Gas chromatographic retention index system for polychlorinated biphenyls: possibilities and limitations

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Abstract

The identification of individual polychlorinated biphenyls (PCBs) congeners would be easier if a proper reference series were available to calculate retention indices when using electron capture detectors, which are insensitive to the n-alkanes. In a previous work, a mixture of PCBs showing a linear dependence of retention on the number of chlorine atoms in both isothermal and programmed temperature analysis was suggested for reference purposes. Retention index values referred to this series, I_{PCB}, can be calculated and used for identification. In this work a wide collection of columns was tested in order to check the linearity of the proposed reference series. The effect of the initial temperature and heating rate of the programmed runs on the reproducibility of the results was investigated. Retention data of the congeners belonging to the reference series are given together with retention indices of toxic PCB congeners calculated for various columns. The inversion of the elution order or the shift of retention values on columns of different polarities are shown.   © 1997 Elsevier Science B.V.

Keywords: Retention indices; Stationary phases, GC; Polychlorinated biphenyls

1. Introduction

The need to identify all of the 209 polychlorinated biphenyl (PCB) congeners separated by capillary gas chromatography is due to the fact that they are characterized by very different levels of toxicity. The evaluation of the risk connected to their presence in the environment may be very incorrect if based on the total amount of PCBs in a sample without taking into account the different toxicity values. Moreover, the behavior of PCBs in the environment is congener dependent, and information on this phenomenon cannot be obtained when commercial PCB mixtures are used as the reference material for qualitative and

quantitative analysis without identifying the individual components of the sample [1–7]. Retention data of the complete series of PCB congeners are available, but they are reported with different retention parameters, that cannot be easily used for identification in other laboratories when the analytical conditions differ. A uniform parameter for the comparison of chromatographic data is the retention index system, but some difficulties arise when the classical Kováts' calculation method [8] is applied. In a previous work, a wide discussion about the possibility of calculating retention index of PCB congeners has been reported [9]. The homologous series of n-alkanes cannot be used when electron-capture detection (ECD) is used, whereas the n-iodides, which were successfully used as the refer-

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ence series with this specific detector [10–12] are not available in the range of boiling points and retention values necessary to bracket the heaviest and later eluting PCBs. A multifunctional standard of alkyl bis(trifluoromethyl)phosphinesulphides (designated as M series) detectable by several detection methods [flame ionization, ECD, flame photometric, photo ionization (PID) and alkaline thermionization] was introduced as a homologous reference series by Manninen et al. [13]. A reference series based on 10 PCB congeners was proposed by us [9], whose terms show a linear dependence of retention as a function of the number of chlorine atoms on both isothermal and programmed temperature analysis (see Table 1). The compounds included in the reference mixture are commercially available as pure samples, have low toxicity and exhibit linear behavior with respect to the number of Cl atoms of absolute, adjusted or relative retention times in programmed temperature analysis and of the logarithm of these values in isothermal analysis. A similar approach was suggested [14] by using eight PCBs (IUPAC numbers 8, 31, 44, 101, 138, 180 and 194). The linearity condition is the most important one and should be satisfied on every column used for PCB separation. This work shows the results of the control of the linear behavior of the reference series proposed by us on many non-polar and semipolar columns by using data taken from the literature and experimentally measured.

Retention data of all of the 209 congeners and chromatograms of technical PCB mixtures on a number of commercial stationary phases have been

published and reviewed in several papers [14–23], most of them published by the producers of capillary columns in order to check and publicize the performance of new columns for PCB separation. Other literature, which does not show the separation of all the 209 PCBs, reports retention data for several different congeners among which we have found data of some compounds belonging to the reference series [24–27].

2. Experimental

The analyses of PCB mixtures were carried out with glass capillary columns SPB-5 (Supelco, Bellefonte, PA, USA) and DB-5 (J&W Scientific, Folsom, CA, USA). The data of these columns are shown in the first lines of Table 2. Analysis of linear alkanes was also carried out with a poly(dimethylsiloxane) DB-1 capillary column (J&W), 60 m×0.32 mm I.D. with 3 μm film thickness. The columns were installed in Varian Model 3700 and 3400 gas chromatographs (Varian, Palo Alto, CA, USA) equipped with constant mass flow controls, ^{63}Ni pulsed type ECD and with capillary on-column and split–splitless injectors. Pure nitrogen was used as the carrier gas. The injector and detector temperatures were 250 and 300°C, respectively. The analyses were carried out in isothermal conditions at various temperatures or with temperature programming. Different initial temperature and program rates were tested. The start of the temperature program was simultaneous with the injection of the sample; all the analyzed compounds were eluted during the linear increase of temperature. Samples of PCBs of the reference series with a concentration of 35 μg/ml in iso-octane were used (AccuStandard, New Haven, CT, USA); linear alkanes were purchased from Supelco. The retention times were measured with an accuracy of ±0.01 min by using Varian Vista 402 and Varian DS-651 Data Systems.

