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Real-time controlled multidimensional gas chromatography with electronic pressure control: application to chlorobiphenyl analysis

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Abstract

Multidimensional gas chromatography was applied to the quantitative determination of planar chlorobiphenyls (PCBs) in commercial PCB mixtures. The instrumental setup is based on real-time controlled column switching in which the heartcuts are elucidated through retention indices calculated by linear extrapolation from the retention times of *n*-alkanes as reference compounds. Electronic pressure control providing pressure programming was used to adjust the pressure at the injector and at the mid-point of the column tandem.

1. Introduction

The high resolving power of multidimensional gas chromatography (MDGC) is based on the large peak capacity, n_c, which results from the transfer of an unresolved fraction from the first column to a second column of different selectivity [1]. The resolving power of an MDGC system resembles the selectivity of gas chromatography–low resolution mass spectrometry [2].

Selectivity in MDGC is inversely proportional to the width of the fraction transferred to the second column [3,4]. Thus, the peak capacity in conventional MDGC is often significantly less than optimum due to the relatively broad heartcuts needed to eliminate small variations in absolute retention times of peaks to be transferred. Real world samples can cause harmful inaccuracies in absolute retention times like for example by the occurrence of large and small peaks. Also aging of the first column reduces reproducibility of retention times. Regular calibrations are therefore needed in conventional MDGC to ensure precise heartcuts.

A computer method based on indices (*I*) instead of on retention times has recently been developed to overcome these problems and to enable reliable quantitation while exploiting all the selectivity a multidimensional high-resolution gas chromatograph can offer [5]. In this system reference compounds, e.g. *n*-alkanes, are co-injected with the sample. Absolute retention times of the reference compounds are used to predict the retention times of the compounds to be heartcut. This is achieved by real-time extrapolation of the time scale using retention

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indices. The details of the technique are described in Ref. [5].

Tuning of an MDGC system for a particular application is a time-consuming procedure: the pressure balance between the first column and the mid-point of the column tandem is critical and very much dependent on the dimensions of the columns applied and thus needs careful adjusting. Moreover the use of temperature programming strongly affects the pressure balance. Even flow-controlled systems fail in compensating the changes in mid-point pressure caused by a temperature gradient.

Electronic pressure control (EPC), which nowadays is available on modern GC instrumentation, was applied to program and thus to optimize the inlet pressure and the mid-point pressure during temperature programming.

Chlorobiphenyls (PCBs) are a complex group of 209 congeneric compounds, several tens of which have become environmental pollutants [6,7]. Their congener specific separation is known to be a demanding task even for high-resolution chromatographic systems [8,9]. In this contribution the system described above was applied to the separation of the most toxic coplanar chlorobiphenyl congeners from commercial PCB mixtures.

2. Experimental

A single-oven HP 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA) was modified to the multidimensional mode by an SGE Column Switching System (Scientific Glass Engineering, Ringwood, Victoria, Australia). The data system was an HP 3365 Series II ChemStation. A schematic illustration of the instrument is presented in Fig. 1.

Real-time control of the heartcutting was accomplished by a tailor-made computer program, FERRET (Fractionation by Estimation of Retention through Real-time Extrapolation of the Timescale, VTT, Espoo, Finland) [5]. Connection between the gas chromatograph and personal computer (HP Vectra 486/33N, Hewlett-Packard) was made via a DT-2802 chromatography board (Data Translation, Marlboro, MA, USA).

The pressures for column inlet and mid-point of the column tandem were controlled by two EPC units: a back pressure regulated unit was used for the splitless injector, which was operated under constant pressure mode. The mid-point pressure was controlled by a forward pressure regulated EPC unit of a cool on-column injector.

A capillary column, 25 m × 0.20 mm I.D. Ultra-1 (Hewlett-Packard), coated with a 0.11-μm film of methyl silicone was used as first column. The final separation was carried out on a 30 m × 0.18 mm I.D. capillary column coated with 0.25-μm home-synthesized polycarbonate stationary phase [10].

Retention times of the reference compounds were monitored by a flame ionization detector. The separation in the second column was recorded by an electron capture detector.

The temperatures of injector and detectors were 300 and 320°C, respectively, in all runs. Splitless time for 1-μl injections was adjusted to 1 min. Helium was used as the carrier gas. Initial inlet pressure and the pressure at the mid-point of the columns were 0.420 and 0.305 MPa at 80°C producing 25 and 42 cm/s linear carrier gas velocity on the first and second column, respectively.

