

Enhanced possibilities for identification using series-coupled capillary gas chromatographic columns

II. Retention indices as an identification tool in selectivity tuning

T. MAURER, W. ENGEWALD* and A. STEINBORN

Department of Chemistry, Analytical Centre, Karl Marx University Leipzig, Talstrasse 35, DDR 7010 Leipzig (G.D.R.)

ABSTRACT

For calculating retention (t_R) times from retention indices in series-coupled column systems with different flow-rates in the individual columns, the dependence of the dead time and of the parameters a and b of the $\log t_R$ versus carbon number plots for n -alkanes on the pressure of the medium is discussed. It is shown that the intercept a of the $\log t_R$ versus carbon number plots is composed additively of an easily determinable flow-independent term and a flow-dependent term. On this basis, a method was developed for the precalculation of the retention times of any substances from their retention indices under the conditions of selectivity tuning by varying the flow-rates in a system of series-coupled GC columns. The method allows statements on the presence or absence of compounds of interest by comparing calculated and measured system retention times. By repeating the identification algorithm at different pressure settings and, consequently, flow-rates, the peak shifts expected in selectivity tuning can be pursued on the one hand; on the other, a degree of certainty about the positive identification of compounds can be achieved owing to the feasible avoidance of peak overlapping, which is not possible by means of the classical retention index concept.

INTRODUCTION

High-resolution gas chromatography (HRGC) implies the optimization of the column efficiency, the selectivity and the capacity ratio. With the introduction of capillary columns, the main emphasis was laid on their high efficiency, whereas the optimization of the selectivity was not so closely observed for a long period. Only when it was recognized that many samples were more complex than had been originally thought did the necessity arise to produce capillary columns with specialized selectivity. A system with specialized selectivity can be obtained by selectivity tuning.

Sandra *et al.*¹ suggested that in capillary GC selectivity tuning can be accomplished in one of three ways: by using tailor-made stationary phases; by using mixed phases; or by serial coupling of columns with different stationary phases.

The last variant seems to be the most elegant method for producing a tunable selectivity because, in addition to the column length and the phase ratio, β , as variables, the very easily accessible variables temperature and/or carrier gas flow-rate can be used for setting the desired selectivity, and this method offers the chance to use standardized stationary phases with known retention properties and retention values.

However, the use of selectivity tuning for changing the selectivity in a series-coupled system places high demands on the identification in the sense of refinding the peaks in the individual chromatograms because in the case of selectivity changes it is always necessary to take account of a change in the retention sequence of components and, consequently, also of the removal of peak overlappings and the occurrence of new interferences.

Therefore, considerable experience and possibly also a certain amount of intuition are necessary for analysing complex mixtures if optimum separation is to be achieved. This especially applies to series-coupled systems in which the number of values to be varied is greater. This makes searching for a resolution optimum a very time-consuming process and it is desirable that the extent of experiments for finding this optimum should be considerably reduced by suitable simulation programs.

One precondition for optimization is the knowledge of the connection between retention and pressure of the medium or flow-rates in the column system used. This topic has been discussed in a few papers (see refs. 2 and 3 and papers cited therein). Another possibility is the use of system retention indices in directly series-coupled systems for identification⁴⁻⁷. However, these system indices have the disadvantages that they are not directly correlated with a thermodynamic value, that they are dependent on the behaviour of the flow-rates in the two columns and that the function on which their determination is based is not linear but, depending on the individual columns used, a more or less bent curve⁸. As an alternative to this we suggest that the system retention times should be calculated in advance by means of known (tabulated) retention index values of the substances on the two individual columns and that identification be achieved by comparing measured and precalculated retention times at different pressure settings of the medium.

In this way it should be possible to use with advantage the existing valuable experience with retention values for many substances on different stationary phases in the form of Kováts retention index libraries, in spite of the known disadvantages of the retention index system².

For this purpose, the fundamentals for the conversion of tabulated index data into retention times are presented. In the following, it will be shown how the identification of sample components (in the sense of refinding already known compounds or for establishing the presence or absence of compounds of interest) can be achieved under the conditions of selectivity tuning by comparing measured and precalculated system retention times.

