Optimization of Individual Column Length of a Tandem Column System for the Isomer- and Enantiomer Selective Separation of Toxaphenes

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Introduction

Only some congeners present in technical toxaphene are bioaccumulated. However, the remaining number in e.g. marine biota is still large enough to cause severe signal overlaps when separating them simultaneously into isomers and enantiomers by gas chromatography (GC). Tandem columns allow to improve such separations. However, the empirical optimization of such a system (capillary lengths, temperature program) for a complex mixture is very time consuming, and the chance to eliminate critical co-elutions by this approach is rather small [1]. A theory was developed by Purnell et al. [1-4] to optimize the length combination of coupled columns for isothermal separations. This theory was applied to the separation of 22 toxaphenes by a tandem system consisting of a column with a newly developed achiral stationary phase and a enantioselective column with a modified β-cyclodextrine. Here, only a brief description of the approach is given. More details will be published elsewhere.

Material and Methods

Theory: The complete theory is presented in ref. 1-4. Therefore, only a short summary is given here. For two columns coupled in series, the total retention time t_R of an analyte is the sum of the times spent in the front (F) and back (B) column: $t_R=t_{RF}+t_{RB}$. According to [1] the overall retention factor k_{ov} can be expressed as $k_{ov}=[(t_{DF}/t_{DB})k_F+k_B]/[(t_{DF}/t_{DB})+1]$ where t_D is the dead time of the single columns. To predict k_{ov} for a congener, k_F and k_B of the individual columns have to be known as well as the ratio (t_{DF}/t_{DB}). However, the latter is not easily available for a tandem system because of the gas compressibility. Hildebrand and Reilley [5] defined a function R_F (resistance to flow), which allows to calculate the pressure p at the junction between front and back column from the inlet pressure p_i, the outlet pressure p_o, the length fraction $l_F=L_F/(L_F+L_B)$ (L_F and L_B are the lengths of the individual columns) and the total volume of the mobile phase V_M [1]. With these values k_{ov} for each congener can be calculated.

However, the overall capacity factor is usually not linear to l_F due to the non-linearity of the pressure drop. Therefore, a coefficient f has to be introduced, which linearly relates k_{ov} to the k values of the individual columns. On condition that $f_F+f_B=1$, k_{ov} can be expressed as $k_{ov} = k_F f_F + f_B k_B$. Both f_F and l_F can also be described as a function of p_o, p, p_i and other column parameter such as R_F, L and V_M. A plot of k_{ov} over the range of $f_F=0$-1 can be constructed, which connects the k values of each compound obtained from the individual columns (see Figure 2 above). This plot allows to determine relative retentions $\alpha=k_2/k_1$ at a given f_F for compounds to be separated. By plotting the α values for the most difficult separation vs. f_F, a window diagram can be constructed (see Figure 2 and ref. 4). The f_F-value at the maximum window height and/or the

most suitable window gives the α for the calculation of the best I_F value. The minimum number of theoretical plates required for a base-line separation N_{req} is obtained by the equation $N_{req}=36[\alpha/(\alpha-1)]^2\ [(1+k)/k]^2$. Together with the previously obtained results, this can be used to calculate both the overall tandem and individual column lengths.

Standards and chemicals: Solutions containing all 22 toxaphene congeners (Parlar no. 11, 12, 15, 21, 25, 26, 31, 32, 38, 39, 40, 41, 42, 44, 50, 51, 56, 58, 59, 62, 63 and 69) or only single congeners were used as described in [5]. Parlar no. 69 was not included into the calculations since no interferences occur to this last eluting congener. To facilitate the readability of Figure 1 and 2, the compounds were numbered according to the elution order on the achiral column:

No.	x	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Parlar no.	12,15,16	21	26	25	31	32	38	39	41	40	42.1	42.2	44	50	51	56	58	59	62	63

Instrumentation: Separations were carried out on a Hewlett-Packard 6890 gas chromatograph equipped with a ^{63}Ni electron capture detector. Helium was used as carrier gas and nitrogen as make-up gas at a flow rate of 100 ml/min. The temperature of the split/splitless injector was set to 160°C (see [6]) and of the ECD to 250°C. Splitless injections of 1-2 µl were performed manually at 100°C column temperature. The isothermal and splitless period was 1 minute followed by a steep ramp of 100°C/min to 230°C. This temperature was kept until the last congener eluted. The constant flow modus was used at an initial pressure of 1.6 bar. For chromatogram D in Figure 1 a heating rate of 5°C/min and an initial pressure of 1.27 bar was applied.