The column oven temperature was programmed at 6°C/min to 270°C after a 1-min isothermal part at the initial temperature of 80°C. All heartcuts were made during this gradient. The fractions were collected in a cold trap situated at the inlet of the second column. A negative temperature gradient of 20°C/min was carried out in order to cool the columns down to 180°C, the temperature at which the fractions were re-injected into the second column. A second separation was done under a program rate of 8°C/min to the final temperature (300°C). The retention time of the last eluting analyte in the second column was ca. 53 min after sample introduction.

Alkanes having even carbon numbers and ranging from dodecane to hexacosane used as reference compounds for retention index calcula-

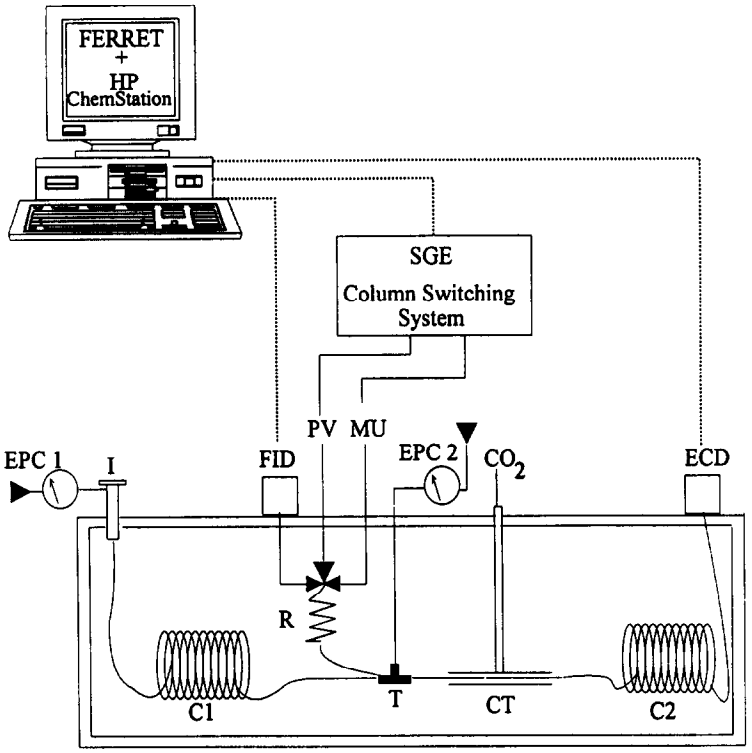

Fig. 1. Key parts of the multidimensional gas chromatograph used in the present study: EPC = electronic pressure control unit; I = injector; C1 and C2 = first and second capillary column; FID = flame ionization (monitor) detector; PV = pneumatic valve; MU = helium make up gas; R = capillary flow restrictor; T = coupling T-piece for the outlet of the first column, inlet of the second column and the restrictor capillary; CT = cold trap with liquid CO_2 cooling; ECD = electron capture (main) detector. Solid lines indicate gas tubings and dotted lines data cables.

tions were purchased from Alltech (Laarne, Belgium). Standard solutions (10 ng/μl) of non-*ortho* chlorinated 3,3',4,4'-tetrachloro-, 3,3',4,4',5-pentachloro-, 3,3',4,4',5,5'-hexachlorobiphenyl and mono-*ortho* chlorinated 2,3,3',4,4'-pentachlorobiphenyl (IUPAC nos. 77, 126, 169 and 105, respectively) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). A 10 ng/μl solution of 1,2,3,4-tetrachloronaphthalene used as internal standard and PCB mixtures Aroclor 1242, 1248 and 1260, all in 100 ng/μl concentration were purchased from the same source. All samples were dissolved in nanograde quality 2,2,4-trimethyl pentane (Rathburn, Walkerburn, UK).

Four calibration mixtures containing 1, 10, 100 and 1000 pg/μl of each congener were prepared from the individual PCB stock solutions. Tetrachloronaphthalene was added as an internal standard to the calibration mixtures and the Aroclor solutions giving a concentration of 1000 pg/μl. Finally, a mixture of *n*-alkanes was added into all solutions as retention index markers giving a concentration level of 10 ng/μl per component.