The characteristics of the columns whose data were taken from the literature are shown in Table 2. The CP-Select for the PCB column is a prototype column whose behaviour has been described by Vetter and co-workers [17,18]. A capillary column with very similar properties, the non-polar CP-Sil2 (Chrompack) is commercially available. Both col-

Table 1
IUPAC numbering and structure of PCB congeners belonging to the proposed reference series which show a linear behaviour of the retention as a function of the number of chlorine atoms

Congener no. (IUPAC)	Chlorine atom position
1	2
9	2,5
27	2,3',6
69	2,3',4,6
121	2,3',4,5',6
151	2,2',3,5,5',6
178	2,2',3,3',5,5',6
200	2,2',3,3',4,5,6,6'
207	2,2',3,3',4,4',5,6,6'
209	2,2',3,3',4,4',5,5',6,6'

Table 2

Columns used to verify the linear trend of the proposed PCB reference series by using experimental and literature data

Type of column	Stationary phase	Length (m)	Diameter (mm)	Film (μm)	Polarity	Refs.
SE-54	poly(5% phenyl/ 1% vinyl/95% methylsiloxane)	50	0.20	unknown	slightly polar	Mullin et al. [15]
BPX5	5% phenyl (equiv.) polysilphenylene polysiloxane[a]	25	0.22	0.25	slightly polar	RMIT Dept. [21]
BPX35	35% phenyl (equiv.) polysilphenylene polysiloxane[a]	25	0.22	0.25	moderately polar	RMIT Dept. [21]
HT8	8% phenyl (equiv.) polysiloxane— carborane[a]	25	0.22	0.25	slightly polar	RMIT Dept. [21]
SPB-5	poly(5% diphenyl/ 95% dimethylsiloxane)	60	0.75	1.0	slightly polar	Experimental
SPB-5	same as above	60	0.75	1.0	slightly polar	Experimental
DB-5	poly(5% diphenyl/ 95% dimethylsiloxane)	30	0.32	0.25	slightly polar	Experimental
DB-5	same as above	30	0.32	0.25	slightly polar	Experimental
APIEZON L	paraffinic hydrocarbons mixture				non-polar	Bush et al. [26]
CP SELECT for PCB	see text	52	0.25	0.12	non-polar	Vetter [18]
CP-Sil5 CB	poly(dimethylsiloxane)	50	0.25	0.25	non-polar	Chrompack [22]
CP-Sil5/C18 CB	poly(methyloctadecyl_ siloxane)	100	0.25	0.10	non-polar	Chrompack [22]
CP-Sil8 CB	poly(5% diphenyl/ 95% dimethylsiloxane)	50	0.25	0.25	slightly polar	Chrompack [22]
CP-Sil8/C18 CB	poly(5% diphenyl/ 95% methyloctadecylsiloxane)	50	0.25	0.10	sligtly polar	Chrompack [22]
CP-Sil13 CB	poly(14% diphenyl/ 86% dimethylsiloxane)	50	0.25	0.20	moderately polar	Chrompack [22]
CP-Sil19 CB	poly(14% cyanopropylphenyl/ 86% dimethylsiloxane)	50	0.25	0.20	medium polar	Chrompack [22]

[a] Manufacturer's description.

umns consist of chemically bonded and cross-linked (4%) silicon-containing high-molecular-mass (<10 000) hydrocarbons similar to squalane [19]. The t_R values of the congeners of the proposed reference series for the CP-Select for PCB columns were obtained according to the method proposed by Vetter and Luckas [17].

