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## SOME FACTORS AFFECTING THE PRECISION IN THE DETERMINATION OF RETENTION INDICES ON POLAR CAPILLARY COLUMNS FOR GAS CHROMATOGRAPHY

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### SUMMARY

Three series of homologues were evaluated for use as standards for the determination of retention indices on capillary columns coated with a silicone with 60% cyanopropyl substitution. The homologous series tested were *n*-alkanes according to Kováts, 2-ketones and fatty acid methyl esters. Using a highly polar stationary phase, *n*-alkanes were adsorbed at the gas-liquid interface. The presence of such an additional retention mechanism resulted in *n*-alkane retention times being highly dependent on the stationary phase area to volume ratio. The retention of the polar homologues was, however, relatively insensitive in this respect, and the reproducibility of the retention index determinations was thereby highly improved.

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### INTRODUCTION

It is well documented that gas chromatographic (GC) retention data can be of great value in qualitative analysis. Such data are influenced by several factors, and in order to be useful in qualitative analysis they should be measured under standardized conditions and reported in a uniform manner. A relatively high insensitivity towards experimental parameters may be achieved when retention is measured under isothermal conditions as relative retention. A retention index system fulfilling these requirements was defined by Kováts<sup>1-3</sup> by reference to two standard *n*-alkanes, the peaks of which bracket the band of the compound of interest. The retention index is given by

$$I(x) = 100z + 100 \frac{\log t'_{R(x)} - \log t'_{R(P_z)}}{\log t'_{R(P_{z+1})} - \log t'_{R(P_z)}} \quad (1)$$

where  $I(x)$  is the retention index of compound  $x$ ,  $t'_{R(x)}$  is the adjusted retention time of compound  $x$  and  $P_z$  and  $P_{z+1}$  are the two *n*-alkanes which are eluted just before and just after the peak of compound  $x$ . The adjusted retention time is  $t'_R = t_R - t_m$ , where  $t_R$  is the retention time and  $t_m$  is the retention time of a non-retained substance. The

retention index scale elaborated by Kováts is founded on the relationship between  $\log t'_R$  and the carbon number,  $z$ , of the members of the homologous series of  $n$ -alkanes:

$$\log t'_R = az + b \quad (2)$$

where  $a$  and  $b$  are constants. Eqn. 2 is based on the assumption that each methylene group makes a constant contribution to the retention, this not being valid, however, for the lower homologues. Wainwright and Haken<sup>4</sup> showed non-linearity for methane and ethane; some workers consider that the equation shows linearity from  $n$ -pentane<sup>5,6</sup> onwards and others from  $n$ -heptane onwards<sup>7</sup>. Rohrschneider<sup>8</sup> and Golovnya and Grigoryeva<sup>9</sup> indicated linearity from  $n$ -nonane. A further complication may be a possible oscillation in the chromatographic properties of successive members of the homologous series. In order to avoid such problems, Kováts first defined the retention index on the basis of even-carbon  $n$ -alkanes. This was experimentally found to be unnecessary, and Kováts redefined the retention index and proposed an increment of 100 between  $n$ -alkanes<sup>3</sup>. The influence of odd or even carbon numbers on the retention of  $n$ -alkanes was later discussed by Vigdergauz and co-workers<sup>10-12</sup>.

It is well known that several compounds can have the same retention index on a given column, so retention data cannot be taken as a positive identification. Nevertheless, in combinations of GC and other analytical methods such as mass spectrometry and Fourier transform infrared spectrometry, retention data can be of considerable help. The utility of the retention data in this connection is, of course, dependent on their reliability.

The Kováts retention index system is very widely used, as demonstrated in recent reviews<sup>7,13</sup>. It can be applied to all possible combinations of solutes and stationary phases; the reliability is, however, not equally acceptable in all situations.

The reproducibility of retention indices between different laboratories is generally considered to be within one index unit for non-polar columns and within a few index units for polar columns. It is the opinion of the present authors that the precision obtained on polar columns could be improved. To achieve this, the sources of error should be identified and minimized.

Accurate values of the retention index are thought to result, in an ideal case, when retention is due solely to dissolution of the sample constituents in the pure stationary phase, thus reflecting only the distribution constants between the stationary and mobile phases. Other retention mechanisms, such as adsorption at the support surface and at the gas-liquid interface, should thus be suppressed. Capillary deactivation methods have been much improved in recent years. Now, typical polar solutes can be successfully chromatographed when present in at least nanogram amounts. Adsorption at the gas-liquid interface is, however, almost inevitable for the combination of  $n$ -alkanes and polar stationary phases<sup>14</sup>. The sample capacity for  $n$ -alkanes here becomes so low that, in practice, column overloading cannot be avoided, thus leading to retention times that are dependent on sample amounts in the range normally encountered in GC with flame ionization detection (FID). The retention of the reference substances,  $n$ -alkanes, thus becomes ill-defined and thereby also the retention indices. The use of more polar standards has been suggested for improving the precision<sup>3,15-17</sup>, e.g., 2-alkanones<sup>16,18</sup>,  $n$ -aldehydes<sup>19</sup> and methyl esters of saturated fatty acids (equivalent chain length)<sup>20,21</sup> have been investigated. The

function of the polar standards in isothermal analysis is primarily to provide a reference system based on dissolution in the stationary phase. In temperature-programmed analysis, the relative insensitivity of the retention index towards small fluctuations in experimental conditions is lost<sup>13</sup>. The distribution coefficients of different compounds are differently affected by changes in temperature. This sensitivity towards fluctuations in experimental conditions can be partly compensated, however, by the use of reference compounds having structures similar to those of the sample components, provided, of course, that the sample is homogeneous. The reasons for the use of alternative standards are therefore different in isothermal and temperature-programmed analysis.

