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## OPTIMIZATION OF SELECTIVITY BY TUNING COLUMN TEMPERATURES FOR SERIES-COUPLED CAPILLARY COLUMNS IN DUAL-OVEN GAS CHROMATOGRAPHIC SYSTEMS

D. REPKA, J. KRUPČÍK and E. BENICKÁ

*Slovak Technical University, Faculty of Chemistry, Department of Analytical Chemistry, Radlinského 9, 812 37 Bratislava (Czechoslovakia)*

and

P. A. LECLERCQ and J. A. RIJKS\*

*Eindhoven University of Technology, Department of Chemical Engineering, Laboratory of Instrumental Analysis, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)*

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### SUMMARY

A method has been developed and evaluated for optimization of the selectivity of series-coupled capillary columns in a twin-oven gas chromatographic system in the shortest possible analysis time. The temperatures of either one or both columns were varied independently (partial optimization) or simultaneously (overall optimization), in order to separate the maximum number of sample components. The method is based upon computer-aided calculation of Kováts indices of the coupled columns by means of second and third order (polynomial) functions of the temperatures of the individual columns. The applicability and limitations of the method are discussed and illustrated with the separations of a synthetic hydrocarbon mixture of 33 components.

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### INTRODUCTION

Although high resolution gas chromatography (GC) is the method of choice for the separation of complex mixtures, the frequency of peak overlap is appreciable. There is increasing interest in the development of mathematical models and methods that do allow the prediction of the probable number of peaks that can maximally be distinguished in a given range of a chromatogram.

Obviously it would be highly desirable to reach the ultimate aim of capillary GC separation: maximum resolution and separation efficiency within the shortest time. Unfortunately, general optimization procedures aiming at these goals are rather complex, tedious and time consuming, particularly for complex samples. Therefore, in most optimization procedures only a limited number of these variables is included. In daily laboratory practice, the optimization of the separation temperature is an attractive and simple approach for tuning of columns with an appropriate selectivity and efficiency in isothermal GC. The column temperature has an effect on both efficiency and selectivity, as well as on solute retention. The effect of temperature on

selectivity is significant, particularly for polar phases and compounds. Therefore, the selectivity can be tuned by temperature control of series-coupled non-polar and polar capillary columns, whether they are coupled in a single<sup>1-3</sup> or a dual-oven GC system<sup>1,4,5</sup>.

The algorithms for temperature optimization of single and series-coupled columns in a one-oven GC system are fundamentally similar. Recently introduced procedures<sup>6-8</sup> for the optimization of the selectivity by selection of the best column temperature can be applied to both single as well as for series-coupled columns in a one-oven system. While these procedures are based upon Kováts retention indices, obviously literature data can be used for this purpose.

For selectivity tuning with series-coupled columns in different GC ovens, so far no procedures are available, although the potential of this approach has been recognized<sup>2-5</sup>. In this paper, a computer-aided method is introduced for the optimization of the selectivity of series-coupled columns in a twin-oven GC instrument. This method is based on tuning the temperatures of the individual columns with the aim to separate the maximum number of solutes in the shortest possible time.

## THEORETICAL

As was previously shown<sup>7</sup>, the optimization of the column temperature for series-coupled columns in a single-oven system appears rather simple. This procedure is based upon Kováts retention indices,  $I$ , which are considered<sup>9</sup> to depend linearly on column temperature,  $T$ .

$$I_{i,T} = A_i + B_i T \quad (1)$$

where  $A_i$  and  $B_i$  are constants and  $i$  is the solute under investigation.

For the difference in retention indices,  $\delta I_{j,i}$ , between two adjacent peaks  $j$  and  $i$ , it follows from eqn. 1:

$$\delta I_{j,i} = A_j - A_i + (B_j - B_i)T \quad (2)$$

This difference is related to the resolution,  $R_{j,i}$ , according to<sup>7</sup>

$$\delta I_{j,i} = \frac{400 R_{j,i}}{\sqrt{N_j} \cdot \ln \alpha} \quad (3)$$

where  $N_j$  is the effective plate number for peak  $j$ , and  $\alpha$  the relative retention of two consecutive  $n$ -alkanes.