3. Results and discussion

Table 3 shows the temperature conditions used for analysis and the type of data obtained: retention relative to octachloronaphthalene, RRT(OCN), ad-
justed retention time t_R' or its logarithm, retention relative to the average value of retention times of PCB 52 and PCB 180 (IUPAC classification). The parameters (intercept and slope) of the straight line obtained by plotting the retention values of the PCBs belonging to the proposed reference series as a function of the number of chlorine atoms, n_{Cl}, are also shown (see Fig. 1 and Table 4). Independent of the column type and kind of retention data, a fair linearity was observed, with correlation coefficient very close to unity. One must consider the fact that in many sets of data taken from the literature the analyses have been carried out with a double-step temperature-programming condition that is not ideal

Table 3
Linear regression coefficients ($y = ax + b$ type) and correlation coefficient R^2 of the different kinds of retention data of the proposed PCB reference series on the columns tested

Type of column	Temperature	Retention	a	b	R^2
SE54	from 100 to 240°C at 1°C/min	RRT[a]	0.0991	0.0562	0.9999
BPX5	70°C (1 min) to 140°C at 20°C/min, to 295°C at 3°C/min	t_R	4.0594	5.9313	0.9985
BPX35	70°C (1 min) to 140°C at 20°C/min, to 295°C at 3°C/min	t_R	4.3315	9.4487	0.9990
HT8	70°C (1 min) to 140°C at 20°C/min, to 295°C at 3°C/min	t_R	4.5419	8.7273	0.9996
SPB5	from 120 to 290°C at 5°C/min	t_R	2.6679	11.403	0.9991
SPB5	isothermal 240°C	$\ln t_R'$	0.4016	−0.3119	0.9993
DB-5	from 120 to 290°C at 3°C/min	t_R	4.4871	10.018	0.9998
DB-5	isothermal 200°C	$\ln t_R'$	0.4815	0.2225	0.9995
APIEZON-L	from 70 to 130°C at 10°C/min to 230°C at 4°C/min	t_R	2.5646	2.5564	0.9972
CP SELECT for PCB	75 (2 min) to 150°C at 15°C/min, to 275°C at 1.5°C/min	t_R	8.5847	−2.4687	0.9972
CP-Sil5 CB	75 (2 min) to 150°C at 15°C/min, to 300°C at 1.5°C/min	RRT[b]	0.0762	0.0567	0.9964
CP-Sil5/C18 CB	75 (2 min) to 150°C at 15°C/min, to 300°C at 0.75°C/min	RRT[b]	0.0820	0.0140	0.9955
CP-Sil8 CB	75 (2 min) to 150°C at 15°C/min, to 300°C at 1.5°C/min	RRT[b]	0.0714	0.0817	0.9982
CP-Sil13 CB	75 (2 min) to 150°C at 15°C/min, to 300°C at 1.5°C/min	RRT[b]	0.0770	0.0484	0.9961
CP-Sil19 CB	75 (2 min) to 150°C at 15°C/min, to 275°C at 1.5°C/min	RRT[b]	0.0704	0.0776	0.9983
CP-Sil8/C18 CB	75 (2 min) to 150°C at 15°C/min, to 275°C at 1.5°C/min	RRT[b]	0.0705	0.0791	0.9991

[a] RRT with respect to octachloronaphtalene.
[b] RRT with respect to (t_R PCB5 + t_R PCB180).

for the linear dependence of retention values on the number of chlorine atoms and consequently for a correct calculation of the retention indices. However, the plots of the retention values of the proposed PCBs shown in Fig. 1 have linear correlation coefficients ever greater than 0.99, which permit their

use as the reference compounds for calculation of retention indices relative to selected PCBs, I_{PCB}.

On all the columns tested, the behaviour of the proposed PCB reference series under isothermal conditions resembles strictly that of a typical homologous series. As an example, the equation,
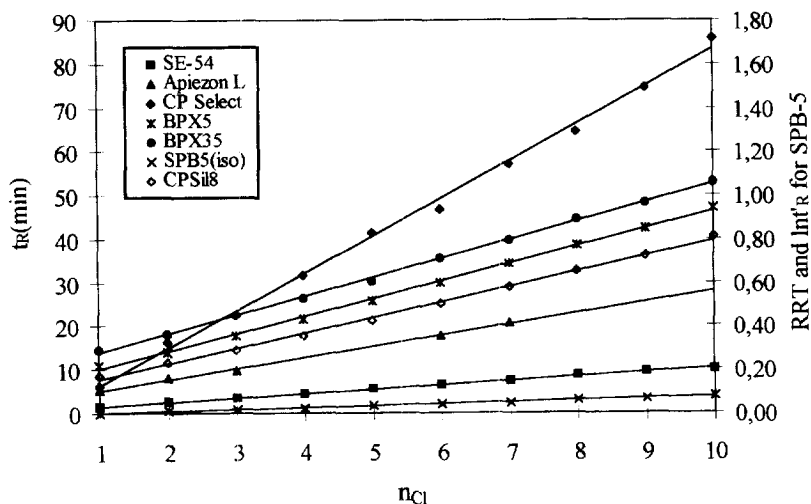

Fig. 1. Retention values of the proposed PCB reference series on different columns as a function of the number of chlorine atoms, n_{Cl}. Scale on the left, retention times, t_R; scale on the right, retention relative to octachloronaphthalene for column SE-54, and relative to PCB 52 and 180 for CPSil column; logarithm of adjusted retention times for SPB5 column used in isothermal run at 240°C (see Table 3).