A method for the calculation of invariant retention indices was described by Berezkin<sup>22,23</sup>:

$$I_i = I_0 + a_i (1/V_L) \quad (3)$$

where  $I_0$  is the limiting value of the retention index determined only by dissolution of a compound  $i$  in the stationary phase,  $a_i$  is a constant characterizing adsorption of this compound in the given system (the assumption being that the retention of the standard solute is due only to dissolution in the stationary phase) and  $V_L$  is the stationary phase volume in the column. This equation was suggested to be applied if the retention index was measured on packed columns. A similar equation, intended for use when retention indices were measured on capillary columns, was also described by Berezkin<sup>23,24</sup>:

$$I_i = I_0 + a_{ij} (1/k'_{st}) \quad (4)$$

where  $k'_{st}$  is the capacity ratio for a standard compound, being unaffected by adsorption effects. In our experience, it is possible to obtain linearity of eqn. 3, even though the standards are adsorbed on the gas-liquid interface, if the solute to be indexed is unaffected by adsorption. The constant will then be  $a_{st}$ , representing the adsorption on the gas-liquid interface of the standard compounds. It is worth noting that the constants  $a_i$  and  $a_{ij}$  in eqns. 3 and 4 includes the stationary phase surface area, the assumption for the validity of eqns. 3 and 4, thus being that with changes in the amount of stationary phase in the column, the gas-liquid interfacial area remains more or less unchanged<sup>25</sup>. This assumption is, however, not valid in this work, where columns of different dimensions and film thicknesses have been used. Therefore, a modified version of eqn. 3 has been applied:

$$I_i = I_0 + a_{st} (A_L/V_L) \quad (5)$$

where  $A_L$  is the gas-liquid interfacial area, the assumption being, in this instance, that the film thickness corresponds to the concentration of the coating solution and that the adsorption of the solute is negligible. For capillary columns used in GC,  $d_f$  is much smaller than  $r$ , and  $A_L/V_L$  is then approaching  $1/d_f$ .

In this work, indexing of retention data obtained on capillary columns coated with a polar stationary phase was compared for three different systems,  $n$ -alkanes, 2-ketones and methyl esters of saturated fatty acids. Further, the influence on the retention index of the method used for measurement of the gas hold-up time was

studied. The possible existence of an oscillation of the chromatographic properties of odd- and even-carbon *n*-alkanes was also examined.

## EXPERIMENTAL

### Columns

Five fused-silica capillary columns were coated with a silicone gum stationary phase (60-CN) having *ca.* 60% cyanopropyl, 37% methyl and 3% vinyl substitution. The synthesis of this stationary phase has been described previously<sup>26</sup>. One column was coated with a poly(dimethylsiloxane)-(5–6% diphenyl)-(0.1–0.3% methylvinylsiloxane) copolymer gum, PS264 (Fluka, Buchs, Switzerland) and used as a non-polar reference column.

### Reagents

For the silylation of the polar columns a biscyanopropylcyclosiloxane mixture<sup>26,27</sup> was used, and the non-polar column was silylated by reaction with decamethylcyclopentasiloxane<sup>28</sup>.

### Column preparation

*Polar columns.* Fused-silica capillary tubing (20 m × 0.32 mm, 26.5 m × 0.22 mm and 26.5 m × 0.25 mm I.D.) (Chrompack, Middelburg, The Netherlands) was used as the column material. Prior to silylation, the capillaries were pretreated according to a previously described method<sup>26</sup>, including leaching with 20% (v/v) hydrochloric acid, rinsing with hydrochloric acid (pH 3) and methanol and dehydration of the leached fused silica. Immediately after the dehydration step, the capillaries were deactivated with the biscyanopropylcyclosiloxane mixture [2% (w/v) in dichloromethane] and silylated at 395°C in an inert atmosphere<sup>26,27</sup>. The silylation was followed by rinsing with at least 10 ml of dichloromethane and further drying with nitrogen for several hours. Before coating, the stationary phase was dissolved in dichloromethane (analytical-reagent grade, freshly distilled) at concentrations giving film thicknesses of 2.0, 1.0, 0.16 and 0.20 μm. All the columns were coated by the static method at room temperature. When the solvent evaporation was complete, the columns were flushed with dry nitrogen overnight in order to remove residues of dichloromethane.

*Non-polar column.* Fused-silica capillary tubing (18 m × 0.32 mm I.D.) from Quartz et Silice (Paris, France) was used as the column material. The capillary was first flushed with nitrogen and then deactivated by reaction with decamethylcyclopentasiloxane at 370°C for 2 h<sup>28</sup>, followed by rinsing with dichloromethane and drying with nitrogen. PS264 was dissolved in dichloromethane at a concentration giving a film thickness of 0.5 μm. The coating procedure was the same as for the polar columns. The column dimensions are given in Table I.

