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GC×GC METHOD DEVELOPMENT FOR ANALYSIS OF SEVENTEEN 2,3,7,8- SUBSTITUTED DIOXINS, FURANS AND TWELVE DIOXIN-LIKE PCBs

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Introduction

The European research project DIAC ("Dioxin Analysis by Comprehensive Multi-dimensional Gas Chromatography") is focussed on the development of an alternative method for analysis of polychlorinated dioxins, furans and dioxin-like PCBs. Normally, high resolution mass spectrometry (HRMS) is used for detection in the gas chromatographic (GC) determination of these analytes due to its high selectivity and sensitivity. However, this method is relatively expensive. Given the need for a regular monitoring of dioxins and PCBs in Europe, which is further enhanced by the new maximum residue values for dioxins in food and animal feed per 1 July 2002, cheaper, but reliable methods would be most welcome.

Comprehensive two-dimensional GC, or GC×GC, has emerged as an extremely powerful separation technique. Much higher peak capacities can be obtained than in conventional GC, because each successive small fraction eluting from the conventional-size first-dimension column is subjected, in real time, to a second, orthogonal separation, on a relatively short (*ca.* 1 m) second-dimension column with different separation characteristics. In addition, an increase in the S/N ratios as a result of the focusing effect of the modulation is achieved what results in lower limit of detection compared to one-dimensional GC[1]. Both these characteristics make GC×GC technique highly promising for analysis of dioxins, furans and dioxin-like PCBs[2].

An essential aspect of GC × GC is the so-called modulation. Different ways of modulating have been reported[3]. Therefore, the first step of the GC×GC method development was evaluation of different modulators for dioxins, furans and PCBs analysis presented in a previous paper[4]. The next step in GC×GC method development, presented in this study, is the selection of the stationary phases in the first- and second-dimension columns, because it is one of the most important tasks when designing a GC×GC separation system. The main goal is to achieve separation of all seventeen 2,3,7,8-substituted PCDD/Fs and twelve dioxin-like PCBs from interfering sample constituents and all PCDD/Fs and PCBs congeners with different TEF values from each other to be able to calculate TEQ value required by European legislation[5].

Methods and Materials

The GC×GC system was built from a HP 6890 (Agilent Technologies, CA, USA) gas chromatograph equipped with a single carbon dioxide jet modulator (Zoex Corp., NE, USA) consisting of a holder of the modulator loop and one cold and one hot jet, with the nozzles mounted orthogonal to each other. Three columns were used as first-dimension columns: 60m ×

0.25 mm × 0.25 μm DB-DIOXIN from Agilent Technologies, 30 m × 0.25 mm × 0.25 μm HP-1 (100%-dimethylpolysiloxane) from Agilent and 30 m × 0.25 mm × 0.1 μm HT-5 (5% phenyl (equiv.) polycarborane-siloxane) from SGE International (Australia). Five columns were used as second-dimension columns: 1 m × 0.1 mm × 0.1 μm HT-8 (8%-phenyl (equiv.) polycarborane-siloxane), 1 m × 0.1 mm × 0.1 μm BPX-50 (50%-phenyl (equiv.) polysilphenylene-siloxane) both from SGE International, 1 m × 0.1 mm × 0.1 μm OV1701 (14%-cyanopropyl-siloxane) from Quadrex Corp. (IL, USA), 1 m × 0.1 mm × 0.1 μm 90%-cyanopropyl-siloxane (Varian-Chrompack, the Netherlands) and 1 m × 0.1 mm × 0.1 μm SupelcoWax-10 (polyethylene glycol) from Supelco (PA, USA). A 1.5 m × 0.1 mm deactivated column (BGB Analytik, Switzerland) was used as modulator loop. The head of the first column was connected directly to the injector and its outlet, to the modulator loop. The head of the second-dimension column was connected to the modulator loop and its end to the detector. Mini press-fits (Techrom, the Netherlands) were used for the connections. Helium gas (Hoek Loos, the Netherlands) with a purity of 99.999% was used as carrier gas through the GC×GC system at an inlet pressure of 45 psi for DB-1 and HT-5 column and 70 psi for DB-DIOXIN column. The micro ECD (Agilent) was operated at 300°C, with 99.999% pure nitrogen (Hoek Loos) as make-up gas at a flow of 150 ml/min. 1-μl samples were injected manually into a split/splitless inlet port (Agilent) operated in the splitless mode at 280°C; the purge time was 2 min. The temperature of the cold jet was adjusted before each run to be –10°C at room temperature. The temperature of the hot jet was 400°C during entire temperature programme. The modulation period was 7 s, and the hot air pulse duration was 200 ms. Chemstation software (Agilent) was used for data acquisition. The data acquisition rate was 50 Hz. Transform software (Fortner Research, VA, USA) was used for data visualisation and evaluation.
A standard mixture containing 17 PCDDs/Fs and 12 dioxin-like PCBs (for composition see Figure 1) in n-nonane was prepared from two commercial standard mixtures, EPA 8290 STN and WP-STK from Wellington (Ontario, Canada). Samples after extraction were purified in a multilayer silica column. The purified extract was fractionated in SPE carbon tubes. Two fractions were obtained: the first one, containing PCBs and the second one containing PCDD/F. The second fraction did not need any additional clean-up. PCB fraction was fractionated on a HPLC pyrenyl column in order to separate the bulk of the PCBs into one fraction and the 12 dioxin-like PCBs into the second fraction.