Table 4
Retention of PCB congeners (IUPAC numbering) of the proposed reference series on columns tested for linearity

Column	Units	1	9	27	69	121	151	178	200	207	209
SE54	RRT	0.1544	0.2570	0.3521	0.4510	0.5518	0.6499	0.7537	0.8494	0.9423	1.0496
BPX5	t_R	11.000	14.036	17.724	21.707	25.743	30.023	34.264	38.541	42.448	47.100
BPX35	t_R	14.500	17.947	22.482	26.391	30.341	35.503	39.694	44.530	48.261	53.082
HT8	t_R	12.80	17.762	22.467	27.114	31.823	36.059	40.846	44.928	49.170	54.115
SPB5 (prgT)	t_R	13.90	16.81	19.60	22.22	24.75	27.36	29.97	32.57	35.01	38.58
SPB5 (isoT)	$\ln t_R'$	0.1231	0.4983	0.9079	1.2868	1.6575	2.0645	2.4748	2.8972	3.2892	3.7679
DB-5 (prgT)	t_R	14.205	19.254	23.575	27.800	32.571	36.966	41.565	45.969	50.115	54.948
DB-5 (isoT)	$\ln t_R'$	0.7324	1.2018	1.6780	2.1411	2.5989	3.0725	3.5723	4.0594	4.5382	5.1116
APIEZON-L	t_R	5.33	7.86	9.79	—	—	17.66	20.87	—	—	—
CP SELECT	t_R	6.18	16.34	22.50	31.81	41.54	46.91	57.14	64.59	74.74	85.72
CP-Sil5 CB	RRT	0.1573	0.2169	0.2763	0.3476	0.4244	0.5000	0.5859	0.6655	0.7467	0.8388
CP-Sil5/C18 CB	RRT	0.1261	0.1833	0.2418	0.3292	0.4205	0.4871	0.5858	0.6589	0.7587	0.8565
CP-Sil8 CB	RRT	0.1688	0.2301	0.2906	0.3577	0.4279	0.5008	0.5782	0.6539	0.7252	0.8085
CP-Sil13 CB	RRT	0.1504	0.2099	0.2750	0.3407	0.4130	0.4990	0.5811	0.6701	0.7455	0.8372
CP-Sil19 CB	RRT	0.1617	0.2214	0.2887	0.3471	0.4147	0.4969	0.5715	0.6450	0.7083	0.7911
CP-Sil8/C18 CB	RRT	0.1583	0.2225	0.2811	0.3547	0.4304	0.4969	0.5770	0.6465	0.7218	0.7774

which describes the behaviour at 200°C on a DB-5 column, 30 m long is:

$$\ln t_R' = 0.4815 n_{Cl} + 0.2225 \qquad (1)$$

with a correlation coefficient $R^2 = 0.9995$; similar values were obtained at different isothermal temperatures. Under temperature-programming conditions the linear behaviour of retention times against the number of chlorine atoms is quite good and near independent on the program rate as shown in Fig. 2 for the same DB-5 column as above. Good correlation coefficients were also obtained on a 60-m long SPB-5 capillary column. However the linearity de-

creases when the initial temperature of the program increases (Fig. 3). The discussion on the validity of the I_{PCB} calculation under different temperature-programming conditions must take into account the general debate about the correspondence between isothermal (I_1) and programmed temperature (I_{PR}) retention indices. The I_1 values are calculated with the classical Kováts' method [8] by using the logarithm of the adjusted retention values; the IPR values with the Van den Dool and Kratz equation [28] which employs the retention temperatures or the retention times in a non-logarithmic equation. In
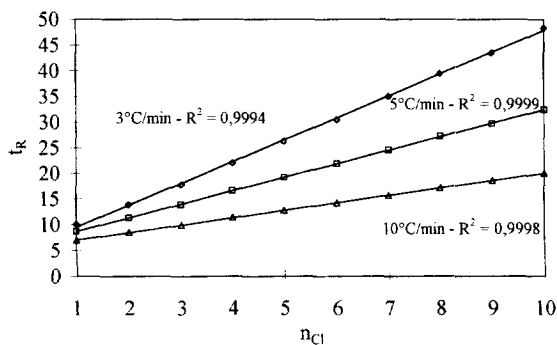