EXPERIMENTAL

The schematic representation of a series-coupled system in Fig. 1 shows the values necessary for its description and their indices.

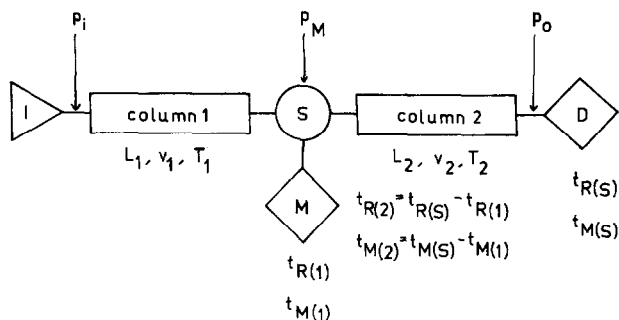


Fig. 1. Schematic diagram of the series-coupled two-column system used without intermediate trapping. I = Sample injection; S = coupling device (live T-piece) with additional carrier gas supply; M = monitor detector (FID 1); D = main detector (FID 2).

The instrument used was a Sichromat 2 (Siemens, Karlsruhe, F.R.G.), equipped with a split injector, a live T-piece and two flame ionization detectors. The carrier gas was hydrogen.

In system A (*a* values), column 1 was fused silica, 40 m × 0.32 mm I.D., containing WG-11 (WGA, Pfungstadt, F.R.G.) and column 2 was fused silica, 50 m × 0.32 mm I.D., containing WG-PB1 (OV-1), with temperatures and pressures as indicated in Table I. In system B (prediction), column 1^a was glass, 67 m × 0.24 mm I.D., containing 2-[4-(4-pentylbenzoyloxy)phenyl]-5-hexylpyrimidine (PBHP)⁹ and column 2 was glass, 32 m × 0.32 mm I.D., containing OV-1, with the temperatures and pressures as indicated in Fig. 4 and Table II.

RESULTS AND DISCUSSION

Dead times in series-coupled column systems

As is known, in a series-coupled column system without intermediate trapping, both the retention times (t_R) and the dead times (t_M) for the total system are composed additively of the contributions for the individual columns:

$$t_{M(S)} = t_{M(1)} + t_{M(2)} \quad (1)$$

$$t_{R(S)} = t_{R(1)} + t_{R(2)} \quad (2)$$

$$t'_{R(S)} = t'_{R(1)} + t'_{R(2)} \quad (3)$$

The dead time of a capillary separation column is dependent on its geometrical dimensions and the average linear flow-rate of the carrier gas, which is determined by the difference in the pressure existing at the inlet and the outlet of the column. The dead time can be calculated according to the following equation³:

$$t_M = \frac{32 L^2 \eta(T)}{3 r^2} \cdot \frac{p_i^3 - p_o^3}{(p_i^2 - p_o^2)^2} \quad (4)$$

^a The column was made available by courtesy of Dr. G. Kraus of the Chemistry Department of the Martin Luther University, Halle, G.D.R.

where L is column length, $\eta(T)$ is carrier gas viscosity, r is column radius, p_i is inlet pressure and p_o is outlet pressure.

It will be useful to insert flow-dynamically ascertained values for the length or the inside diameter of the columns in eqn. 4. If the manufacturer's data on the length and inside diameter are used, they should be checked experimentally by comparing the calculated and the measured dead times under defined pressure conditions.

In principle, eqn. 4 is also valid for calculating the dead times of the individual columns in a system of series-coupled columns. In this instance, the pressure of the medium, p_M , set at the coupling piece should be inserted as the column outlet pressure of the first and as the column inlet pressure of the second separation column.

If the pressure of the medium, p_M , is increased by feeding additional carrier gas into the connecting piece, the flow-rate of the mobile phase in column 2 will increase while the flow-rate in column 1 will correspondingly be reduced. Consequently, the dead time $t_{M(1)}$ becomes greater and $t_{M(2)}$ smaller when p_M is increased (Fig. 2). Therefore, the schematic representation of the system dead time $t_{M(S)}$ as a function of the pressure of the medium shows a minimum, the position of which is dependent on the parameters of both columns.