3. Results

Traditionally the pressure balancing in MDGC is, more or less, searching for a good compromise between the optimum pressures at different temperatures. MDGC instruments from different manufacturers slightly differ in details but the basic principle is the same in all cases: heartcuts producing a total transfer of the fractions throughout the temperature range require balancing of the system at the highest temperature used in the current application. This is a

Table 1
Retention indices in index units (i.u.) and heartcut widths of the planar chlorobiphenyls and the internal standard 1,2,3,4-tetrachloronaphthalene (TCN)

Compound	Interpolated I (i.u.)	Extrapolated I (i.u.)	Peak width (s)	Heartcut width (s)
TCN	1919.75	1910.14	10.2	14
PCB-77	2122.67	2112.78	9.7	12
PCB-105	2250.31	2245.64	9.4	12
PCB-126	2324.83	2313.25	9.6	12
PCB-169	2506.19	2497.53	9.0	12

Interpolated retention indices were calculated as originally published by Van den Dool *et al.* [11]. Extrapolated indices were calculated on the basis of the last two *n*-alkanes preciding each analyte [5]

drawback as the optimal carrier gas flow-rate in the first column usually requires the smallest possible backpressure at the mid-point. This problem can be overcome if pressure programming is applied.

The inlet pressure (0.420 MPa) was first chosen to give a relatively high flow-rate in the second column. The pressure at the mid-point was monitored by EPC readout while the vent line of the on-column injector was plugged. The mid-point pressure was recorded during a slow temperature program. An almost linear pressure increase of 0.020 MPa was observed through the temperature gradient. The mid-point pressure was then adjusted at initial temperature to give a total transfer of the heartcut. The change in pressure during the temperature gradient was taken into account in the final mid-point pressure program: 0.305 MPa (1 min), $8.0 \cdot 10^{-4}$ MPa/min to 0.325 MPa, the final pressure.

Retention indices of the congeners to be heartcut and the heartcut widths were first determined by running a semi-quantitative ca. 10 ppm PCB mixture also containing the *n*-alkanes as reference compounds. Peak widths defined as 6σ were estimated as an average of the widths in three runs. The heartcut widths were chosen to be ca. 1.2 times the corresponding peak width due to slight tailing most probably caused by the T-piece of the SGE system. As the widths of heartcuts were very narrow, the delay for a solute to elute from the T-piece to monitor detector, 1 s, was also taken into consideration. The obtained index values calculated by the linear extrapolation method [5] and the heartcut widths are given in Table 1.

Table 2
Concentrations of planar chlorobiphenyls and their relative standard deviations (R.S.D.) in Aroclor mixtures on the basis of four determinations expressed as % (w/w)/R.S.D. (%); results of the present study are compared with literature values [12,13]

		PCB-77	PCB-105	PCB-126	PCB-169
Aroclor 1242	Present study	0.31/0.32	0.19/1.1	0.0077/5.0	– [a]
	Ref. [12]	0.50	0.33	–	–
	Ref. [13]	0.25	0.43	0.0037	–
Aroclor 1248	Present study	0.31/0.48	1.0/0.50	0.01/0.48	–
	Ref. [12]	0.30	0.55	–	–
	Ref. [13]	0.40	1.0	0.011	–
Aroclor 1260	Present study	0.024/1.7	0.07/1.0	0.0096/1.2	–
	Ref. [12]	–	0.08	–	0.05
	Ref. [13]	0.0038	0.045	0.0004	–

[a] Not observed.

A multipoint calibration containing four concentration levels in the range of 1–1000 pg/μl was made for all analytes on the basis of duplicate injections at each concentration level. Correlation coefficients of 1.000 were obtained in each case. Measured concentrations of toxic

