Journal of Chromatography A, 1217 (2010) 1821–1829

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

The non-modulated transfer of total effluent for serially coupled columns in gas chromatography

Ján Krupčík[a,*], Janka Mydlová-Memersheimerová[a], Pavel Májek[a], Michal Zapadlo[b], Pat Sandra[c]

[a] Institute of Analytical Chemistry, Faculty of Chemical and Food and Technology, STU, Radlinského 9, 812 37 Bratislava, Slovakia
[b] Department of Analytical Chemistry, Faculty of Science, Palacky University, Trida 17, Listopadu 1192/12, CZ-77146 Olomouc, Czech Republic
[c] Research Institute for Chromatography, Kennedypark 26, B-8500 Kortrijk, Belgium

ARTICLE INFO

Article history:
Received 4 August 2009
Received in revised form
23 December 2009
Accepted 7 January 2010
Available online 15 January 2010

Keywords:
Serially coupled capillary columns
Non-modulated total transfer of ^{1}D effluent
Separation of PCBs
Construction of 2D and 3D images

ABSTRACT

The non-modulated transfer (NMT) of the total effluent of the separation of polychlorinated biphenyls (PCBs) on two columns coupled in series has been studied. A non-polar poly(5%-phenyl-95%-methyl)siloxane column (40 m × 100 μm × 0.1 μm) and a polar 70%-cyanopropyl-polysilphenylene-siloxane column (4 m × 0.1 mm × 0.1 μm) were used as ^{1}D and ^{2}D columns, respectively. The effluents from both the ^{1}D column and the ^{1}D + ^{2}D column series were monitored independently by two FIDs. Data from the ^{1}D and ^{1}D + ^{2}D integration reports were used to evaluate the NMT experiment. ^{1}D retention times, $t_{R,i,^1D}$, were directly accessible from ^{1}D integration report while ^{2}D retention times, $t_{R,i,B}$, were calculated for all corresponding peak pairs from ^{1}D and ^{1}D + ^{2}D integration reports as a difference $t_{R,i,^2D} = t_{R,i,^1D+^2D} - t_{R,i,^1D}$. Search for corresponding peaks of PCB congeners in the ^{1}D and ^{1}D + ^{2}D chromatograms is elucidated in detail on standard PCB samples and on PCB congeners present in the technical formulation Arochlor 1242. Both retention times ($t_{R,i,^1D}$ and $t_{R,i,^2D}$) as well as peak widths at half height ($w_{h,i}$) and peak heights (h_i) obtained from integration reports were used to construct 2D and 3D images for PCB NMT separations on serially coupled columns. The performance of the NMT procedure is illustrated by the separation of (i) standard PCB solutions, (ii) a mixture of the 209 PCBs, and (iii) a mixture of Arochlor 1242 and hydrocarbons on the DB-5 + BPX-70 column series.

1. Introduction

The serial coupling of two columns of different polarities to improve the separation of complex mixtures is a common practice in GC. The possibilities to couple two columns in series in capillary GC are dependant on the interface [1–6] and the following combinations exist (i) dual column gas chromatography, (ii) two-dimensional gas chromatography, and (iii) comprehensive two-dimensional gas chromatography.

(i) In *dual column gas chromatography*, two capillary columns of different polarities are directly coupled in series. The overall selectivity of the capillary column tandem depends on the contribution of the individual column selectivity and due to gas compressibility is a function of the column order. The overall separation selectivity of the column series can easily be tuned by changing the carrier gas flow rates in the individual columns. Serial coupled columns are the simplest approach of multidimensional chromatography. This experimental design, however, does not yield comprehensive separations as the final separation is a convolution of the used two columns' separations [1–6].

(ii) Conventional *two-dimensional gas chromatography* may be considered as a combination of two regular GC columns. In this technique one or several discrete parts of the first column effluent are directed to the second column. Because only a few selected peaks enter the second column, interference from other peaks that pre- or precede the heartcut is eliminated, and the second column's separation becomes largely independent of the first's [1–6].

(iii) In *comprehensive GC × GC* each peak transits the first column (first dimension, ^{1}D), is trapped at the end of the first or the beginning of the second column and is released for the second column separation (second dimension, ^{2}D). Aliquot parts of effluent from the ^{1}D column are repeatedly transferred to the ^{2}D column, at a fast synchronized interval called the modulation period (P_M) for separation on the ^{2}D column. Each peak eluting from the ^{1}D is sampled on the ^{2}D multiple times. Compounds that overlap on ^{1}D may be separated on ^{2}D if the selectivity of ^{2}D differs enough from that of ^{1}D, and peak widths on ^{2}D are rather small compared to ^{2}D separation time. The composition of the ^{2}D effluent is usually monitored by a

* Corresponding author. Tel.: +421 2 59325314; fax: +421 2 52926043.
E-mail address: jan.krupcik@stuba.sk (J. Krupčík).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.01.012

flame ionization detector (FID) or a mass spectrometer (MSD). The major difference between GC × GC and heart-cutting two-dimensional GC is that for GC × GC the trapping time and second column separation speed are fast enough so that many heart-cuts can be analyzed in the second column during the course of a single first column run [1,2,4–6].

In the framework of two-dimensional GC and comprehensive GC × GC separations, a question arose: "Is there any possibility to perform two-dimensional GC separations on serial coupled columns using ^{1}D non-modulated transfer (NMT) column effluent?" In the positive case, both ^{1}D and ^{1}D + ^{2}D analyses could be treated as classical one-dimensional separation using various detector combinations.

In this paper results obtained by NMT of total ^{1}D effluent separations of PCBs on DB-5 {40 m × 100 μm coated with 0.1 μm of poly(5%-phenyl-95%-methyl)siloxane} and BPX-70 {4 m × 0.1 mm coated with 0.1 μm of 70% cyanopropyl-polysilphenylene-siloxane} coupled columns are presented. Effluents from both ^{1}D and ^{1}D + ^{2}D separations were monitored independently by two FIDs.

2. Experimental

2.1. Instrument

A schematic of the instrument used in this study for NMT of ^{1}D total effluent on serial coupled columns is depicted in Fig. 1. The instrument consisted of an Agilent 6890 Gas Chromatograph and an Agilent 7673 automatic liquid sampler (Agilent Technologies, CA, USA). The instrument was equipped with electronic pressure control units (EPC), split/splitless and PTV injectors and two flame ionization detectors (FIDs) capable of producing a digital signal at a sampling rate of 200 Hz. Helium was used as a carrier gas.

DB-5 {40 m × 100 μm I.D. coated with 0.1 μm film thickness of poly(5%-phenyl-95%-methyl)siloxane phase from Agilent J&W GC Columns, Agilent Technologies, CA, USA} was used as ^{1}D column. This column was coupled by means of a fused silica T – press-fit connector to BPX-70 {4.0 m × 100 μm I.D. coated with 0.1 μm film thickness of 70% cyanopropyl-polysilphenylene-siloxane phase from Scientific Instrument Services, NJ, USA} as ^{2}D column as well as to a retention gap (4.0 m × 100 μm I.D. silylated fused silica capillary). Inlet pressure p_i = 667.6 kPa and intermediate pressure p_m = 157.3 kPa were set up using the EPCs of the split/splitless and PTV injectors, respectively. The outlet pressure of the restrictor was atmospheric (p_o = 101.3 kPa).