### Cross-linking

The 60-CN stationary phase was immobilized by the use of azo-*tert.*-butane (ATB) (Ventron, Karlsruhe, F.R.G.). Columns coated with film thicknesses of 1 and 2 μm were flushed with ATB-saturated nitrogen for 1 h, first from one end and then from the other, while the columns coated with film thicknesses of 0.16 and 0.20 μm

TABLE I  
COLUMN DIMENSIONS

Column No.	Length (m)	I.D. (mm)	$d_f$ ( $\mu\text{m}$ )	Stationary phase
1	20.0	0.32	2.0	60-CN
2	20.0	0.32	1.0	60-CN
3	26.5	0.22	0.16	60-CN
4	26.5	0.25	0.20	60-CN
5	26.5	0.25	0.20	60-CN
6	18.0	0.32	0.5	PS264

were flushed for 30 min, also from each end. After flame sealing, the columns were cured at 220°C for 1 h after programming at a rate of 4°C/min. The non-polar stationary phase was not immobilized.

#### Testing conditions

In order to evaluate the stationary phase properties, and especially the retention indices, the polar columns were tested three times according to the following procedure: (a) after a short conditioning at 220°C for 30 min, programming rate 5°C/min; (b) after immobilization with ATB and conditioning as in (a), and (c) after rinsing with 10 ml of dichloromethane and conditioning as in (a).

#### Test mixtures

Three test mixtures were used: (1) *n*-alkane and 2-methylnaphthalene; C<sub>13</sub>–C<sub>20</sub> *n*-alkanes were used for the polar columns and C<sub>10</sub>–C<sub>16</sub> *n*-alkanes for the column coated with PS264; (2) 2-ketones (C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub> and C<sub>15</sub>) and 2-methylnaphthalene; (3) Saturated fatty acid methyl esters (FAME) (C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub> and C<sub>14</sub>) and 2-methylnaphthalene.

In standard 2, the ketone standard (C<sub>10</sub>) was 2-decanone, so that all carbon atoms were included when this was set to have retention index value of 1000 i.u. In standard 3, the FAME standard (C<sub>10</sub>) was methyl decylate, and therefore the methyl ester carbon was not included when this was set to have a retention index value of 1000 i.u.

The concentration of the *n*-alkanes in the standard intended for the polar columns was *ca.* 20 ng/ $\mu\text{l}$  and all other components were in the concentration range 100–200 ng/ $\mu\text{l}$ .

#### Apparatus

All chromatographic measurements were performed on a Carlo Erba (Milan, Italy) Mega gas chromatograph equipped with a flame ionization detector. Hydrogen was used as the carrier gas and the gas velocity was 40–50 cm/s at the oven temperature for the run. The injector temperature was held at 220°C, the detector temperature was 250°C and the injections were performed at column temperatures of 115, 125 and 135°C. A splitting ratio of 1:100 was used for all columns and temperatures and the injection volume was 1 or 2  $\mu\text{l}$ .

A laboratory data system, ELDS881 (Chromatography Data System, Kungs-

hög, Stenhamra, Sweden) was connected to the gas chromatograph for signal registration and treatment. The sampling frequency was set to give 40–400 data points over a chromatographed peak.

#### *Column dead-time measurement*

The column dead time was measured either by injection of methane or by the mathematical method described by Guardino *et al.*<sup>29</sup>.

### RESULTS AND DISCUSSION

#### *Determination of column dead time, $t_m$*

The retention indices were established using eqn. 2, by iteration of  $t_m$ , to obtain the the best fit to a straight line for carbon number *versus*  $\log t'_R$  according to Guardino *et al.*<sup>29</sup>. However, as discussed by Smith and co-workers<sup>30,31</sup> and Ettre<sup>32</sup>, this value of the mathematical dead time may not be the real column dead time, but a function of it. The different homologous series used here thus resulted in different  $t_m$  values. For example, on column 2 (Table I) at 125°C, the  $t_m$  value obtained by injection of methane was 41.4 s, the mathematical dead time obtained with *n*-alkane homologues was 40.8 s and with 2-ketone and the FAME homologues 34.2 and 36.6 s, respectively. In this work, the retention indices were calculated using the mathematical dead time obtained with the homologous series used for indexing. For the calculation of  $k'$  values, the dead time obtained by injection of methane was used.

#### *Column efficiency*

Polar columns are known to be less efficient than non-polar columns, especially when the stationary phase film is thicker. This is partly due to a lower diffusion coefficient in the polar stationary phases, but also to the difficulty in preparing columns with an even stationary phase film. In Table II the column efficiencies are represented by the height equivalent to a theoretical plate (HETP) values for *n*-heptadecane, *n*-eicosane, 2-pentadecanone, methyl myristate and 2-methylnaphthalene at 125°C. As expected, the HETP values for the *n*-alkanes are significantly higher than those for the other solutes. This is mainly due to the poor solubility of these non-polar compounds in the polar stationary phase, the sample capacity being low in this instance. As has been shown in an earlier evaluation of the 60-CN phase<sup>33</sup>, an overloading of *n*-alkanes on this stationary phase will lead to concentration-dependent retention indices. One way of avoiding overloading is, of course, to inject smaller amounts of *n*-alkanes on to the column, but detectability may then become a problem; the consequence of a low signal-to-noise ratio will be ill-defined retention times and hence possible uncertainty in the retention index determination. Table II also shows that the ATB treatment has a significant effect on the column efficiency. All the test solutes are affected here, but the *n*-alkanes give by far the greatest decrease in efficiency. This decrease is even more pronounced with increasing stationary phase film thickness. In Fig. 1, the chromatograms obtained before ATB treatment of the columns, using a test mixture containing *n*-alkanes, are shown for (a) column 2 at 135°C, (b) column 3 at 125°C and (c) column 5 at 125°C. The corresponding chromatograms obtained after ATB treatment are shown in Fig. 2a, b and c.

