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Hold-up time in gas chromatography[1] IV. Improved determination of Kováts' retention indices

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Abstract

Kováts' retention indices of *n*-alkanes eluted under isothermal conditions, in nine columns (packed and capillary), at temperatures between 60 and 150°C are calculated by three different methods, and the results are compared with their theoretical values. Two of the methods are based on the linearity assumption of the plot of the logarithms of the adjusted retention times versus carbon number. The third one does not accept the linearity assumption. Results show that methods based on the linearity assumption may produce a clear deviation from the true value of the retention index, while the third method tested, considerably improves the accuracy of the results, showing a similar precision than that obtained by the classical procedures. © 1998 Elsevier Science B.V.

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1. Introduction

Gas chromatography (GC), one of the most powerful tools in analytical chemistry, produces a single parameter which may be used for identification: the retention time of the compound under well-defined conditions. This parameter depends too much on the particular experimental conditions and has little value for interlaboratory comparisons. For this reason, relative values are preferred to this absolute value.

An early observation in GC was the fact that, in isothermal chromatograms, the logarithms of adjusted retention volumes (and times) of homologous series lied on a straight line when plotted versus the chain length or number of carbon atoms (hereafter, the semilog plot), with a slope that, for a given compound type, depended both on temperature and the nature of the stationary phase (SP). This observation has led, among other things, to the establishment of the retention index system proposed by Kováts [1], which has gained universal acceptance. Retention indices (I) have been used for interlaboratory comparisons, identification of components of complex mixtures, characterization of SPs, etc. [2–8]. They are related to structural and physicochemical properties of both the compound and the SP of the column. The value of I of a particular substance on a given SP, similarly to what happens to the specific retention volume (V_g), does not depend on the particular gas chromatograph or column type, nor on the experimental conditions, with the exception of temperature.

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Kováts used the *n*-alkanes as standards and defined the retention index of a *n*-alkane as one hundred times the number of carbon atoms. Then, he defined the *I* of any analyte eluted under any chromatographic conditions, as one hundred times the apparent number of carbon atoms of a hypothetical *n*-alkane that would have the same retention time as the analyte, under identical chromatographic conditions. Kováts' expression to find out the value of *I* of compound X is based on the mentioned observed linearity of the semilog plot:

$$I_X^{SP} = 100z + 100n \cdot \frac{\log V_g(X) - \log V_g z}{\log V_g(z+n) - \log V_g z} \quad (1)$$

where I_X^{SP} is the Kováts retention index of substance X on the stationary phase SP; $V_g(X)$ is the specific retention volume, and z and $z+n$ refer to the *n*-alkanes of z and $z+n$ carbons atoms eluted before and after the compound X. In the original proposal, z was an even number and n was taken as 2 because it was erroneously thought that not all *n*-alkanes behaved similarly, although it has later been observed that all *n*-alkanes do [9]. Kováts used decimal logarithms of V_g values; however the equation works equally well with natural logarithms and with other retention parameters related to V_g. Considering that V_g values are not simple to obtain, the normal practice is the use of adjusted retention times (t'_R) deduced from the experimental retention times (t_R) and the retention of an 'unretained' substance (the hold-up time, t_M).

The expression for I_X^{SP} has been generalized, and it is now accepted [6] that a greater accuracy in the estimation of I_X^{SP} is obtained if it is deduced from the straight line defined by the semilog plot of a minimum of four *n*-alkanes. Various methods for deducing retention indices based on this principle have been in use for years. The minimum accepted number of carbon atoms of the lightest *n*-alkane which may be included in the linearity plot is five or six, considering that the effect of the addition of a methylene group to shorter *n*-alkanes should not be independent of the chain length. Experience shows, however, that it seems to depend on the conditions of the chromatogram (stationary phase polarity, column temperature, etc. [10]). Results of experiments have been published in which a straight line may be drawn using retention data of *n*-alkanes from three to seven [11] and eight [12] carbon atoms. The linearity of the semilog plot would indicate that the retention times of *n*-alkanes in an isothermal chromatogram may be described by Eq. (2):

$$t_R(z) = A + \exp(B + Cz) \quad (2)$$

The precision with which *I* values may be obtained is normally accepted as no better than one or two index units. A good procedure for calculating retention indices would represent an improvement of the possibilities of GC as an identification procedure. Retention times can be measured with great precision, particularly with capillary columns, and other experimental parameters can nowadays be controlled reasonably well. If a reliable procedure of calculating retention indices was available, this would represent an important step forward in the use of GC for identification purposes or physicochemical studies, the latter becoming more and more important every day, as deduced from published papers on inverse gas chromatography (IGC).