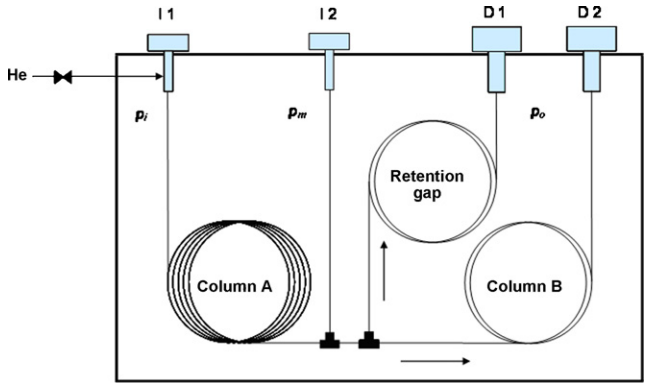

Fig. 1. Schematic of GC instrument used for NMT experiments. I1 – split/splitless injection port; I2 – PTV injection port; D1 and D2 – FIDs monitoring ^{1}D column and ^{1}D + ^{2}D column series effluents, respectively, p_i, p_m and p_o – inlet, ^{1}D and ^{2}D columns join point, and outlet carrier gas pressures, respectively.

Table 1
Data characterizing columns applied in this study for NMT separation of PCBs.

Data	^{1}D	^{2}D
Column length (m)	40	4
Column I.D. (mm)	0.1	0.1
Stationary phase film thickness (μm)	0.1	0.1
Retention time interval	57.1 min	60 s
Average peak width at base (s)	4.7	4.8
Theoretical peak capacity	690	12

The mean carrier gas velocities were 44 cm/s and 116 cm/s in the ^{1}D and the ^{2}D column, respectively. 1 μL was injected into the ^{1}D column via the splitless injector heated at 250 °C. The FID was set at 300 °C. The temperature of the columns was programmed from 80 °C (2 min) to 250 °C (30 min) at 3 °C/min.

The signal of the FIDs was processed by Chemstation Software (Agilent Technologies, CA, USA). The data files were exported into OriginPro 7.5 software (http://www.originlab.com) to deconvolute overlapping peaks.

2.2. Analytes

The PCB standard solutions C-CS-01–C-CS-09 containing all 209 PCBs at a concentration 10 μg/mL in isooctane were purchased from AccuStandard Inc. (New Haven, CT, USA). All nine multi-congener solutions were mixed to produce a solution containing all 209 congeners at a concentration 1 μg/mL. Arochlor 1242 was obtained from Monsanto (St. Louis, MO, USA). 10 μg of Arochlor 1242 was dissolved in 1 mL of isooctane. Odd numbered C15-C25 *n*-alkanes were prepared in VURUP (Slovnaft, Bratislava, Slovakia) at a concentration 10 μg/mL.

2.3. Description of NMT of ^{1}D total effluent separation on serially coupled columns

NMT separation was performed on two columns coupled in series where effluents from ^{1}D column and ^{1}D + ^{2}D column series were monitored independently by two detectors. Table 1 describes ^{1}D and ^{2}D column dimensions used for NMT PCB separations in this study. Non-polar capillary column DB-5 (40 m × 100 μm × 0.100 μm) was used as the first column. The column showed a very high theoretical peak capacity (690), since average peak width at the base was relatively small (4.7 s). The polar capillary column BPX-70 (4 m × 100 μm × 0.100 μm) was used as the second column. Since ^{2}D column characteristics determine retention time window in NMT experiment, they should be carefully chosen, since peaks separated the ^{1}D column may re-mix in the ^{2}D column and preclude to search for corresponding peak pairs on the ^{1}D and ^{1}D + ^{2}D chromatograms.

It was found that the retention time window of the ^{2}D column used in this study was for PCBs relatively high (0.2–1.0 min) [7]. Optimization of the ^{2}D column length and pressure drop tuning in ^{1}D and ^{2}D columns is currently studied and shall be published separately [8].

Both ^{1}D column and ^{1}D + ^{2}D column series chromatograms represent one-dimensional separation and can therefore be integrated by any proper ^{1}D integration software. In this work Agilent ChemStation Software was used (Agilent Technologies, CA, USA).

Note that the NMT application described in this study uses two FIDs. However any combination of two detectors can be used to monitor ^{1}D and ^{1}D + ^{2}D effluents.

The data processing of NMT experiments consists of following steps:

(i) Reading data (particularly retention times, $t_{R,i,^1D}$; peak areas, $A_{i,^1D}$, $A_{i,^1D+^2D}$; peak widths at half height, $w_{h,i,^1D}$, $w_{h,i,^1D+^2D}$

and peak heights $h_{i,^1D}$, $h_{i,^1D+^2D}$) from the ^1D and $^1D+^2D$ integration results, respectively.

(ii) Searching for corresponding peaks (i) on ^1D column and $^1D+^2D$ column series chromatograms.

(iii) Mapping data for ^2D column:
- for all corresponding peaks (i), retention times, $t_{R,i,^2D}$, are calculated from the ^1D and $^1D+^2D$ integration results: $t_{R,i,^2D} = t_{R,i,^1D+^2D} - t_{R,i,^1D}$
- peak areas, $A_{i,^1D}$, $A_{i,^1D+^2D}$ and peak widths at half height $w_{h,i,^1D}$, $w_{h,i,^1D+^2D}$ are searched comparing the ^1D and $^1D+^2D$ integration results.

Data found for all corresponding peaks on ^1D and ^2D columns and $^1D+^2D$ column series {retention times, $t_{R,i,^1D}$, $t_{R,i,^2D}$, peak widths $w_{h,i,^1D}$, $w_{h,i,^1D+^2D}$, and peak heights h_i} are used for off-line construction of 2D and 3D images.

The NMT procedure is explained in detail on examples obtained by the separation of model mixtures of PCBs and a mixture of Arochlor 1242 and C15-C25 alkanes.

2.4. Construction of 2D and 3D images

Two-dimensional (2D) and three-dimensional (3D) images were constructed in modified MATLAB software using integration data obtained for chromatograms recorded by the FIDs for the first column as well as the column series (http://www.mathworks.com). ASCII data of retention times on ^1D and ^2D columns ($t_{R,i,^1D}$ and $t_{R,i,^2D}$), peak widths at half height on ^1D column and $^1D+^2D$ column series ($w_{h,i,^1D}$ and $w_{h,i,^1D+^2D}$) and peak heights (h_i) were inputs for the calculation of elliptical Gaussians using a home made m-file function. Calculation of the two-dimensional Gaussian function was performed using Eqs. (9) and (10) defined in the standard 2D Gaussian model described in http://mathworld.wolfram.com/GaussianFunction.html.

2D and 3D images were constructed using standard MATLAB plot functions. The calculation and plotting of neat 2D or 3D images is, however, too demanding for the computer, especially on processor, memory and video card.

3. Results and discussion

As stated above, the key of the NMT separation is the search for corresponding peak pairs on the ^1D and $^1D+^2D$ chromatograms. The search procedure is illustrated by the chromatograms in Fig. 2 in which the separation of 21 PCBs on the DB-5 column as well as on the DB-5 + BPX-70 column series is depicted.