The system dead-time curve can be described mathematically either by an approximation polynomial of the fourth degree⁷ or in a simpler way by making use of eqns. 1 and 4:

$$t_{M(S)} = \frac{32 L_1^2 \eta_1 (p_i^3 - p_M^3)}{3 r_1^2 (p_i^2 - p_M^2)^2} + \frac{32 L_2^2 \eta_2 (p_M^3 - p_o^3)}{3 r_2^2 (p_M^2 - p_o^2)^2} \quad (5)$$

Parameters of the $\log t_R$ versus carbon number plot for *n*-alkanes

The possibility of calculating retention times on the basis of retention indices with the aid of the coefficients a and b of the $\log t_R$ versus carbon number plot was briefly dealt with in Part I¹⁰. However, as usually the opposite method is used, *i.e.*,

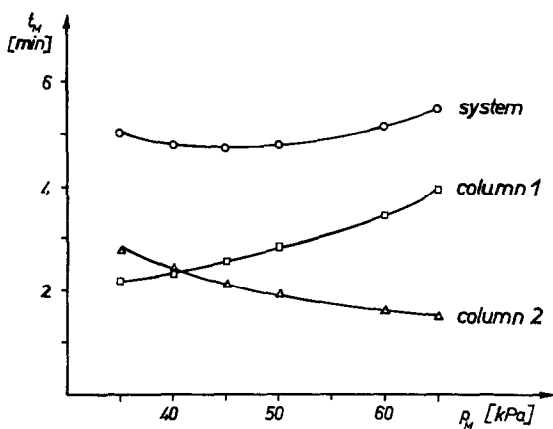


Fig. 2. Dependence of the carrier gas hold-up times $t_{M(1)}$, $t_{M(2)}$ and $t_{M(S)}$ on the pressure of the medium, obtained by using column system A. The $t_{M(1)}$ and $t_{M(S)}$ values are measured using the monitor and main detector and the $t_{M(2)}$ values are obtained according to eqn. 1.

calculating retention indices by means of retention times, the theoretical foundations of this process will be briefly described here.

The retention index concept according to Kováts is based on the, for higher n -alkanes, undisputed linear increase in the logarithm of the adjusted retention time (t'_R) with increase in carbon number for higher n -alkanes:

$$\log t'_R = a + bZ \quad (6)$$

where Z is the carbon number of the n -alkane used and the constants a and b are the intercept and the slope, respectively, of the n -alkane plot.

By means of regression methods¹¹, the constants a and b can be easily calculated from the retention times of an n -alkane mixture.

The slope $b = \log [t'_{R(Z+1)}/t'_{R(Z)}]$ is correlated with the partial molar free sorption energy of the methylene group in the n -alkane¹². According to this, b is dependent on the structure of the stationary phase and on the column temperature¹³ but is independent of the set flow-rate.

Nothing has been said in the literature so far about the thermodynamic importance of constant a . However, it can be easily shown that, amongst other things, the dependence of the retention times on the set flow-rate will be incorporated in constant a . For the precalculation of retention times under the conditions of selectivity tuning by varying the flow-rates, it is therefore necessary to know the dependence of the a value on the carrier gas flow-rate or a value proportional to it.

If on both sides of eqn. 6 the term $\log t_M$ is subtracted:

$$\log t'_R - \log t_M = a - \log t_M + bZ \quad (7)$$

the logarithm of the k' value of the respective n -alkane will be obtained on the left-hand side of eqn. 7. As this is a flow-independent value, the term $a - \log t_M$ on the right-hand side must also be independent of flow-rate. If this flow-independent "basic value" is termed a_k , the following connections are valid for an n -alkane of carbon number Z :

$$\log k' = a_k + b + Z \quad (8)$$

$$\log t'_R = a + b + Z \quad (9)$$

$$a = a_k + \log t_M \quad (10)$$

According to eqn. 10 a is composed additively of a flow-independent term a_k and the flow-dependent term $\log t_M$.