Flow rates were determined with a soap bubble meter. The inlet pressure was measured with a precision pressure gauge (No. 312.20.160, Manometer AG, Hitzkirch) and the outlet pressure with a MKS Baratron PDR-C-1B (Burlington, MA) barometer. Capacity factors were determined by replicate injections on the two columns. Dead times t_D were determined by butane injections. Repetitive measurement (n=15-30) were made to obtain average values of flow, the dead times, inlet and outlet pressures. Standard deviations were in the range of 0.15 %.

Capillary columns: The following columns were employed: 1) *Optima Delta 3 (OPT)*: 23.5 m length x 0.2 mm ID, coated with 0.35 µm methylbiphenylpolysiloxane (Macherey-Nagel, Switzerland). 2) *Enantioselective capillary (CD):* 30.35 m length x 0.25 mm i.d., coated with 0.2 µm of OV-1701-OH with 10% heptakis(2,3,6-O-t-butyldimethylsilyl)-β-cyclodextrin [7]. The columns were connected with a Connex Column Connector System (J & W Scientific).

Results and Discussion

The theory of Purnell et al. allows to optimize the relative length of tandem columns by using data obtained from single columns [1]. However, it requires an isothermal separation. This is not feasible for the toxaphenes. Therefore a very fast and short heating ramp (100°C/min) was applied to obtain isothermal conditions during most time of the separation.

In Figure 1 the chromatograms (A and B) of the single columns are shown. The "Opt" capillary is able to separate all toxaphene congeners. Except Parlar 58 (signal 17), the CD phase allows a complete separation into enantiomers. However, co-elutions of different enantiomers are observed. Figure 1C shows the chromatogram of the tandem combination CD-OPT with the capillaries of original lengths. Compared to B, a partial improvement was obtained such as an undisturbed separation of Parlar 50 (signal 14). However, due to an overall length of more than 50 m, very long retention times were observed and a partial or complete degradation of some congeners such as as signal no. 16 (Parlar 56), no. 17 (Parlar 58), no. 18 (Parlar 59) and no. 19 (Parlar 62).