The number of peaks,  $m$ , that can be distinguished in the range of interest in the chromatogram is the main criterion for optimization. Whether peaks are considered separated depends on a minimum difference in Kováts indices,  $\delta_{\min} = \delta I_{j,i,\min}$ , compatible with the required (desired) resolution. The column temperature (one-oven system) or combination of column temperatures (twin-oven system), corresponding to the maximum number of peaks,  $m_{\max}$ , is considered optimal. If more than one (partial) optimum is observed, the one corresponding to the shortest retention time is considered to be the best choice. As expected, and according to recently published

papers<sup>2,4,5,10</sup>, the temperature dependence of Kováts retention indices of series-coupled columns in a twin-oven GC system is not linear. The separation of a given sample can be optimized, in the sense described above, by trial-and-error or simplex sequential methods. However, many experiments are needed. Therefore, and for reasons of efficiency, suitable polynomial equations with empirically determined constants were selected, in order to calculate overall retention indices of the series-coupled columns at any temperature or combination of temperatures of the individual columns. These polynomials can be used to predict the number of peaks in a defined region of the temperature ranges of the individual columns.

## EXPERIMENTAL

### *Instrumentation*

The GC system with two independently controlled ovens consisted of two instruments, a Fractovap 2350 and a Fractovap 4180 respectively (Carlo Erba Instrumentazione, Milan, Italy). They were interfaced using a separately heated stainless-steel tube (150 mm × 1.5 mm I.D., wall thickness 0.3 mm), inserted in a glass tube (120 mm × 2.5 mm I.D., wall thickness 1.0). The column inlet of the first column (column A, placed in the Fractovap 2350 gas chromatograph) was connected to an all-glass inlet stream splitter injection port. The outlet of column A was led through the interface to the Fractovap 4180 gas chromatograph, where it was coupled to the inlet of column B using PTFE shrinkable tubing. The outlet of column B was inserted into the jet of the Fractovap 4180 flame ionization detector. The detector signal was fed to an electrometer model EL 480 (Carlo Erba), and recorded by a computing integrator Chromatopac C-R3A (Shimadzu, Kyoto, Japan). Nitrogen was used as the carrier gas with a constant inlet pressure of 170 kPa (absolute).

The inlet splitter was operated with a splitting ratio of 1:100. A sample of hydrocarbons (see Table I) was diluted in acetone to 0.2% (w/w) per compound. A 1- $\mu$ l volume of the diluted sample was injected. The gas hold-up time was measured by injecting methane.

Curve fitting and optimization calculations were performed on an HP 85B microcomputer (Hewlett-Packard, Palo Alto, CA, U.S.A.). The facilities of the BASIC language were enlarged using a Matrix ROM, an Advanced Programming ROM and a Printer/Plotter ROM (all from Hewlett-Packard).

### *Columns*

Two glass capillary columns were used, both made of soda lime glass (Unihost, Teplice, Czechoslovakia). The inner glass surface was etched with gaseous hydrogen chloride at 330°C during 24 h, prior to coating. Column A (40 m × 0.3 mm I.D.) was statically coated with a 0.2% solution of SE-30 in dichloromethane. Column B (50 m × 0.3 mm I.D.) was coated dynamically with a 5% solution of Carbowax 20M in dichloromethane, using a mercury plug forced by compressed nitrogen.

## RESULTS AND DISCUSSION

Generally there are three modes for the optimization of the selectivity of double-oven tandem capillary GC columns by variation of the column temperatures of