The validity of this relationship was examined using a C_6 - C_{11} n -alkane mixture. The a_k values of the individual columns were determined at $p_M = 50$ kPa according to eqn. 8 from the capacity factors of the n -alkanes. Table I shows the high degree of agreement of the a values precalculated for various pressures of the medium with the corresponding experimental values.

Using as a basis the well known fact that the retention index (I) of any chosen substance i can be considered to be 100 times the carbon number of a hypothetical n -alkane with the same retention time, Haken *et al.*¹⁴ formulated:

$$I_i = 100(\log t'_{R,i} - a)/b \quad (11)$$

TABLE I

DEPENDENCE OF THE DEAD TIMES $t_{M(1)}$ AND $t_{M(2)}$ ON THE PRESSURE OF THE MEDIUM, p_M , AND COMPARISON OF PREDICTED AND MEASURED a VALUES WITH COLUMN SYSTEM A AT $T_1 = T_2 = 100^\circ\text{C}$ AND $p_i = 100$ kPa

p_M (kPa)	$t_{M(1)}^a$ (min)	$t_{M(2)}^a$ (min)	$a_{k(1)}$	Log $t_{M(1)}$	$a_{t(1)calc.}$	$a_{t(1)meas.}$	$a_{k(2)}$	Log $t_{M(2)}$	$a_{t(2)calc.}$	$a_{t(2)meas.}$
35	2.21	2.80		0.79	-6.14	-6.17		1.02	-4.32	-4.31
40	2.39	2.45		0.87	-6.06	-6.11		0.90	-4.44	-4.47
45	2.58	2.19		0.95	-5.98	-5.95		0.78	-4.56	-4.57
50	2.85	1.98	-6.93	1.05	-5.88	-5.88	-5.34	0.68	-4.66	-4.66
60	3.49	1.67		1.25	-5.68	-5.70		0.51	-4.83	-4.85
65	3.97	1.53		1.38	-5.55	-5.60		0.43	-4.91	-4.91

^a Dead times $t_{M(1)}$, $t_{M(2)}$ were calculated by a regression method according to Kaiser and Rackstraw¹¹.

The inversion of this equation and combination with eqn. 10 results in

$$\log t'_{R,i} = a + 0.01bI_i \quad (12)$$

$$\log t'_{R,i} = a_k + \log t_M + 0.01bI_i \quad (13)$$

This offers a chance of the precalculation of retention times of any substances from their retention indices at different flow-rates, on the condition that the parameters a_k and b of the n -alkane straight line were determined at one given flow-rate.

Precalculation of system retention times at different pressure settings in a series-coupled GC system

On the basis of the fundamentals presented above for the calculation of dead time from column parameters and of retention times from tabulated retention indices, a program was developed which allows the prediction of system retention times in a series-coupled column system at different pressure settings, thus offering new possibilities both for pursuing the peak shifts required in selectivity tuning and for the reliable proof of the presence or absence of substances of interest in complex mixtures.

The program is based on the following conditions: the geometrical parameters, *i.e.*, length and inside diameter, of the separation columns used are sufficiently accurately known or have been determined according to flow-dynamic methods; the stationary phases used are standard phases with different retention properties, which can be generated in a reproducible form and for which retention index libraries are available which should be as comprehensive as possible; and on each individual column the chromatogram of an n -alkane mixture was recorded at the operating temperature and at one defined inlet and outlet pressure of the column. Fig. 3 shows the structure of the program in principle.

