

Application of Multidimensional (Heart-Cut) Gas Chromatography to the Analysis of Complex Mixtures of Organic Pollutants in Environmental Samples

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Abstract: This contribution presents some applications developed in our laboratory of multidimensional (heart-cut) gas chromatography (MDGC) applied to the analysis of complex mixtures of pollutants in environmental samples. Practical aspects of this technique as well as qualitative and quantitative examples are discussed. Determination of trace levels of pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) with MDGC-ECD/ECD and MDGC-ECD/MS in fish, mussels, or sewage sludge is presented.

Keywords: Environmental pollutants · Heart-cut · Multidimensional gas chromatography · PBDEs · PCBs

Introduction

The generalization of using capillary columns remarkably enhances resolution in gas chromatography. Recent development of this technique offers to the users a wide range of column phases, lengths and internal diameters enabling almost all problems of identification of different analytes in various matrices to be overcome. However, single column chromatographic techniques cannot always provide sufficient separation of all components of organic pollutants in complex mixture, especially at trace levels. Therefore, for good laboratory practices in trace residue analysis, it is mandatory to work with at least two columns with different polarities. This will enable the isolation of some compounds from a group (one peak) but there is a risk of regrouping oth-

ers which may have been previously separated.

In order to enhance peak capacity, multidimensional gas chromatography (MDGC) was proposed many years ago. Marriott and Shellie [1] define multidimensional analysis in chromatography as 'any technique that combines two or more distinct separation/analysis steps, where at least one of the steps or dimensions involves a chromatographic separation'.

Today, there are two kinds of MDGC: conventional or 'heart-cutting' MDGC and 'comprehensive' two-dimensional gas chromatography (GCxGC).

In the *heart-cutting MDGC technique*, one or more unresolved fractions from a first column (first dimension) are transferred to a second one having a different polarity (second dimension) where the separation of the compounds will be achieved. In general, the first column is non-polar with a length of 30 to 60 meters, while the second is 30 meters long with a higher polarity. The heart-cut can be directly transferred to the second column or it can be trapped on a cryogenic device and transferred later. Thus, it is possible to enrich the trap with many heart-cuts of the same analyte coming from sequential injections.

In *comprehensive two-dimensional gas chromatography (GCxGC)*, the entire

sample, and not only fractions, are separated on two different columns. The columns are shorter, typically 15 to 30 meters for the first and only 1 to 2 meters for the second. The short length of the second column will enable very fast separations while collecting the fractions from the first column. The most important component of the system is the 'modulator', which will accumulate the fractions coming from the first column on a short segment of column, and then release it quickly into the second one. There are different kinds of modulators but in general the mechanisms involve alternate cryofocusing and thermal desorption of the analytes trapped [1].

Due to the very fast separations, GCxGC also needs very fast detection systems. Generally used are fast-FID (flame ionization detector), micro-ECD (electron capture detector) and TOF-MS (time-of-flight mass spectrometer). Finally, it is important to have a good software program to treat the data generated by the system.

Lack of rapid detection systems and of appropriate software for data treatment are the main inconveniences of this technique [4].

Extensive reviews on the principles and applications of MDGC and GCxGC have been recently presented by different authors [1–7].

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MDGC Technique

Despite the great interest and possibilities of comprehensive MDGC (GCxGC), this technique has not yet been widely introduced in analytical laboratories for the reasons stated above. In our laboratory, we started almost ten years ago to work with the conventional heart-cutting system. The techniques used will be described below in more details.

Basically, to perform MDGC, one GC oven, two columns, two detectors and one switching system are needed. The disadvantage of this simplest configuration is that if a temperature program is needed to separate different peaks of one sample, the program will be the same for both columns, losing peak capacity. To solve this problem one can install a cryogenic trap inside or outside the GC. In this case, analytes will be trapped there for some time and will be released later to the second column.

Another possibility is to work with two independent ovens. One GC with two ovens can be used (for example the well-known Sichromat 2-8 from Siemens, unfortunately no longer manufactured). Or, like in our laboratory where two GCs are connected together by means of a heated transfer line. When working with two ovens, it is not necessary to use a cryogenic trap. In this case, the heart-cut from the first column is sent to the second oven where the temperature is lower and then the analytes will be trapped at the beginning of the column until the temperature program starts.

