

FEASIBILITY OF MULTIDIMENSIONAL GC TECHNIQUES (HEART-CUT MDGC AND GC × GC) FOR THE ANALYSIS OF THE ENANTIOMERS OF CHIRAL TOXAPHENES

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Introduction

The enantioselective analysis of complex mixtures of chiral pollutants is still a challenge. Besides the difficulties in finding appropriate enantioselective stationary phases able to resolve each chiral compound into its constituent enantiomers, there is an increased risk of co-elution due to the higher number of peaks in real samples. In the case of the pesticide toxaphene, one of the most heavily used chlorinated pesticides world-wide distributed; most of the congeners found in real samples are chiral¹. In order to provide a more comprehensive understanding of their metabolism and the toxicity associated with their presence, the enantioselective analysis has become a relevant tool in the environmental and food fields.

The most commonly used analytical methodologies for the determination of toxaphene congeners are based on an extraction followed by an extensive clean-up and fractionation step¹. Further instrumental analysis has usually been carried out by gas chromatography (GC) with electron-capture detector (ECD). However, the lack of selectivity of ECD considerably increases the risk of co-elution between congeners. This limitation can be circumvented by the use of the more selective mass spectrometric (MS) detection² or, as an alternative, multidimensional GC techniques (MDGC), such as heart-cut MDGC and comprehensive two dimensional GC (GC × GC). The two independent GC separations provide an enhanced peak capacity that, when using an ECD, contributes to compensate its limited selectivity.

The aim of this study is to assess the feasibility of both multidimensional GC techniques, heart-cut MDGC and GC × GC, for the accurate enantioselective determination of 5 chiral toxaphenes typically found in environmental samples, namely Parlar 26, 32, 40, 44 and 50. In the environmental and food field, heart-cut MDGC has been used for the congener specific analysis of toxaphenes^{3,4}. However, to the best of our knowledge, the determination of chiral toxaphenes by GC × GC has not been reported so far. Thus, different column combinations have been tested and, once the best combination has been selected, the enantiomeric fraction (EF) of the above-mentioned congeners has been determined in commercial fish oil samples. The results have been compared to those obtained by heart-cut MDGC.

Materials and Methods

A working standard solution containing Parlar 26, 32, 40, 44 and 50 was prepared from individual toxaphene standards (Dr. Ehrenstorfer GmbH, Augsburg, Germany). A BGB-172 (25% 2,3,6-*tert*-butyldimethylsilyl β-CD, 30 m × 0.25 mm I.D., 0.18 μm film thickness) from BGB Analytik (Adliswil, Switzerland) was used as chiral column. In the case of the heart-cut MDGC system it was placed in the second dimension (main column) while a DB-5 (30 m × 0.25 mm, 0.25 μm film thickness; J&W Scientific, USA) was used as pre-column. In the case of the GC × GC experiments, the chiral column was installed as first dimension while three non-enantioselective columns with different polarity were tested as second dimension columns: a 2 m HT-8 (8% phenyl polysiloxane-carborane) and 2 m BPX-50 (50% phenyl polysilphenylene siloxane) both from SGE (Darmstadt, Germany) and a 1 m Supelcowax-10 (polyethylene glycol) from Supelco (Bellefonte, USA), all of them 0.10 mm I.D., 0.10 μm film thickness. Fish oil samples were purchased from supermarkets in Madrid (Spain).

GC × GC analyses were performed on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, USA) equipped with a μECD and a cryogenic loop modulator KT2003 (Zoex Corporation, Lincoln, Nebraska). Details of the loop modulator principles have been described elsewhere⁵. Columns were connected to the

modulator loop via mini press-fits (Techrom, Purmerend, The Netherlands). A secondary oven was installed inside the main oven, hosting the second dimension column. Helium (99.999%, Praxair, Madrid, Spain) was used as carrier gas in the constant flow mode at an initial head pressure of 310 kPa. Manual injections were performed in the splitless mode (1 μ L; splitless time, 0.75 min) at 250 °C. The μ ECD was maintained at 300 °C and nitrogen was used as make-up gas at a flow rate of 150 mL/min. The modulation period was set at 6 s with a 200 ms hot jet pulse duration. The temperature program for the first dimension (*i.e.* BGB-172) was: 90 °C (2 min), at 25 °C/min to 190 °C (2 min), at 2 °C/min to 210 °C (27 min) and at 10 °C/min to 240 °C. The second-dimension oven was programmed to track the main oven in the case of HT-8 and Supelcowax-10. When using BPX-50, a temperature off-set of 20 °C between both ovens was applied and the temperature program was as follows: 210 °C (8 min), at 2 °C/min to 230 °C (27 min) and at 10 °C/min to 260 °C. The temperature of the hot jet heater had an offset of 80 °C over the temperature of the main oven during the whole chromatographic run. Data acquisition rate was set at 50 Hz. ChemStation software was used for acquiring the raw data, which was further exported as comma separated values to the GC Image v1.4 program (University of Nebraska, Lincoln, USA) for further data analysis. Peak volumes were manually integrated.