TABLE I  
COMPOSITION OF THE HYDROCARBON MIXTURE USED

Peak No.	Name
1	2,3,4-Trimethylpentane
2	2,2,5-Trimethylhexane
3	<i>n</i> -Octane
4	2,3,5-Trimethylhexane
5	2,4-Dimethylheptane
6	4,4-Dimethylheptane
7	3,5-Dimethylheptane ( $\alpha,\beta$ )
8	3,3-Dimethylheptane
9	2,3-Dimethylheptane
10	3,4-Dimethylheptane ( $\alpha$ )
11	3,4-Dimethylheptane ( $\beta$ )
12	3,3-Diethylpentane
13	Isopropylbenzene
14	<i>n</i> -Nonane
15	4,4-Dimethyloctane
16	<i>n</i> -Propylbenzene
17	2,6-Dimethyloctane
18	3,3-Dimethyloctane
19	3,4-Diethylhexane
20	2-Methyl-3-ethylpentane
21	1,3,5-Trimethylbenzene
22	1,2,4-Trimethylbenzene
23	<i>tert.</i> -Butylcyclohexane
24	1,2,3-Trimethylbenzene
25	<i>n</i> -Decane
26	<i>sec.</i> -Butylcyclohexane
27	1,3-Diethylbenzene
28	<i>n</i> -Butylbenzene
29	<i>n</i> -Butylcyclohexane
30	1,4-Diethylbenzene
31	1,2-Diethylbenzene
32	1,3-Dimethyl-4-ethylbenzene
33	<i>n</i> -Undecane

one or both columns: (1) varying only the second column temperature; (2) varying only the first column temperature; (3) simultaneous variation of both column temperatures. For the first two modes, the dependences of Kováts indices,  $I_{AB}$ , upon temperatures,  $T_A$  or  $T_B$ , have been reported<sup>4,5</sup>. The last mode had not been presented. All these possibilities are discussed in the following.

#### *Optimization by varying only the second column temperature*

Keeping the first column temperature constant,  $T_A = 60^\circ\text{C}$ , the temperature of the second column was varied between 50 and  $150^\circ\text{C}$  in  $10^\circ\text{C}$  steps. Kováts indices,  $I_{AB,i}$ , of the hydrocarbons of the synthetic mixture (33 components) were measured on the coupled columns at any temperature of the second column. The experimental results are presented in Fig. 1.

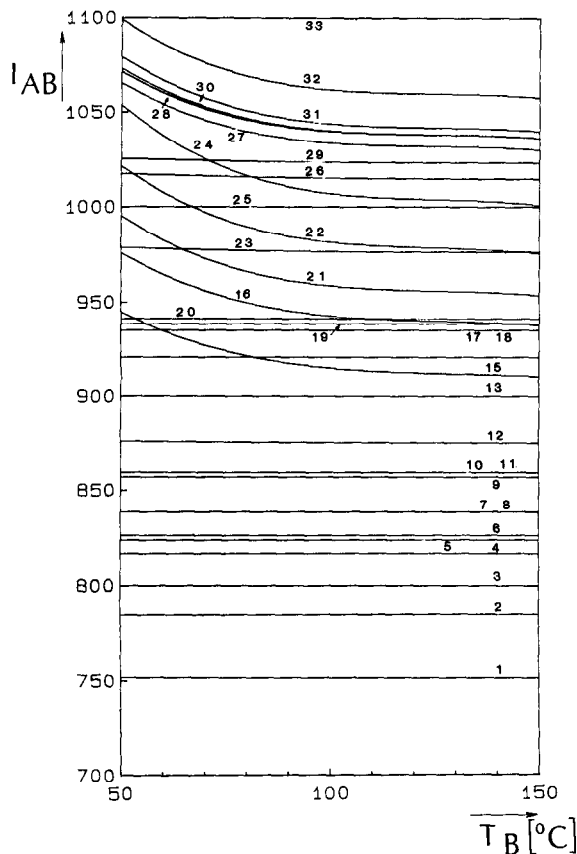


Fig. 1. Plot of Kováts retention indices of series-coupled columns in a twin-oven GC system versus column temperature of the second (polar) column at a constant ( $T_A = 60^\circ\text{C}$ ) temperature of the first (non-polar) column. The line numbers correspond to the component numbers given in Table I.