The search procedure starts with the first peak ascertained on the DB-5 chromatogram. Initially it has been supposed that the first peaks on the DB-5 and DB-5 + BPX-70 chromatograms correspond. Retention time of the first peak on the BPX-70 (^2D) column calculated from the integration data listed in Table 2 ($t_{R,1,^2D} = t_{R,1,^1D+^2D} - t_{R,1,^1D} = 0.356$ min) proved the correctness of this approach, since the comparison of the first peak on DB-5 and the second peak on the DB-5 + BPX-70 chromatograms showed a retention time in the ^2D column ($t_{R,1,^2D} = t_{R,1,^1D+^2D} - t_{R,1,^1D} = 2.854$ min), which is far away from the retention time window determined experimentally for PCBs on BPX-70 column in considered column series ($t_{R,i,^2D} = 0.2$–1.0 min).

Table 2 lists data for both chromatograms needed to process the NMT experiment. Comparison of peak abundances on ^1D and $^1D+^2D$ chromatograms was used to support the search for corresponding peak pairs, too. Since the peak areas registered for

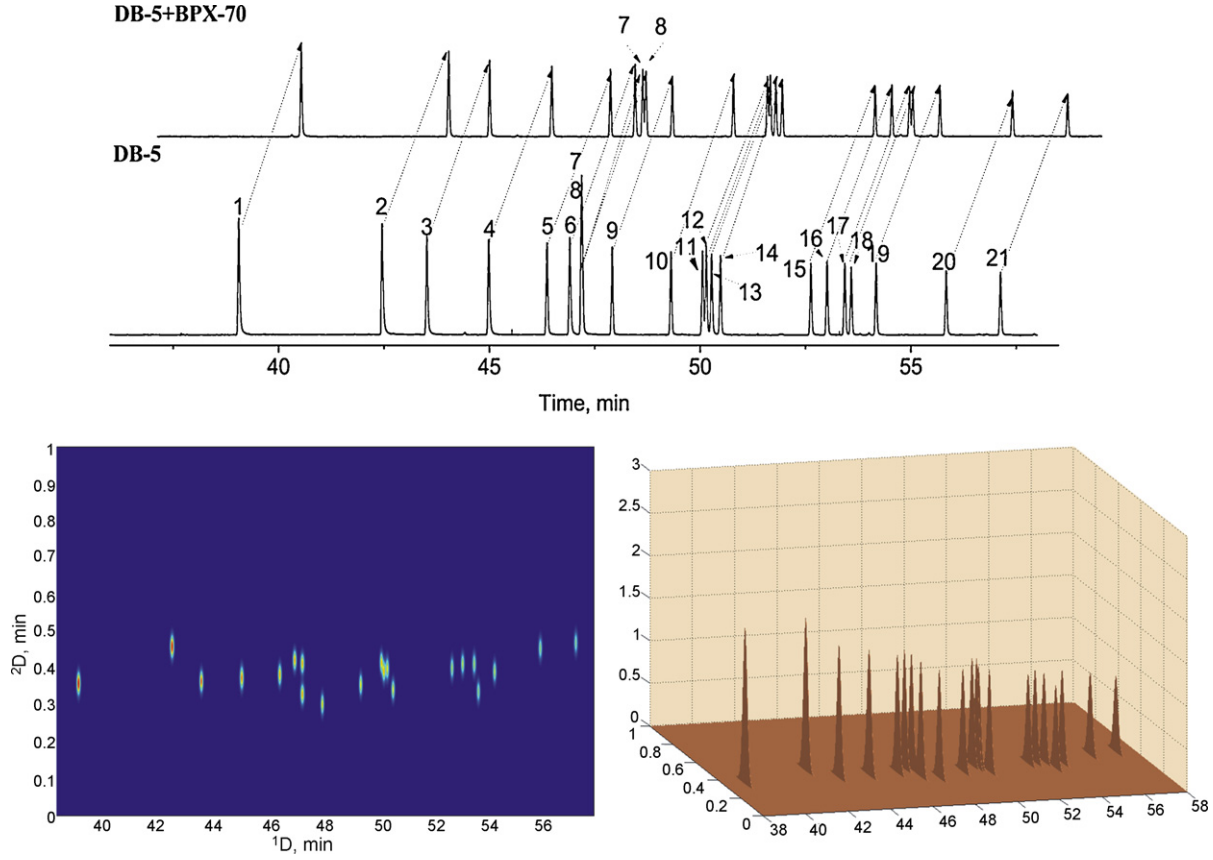

Fig. 2. FID chromatograms obtained by separation of 21 PCBs present in C-CS-09 PCB standard mixture on the DB-5 (^1D) column and DB-5 (^1D) + BPX-70 (^2D) column series and 2D as well as 3D images constructed using MATLAB software.

Table 2
Some adapted integration data of 21 PCBs on DB-5 (^{1}D) column and DB-5 + BPX-70 (^{1}D + ^{2}D) column series.

Peak #	DB-5			Peak #	DB-5 + BPX-70		
	$t_{R,i}$	$w_{h,i}$	A_i (%)		$t_{R,i}$	$w_{h,i}$	A_i (%)
1	39.057	0.046	6.68	1	39.413	0.042	6.98
2	42.457	0.046	6.36	2	42.911	0.043	6.89
3	43.519	0.045	5.53	3	43.882	0.040	5.75
4	44.987	0.046	5.47	4	45.357	0.043	5.66
5	46.367	0.043	5.09	5	46.748	0.040	5.09
6	46.910	0.046	5.66	6	47.335	0.042	5.66
7	47.191	0.050	10.05	7	47.514	0.040	5.09
8				8	47.589	0.042	5.09
9	47.914	0.042	4.64	9	48.216	0.040	4.62
10	49.308	0.044	4.64	10	49.665	0.040	4.43
11	50.058	0.043	4.64	11	50.474	0.041	4.62
12	50.144	0.043	4.90	12	50.540	0.041	4.81
13	50.273	0.046	4.71	13	50.671	0.043	4.53
14	50.482	0.042	4.33	14	50.824	0.040	4.25
15	52.627	0.044	4.01	15	53.027	0.041	3.96
16	53.012	0.044	4.07	16	53.424	0.041	3.96
17	53.431	0.045	4.13	17	53.845	0.043	4.06
18	53.584	0.044	3.82	18	53.920	0.042	3.68
19	54.174	0.042	3.94	19	54.564	0.041	3.87
20	55.836	0.046	3.75	20	56.285	0.042	3.58
21	57.126	0.045	3.56	21	57.594	0.042	3.40

Retention times (t_R) and half height peak widths (w_h) are given in min in all tables.

Table 3
Data used for off-line construction of 2D and 3D images for the NMT GC × GC separation of 21 PCBs on DB-5 and BPX-70 column series.