In all cases the most important part is the switching system, which will transfer the analytes from one column to the other. The most widely employed systems today are the mechanical valves and the DEANS valveless pressure switch (named after its inventor, D.R. Deans, around 1981).

Both switching systems are commercially available. Valves could present problems such as dead volumes, sample adsorption and bad resistance to high temperatures as well to the heating-cooling process if installed inside the GC oven (leaks). If possible, the valve should be placed in a vacant detector oven. Valves are easy to use and relatively cheap.

The valveless switch eliminates these problems but it is more time consuming to fit. The flow direction during the heart-cut in the DEANS three 'live T-pieces' is caused by pressure changes on the different 'Ts', and for this reason, many pressure adjustments are necessary before being operational. As for the second dimension the total length of columns is the addition of both columns, *i.e.* 80 to 90 meters, the flow will be lower than for the first dimension, which

only has a column of 50 to 60 meters. To balance this, we need to add to the column of the first dimension an empty column with the same internal diameter and with a length of 25 to 30 meters.

The column switching systems are generally controlled by the GC itself (on-off relay) or by external modules like the one proposed by SGE International, the MDS series for one GC [8]. The need for an external device will enhance the cost of the whole system.

It is also possible to work with two completely independent chromatographic systems. In order to increase the amount of analytes, Glausch *et al.* trapped the cut of three different injections from a GC-ECD on a cooled piece (50cm) of a capillary column, and transferred it afterwards to a GC-MS to perform the detection, coupled with the analytical column [9].

No specific detectors are needed to perform heart-cutting MDGC. The most frequently used detectors are standard FID, ECD, and MS.

However, to perform good MDGC, organic extracts need to be clean, otherwise impurities will induce changes in the retention time, and it will be difficult to correctly program the 'window' for the cut-on and cut-off time. Internal standards can be used for the correction of cutting times and for the correction of detector responses when sent to the second dimension with an analyte.

Heart-Cutting MDGC at the CECOTOX Laboratory

MDGC-ECD/ECD

A scheme of our equipment is shown in Fig. 1a. In order to take advantage of the existing equipment in our laboratory, we chose to connect two GCs by means of a heated transfer-line. With technical help from Varian Inc (Zug, Switzerland) [10], we put together a Varian 3400 (first column) equipped with a pneumatic six-way VALCO valve and a Varian 3300 (second