Regression analysis has shown that each curve in Fig. 1 can be described by the following polynomial:

$$I_{AB,i}(T_B, 60^\circ\text{C}) = A_{0,i} + A_{1,i}T_B + A_{2,i}T_B^2 + A_{3,i}T_B^3 \quad (4)$$

The coefficients were determined, for each compound  $i$ , by fitting eqn. 4 to the experimental results. Subsequently, retention indices were calculated for all the sample constituents in the range of 50–150°C in 1°C steps for column B. The number of peaks that can be distinguished was also calculated, using a threshold,  $\delta_{\min} = \delta I_{j,i,\min}(60^\circ\text{C}, T_B)$ , corresponding to a resolution,  $R_{j,i,\text{req}} = 1.0$ . The average  $\delta_{\min}$  value used for the determination of the number of peaks separated appeared to be about 2 I.u. for capacity factors larger than 2. The maximum number of peaks that can be separated at different temperatures of the second column is shown in Fig. 2. Obviously, according to the main criterion given in the Theoretical, more than one optimum is observed. Taking into account the second criterion, *i.e.*, minimum separation time, the optimum

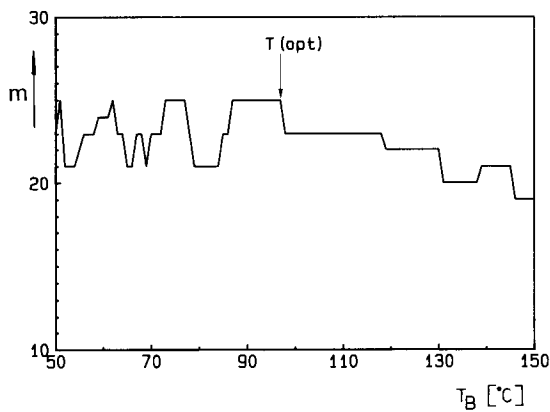


Fig. 2. Number of separated peaks,  $m$ , versus temperature of the second column,  $T_B$ , at constant temperature ( $T_A = 60^\circ\text{C}$ ) of the first column.

temperature,  $T_{B,\text{opt}}$ , in this case is  $97^\circ\text{C}$ . The chromatogram obtained under these conditions is depicted in Fig. 3.

#### Optimization by varying only the first column temperature

Following a similar procedure to that described above, the temperature of the first column,  $T_A$ , was varied from 60 to  $130^\circ\text{C}$  in  $10^\circ\text{C}$  steps at a constant temperature,  $T_B = 50^\circ\text{C}$ , of the second column. An equation similar to eqn. 4 was fitted to the experimental results. Calculated Kováts indices [ $I_{AB}(T_A, 50^\circ\text{C})$ ] at  $1^\circ\text{C}$  intervals are plotted versus the second column temperature in Fig. 4. The maximum number of peaks for different temperatures of the first column is presented in Fig. 5. Also in this case different optima are observed. The criterion of the shortest analysis time shows  $T_A = 80^\circ\text{C}$  to be the optimum temperature for the first column. The chromatogram

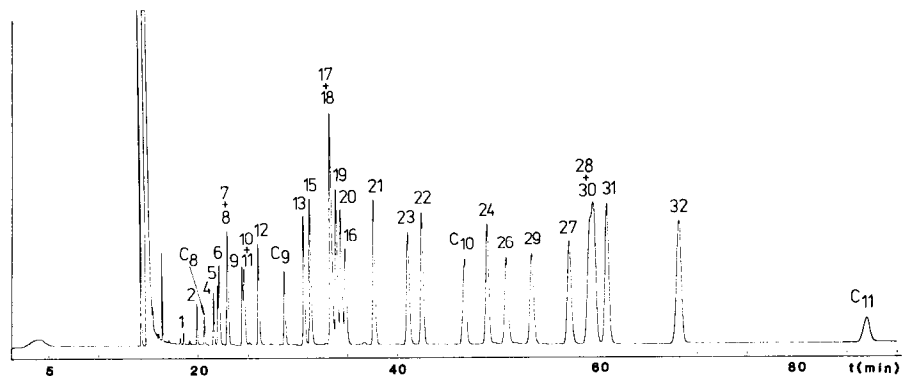


Fig. 3. Separation of the hydrocarbon sample in tandem capillary columns at the optimum temperature of the polar column,  $T_{B,\text{opt}} = 97^\circ\text{C}$ , at a constant temperature of the non-polar column ( $T_A = 60^\circ\text{C}$ ). For peak numbers see Table I.