TABLE II  
COLUMN EFFICIENCIES AT 125°C

Column No. <sup>a</sup>	Treatment <sup>b</sup>	HETP (mm)				
		<i>n</i> -Heptadecane	<i>n</i> -Eicosane	2-Pentadecanone	Methyl-myristate	2-Methyl-naphthalene
1	a	3.39	1.35	0.78	0.96	0.80
2	a	1.71	1.15	0.54	0.67	0.55
3	a	1.27	1.14	0.42	0.50	0.29
4	a	0.48	0.58	0.28	0.32	0.27
5	a	0.45	0.34	0.28	0.29	0.27
6	a	—	—	0.36	0.38	0.31
1	b	5.14	2.78	0.94	1.21	0.95
2	b	4.96	3.26	0.96	1.25	0.81
3	b	1.69	2.00	0.55	0.68	0.36
4	b	3.27	7.71	0.80	1.11	0.30
5	b	1.04	1.79	0.39	0.44	0.28

<sup>a</sup> See Table I.

<sup>b</sup> a = Before ATB treatment, after conditioning at 220°C for 30 min; b = after ATB treatment and additional conditioning at 220°C for 30 min.

#### Comparison between retention index systems

Three retention index systems were evaluated for use on polar columns. The retention indices were determined by use of the equation  $\log t'_R = aI + b$ , where the constants  $a$  and  $b$  are dependent on the stationary phase and on the nature of the chemical group bound to the alkyl chain<sup>34</sup>, and  $a$  should deviate only slightly for all

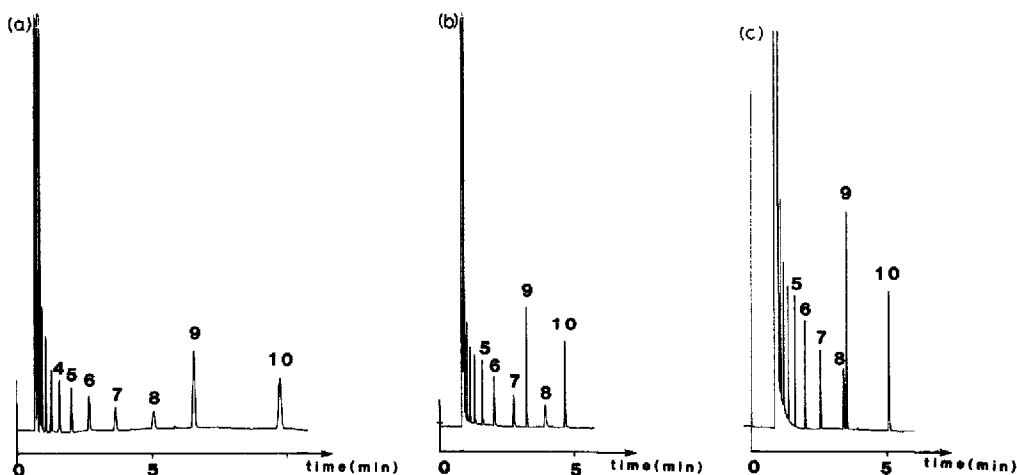


Fig. 1. Gas chromatograms (FID) of a mixture containing *n*-alkanes, 2-methylnaphthalene and biphenyl on three capillary columns coated with 60-CN stationary phase. Conditions: split injections at 135 or 125°C after the columns had been conditioned at 220°C for 30 min. (a) Column 2 at 135°C; (b) column 3 at 125°C; (c) column 5 at 125°C. Peaks: 1 = *n*-tridecane; 2 = *n*-tetradecane; 3 = *n*-pentadecane; 4 = *n*-hexadecane; 5 = *n*-heptadecane; 6 = *n*-octadecane; 7 = *n*-nonadecane; 8 = *n*-eicosane; 9 = 2-methylnaphthalene; 10 = biphenyl.

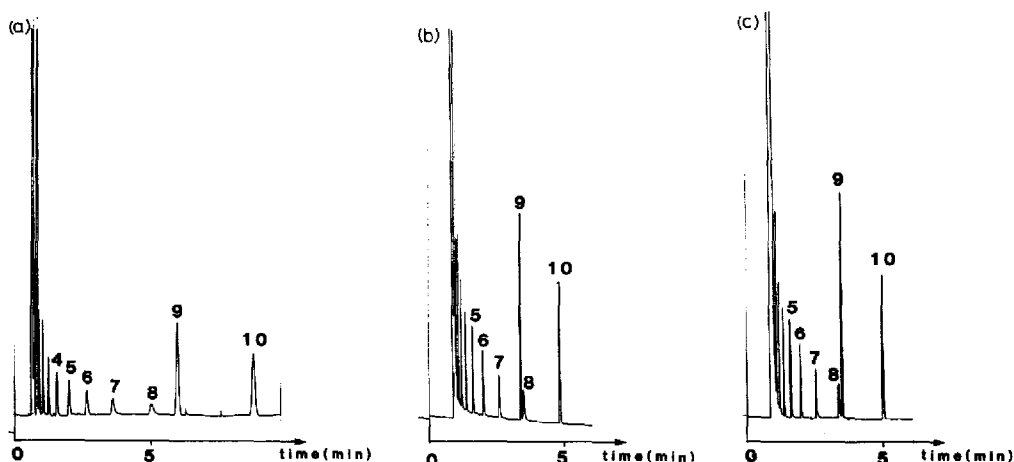


Fig. 2. Gas chromatograms (FID) of a mixture containing *n*-alkanes, 2-methylnaphthalene and biphenyl on three capillary columns coated with 60-CN stationary phase. Conditions: split injections at 135 or 125°C after ATB treatment and conditioning at 220°C for 30 min. (a) Column 2 at 135°C; (b) column 3 at 125°C; (c) column 5 at 125°C. Peaks as in Fig. 1.