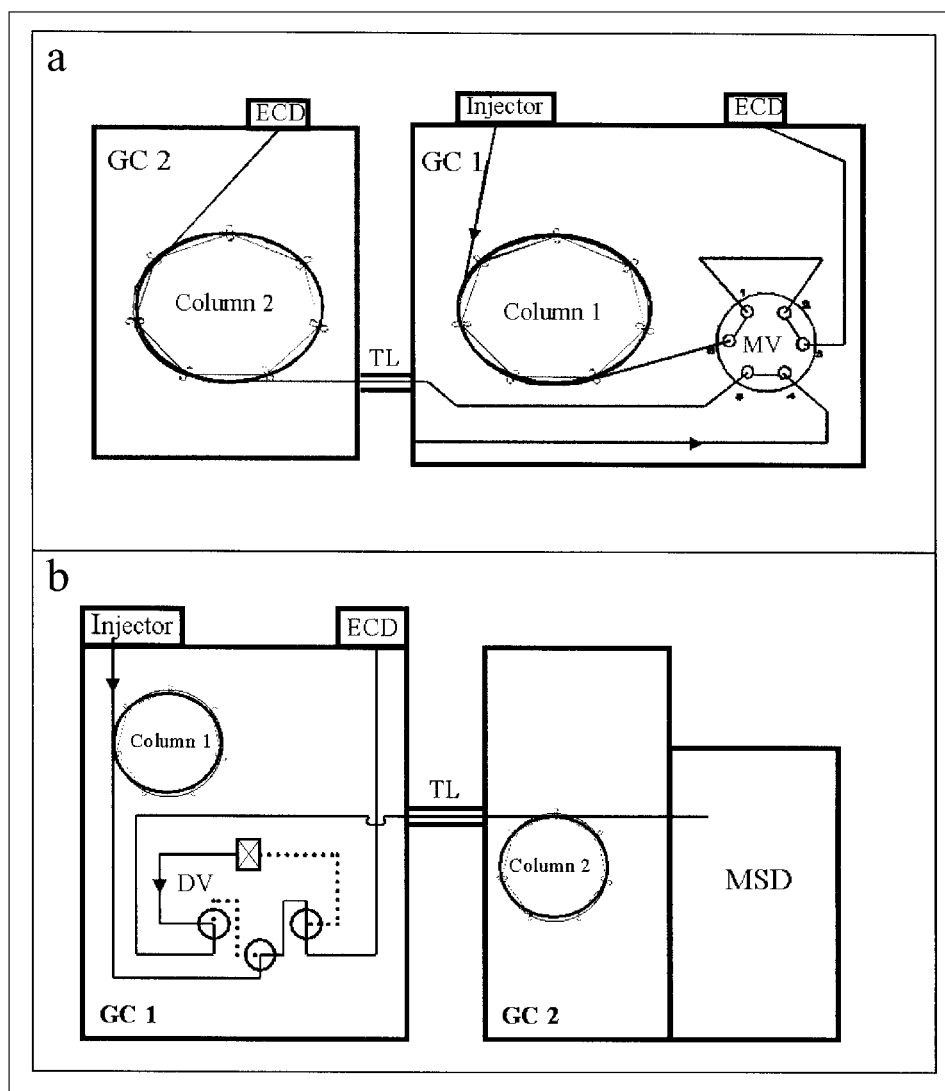


Fig. 1. Schematic representation of MDGCs: a) MDGC-ECD/ECD and b) MDGC-ECD/MSD. (MV: mechanical valve, DV: DEANS valveless switch, TL: transfer line).

column). Both GCs were equipped with ECD detectors. The instrument keyboard of the Varian 3400, without the help of any specific software, controlled both GCs. Later on, we coupled the MDGC to a computer, which then controlled the whole system. With such a system both GCs can work alone, if necessary, by simply disconnecting the columns from the switch valve.

General working conditions are presented in the Table.

This configuration is relatively easy to install. To maintain some equilibrium of the carrier gas pressure between the cuts, one meter of an empty capillary column was installed in the valve, which works like a restrictor.

The weak point of the system is the valve itself. Although the rotor material (polyamide/PTFE/carbon) is inert and the contact time very short, slight adsorption occurs and peaks from the first dimension are broader at base line than under normal conditions. Moreover, as the valve is inside the oven, leaks can occur with time, caused by the heating and cooling process during the temperature program.

But, as shown in the next chapter, the heart-cutting times are precise and the system gives satisfactory results.

MDGC-ECD/MS

More recently, we acquired a new MDGC equipment from Varian Inc. Two 3800GCs were connected together; a scheme of the equipment is shown in Fig. 1b. The first GC is equipped with a FID and an ECD detector. The second is directly connected to a 1200L mass spectrometer detector (quadrupole). The switch is a DEANS valveless system. As explained before, this switch was more time consuming to adjust, the problem being the influence of the vacuum produced by the MS on the columns. To obtain the same sensitivity for organohalogenated compounds as observed with ECD, it is necessary to work with NCI-SIM detection. Details of the working conditions are presented in the Table.

With this configuration, unknown peaks from FID or from ECD runs can be easily sent to the MS and can be identified if the amounts are sufficient enough to be detected.

An additional advantage of MDGC in MS analysis is that, with the heart-cut system, only very small amounts of the extract are sent to the MS detector, avoiding contamination of the system with impurities or other pollutants present in the extract.

Application of MDGC to 'Real-Life' Analysis

Despite the great potential of this technique in the analysis of mixtures of organic substances, it seems that the applications of MDGC in routine laboratory analysis are still limited (the only exception might be petrochemical laboratories).

In the case of our laboratory, which performs analysis of pollutants at trace levels in environmental samples, MDGC helps us to solve problems linked to the analysis of complex mixtures of pollutants such as, for example, organohalogenated pollutants. Some examples of applications are presented below.