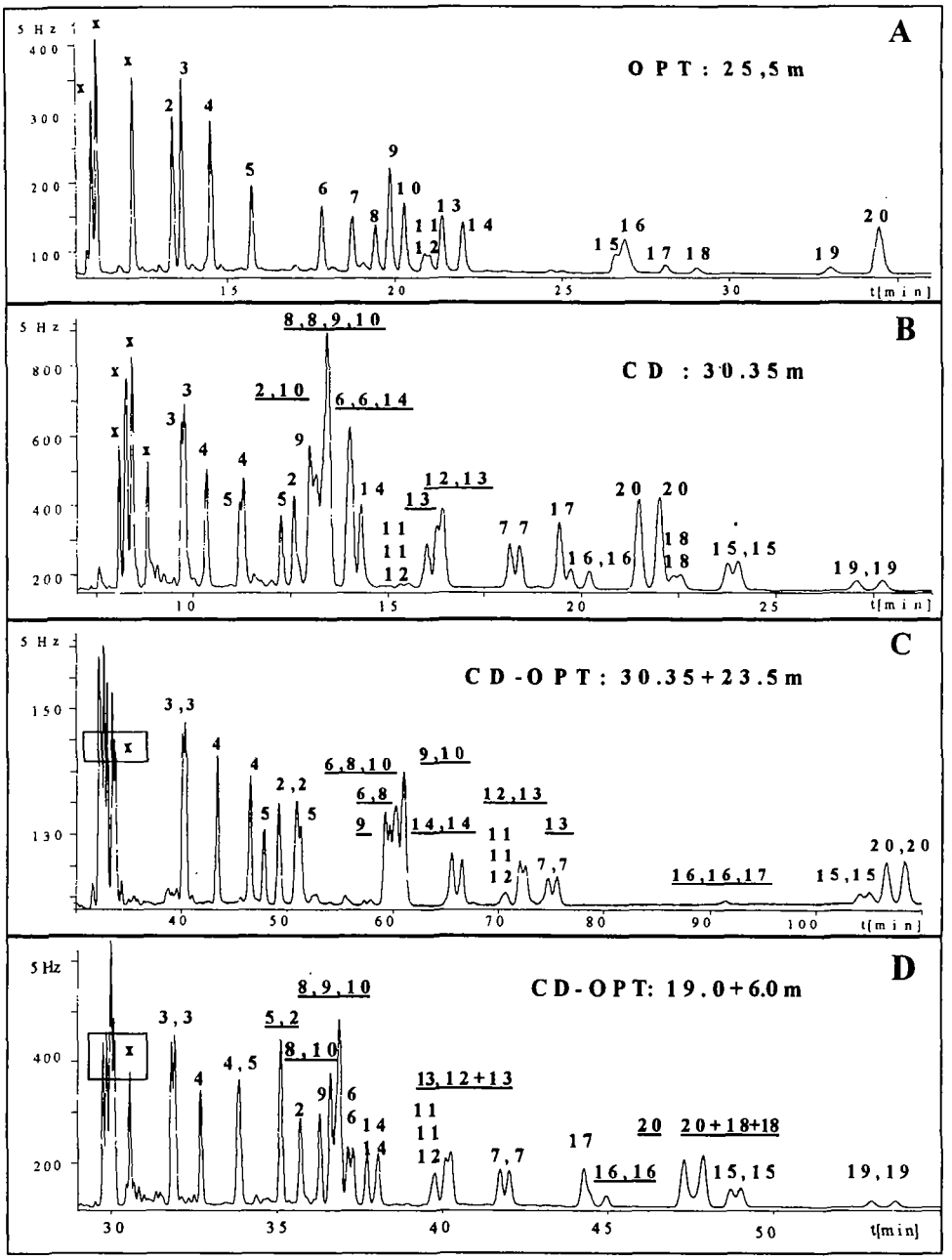

Figure 1: Gas chromatograms of the separation of the 22 toxaphene congeners on the single columns (A, B), on the tandem columns with the original single column length combination (C) and with the optimized length combination (D).

The k_{ov}-f_F plot given in Figure 2 (top) yields various information. Each f_F value corresponds to a length fraction and corresponding length combinations can be calculated as described above. The elution pattern obtained for a selected length combination corresponds to the intersection points of the k_{ov} lines with the vertical f_F-line. For instance, the vertical lines 1 and 2 are in accordance with the elution order of the chromatograms C and D.

Furthermore, an intersection of k_{ov} lines means an identical k_{ov} value for two substances and hence coelution. For instance, for the OPT capillary alone ($\rightarrow f_F=0$), all lines for enantiomers meet, which means no enantioselectivity. It is obvious from Figure 2 (top) that it is nearly impossible to find an f_F value and a respective length combination, which allows a complete separation of all isomers and enantiomers with the applied column system. This is confirmed by the window diagram in Figure 2 (bottom). The α values at maximum window height are very low and the windows are very narrow. For example, at f_F=0.4 (α=1.003) a total length of the tandem system of about 350 m is needed for a baseline separation of all congeners and enantiomers. Therefore, it was looked for a more realistic compromise. At f_F = 0.9 and α = 1.027 a simultaneous isomer and enantiomer selective separation of the so-called indicator congeners [8] should be achievable. Using the approach described under Theory, corresponding capillary lengths of 20 m (CD) and of 6 m (Opt) were calculated. Since each single column is cut into two pieces of different length, totally 8 combinations and corresponding elution patterns are possible.

However, as seen from the k_{ov}-f_F plot in Figure 2 (top), vertical line 2, for f_F = 0.9 the k_{ov}-lines are sometimes rather close together. The separation could be improved further by optimizing the temperature and pressure program (Figure 1D). Compared to the single CD column, the enantiomer signal pairs no. 6 (Parlar no. 32) and no. 14 (Parlar no. 50) are now completely undisturbed allowing an enantioselective determination of the four so-called indicator congeners. The price of this compromise are some new co-elutions, which showed up for other congeners. However, at a smaller heating rate (1°C/min) the congeners no. 2, 4, 5, 9 and 16 (Parlar no. 21, 25, 31, 41 and 56) can be well separated from the other congeners. The complete results of all the possible combinations CD-Opt and the inverted combinations will be presented later.