series. However, this seems to be true for the non-polar column and for column 1, but for the other columns the *a* values for the *n*-alkane homologous series deviate considerably from the *a* values for the 2-ketone and FAME series (Table III). The greatest deviation was observed for column 3, where the adsorption effects for the

TABLE III  
CONSTANTS *a* AND *b* IN  $\text{LOG } t'_R = aI + b$ , AT 125°C (BEFORE ATB TREATMENT)

Column No.	Standard	$t_m$ (min)		$a \cdot 10^2$	<i>b</i>
		Mathematical	Methane		
1	<i>n</i> -Alkanes	0.675	0.690	0.172	-2.334
2	<i>n</i> -Alkanes	0.706	0.688	0.178	-2.713
3	<i>n</i> -Alkanes	0.873	0.855	0.202	-3.554
4	<i>n</i> -Alkanes	0.947	0.917	0.182	-3.221
5	<i>n</i> -Alkanes	0.932	0.907	0.182	-3.231
6	<i>n</i> -Alkanes	0.523	0.535	0.243	-2.656
1	2-Ketones	0.584	0.688	0.173	-1.076
2	2-Ketones	0.657	0.685	0.174	-1.364
3	2-Ketones	0.840	0.852	0.176	-1.927
4	2-Ketones	0.933	0.920	0.176	-1.873
5	2-Ketones	0.916	0.907	0.175	-1.877
6	2-Ketones	0.506	0.537	0.243	-2.179
1	FAME	0.605	0.688	0.173	-1.051
2	FAME	0.659	0.685	0.173	-1.339
3	FAME	0.841	0.830	0.177	-1.921
4	FAME	0.935	0.922	0.175	-1.847
5	FAME	0.917	0.906	0.175	-1.853
6	FAME	0.469	0.537	0.242	-1.847



TABLE IV  
*I* VALUES OF THE DIFFERENT STANDARDS IN THE DIFFERENT SYSTEMS, CALCULATED FROM  $\text{LOG } i_k^* = aI + b$ , AT 125°C (BEFORE ATB TREATMENT)

Column No.	<i>I</i> in the <i>n</i> -alkane system <sup>a</sup>			<i>I</i> in the 2-ketone system <sup>a</sup>						<i>I</i> in the FAME system <sup>a</sup>		
	<i>Ket</i> <sub>10</sub>	<i>Ket</i> <sub>11</sub>	<i>Ket</i> <sub>12</sub>	<i>FAME</i> <sub>10</sub>	<i>FAME</i> <sub>11</sub>	<i>FAME</i> <sub>12</sub>	<i>FAME</i> <sub>10</sub>	<i>FAME</i> <sub>11</sub>	<i>FAME</i> <sub>12</sub>	<i>FAME</i> <sub>10</sub>	<i>Ket</i> <sub>11</sub>	<i>Ket</i> <sub>12</sub>
1	1734.5	1836.9	1939.2	1749.4	1851.3	1952.8	1017.0	1116.6	1216.5	986.8	1087.3	1188.1
2	1728.6	1824.5	1926.4	1740.3	1839.0	1937.5	1009.9	1109.4	1209.0	989.4	1089.9	1190.2
3	1665.0	1755.9	1845.8	1676.5	1767.6	1857.7	1011.4	1112.0	1212.8	989.5	1089.2	1189.1
4	1701.5	1799.4	1897.0	1715.2	1812.8	1910.0	1012.1	1111.5	1211.1	988.3	1088.8	1189.5
5	1702.5	1800.5	1898.1	1716.3	1814.0	1911.3	1016.3	1116.2	1216.3	988.1	1088.4	1188.9
6	1195.2	1296.0	1396.7	1325.6	1426.1	1526.4	1130.1	1230.2	1330.4	871.2	970.1	1069.9

<sup>a</sup> *Ket*<sub>10</sub>, *Ket*<sub>11</sub>, *Ket*<sub>12</sub> = 2-decanone, 2-undecanone, 2-dodecanone; *FAME*<sub>10</sub>, *FAME*<sub>11</sub>, *FAME*<sub>12</sub> = methyl decylate, methyl undecylate, methyl dodecylate.

*n*-alkanes were pronounced (Fig. 1b). An effect of this deviation is that the increase between two consecutive members in the homologous series is no longer 100 i.u. In Table IV, the retention indices of 2-decanone, 2-undecanone, 2-dodecanone, methyl decylate, methyl undecylate and methyl dodecylate are given. The values were calculated for the *n*-alkane standard system and for the 2-ketone and FAME systems, respectively. As expected, on column 3 the difference between two consecutive homologues was only *ca* 90 i.u. when the *n*-alkanes were used as standards, whereas a value of 100 i.u. was found with the other two systems. As pointed out by Kováts<sup>3</sup>, "a homologous series is an ideal secondary reference series when the indices of its consecutive members increases by 100...". Obviously, this rule fails in systems where interfacial adsorption contributes seriously to the retention<sup>35</sup>.

#### *Influence of odd/even n-alkanes*

It has been suggested that plots of eqn. 2 would show an oscillating shape owing to differences in the molecular structure of odd- and even-carbon homologues<sup>10-12,36</sup>. In this work, looking at fine details, no such oscillation could be detected (Table V).