Determination of Chlorobiphenyl Congeners in Environmental Samples with MDGC-ECD/ECD

There is no single capillary column able to separate all individual PCB congeners (209). Only a few congeners are toxic and are therefore surveyed for human or ecosystem protection. Toxic congeners often overlap with peaks of other congeners or other significant coextracted interferences. GC-MS cannot be used to distinguish co-eluting congeners with the same

Table. General characteristics of the two MDGC systems presented in this work

	System 1 (MDGC - ECD/ECD)		System 2 (MDGC - ECD/MS)	
	Varian 3400 Cx	Varian 3300	Varian CP 3800	Varian CP 3800
Injector	Auto sampler - SPI 85 °C, 0.2 min, 100 °C/min to 250 °C for 98min	None	Auto sampler - SPI 85 °C, 0.1 min, 150 °C/min to 250 °C,	None
Column type	Rtx-5 (5% phenyl-95% methyl polysiloxane)	DB-17 (50% phenyl-50% methyl polysiloxane)	CP Sil-8 (5% phenyl-95% methyl polysiloxane)	CP Sil-24 (50% phenyl-50% methyl polysiloxane)
Column dimensions	60 m × 0.25 mm × 0.25 µm	30 m × 0.25 mm × 0.25 µm	60 m × 0.25 mm × 0.25 µm	30 m × 0.25 mm × 0.25 µm
Detector / Make-up gas	ECD 350 °C Nitrogen	ECD 350 °C Nitrogen	ECD 350 °C Nitrogen	MS 1200L (quadrupole) EI 70ev, 200 °C. T.-L. 250 °C. SIM M/Z 358/360/362
Valve type	6 ports valve (VALCO) Rotor Valcon T (max. 300 °C) polyimide/PTFE/carbonate	-	DEANS Switching system	-
Carrier gas	Helium, 24 cm/sec	Helium, 24 cm/sec	Helium, 26 cm/sec	Helium 26 cm/sec
Transfer line	150 °C isothermal	-	280 °C isothermal	-
Oven temperature programs (PCBs)	80 °C for 30 sec, then 50 °C/min to 150 °C for 1 min, then 2.5 °C/min to 280 °C for 45 min	150 °C for 60 min, then 4 °C/min to 280 °C for 25 min	80 °C for 30 sec, then 50 °C/min to 150 °C for 0 min, then 5 °C/min to 280 °C for 42 min	150 °C for 40 min, then 10 °C/min to 280 °C for 17 min
(PBDE)	80 °C for 30 sec, then 30 °C/min to 200 °C for 1min, then 10 °C/min to 300 °C for 65 min	150 °C for 35 min, then 15 °C/min to 280 °C for 36 min		
(o,p'-DDT)	80 °C for 30 sec, then 50 °C/min to 150 °C for 1 min, then 2.5 °C/min to 280 °C for 33 min	(Chiral column) 80 °C for 45 min, then 50 °C/min to 160 °C for 0 min, then 3 °C/min to 260 °C for 9 min.		

chlorine number (isomers), as the MS spectra of these are identical or very similar. Even if one co-eluting compound has a different number of chlorines than another, but is present at a very different concentration, identification will be difficult due to the presence of the less concentrated one [2]. Therefore, working with at least two columns with different polarities is necessary for the correct identification of the CBs.

Our lab has been participating since 1990 in the certification of reference materials for the European Bureau of Reference (BCR, European Union). In the case of PCBs, the labs are asked to analyze twelve CB congeners. We used our MDGC-ECD successfully in the certification of CB congeners IUPAC no. 28, 52, 101, 105, 128, 118, 138, 149, 153, 156, 170, and 180 in matrices like fish (BCR 718) and mussels (BCR 682). Moreover, we also participated in the certification of the most toxic non-ortho (planar) CBs, IUPAC no. 77, 81, 126, and 169 in a chub (fish) sample (BCR 719).

In almost all cases and for all congeners our results were in very good agreement with those from the other participants, who were among the most skilled labs performing PCB analysis in Europe.

MDGC was able to show the presence of co-eluting congeners or non-identified substances in one peak. This could explain the differences between the results of some participants, depending on the conditions used for identification and quantification.

An example is presented in Fig. 2, where we performed two heart-cuts for two CB identifications. Heart-cut 1 showed the presence of congener CB 167 and CB 128, and heart-cut 2 separated congeners CB 202 and CB 171 from CB 156.

Repeatability of the heart-cuts was very good. Standard mixtures of twelve PCB congeners injected four times over night by the autosampler, during a sequence of samples, showed a standard deviation (SD) for retention time of between 0.01 and 0.17%. Peak area presented a SD between 1.8 and 11.5% depending on the congener.

Practically, for the certification of twelve CBs in a biological matrix such as herring, for example, we injected the extract three times and we performed four heart-cuts each time. Typical heart-cut windows were between 10 and 20 sec. Total time for one analysis was 300 min. In comparison, injection on a single column will take 90 min.