First, the parameters a_k and b of the n -alkane plot are calculated for both columns on the basis of the retention times of the n -alkane runs. Then the dead times for the individual columns are precalculated for the pressure settings of the coupled system for which the retention times of any substances are intended to be precalcu-

INPUT

```

column parameters for the
single columns:
length: L1, L2
column radius: r1, r2
temperature: T1, T2

retention indices of the compounds
on both single columns:
I11 . . . I1N1
I12 . . . I1X2

retention times of a single
n-alkane-run on both columns:
tR11 . . . tRN1

tR12 . . . tRN2

inlet , medium and outlet pressure
used for the n-alkane run
p1, pM, p0

inlet , medium and outlet pressure
settings for the prediction of
system retention times:
p1*, pM*, p0*

```

CALCULATIONS

```

-regression parameters for the n-
alkane plots at p1, pM, p0 (on both
columns) a1, a2, ak1, ak2, b1, b2:
log tR = a + b / Z
log k' = ak + b / Z

-dead times for the first and second
column:

tM(1) = 32 L1^2 η p1^3 - pM^3 /
3 r1^2 (p1^2 - pM^2)^2

tM(2) = 32 L2^2 η pM^3 - p0^3 /
3 r2^2 (pM^2 - p0^2)^2

-calculation of a values for the
calculated dead times:
a1 = ak1 + log tM(1)
a2 = ak2 + log tM(2)

-predicted retention times on the
single columns:
tR(1) = exp(a1 + b1 / I11 / 100) + tM(1)
tR(2) = exp(a2 + b2 / I12 / 100) + tM(2)

-predicted system retention times:
tR(S) = tR(1) + tR(2)

```

OUTPUT

```

system retention
times of the
compounds at the
pressure settings
p1*, pM*, p0*

```

Fig. 3. Computer program for calculation of retention times for series-coupled columns.

lated. Then follows the calculation of the a values from the a_k values according to eqn. 10.

All the conditions have been given for calculating the retention times on the individual columns for all the components of interest in the mixture (whose retention indices must, of course, be known on both individual columns). The addition of these precalculated adjusted retention times to the likewise precalculated dead times for the two columns yields the system retention times to be expected for the substances of interest at the chosen pressure setting.

Fig. 4 shows the chromatogram of an alkylbenzene test mixture at different pressure settings of the medium, p_M , and constant inlet pressure, p_i .

The applied column system B is a combination of two glass capillary columns with a significantly different separation powers. The stationary phases used included a liquid-crystalline phase (PBHP⁹) in which the solute retention is determined by its geometry (shape or length to breadth ratio), and a methylsilicone (OV-1) stationary phase in which the retention is determined by the vapour pressure (boiling point) of the solute. The retention indices given in the legend to Fig. 4 demonstrate the different elution sequences resulting for the test substances used. The liquid-crystalline stationary phase has an excellent selectivity for *meta* and *para* isomers which, as is known, cannot be or can hardly be separated on non-polar stationary phases.

The chromatograms in Fig. 4 show some different selectivities of the system which can be achieved with the indicated values of the pressure of the medium, p_M , i.e., with different shares of the two columns to the total selectivity of the system. A retention inversion of the *o*-xylene-isopropylbenzene pair can be observed. In