Fig. 2. Linear regression of retention time vs. number of chlorine atoms of the PCB reference series analysed under temperature programming at three different heating rates (3, 5 and 10°C/min, column DB-5, 30 m×0.32 mm I.D., 0.25 μm film thickness).
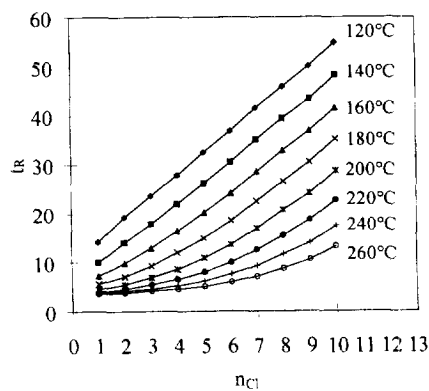

Fig. 3. Behaviour of retention times of the PCB belonging to the proposed reference series under programmed-temperature conditions as a function of the initial temperature of the program, T_0 (column DB-5, 30 m×0.32 mm I.D., 0.25 μm film thickness).

general [8] the retention index equation can be written:

$$I = 100i \cdot \frac{X - M_{(n)}}{M_{(n+i)} - M_{(n)}} + 100n \qquad (2)$$

where n is the number of molecular groups used to characterise the homologous series (i.e. carbon atoms in alkanes and chlorine atoms in PCBs) and i is the difference between the two reference probes used for the calculation of the retention index. In isothermal conditions the I_I value is found by substituting for X, $M_{(n)}$ and $M_{(n+i)}$ the logarithms of the adjusted retention volume or of the adjusted retention times of the selected compound and both markers, respectively. In programmed temperature analysis for X, $M_{(n)}$ and $M_{(n+i)}$ either the adjusted, t'_R, or the gross, t_R, retention times may be used. In this work and in the papers whose data were used for comparison, programmed temperature analyses were evaluated by using the t_R values. It is generally assumed that I_{PR} changes insignificantly over a wide range of experimental conditions, such as the initial temperature, T_0, the program rate, r, and the carrier gas flow-rate, mainly when the substances under investigation have a small temperature gradient of the retention index and the stationary phase used has low polarity [29–33]. However, it was observed [34] that I_{PR} may be influenced considerably by some change of experimental conditions, and attempts were made to correlate the I_{PR} with the I_I [32,33,35].

The non-logarithmic equation of Van den Dool and Kratz has received much attention owing to the extensive use of temperature-programmed analysis and it was found that the indices obtained are much more variable than isothermal results [34,35]. In order to verify the validity of I_{PCB} values calculated with the two methods, some experiments were carried out under isothermal conditions and in programmed-temperature runs by using both the proposed PCB series and n-alkanes with 11–18 carbon atoms. On the basis of experimental data shown in Fig. 1 and in Table 3 it is possible to confirm that the accuracy and the reproducibility of the I_{PCB} values calculated in isothermal conditions with the Kováts formula is very high. The I_{PCB} obtained in programmed-temperature analysis with the Van den Dool and Kratz formula are also accurate and

reproducible and nearly independent on the programming rate, as confirmed by many authors who found that changing r does not change the retention index values if the initial, T_0, and final, T_F, temperatures remain the same. Whereas the effect of changing r was investigated widely [36–43], the effect of changing the value of T_0 received less consideration [41,43.44] because the procedure followed generally in order to find the correct parameters of the best programmed run for a given separation takes as T_0 value a temperature below the boiling point of the most volatile component of the sample. In this instance, the behaviour of t_R as a function of the increasing number of carbon atoms, n_C, in the n-alkane homologous series or of chlorine atoms, n_{Cl}, in the proposed series of PCBs is fairly linear as shown by the plots at 120 and 140°C in Figs. 3 and 4 and by the linearity correlation coefficient R^2 in Table 5. When T_0 is increased, keeping constant both T_F and r, the retention times of the compounds eluted at the beginning of the analysis decrease quickly, and non-linear plots are obtained. With the columns and programming rate used in the experiments described here, the plots obtained with T_0 values between 120 and 150°C can be approximated well enough by straight lines whereas, when T_0 is greater, the plots show a marked inflection and a nearly exponential behaviour is found (Table 5). The experiments carried out with n-alkanes (see Fig. 4) have shown that this behaviour does not depend on
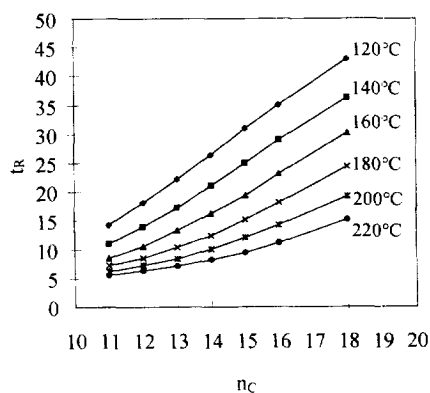

Fig. 4. Behaviour of retention times of linear alkanes under programmed-temperature conditions as a function of the initial temperature of the program, T_0 (column DB-1, 60 m×0.32 mm I.D., 3 μm film thickness).

