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OPTIMIZATION OF EXPERIMENTAL CONDITIONS FOR THE ANALYSIS OF COMPLEX MIXTURES BY GAS CHROMATOGRAPHY

J. KRUPČÍK, J. MOCÁK, A. ŠIMOVÁ and J. GARAJ

Slovak Technical University, Chemical Faculty, Department of Analytical Chemistry, 880 37 Bratislava (Czechoslovakia)

and

G. GUIOCHON*

École Polytechnique, Laboratoire de Chimie Analytique Physique, 91128 Palaiseau Cedex (France)

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SUMMARY

An optimization procedure for the gas chromatography of complex mixtures is described and has been applied to the analysis of a mixture of 40 polychlorobiphenyls on a column made by connecting an Apiezon L-coated column with a Carbowax 20M-coated column.

INTRODUCTION

Open-tubular gas chromatographic columns are used mainly for the analysis of complex mixtures of organic compounds. Their development in the last 12 years has been speeded up by pressing needs for trace analysis, the identification of components of complex mixtures and faster analyses. It was made possible by the ease with which such columns can be prepared from glass tubes.

Analytical results are strongly dependent upon working conditions and up to now the adjustment of experimental parameters has been empirical. The analysis of complex mixtures is long, however, and empirical optimization is tedious and time consuming and the results depend heavily on the experience and skill of the analyst.

The optimization of analytical conditions for the separation of a binary mixture is easy to achieve or even to formulate, as we have to maximize only the resolution, R_{ij}, between the two peaks:

$$R_{ij} = \frac{\sqrt{n_i}}{4} \cdot \frac{k_j}{1 + k_j} \cdot \frac{\alpha_{ij} - 1}{\alpha_{ij}} \tag{1}$$

(see list of symbols) and to adjust the analysis time to achieve the necessary resolution.

The problem becomes more complicated when we have to separate a multicomponent mixture, where there are always several pairs of compounds the resolution

of which is more critical than others. Morgan and Deming[1], for example, have shown how to optimize the temperature and the carrier gas flow-rate in the analysis of two- to five-component mixtures using a sequential simplex method and carrying out calculations with a computer. In the cases they discussed, however, the elution order remained unchanged. This is not what happens in actual situations of current interest when we attempt to optimize the temperature, the phase ratio and the selectivity of the stationary phase to separate a complex mixture with a large number of components whose retention order will change markedly in the range of experimental conditions that can be achieved. For each such change there is a corresponding narrow range of conditions in which total resolution of the mixture is impossible in practice. A systematic approach to the problem is extremely difficult and time consuming, and has not been achieved up to now, although Laub et al.[2] have suggested an interesting scheme with mixed stationary phases. We have previously explained why we prefer to use series of columns prepared with different stationary phases and how it is possible to predict retention data on such series of columns[3].

We describe here a systematic approach using some simple approximations and demonstrate its application to the analysis of mixtures of 40 polychlorinated biphenyls (PCBs) by optimizing the column temperature and the length of the second column of a series of two columns.

EXPERIMENTAL

Samples were prepared by dissolving 100 mg of Aroclor 1242 (Monsanto, St. Louis, MO, U.S.A.), which is a mixture of PCBs containing 42 % (w/w) of chlorine, in 1 ml of n-hexane.

Open-tubular columns manufactured from soda-lime glass were used. The inner surface of the glass tube was treated with gaseous hydrogen chloride using the procedure described previously[4]. The columns were coated with stationary phase using a dynamic procedure[5].

Column A

Apiezon L was purified by column liquid chromatography on alumina[6], and a 114-m glass capillary column of 0.25 mm I.D. was coated with 0.3 ml of a 10% solution of Apiezon L in n-hexane using the dynamic procedure with a mercury plug[5]. The column was divided into six 19-m long sections. The thickness of the stationary phase film was checked by measuring the capacity ratio for 2,5,2'-trichlorobiphenyl. The capacity ratios in the four middle parts were 1.70 ± 0.085. These four parts were connected by shrinkable Teflon tubing and a 75.6-m long column (A) was obtained.