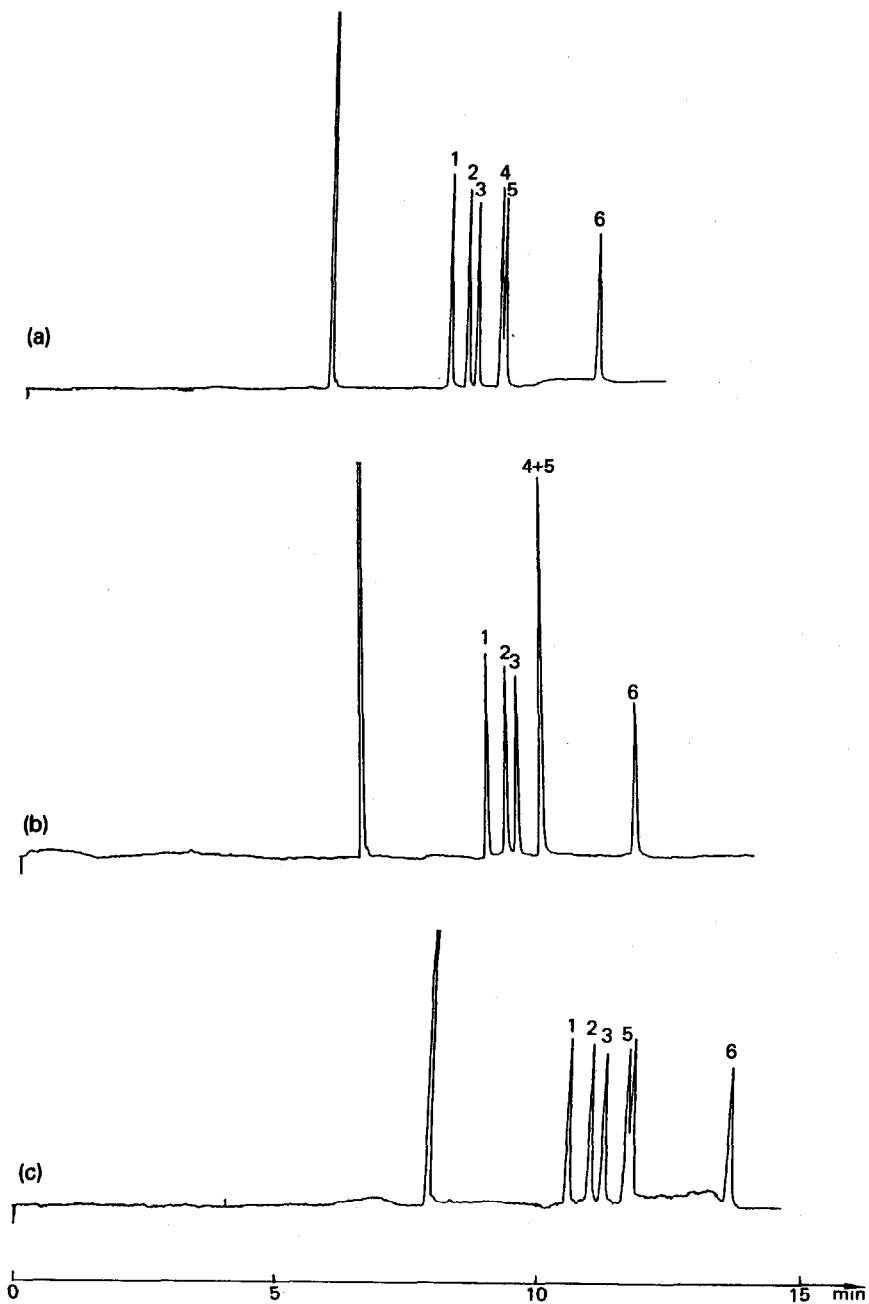


Fig. 4. Chromatograms of some alkylbenzenes obtained with column system B at $T_1 = T_2 = 90^\circ\text{C}$ by means of the main detector. $p_1 = 110$ kPa; $p_M =$ (a) 40, (b) 50 and (c) 58 kPa.

Peak No.	Compound	t^{OV-1} (90°C)	t^{PBHP} (90°C)
1	Ethylbenzene	857.3	951.5
2	<i>m</i> -Xylene	865.2	974.1
3	<i>p</i> -Xylene	866.7	986.9
4	<i>o</i> -Xylene	887.3	1005.1
5	Isopropylbenzene	920.7	991.2
6	<i>tert.</i> -Butylbenzene	980.9	1043.9

TABLE II

COMPARISON OF PREDICTED AND MEASURED SYSTEM RETENTION TIMES, $t_{R(S)}$, FOR THE SERIES-COUPLED COLUMN SYSTEM B AT DIFFERENT PRESSURE SETTINGS OF THE MEDIUM

For experimental conditions, see Fig. 4.

Compound	$p_M = 40 \text{ kPa}$			$p_M = 50 \text{ kPa}$			$p_M = 58 \text{ kPa}$		
	$t_{R(S)} \text{ meas.}$ (min)	$t_{R(S)} \text{ calc.}$ (min)	Δt^a (s)	$t_{R(S)} \text{ meas.}$ (min)	$t_{R(S)} \text{ calc.}$ (min)	Δt^a (s)	$t_{R(S)} \text{ meas.}$ (min)	$t_{R(S)} \text{ calc.}$ (min)	Δt^a (s)
Ethylbenzene	10.525	10.57	3	11.297	11.35	3	12.395	12.30	5
<i>m</i> -Xylene	10.958	10.99	2	11.759	11.71	3	13.010	12.92	5
<i>p</i> -Xylene	11.188	11.22	2	12.022	11.98	3	13.314	13.23	5
<i>o</i> -Xylene	11.765	11.83	4	12.589	12.58	1	13.938	13.90	3
Isopropylbenzene	11.869	11.95	5	12.589	12.58	1	13.826	13.80	2
<i>tert.</i> -Butylbenzene	14.294	14.33	2	14.993	15.06	4	16.191	16.08	6