For the heart-cut MDGC analysis the system used consisted of two GC systems (Varian CP3800, Varian Iberica, Spain), each one equipped with an ECD. A Deans switching system, placed in the first oven, was used to transfer the selected fractions containing the compounds of interest. The transfer-line ($T=280$ °C) was directly connected to the main column using a press-fit connection. Manual injections (1 μ L) were performed at 270 °C in the splitless mode (splitless time, 1 min). Monitor ECD was maintained at 300 °C, while the temperature of the detector placed after the main column was 250 °C. Nitrogen was used as carrier gas at 209 kPa column head pressure. Oven temperature programs were as follows: pre-column (DB-5): 80 °C (1 min), at 30 °C/min to 185 °C (3 min), at 1.9 °C/min to 234 °C (25 min), at 2 °C/min to 270 °C. Main column (BGB-172): 90 °C (1 min), at 15 °C/min to 170 °C (25 min), at 2 °C/min to 180 °C (30 min), at 2 °C/min to 210 °C.

Results and discussion

GC \times GC column selection

Up to now, enantioselective stationary phases based on *tert*-butyldimethylsilyl β -CD have provided the best results for the separation into enantiomers of toxaphene congeners⁶. In fact, BGB-172 is able to resolve into enantiomers the five toxaphene congeners studied, namely Parlar 26, 32, 40, 44 and 50. Nevertheless, overlapping between the first enantiomer of Parlar 32 and the first enantiomer of Parlar 50 was observed. In the case of the GC \times GC experiments, three capillary non-enantioselective columns with different polarity were evaluated as second dimension in combination with BGB-172: HT-8, an apolar column used mainly for the separation of PCBs, BPX-50, a semi-polar phase, and the polar Supelcowax-10, which has shown to be also a good alternative for PCB separation in GC \times GC⁷. All three column combinations adequately solved the above-mentioned co-elution between Parlar 32 and 50. Nevertheless, the use of Supelcowax-10 as second dimension column was discarded because partial (for Parlar 26, 40 and 50) or total (Parlar 32) degradation was observed for some congeners⁸. For the two column combinations assayed, BGB-172 \times HT-8 and BGB-172 \times BPX-50, some bleeding from the enantioselective stationary phases was observed when the temperature program reached a value close to the maximum operation temperature of this enantioselective column (240 °C). When using HT-8 as second dimension and with the above-mentioned experimental conditions, Parlar 26 eluted close to this band, a fact that could hinder its determination especially when dealing with low concentrations in complex matrices. In the case of BPX-50, all enantiomers eluted far away from the bleeding band. Therefore this column combination, BGB-172 \times BPX-50, was selected for further analysis. Satisfactory repeatability and reproducibility of the retention times in both the first and the second dimension were observed for all target compounds (RSDs below 0.8%, $n=4$). Linear responses in the tested range of 10-200 pg/ μ L and limits of detection in the range of 2 to 6 pg/ μ L were obtained.

EF determination by heart-cut MDGC and GC \times GC

The enantiomeric fraction (EF), defined as the area of the first eluted enantiomer (E1) divided by the sum of the area of the first and second eluted enantiomer (E2), $EF = E1/(E1+E2)$, was used to calculate the enantiomeric composition of the selected toxaphenes in the fish oil samples. In the case of the GC \times GC determinations peak

volume was used instead of peak area. The repeatability and reproducibility at a concentration of 100 pg/ μ L, evaluated as the RSDs of the enantiomeric fraction (EF), was better than 11% ($n = 4$) in all instances.

In the GC \times GC contour plots, all detected congeners, except Parlar 26, eluted in the latter part of the chromatogram, well separated from the rest of analytes present in the extract. Parlar 26 eluted in the middle part where a large number of analytes also eluted (**Figure 1**). Most of the congeners showed a racemic composition (EF Parlar 26 = 0.54-0.57, EF Parlar 44 = 0.43-0.60, EF Parlar 50 = 0.46-0.49) while for Parlar 40, an apparently clear enrichment of the first eluting enantiomer could be observed as the second eluting enantiomer was found to be below the LOD in all samples. However, a detailed inspection of the chromatogram revealed a possible coelution of the last eluting enantiomer of Parlar 40 with another compound present in the extract.