Column B

A 90-m glass capillary column of 0.25 mm I.D. was coated with 0.3 ml of a 5% solution of polyethylene glycol 20.000 (Carbowax 20M) in dichloromethane, using the dynamic procedure with a mercury plug. The column was divided into six parts. The four middle parts formed columns B_1 (15.5-m long), B_2 (14.3 m), B_3 (14.7 m) and B_4 (12.1 m). These individual parts were successively connected with column A by shrinkable Teflon tubing to make series of two columns with a variable length of the second column[3].

TABLE I

CHARACTERISTICS OF THE COLUMNS USED, MEASURED WITH 2,5,2'-TRICHLOROBIPHENYL AT 180°C

Column designation	Characteristics*			
	$\bar{u}$ (cm/sec)	k'	n	N
A	13.90	1.70	287,500	114,000
A+B$_1$	12.60	1.76	349,500	142,000
A+B$_1$+B$_2$	12.86	1.86	387,000	164,000
A+B$_1$+B$_2$+B$_3$	13.60	1.92	419,500	181,000
A+B$_1$+B$_2$+B$_3$+B$_4$	14.00	2.04	452,500	204,000

* n = number of theoretical plates found for 2,5,2'-trichlorobiphenyl; $\bar{u}$ = average carrier gas (nitrogen) velocity; k' = capacity ratio for 2,5,2'-trichlorobiphenyl; N = number of effective plates $\left[N = n\left(\dfrac{k'}{1 + k'}\right)^2 \right]$.

The characteristics of the columns are summarized in Table I.

Equipment

A Fractovap Model 2300 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame-ionization detector and a sampling splitter was used, which allowed the easy use of glass capillary columns. The end of the column was carefully inserted into the detector jet to minimize the equipment contribution to band broadening. Nitrogen was used as the carrier gas.

Retention times for the calculation of Kováts retention indices were measured with a stopwatch.

The calculation of the optimal values of the temperature and column selectivity was performed using a Siemens 4004-150 computer and a FORTRAN program called OPTIM[7].

THEORETICAL

The optimal carrier gas velocities of the columns used were in the range 7–10 cm/sec for nitrogen and 25–30 cm/sec for hydrogen, in agreement with the findings of Schomburg et al.[8]. Further, a change in the velocity of nitrogen between 7 and 14 cm/sec caused a relative decrease in resolution of less than 10%, which is approximatively equal to the error of the resolution measurement and to the difference between the resolutions obtained with hydrogen and nitrogen. There is little need to optimize further that velocity except in an attempt to minimize the analysis time, which is not the topic of this work. Therefore, all the experiments were made using a stream of nitrogen at an average velocity around 13–14 cm/sec (cf., Table I).

The last two factors on the right-hand side of eqn. 1 depend greatly on the column temperature. The influence of temperature on the effective plate number:

$$N_j = n_j \left(\frac{k'_j}{1 + k'_j}\right)^2 \tag{2}$$

has been discussed previously[9].

The selectivity of the stationary phase is also a function of temperature. It can also be changed by using another liquid phase. Unfortunately, only a small number of liquid phases can be used to prepare good, efficient capillary columns. They do not mix easily and the preparation of mixed-phase open-tubular columns is impaired by wettability problems; glass has to be treated in different ways in order to be wetted properly by different stationary phases. The approach used by Laub et al. with packed columns[2] cannot, therefore, be used with capillary columns, whether well founded or not[3].

On the other hand, the ease with which open-tubular columns can be connected without any loss in efficiency, using shrinkable Teflon tubing[4], allows the systematic use of series of columns prepared with different stationary phases. This permits a virtually continuous change in the selectivity of column series, by changing the length of the second column[3]. The only problem will be that the longer the second column, the higher the efficiency of the column series, and this will have to be taken into account in the calculations.

