

CHROM. 7202

CALCULATION AND APPLICATION OF THE RETENTION INDICES IN PROGRAMMED-TEMPERATURE GAS CHROMATOGRAPHY

P. MAJLÁT

State Institute for Pharmacy, Budapest (Hungary)

and

Z. ERDÖS and J. TAKÁCS

Institute for General and Analytical Chemistry, Technical University, Budapest (Hungary)

SUMMARY

Temperature programming, consisting of programmed and isothermal periods, is often used in gas chromatography, and in this paper it is shown how to calculate retention indices according to each period in combined programmed-temperature and isothermal gas chromatography.

INTRODUCTION

It is well known that the lack of pure standards makes the qualitative evaluation of chromatograms very difficult if one does not possess the instruments (IR spectrometer, mass spectrometer, etc.) associated with the gas chromatograph that aid in qualitative identifications by giving specific signals for different materials. Because of the high price of such instruments, more simple methods are usually employed in qualitative evaluations and the retention index system of Kováts¹ has proved to be especially useful. In recent years, many researchers have dealt with various problems of this system, and particularly important is Van den Dool's and Kratz's work² on the application of Kováts's retention indices in programmed-temperature gas chromatography, and also the work of Harris and Habgood³. In practice, the number of gas chromatographic analyses carried out by using temperature programming, generally combined programmed-temperature and isothermal gas chromatography, is increasing rapidly. For the analysis of wide boiling range mixtures, including samples of natural origin, the most suitable programmed-temperature period(s) and isothermal period(s) for the composition of the samples must be established. In such instances, qualitative evaluations of the chromatograms become extremely difficult without using pure standards, and we have therefore studied the application of Kováts's retention indices under these conditions.

THEORETICAL

Before studying the theoretical basis of the problem, we must consider the

various possible combinations, as these determine the calculation procedure. In principle, there are the following possibilities:

- (1) programmed heating (generally linear) followed by isothermal elution at the optimal temperature;
- (2) isothermal elution followed by programmed heating;
- (3) isothermal elution and programmed heating with the same or different heating rates alternately;
- (4) a programme involving different heating rates in succession;
- (5) isothermal elution preceding or following programmed heating at different heating rates.

There appear to be a large number of variations, but virtually all of them consist of the same elements, *viz.* isothermal and programmed-temperature steps, so that if the influence of these elements on the retention indices can be established, we shall have made a significant contribution to the solution of the problem.

There are two equations of theoretical importance:

(1) for calculating retention indices under isothermal conditions, the Kováts's equation:

$$I = 100 \left[\frac{\log V_{N(x)} - \log V_{N(z)}}{\log V_{N(z+1)} - \log V_{N(z)}} + z \right] \quad (1)$$

(2) the equation derived by Van den Dool and Kratz² for conditions of linear temperature programming:

$$I_{PTGC} = 100 \left[\frac{T_{r(x)} - T_{r(z)}}{T_{r(z+1)} - T_{r(z)}} + z \right] \quad (2)$$

where

I = the isothermal retention index (index units);
 V_N = the net retention volume of the carrier gas (ml):

$$V_N = jF(t_R - t_M) \quad (3)$$

j = compressibility factor according to James and Martin⁴;
 F = flow-rate of carrier gas (ml/min);
 t_R = retention time (min);
 t_M = retention time of an inert gas (gas hold-up time) (min);
 x = symbol for the unknown component;
 z and $z+1$ = symbols for normal alkanes with z and $z+1$ carbon atoms, respectively;
 T_r = retention temperature in programmed-temperature gas chromatography.

In addition to using these equations, we can begin with one of the basic premises of the retention index system, namely that only the gas chromatographic behaviours of three components (the component of interest and the two normal alkanes eluted immediately before and after this component) have to be followed. In connection with the elution of normal alkanes, it should be noted that the retention index system includes the possibility that the component of interest and one of the two normal alkanes are eluted at the same time.

Considering the theoretical possibilities of combined programmed-temperature and isothermal gas chromatography, we can ascertain the following:

(1) All three components emerge during the same period; this is the most favourable and the simplest case;

(2) two components emerge during the same period but the third is eluted during the previous or the following period; examination of this case is comparatively difficult;

(3) each component emerges during different periods; this is the most difficult case but luckily it occurs very rarely.

These three cases plus two others, and the different possibilities within each, are considered below.

(1) In the first case, there are the following possibilities:

(a) All three components are eluted during the isothermal step which follows a programmed-temperature step started at the beginning of the run.

The symbols for this temperature programming are shown in Fig. 1a. (We shall not discuss the case when all components are eluted during the initial programmed-temperature step because the Van den Dool and Kratz equation² can be applied in this case.)