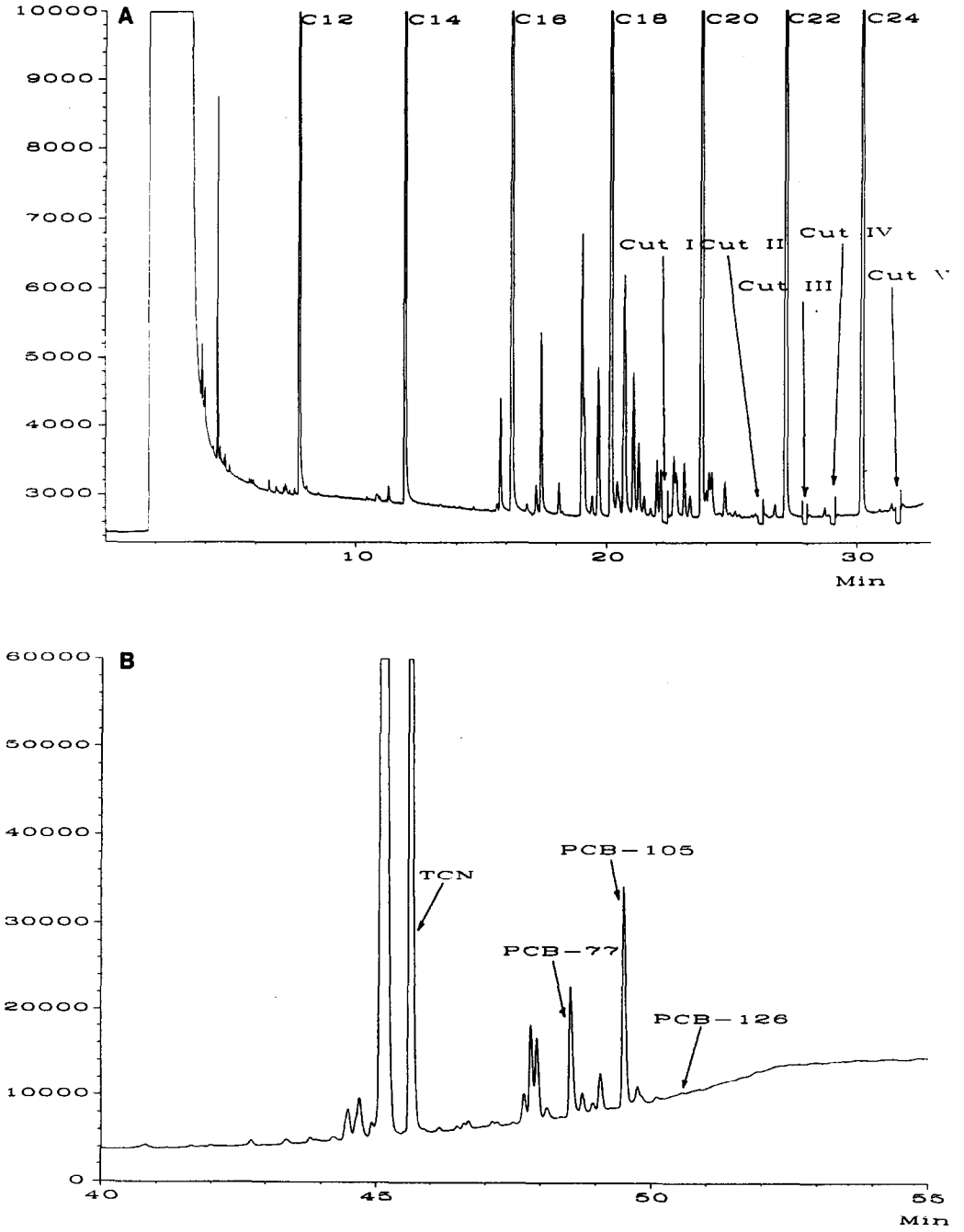

Fig. 2. Chromatograms illustrating the separation of coplanar chlorobiphenyls from Arochlor 1242 in the first (A) and second (B) column. *y*-Axis represents detector response in abundance units.

chlorobiphenyl congeners as averages of four injections are given in Table 2. Relative standard deviations (Table 2) were less than 5% for all determinations. Chromatograms illustrating the separation in the first and the second column are presented in Fig. 2. The results of the present study are well compatible with those published in Refs. [12,13] considering that some batch-to-batch variation in the composition of chlorobiphenyls is commonly known to exist. Small deviations can also be caused by the different selectivity of the polycarbonate column compared to more conventional stationary phases.

4. Discussion

Multidimensional gas chromatography utilizing real-time controlled column switching has been shown to be applicable for complex samples. The system does not need regular calibrations in order to have repeatable and reproducible heartcuts, i.e. very narrow heartcuts maximizing selectivity of the system, can be used without the danger of loosing parts of the analytes. The repeatability ($n = 47$) of retention times and retention indices was 0.15 and 0.015%, respectively. Electronic pressure control seems to be a very promising tool for multidimensional gas chromatography. For example in this study, the instrumental setup was twice dismantled and rebuilt using the original chromatographic method containing pressure settings. No recalibrations or test runs were required which was proved by running known test samples. Pressure balancing could even be automated providing an instrument with the same user-friendly characteristics as a classical gas chromatograph.

In addition to the high selectivity, the system also provides reliable compound identification as retention parameters are drawn from two columns with different characteristics. The presence of the analyte peak at the second detector indicates that the compound has a retention index in the first column within a narrow I window (in this case ca. 13 index units) corresponding to the width of the heartcut. On the other hand the absolute retention time of the analyte peak in the second column of different selectivity and calculated from the re-injection from the cold trap is an independent variable. Thus, even if the analyte peak has not been observed at the monitor detector two independent retention variables are obtained and their combination used as the basis for the compound identification is analogous to the more traditional application of two-channel gas chromatography utilizing two parallel columns of different selectivity.

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