Finally, our previous work has shown that, in practice, retention indices of many compounds increase linearly with the length of the second column, provided that this column is shorter than the first one[3]. This fact will make the calculations fairly simple. The retention index of a compound i, I_i, is defined as

$$I_i = 100z + 100 \cdot \frac{\log (t'_{R,i}/t'_{R,z})}{\log (t'_{R,z-1}/t'_{R,z})} \tag{3}$$

If compounds i and j are both eluted between the n-alkanes with z and $z + 1$ carbon atoms, the difference between their retention indices is

$$\Delta I = I_j - I_i = 100 \cdot \frac{\log \alpha_{ij}}{\log (t'_{R,z-1}/t'_{R,z})} \tag{4}$$

Combination of eqns. 1, 2 and 4 gives

$$R_{ij} = \frac{\sqrt{N_j}}{4} \left[1 - \left(\frac{t'_{R,z}}{t'_{R,z-1}} \right)^{\Delta I/100} \right] \tag{5}$$

When ΔI becomes small, R_{ij} tends towards zero. For most stationary phases $t'_{R,z-1}/t'_{R,z}$ is around 1.5. Hence we can approximate eqn. 5, for small differences between retention indices, by

$$R_{ij} \approx \frac{\sqrt{N_j}}{4} \cdot \frac{\Delta I}{100} \cdot \ln \left(\frac{t'_{R,z-1}}{t'_{R,z}} \right) \approx 10^{-3} \, \Delta I \sqrt{N_j} \tag{6}$$

This shows that a resolution of unity can be obtained between two compounds whose retention indices differ only by 1 if the effective plate number is 10^6, which is very high[8].

As we have shown above, the retention index can be written as a linear function of temperature and of the length of the second column of the series:

$$I_i = A_i + B_i T + C_i L + D_i \, LT \tag{7}$$

As the two partial differentials of I depend only on the other parameter, it is possible to calculate the four coefficients of eqn. 7 for a given compound from experimental data using the linear least-squares method.

If the components of the mixture are arranged in order of increasing retention indices, the difference

$$\Delta I_i = I_{i+1} - I_i \tag{8}$$

between the retention indices of two adjacent compounds gives an estimate of their resolution for a given system. As a first approximation it can be predicted that the two compounds will be resolved if ΔI_i exceeds a threshold δ, determined as a function of the desired resolution and of the range of the effective plate number of the column used for the separation of the components of the mixture. We know that for capillary columns the effective plate number increases very rapidly for column capacity factors between 0 and 1 and then much more slowly, so that the approximation will be valid for compounds that are significantly retained, but not for those which are eluted early and are difficult to separate anyway[10].

The basic tasks of the program will thus be as follows: (i) to calculate the retention indices of all compounds in a given set of conditions; (ii) to arrange compounds in order of increasing retention indices; (iii) to calculate all retention indices increments, ΔI_i; (iv) to determine how many increments are below the threshold and what is the lowest increment above the threshold.

Optimal conditions are those in which there is the smallest number of unresolved pairs of compounds. If there are several sets of conditions in which the numbers of such pairs are equal, the optimal conditions are those in which the smallest increment above the threshold is the largest (the compounds with an increment below the threshold are unresolved so that it is pointless to use their increments, unless the column can be made longer and therefore the threshold decreased).

Using experimental data covering a given range of temperature and column length (cf., eqn. 7), it is possible to calculate retention indices for any combination of T and L in this range. Thus, the calculation of optimal conditions is possible using a variety of techniques. It must be emphasized, however, that altough eqn. 7 is linear in L and in T, ΔI_i is not regular but exhibits discontinuities each time the order of elution of the components of the mixture is changed. The use of conventional optimization methods such as the simplex method becomes difficult and has not been contemplated at this stage.

Main characteristics of the calculation program

The main operations carried out by the program are the following:

(i) Reading retention indices measured for all components of the mixture at different temperatures and second column lengths. Calculation of the coefficients of eqn. 7 for each component.

(ii) For each selected value of temperature and the second column length chosen for this investigation, calculation of retention indices of all components. The compounds are then ordered according to increasing retention index and differences ΔI_i are calculated. Usually the network chosen for these calculations is regular, dense and contained in the range of temperatures and column lengths investigated in the measurements of experimental data.