Peak #	$t_{R,i,^1D}$	$w_{h,i,^1D}$	$w_{h,i,^1D+^2D}$	$t_{R,i,^2D}$	h^*
1	39.053	0.049	0.048	0.359	2.00
2	42.452	0.050	0.049	0.459	1.97
3	43.517	0.045	0.044	0.365	1.71
4	44.984	0.047	0.047	0.374	1.61
5	46.366	0.047	0.047	0.383	1.53
6	46.907	0.049	0.050	0.422	1.52
7	47.187	0.050	0.050	0.330	1.49
8	47.187	0.046	0.047	0.413	1.49
9	47.913	0.043	0.044	0.303	1.39
10	49.309	0.045	0.046	0.356	1.33
11	50.058	0.045	0.045	0.416	1.38
12	50.148	0.045	0.046	0.392	1.40
13	50.273	0.047	0.048	0.399	1.29
14	50.483	0.047	0.048	0.342	1.30
15	52.626	0.046	0.047	0.401	1.15
16	53.013	0.047	0.048	0.412	1.19
17	53.433	0.048	0.048	0.412	1.14
18	53.585	0.047	0.048	0.337	1.06
19	54.174	0.047	0.048	0.391	1.19
20	55.835	0.049	0.051	0.454	1.05
21	57.127	0.048	0.052	0.469	0.97

h^* is expressed in mV.

corresponding peaks on ^{1}D and ^{1}D + ^{2}D chromatograms differ, as a consequence of differences of retention gap capillary and ^{2}D column flow resistances and FID responses, relative peak areas, (A_i in %) were used to assess the abundances of corresponding peaks on both chromatograms, particularly to search overlapping of peaks with different abundances.

The slopes of dotted lines depicted in Fig. 2 may also help to search for corresponding peaks on ^{1}D and ^{1}D + ^{2}D chromatograms visually.

Data listed in Table 3 were extracted from integration results and used to construct 2D and 3D images depicted in Fig. 2. More details on the construction procedure are described in Section 2.4.

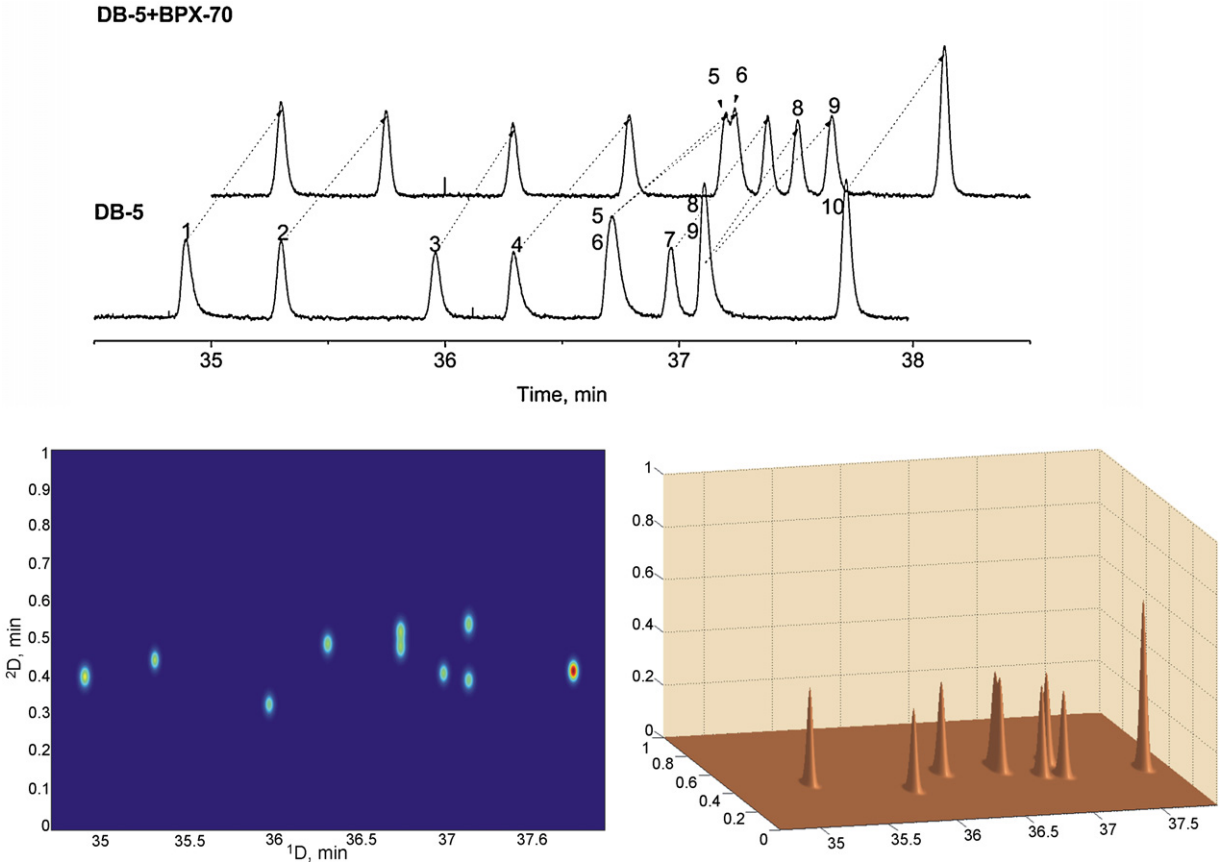

Fig. 3. Parts of FID chromatograms illustrating separations of PCBs eluted from a mixture of all 209 PCBs between 34.5–41.5 min and 35.0–38.5 min on DB-5 (^{1}D) column and DB-5 + BPX-70 (^{1}D + ^{2}D) column series, respectively, as well as 2D and 3D images constructed using MATLAB software.

Table 4
Data of PCB congeners eluted from a mixture of all 209 PCBs between 34.5–41.5 min and 35.0–38.5 min on DB-5 (^{1}D) column and DB-5 + BPX-70 (^{1}D + ^{2}D) column series, respectively.

Peak #	$t_{R,i,^1D}$	$w_{h,i,^1D}$	$A_{i,^1D}$ (%)	$t_{R,i,^1D+^2D}$	$w_{h,i,^1D+^2D}$	$A_{i,^1D+^2D}$ (%)	$t_{R,i,^2D}$	h^*
1	34.896	0.049	10.71	35.300	0.045	10.84	0.404	0.40
2	35.300	0.041	9.00	35.748	0.040	8.91	0.448	0.37
3	35.960	0.045	8.32	36.291	0.042	8.25	0.331	0.31
4	36.298	0.049	9.04	36.788	0.045	9.75	0.490	0.35
5	36.718	0.064	18.74	37.198	0.045	9.18	0.480	0.33
6				37.244	0.045	9.45	0.526	0.34
7	36.967	0.044	8.99	37.379	0.041	8.71	0.412	0.34
8	37.112	0.048	17.86	37.508	0.042	8.39	0.396	0.32
9				37.654	0.046	9.81	0.542	0.34
10	37.716	0.044	17.34	38.135	0.041	16.70	0.419	0.64

h^* is the peak height, expressed in mV.

The search for corresponding peaks on ^{1}D and ^{1}D + ^{2}D chromatograms obtained for the separation of all 209 PCB is, however, more complex than for the C-CS-09 standard mixture, since: (i) chromatograms recorded by FIDs were showed much more noise since the sample of the 209 PCBs was ten times more diluted compared to the C-CS-09 standard, (ii) many of the peaks overlapped on both ^{1}D and ^{1}D + ^{2}D chromatograms, (iii) both chromatograms were too long to process them manually at the spot and therefore they had to be divided into sub-chromatograms.