$$^a \Delta t = t_{R \text{ meas.}} - t_{R \text{ calc.}}$$

Table II the predicted retention times are compared with the measured retention times.

The good agreement between the precalculated and the measured values confirms the fundamental suitability of the method presented. The error in predicting the retention time is less than 2% and is partially due to the fact that for this first example the outlet pressure p_o was considered to be constant and equal to the standard pressure.

CONCLUSIONS

The method presented for the precalculation of the retention times of any substances under the conditions of selectivity tuning by varying the flow-rates in a system of series-coupled GC columns allows the identification of components expected in a mixture by comparing the precalculated and the measured system retention times.

By repeating the identification algorithm at different pressure settings and, consequently, flow-rates it is possible, on the one hand, to pursue the peak shifts expected in selectivity tuning (identification in the sense of a refinding of already known compounds under changed chromatographic conditions according to the repetition of the analysis on a second and third column with changed polarity). On the other hand, owing to the avoidability of overlapping peaks, a degree of reliability can be reached in the positive identification of compounds which is not possible with the classical retention index concept. The combination of such a GC identification system with a spectrometric detector should permit a reliable qualitative analysis in many instances.

A clear advantage of the method suggested is the unlimited applicability of already existing retention index libraries for single-column systems.

An extension of the method by including selectivity tuning by varying the column temperatures is currently under investigation. Further, there are interesting

possibilities of coupling this method with an optimization algorithm for finding the column combinations and pressure settings that allow the maximum separation of a mixture of known composition.

REFERENCES

- 1 P. Sandra, F. David, M. Proot, G. Dirricks, M. Verstappe and M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 782.
- 2 J. V. Hinshaw and L. S. Ettre, *Chromatographia*, 21 (1986) 561, 669.
- 3 J. H. Purnell and P. S. Williams, *J. Chromatogr.*, 292 (1984) 197.
- 4 J. Krupcik, G. Guiochon and J. M. Schmitter, *J. Chromatogr.*, 213 (1981) 189.
- 5 T. Toth and F. Garay, in P. Sandra (Editor), *Proceedings of the 8th International Symposium on Capillary Chromatography, Riva del Garda, May 19–21, 1987, 1987*, pp. 585–595.
- 6 D. Repka, J. Krupcik, E. Benicka, P. A. Leclercq and J. A. Rijks, *J. Chromatogr.*, 463 (1989) 243.
- 7 R. E. Kaiser and R. I. Rieder, *Labor-Praxis*, 9 (1985) 1465.
- 8 T. Maurer, Th. Welsch and W. Engewald, *J. Chromatogr.*, 471 (1989) 245.
- 9 E. Matisova, E. Kovacicova, J. Garaj and G. Kraus, *Chromatographia*, 27 (1989) 494.
- 10 W. Engewald and T. Maurer, *J. Chromatogr.*, 520 (1990) in press.
- 11 R. E. Kaiser and A. J. Rackstraw, *Computer Chromatography*, Vol. 1, Hüthig, Heidelberg, 1983.
- 12 R. V. Golovnya, *Chromatographia*, 12 (1979) 533.
- 13 G. Tarjan, S. Nyiredy, M. Györ, E. R. Lombosi, T. S. Lombosi, M. V. Budahegyi, S. Y. Meszaros and J. M. Takacs, *J. Chromatogr.*, 472 (1989) 1.
- 14 J. K. Haken, M. S. Wainwright and R. S. Smith, *J. Chromatogr.*, 133 (1977) 1.