min → 110°C, 1°C/min → 250°C (5min) and 110°C (2min), 5°C/min → 130°C, 1°C/min → 270°C (5min), respectively. The optimised temperature program for HP-1 – SupelcoWax-10 column set-up was 90°C (2min), 1°C/min → 230°C (24min) for the 1st oven and 140°C (2min), 1°C/min → 280°C (24min) for the 2nd oven. The connections among the columns were made by micro and mini press-fits (Techrom, Purmerend, The Netherlands). The slotted heater temperature was set 100°C above the 1st oven temperature. Comprehensive two-dimensional gas chromatography operating program, version 2.0z (Zoex Corp., Lincoln, NE, USA) was used for the sweeper control and data acquisition. The modulation period was 6.5 s at the rotating speed 0.15 rev.s^{-1} and pause time 0.4 s. Data acquisition rate was set at 50 Hz. Transform software (Fortner Research, Sterling, VA, USA) was used for data visualization.

A mixture consisted of 90 PCB congeners (4, 5, 7, 10, 11, 12, 16, 24, 26, 28, 29, 31, 33, 37, 40, 44, 47, 49, 50, 52, 53, 54, 56, 60, 61, 65, 66, 69, 70, 72, 74, 75, 77, 78, 80, 81, 82, 84, 85, 87, 88, 92, 95, 97, 99, 101, 103, 105, 110, 114, 116, 118, 119, 121, 123, 124, 126, 128, 129, 136, 137, 138, 140, 141, 149, 151, 153, 154, 155, 156, 157, 163, 167, 169, 170, 171, 173, 180, 183, 185, 187, 189, 194, 195, 198, 200, 202, 206, 207 and 208)[9] in iso-octane (Promochem, Wesel, Germany) was used for all experiments. The concentrations of all congeners in the mixture were ca. 1ng/ml.

Results and Discussion

Two different stationary phases in the second column were tested in this study: 8%-phenyl-polycarborane-siloxane (HT-8) and polyethylene glycol (SupelcoWax-10). Figure 1 presents the chromatogram obtained with HP-1 – HT-8 column combination under optimised conditions. Out of all 90 CBs present in the mixture, 84 peaks were observed in the chromatogram. This means, that 6 critical pairs were not separated (4-10, 28-31, 52-69, 74-61, 56-60 and 138-163). However, all 12 CBs mentioned in the WHO list (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) were baseline separated. Moreover, this column combination delivers structured chromatograms with chemically meaningful groups of peaks. Each group of peaks contains CBs with the same number of chlorines in the molecule and the position of a peak within a group is determined by the pattern of chlorines on the biphenyl structure. Carborane phase exhibits a dual nature. For CBs with two or more *ortho*-chlorines the phase act as non-polar, while for non- and

mono-*ortho* substituted CBs act as polar. Therefore all 12 WHO non- and mono-*ortho*- PCB congeners have the highest retention time in the second dimension and are situated always on the top of the group. The separation of PCBs into the groups with the same chlorine number were theoretically predicted for the 100%-dimethylpolysiloxane and p,p-cyanobiphenyl stationary phase combination[9], but to date no experimental results have been published.

A separation of more PCB congeners was achieved by using a polyethylene glycol phase in the second column (Figure 2). With this column set-up 87 peaks were observed in the chromatogram out of all 90 PCBs present in the mixture. Only three critical pairs were non-separated (28-31, 56-60 and 80-88). As well as with the HT-8 column, all 12 PCB congeners mentioned in the WHO list were baseline separated. However, at this column combination no structured chromatogram with meaningful pattern was observed. As can be seen from the comparison of the Figures 1 and 2, the polyethylene glycol has higher selectivity for PCBs than carborane phase – peaks eluted from polyethylene glycol column are spread over the entire chromatographic plane while peaks eluted from carborane column are grouped more together. The determining factor of PCB retention in the polyethylene glycol stationary phase is again the degree of *ortho*-substitution. The retention time is higher for CBs with none or a few *ortho*-substitution than for CBs with many *ortho*-substitution. However, the degree of *ortho*-substitution is apparently not the only determining factor. Therefore, the congeners 123, 118, 114, 167 appeared in the middle of chromatographic plane.

Both used column combinations allow the separation of all 12 toxic non-*ortho* and mono-*ortho* congeners mentioned in the WHO list from the test mixture without any pre-separation step. Moreover, a HP-1 – HT-8 column combination delivers structured chromatograms with PCBs distributed over the retention plane in chemically meaningful patterns, what makes the identification more reliable. All these characteristics, together with a good quality quantitation for PCBs, which was confirmed before[10], make the GC×GC technique highly suitable for a congener-specific PCB determination.