The search procedure for corresponding peak pairs on ^{1}D and ^{1}D + ^{2}D sub-chromatograms is, however, in principle equal to above discussed example. Two series of sub-chromatograms are discussed in detail.

The upper part of Fig. 3 shows sub-chromatograms illustrating the separations of PCBs eluting from the mixture of all 209 PCBs between 34.5–41.5 min and 35.0–38.5 min on the DB-5 (^{1}D) column and on the DB-5 + BPX-70 (^{1}D + ^{2}D) column series, respectively.

The search for corresponding peaks on both chromatograms starts again with the comparison of integration data for the first peaks on ^{1}D and ^{1}D + ^{2}D chromatograms. Relative peak areas listed for the first peaks in Table 4 ($A_{i,^1D} = 10.71$ and $A_{i,^1D+^2D} = 10.84$) confirm this decision. Consecutive comparison of retention times and peak areas listed in Table 4 as well as visual inspection of slopes of lines connecting peaks in Fig. 3 allows finding all corresponding peaks on both chromatograms. Areas of overlapping peaks 5 and 6 on ^{1}D + ^{2}D chromatogram listed in Table 4 were determined by a deconvolution of this cluster using the Peak Fitting Module of the

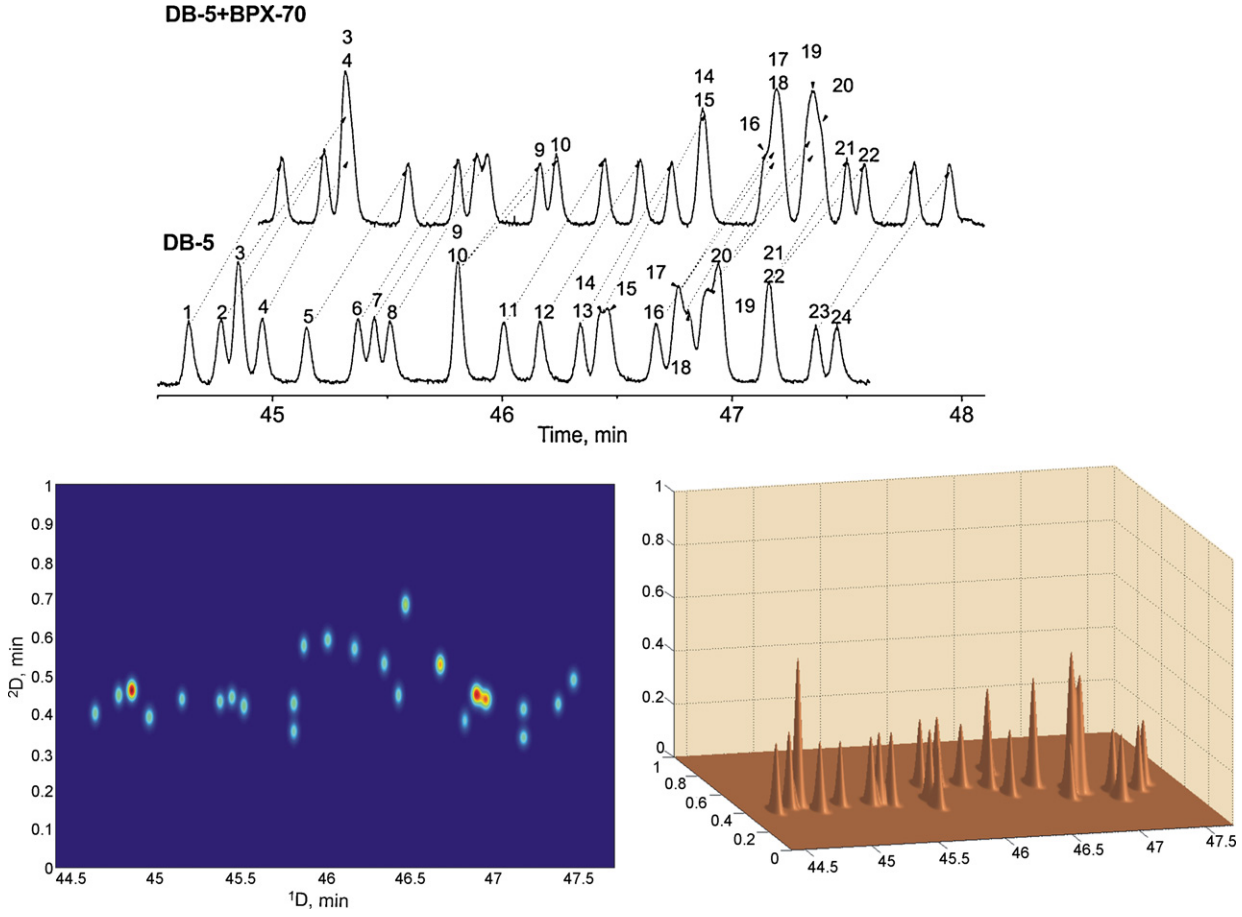

Fig. 4. Parts of FID chromatograms illustrating separations of PCBs eluted from a mixture of all 209 PCBs between 44.5–47.7 min and 44.9–48.1 min on DB-5 (^{1}D) column and DB-5 + BPX-70 (^{1}D + ^{2}D) column series, respectively, as well as 2D and 3D images constructed using MATLAB software.

Table 5
Data of PCB congeners eluted from a mixture of all 209 PCBs in time interval 44.5–47.7 min and 44.9–48.1 min on ^{1}D column and ^{1}D + ^{2}D column series, respectively.

Peak #	$t_{R,i,^1D}$	$w_{h,i,^1D}$	$A_{i,^1D}$ (%)	$t_{R,i,^1D+^2D}$	$w_{h,i,^1D+^2D}$	$A_{i,^1D+^2D}$ (%)	$t_{R,i,^2D}$	$h_{i,^1D+^2D}$
1	44.637	0.043	3.62	45.041	0.043	3.70	0.401	0.258
2	44.776	0.043	3.85	45.226	0.040	3.86	0.446	0.287
3	44.853	0.047	7.63	45.324	0.058	11.68	0.474	*0.392*
4	44.956	0.050	4.12				0.364	*0.204*
5	45.149	0.044	3.18	45.590	0.040	3.27	0.440	0.240
6	45.373	0.045	3.84	45.807	0.040	3.43	0.437	0.258
7	45.443	0.042	3.68	45.888	0.040	3.57	0.448	0.263
8	45.514	0.049	3.90	45.936	0.045	4.05	0.426	0.269
9	45.808	0.044	7.07	46.164	0.039	3.18	0.354	0.242
10				46.236	0.043	3.83	0.426	0.265
11	46.008	0.048	3.71	46.446	0.041	3.47	0.436	0.250
12	46.166	0.047	3.69	46.602	0.042	3.57	0.432	0.250
13	46.341	0.042	3.25	46.738	0.040	3.24	0.398	0.240
14	46.423	0.044	3.62	46.874	0.051	7.80	0.444	*0.223*
15	46.464	0.045	4.01				0.404	*0.233*
16	46.672	0.046	3.56	47.149	0.050	3.60	0.479	0.272
17	46.7621	0.052	6.10	47.201	0.050	10.23	0.441	*0.325*
18	46.811	0.048	4.20				0.391	*0.222*
19	46.884	0.051	6.09	47.355	0.080	14.36	0.471	0.461
20	46.940	0.054	8.41				0.415	0.390
21	47.162	0.048	6.43	47.502	0.039	3.25	0.342	0.244
22				47.576	0.041	3.25	0.416	0.238
23	47.366	0.043	3.04	47.793	0.040	3.27	0.423	0.240
24	47.457	0.047	3.30	47.948	0.043	3.38	0.488	0.236

*h** is expressed in mV.