(iii) The smallest ΔI_i is divided by the threshold δ, selected for the particular analysis. If the result is larger than 1, this result minus one $(\Delta I_i/\delta) - 1$ is the value of the optimization criterion for this particular set of conditions. If the result is smaller than 1, a value equal to -10 is taken for the optimization criterion and then the same operation is repeated for the second smallest ΔI_i. Again, if the result is larger than 1, $(\Delta I_i/\delta) - 1$ is added to the criterion and the program passes on to the next set of conditions; if the result is smaller than 1, -10 is added to the criterion and the next larger ΔI_i is considered.

The final value of the optimization criterion for a given set of experimental conditions is equal to -10 multiplied by the number of unresolved pairs $(\Delta I_i \leqslant \delta)$ plus the term $(\Delta I_i/\delta) - 1$ corresponding to the smallest I_i larger than the threshold.

(iv) The procedure described under operations (ii) and (iii) is repeated for all the sets of experimental conditions corresponding to the network defined by the instructions given to the computer.

(v) When the calculation is finished, the computer prints out the optimal experimental conditions (T and L), the corresponding value of the optimization criterion and the list of retention indices of all components of the mixture in increasing order under these optimal conditions.

The values of the optimization criterion under other conditions (for example, optimal column length at any given temperature), retention indices under any given conditions, regression coefficients or standard deviations can also be printed on request.

RESULTS AND DISCUSSION

This optimization procedure was applied to the analysis of the mixture of polychlorinated biphenyls (Aroclor 1242) on the glass capillary columns described above. Measurements were made on Apiezon L column A alone and then on series of columns made by connecting to column A the four sections of the Carbowax column B as indicated in Table I. All measurements were carried out at 170, 175 and 180°C.

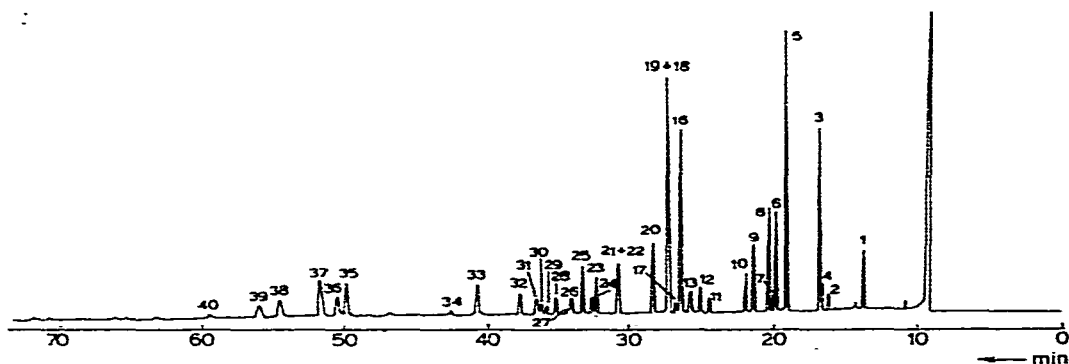As an example, a chromatogram showing the separation of the mixture of