The idea of a straight line defined as mentioned above has recently been questioned [13–15], as it was found that the retention times of the *n*-alkanes in an isothermal chromatogram are better defined by Eq. (3):

$$t_R(z) = A + \exp(B + Cz^D) \quad (3)$$

Therefore, if Eq. (3) rather than Eq. (2) represents the behaviour of the *n*-alkanes in an isothermal chromatogram, the value of *I* of any substance should be obtained from the apparent carbon number of a hypothetical *n*-alkane having the same retention time as the substance, as deduced from Eq. (3), and not from Eq. (2), as is the normal practice at present.

In this paper we shall compare values of retention indices as deduced with Eq. (3), with those obtained with well-established procedures based on Eq. (2), both according to their normal procedure. The only substances with known retention indices are the *n*-alkanes; therefore, experimental retention times of *n*-alkanes have been used.

2. Experimental

2.1. Apparatus and chromatograms

The apparatus, chromatographic columns and other experimental conditions have been described in a previous publication [14]. Attention is paid here to a printing error in Table 5 of that publication: the t_R values of *n*-tetradecane to *n*-heptadecane assigned to column 8 in the table do actually belong to column 9.

2.2. Mathematical treatment

The experimental retention times of all *n*-alkanes eluted in the same chromatogram were used to calculate the parameters *A*, *B*, *C* and *D* of Eq. (2) or Eq. (3), as applicable, and their *I* values were then deduced from the corresponding equation, by application of computer programs, as follows.

2.2.1. Methods based on linearity

Two programs were used: the method of García-Domínguez et al. [16] was applied with a program written in Fortran by the authors. The program used with the method of Guardino et al. [17] was written in Basic by Furr [18]. In both cases the program calculates the hold-up time (parameter *A* of Eq. (2)). *I* values are then deduced by Eq. (4):

$$I_X = 100 \cdot \frac{[\ln t_R(X) - A] - B}{C} \quad (4)$$

where $t_R(X)$ is the retention time of compound X, and *A*, *B* and *C* are the parameters of Eq. (2).

2.2.2. The LQG method

Data were fitted by a regression procedure that obtains least-squares estimates of the parameters of Eq. (3) in a nonlinear regression model by minimizing the residual sum of squares [13,14], with a program written in Fortran. Bearing in mind the definition of *I*, the expression used is:

$$I_X = 100 \cdot \sqrt[D]{\frac{\{\ln[t_R(X) - A]\} - B}{C}} \quad (5)$$

The LQG method (Lebrón–Quintanilla–García [14]) has been applied to all the *n*-alkanes of the chromatogram, but methane was not included because, contrary to what happens with the 'linearity methods', the hold-up time is not necessary to deduce *I* values.

Note that Eqs. (2) and (4) are the same as Eqs. (3) and (5), respectively, for a value of $D = 1$.

3. Results

Results obtained for a total of 115 chromatograms of mixtures of *n*-alkanes, in nine columns of different polarities, served to check the validity of Eqs. (2) and (3) for the deduction of *I* values, and were used to carry out a study of the accuracy and precision of the methods. Table 1 presents the experimental retention times used to elaborate Table 2 and Figs. 1 and 2, chosen as an example of the rest of the chromatograms.

3.1. Validity of the linearity assumption

Retention indices of *n*-alkanes, calculated according to Eqs. (4) and (5) may be compared with those defined by Kováts for the same substance. The validity of the corresponding assumption will be deduced from the differences between calculated and theoretical values. A random distribution round a value of zero would indicate that the model is correct. Fig. 1 shows an example of the type of plots obtained by this test. The example corresponds to experimental retention times of one chromatogram (Table 1), not average values of various runs; but the plots deduced for all other chromatograms are similar. It may be observed that the residuals of the model based on Eq. (3) indicate a good correlation between experiments and model. However, in the case of models based on Eq. (2), the residuals show a trend that clearly indicates a lack of fit. It may be deduced that methods based on Eq. (2) are not the best choice for determination of *I* values.