Microcal Origin 7.5 software. 2D and 3D images depicted at the bottom part of Fig. 3 were constructed using data listed in Table 4.

The upper part of Fig. 4 shows sub-chromatograms illustrating the separations of PCBs eluting from the mixture of all 209 PCBs between 44.5–47.7 min and 44.9–48.1 min on DB-5 (^{1}D) column and DB-5 + BPX-70 (^{1}D + ^{2}D) column series, respectively. The search for corresponding peaks on both chromatograms was again based on the visual inspection of chromatograms assuming that labels of the first peaks are equal as well as on comparison of retention times and relative peak areas listed in Table 5. Comparison of peak areas confirmed the correctness of (i) labeling of the first two peaks on both chromatograms, (ii) overlapping of peaks 3 and 4 on ^{1}D + ^{2}D chromatogram and (iii) separation of peaks 9 and 10 as well as 21 and 22 on ^{1}D + ^{2}D chromatogram. Problems were encountered with the comparison of relative peak areas of poorly separated peaks 16–20 in the data obtained for integration of original chromatograms by ChemStation Software. Data in Table 5 illustrate that fitting of peak areas for peaks 16, 17 and 18 on ^{1}D and ^{1}D + ^{2}D chromatograms is acceptable only after deconvolution and reintegration of peak clusters by the Peak Fitting Module build of the Origin software. Deconvolution of the peak cluster consisting of peaks 19 and 20 was, however, not successful on ^{1}D + ^{2}D chromatogram. This cluster was therefore considered as non-separated, in spite of that a shoulder is detected on the descend part of the peak cluster. Overlapping of peaks 19 and 20 may influence quantitative analysis, but not the search for corresponding peaks, since the peak areas of the clusters on ^{1}D and ^{1}D + ^{2}D chromatograms (14.50% and 14.36% in Table 5) are corresponding.

2D and 3D images depicted at the bottom of the Fig. 4 were constructed using the data listed in Table 5. Peak heights determined on the ^{1}D + ^{2}D chromatogram were used for resolved peaks. The peak heights for peaks overlapping in clusters on the ^{1}D + ^{2}D chromatogram, written in Tables 5 and 6 in italics, were recalculated from the separated peaks on the ^{1}D chromatogram.

Supplement A contains all eleven ^{1}D and ^{1}D + ^{2}D sub-chromatograms on which the search of corresponding peaks as well as 2D and 3D images are depicted for the NMT separation of 209 PCBs on DB-5 + BPX-70 column series. UIPAC PCB numbers were used to label peaks on sub-chromatograms. Retention data and MS spectra we have recently published on DB-5 capillary column (80 m × 100 μm × 0.1 μm) were used for identification purposes [9].

The last examples that demonstrate the search of corresponding peaks in ^{1}D and ^{1}D + ^{2}D chromatograms and construction of 2D and 3D images, consider a model mixture of some hydrocar-

Table 6
Data of hydrocarbons and PCB congeners eluted from Arochlor 1242 in time interval 34.5–41.5 min and 35.0–41.5 min on ^{1}D column and on ^{1}D + ^{2}D column series, respectively.

Peak #	$t_{R,i,^1D}$	$w_{h,i,^1D}$	$A_{i,^1D}$ (%)	$t_{R,i,^1D+^2D}$	$w_{h,i,^1D+^2D}$	$A_{i,^1D+^2D}$ (%)	$t_{R,i,^2D}$	h^*
1	34.869	0.055	3.4	35.222	0.042	8.1	0.353	*0.286*
2	35.187	0.039	4.9				0.036	*0.587*
3	35.665	0.047	5.4	36.058	0.045	5.2	0.392	0.521
4	36.124	0.052	23.0	36.526	0.051	22.5	0.402	2.000
5	37.006	0.044	1.2	37.110	0.045	1.3	0.104	0.131
6	37.584	0.047	2.6	37.985	0.043	2.4	0.402	0.249
7	39.010	0.059	1.6	39.496	0.082	1.8	0.486	0.097
8	39.292	0.052	22.8	39.653	0.052	22.7	0.361	1.975
9	39.436	0.045	14.3	39.785	0.041	9.1	0.349	1.002
10				39.947	0.052	5.5	0.511	0.478
11	40.028	0.046	1.6	40.397	0.046	1.7	0.369	0.161
12	40.632	0.050	14.2	41.031	0.054	14.5	0.400	1.217
13	41.209	0.038	5.0	41.244	0.038	5.2	0.035	0.622

*h** is expressed in mV.