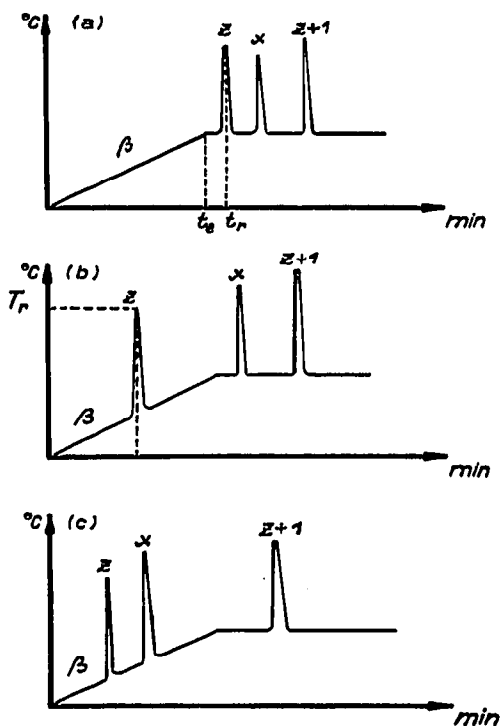


Fig. 1. (a) The three components are eluted during the isothermal step that follows a programmed-temperature step started at the beginning of the run. (b) One of the components emerges during the programmed-temperature period started at the beginning of the analysis while the other two components are eluted during the isothermal period. (c) Two components emerge during the programmed-temperature period started at the beginning of the analysis while one component is eluted during the isothermal period.

In case (a), supposing the flow-rate of carrier gas does not change during the temperature programming, the net retention volumes of components can be calculated according to the equation

$$V_N = F \left[\frac{\int_{P_0}^{P_c} j dP \cdot (t_e - t_{M^p}) + j_r t_r}{P_c - P_0} \right] \quad (4)$$

where

F = flow-rate of carrier gas (ml/min);

P_0 = relative pressure drop (P) belonging to the initial temperature of the programme;

P = p_i/p_0 ;

p_i = carrier gas pressure at column inlet (kp/cm²);

p_0 = carrier gas pressure at column outlet (kp/cm²);

P_c = relative pressure drop belonging to the terminal temperature of the programme;

j = gas compressibility correction factor according to James and Martin⁴;

t_e = the time from the introduction of the sample to the end of the programme (min);

t_{M^p} = the time taken for the inert gas to traverse the whole system under programmed-temperature conditions (min);

j_r = the compressibility correction factor of the isothermal period that follows the programmed-temperature period;

t_r = the time from the end of the programme to the appearance of the component in maximum concentration (min).

The value of the integral of eqn. 4 has been given in one of our recent papers⁵ and we have pointed out that the value of this integral can generally be substituted by the arithmetical mean value of the corresponding compressibility factors.

(b) One (Fig. 1b) or two (Fig. 1c) of the components emerge(s) during the programmed-temperature period started at the beginning of the analysis, while the other two or one of the three components are eluted during the isothermal period.

The net retention volumes of the components eluted during the programmed-temperature step can be calculated according to the equation

$$\log V_{N(\text{PTGC})} = \frac{\int_{T_0}^{T_r} \log V_N dT}{T_r - T_0} = A + \frac{2.3B \log \left(\frac{T_r + C}{T_0 + C} \right)}{T_r - T_0} \quad (5)$$

where

T = the column temperature (°K);

A , B and C = constants of equations describing the dependence of the logarithms of the net retention volumes on column temperature⁶.

The calculation of the net retention volume(s) of the component(s) eluted during the isothermal period that follows a programmed-temperature step has already been discussed (eqn. 4).

(2) In the second case, there are the following possibilities:

(a) All three components are eluted during the programmed-temperature period that follows the isothermal step (Fig. 2a).

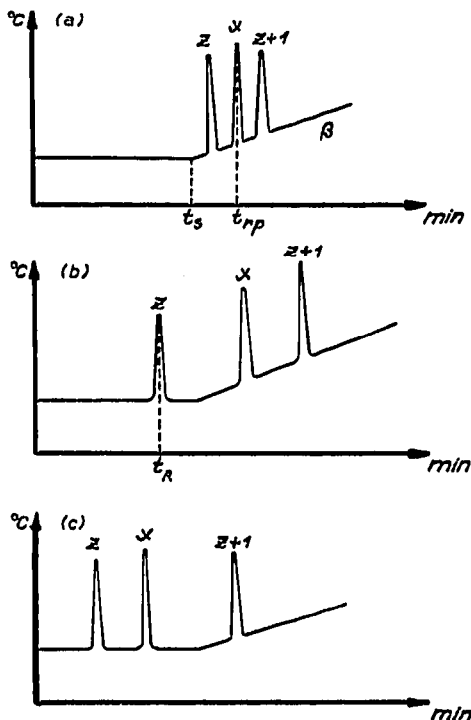


Fig. 2. (a) The three components are eluted during the programmed-temperature period that follows the isothermal step. (b) One component is eluted during the isothermal period while the other two components emerge during the programmed-temperature period that follows the isothermal step. (c) Two components are eluted during the isothermal period while one component emerges in the programmed-temperature period.