3.2. Accuracy

A general quantitation of the accuracy of the methods was carried out by examining the mean

Table 1
Retention times of *n*-alkanes used to elaborate Figs. 1 and 2, and parameters *A*, *B*, *C* and *D* of Eqs. (2) and (3)

	t_R (s)					
	HP-INNOWAX, 120°C	CPSIL-5CB, 150°C				
Alkane						
n-Pentane		243.54,	244.08,	244.14,	244.38,	244.50
n-Hexane		249.66,	250.14,	250.20,	250.44,	250.62
n-Heptane		259.62,	260.04,	260.16,	260.40,	260.58
n-Octane	370.98	276.06,	276.42,	276.60,	276.72,	276.96
n-Nonane	379.56	302.76	303.18,	303.36,	303.54,	303.78
n-Decane	394.14	346.56,	346.86,	347.10,	347.34,	347.58
n-Undecane	418.32	417.66,	418.14,	418.32,	418.62,	418.80
n-Dodecane	458.82	533.46,	533.88,	534.24,	534.54,	534.78
n-Tridecane	526.38	721.14,	721.86,	722.16,	722.52,	722.64
n-Tetradecane	638.64	1025.04,	1025.70,	1026.12,	1026.60,	1026.72
n-Pentadecane	825.30	1516.14,	1517.16,	1516.98,	1518.00,	1517.94
n-Hexadecane	1134.78					
n-Heptadecane	1647.48					
Values of the parameters						
LQG						
A	358.423	234.470,	235.056,	235.190,	235.365,	235.474
B	−1.90044	−0.50578,	−0.54300,	−0.56751,	−0.55241,	−0.53446
C	0.61467	0.59410,	0.60451,	0.61293,	0.60817,	0.60328
D	0.94973	0.94420,	0.93959,	0.93565,	0.93783,	0.93995
Ref. [16]						
A	357.963	233.763,	234.395,	234.416,	234.677,	234.730
B	−1.52374	−0.16168,	−0.17687,	−0.17088,	−0.17434,	−0.16345
C	0.51125	0.48835,	0.48949,	0.48909,	0.48937,	0.48855
Ref. [17]						
A	357.836	233.682,	234.282,	234.296,	234.563,	234.645
B	−1.51018	−0.15308,	−0.16475,	−0.15800,	−0.16218,	−0.15436
C	0.51036	0.48768,	0.48855,	0.48809,	0.48842,	0.48784

square deviation (MSD) from five chromatograms run under identical experimental conditions at each temperature, according to the following expression:

$$\mathrm{MSD} = \frac{1}{m}\sum_{i=1}^{m}\left(\bar{I}_{i\,(\mathrm{exp})} - I_{i\,(\mathrm{theo})}\right)^2 \qquad (6)$$

where m is the number of *n*-alkanes in the chromatogram, $\bar{I}_{i(\mathrm{exp})}$ is the mean value of I of *n*-alkane i (from the five runs), and $I_{i(\mathrm{theo})}$ is the theoretical value of I for that *n*-alkane; i represents the order of elution of the *n*-alkanes in the same chromatogram. Table 3 shows that the LQG model produces MSD values considerably smaller than the other two models. It becomes clear that I values deduced with Eq. (5) are much more accurate than those based on Eq. (4).