Acknowledgements

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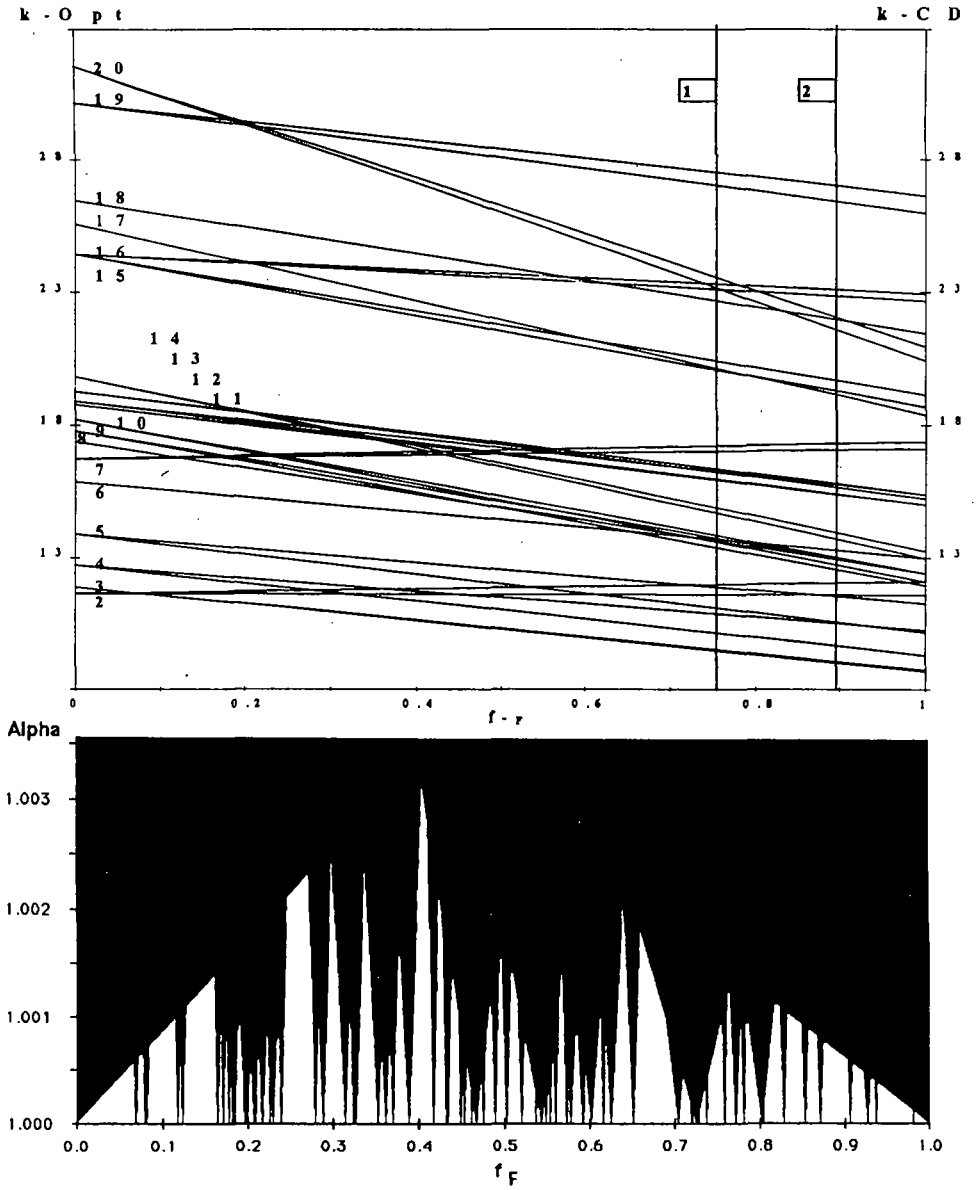

Figure 2: Plot of the retention factors k_F (front column = CD) vs. f_F (top) and plot of the relative retentions α vs. f_F (bottom) for the mixture of 22 toxaphenes.