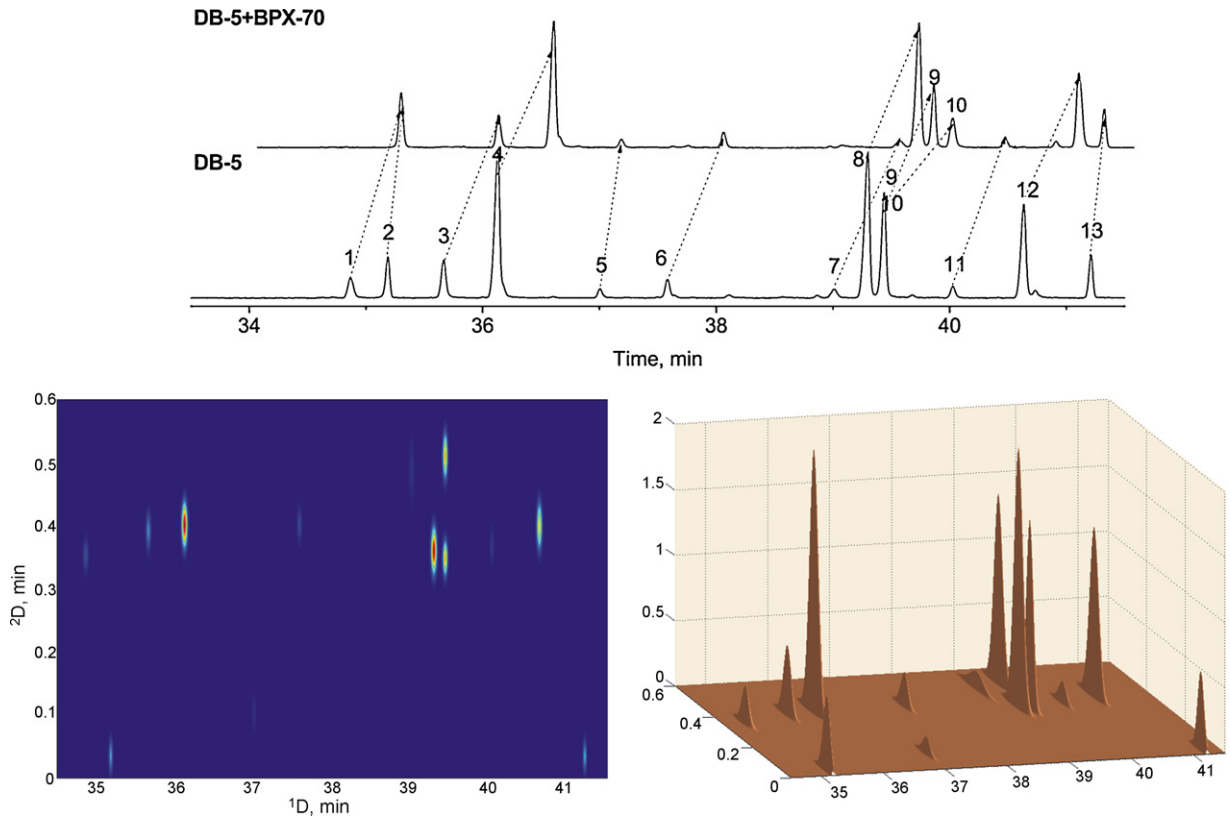

Fig. 5. Parts of FID chromatograms illustrating both separations of hydrocarbons and PCB congeners eluted from Arochlor 1242 in time interval 34.5–41.5 min and 35.0–41.5 min on ^{1}D column and on ^{1}D + ^{2}D column series, respectively, as well as 2D and 3D images constructed using MATLAB software.

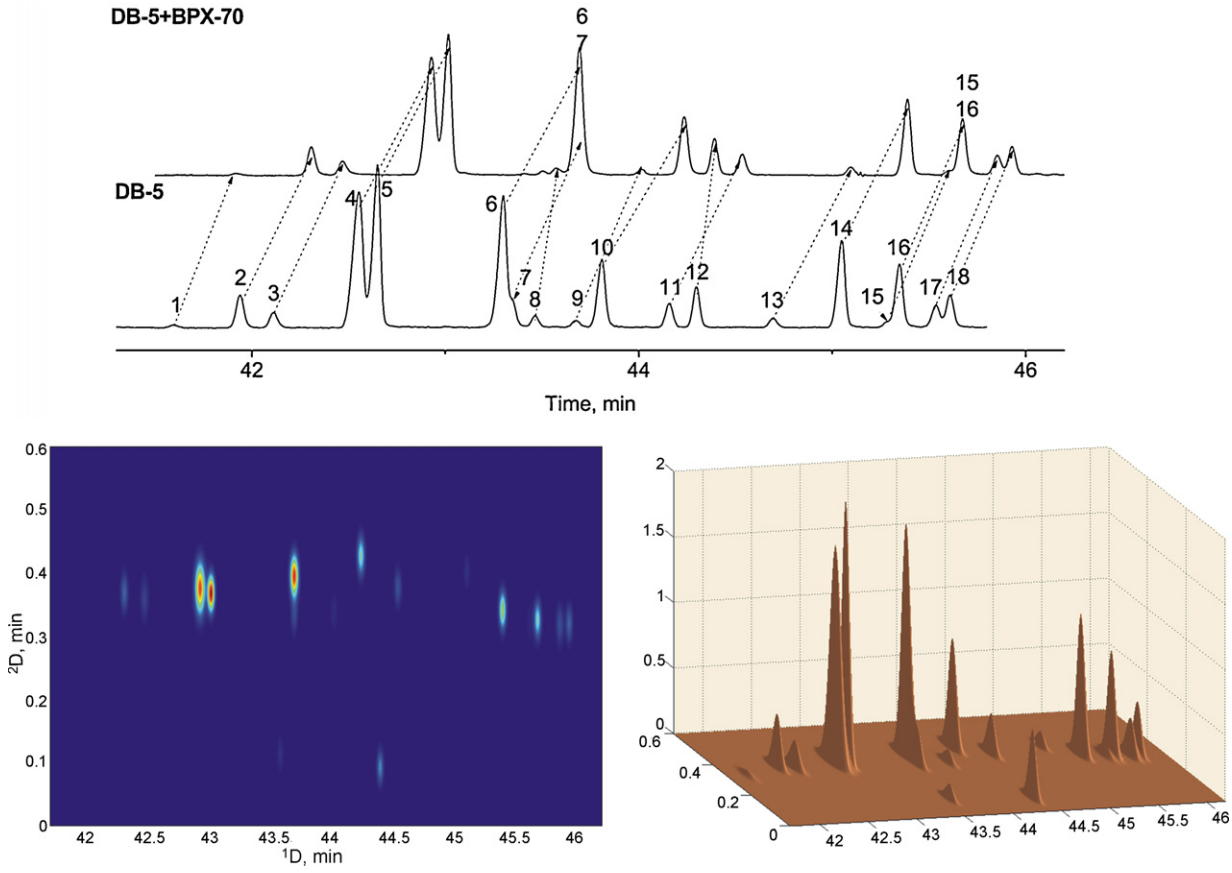

Fig. 6. Parts of FID chromatograms illustrating both separations of hydrocarbons and PCB congeners eluted from Arochlor 1242 in time interval 41.5–45.8 min and 41.5–46.3 min on ^{1}D column and on ^{1}D + ^{2}D column series, respectively, as well as 2D as well as 3D images constructed using MATLAB software.

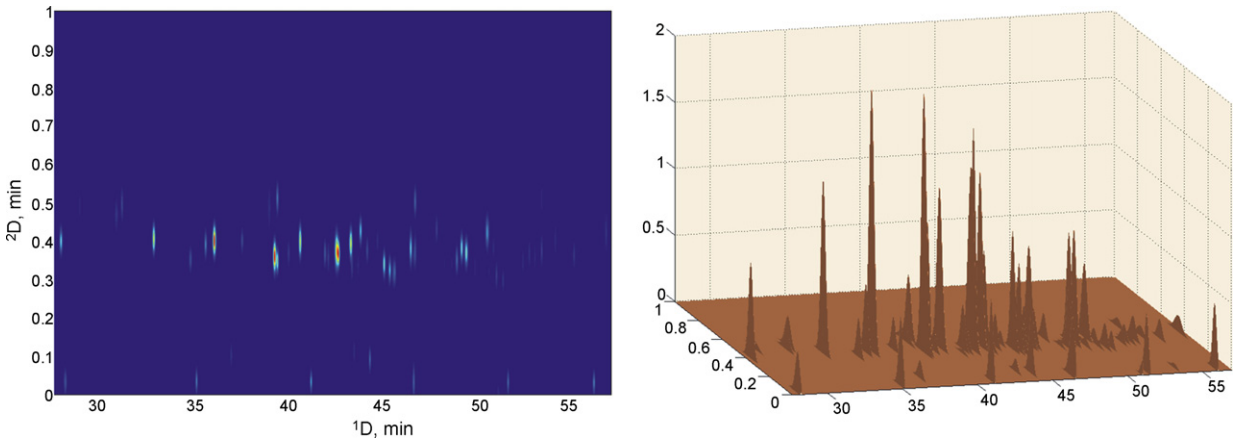

Fig. 7. 2D and 3D images constructed using MATLAB software from the data obtained by the separation of hydrocarbons and PCB congeners present in Arochlor 1242 by NMT separation on DB-5 (^{1}D) column and BPX-70 (^{2}D) column series.

bons and Arochlor 1242, in which PCB congeners drastically differ in concentration.