(We shall not discuss the case when all three components emerge during the isothermal period as the retention indices can be calculated by eqn. 1 in this case.)

As the same amounts of carrier gas have flowed through the system relative to all three components until the time of the starting of the programme, the corrected and reduced partial values of the net retention volumes will also be the same. The calculation of the net retention volume(s) of the component(s) eluted during the programmed-temperature period can be solved by the equation

$$V_N = F \left[j_0 (t_s - t_M) + (t_{rp} - t_s) \int_{P_0}^{P_{rp}} \frac{j dP}{P_{rp} - P_0} \right] \quad (6)$$

where

- j_0 = the compressibility factor corresponding to the isothermal period;
- t_s = the time from the sample introduction to the start of the programme (min);

t_{rp} = the time from the start of the programme to the appearance of the component in maximum concentration (min).

(b) One (Fig. 2b) or two (Fig. 2c) of the three components are eluted at the temperature of sample introduction during the isothermal period while the two other or one of the three components are eluted during the programmed-temperature period that follows the isothermal step.

This case can be calculated by using eqns. 3 and 6.

(3) The cases belonging to the third theoretical possibility are combinations of the cases already discussed above, and therefore the retention indices can be calculated by applying the corresponding data. For example, consider the following case: a linear programmed-temperature period with a heating rate of β_1 is followed by an isothermal period at temperature T_1 and the run ends with a linear programmed-temperature period with a heating rate of β_2 (Fig. 3a). According to the peak distribution, there are the following six possibilities:

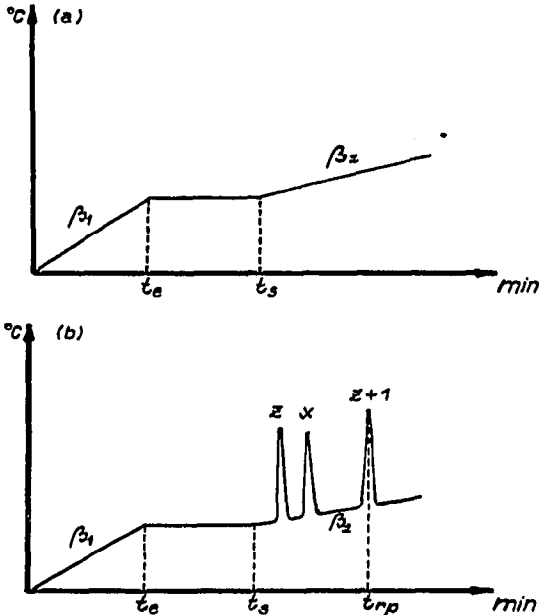


Fig. 3. (a) A linear programmed-temperature period with a heating rate of β_1 is followed by an isothermal period at temperature T_1 and the run ends with a linear programmed-temperature period with a heating rate of β_2 . (b) The three components of interest emerge during the second programmed-temperature period.

(a) All three components emerge during the first programmed-temperature step, and eqn. 2 can be applied.

(b) All three components are eluted in the isothermal period that follows the first programmed-temperature period, and eqn. 4 can be applied.

(c) All three components emerge during the second programmed-temperature period (Fig. 3b), and eqn. 7 can be used.

$$V_N = F \left[\int_{P_0}^{P_e} \frac{j dP}{P_e - P_0} (t_e - t_M^P) + j_e(t_s - t_e) + \int_{P_s}^{P_{rp}} \frac{j dP}{P_{rp} - P_s} (t_{rp} - t_s) \right] \quad (7)$$

where

t_s = the starting time of the second temperature programming taking the time of the sample introduction as $t = 0$;

t_{rp} = the time corresponding to the appearance of the component that is eluted during the second programmed-temperature period in maximum concentration;

P_{rp} = the inlet pressure corresponding to the time t_{rp} .

(d) One (Fig. 4b) or two (Fig. 4a) component(s) are eluted in one of the two programmed-temperature steps and two or one emerge(s) in the isothermal step, and eqns. 4 and 7 can be applied.

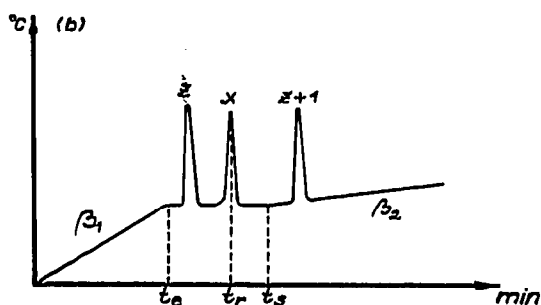
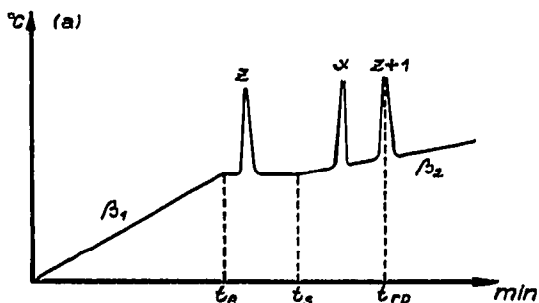


Fig. 4. (a) Two components are eluted during one of the two programmed-temperature steps and one component emerges during the isothermal step. (b) One component is eluted during one of the two programmed-temperature steps and the other two emerge during the isothermal step.