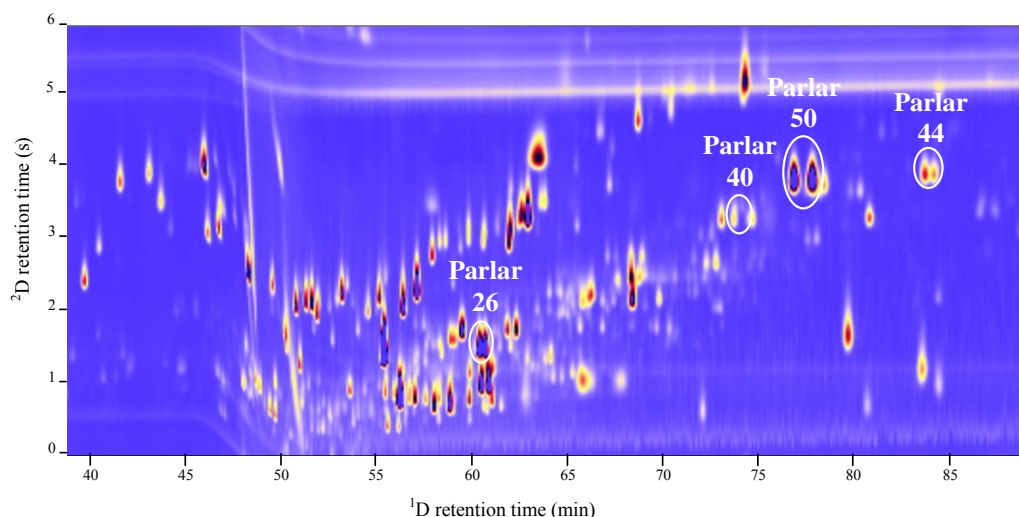


Figure 1. GC \times GC contour plot of one of the fish oil samples analysed.

When the same extracts were analysed by heart-cut MDGC, the toxaphene congeners were separately transferred to the enantioselective column by four consecutive injections of the extract. The results from the EF calculation using MDGC agreed with the racemic composition obtained by GC \times GC in the case of Parlar 26 (0.48-0.50), Parlar 44 (0.52-0.61) and Parlar 50 (0.49-0.52). For Parlar 40, similarly to that observed by GC \times GC, some tailing was observed in the second eluting enantiomer of Parlar 40 which could be associated to a coelution with another compound present in the extract. When using an enantioselective 30% *tert.*-butyldimethylsilyl β -CD column, the enantiomers of Parlar 40 have been reported to be surrounded by the enantiomers of Parlar 41⁶. In this case, the two peaks surrounding Parlar 40 could tentatively be assigned to the enantiomers of Parlar 41, being the tailing observed for the second eluted enantiomer of Parlar 40 the coelution with the second enantiomer of Parlar 41 (**Figure 2**). In addition, Parlar 40 and Parlar 41 have been reported to coelute in DB-5 type columns⁴, a fact that supports this statement and would explain why the coelution observed in GC \times GC was not solved when using heart-cut MDGC. Nevertheless, because of the complexity of the extracts investigated, possible coelution with a different compound present in the matrix cannot be ruled out either.

The number of studies dealing with the determination of the enantiomeric composition of toxaphenes in fish and fish oil is limited and with somehow differing results. While Parlar *et al.* reported a close to racemic composition for Parlar 26, 44, 50 and 62 in different fish species⁹, Angerhöfer *et al.* found enantioenrichment for Parlar 44 and 62 in fish and cod liver oil, while no deviation from the racemic value was observed for Parlar 50¹⁰. Changes in the enantiomeric composition are not well understood yet and further investigation in this direction may be recommended.

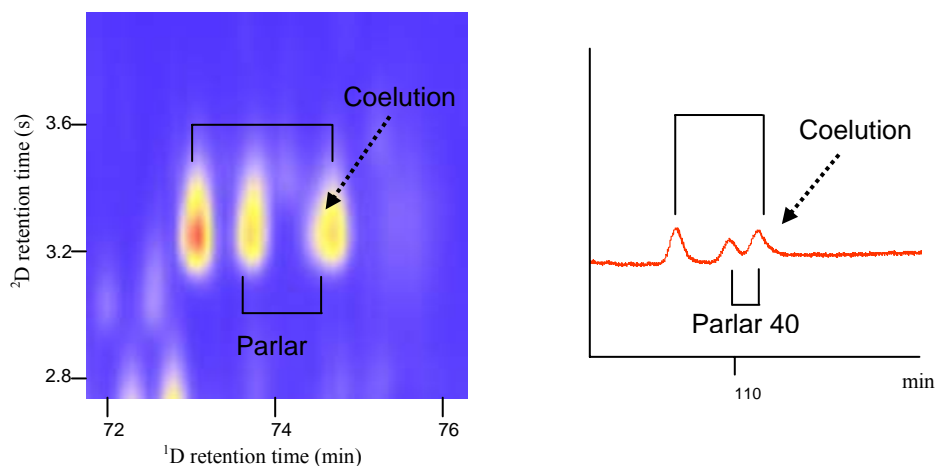


Figure 2. Detail of the elution profile of Parlar 40 in one of the fish oil samples obtained by (a) GC \times GC- μ ECD (column combination BGB-172 \times BPX-50) and (b) heart-cut MDGC (column combination DB-5 – BGB-172).

In conclusion, a GC \times GC- μ ECD method using the enantioselective BGB-172 column in the first dimension and a medium polar column in the second dimension, BPX-50, has been developed and applied to the determination of the EF of some of the most abundant chiral toxaphenes congeners in environmental samples (Parlar 26, 32, 40, 44 and 50). Its feasibility for real-life analysis has been demonstrated by determining the EF in commercially available fish oil samples and successful comparison of the results with those obtained by heart-cut MDGC using the same enantioselective column. Just in the case of Parlar 40, coelution with another compound present in the extract cannot be ruled out, a fact that could prevent from accurate determination of the enantiomeric ratio for this particular congener in this type of extracts, also when using heart-cut MDGC.

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