As was stated above, ^{1}D and ^{1}D + ^{2}D chromatograms representing the separation of the Arochlor 1242 congeners and the C15-C25 alkanes were again too long to process them manually at the spot and, therefore, they were divided into 5 sub-chromatograms. Processing of two sub-chromatograms is described in detail. Search results for corresponding peaks on ^{1}D and ^{1}D + ^{2}D chromatograms as well as 2D and 3D images for all sub-chromatograms are included in Supplement B.

Fig. 5 shows ^{1}D and ^{1}D + ^{2}D separations of hydrocarbons and Arochlor 1242 congeners eluting in the time interval 34.5–41.5 min and 35.0–41.5 min on the ^{1}D column and the ^{1}D + ^{2}D column series, respectively. Table 6 shows labeling of the corresponding peaks on both chromatograms, identification of peaks, and data derived from integration results. Visual inspection of the line-slopes that connect peaks and comparison of relative peak areas helped to find corresponding peaks on both ^{1}D and ^{1}D + ^{2}D chromatograms. Similar assessment was made (Fig. 6 and data included in Table 7) for the hydrocarbons and Arochlor 1242 congeners eluting in the time interval 41.5–45.8 min and 41.5–46.3 min on ^{1}D column and ^{1}D + ^{2}D column series, respectively.

There, however, are several small peaks in both chromatograms of Figs. 5 and 6 which peak areas are not sufficiently precise for unambiguous designation of corresponding peaks in the ^{1}D and ^{1}D + ^{2}D chromatograms. Problems start with PCB congeners which content in Arochlor 1242 is bellow 0.1% [10]. Identification of these congeners on ^{1}D and ^{1}D + ^{2}D chromatograms was performed using published retention data on DB-5 column [9]. The search of corresponding peaks for such small peaks was in some cases problematic. The NMT separation of trace PCB congeners in Arochlor 1242 (with content 0.01–0.1%) on the DB-5 + BPX column series is currently studied and shall be published later [11]. 2D and 3D images located at the bottom of Figs. 5 and 6 show expected structuralized separation of hydrocarbons and PCB congeners.

Supplement B presents separation and the corresponding peaks on all five ^{1}D and ^{1}D + ^{2}D sub-chromatograms, as well as 2D and 3D images constructed for the NMT separation of hydrocarbons and Arochlor 1242 congeners on DB-5 + BPX-70 column series.

Fig. 7 shows 2D and 3D images of the NMT separation of C15–C25 hydrocarbons and PCB congeners present in Arochlor 1242 with content higher than 0.1%.

Table 7
Data of hydrocarbons and PCB congeners eluted from Arochlor 1242 in time interval 41.5–45.8 min and 41.5–46.3 min on ^{1}D column and on ^{1}D + ^{2}D column series, respectively.

Peak #	$t_{R,i,^1D}$	$A_{i,^1D}$, %	$t_{R,i,^1D+^2D}$	$A_{i,^1D+^2D}$, %	h^*	$t_{R,^2D}$
1	41.596	0.3	41.922	0.4	0.03	0.326
2	41.938	3.5	42.307	3.6	0.40	0.369
3	42.112	1.7	42.471	2.0	0.20	0.359
4	42.549	19.9	42.924	19.9	1.65	0.376
5	42.645	17.1	43.013	16.8	2.00	0.368
6	43.296	15.8	43.691	1.9	1.77	0.395
7	43.357	1.9			0.25	0.334
8	43.464	1.3	43.578	1.0	0.11	0.114
9	43.676	0.9	44.014	0.8	0.09	0.338
10	43.807	7.5	44.234	7.6	0.83	0.427
11	44.158	2.5	44.535	2.6	0.30	0.377
12	44.298	3.9	44.391	4.2	0.52	0.093
13	44.695	1.0	45.097	1.0	0.11	0.402
14	45.047	9.4	45.388	9.3	1.07	0.341
15	45.291	0.7	45.612	0.7	0.06	0.321
16	45.347	6.6	45.673	6.8	0.79	0.326
17	45.536	2.6	45.854	2.6	0.28	0.318
18	45.609	3.4	45.930	3.4	0.40	0.320

4. Conclusions

Results in this paper enable to formulate following conclusions:

1. Non-modulated transfer (NMT) of ^{1}D effluent on the serially coupled columns GC, where the first column is narrow bore with high separation efficiency and second column has similar dimensions to that used in modulated comprehensive GC × GC, may be considered as two-dimensional GC.
2. The use of NMT of ^{1}D effluent on the serially coupled columns GC was tested by the separation of PCBs on DB-5 + BPX-70 column series. There were no problems with processing of NMT separation of model samples of PCBs where equal content of congeners were applied. There were, however, problems with the processing of NMT experiment for minor contents PCB congeners present in Arochlor 1242.
3. Since the manual peak pairing procedure in NMT of ^{1}D effluent on the serially coupled columns GC is too laborious, this method might be predominantly used for GC analyses of samples where qualitative and quantitative data for sample constituents are required.
4. The NMT separation of PCBs on serially coupled GC columns might be a perspective method for PCB congener specific analyses since it enables to monitor effluents of ^{1}D column and ^{1}D + ^{2}D column series with two different detectors (e.g. ECD and MSD).

Acknowledgements

Authors acknowledge the Grant Agency of the Slovak Republic for a VEGA 1/0827/08 Grant and the Agency for International Science and Technology Cooperation in Slovakia for APVV-20-035-205 and APVV-0319-07 Grants and grant of Ministry of Education, Youth and Sports of the Czech Republic (MSM 6198959216).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.01.012.

References

[1] W. Bertsch, J. High Resolut. Chromatogr. 22 (1999) 647.
[2] W. Bertsch, J. High Resolut. Chromatogr. 23 (2000) 167.
[3] J.C. Giddings, Anal. Chem. 6 (1984) p.1258A.
[4] P.J. Marriott, P.D. Morrison, R.A. Shellie, M.S. Dunn, E. Sari, D. Ryan, LC–GC Europe 16 (2003) 23.
[5] J. Harynuk, T. Górecki, J. Sep. Sci. 27 (2004) 431.
[6] J.V. Hinshaw, LC–GC Europe 17 (2004) 2.
[7] M. Zapadlo, J. Krupčík, P. Májek, P. Sandra, D.W. Armstrong, under review.
[8] M. Zapadlo, J. Krupčík, P. Májek, D.W. Armstrong, in preparation.
[9] J. Mydlová-Memersheimerová, F. David, B. Tienpont, J. Krupčík, P. Sandra, J. Chromatogr. A 1216 (2009) 6043.
[10] G.M. Frame, Fresenius J. Anal. Chem. 357 (1997) 714.
[11] J. Krupčík, M. Zapadlo, P. Májek, P. Sandra, D.W. Armstrong, in preparation.