(e) One (Fig. 5a) or two (Fig. 5b) of the three components are eluted in the first programmed-temperature period and two or one emerge(s) in the second programmed period, and eqns. 5 and 7 can be used.

(f) Each peak appears during different periods. The symbols for this temperature programming are illustrated in Fig. 6. The values of the net retention volumes can be calculated according to eqns. 4, 5 and 7.

(4) In the fourth case, a new equation is required when one (Fig. 7a) or two (Fig. 7b) of the three components emerge(s) in a programmed-temperature step at a different heating rate to the other two or one component(s) eluted. When the components are eluted during the first step, the net retention volumes can be calculated

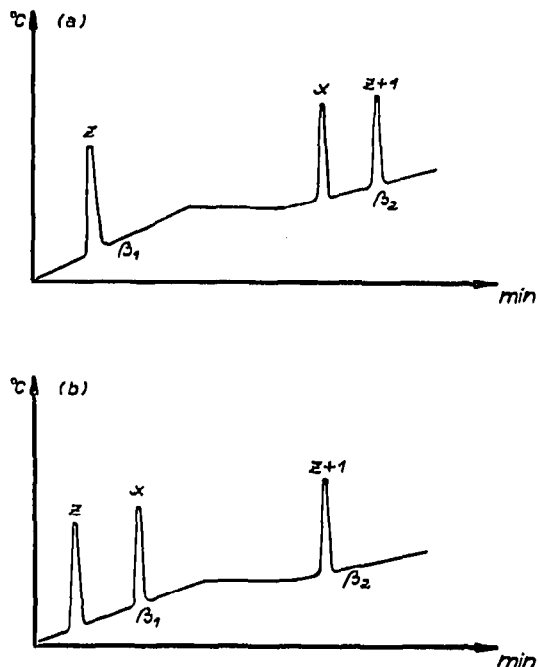


Fig. 5. (a) One component is eluted during the first programmed-temperature period and the other two emerge during the second programmed-temperature period. (b) Two components emerge during the first programmed-temperature period and one component is eluted during the second programmed-temperature period.

according to eqn. 5. Concerning the components eluted during the second step, we have derived the following equation:

$$V_N = F \left[\int_{P_0}^{P_{es}} \frac{j dP}{P_{es} - P_0} (t_{es} - t_M^P) + \int_{P_{es}}^{P_{rp}} \frac{j dP}{P_{rp} - P_{es}} (t_{rp} - t_{es}) \right] \quad (8)$$

When three programmed-temperature periods with different heating rates follow each other (including the possibility that the heating rates of the first and third periods are the same), there are the following three possibilities:

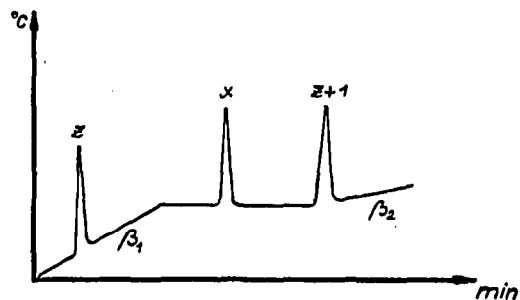


Fig. 6. Each peak appears during different periods.

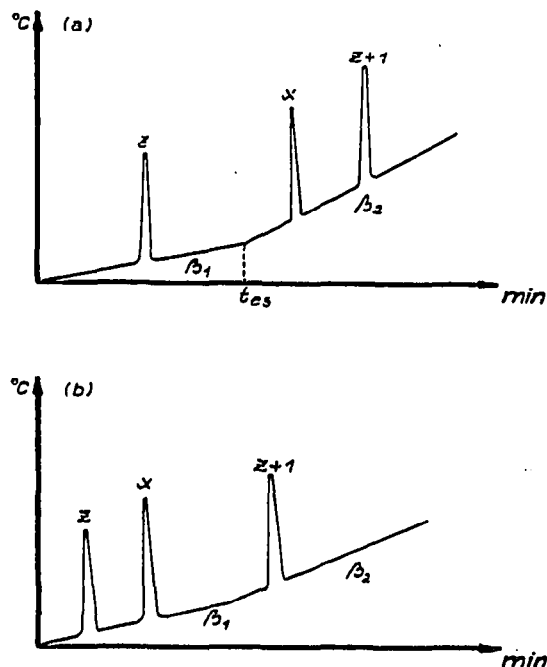


Fig. 7. (a) One component emerges during the first programmed-temperature step and the other two are eluted during the second programmed-temperature step. The two programmed-temperature steps have different heating rates. (b) Two components emerge during the first programmed-temperature step while the third component is eluted during the second programmed-temperature step.