#### *Influence of film thicknesses and column dimensions on retention indices*

The peculiar behaviour of *n*-alkanes on the polar stationary phase can also be considered in terms of changes in retention indices with column dimensions and film thicknesses. Table VI shows the column capacity factors observed after a short column conditioning, and the ratio of gas-liquid surface area to stationary phase volume in the columns. In Table VII the corresponding retention indices are listed. These data are also presented in Fig. 3 as a plot of *I* vs.  $A_L/V_L$  according to eqn. 5. In the *n*-alkane reference system (Fig. 3a), a large effect was observed on the retention index for 2-methylnaphthalene when the area to volume ratio increased. The difference was more than 100 i.u. on comparing columns 1 and 3. The value of  $a_{st}$  in eqn. 5 was  $-14.1 \cdot 10^{-3}$ . However, the same trend was observed when 2-ketones and FAME were used as standards (Fig. 3b and c), but to a far smaller extent, the values of  $a_{st}$  being  $-1.15 \cdot 10^{-3}$  and  $-1.90 \cdot 10^{-3}$ , respectively, indicating that adsorption represents a significant contribution to the retention for the *n*-alkanes on this highly polar stationary phase.

TABLE V

EFFECT OF THE LINEARITY AND THE RETENTION INDEX OF 2-METHYLNAPHTHALENE AT 115°C, USING ODD- OR EVEN-CARBON *n*-ALKANES (BEFORE ATB TREATMENT)

Column No.	Carbons	$t_m$ (min)		<i>I</i>	$r^2$
		Methane	Mathematical		
1	13-20	0.678	0.667	2018.6	0.9999980
1	Odd: 13-19		0.666	2018.4	0.9999997
1	Even: 14-20		0.685	2018.5	0.9999994
3	13-20	0.860	0.899	1914.0	0.9999800
3	Odd: 13-19		0.896	1914.0	0.9999928
3	Even: 14-20		0.908	1913.6	0.9999915

TABLE VI

COLUMN CAPACITY FACTORS AT 125°C AND AREA TO VOLUME RATIO (BEFORE ATB TREATMENT)

 $t_m$  values used for the calculations were measured by methane injections.

Column No.	$k'$ values				$(A_L/V_L) \cdot 10^{-3}$ ( $\text{mm}^{-1}$ )
	<i>n</i> -Eicosane	2-Pentadecanone	Methyl myristate	2-Methyl naphthalene	
1	18.9	48.4	34.2	23.4	0.50
2	10.2	25.4	17.8	12.2	1.00
3	3.6	6.0	4.4	2.8	6.29
4	2.8	6.3	4.4	2.9	5.00
5	2.8	6.3	4.4	2.9	5.00
6	—	54.6	64.2	6.2	2.04

*Influence of temperature on retention indices*

In Table VIII, the changes in retention indices for 2-methylnaphthalene in the three reference systems are given. The temperature difference was 10°C (125–135°C). On the polar columns, the *n*-alkane reference system was the most sensitive,  $dI/10^\circ\text{C}$  being 36–33 i.u., whereas in the 2-ketone system it was only *ca.* 15 i.u. The retention

TABLE VII

RETENTION INDICES OF 2-METHYLNAPHTHALENE AT 125°C IN THE THREE DIFFERENT STANDARD SYSTEMS (AVERAGE OF TWO OR THREE MEASUREMENTS)

Column No.	Treatment <sup>a</sup>	<i>n</i> -Alkane		2-Ketone		FAME	
		<i>I</i>	<i>S.D.</i>	<i>I</i>	<i>S.D.</i>	<i>I</i>	<i>S.D.</i>
1	a	2053.4	0.62	1317.4	0.13	1307.4	0.13
2	a	2042.4	0.20	1316.9	0.10	1306.2	0.28
3	a	1946.9	0.12	1311.7	0.09	1296.7	0.01
4	a	2010.0	0.06	1311.1	0.05	1298.0	0.05
5	a	2010.6	0.08	1311.6	0.08	1298.6	0.04
6	a	1305.2	0.03	1111.8	0.07	982.6	0.02
1	b	2050.2	0.79	1320.6	0.29	1308.9	0.06
2	b	2014.7	0.09	1312.0	0.30	1300.1	0.04
3	b	1990.7	0.46	1319.5	0.01	1306.9	0.03
4	b	2019.4	1.10	1319.1	0.03	1307.5	0.09
5	b	2012.8	0.48	1313.6	0.05	1299.7	0.02
1	c	2011.1	0.53	1316.9	—	1307.0	—
2	c	1951.7	1.74	1302.6	0.81	1288.8	0.65
3	c	1986.1	0.16	1306.6	0.02	1290.1	0.03
4	c	1999.4	0.51	1309.9	0.09	1295.2	0.04
5	c	1996.6	0.22	1308.0	0.02	1293.1	0.01

<sup>a</sup> a = Before ATB-treatment, after conditioning at 220°C for 30 min; b = after ATB treatment and additional conditioning at 220°C for 30 min; c = after extraction with dichloromethane and additional conditioning at 220°C for 30 min.

indices on the non-polar column (column 6) showed the smallest dependence on temperature and all three reference systems were changed to the same extent. A linear relationship between  $I$  and temperature was obtained in the temperature range examined, *i.e.*, 115–135°C.