Results and Discussion

The DB-DIOXIN column was our first choice as the first-dimension column, because it separates all priority congeners from each other. However, due to the high bleeding of this column, 10 times higher noise was observed for this column compared to non-polar stationary phase (100-Hz noise for DB-DIOXIN *vs.* 10-Hz noise for DB-1 column) what has a negative influence on the limit of detection. Therefore, our next choice was the low bleeding non-polar DB-1 column.
As can be seen from Figure 1, the DB-1 column itself offers a relatively good separation for the analytes of interest. In the first dimension, two critical pairs are not separated (OCDD/OCDF and CB169/1,2,3,7,8 PeCDD) and four pairs are partly overlapping (CB123/CB118, CB156/CB157, 1,2,3,4,7,8 HxCDD/1,2,3,6,7,8 HxCDD and 1,2,3,7,8,9 HxCDD/1,2,3,7,8,9 HxCDF). In fact, only co-elution of CB169 and 1,2,3,7,8 PeCDD prevents to calculate a TEQ value, because the rest of co-eluting pairs have the same TEF value. Thus, in order to be able to calculate the TEQ value, the second dimension column has to provide separation of CB169 from 1,2,3,7,8 PeCDD. In this study, five stationary phases were tested in combination with DB-1 column: 8%-phenyl (equiv.)

polycarborane-siloxane (HT-8), 50%-phenyl (equiv.) polysilphenylene-siloxane (BPX-50), polyethylene glycol (SupelcoWax-10), 14%-cyanopropyl-siloxane (OV1701), 90%-cyanopropyl-siloxane. The first two columns, HT-8 and BPX-50 do not separate critical pair CB169/1,2,3,7,8-PeCDD which is required to calculate TEQ. Therefore, these are not suitable for combination with DB-1. However, it is important to notice, that BPX-50 separates OCDF from OCDD. Polypropylene stationary phase (SupelcoWax-10) offers the highest selectivity. The critical pair for the TEQ value calculation (CB 169 and 1,2,3,7,8-PeCDD) is resolved. Partly co-eluting pairs in the first dimension CB123/CB118 and CB156/CB157 are resolved in the second dimension as well. Even the difficult pairs 1,2,3,4,7,8-HxCDF/1,2,3,6,7,8-HxCDF and 1,2,3,4,7,8-HxCDD/1,2,3,6,7,8-HxCDD are partly separated in the second dimension. Despite all these positive aspects this phase is not suitable for dioxin, furan and planar PCB analysis, because decomposition of OCDF was observed. The cyano stationary phases, 14%-cyanopropyl (OV1701) and 90%-cyano column (Figure 1), are only the phases capable for TEQ determination. The critical pair CB169/1,2,3,7,8-PeCDD is completely separated. The 90%-cyanopropyl column is preferred because of the better overall separation compared to 14%-cyanopropyl.

The last column tested in the first dimension was HT-5. As can be seen from Figure 2, the congeners CB169 and 1,2,3,7,8 PeCDD are separated on this column. That allows the use of the BPX-50 column in the second dimension, and to achieve also separation of OCDD and OCDF. The HT-5 × BPX-50 is thus the second column combination suitable for determination of the TEQ value.

The two column combinations capable to determine the TEQ value (DB-1 × 90%-cyanopropyl and HT-5 × BPX-50) were further evaluated for the separation of all seventeen 2,3,7,8-substituted PCDD/Fs and twelve dioxin-like PCBs from interfering sample constituents. Milk, fish oil and pork fat samples were extracted, cleaned and pre-separated in the same way as for GC-HRMS determination. Three fractions were obtained: i) dioxin's, ii) non- and mono- ortho PCB's, and iii) remaining PCBs. The first two fractions were spiked with 17 PCDDs/Fs (concentration range: 2.6 – 13.0 pg/μl) and 12 dioxin-like PCBs (concentration 26.1 pg/μl) and co-elution was studied. It was found that both tested column combinations are able to separate all priority analytes from the sample constituents present in both fractions. An example of a spiked dioxin fraction of pork fat extract is shown in Figure 2.