(a) One (Fig. 8a) or two (Fig. 8b) peak(s) emerge(s) in the first step (eqn. 5) and two or one peak(s) in the third step. For calculating the net retention volume(s) of component(s) that appear in the third step, eqn. 9 can be applied:

$$V_N = F \left[\int_{P_0}^{P_{e,1}} \frac{j dP}{P_{e,1} - P_0} (t_{es1} - t_{Mp}) + \int_{P_{e,1}}^{P_{e,2}} \frac{j dP}{P_{e,2} - P_{e,1}} (t_{es2} - t_{es1}) + \int_{P_{e,2}}^{P_{rp}} \frac{j dP}{P_{rp} - P_{e,2}} (t_{rp} - t_{es2}) \right] \quad (9)$$

(b) One (Fig. 8d) or two (Fig. 8c) component(s) are eluted during the second programmed-temperature period and two or one in the third step, and eqns. 8 and 9 can be applied.

(c) Each component elutes at different periods (Fig. 8e), and eqns. 5, 8 and 9 can be used.

(5) Cases belonging to the fifth theoretical possibility can be constructed from the other cases discussed above.

We should like to emphasize that we have shown only the calculation of net retention volumes in this paper, as retention indices can then be calculated using eqn. 1 from the values of the net retention volumes.

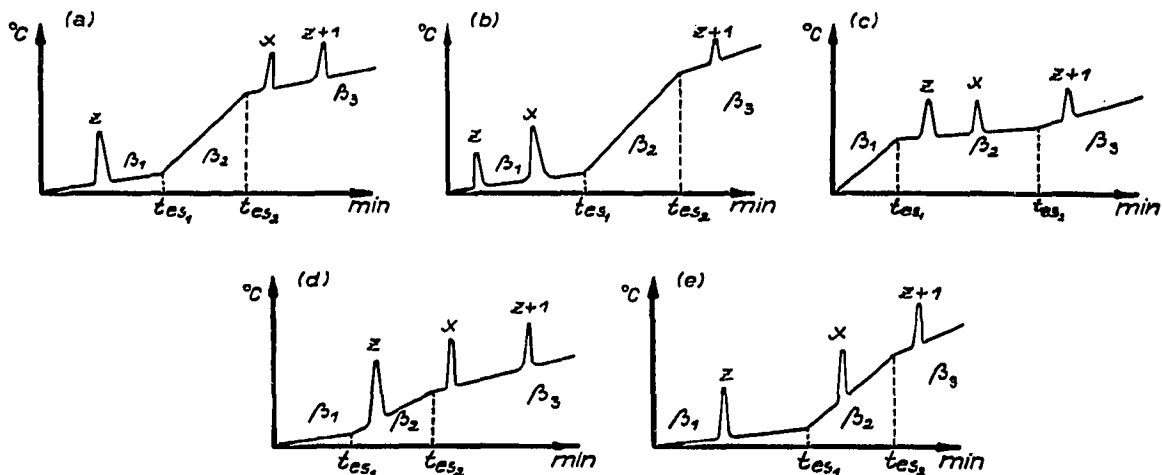


Fig. 8. (a) One component emerges during the first programmed-temperature step and the other two components are eluted during the third programmed-temperature step. (b) Two components emerge during the first programmed-temperature step and one component is eluted during the third programmed-temperature step. (c) Two components are eluted during the second programmed-temperature period and one component emerges during the third programmed-temperature period. (d) One component emerges during the second programmed-temperature period and the other two are eluted during the third programmed-temperature period. (e) Each component is eluted during different periods.

EXPERIMENTAL AND RESULTS

The analyses were carried out with Carlo Erba Model D, GV and GI-452 gas chromatographs, with flame ionization detectors, nitrogen as carrier gas and hydrogen and oxygen as auxiliary gases. Mixtures containing the examined substances and the *n*-alkanes were injected into the columns with Hamilton syringes. Speedomax G and W recorders were used (1 mV at full scale; response time 1 sec; chart speed 1.27 cm/min). Table I shows the characteristic data of the columns used.