Table 5

Linear and exponential regression of t_R vs. n_C (n-alkanes, column DB-1, 60 m×0.32 mm I.D., 3 μm film thickness) and t_R vs. n_{Cl} (PCB reference series, column DB-5, 30 m×0.32 mm I.D., 0.25 μm film thickness) obtained under temperature-programming conditions with different initial temperatures (T_0)

PCB reference series			n-Alkanes		
T_0 (°C)	Linear	Exponential	T_0 (°C)	Linear	Exponential
120	$y=4.4871x+10.018$ $R^2=0.9998$	$y=14.611e0.1427x$ $R^2=0.9625$	120	$y=4.1604x-31.66$ $R^2=0.9996$	$y=2.755e0.1575x$ $R^2=0.9729$
140	$y=4.2622x+5.2477$ $R^2=0.9994$	$y=10.246e0.1675x$ $R^2=0.9635$	140	$y=3.6743x-29.981$ $R^2=0.9981$	$y=1.8136e0.1713x$ $R^2=0.9801$
150	$y=4.1039x+3.1328$ $R^2=0.9976$	$y=8.3637e0.1812x$ $R^2=0.9689$	160	$y=3.1409x-27.052$ $R^2=0.9924$	$y=1.1951e0.1834x$ $R^2=0.9888$
160	$y=3.8854x+1.7037$ $R^2=0.9944$	$y=6.9981e0.1915x$ $R^2=0.9749$	180	$y=2.4752x-21.237$ $R^2=0.9805$	$y=1.0258e0.178x$ $R^2=0.9969$
180	$y=3.3421x-0.1629$ $R^2=0.9818$	$y=4.9661e0.2066x$ $R^2=0.9881$	200	$y=1.8632x-15.257$ $R^2=0.9719$	$y=1.0243e0.1635x$ $R^2=0.9987$
200	$y=2.6991x-0.7793$ $R^2=0.9598$	$y=3.7299e0.21x$ $R^2=0.9974$	220	$y=1.8632x-15.257$ $R^2=0.9719$	$y=1.1836e0.1394x$ $R^2=0.9981$
220	$y=2.0415x-0.4384$ $R^2=0.9332$	$y=3.0771e0.1989x$ $R^2=0.9966$			
240	$y=1.2538x+1.1331$ $R^2=0.9162$	$y=2.8837e0.1688x$ $R^2=0.9834$			
260	$y=1.0031x+1.1663$ $R^2=0.8793$	$y=2.7244e0.1458x$ $R^2=0.9654$			

the use of the proposed PCB series as reference compounds, but is common to all the homologous series when the initial temperature of the programmed run increases and approaches or overcomes the boiling point of the first terms.

The calculation of the I_{PCB} values in programmed-temperature runs with the Van den Dool and Kratz formula also yields reproducible values if non-contiguous terms of the reference series are used, when the dependence of t_R on n_C or n_{Cl} follows a straight line. When, owing to high T_0 values, the plots show curvature, the linear interpolation between non-consecutive terms results in non-negligible errors of the index values. This problem is also observed when using the relative retention times of PCB 52 and PCB 180 [15] if the temperature programming is not linear, or the initial temperature is too high. However, if the curvature of the plot is small, linear interpolation between contiguous reference terms yields the same accuracy as the classical method of Van den Dool and Kratz. In order to test if the interpolation between contiguous terms of the reference series yields tolerable deviations from true retention index values, the retention indices of the various congeners were calculated assuming each congener, $M_{(n)}$, as an unknown component and using as the reference the two contiguous terms, $M_{(n-1)}$ and $M_{(n+1)}$, taken with their theoretical retention index values $100(n-1)$ and $100(n+1)$ respectively. The interpolation in this instance was made on a range of 200 retention index units (i.u.), and under isothermal conditions the range of variation of the

retention index of the $M_{(n)}$ term calculated by this way was 2–3 i.u., whereas in programmed-temperature runs this variation was about 4 i.u. Similar values were found when the same method was applied to n-alkanes. The uncertainty in the determination of the retention index of an unknown compound bracketed between two contiguous reference terms can therefore be about 1 i.u. in isothermal and 2 i.u. in programmed runs. It was reported [36] that for routine operations a reproducibility of about 1 i.u. may be expected with non-polar phases and 2–3, possibly 5, with highly polar phases.