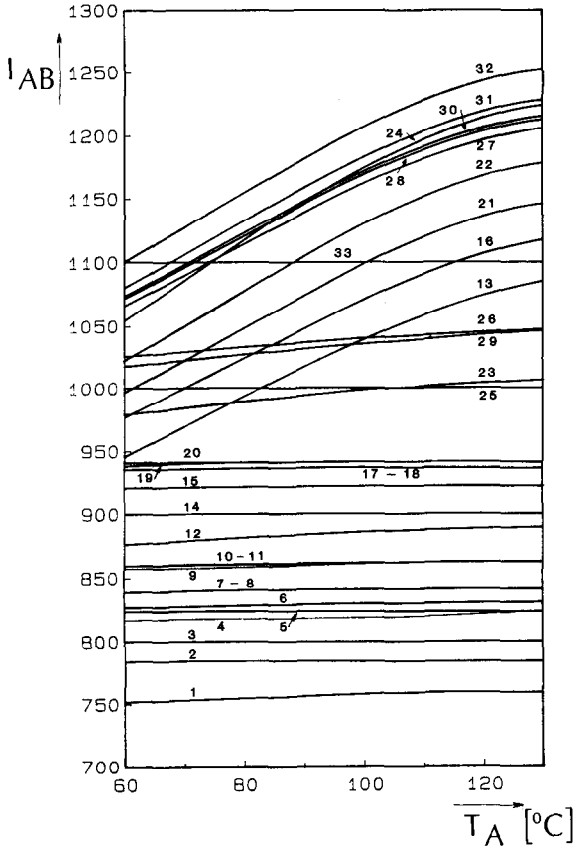


Fig. 4. Plot of Kovats retention indices of series-coupled columns in a twin-oven GC system *versus* column temperature of the first (non-polar) column at a constant temperature ( $T_B = 50^\circ\text{C}$ ) of the second (polar) column. The line numbers correspond to the component numbers given in Table I.

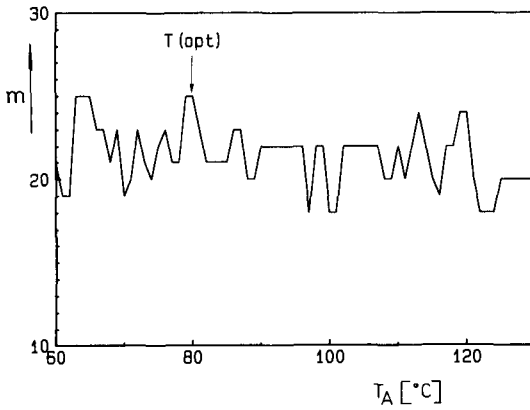


Fig. 5. Number of separated peaks,  $m$ , *versus* temperature of the first column,  $T_A$ , at a constant temperature of the second column,  $T_B = 50^\circ\text{C}$ .

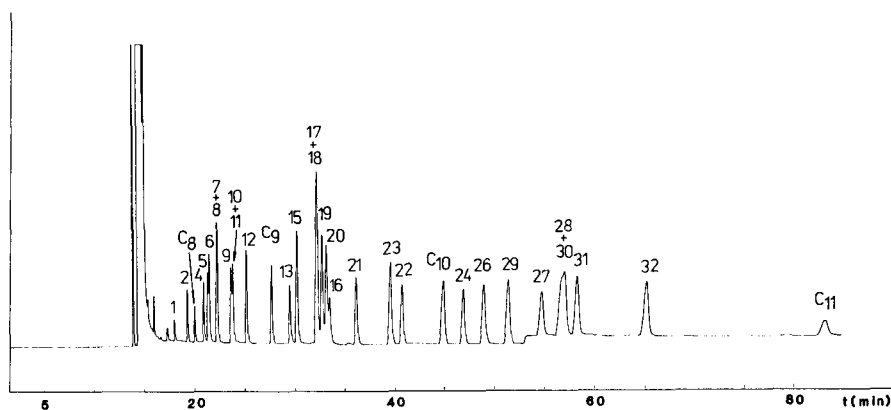


Fig. 6. Chromatogram of the hydrocarbon mixture at the optimum temperature of the non-polar column,  $T_A = 80^\circ\text{C}$ , at a constant temperature of the second column,  $T_B = 50^\circ\text{C}$ . Peak numbers correspond to the numbers given in Table I.

corresponding to this optimum is given in Fig. 6. Comparing the chromatograms under optimum conditions, Figs. 3 and 6 show that obviously 25 peaks are separated in both cases. However, different peak pairs are considered separated according to the criteria given in the Theoretical, in both cases.