TABLE I
CHARACTERISTIC DATA FOR THE COLUMNS USED

Column	Material	Length (m)	I.D. (mm)	Stationary phase	Proportion of liquid (% w/w)	Support (mesh)
1	Glass	2.1	3.0	Squalane	10.0	Chromosorb W (60-80)
2	Aluminium	2.0	4.0	DC-550	12.5	Chromosorb W (60-80)
3	Stainless steel	2.0	4.0	SE-30	10.0	Chromosorb W (60-80)
4	Aluminium	2.0	4.0	PEG-1540	10.0	Chromosorb W (60-80)
5	Aluminium	2.0	4.0	PEG-20M	10.0	Chromosorb W (60-80)
6	Stainless steel	2.0	4.0	DC-702	10.0	Chromosorb W (60-80)
7	Stainless steel	2.0	4.0	Porapak Q	—	Chromosorb W (60-80)

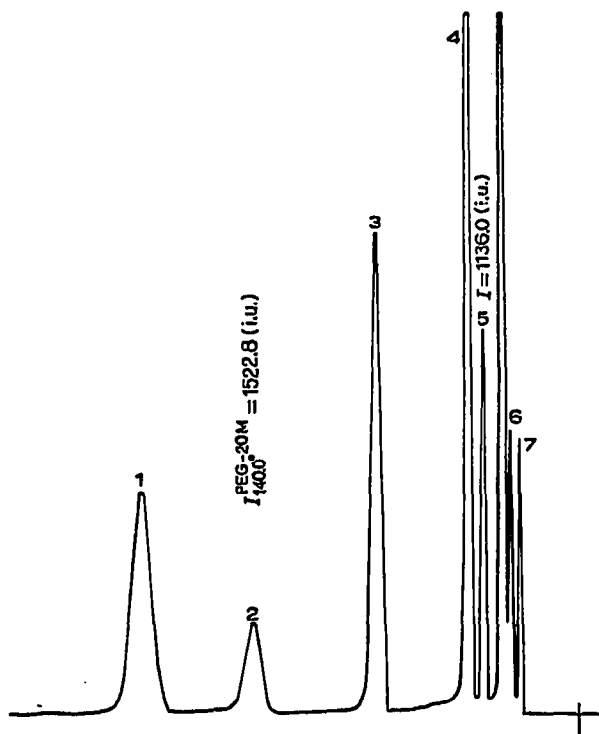


Fig. 9. Isothermal chromatogram obtained at 140° . 1 = Hexadecane, 2 = 2,2'-dichlorodiethyl ether, 3 = tetradecane, 4 = dodecane, 5 = 2-chloroethyl vinyl ether, 6 = ethanol, 7 = vinyl ether.

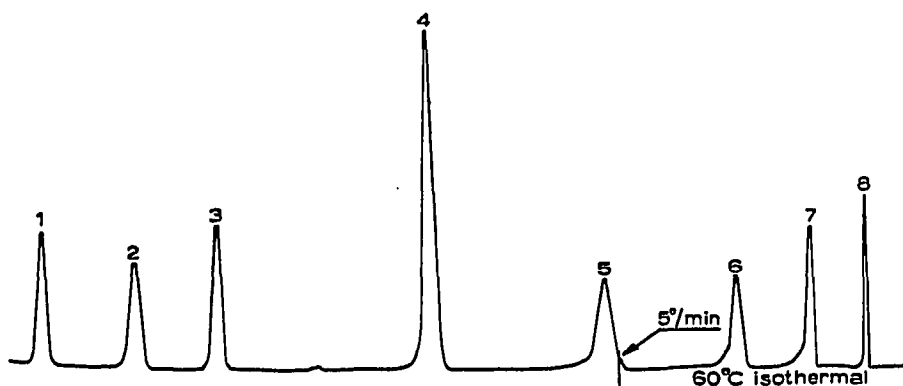


Fig. 10. Programmed-temperature chromatogram obtained with the sequence of 60° isothermal for 10 min followed by temperature-programming with a heating rate of $5^\circ/min$. 1 = Hexadecane, 2 = 2,2'-dichlorodiethyl ether, 3 = tetradecane, 4 = dodecane, 5 = 2-chloroethyl vinyl ether, 6 = decane, 7 = ethanol, 8 = vinyl ether.