When the plots obtained by starting from high T_0 values are not linear, their exponential behaviour permits the determination of the I_{PCB} value. By applying an exponential regression procedure to the available experimental data obtained by injecting the reference series, the numerical parameters C and D of the equation:

$$t_R = C\,e^{Dn_{Cl}} \tag{3}$$

can be obtained. The exact I_{PCB} value can then be calculated with the formula:

$$I_{PCB} = \frac{\ln t_R - \ln C}{D} \times 100 \tag{4}$$

which is a general expression for the retention index when the dependence of t_R on the number of carbon or chlorine atoms of the reference compounds follows with good approximation a logarithmic law, as postulated by Kováts and generally found in isothermal conditions. The I_{PCB} values can be converted

into classical Kováts I values when the proposed PCB reference series and n-alkanes are analysed on the same column and are available with a number of carbon atoms great enough to cover the entire range of retention times of all PCB congeners (on non-polar or slightly-polar columns n-alkanes with 14–29 carbon atoms are necessary).

A possible source of error may be the change in polarity of the column with increasing temperature, which may influence the slope and linearity of the plots shown in Fig. 1. The stationary phase which shows the greatest slope, and theoretically the best separation between different congeners, is the CP-Sil8/C18 CB poly(5% diphenyl/95% methyl-octadecyl siloxane) column. It was observed that for methyl-octadecyl phases the polarity changes with changing temperature in a non-linear way. The value of the ΔC parameter, which is a measure of the polarity contribution to the solute–solvent interaction, and is obtained by comparing the behaviour of n-alkanes and n-alcohol on a given stationary phase [45,46], changes from 2.20 at 60°C to 2.39 at 200°C. As the analyses used to obtain the data of Table 3 were carried out with temperature programming, the polarity change may have influenced the retention of compounds eluting later and increased the slope of the line. All other columns tested show a small

polarity change with temperature; some of them (poly(dimethylsiloxane) and poly(methyl-phenylsiloxane) with a small amount of phenyl groups), do not appreciably change their polarity in the temperature range between 60 and 200°C [47].

In order to check the application of the proposed method, the I_{PCB} values of some PCB congeners, considered generally as the most toxic ones, are shown on eight columns under programmed-temperature analysis conditions (see Table 6). The stationary phases tested, all having a low polarity, elute all the PCB congeners in a similar way, as shown by the high correlation coefficients, R^2, obtained by plotting the I_{PCB} values of toxic PCBs measured on all columns with respect to those obtained on poly(dimethylsiloxane) (CP-Sil5). The differences in I_{PCB} values are great enough in some cases to confirm the identification of congeners by comparing the results obtained on columns of different composition. By using literature retention data of the 209 PCB congeners on different columns (Table 2) and the proposed PCB reference series, retention indices of all PCB congeners were obtained. Then the retention index value of each congener was subtracted from the following one; in this way it was possible to evaluate that, in all the columns considered, the I_{PCB} values of about 75% of the congeners

Table 6
Retention indices, with reference to the proposed PCB reference series, of PCB toxic congeners on different columns, and parameters of the linear regression of I_{PCB} on various columns with respect to I_{PCB} on CP-Sil5 column

Congener (IUPAC)	CP-Sil5	CP-Sil8	SE-54	CP-Sil13	CP-Sil8/C18	BPX5	BPX35	HT8
77	572.09	580.52	579.20	589.77	590.98	591.26	601.29	615.23
126	691.39	698.84	697.59	708.31	712.23	710.38	722.95	740.45
169	809.85	812.06	811.63	811.54	837.32	821.86	826.21	869.97
37	427.99	435.61	434.52	448.41	438.18	447.72	468.58	456.55
81	558.99	566.53	564.32	574.65	576.84	576.12	585.35	595.49
105	644.70	653.88	652.99	666.38	658.30	661.85	678.74	682.89
114	627.71	633.20	631.70	640.44	641.82	639.14	648.56	654.88
118	614.78	618.60	618.69	621.68	629.96	625.30	627.89	644.24
123	611.53	615.50	615.32	618.88	624.34	619.17	620.40	638.27
156	753.39	758.65	759.35	764.04	774.24	766.14	774.09	803.63
157	759.30	766.05	767.61	773.82	779.28	772.78	782.20	812.99
167	725.00	727.34	728.94	728.99	744.32	735.14	735.38	766.12
189	863.67	867.04	857.55	869.76	885.79	875.76	884.48	921.01
a		0.935	1.295	1.0107	0.9218	53.174	56.689	59.310
b		0.0291	−0.0152	−0.0089	0.0279	2.9505	6.2916	5.4802
R^2		0.9997	0.9965	0.9997	0.9971	0.9993	0.998	0.9941