A *t*-Student test was applied to the different groups of data, in order to find out whether any of the methods showed any bias. One example of what is obtained is shown in Table 2. It may be observed that at a 5% significance level, the LQG method does not present significant differences between experimental and theoretical values for any of the *n*-alkanes, while the other two methods present a significant bias, as expected from an examination of Fig. 1. In other words: the LQG method allows the accurate determination of I values, if a sufficient

Table 2
Accuracy of the methods. Student's *t*-test applied to results on column CPSIL-5CB at 150°C

Alkane	LQG			Ref. [16]			Ref. [17]		
	$\bar{I}\pm$S.D. ($n=5$)	t_{cal} [a]	A/R [b]	$\bar{I}\pm$S.D. ($n=5$)	t_{cal} [a]	A/R [b]	$\bar{I}\pm$S.D. ($n=5$)	t_{cal} [a]	A/R [b]
n-Pentane	499.53±0.469	−2.239	A	500.00		–	500.77±0.136	12.702	R
n-Hexane	600.18±0.208	1.972	A	599.31±0.229	−6.699	R	599.44±0.209	−6.020	R
n-Heptane	699.98±0.123	−0.414	A	699.11±0.112	−17.859	R	698.89±0.130	−19.092	R
n-Octane	800.12±0.200	1.246	A	799.81±0.187	−2.267	A	799.46±0.185	−6.565	R
n-Nonane	900.00±0.070	0.059	A	900.37±0.159	5.170	R	899.99±0.095	−0.141	A
n-Decane	1000.02±0.055	0.676	A	1000.91±0.103	19.702	R	1000.59±0.059	22.461	R
n-Undecane	1099.95±0.042	−2.588	A	1101.08±0.154	15.622	R	1100.86±0.122	15.860	R
n-Dodecane	1200.00±0.020	−0.080	A	1201.00±0.074	30.300	R	1200.92±0.061	33.432	R
n-Tridecane	1300.02±0.022	1.668	A	1300.51±0.034	33.879	R	1300.58±0.044	29.496	R
n-Tetradecane	1399.99±0.013	−1.008	A	1399.59±0.078	−11.640	R	1399.81±0.047	−9.024	R
n-Pentadecane	1500.00±0.003	0.378	A	1498.32±0.172	−21.899	R	1498.70±0.107	−27.303	R

[a] If $t_{cal} > t_{tab}$, the calculated retention index is significantly different from the theoretical (the null hypothesis is rejected). t_{tab} (0.05, 4) = 2.776.
[b] A, accepted; R, rejected.

number of injections are performed (five should be enough in the worst case), while the other two methods would never give the correct value of *I*, independently of the number of injections. The situation may be clearly appreciated from Fig. 2, which is the representation of the individual values used to elaborate Table 2. The LQG values lie round the theoretical value, while in the other cases they are distributed round values which lie to one or the other side, depending on the region of *n*-alkanes considered.

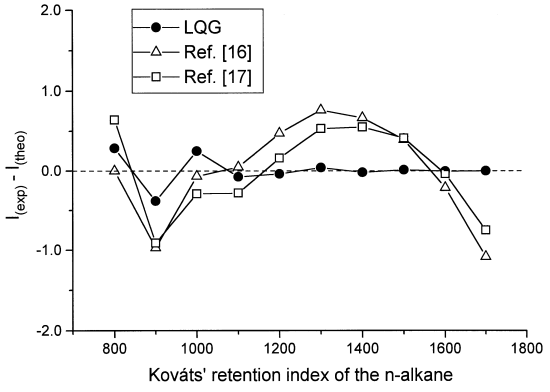

Fig. 1. Residuals of the retention indices calculated according to methods based on Eqs. (2) and (3). The plot corresponds to a single chromatogram on a HP-INNOWAX (crosslinked polyethyleneglycol) capillary column (60 m×0.25 mm, 0.25 μm) at 120°C. $I_{(exp)}$, value deduced according to the model. $I_{(theo)}$, theoretical value (100*z*).

3.3. Precision

The mean variance of each one of the three

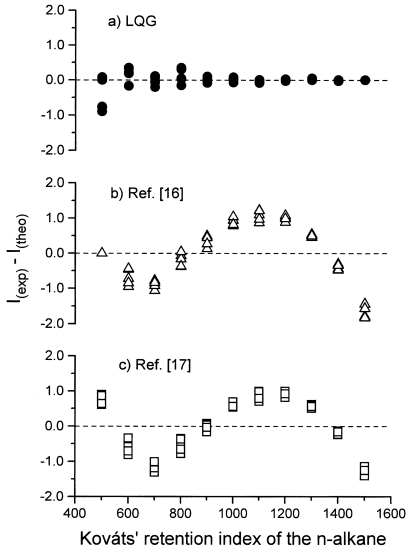

Fig. 2. Residuals of the retention indices calculated according to methods based on Eqs. (2) and (3), for five chromatograms run under identical conditions, on a CPSIL-5CB (polydimethylsiloxane) capillary column (50 m×0.32 mm, 0.43 μm) at 150°C.