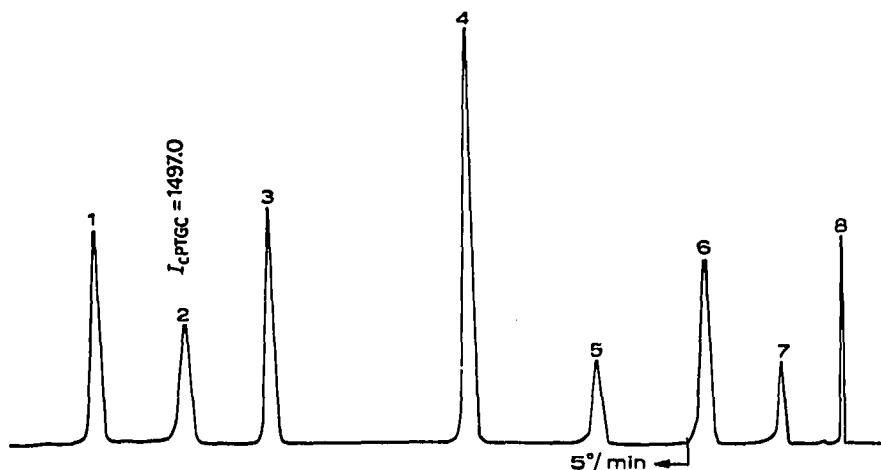


Fig. 11. Chromatogram obtained with the sequence of 60° isothermal for 7 min followed by temperature programming with a heating rate of 5°/min. 1 = Hexadecane, 2 = 2,2'-dichlorodiethyl ether, 3 = tetradecane, 4 = dodecane, 5 = 2-chloroethyl vinyl ether, 6 = decane, 7 = ethanol, 8 = vinyl ether.

The optimum operating conditions (inlet pressure and flow-rate of carrier gas, column temperature, heating rate, etc.) were worked out separately in each instance.

Figs. 9–13 show some of the chromatograms obtained. The operating parameters for these chromatograms were as follows:

Gas chromatograph: Carlo Erba Fractovap Model GV.

Detector: flame ionization.

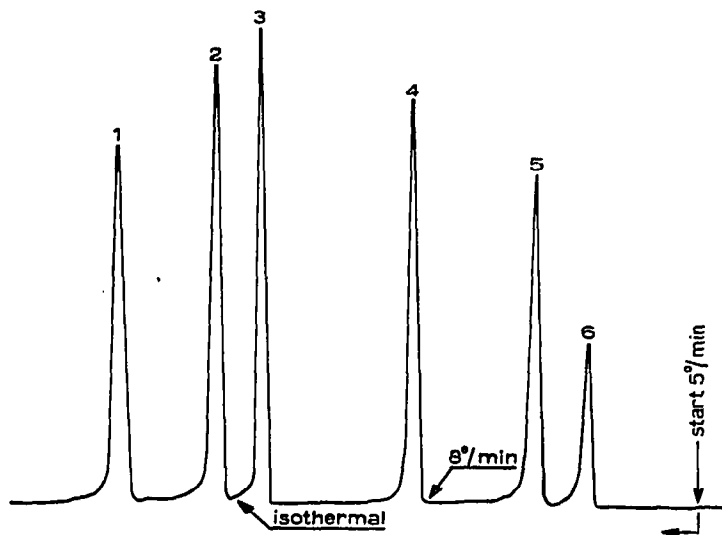


Fig. 12. Chromatogram obtained with the sequence of 60° isothermal for 3 min followed by temperature programming with a heating rate of 5°/min for 9 min and then 8°/min for 6 min, the run ending with a final isothermal period. 1 = Hexadecane, 2 = 2,2'-dichlorodiethyl ether, 3 = tetradecane, 4 = dodecane, 5 = 2-chloroethyl vinyl ether, 6 = decane.

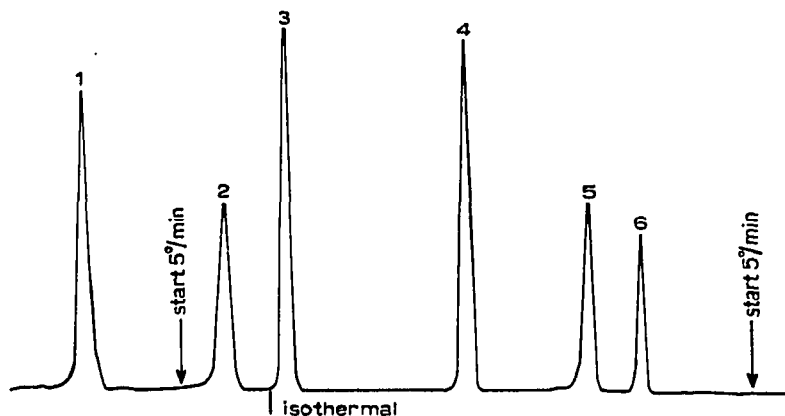


Fig. 13. Chromatogram obtained with the sequence of 60° isothermal for 3 min followed by temperature programming with a heating rate of 5°/min for 16 min and then an isothermal step for 3 min, the run ending with temperature programming with a heating rate of 5°/min. 1 = Hexadecane, 2 = 2,2'-dichlorodiethyl ether, 3 = tetradecane, 4 = dodecane, 5 = 2-chloroethyl vinyl ether, 6 = decane.

Column: aluminium, 2 m × 4 mm I.D.

Reference column: the same as the column.

Packing: 10% (w/w) of PEG-20M on Chromosorb W (60–80 mesh).

Column conditioning: conditioning for 24 h at 170° with nitrogen passing through the column at the rate of 25 ml/min.

Carrier gas: nitrogen at the flow-rate of 25 ml/min.

Inlet pressure of carrier gas: 1.30–1.50 kp/cm².

Inlet pressure of hydrogen: 1.85 kp/cm².