#### *Optimization by simultaneous variation of both column temperatures*

In order to limit the number of experiments, in this case both column temperatures were varied in the interval  $60\text{--}100^\circ\text{C}$  according to a three level orthogonal design as shown in Table II. For the calculation of the Kováts indices the following equation was used to approximate the experimental data:

$$I_{AB,i}(T_A, T_B) = A_{0,i} + A_{1,i}T_A + A_{2,i}T_A^2 + B_{1,i}T_B + B_{2,i}T_B^2 + C_iT_AT_B \quad (5)$$

In the same way as discussed above, optimum temperatures calculated for the first and

TABLE II

TEMPERATURES OF THE FIRST ( $T_A$ ) AND THE SECOND ( $T_B$ ) COLUMNS AS PROPOSED BY A THREE LEVEL ORTHOGONAL DESIGN

<i>Exp. No.</i>	$T_A$ ( $^\circ\text{C}$ )	$T_B$ ( $^\circ\text{C}$ )
1	80	80
2	100	100
3	100	60
4	60	100
5	60	60
6	80	100
7	80	60
8	100	80
9	60	80



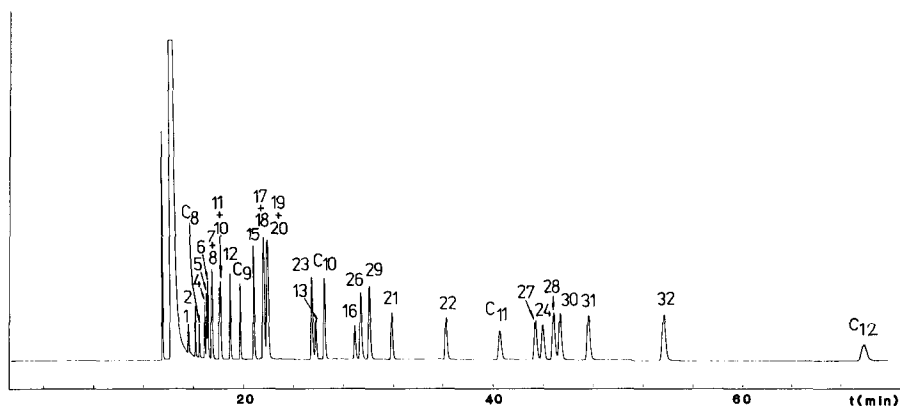


Fig. 7. Separation of the hydrocarbon sample in tandem capillary columns at the optimum selectivity tuned by simultaneous optimization of the temperatures of the non-polar column,  $T_{A,opt} = 62^{\circ}\text{C}$ , and the polar column,  $T_{B,opt} = 100^{\circ}\text{C}$ . Peak numbers correspond with the numbers given in Table I.

second columns appeared to be  $62^{\circ}\text{C}$  ( $T_{A,opt}$ ) and  $100^{\circ}\text{C}$  ( $T_{B,opt}$ ). The separation of the hydrocarbon mixture under these conditions is shown in Fig. 7.

As with the foregoing methods, optimization procedure 3 accidentally also yields an optimum where 25 hydrocarbons were separated with  $R_{j,i,req} = 1.00$  or better, but the analysis time was shorter. The equations presented proved to be very useful to approximate Kováts retention indices; the agreement between calculated and experimental indices in all instances appeared to be within  $\pm 1$  I.u. From Figs. 3, 6 and 7, changes in peak retention order at the respective optimum temperatures are obvious, cf., peak pairs 19–20 and 28–30.

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