Fig. 1. Separation of Aroclor 1242 on a glass capillary column coated with Apiezon L at 180°C. Peak numbers refer to the same compounds as those in all other figures and in Table II, except for Fig. 3. Peak identification is given in ref. 11.
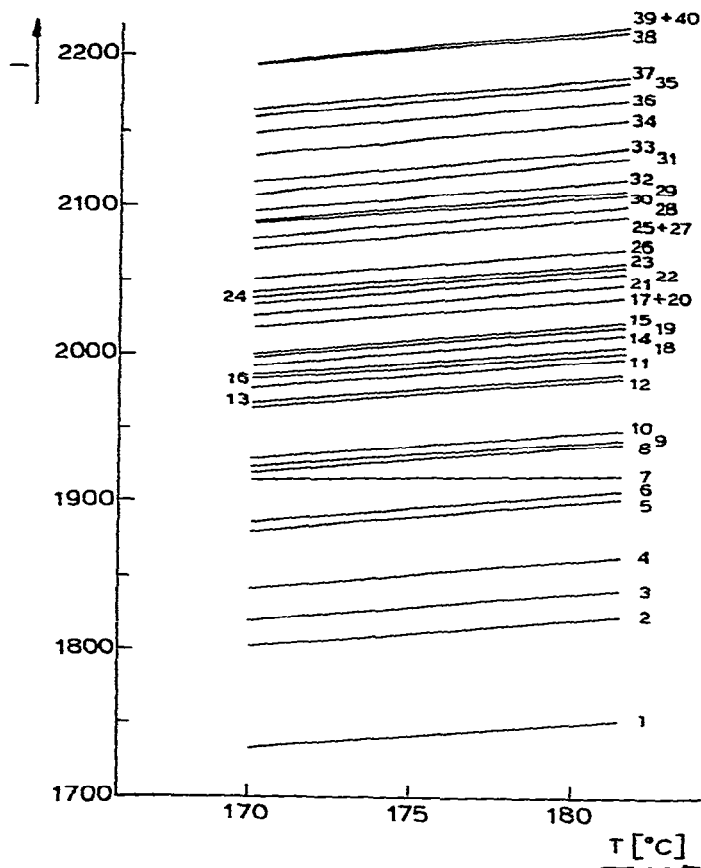
Fig. 2. Plots of retention indices (*I*) *versus* temperature (*T*) for PCBs, using column system A + B$_1$ + B$_2$ + B$_3$ + B$_4$ (*cf.*, Table I).

chlorinated biphenyls on column A at 180°C is shown in Fig. 1. Forty components of the mixture have been identified, but a few trace components have not. The numbers used are the same as those given elsewhere[3]. Several peaks on this chromatogram overlap and the resolution of a few other pairs is inadequate.

The influence of temperature on the separation of the 40 components identified in Aroclor 1242 is illustrated in Fig. 2, where plots of retention indices on columns A + B *versus* temperature are given for these compounds. We observe that although the resolution of several pairs of components could be improved by a decrease in temperature, a significant improvement can be obtained only by a marked decrease in temperature resulting in an excessively long analysis time; most of the straight lines in Fig. 2 are almost parallel.

When Aroclor 1242 is analysed on column B (B$_1$ + B$_2$ + B$_3$ + B$_4$), the separation of some pairs of PCBs improves in comparison with that obtained by analysis on the apolar column A but, as shown in Fig. 3, other components overlap. Note that the numbers in Fig. 3 do not correspond to those in Figs. 1 and 2 or those used below, because the peaks have not been all identified in this chromatogram.
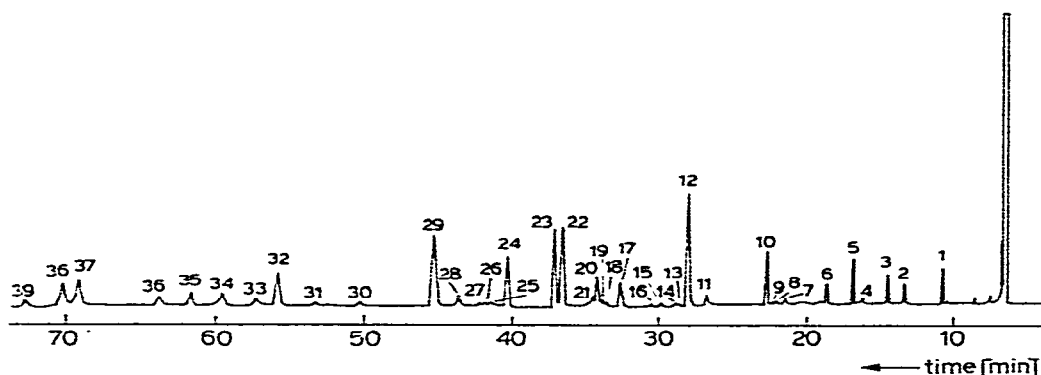
Fig. 3. Separation of PCBs on a glass capillary column coated with Carbowax 20M at 180°C. Peaks are still unidentified.