Inlet pressure of oxygen: 2.00 kp/cm².

Column temperatures:

Fig. 9: 140°.

Fig. 10: 60° for 10 min followed by temperature programming with a heating rate of 5°/min.

Fig. 11: 60° for 7 min followed by temperature programming with a heating rate of 5°/min.

Fig. 12: 60° for 3 min followed by temperature programming with a heating rate of 5°/min for 9 min and then 8°/min for 6 min, the run ending with a final isothermal period.

Fig. 13: 60° for 3 min followed by temperature programming with a heating rate of 5°/min for 16 min and then an isothermal step for 3 min, the run ending with temperature programming with a heating rate of 5°/min.

Detector temperature: 180°.

Recorder: Speedomax G.

Chart speed: 1.27 cm/min.

EXAMPLES OF CALCULATION

(1) The three components of interest were eluted with a linear temperature-programming step started at the beginning of the run. The retention temperatures of the components were as follows: n -C₁₄ alkane, 174.0°; 2,2'-dichlorodiethyl ether, 211.5°; and n -C₁₅ alkane, 213.5°. By substitution of the corresponding data in eqn. 2:

$$\begin{aligned} I_{\text{PTGC}}^{\text{PEG-20M}} &= 1400 + 100 \cdot \frac{211.5 - 174.0}{213.5 - 174.0} \\ &= 1400 + \frac{37.5}{39.5} 100 \\ &= 1494.9 \text{ index units.} \end{aligned}$$

(2) The following example shows the calculation of net retention volumes. The analysis was carried out by applying combined sequence PTGC consisting of four steps. An isothermal period at 60° lasting for 3 min was followed by temperature programming with a heating rate of 5°/min for 9 min and 8°/min for 6 min, run ending with a final isothermal step. The component of interest and the C₁₆ n -alkane were eluted just after the component emerged in the final isothermal step, and the C₁₄ n -alkane was eluted just before the component appeared in the temperature-programming period that preceded the final isothermal step. By substitution of the corresponding data in eqns. 10 and 11:

$$V_N = F \left[j_0(t_s - t_m) + \int_{P_0}^{P_e} \frac{j dP}{P_e - P_0} (t_{es} - t_s) + \int_{P_e}^{P_{rp}} \frac{j dP}{P_{rp} - P_e} (t_{rp} - t_{es}) \right] \quad (10)$$

$$\begin{aligned} V_N = F \left[j_0(t_s - t_M) + \int_{P_0}^{P_e} \frac{j dP}{P_{e1} - P_0} (t_{es} - t_s) + \right. \\ \left. + \int_{P_{e1}}^{P_{e2}} \frac{j dP}{P_{e2} - P_{e1}} (t_e - t_{es}) + j_e(t_{RP} - t_e) \right] \quad (11) \end{aligned}$$

then

$$V_{N(nC_{14})} = \left[0.8798 (3.00 - 0.49) + 9 \int_{1.27}^{1.33} \frac{j dP}{0.06} + 5.43 \int_{1.33}^{1.39} \frac{j dP}{0.06} \right] \cdot 25.0$$

$$\begin{aligned} V_{N(x)} = \left[0.8798 (3.00 - 0.49) + 9 \int_{1.27}^{1.33} \frac{j dP}{0.06} + 6 \int_{1.33}^{1.40} \frac{j dP}{0.07} + \right. \\ \left. + 0.8257 \cdot 0.94 \right] \cdot 25.0 \end{aligned}$$

$$V_{N(nC_{16})} = V_{N(x)} + 3.15 \cdot 0.8257 \cdot 25.0$$

$$V_{N(nC_{14})} = 364.3 \text{ ml of nitrogen}$$

$$V_{N(x)} = 395.8 \text{ ml of nitrogen}$$

$$V_{N(nC_{16})} = 460.8 \text{ ml of nitrogen}$$

$$I_x^{\text{PEG-20M}} (\text{PTGC}) = 1400 + 200 \left(\frac{\log 395.8 - \log 364.3}{0.102} \right)$$

$$= 1470.5 \text{ index units}$$

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Dr. Ernő Pungor and Prof. Dr. István Bayer for ensuring the possibilities of research and for useful cooperation.

REFERENCES

- 1 E. Kováts, *Helv. Chim. Acta*, 41 (1958) 1915.
- 2 H. van den Dool and P. D. Kratz, *J. Chromatogr.*, 11 (1963) 463.
- 3 W. E. Harris and H. W. Habgood, *Programmed-Temperature Gas Chromatography*, Wiley, New York, 1966.
- 4 A. T. James and A. J. P. Martin, *Biochem. J.*, 50 (1952) 679.
- 5 J. M. Takács, M. Rockenbauer and J. Hári, *J. Chromatogr.*, 81 (1973) 136.
- 6 E. B. Molnár, P. Móritz and J. Takács, *J. Chromatogr.*, 66 (1972) 205.