*Influence of immobilization with ATB*

Treatment with ATB did not give reproducible results (Table VII). When using 2-ketones or FAME for indexing, the treatment resulted in an increase in retention

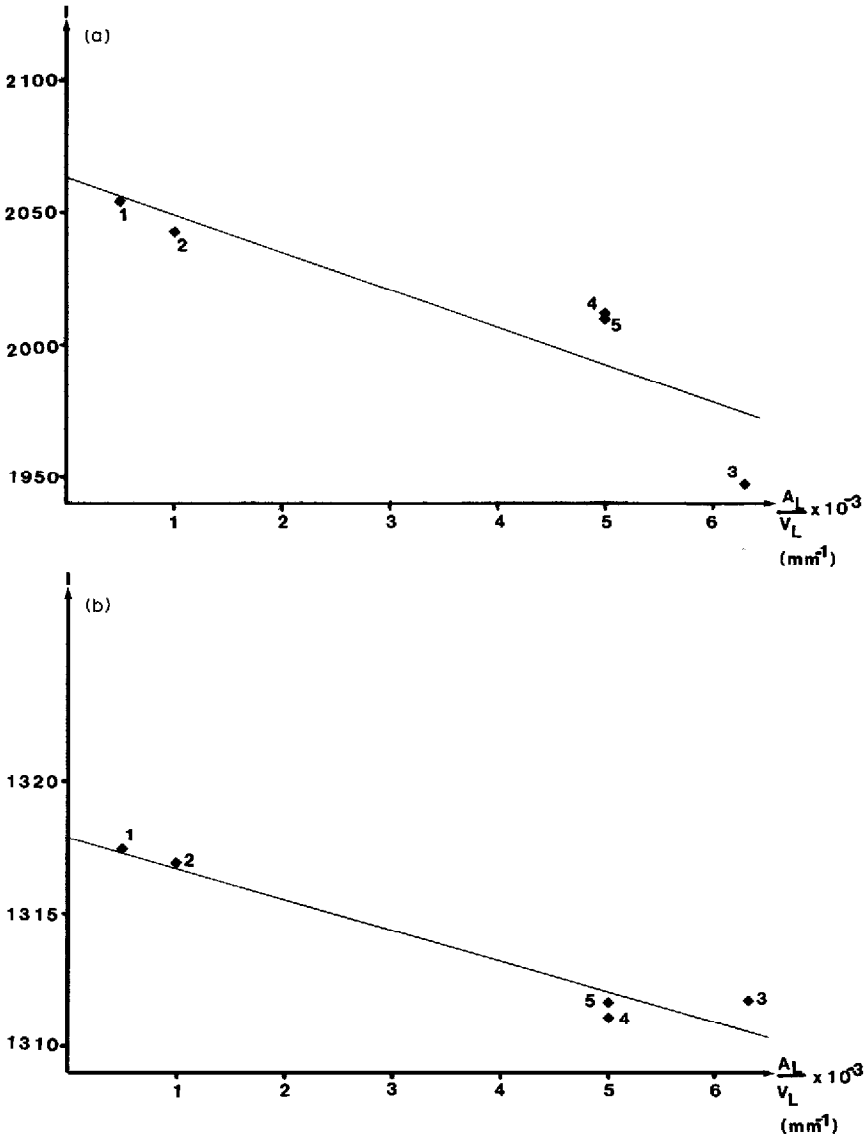


Fig. 3

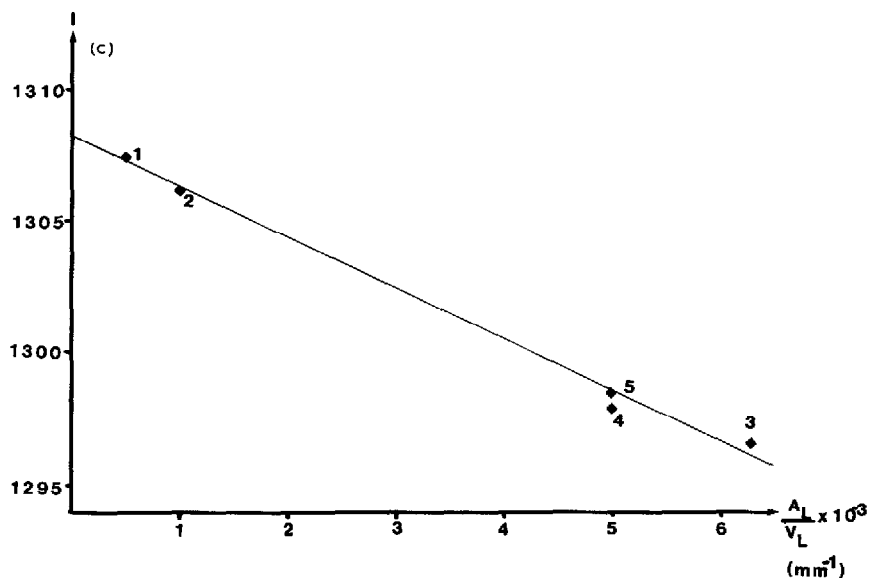


Fig. 3. Dependence of the retention index of 2-methylnaphthalene on the stationary phase area to volume ratio ( $A_L/V_L$ ). Conditions: split injections at 125°C after the columns had been conditioned at 220°C for 30 min. (a) *n*-Alkane standard,  $I = 2063 - 14.1 \cdot 10^{-3} (A_L/V_L)$ ,  $r = 0.890$ ; (b) 2-ketone standard,  $I = 1318 - 1.15 \cdot 10^{-3} (A_L/V_L)$ ,  $r = 0.968$ ; (c) FAME standard,  $I = 1309 - 1.90 \cdot 10^{-3} (A_L/V_L)$ ,  $r = 0.996$ . Numbers correspond to column numbers in Table I.

indices for 2-methylnaphthalene for all columns except for column 2. The same result was obtained when *n*-alkanes were used as standards; however, column 1 gave a slightly lower retention index in this system.