differ from that of the contiguous peak by more than 1 i.u. and can therefore permit the identification of the compound by using tabulated retention index values. The congeners that show a difference of smaller than 1 i.u. are not the same in all the columns and their separation and identification can be carried out by using different columns which, owing to different polarity, can permit a better resolution of the compounds of interest and sometimes the inversion of the elution order.

The separation power of different columns can be easily compared when the retention data are normalised by using the retention index system proposed, whereas the comparison of different kinds of retention values is almost impossible when a great number of peaks is obtained under different analytical conditions. If the I_{PCB} values of the 209 congeners obtained with one column are subtracted from those obtained with another stationary phase, the positive or negative sign of the difference indicates if the given congener is more or less retained, and this can be correlated with its structure, with the composition of the two stationary phases and with the conditions of analysis. The upper-right portion of Table 7 shows the number of negative values of the difference obtained by subtracting the I_{PCB} values (measured under the experimental conditions described above (Tables 2 and 3) in the columns listed in the vertical array from those obtained on the columns listed horizontally at the top of the table. The lower-left portion of Table 7 lists the number of congeners which show an inversion of

the elution order when the two corresponding columns are compared. Surprisingly, notwithstanding the fact that the range of polarity of the used columns covers a restricted interval (from the nonpolar CP-Sil5 to the moderately polar BPX35) a great number of negative differences and of inversions of the elution order was found. Only if the number of negative differences and of peak inversions observed when two columns are compared is small, can the two stationary phases be considered equivalent for the separation of PCB, and the retention data reported in the literature for one of the two columns can be used for identification purposes in analyses carried out with the other. Conversely, when two columns elute the PCB congeners with a great number of peak inversions, the results can be used in order to confirm the identification of selected compounds.

4. Conclusions

The proposed method for the determination of homogeneous retention data for PCBs through the calculation of retention indices relative to a selected series of PCBs with increasing number of chlorine atoms is suitable for application to the data obtained with all of the tested columns, independent of the units used in the original measurement of the retention values. When the initial temperature of the programmed run is low and, as a consequence, the plot of the retention times of the congeners belong-

Table 7
The upper-right portion of the table shows the number of negative values of the differences between the I_{PCB} values measured on the columns listed at the top of the table and those listed at the left; the lower-left portion lists the number of congeners whose peaks show an inversion of the elution order when two stationary phases are compared

	CP-Sil5	SE-54	CP-Sil5/C18	CP-Sil8/C18	CP-Sil8	CP-Sil13	BPX5	BPX35	HT8
CP-Sil5	0	45	75	48	38	42	33	37	41
SE-54	103	0	108	89	93	55	39	47	60
CP-Sil5/C18	173	186	0	108	102	102	95	75	56
CP-Sil8/C18	154	162	176	0	95	102	86	65	37
CP-Sil8	93	69	194	165	0	44	32	34	51
CP-Sil13	144	140	200	179	140	0	116	42	62
BPX5	148	149	194	146	130	149	0	31	73
BPX35	170	176	197	195	174	151	166	0	98
HT8	170	193	190	181	196	191	199	186	0

ing to the reference series is linear as a function of the number of chlorine atoms in the molecule, the calculation of the retention index values can be carried out by using the classical Van den Dool and Kratz formula. When the plots show appreciable curvature due to high initial temperature of the programmed run, the determination of retention indices can be done with exponential formulas. By using non-polar or slightly polar columns, the comparison of the retention indices differing by more than 1 i.u. from tabulated values or from the chromatograms of known standards permits the preliminary identification of about 75% of the congeners. Coincident or less-separated congeners can be identified by using the data obtained with different columns as the coincidence is not the same on all the tested stationary phases. The I_{PCB} values also permit to select columns with similar behaviour and to employ literature data obtained under different analysis conditions. Conversely, columns showing a great number of peak inversions can be used to confirm the identification of many PCB congeners.

Acknowledgements

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