Table 3
Accuracy of the methods. Mean squares deviations (MSD) from five chromatograms at each temperature

Column	Type	T (°C)	MSD		
			LQG	Ref. [16]	Ref. [17]
Squalane	Packed	60	0.0004	0.0028	0.0026
PS-255	Packed	90	0.0000	0.0018	0.0017
		120	0.0116	0.0223	0.0213
		150	0.0006	0.0954	0.2666
OV-3	Packed	90	0.0002	0.0076	0.0068
		120	0.0079	0.0107	0.0096
OV-11	Packed	60	0.0000	0.0001	0.0001
		90	0.0000	0.0087	0.0078
		120	0.0031	0.0182	0.0166
QF-1	Packed	60	0.0000	0.0012	0.0011
		90	0.0011	0.0080	0.0069
SUPEROX 20M	Packed	60	0.0126	0.0450	0.0397
		90	0.0013	0.0811	0.0755
CPSIL-5CB	Capillary	60	0.0002	0.0411	0.0369
		90	0.0048	0.2264	0.1933
		120	0.0352	0.3017	0.2518
		150	0.0247	0.6978	0.5845
HP-5	Capillary	60	0.0002	0.0215	0.0195
		90	0.0098	0.0603	0.0534
		120	0.0133	0.1184	0.1025
		150	0.0328	0.3746	0.3130
HP-INNOWAX	Capillary	90	0.0011	0.0226	0.0195
		120	0.0074	0.1722	0.1418
Average			0.0073	0.1017	0.0945

methods was used as an indicator of their precision. The expression employed was:

$$\overline{s^2} = \frac{1}{m}\sum_{i=1}^{m} s_i^2 \qquad (7)$$

In this expression $\overline{s^2}$ is the calculated mean variance, m is the number of n-alkanes in the chromatogram, s_i^2 is the variance of the retention index calculated for the n-alkane i in the chromatograms of that column and temperature (five in all cases), and i is the order of elution of the n-alkanes in the chromatogram.

Table 4 shows results for all columns at each temperature. The values indicate that there is no difference in the precision obtained by any of the three methods, perhaps with a slightly worse result for the case of the LQG method; differences are, however, minimal. Two other facts may be observed: results on packed columns are, as a rule, better. This is due to the fact that on the former, fewer n-alkanes have been injected, and also retention time differences between consecutive n-alkanes tend to be larger than on the equivalent capillary column at the same temperature. The second observation is that, for any one column, the variance increases with increasing temperature, again a reasonable consequence of the reduction of retention times.

In order to check whether the differences observed among the three methods were significant, a Fisher–Snedecor F-test applied to variances was carried out for each n-alkane. Results, at a 5% significance level, show that in general there is no difference between the variances of the I values deduced by any of the

Table 4
Precision of the methods. Comparison of mean variances under all conditions

Column	Type	T (°C)	Mean variance ($\bar{s}^2$)		
			LQG	Ref. [16]	Ref. [17]
Squalane	Packed	60	0.0022	0.0063	0.0057
PS-255	Packed	90	0.0000	0.0050	0.0048
		120	0.0014	0.0064	0.0033
		150	0.0185	0.0114	0.0103
OV-3	Packed	90	0.0080	0.0045	0.0042
		120	0.0220	0.0113	0.0104
OV-11	Packed	60	0.0000	0.0007	0.0007
		90	0.0021	0.0050	0.0044
		120	0.0231	0.0122	0.0115
QF-1	Packed	60	0.0037	0.0102	0.0094
		90	0.0225	0.0299	0.0265
SUPEROX 20M	Packed	60	0.0095	0.0041	0.0039
		90	0.0116	0.0114	0.0395
CPSIL-5CB	Capillary	60	0.0008	0.0021	0.0019
		90	0.0093	0.0045	0.0036
		120	0.0373	0.0085	0.0066
		150	0.0302	0.0183	0.0145
HP-5	Capillary	60	0.0041	0.0030	0.0027
		90	0.0242	0.0280	0.0235
		120	0.0270	0.0204	0.0173
		150	0.1426	0.1477	0.1174
HP-INNOWAX	Capillary	90	0.0037	0.0019	0.0018
		120	0.1471	0.1263	0.0915
Average			0.0249	0.0217	0.0187