References

1. WHO meeting in Stockholm, Sweden, 15-17 June 1997.
2. Larsen B.R. (1995) J. High Resolut. Chromatogr. 18, 141.
3. Phillips J., Beens J. (1999) J. Chromatogr. A 856, 331.
4. Dimandja J.-M. D., Stanfill S.B., Grainger J., Petterson D.G. (2000) J. High Resolut. Chromatogr. 23, 208.
5. de Geus H.-J., Aidos I., de Boer J., Luten J.B., Brinkman U.A.Th. (2001) J. Chromatogr. A 910, 95.
6. de Geus H.-J., de Boer J., Phillips J.B., Ledford E.B., Brinkman U.A.Th. (1998) J. High Resolut. Chromatogr. 21, 411.
7. Phillips J.B, Gaines R.B., Blomberg J., van der Wielen F.W.M., Dimandja J.-M., Green V., Granger J., Patterson D., Racovalis L., de Geus H.-J., de Boer J., Haglund P., Lipsky J., Sinha V., Ledford E.B. (1999) J. High Resolut. Chromatogr. 22, 3.
8. Ballschmiter K., Bacher R., Mennel A., Fischer R., Riehle U., Swerev M. (1992) J. High Resolut. Chromatogr. 15, 260.
9. Phillips J.B., Xu J. (1997) Organohalogen Compounds 31, 199.
10. de Geus H.-J., Schelvis A., de Boer J., Brinkman U.A.Th. (2000) J. High Resolut. Chromatogr. 23, 189.

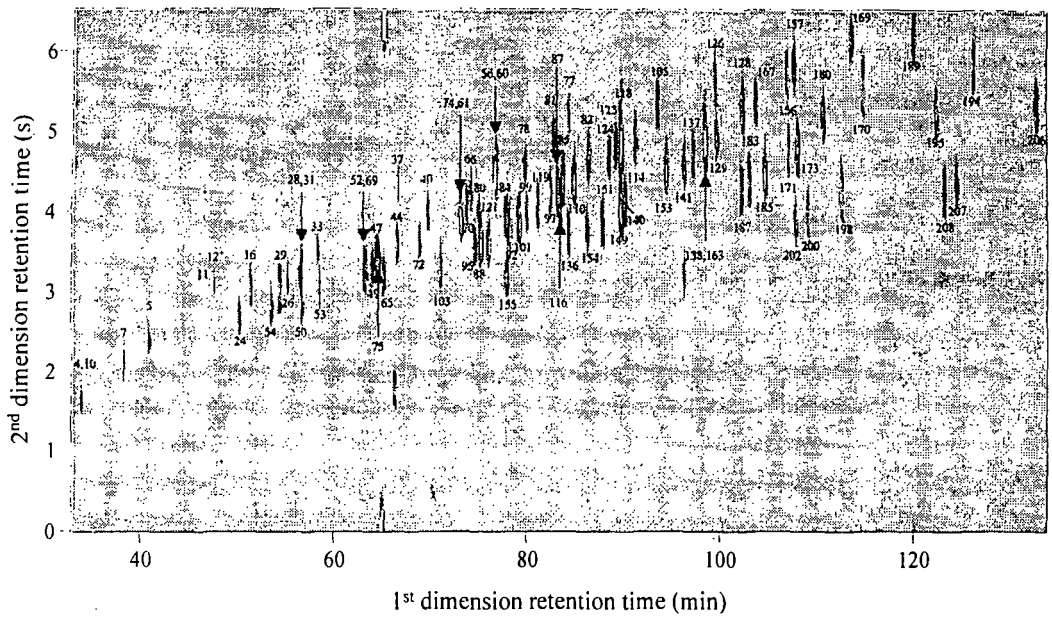

Figure 1. GC×GC-μECD chromatogram of a mixture containing 90 PCB congeners (1ng/ml) achieved on HP-1 - HT-8 column set-up.

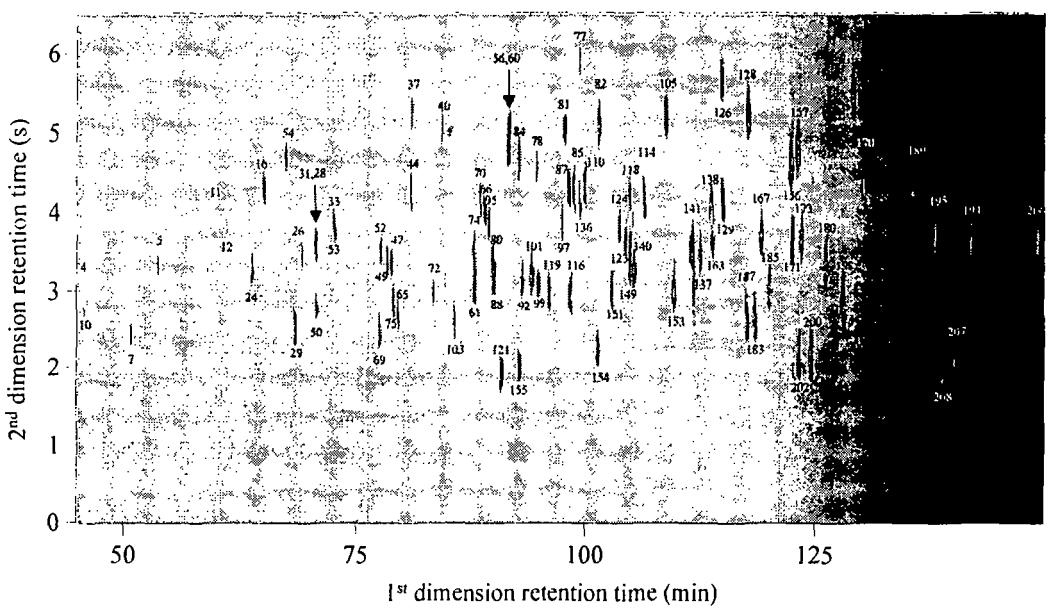

Figure 2. GC×GC-μECD chromatogram of a mixture containing 90 PCB congeners (1ng/ml) achieved on HP-1 - SupelcoWax-10 column set-up.