Determination of Non-Ortho CBs in Fish With MDGC-ECD/ECD

The most impressive performance of our system was the certification of non-or-

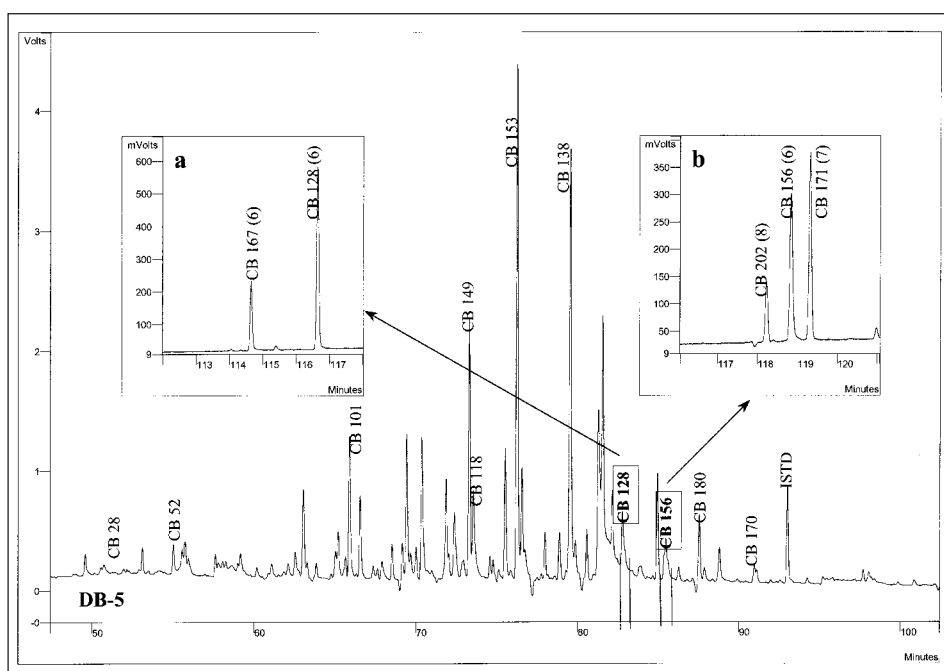


Fig. 2. Chromatogram of a mussel extract on a DB-5 column and heart-cuts of peaks corresponding to CB 128 (a) and CB 156 (b) from the DB-17 column. (For conditions see Table. In brackets the number of chlorine atoms).

tho CBs in fish material. These congeners are very difficult to determine because of possible interferences with other CBs and because the expected levels of contamination were very low. For that reason, the eleven other participants in the certification used GC-MS for the identification and detection. CB 169 was certified at a concen-

tration as low as 1.8 ± 0.23 ng/kg. Fig. 3 presents the chromatogram of the heart-cut of the four non-ortho CBs certified in the fish extract.

MDGC is recommended as the most suitable technique for direct determination of CBs without a preparation/fractionation of non-ortho CBs from other CBs [2].