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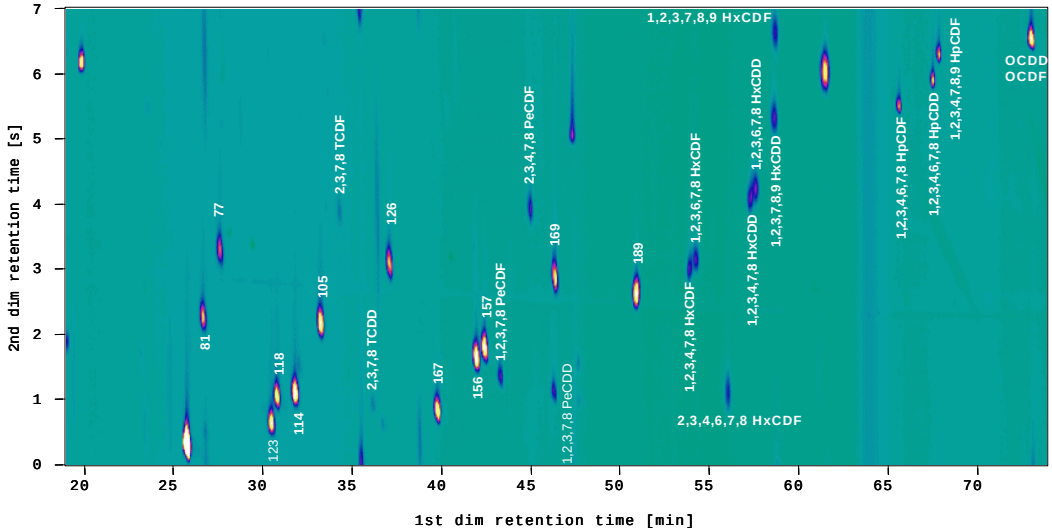

Figure 1 GC×GC–ECD chromatogram of the mixture of 17 PCDDs/Fs and 12 dioxin-like PCBs with DB-1 × 90%-cyanopropyl column combination. Temperature programme: 90°C (2 min), at 30°C/min to 190°C (15min), then at 1.5°C /min to 230°C (16 min), then at 20°C/min to 270°C (0 min), then at 1.5°C/min to 290°C (10 min)

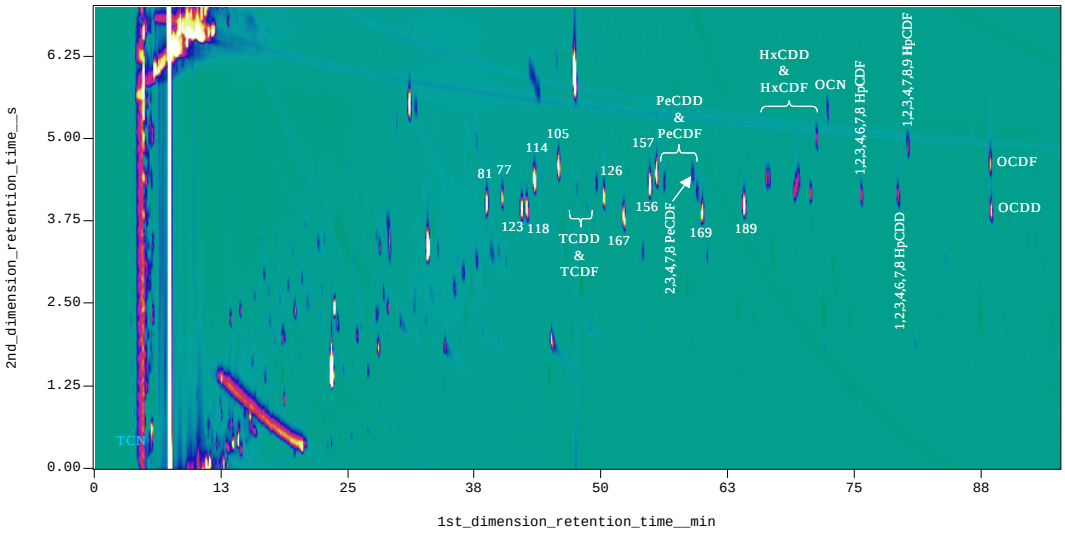

Figure 2 GC×GC–ECD chromatogram of the dioxin fraction of the pork fat extract spiked with 17 PCDDs/Fs and 12 dioxin-like PCBs with HT-5 × BPX-50 column combination. Temperature programme: 90°C (2 min), at 30°C/min to 170°C (5min), then at 1.5°C /min to 320°C (0 min))