Similar problems were observed in the analysis of Aroclor 1242 on glass capillary columns coated with OV-101 silicone oil or with the hydrocarbon C_{87} phase[2,11]. Further, a considerable disadvantage in changing the stationary phase is the necessity to repeat the qualitative analysis of the mixture whenever a new phase is used. Moreover, the change in the stationary phase selectivity is discontinuous and cannot be controlled.

When the selectivity of the stationary phase is changed by connecting different column sections of increasing length but coated with the same polar stationary phase to a fixed, non-polar column, the selectivity changes with the length and film thickness of the added column. When sufficiently short parts of the polar column are used, the selectivity of the resulting column varies almost continuously. If the properties of the added columns (k') remain unchanged when their length is increased, the selectivity of the resulting column increases linearly with increasing length of the added column, as can be seen in Fig. 4, which shows graphs of retention indices of a column series versus the length of the Carbowax 20M column added to the fixed Apiezon L column.

The plots in Figs. 2 and 4 exhibit perfect linearity, in agreement with the correlation coefficients of both dependences, which are greater than 0.998 for all components. The difference in the slopes of the lines can be explained, inter alia, by the different number of chlorine atoms and by their various positions in the PCB molecules.

For the optimization of column temperature and the selectivity of the stationary phase system, we measured retention indices corresponding to fifteen different experimental conditions: three temperatures (170, 175 and 180°C) and five different lengths of the added column (cf., Table I). The network $T \times L$ was defined by the temperature range (170–180°C), the increment $\Delta T = 1$°C (altogether eleven chosen T-values), the range of length of the added column (0.0–60.0 m) and the increment $\Delta L = 0.5$ m (121 chosen L-values in all). Thus the investigated network consisted of 1331 points for each of the 40 components, and 53,240 values of retention indices were calculated by linear regression from 600 experimental measurements.

The threshold δ was derived from chromatograms recorded as follows. The
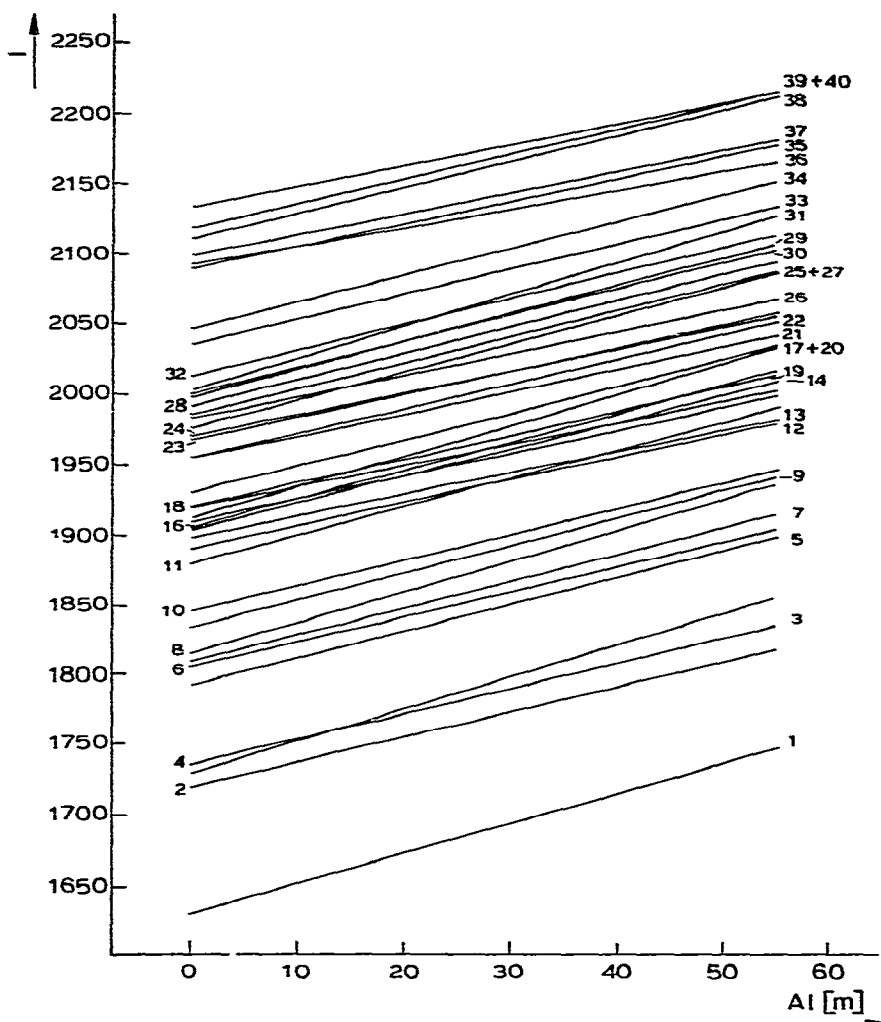
Fig. 4. Plots of retention indices (I) *versus* length of the Carbowax 20M column (A) coupled to a fixed Apiezon L glass capillary column at 180°C.