#### *Influence of column rinsing with dichloromethane*

The influence of extraction of the ATB-treated columns is demonstrated in Table IX. For the thick-film columns (columns 1 and 2), the degree of immobilization was approximately 70%. Column 3 was almost completely immobilized, and columns

TABLE VIII

TEMPERATURE DEPENDENCE OF THE RETENTION INDEX FOR 2-METHYLNAPHTHALENE IN THE THREE DIFFERENT STANDARD SYSTEMS (BEFORE ATB TREATMENT)

Column No.	$dI/10^\circ C (125-135^\circ C)$		
	<i>n</i> -Alkane	2-Ketone	FAME
1	36.2	15.6	22.4
2	34.5	15.2	21.7
3	33.1	14.9	21.1
4	33.5	14.9	21.2
5	33.8	15.0	21.2
6	6.9	6.2	7.0

TABLE IX  
PERCENTAGE DECREASE IN COLUMN CAPACITY FACTORS

Column No.	Temperature (°C)	Decrease in $k'$ values after extraction with $\text{CH}_2\text{Cl}_2$ (%)			
		<i>n</i> -Eicosane	2-Pentadecanone	Methyl myristate	2-Methylnaphthalene
1	125	17.3	30.8	32.0	30.3
1	135	19.4	42.0	31.1	29.5
2	125	1.0	28.2	27.5	33.2
2	135	—	27.9	28.1	32.2
3	125	20.7	0.5	0.2	4.6
3	135	17.8	0.5	1.1	3.6
4	125	9.5	11.7	11.1	12.6
4	135	8.8	11.2	11.0	12.1
5	125	—	17.7	17.7	19.7
5	135	17.2	20.7	20.3	21.9

4 and 5 were immobilized to *ca.* 80–90%. Solvent rinsing resulted in a similar decrease in  $k'$  values for 2-pentanone, methyl myristate and 2-methylnaphthalene, whereas the decrease for *n*-eicosane showed a significant deviation. Comparison of the retention indices before and after extraction in Table VII shows that the result of column rinsing is a lowering of column polarity, for all three reference systems. As reported earlier<sup>26,27</sup>, there may be an over-representation of polar or moderately polar fragments in the mixed cyclic compounds that are removed from the column on rinsing.

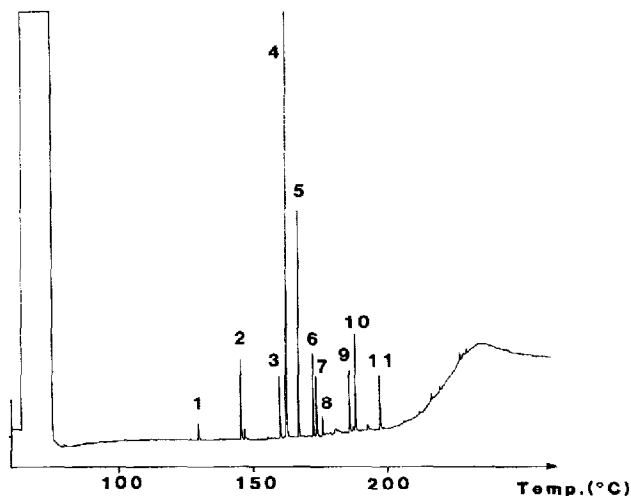


Fig. 4. Gas chromatogram (FID) of a fatty acid methyl ester mixture (rapeseed oil, Supelco) on a fused-silica capillary column coated with 60-CN stationary phase (column 4, Table I). The chromatogram was obtained after ATB treatment, rinsing with dichloromethane and conditioning at 220°C for 10 h. Conditions: splitless injection at 70°C and, after 2 min, programming at 5°C/min to 220°C. Peaks: 1 = 14:0; 2 = 16:0; 3 = 18:0; 4 = 18:1; 5 = 18:2; 6 = 18:3; 7 = 20:0; 8 = 20:1; 9 = 22:0; 10 = 22:1; 11 = 24:0.

### Applications

The suitability of the 60-CN stationary phase for the separation of FAME is demonstrated in Fig. 4 for a rapeseed oil sample on column 4.

### CONCLUSIONS

It may be considered that the greatest advantage of the Kováts retention index system is that it permits a comparison between results obtained in different laboratories. This universal role is hindered by the use of different systems for data presentation, including other retention index systems<sup>7</sup>. It is well known that the reproducibility of retention index determinations is much lower on polar than on non-polar columns. It seems that the explanation mainly involves the use of *n*-alkanes as reference substances. For the *n*-alkanes, a large proportion of the retention on polar columns is due to adsorption at the gas-liquid interface. Retention based on such a mechanism is highly dependent on the experimental conditions, such as amount injected, stationary phase surface area to volume ratio and small alterations in stationary phase properties. In this work, it has been shown that, when using polar columns, a relatively high insensitivity to experimental conditions in isothermal analysis can be obtained when utilizing 2-ketones or fatty acid methyl esters as standards. The merit of these, in this context, is that they interact with the stationary phase primarily by dissolution. The reproducibility of retention index determinations is thereby much improved. It seems that the universality of the retention index must be sacrificed for the improvement of reproducibility in cases where adsorption may contribute significantly to the retention.

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