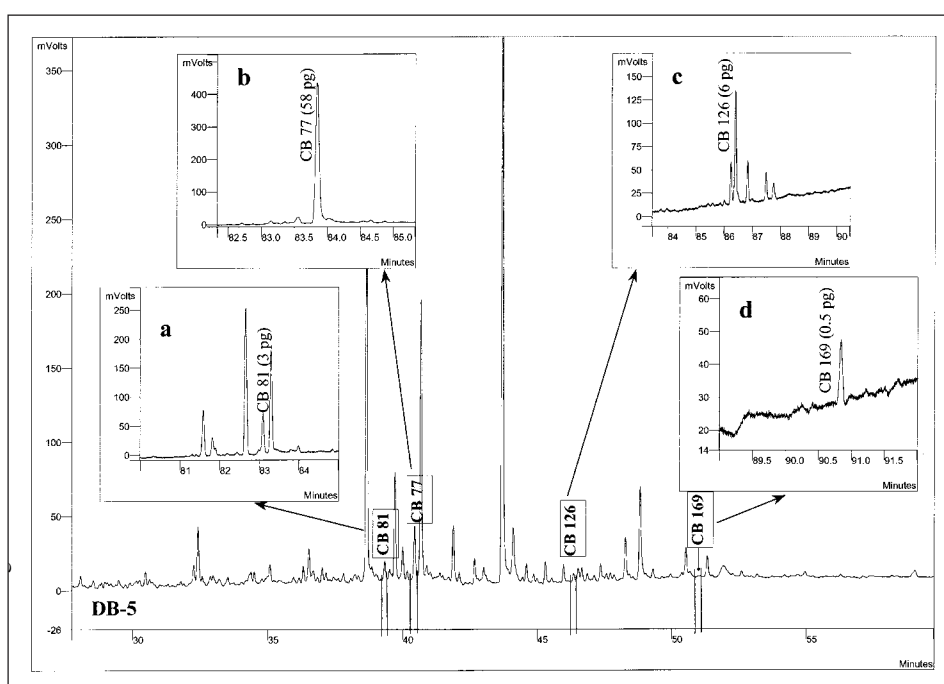


Fig. 3. Chromatogram of a fish extract (chub) on a DB-5 column and heart-cuts of peaks corresponding to planar-CBs 77, 81, 126 and 169 from the DB-17 column. (For conditions see Table. In brackets, injected amounts).

Determination of PBDEs Congeners in Environmental Samples with MDGC-ECD/IED

At the beginning of 2003, our laboratory participated in the feasibility study for the certification of eleven congeners of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) in a fish (flounder) homogenate and in a marine sediment, organized by the European Bureau of Reference (BCR). The PBDE congeners to be determined were no. 28, 47, 49, 66, 85, 99, 100, 153, 154, 183, and 209. The less brominated congeners have almost the same retention time as the highly chlorinated biphenyls congeners, and co-elutions are possible. Therefore, it is important to separate them well before identification by GC, especially when using an ECD.

Our results were in good agreement with those from other laboratories, especially those working with MS detectors.

Repeatability of the heart-cuts was very good. Standard mixtures of eight PBDE congeners injected four times over night by the autosampler during a sample sequence showed a standard deviation (SD) of retention time comprised between 0.01 and 0.03%. Peak area presented a SD between 2.5 and 8.6% depending on the congener.

Another example of the usefulness of MDGC in PBDE analysis is presented in Fig. 4, where we can see the DB-5 chromatogram of a Swiss sewage sludge extract and two heart-cuts corresponding to windows of peaks BDE-28 and BDE-49. Figs 4a and 4b show that interferences correspond to PCB congeners.

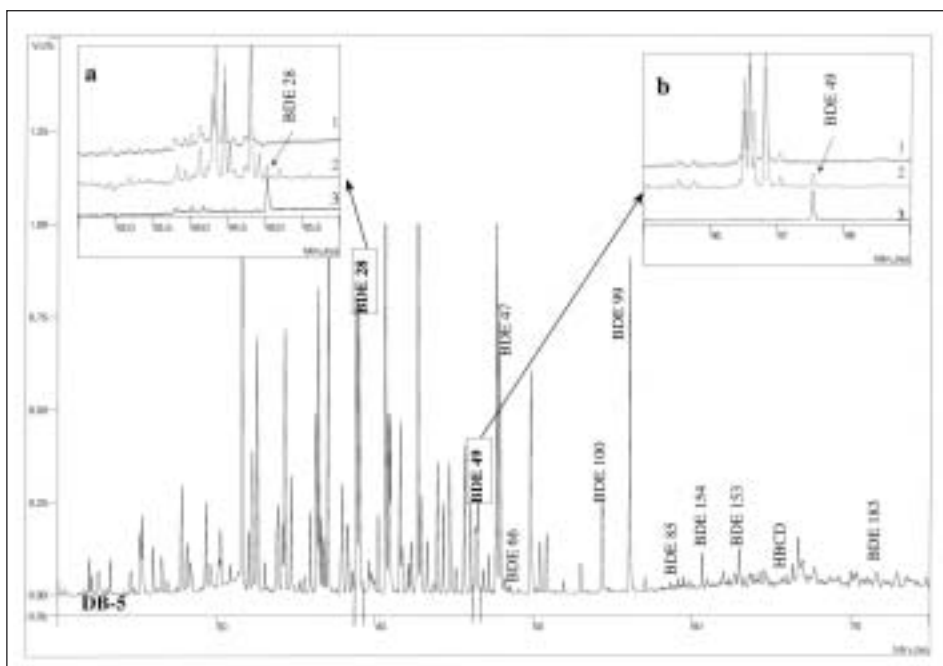


Fig. 4. Chromatogram of a sewage sludge extract on a DB-5 column and heart-cuts of peaks corresponding to PBDE 28 (a) and PBDE 49 (b) from the DB-17 column. For comparison: 1. Mixture of commercial PCB, 2. sludge extract, 3. BDE 28 or 49 standards. (For conditions see Table).