minimum degree of resolution between two peaks of equal size at which there appears a valley is 0.50. When we add sections of a polar column to increase selectivity we also increase efficiency. On column A the first serious interference problem occurs for $k' = 1.7$, and then $N = 114,000$ (cf., Table I). Thus, if we increase the column length and the apparent k' (ref. 3), the effective plate number will increase and we shall be able to resolve compounds with closer retention indices. With column A (Apiezon L), we found that at 170°C, log $(t'_{R,z+1}/t'_{R,z}) = 0.192$, and hence ln $(t'_{R,z+1}/t'_{R,z}) = 0.442$. Then for $k_j = 1.7$ and $N_j = 114,000$, to the R_{ij} value of 0.50 there corresponds a value $\Delta I_i = I_{i+1} - I_i = 1.34$, which was used as the threshold δ. The consequence of this approach is that the optimization procedure used tends to produce a greater polar column length than would be necessary simply on the basis of selectivity.

TABLE II

KOVÁTS RETENTION INDICES (I_i) OF PCBs FOUND UNDER OPTIMAL CONDITIONS (T_{opt} = 170°C, L_{opt} = 40.0 m): OUTPUT FROM COMPUTER

i	Peak reference number	I_i	ΔI^*
1	1	1701.80	
2	2	1777.50	6.70
3	3	1794.10	16.60
4	4	1809.03	14.93
5	5	1854.32	45.29
6	6	1862.12	7.80
7	7	1876.79	14.67
8	8	1888.46	11.67
9	9	1896.19	7.73
10	10	1902.21	6.02
11	12	1940.46	38.25
12	13	1943.93	3.47
13	11	1947.83	3.90
14	16	1959.11	11.28
15	18	1964.09	4.98
16	14	1965.57	1.48
17	15	1970.25	4.68
18	19	1972.95	2.70
19	17	1985.10	12.15
20	20	1990.67	5.57
21	21	2003.32	12.65
22	22	2009.44	6.12
23	24	2016.30	6.86
24	23	2018.07	1.77
25	26	2028.55	10.48
26	25	2041.77	13.22
27	27	2043.21	1.44
28	28	2051.18	7.97
29	29	2060.25	9.07
30	30	2061.19	0.94
31	32	2069.99	8.80
32	31	2075.83	5.84
33	33	2090.86	15.03
34	34	2107.41	16.55
35	36	2129.97	12.56
36	35	2137.79	7.82
37	37	2142.85	5.06
38	38	2168.45	25.60
39	39	2170.71	2.26
40	40	2174.82	4.11

* $\Delta I = I_{i-1} - I_i$.