three methods. In some cases in which a significant difference could be appreciated, always a greater precision was attributed to the LQG method, with the exception of the first *n*-alkane eluted, which in some cases showed a greater variance in the results of this method. An examination of the factors affecting precision has not been carried out in this paper because they are mainly experimental factors affecting precision of retention times, and in any case they affect all methods in the same way.

4. Conclusions

Results show that the methods based on the linearity of the 'semilog plot' of the *n*-alkanes present a clear lack of fit, producing *I* values of the *n*-alkanes (and any other substance eluted in the same chromatogram) which will show a clear deviation from the true value, the error depending on the zone of the chromatogram which is being considered.

On the other hand, it is once more confirmed that Eq. (3) describes the behaviour of *n*-alkanes in isothermal chromatograms with a high degree of reliability. Retention indices deduced from this equation may be obtained without the need for knowing the hold-up time of the chromatogram (necessary with the other two methods) and only four *n*-alkanes may be used. The *n*-alkanes chosen need not be consecutive, a clear advantage in the case of complex samples. *I* values obtained by this method show a similar precision to other classical methods, but much higher accuracy.

As a general rule, it is again confirmed that higher retention times (lower temperature or carrier-gas linear velocity, longer or more heavily loaded columns, etc.) improve precision.

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References

[1] E.sz. Kováts, Helv. Chim. Acta 41 (1958) 1915.
[2] G. Schomburg, G. Dielmann, J. Chromatogr. Sci. 11 (1973) 151.
[3] L.S. Ettre, Chromatographia 6 (1973) 489.
[4] M.V. Budahegyi, E.R. Lombosi, T.S. Lombosi, S.Y. Mészáros, S. Nyiredy, G. Tarján, I. Timár, J.M. Takács, J. Chromatogr. 271 (1983) 213.
[5] G. Tarján, S. Nyiredy, M. Györ, E.R. Lombosi, T.S. Lombosi, M.V. Budahegyi, S.Y. Mészáros, J.M. Takács, J. Chromatogr. 472 (1989) 1.
[6] V. Pacáková, L. Feltl. Chromatographic Retention Indices. An Aid to Identification of Organic Compounds, Ellis Horwood, Chichester, 1992.
[7] C.T. Peng, J. Chromatogr. A 678 (1994) 189.
[8] W.O. McReynolds, J. Chromatogr. Sci. 8 (1970) 685.
[9] E.sz. Kováts, Adv. Chromatogr. 1 (1965) 229.
[10] R.V. Golovnya, D.N. Grigoryeva, Chromatographia 17 (1983) 613.
[11] L.S. Ettre, Chromatographia 13 (1980) 73.
[12] M.R. Becerra, E. Fernández-Sánchez, J.A. García-Domínguez, J. García-Muñoz, M.J. Molera, J. Chromatogr. Sci. 20 (1982) 363.
[13] R. Lebrón-Aguilar, J.E. Quintanilla-López, J.A. García-Domínguez, J. Chromatogr. A 760 (1997) 219.
[14] J.E. Quintanilla-López, R. Lebrón-Aguilar, J.A. García-Domínguez, J. Chromatogr. A 767 (1997) 127.
[15] J.A. García-Domínguez, J.E. Quintanilla-López, R. Lebrón-Aguilar, J. Chromatogr. A 803 (1998) 197.
[16] J.A. García-Domínguez, J. García-Muñoz, E. Fernández-Sánchez, M.J. Molera, J. Chromatogr. Sci. 15 (1977) 520.
[17] X. Guardino, J. Albaigés, G. Firpo, R. Rodríguez-Viñals, M. Gassiot, J. Chromatogr. 118 (1976) 13.
[18] H.C. Furr, J. Chromatogr. Sci. 27 (1989) 216.