Determination of *o,p'*-DDT Enantiomers in Fish Samples by Chiral Separation and MDGC-ECD/IED

In 1996, Ceschi *et al.* showed that fish from lake Maggiore (Verbano), on the border between Switzerland and Italy, were highly contaminated by DDT (isomer and metabolites) [11]. Following this study, we investigated the enantiomeric ratio (ER) of *R*-(-)-*o,p'*-DDT and *S*-(+)-*o,p'*-DDT in two species of fish and in sediments from the lake [12]. The (-)-enantiomer is more estrogenically active than the (+)-enantiomer [13].

For this work, the first column of the MDGC-ECD was a DB-5 (60 m × 0.25 mm × 0.25 μm) and the second one a BGB 172 (OV1701 with 20% of tert-butylidimethylsilylated β-cyclodextrine, 30 m × 0.25 mm × 0.25 μm). A chromatogram of the chiral separation of the *o,p'*-DDT heart-cut from a whitefish (*Coregonus sp.*) sample is presented in Fig. 5.

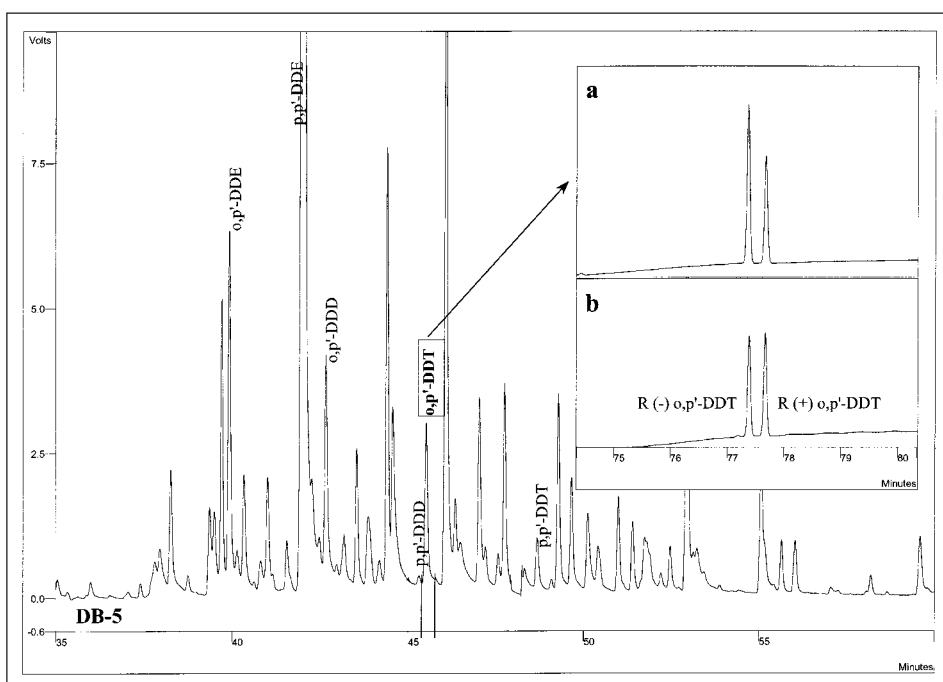


Fig. 5. Chromatogram of fish extract (*Coregonus sp.*) on a DB-5 column and heart-cut of the peak corresponding to *o,p'*-DDT from the chiral column (a). A heart-cut of the standard technical *o,p'*-DDT is also presented for comparison (b). (For conditions see Table).

This preliminary study showed that in sediment and in tissues of aloses (*Alosa fallax lacustris*) the ER is not different from that of *o,p'*-DDT in technical mixtures, *i.e.* close to 1 (ER = 0.97, n = 18, for fish). However, in whitefish, ER was higher than 1 (ER = 1.5, n = 7) indicating that this fish accumulates preferably *R*-(-)-*o,p'*-DDT or degrades preferably *S*-(+)-*o,p'*-DDT. This shows that processes of accumulation and degradation are not the same between species.