The optimal conditions found in the analysis of Aroclor 1242 by the computer program are T_{opt} = 170°C and optimal length of added Carbowax column L_{opt} = 40.0 m. The value of the optimization criterion OPTTOT = −9.93 corresponds to one unresolved pair of peaks.
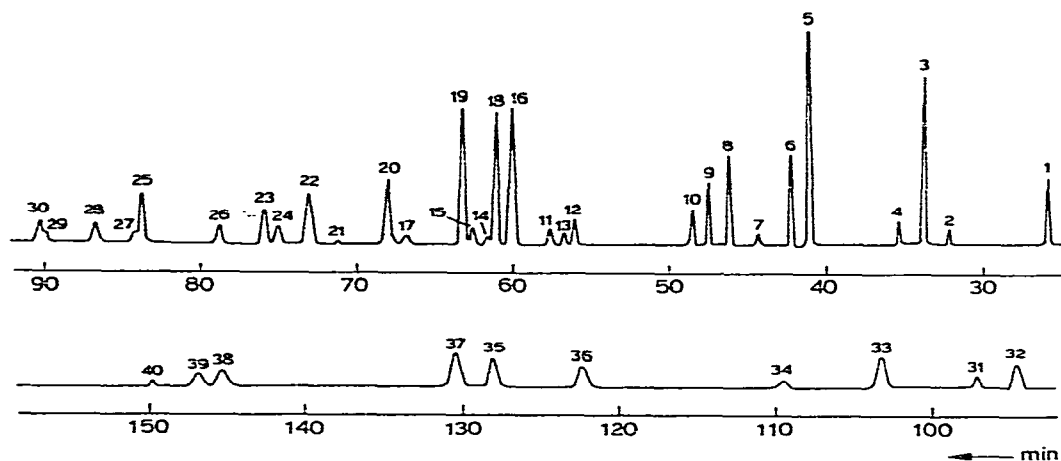
Fig. 5. Separation of Aroclor 1242 at the optimal temperature (170°C) and optimal column polarity (40.0 m Carbowax 20M capillary column connected to a 75.6 m Apiezon L glass capillary column).

Table II lists the retention indices calculated and ordered by the program, corresponding to the components of the PCB mixture under the optimal conditions. The difference between the retention indices corresponding to peaks 29 and 30 is 0.94 (Table II), which is less than the threshold (1.34), so that this pair of peaks is considered to be unresolved, which is in agreement with the poor resolution of these peaks on the chromatogram shown in Fig. 5: calculated $R_{ij} = 0.35$; from the chromatogram it seems that R_{ij}, which is closer to 0.5, may be slightly larger, as can be expected from the use of an almost twice as long column and a value of N_j probably close to 200,000 (Table II). We also observe in Fig. 5 the poor separations of peaks 25 and 27 ($\delta_i = 1.44$, $R_{ij} = 0.54$) and of peaks 18 and 14 ($\delta_i = 1.48$, $R_{ij} = 0.55$). The separation of these last two pairs is further complicated by the different concentration of the two compounds.

The optimization criterion introduced in this paper unambiguously prefers conditions at which the maximal number of peaks is resolved. This is ensured by a sufficiently large contribution to the value of the optimization criterion for each unresolved pair (-10) which, in the separation of PCBs, was considerably greater than the secondary part of the criterion: in the example discussed, OPTTOT = $1 \cdot (-10) + (1.44 - 1.34)/1.34 = -10 + 0.07 = -9.93$. Some difficulty can arise when the contribution of the secondary part is too big [i.e., if $(\delta_i - \delta)/\delta > 10$]; in such a case, however, the optimization problem would simply become optimization of a binary separation.

Finally, if the data are extrapolated to a lower temperature, we find that at 163°C the difference in retention indices for peaks 29 and 30 increases from 0.94 to 1.19, but this marginal improvement in separation is obtained at the cost of an increase in analysis time from 2.5 to almost 4 h.

LIST OF SYMBOLS

A, B, C, D	Coefficients in eqn. 7
I_i	Retention index of compound i (eqn. 3)
k_j	Column capacity factor for compound j (eqn. 1)
L	Column length (eqn. 7)
N_i	Number of effective theoretical plates for compound i (eqn. 2)
n_j	Number of theoretical plates for compounds j (eqn. 1)
R_{ij}	Resolution between the peaks of compounds i and j (eqn. 1)
$t_{R,i}$	Adjusted retention time of compound i (eqn. 3)
T	Temperature (eqn. 7)
z	Number of carbon atoms in a normal alkane (eqn. 3)
α_{ij}	Relative retention of compounds i and j (eqn. 1)
ΔI_{ij}	Difference between retention indices of compounds i and j (eqn. 4)
δ	Retention index difference threshold

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