Determination of Chlorobiphenyl (CB) Congeners in Environmental Samples with MDGC-ECD/MS

As previously stated, MS cannot distinguish two isomers co-eluting in the same peak. One of the PCB congeners that must be surveyed in almost all analysis for legal purposes is the hexachlorobiphenyl CB 138. However, it is impossible to separate CB 138 from another hexa-CB, no. 163, with the current and widely used DB-5-like columns.

Fig. 6 shows an MDGC-ECD chromatogram from a fish extract (herring) with a CP-Sil-8 column and the heart-cut from the MS with a CP-Sil 24. The two CBs are well separated.

Again, repeatability of the heart-cuts was very good. Heart-cuts of the windows corresponding to CBs 163 + 138 from five different injections of the sample extract showed a standard deviation (SD) of the retention time of 0.02% for CB 163 and 0.03% for CB 138. Peak area presented a SD of 4.1% for CB 163 and 3.6% for CB 138.

General Discussion

As the examples given above show, two-oven heart-cut MDGC is a very robust and powerful technique. With new progress in the 'comprehensive MDGC' technique, the possibilities of multidimensional gas chromatography will improve even more.

MDGC (two GCs) is commercially available (custom made) from some manufacturers, such as Varian Inc. Moreover, it is not difficult and relatively inexpensive to adapt existing equipment to perform heart-cut MDGC.

MDGC might be more time consuming than traditional one-dimensional gas chromatography, mainly in the beginning. Valve maintenance can be one of the weak points, because working temperatures in the oven (up to 285 °C) are very high. For applications which do not need high temperatures, problems will be probably less important.

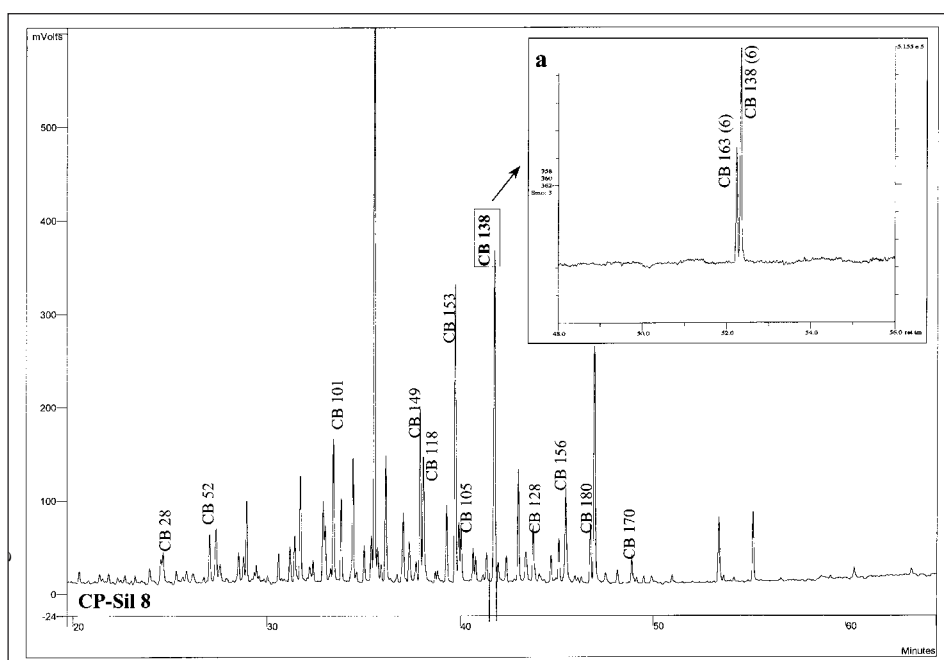


Fig. 6. ECD chromatogram of a fish extract (herring) on a CP-Sil 8 column and heart-cut of the peak corresponding to CB 138 from the CP-Sil 24 column of the MSD (a). (For conditions see Table. In brackets the number of chlorine atoms).

Working with the DEANS switching system will certainly be a better alternative.

Our experiences and results with 'real-life' samples show that this technique is precise and reliable, giving results comparable or better than traditional techniques.

But surprisingly, this technique has not yet been adopted by a larger number of laboratories. Among all the laboratories participating in the presented BCR/EU reference materials certifications, only our lab presented results using MDGC. Other labs had access to MDGC, but for unknown reasons preferred to present their results with traditional one-dimensional GC systems.

Finally, as our results show, MDGC is surely a good technique to help the analytical chemist to solve easily problems when analyzing complexes mixtures of environmental pollutants.

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